

# Blood volume fraction mapping for angiogenesis assessment in a novel human glioblastoma stem cell model

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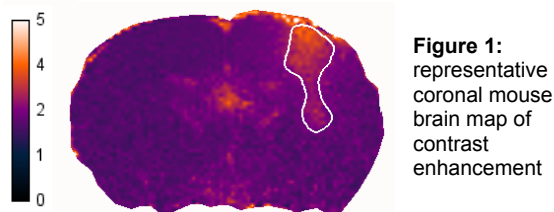
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**Introduction:** It is important in clinics as well as in preclinical studies to identify parameters for tumor angiogenesis that can predict response to antiangiogenic therapies. One such parameter related to angiogenesis is the tumor blood volume. In this study, we quantified the blood volume fraction (BVf) in a novel orthotopic mouse model derived from human glioblastoma stem cells [1] using the Rapid Steady State  $T_1$  (RSST<sub>1</sub>) magnetic resonance MRI technique [2] with Gd-DOTA, a contrast agent approved for clinical use.

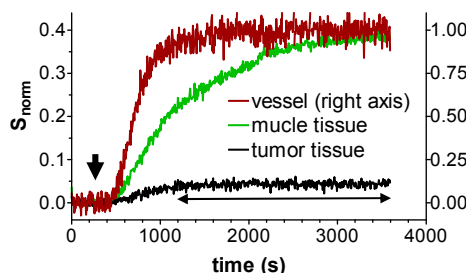
**Material and methods:** Human glioblastoma stem cells ( $5 \times 10^5$  in 5  $\mu$ l phosphate buffered saline) were injected into the right caudate nucleus (Bregma level, 2 mm lateral, 2.5 mm depth) of  $n = 6$  nude mice. One to three months after tumor implantation, they were imaged in a 47/40 Bruker Biospec USR AV III scanner using a homogenous body coil for transmission and a Bruker mouse head surface coil for signal reception with a field of view of  $15 \times 15$  mm<sup>2</sup> and 8 slices of 0.7 mm for all acquisitions. The mice were equipped with an intraperitoneal catheter and injected with 6 mmol/kg (12 ml/kg) Gd-DOTA inside the scanner 5 minutes after the start of the dynamic RSST<sub>1</sub> acquisition (Inversion Recovery prepared Modified-Driven-Equilibrium-Fourier-Transform (MDEFT),  $TR/TR_{\text{echo}}/T_{\text{inversion}}/TE = 750/6.5/303/1.2$  ms, flip angle =  $10^\circ$ , matrix  $32 \times 32$ , duration 6 s per repetition) of 60 minutes duration, followed by  $T_1$  weighted images (Multi-Slice-Multi-Echo,  $TR/TE = 300/6.3$  ms, NA = 8, matrix  $128 \times 128$ ) acquired 60 minutes after Gd-DOTA injection. Prior to contrast agent injection,  $T_2$  weighted (Rapid-Acquisition-Relaxation-Enhanced imaging,  $TR/TE_{\text{effective}} = 3500/33$  ms, NA = 6, matrix  $128 \times 128$ ),  $T_1$  weighted and proton density weighted MDEFT images  $TR/TR_{\text{echo}}/T_{\text{inversion}}/TE = 10$  s/6.5 ms/9 s/1.2 ms, matrix  $32 \times 32$ , duration 1 min 20 s) were acquired. Contrast enhancement was quantified as  $(S_{\text{post}_T1w} - S_{\text{pre}_T1w})/S_{\text{pre}_T1w}$ ,  $S_{\text{post}_T1w}$  and  $S_{\text{pre}_T1w}$  being the  $T_1$  weighted (T1w) pre and post contrast signal, respectively. The BVf maps were obtained according to  $S_{\text{norm}} = (S_{\text{post}} - S_{\text{pre}})/S_0$  [2], where  $S_{\text{pre}}$  and  $S_{\text{post}}$  are the average pre and post contrast RSST<sub>1</sub> images during the steady state phase, while  $S_0$  is the proton density weighted signal. When the contrast agent is confined to the vascular compartment the normalized signal equals the BVf:  $S_{\text{norm}} = \text{BVf}$  [2].

**Results and discussion:** During the 1<sup>st</sup> month, tumor growth was not detectable with either MR technique. During the 2<sup>nd</sup> month, the tumor occurrence was hardly visible on  $T_2$  weighted acquisitions, but contrast enhancement in the order of 50 to 400% could be observed (Figure 1) on  $T_1$  weighted acquisitions. Figure 2 displays typical plots of  $S_{\text{norm}}(t)$  from one mouse. The dynamic RSST<sub>1</sub> acquisition showed no contrast agent leakage in the tumor region, similar to the RSST<sub>1</sub> signal from a major vessel. For comparison, a typical contrast agent leakage profile such as observed in muscle tissue is also shown. Fifteen to at least 35 minutes after contrast agent injection the signal is in a steady state reflecting the thermodynamic equilibrium signal from the vascular compartment. Under the assumption that the contrast agent is confined to the vascular compartment after intraperitoneal injection [3], the BVf in the tumor region is  $0.034 \pm 0.010$  (range 0.020 to 0.043) while it is  $0.023 \pm 0.004$  (range 0.018 to 0.029) contralateral to the tumor. In most mice, clinical signs appeared 3 months after tumor implantation.

**Conclusion:** Similar to clinically encountered tumors, this novel tumor model develops slowly, has an infiltrating growth pattern and it mimics the cellular heterogeneity encountered in clinical tumors. During the 2<sup>nd</sup> month of tumor development, the tumor vasculature is impermeable to Gd-DOTA since dynamic RSST<sub>1</sub> acquisitions revealed no signal time course typical for contrast agent leakage in the tumor tissue. This study demonstrates that quantitative micro- and macrovascular BVf maps for angiogenesis assessment can be acquired noninvasively using the RSST<sub>1</sub> technique with intraperitoneal administration of Gd-DOTA. This novel glioblastoma model is useful for the evaluation of new treatment strategies such as antiangiogenic agents.



**Figure 1:** representative coronal mouse brain map of contrast enhancement



**Figure 2:** typical signal time course ( $n = 1$ ) after intraperitoneal injection of 6 mmol/kg Gd-DOTA (vertical arrow), and resulting steady state interval (horizontal arrow)

[1] Platet et al, Cancer Lett. 2007; [2] Perles-Barbacaru and Lahrech, JCBFM 2007; [3] Moreno, NMR Biomed 2006