

# Evaluation of MR Imaging Biomarkers of the Infiltrative and Vascular Phenotype in Orthotopic Murine RG2 Gliomas

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## Introduction

Despite recent treatment advances, high grade glioma remains a leading cause of tumour-related morbidity and mortality in both children and adults, with 5-year survival rates less than 20%. In diffuse infiltrative gliomas, the most common form of primary brain tumour in adults, cells preferentially invade along the myelinated fibres in white matter tracts and obtain essential nutrients by co-opting existing vasculature. Low grade paediatric gliomas typically present with a similarly infiltrative growth pattern. Appropriate delineation of gliomas using conventional Gd-DTPA enhanced MRI can be problematic, as the blood brain barrier remains intact in areas of infiltrative growth, precluding extravasation of Gd-DTPA contrast agent. Anti-VEGF treatment can also restore the blood brain barrier, resulting in reduced tumour detectability in Gd-DTPA enhanced MRI [1]. Furthermore, such treatment strategies have been shown to promote glioma invasion [2]. The combined use of intravascular USPIO contrast agents may facilitate the detection of both residual tumour and infiltrative areas. In this study, the spatial relationship of quantitative Gd-DTPA and USPIO enhanced MRI was compared in orthotopically propagated RG2 gliomas in mice, previously shown to display an infiltrative pattern of growth within mouse brain [3], and histological correlates sought.

## Methods

RG2 rat glioma cells engineered to stably express firefly luciferase ( $5 \times 10^3$ ) were implanted orthotopically in the brains of female NCr nude mice ( $n=6$ ). The establishment and growth of tumours was monitored by bioluminescence imaging using a Xenogen IVIS<sup>®</sup> 200. MRI was performed on established tumours on a Bruker 7T horizontal bore microimaging system, using a 3cm birdcage <sup>1</sup>H coil over a 2.5cm FOV. Both lateral tail veins were cannulated with heparinised 27G butterfly catheters for the administration of contrast agents. RARE  $T_2$  weighted axial images of the brain were acquired to localise the tumour.  $T_1$  weighted RARE ( $T_{\text{eff}}=7.5\text{ms}$ ,  $T_R=1300\text{ms}$ , 4 averages, matrix= $256 \times 256$ ,  $20 \times 1\text{mm}$  slices) and inversion recovery (IR) true-FISP ( $T_E=1.2\text{ms}$ ,  $T_R=2.5\text{ms}$ , scan  $T_R=10\text{s}$ ,  $T_1=25\text{--}1450\text{ms}$ , 50 inversion times, 8 averages, matrix= $128 \times 128$  1 mm slice) sequences were performed prior to, and following, administration of 0.1mmol/kg Gd-DTPA i.v. (Magnevist, Schering). Multi gradient-recalled echo (MGE) images ( $T_E=6\text{--}28\text{ms}$ ,  $T_R=200\text{ms}$ , 8 echoes, 8 averages, matrix= $256 \times 256$ ,  $9 \times 1\text{mm}$  slices) were acquired prior to, and following, USPIO administration i.v. ( $150\mu\text{mol Fe/kg}$  P904, Guerbet). IR-trueFISP and MGE data were fitted on a voxel-by-voxel basis using in-house software, providing maps of tumour spatial heterogeneity of native  $T_1$  and  $\Delta R_1$  with Gd-DTPA, and  $\Delta R_2^*$  with USPIO, the latter used to synthesise maps of fractional blood volume (fBV, %). The median value of each parameter in each tumour was determined. Following MRI, mice were administered with 15mg/kg of the perfusion marker Hoechst 33342 i.v., and one minute later the whole brain was rapidly excised and snap frozen over liquid nitrogen. The perfused vasculature was subsequently assessed by fluorescence microscopy, and haematoxylin and eosin (H&E) staining performed to assess tumour extent and infiltration.

## Results & Conclusions

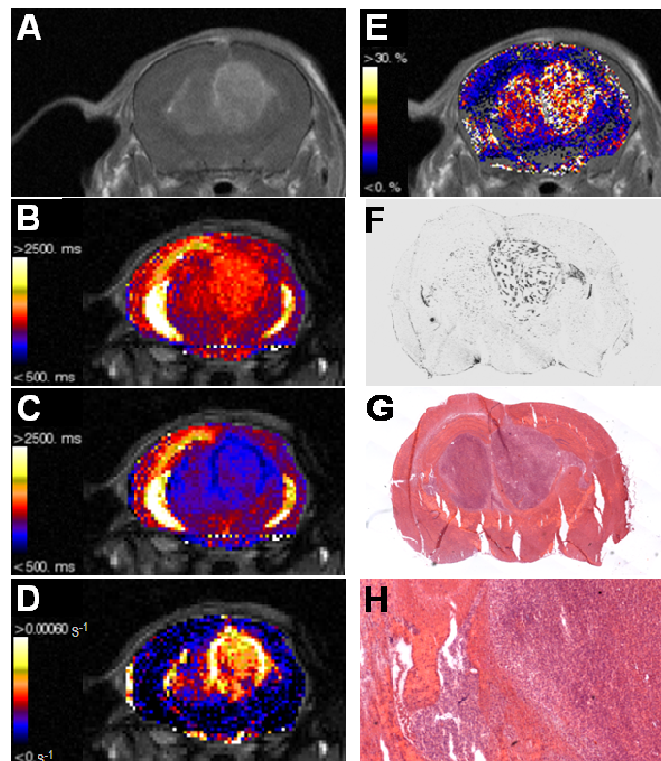
RG2 gliomas were well established at the time of MRI, with a mean tumour volume, as established by segmentation, of  $46 \pm 10\text{mm}^3$ . Parenchymal glioma enhancement seen in  $T_1$  weighted images following administration of Gd-DTPA was spatially heterogeneous, as exemplified in the tumour shown in Figure 1 (1A). Tumour regions exhibiting a shorter native  $T_1$  (1B) typically had a noticeably lower  $\Delta R_1$  (1D). In contrast, tumour areas with longer baseline  $T_1$  were associated with larger  $\Delta R_1$ , and which was typically observed at the tumour boundary. The mean  $\Delta R_1$  for the cohort was  $0.24 \pm 0.04\text{s}^{-1}$ . The second generation USPIO contrast agent P904 was administered to delineate the tumour vasculature and to quantify the fBV of the whole tumour. It was clear from the fBV maps that the blood volume of the RG2 gliomas was substantially higher than the surrounding brain, and that it was also heterogeneously distributed (1E). As expected, tumour regions of relatively high fBV were associated with larger  $\Delta R_1$ . Evaluation of perfusion by Hoechst 33342 uptake (1F) corroborated the blood volume data, with high Hoechst uptake in tumour areas of elevated fBV. It was also noted that the vessels within the well perfused areas were of a markedly larger calibre ( $\sim 50\mu\text{m}$ ) than normal brain vessels ( $\sim 8\mu\text{m}$ ). The overall mean tumour fBV was  $8.1 \pm 0.8\%$ , greater than that reported for orthotopic RG2 gliomas grown in rats [4]. Tumour extent was assessed from composite images of H&E stained tissue sections (1G), and from which it was observed that the tumours grew as partially well encapsulated, partially infiltrative masses, with some local infiltration (1H). Typically the areas of invasion presented as finger-like projections and clusters of tumour cells a small distance from the tumour border. These areas of infiltration correlated with regions of relatively short native  $T_1$ , low  $\Delta R_1$  and small blood volume. The use of contrast enhanced MRI will facilitate the assessment of different growth patterns, functional vascular phenotypes and therapeutic response *in vivo*. This dual contrast method would allow for deeper interrogation of treatment response in orthotopic brain tumours, particularly to anti-angiogenic agents, which have been shown to restore the blood brain barrier, preventing extravasation of Gd-DTPA contrast agents. It may also be possible to evaluate any therapy induced changes in invasion.

## References.

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**Figure 1. Multi-parametric MRI and histological assessment of one RG2 glioma.** (A)  $T_1$  weighted RARE image acquired 1 minute post Gd-DTPA, (B)  $T_1$  maps prior to and (C) post Gd-DTPA, and (D) the resulting  $\Delta R_1$  map. (E) The fractional blood volume map mirrors (F, false colour) Hoechst 33342 uptake. (G) H&E staining delineates tumour extent and (H) areas of invasion.