DCE-MRI evaluation of the temporal evolution of bevacizumab induced anti-vascular effects in colorectal cancer liver metastases

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Introduction Vascular endothelial growth factor (VEGF) plays a crucial role in angiogenesis, enabling tumour growth and survival. Inhibition of VEGF signaling has become an important target in drug development, leading to FDA approval of drugs that target either VEGF or the VEGF receptor (VEGFR)[1]. In particular, phase III trials of the humanized anti-VEGF antibody bevacizumab (Avastin; Genentech, San Francisco, CA) in combination with cytotoxic chemotherapeutics have shown improved overall survival in patients with metastatic colorectal cancer[2]. Dynamic contrast-enhanced imaging has been used widely in phase I/II trials of VEGF inhibitors, to produce pharmacodynamic biomarkers of drug efficacy[3]. However, no detailed imaging studies have been performed to evaluate the magnitude, duration and temporal evolution of drug effect of anti-vascular agents. We sought to provide comprehensive data to define the sequence, magnitude and duration of anti-vascular effects induced by bevacizumab in a study of patients with colorectal cancer liver metastases evaluated using MRI.

Patient recruitment Ethical approval was obtained from the local Research Ethics Committee. Ten patients with liver metastases, with no previous treatment, were recruited in an open label study. Patients with histology proven primary epithelial colorectal carcinoma, aged ≥ 18 years with an Eastern Cooperative Oncology Group score between 0-2, and life expectancy of at least 3 months were eligible. Written informed consent was obtained. Patients received single agent 10 mg/kg bevacizumab over a two week cycle. MRI scans were performed twice at baseline to establish parameter repeatability and at 4 and 48 hours post-treatment and on days 8 and 12.

Image acquisition Data were acquired on a 1.5 tesla Philips Intera system (Philips Medical Systems, Best, Netherlands). After initial anatomical T_1- and T_2-weighted images, DCE-MRI was performed. 3D axial T_1-weighted FFE volumes were consecutively-acquired (TR 4.0 ms, TE 0.82 ms, α =20°, one signal average, FOV 375 mm, matrix 128 x 128, 25 slices with slice thickness 4 mm) following calculation baseline of T_1 (α= 2°/10°/20°; 4 signal averages; identical TR, TE, imaging matrix and slice thickness). Elliptical k-space sampling, partial Fourier encoding, over-contiguous slice spacing and partial echo acquisition were used to improve temporal resolution (4.97 s for the DCE-MRI series). Total imaging time for DCE-MRI was 7 min 33 s. 0.1 mmol/kg of gadodiamide (Omniscan; GEHC, Amersham, UK) was administered intravenously through a Spectris MR (Medrad Inc, Indianola, PA) power injector at 3 ml/s on the sixth dynamic time point. Slice thickness was 4 mm for small target lesions or 8 mm for larger lesions (superior-inferior coverage 100 mm or 200 mm).

Data analysis and Statistical evaluation 3D regions of interest were defined on spatially co-registered high resolution T_1- and T_2-weighted volumes to encompass the entire tumour of interest. Whole tumour volume (WTV) was measured for each lesion. Voxels whose pre- and post-bolus arrival time series had significantly different distributions (where $p < 0.05$ on Mann-Whitney-Wilcoxon rank sum test) were classed as enhancing. DCE-MRI data were analysed using the extended Tofts model[4]. Tumour T_1 was calculated using the variable flip angle method. An arterial input function was measured where possible; alternatively a population-derived function was applied[5]. The following parameters were then calculated for each voxel: (1) tumour volume (2) tumour T_1, (3) enhancing fraction (E_F; the ratio of enhancing voxels to total number of tumour voxels), (4) the volume transfer constant (K^{trans}), (5) blood plasma volume (v_p) and (6) volume of the extracellular extravascular space (v_e).

Relative change from mean baseline values was expressed as a percentage change for each parameter at four and 48 hours and days 8 and 12. A mixed effects model was used to test significance of parameter change across the cohort, since some patients had multiple tumours. Due to the large number of parameters analyzed, p values less than 0.01 were considered statistically significant. All p values were two- tailed and were not formally adjusted for multiple comparisons. Repeatability was examined by measuring percentage within-subject coefficient of variation and percentage smallest detectable change (SDC) in a single tumour.

Results and Discussion Reductions in v_p and E_F were detected throughout the cycle of bevacizumab (Figure 1). In particular, statistically significant reductions in tumour E_F were measured at 48 hours (p=0.004) and maintained at day 8 (p=0.0005) and day 12 (p=0.0026). These changes were accompanied by reductions in v_p at the corresponding time points. No significant changes were detected in any parameter in skeletal muscle at any time points, indicating that it is unlikely that a systemic hemodynamic response – affecting all tissues – accounted for the response measured within the tumours. These changes concur with previous studies which demonstrated reductions in tumour blood flow and blood volume 12 days after infusion of bevacizumab in five patients with rectal cancer[6]. Here, we demonstrate that these changes occur rapidly, are statistically significant within 48 hours and persist throughout the entire cycle of therapy.

Our data demonstrates early statistically significant decrease in median K^{trans} at 4 hours (p=0.0012) and that K^{trans} remained reduced at 48 hours and at day 8. These reductions were not sustained at day 12 (Figure 1). The decoupling of K^{trans} changes from v_p changes at day 12 suggests that K^{trans} is more sensitive to functional changes in the tumour vasculature rather than structural changes.

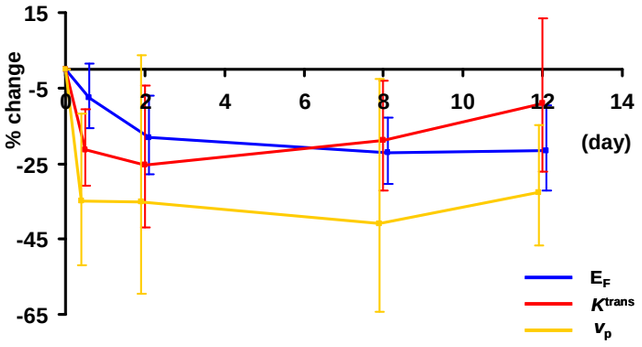

Figure 1 *Temporal evolution of anti-vascular effects of bevacizumab: percentage change in enhancing fraction (E_F), blood plasma volume (v_p) and volume transfer constant (K^{trans}). Error bars are 95% confidence intervals across the group.*

Reduction in tumour volume was detected across the group of 26 tumours by day 12 (Figure 2A) although this change did not reach statistical significance (p=0.0569). No patients achieved partial response as defined by RECIST[8]. The 95% confidence intervals for smallest detectable change (SDC) in volume were calculated to determine whether tumours changed in size from baseline to day 12. The SDC were 76.2% and 131.3%. Using these criteria, 9/26 tumours (in five different patients) exhibited statistically significant volume reduction (Figure 2B). Reductions in tumour volume were accompanied by statistically significant reductions in tumour pre-contrast T_1 at day 12 (p=0.0023) (Figure 2A), likely to represent resolution of tumour oedema caused by reduced vascular permeability. This mirrors similar rapid resolution of oedema observed following inhibition of VEGF with AZD2171 in glioblastoma multiforme[7]. Although a small cytotoxic effect secondary to the early anti-vascular effects of bevacizumab cannot be discounted, these data support the hypothesis that bevacizumab-induced reduction in vessel permeability leads to reduced tumour tissue oedema and is accompanied by reduction in tumour volume.

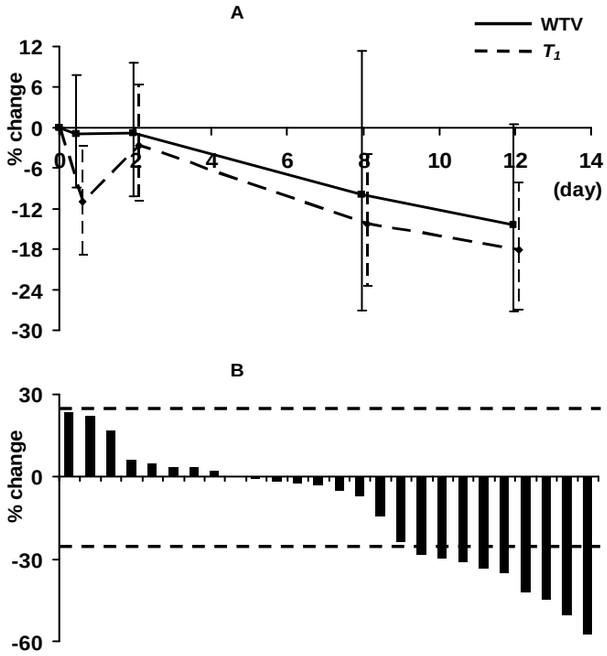

Figure 2 *Evidence for reduction in tumour size with bevacizumab mono-therapy. (A) Change in tumour volume and T_1 from baseline to day 12. (B) Waterfall plot shows percentage reduction in tumour volume from baseline to day 12. The dotted line represents the 95% confidence intervals (smallest detectable change in a single tumour).*

Conclusions Our data demonstrates statistically significant early reductions in the parameters v_p and E_F that remain reduced throughout a single cycle of anti-VEGF mono-therapy. These changes are considered to reflect structural changes in tumour vasculature. In distinction, measured reductions in K^{trans} were transient and represent functional changes. Measured anti-vascular effects led to subsequent resolution of oedema and tumour shrinkage. These data (1) highlight the importance of performing multi-parameter analysis on DCE-MRI data beyond that restricted to K^{trans} and/or *IAUC* alone; and (2) demonstrate the need to optimise measurement timing when applying quantitative imaging to trials of novel therapeutic agents.

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