Apparent diffusion coefficients show good reproducibility but heterogeneous change in response to treatment with combrestation A4 phosphate (CA4P) and bevacizumab in patients with solid abdominal and pelvic tumours

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Introduction To our knowledge, DWI has not been employed in a multicentre clinical setting to evaluate the effects of vascular targeted therapies in Phase I and II drug trials. Diffusion-weighted MRI (DWI) informs on tumour cellularity and microcapillary perfusion, which may also diminish with anti-vascular treatment. In animal studies, treatment with vascular disrupting drug combrestatin-4-phosphate (CA4P) results in an initial decrease of apparent diffusion coefficient (ADC) at six hours after drug administration [1], followed by a rise in ADC at 2 days. However, the effects of CA4P are reversible and the addition of an anti-VEGF antibody (e.g. bevacizumab) can improve the anti-cancer effects [2] by inhibiting neo-vascularization of the tumour rim. Anti-VEGF treatment has also been shown to induce vascular normalisation, which can lead to ADC reduction by decreasing interstitial oedema [3]. We present an assessment of changes in DWI in response to treatment with CA4P combined with bevacizumab in a two centre Phase I clinical trial.

Purpose
To determine the ADC measurement variability in a two centre clinical study, and to track ADC changes in response to the administration of the vascular disrupting drug CA4P and the anti-VEGF antibody bevacizumab.

Materials and Methods
Patients with solid abdominal and pelvic tumours were recruited to a Phase I dose escalation study performed at two UK centres to receive CA4P and bevacizumab. CA4P was administered in three dose cohorts (45mg/m^2, 54mg/m^2, 63mg/m^2), with 3 patients recruited for each cohort unless dose limiting toxicity was observed, in which case the cohort was expanded to 6 patients. At the beginning of treatment, only CA4P was administered. Bevacizumab (10mg/kg) was then administered at 4 hours after the dose of CA4P in subsequent 2 weekly cycles. Dynamic contrast enhanced MRI and DWI were performed six times for the duration of study: twice at baseline (to estimate measurement variability and reproducibility), after CA4P alone (at 4 and 72 hours), and following first cycle of combination treatment with CA4P (4 hrs) and after the added dose of bevacizumab (72 hours). Circulating endothelial cells and progenitors were analysed and serum/ plasma biomarkers were also measured.

DWI evaluation: DWI imaging was performed on 1.5T Siemens' MR systems using common imaging protocols across the two centres. Free-breathing single-shot fat-suppressed echo-planar MRI (*TR = 3500, TE = 72 ms, 340 mm FOV, 112 x 256 matrix, images interpolated to 256 x 256 matrix, 6 mm thickness, Grappa factor 2, Nex = 5)* was performed using 6 b-values (0, 50, 100, 250, 500 and 750 s/mm^2) with diffusion gradients applied in 3 orthogonal directions (phase, frequency and slice select).The images were analysed using *DiffusionView* (Institute of Cancer Research, UK). In each patient, a target lesion measuring ≥ 2 cm diameter was identified. Three contiguous sections through the target lesion at similar levels were evaluated at each time-point. ADCs calculated on a pixel-by-pixel basis were summed and averaged for the three sections. Regions of interests (ROIs) were drawn just within the inner border of the target lesion (~ 1 pixel) on the b = 750s/mm^2 image and copied onto the ADC maps to record their values. The *ADCtotal* (using all b-values), *ADClow* (using b = 0, 50 and 100) and *ADChigh* (using b = 100, 250, 500 and 750) were recorded. Two baseline scans allowed the variability/ reproducibility of ADCs to be evaluated using Bland-Altman statistics. Serial ADCs for each patient were tracked using line-plots.

Results
10 of the 12 scheduled patients have been recruited to date. One target lesion in each patient was evaluated in this analysis. There was excellent reproducibility in the measurement of the perfusion insensitive *ADChigh* and *ADCtotal.* The coefficient of reproducibility for *ADChigh* and *ADCtotal* were 16.6% and 16.5% respectively. The mean *ADChigh* and *ADCtotal* averaged for all lesions across the cohort showed no significant change with treatment (Mann-Whitney test, p > 0.05). Significant changes were observed in the ADCs on a per-patient basis (Figure 1). At 4 hours after initial administration of CA4P alone, 2/10 showed significant increase and 1/10 significant decrease in the *ADChigh* and *ADCtotal*. At 4 hours after second dose of CA4P, 2/10 different patients showed significant increase in *ADChigh* and *ADC total*. After 72 hours treatment with bevacizumab, 2/10 showed significant increase and 1/10 showed decrease in *ADChigh* and *ADC total.*

Discussion
Our study demonstrates that DWI measurements in a two centre clinical trial are highly reproducible. However, using simple analysis of mean ADC values, no clear trends could be observed between drug treatment and ADC changes. This may relate to the variable rates of cell death which occur secondary to vascular shutdown and anti-angiogenic treatment, as well as the temporal tissue responses to the treatment effects. Another reason may relate to the heterogeneity in these tumours. The fact that significant changes in ADC in relation to treatment were observed on some patients indicates that drug effects are occurring. Mechanistically, the administration of CA4P has been shown to acutely reduce ADCs (due to cell swelling), but a rise in ADCs was also observed in our study (possibly related to acute cell lysis or increased interstitial oedema). The introduction of bevacizumab could complicate interpretation of ADC changes since vascular normalization may lead to ADC fall (decreased interstitial oedema), against a background of rising ADCs due to tissue necrosis induced by the anti-vascular agents. A more comprehensive informatics based analysis that includes the DCE-MRI analysis may help to clarify the mechanisms underlying our observations and these are currently underway.

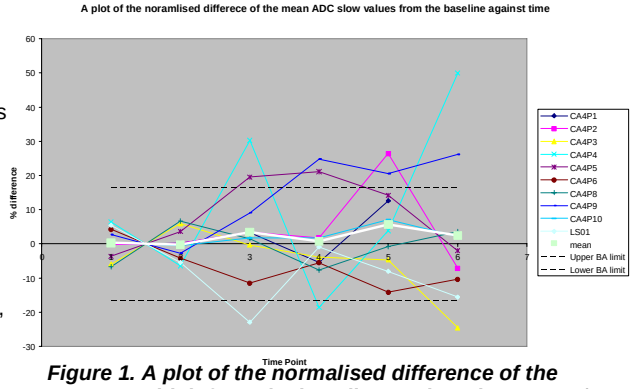

Figure 1. A plot of the normalised difference of the mean ADChigh from the baseline against time. *Dotted black lines indicate the limits of measurement variability in this study cohort. Note significant increase and decrease in ADChigh in a few patients with treatment, but no clear trends could be identified. White line indicates the results of the mean ADChigh across entire cohort.*

Conclusions
ADCs measurements are highly reproducible in a 2 centre clinical trial setting. Although no clear trends were found in the change of mean ADC values across the study cohort, significant changes in ADCs were observed in individual patients. More sophisticated, informatics based analyses are required to quantify the complex changes of ADC in relation to treatment.

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