

Response of a Human Melanoma Xenograft Model to the MEK Inhibitor AZD6244 (ARRY-142886) Evaluated by Diffusion-Weighted MRI

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Background & Aims: BRAF-MEK-ERK1/2 signalling is activated in many human cancers including melanoma, where BRAF is mutated in ~70% of cases (1). This pathway represents a target for novel mechanism-based anti-cancer drugs with agents such as the MEK inhibitor AZD6244 (ARRY-142886) currently in clinical trial. AZD6244 causes inhibition of growth and induction of apoptosis in a range of pre-clinical tumour models (2). Here we assess whether diffusion weighted MRI could be useful in detecting biomarkers of response to AZD6244 in a human melanoma xenograft model, and that may be used as a non-invasive imaging tool to report on the action of this drug in the clinic.

Materials & Methods: WM266.4 (mutant BRAF) human melanoma xenografts were propagated in the flank of female mice by injection of 5×10^6 cells subcutaneously. Mice bearing ~550 mm³ volume tumours were treated with 75mg/kg AZD6244 (AstraZeneca, n=9) or vehicle (n=9) by oral gavage twice daily for 3 days. The effect of treatment on tumour volume was assessed by calliper measurements prior to (day 0) and following (day 3) treatment. MRI was performed pre-treatment and 3 hours after the final dose using a diffusion-weighted spin-echo sequence (TR=1500ms, b values = 6-1000s/mm², 5 b-values, 1 average). Diffusion data were fitted using a novel Bayesian maximum *a posteriori* approach to provide estimates of the native apparent diffusion coefficient (ADC), sensitive to tissue cellularity (3). In addition, native T₁ and T₂ measurements were determined using an inversion recovery (IR) true-FISP sequence (TI=109-2902ms, 25 inversion times, TR=2.4ms, TE=1.2ms, 8 averages, scan TR=10s), and the data fitted using a similar *a posteriori* approach, but which also utilised the dual relaxation sensitivity of the IR true-FISP sequence (4). All data were fitted on a pixel-by-pixel basis using in-house software (ImageView), which provided maps of tumour spatial heterogeneity. The median value of each parameter in each tumour was measured. Following the post-treatment scans, tumours were excised and samples taken for histological analysis of phosphorylated (activated) ERK1/2 levels and for necrosis by H&E staining. Data are expressed as the mean \pm 1 s.e.m. Statistical significance was assessed using Mann-Whitney test with p<0.05 considered to be significant.

Results: Administration of AZD6244 resulted in significant inhibition of tumour growth compared to the vehicle treated cohort, with tumour volumes equal to 103 \pm 6% and 142 \pm 8% of day 0 values, respectively (p=0.001). Whilst there was no significant change in ADC in the vehicle treated group, a highly significant increase in ADC, to 160 \pm 20% relative to day 0, was found in the AZD6244 treated group (p=0.01, Figure 1A&B). There was no correlation between the change in ADC and the change in tumour volume (R²=0.19, p=0.23).

An increase in native T₂ was observed in the drug-treated group (up to 127 \pm 18% relative to day 0, p=0.02) but not the vehicle-treated group (Figure 1B, p=0.26). T₁ values did not change significantly in either treatment group (Figure 1B).

Histological analyses revealed an increase in the necrotic fraction of the AZD6244-compared to the vehicle-treated tumours (Figure 1C) with median values of 60% vs 10%, respectively (p=0.036). Staining for P-ERK1/2 also showed reduced ERK1/2 phosphorylation in the areas of the tumour that remained viable, consistent with MEK signalling down-regulation.

Conclusions: This study shows increases in ADC that are associated with MEK signalling inhibition and induction of tumour necrosis, and which precede tumour volume reduction. Our findings support the use of ADC as an early pharmacodynamic imaging biomarker for assessment of response to AZD6244 and other therapies targeted at BRAF-MEK-ERK1/2 signalling during clinical trials.

References: 1) Davies et al (2002), Nature 417:949-54. 2) Davies et al (2007), Mol Cancer Ther 6: 2209-19. 3) Walker-Samuel et al (2009), Magn Reson Med 62: 420-9. 4) Walker-Samuel et al (2009) Proc. ISMRM, abstract #5595

Acknowledgements: This work was funded by Cancer Research UK [CUK] project grant C1060/A6916, AstraZeneca and The Royal Society. We acknowledge the support received from the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) grant C1060/A10334, also NHS funding to the NIHR Biomedical Research Centre.

