

# APPARENT DIFFUSION COEFFICIENT CORRELATES WITH NECROSIS AND INVERSELY WITH PROLIFERATION: HISTOPATHOLOGICAL QUANTIFICATION IN A PANEL OF HUMAN TUMOUR XENOGRAFTS

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## INTRODUCTION

The water proton Apparent Diffusion Coefficient (ADC) has been proposed as a biomarker of the non-viable fraction in tumours. Our aim was to evaluate the correlation between ADC and histopathology in a diverse panel of human tumour xenografts commonly used in preclinical drug efficacy evaluation. We hypothesised that ADC would correlate with histologically quantified necrosis and oedema, and inversely correlate with histologically quantified proliferation.

## METHODS

Nude mice bearing subcutaneous tumours (n=4 Calu6, n=5 HCT116, n=5 H1975, n=5 BT474c) were used. Anaesthesia was induced with 3% isoflurane in air, then maintained at 1.5-2%. Rectal temperature and respiration rate were maintained at 35-38°C, and 70-80 breaths per minute respectively. MRI was performed at 9.4T (Varian Inova) using a 38mm birdcage transceiver. Multislice fast spin echo (FSE) images with 30mm field-of-view (FOV), 2mm slice thickness and 256\*128 matrix provided anatomical reference. ADC maps were acquired from the same 4 slices using FLASH ( $\alpha=20^\circ$ , TR=21 msec, TE=2.2 msec, 128\*64 matrix) preceded by a stimulated echo preparation module ( $\delta=5$  msec,  $\Delta=30$  msec, b-values 150 - 1000 s.mm<sup>-2</sup>). In a separate set of mice, the dependence of the tumour ADC values on diffusion time and direction of the diffusion gradients was investigated. After imaging, tumours were excised and fixed in 10% neutral buffered formalin for 24 hours. Following routine paraffin processing and sectioning, slides were H&E stained and necrosis scores were determined by eye and using a standard algorithm on a Zeiss KS 400 automated image analysis system. Oedema was rated 0-3 using a by eye analysis. The proliferation index (PI) was determined on Ki67 MIB-1 (Dako M7240) immunohistochemically stained slides by using a standard nuclear cell count algorithm on a Chromavision ACIS II automated image analysis system. ADC maps were created using a combination of ImageJ<sup>1</sup> and Matlab (MathWorks Inc.). Regions of interest (ROI) encompassing the whole tumour were drawn on FSE images and transferred to ADC maps. ADCs were calculated voxelwise, and a mean, weighted by the  $r^2$  from each fitted pixel, was calculated over the tumour ROI. Comparisons between the ADC values and quantified histopathology were performed using JMP (SAS Institute, Cary USA). CoV for ADC was 5.6% in repeat measurements.

## RESULTS

Figure 1 shows a representative ADC map and corresponding  $r^2$  map to show the quality of the fit. ADC ranged from 0.6–1.9 x 10<sup>-3</sup> mm<sup>2</sup>s<sup>-1</sup> (there was one exception of 3.7 x 10<sup>-3</sup> mm<sup>2</sup>s<sup>-1</sup> corresponding to fluid which was evident in FSE: this mouse was excluded from analysis). Auto necrosis scores ranged from 5–54% and proliferation index ranged from 23–87%. Figure 2 demonstrates statistically significant correlations between ADC and necrosis score and proliferation index. There was no correlation between ADC and oedema score. No dependency of ADC on  $\Delta$  or the diffusion gradient direction was found. Including b-values below 150 s.mm<sup>-2</sup> in the fit did not change the correlation data, but in some tumours there was a trend towards slightly higher ADC, possibly reflecting perfusion contributions.

## DISCUSSION AND CONCLUSION

Although ADC appeared to vary with tumour type, we believe this reflects the different necrotic phenotype of the different tumours, not intrinsic tumour differences.

There was significant correlation between ADC and histopathologically quantified necrosis in our panel of human tumour xenografts, consistent with decreased cell density associated with necrosis<sup>2-4</sup>. The reason for the strong inverse correlation between ADC and the proliferation index is less clear, and further work will be required in order to fully understand the mechanism behind this, including analysis of the extent of apoptosis and the extent of this relationship in control vs. treated tumours.

In conclusion, we have demonstrated significant correlations of the ADC with both necrosis and the proliferation index in a range of murine xenograft tumours. This study forms the basis for future longitudinal ADC studies where the effect of candidate oncology drugs will be monitored.

## REFERENCES

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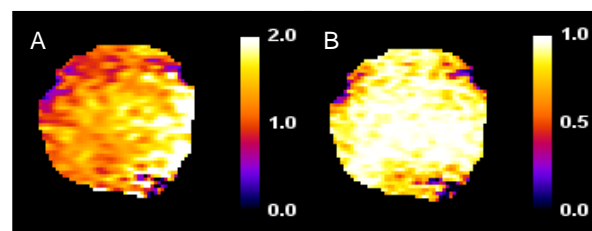


Figure 1. ADC (A) and  $r^2$  (B) maps from an H1975 tumour

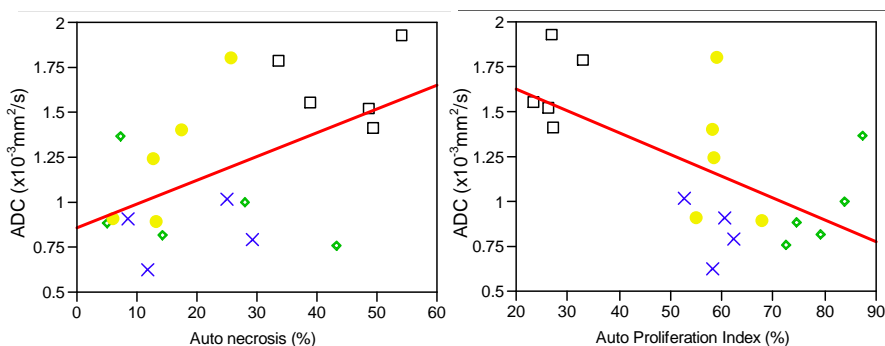


Figure 2. Correlation between ADC and necrosis score ( $r^2 = 0.28$ ,  $p=0.02$ , fig 2A) and proliferation index ( $r^2 = 0.38$ ,  $p=0.005$ , fig 2B) BT474c (●), HCT116 (◆), H1975(□), Calu6 (×)