False-Negative MRI Biomarkers of Tumour Response to Targeted Cancer Therapeutics

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Introduction

Anti-cancer drug discovery and development is now predominantly targeted to cancer-causing genes and the associated pathways. For the evaluation of novel oncology therapeutics, the use of pharmacodynamic biomarkers, including imaging biomarkers, is now desirable (1). Before their deployment in clinical trials, such imaging biomarkers require evaluation, typically through preclinical identification of imaging-pathology correlates. Here we describe two pre-clinical studies in which emerging non-invasive MRI biomarkers were correlated with histology to assess tumour response and target inhibition: **1)** The response of orthotopic PC3 prostate cancer xenografts to the Src kinase inhibitor AZD0530 was investigated using a multiparametric MRI approach, including susceptibility contrast MRI to elucidate the potential anti-angiogenic effect of Src, and diffusion-weighted MRI to assess whether inhibition of Src kinase activity results in restriction of tissue water mobility. **2)** Intrinsic susceptibility MRI was used to evaluate VEGFR2 inhibitor ZD6474-induced temporal changes in baseline tumour R_2* and carbogen-induced ΔR_2* in MNU-induced rat mammary tumours to assess and identify the time window of transient vascular normalisation.

Methods

All MRI was performed on a Bruker 7T horizontal bore microimaging system. ***Study 1:*** Mice bearing established orthotopic PC3 prostate cancer xenografts (2) received either 25mg/kg AZD0530 (saracatinib, AstraZeneca) or vehicle alone p.o. daily for 5 days. MRI was performed 2 hours after the final dose using a 30mm birdcage coil. Apparent diffusion coefficient (ADC), fractional blood volume (fBV) and vessel size index (VSI) were measured using a method previously described using USPIO particles (200μmolFe/kg Sinerem®, ferumoxtran-10, Guerbet) (3). T_1 and T_2 were measured using an IR-trueFISP sequence (4). Hoechst 33342 (15mg/kg) was administered i.v. 1 minute before the animals were killed, and perfusion was subsequently assessed on frozen tumour sections in order to validate the susceptibility contrast MRI biomarkers. Src pathway inhibition was assessed via immunohistochemical detection of phosphorylated focal adhesion kinase (pFak) Y861 and phosphorylated Paxillin (pPaxillin) Y31. ***Study 2:*** Female Sprague Dawley rats were injected with 37.5mg/kg *N*-methyl-*N*-nitrosurea (MNU) i.p., resulting in breast tumours that developed at sites along the mammary fat pad. MRI was performed on established tumours (Day 0) using a 64mm birdcage coil, with a nosepiece positioned for gas delivery. MGE images (8 echoes, T_E=6-28ms, T_R=200ms, 8 averages, matrix=128x128, 3x1.56mm slices) were acquired while the rats first breathed air. The gas supply was then switched to carbogen (95%O_2/5%CO_2), and after a transition time of 2 minutes, a second set of identical MGE images were acquired. Rats received either 30mg/kg ZD6474 (vandetanib, AstraZeneca) or vehicle alone p.o. daily for 5 days, and MRI was repeated on days 2 and 4. Pimonidazole (60mg/kg) was administered i.p. 45 minutes before the rats were killed; hypoxia induced adduct formation was later assessed on frozen tumour sections. VEGF receptor 2 (VEGFR2) expression was also assessed by immunohistochemistry, from which mean vessel density (MVD) was calculated.

Results & Discussion

Study 1: There was no significant difference in tumour volume between the vehicle and AZD0530 treated cohorts at the time of imaging (Table 1). VSI and fBV maps (Figure 1A) demonstrated that uptake of the USPIO particles was typically restricted to the tumour periphery in both cohorts. However, no statistically significant differences were found in any of the quantitative MRI parameters measured between the vehicle and AZD0530 treated cohorts (Table 1). Nevertheless, immunohistochemical analysis revealed a reduction in the expression of activated substrates of Src kinase, pFak Y861 and pPaxillin Y31, in the AZD0530 treated tumours (Figure 1b), confirming target inhibition. In a setting when a treatment has elicited the expected molecular response, but the imaging biomarker failed to show the expected change, we would describe this as a false-negative imaging biomarker response. Hoechst 33342 staining confirmed the fBV observations, demonstrating no significant change in perfusion in response to AZD0530 treatment. In this case the treatment elicited neither the expected molecular response nor the expected biomarker change, so we would describe this as a true-negative imaging biomarker response.

Study 2: Tumours in the vehicle treated group progressed significantly over the study period, whereas ZD6474 induced highly significant anti-tumour activity in all of the treated animals (mean 17±6% reduction in normalised tumour volume at day 2, $p<0.05$; 60±6% at day 4, $p<0.01$). The overall mean baseline tumour R_2* at day 0 was 103.9±15s^{-1}. Figure 2 shows parametric maps of baseline R_2* in a ZD6474 treated animal, and illustrates the anti-tumour effect. Irrespective of treatment, there was no significant difference in tumour R_2* over 5 days (vehicle, 101±17 to 127±20s^{-1} (n=4); ZD6474, 111±27 to 117±32s^{-1} (n=4)). No clear pattern of baseline R_2* or carbogen-induced ΔR_2* response was evident in either cohort over 5 days. However, quantification of VEGFR2 MVD revealed a significant ~80% inhibition in the ZD6474 treated rats compared to vehicle treated animals ($p<0.05$), and hypoxia was significantly lower in treated tumours ($p<0.05$). Here the treatment has anti-tumour efficacy and elicited a pronounced molecular response, but the corresponding imaging biomarker failed to show the anticipated change. We would describe this as a false-negative imaging biomarker response.

Conclusions

While good registries exist for clinical trials, there is no equivalent for animal studies, so if investigators fail to publish "negative" findings in animals, there is a risk that clinical trials using imaging biomarkers may be undertaken with a false expectation of success, or even that promising investigational drugs may be abandoned because a false-negative imaging biomarker response is mistaken for a true-negative.

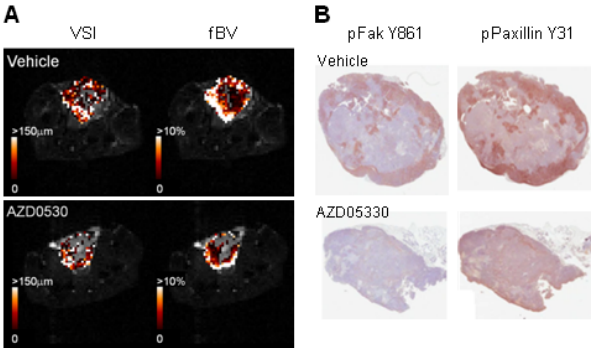

Figure 1A. Representative parametric MRI maps of vessel size index (VSI) and fractional blood volume (fBV) and **B.** whole section images showing immunohistochemical staining for pFak Y861 and pPaxillin Y31 from orthotopic PC3 xenografts following daily treatment with either vehicle or 25mg/kg AZD0530 for 5 days.

	Vehicle	AZD0530
Volume (mm^3)	552 ± 114	632 ± 99
ADC (x10^6 cm^2 s^{-1})	605 ± 48	620 ± 28
Native T_1 (ms)	1999 ± 41	2016 ± 42
fBV (%)	4.08 ± 1	4.46 ± 1
VSI (μm)	52 ± 11	51.2 ± 8

Table 1. Summary of quantitative MRI data from orthotopic PC3 xenografts following daily treatment with vehicle or 25mg/kg AZD0530 for 5 days. Data are mean ± 1 s.e.m, n=7 per cohort.

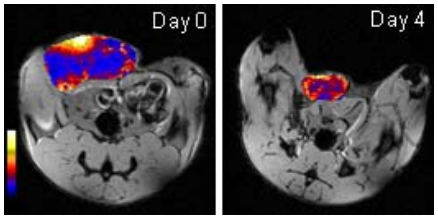

Figure 2. R_2* maps (0-250s^{-1}) overlaid on axial T_2-weighted images acquired from one MNU-induced rat mammary tumour prior to and following daily treatment with ZD6474 for 5 days.

References. **1.** Workman P *et al*. (2006) *J Natl Cancer Inst* 98:580-98. **2.** Walker-Samuel S *et al*. (2011) *Int J Cancer* In press. **3.** Boult JKR *et al*. (2011) *J Pathol* 225:344-52.

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