Evaluating Acute Response to the Novel HIF Inhibitor NSC-134754 in an Orthotopic Prostate Tumour Model by MRI

L. C. Baker[1], S. Walker-Samuel[1], J. K. Boult[1], Y. Jamin[1], M. A. Ashcroft[2], and S. P. Robinson[1]

[1]The Institute of Cancer Research, Sutton, Surrey, United Kingdom, [2]Division of Medicine, University College, London, United Kingdom

Introduction

Tumour hypoxia and angiogenesis are classic hallmarks of the tumour microenvironment. Tumour cells adapt to hypoxic stress through activation of the hypoxia-inducible factor (HIF) pathway, which regulates oxygen homeostasis, energy metabolism, angiogenesis, invasion and cell growth, driving the tumour towards a more malignant phenotype. HIF binding sequences (hypoxia response elements, HRE's), have been identified within the 5' flanking region of human vascular endothelial growth factor (VEGF) and other HIF targets [1]. These HREs are important for transcriptional activation of HIF target genes upon direct binding. HIF-1 is thus a therapeutic target, and small molecule inhibitors are actively being sought. Recently, an *in vitro* screen identified NSC-134754 as a putative HIF-1 inhibitor [2]. The acute physiological effects of HIF-1 inhibition, such as reduced vascular permeability and reduced cellularity, have been measured using biomarkers derived from MRI data [3]. In this study, we investigated the effect of a putative HIF-1 inhibitor, NSC-134754, following acute treatment in PC3 orthotopic prostate tumours. This was undertaken using a novel dynamic contrast-enhanced (DCE) MRI approach, intrinsic susceptibility and diffusion MRI, each of which can provide imaging biomarkers of treatment response. Analysis of the MRI data was performed using a number of novel Bayesian inferential approaches, which have been shown to provide improved accuracy over conventional approaches [4].

Materials and Methods

Animals and Tumours: Tumours were propagated by injecting $1x10^5$ PC3 cells orthotopically into the ventral prostate gland of male NCr nude mice (n=6). Tumours were assessed by MRI once palpable.

Drug Preparation and Administration: NSC-134754 was obtained from the National Cancer Institute (USA), and dissolved in 0.9% saline on the day of treatment. Mice were given 75mg/kg i.p. on day 1 of the study.

MRI Measurements: MRI was performed on day 0 (24hr pre-dose) and day 2 (24hr post dose) on a 7T Bruker horizontal bore magnet. Anaesthetised mice were positioned supine in a 3cm birdcage coil in the magnet, and T_2-weighted, multi-slice morphological data were acquired to provide tumour delineation. A multi gradient-echo (MGE) sequence (T_R=200ms, T_E=6-28ms, 8 echoes, 8 averages) was then used to quantify R_2*. A diffusion-weighed spin-echo sequence (T_R=1500ms, b-value=6-1000s/mm^2, 5 b-values, 1 average) was then used to determine the tumour ADC. Finally, DCE-MRI data using Gd-DTPA (Magnevist, Schering) were acquired using an inversion recovery (IR) true-FISP sequence with one baseline scan (T_I=130-2592ms, 20 inversion times, T_R=4ms, T_E=2ms, 12 averages) and 75 dynamic scans (T_I=130-1037ms, 8 inversion times, T_R=4ms, T_E=2ms, temporal resolution=9s, 2 averages) prior to and following i.v. injection of 0.1mmol/kg Gd-DTPA.

Data Analysis: Diffusion and MGE data were fitted using a novel Bayesian maximum a *posteriori* approach that took into account the Rician distribution of noise in magnitude MR data and provided estimates of the native ADC and R_2* [4]. IR-trueFISP data were fitted using a similar approach, but which also utilised the dual-relaxation sensitivity (T_1 and T_2) of the IR-trueFISP sequence and incorporated the Tofts and Kermode pharmacokinetic model [5]. This provided estimates of K^{trans} (dependent on permeability, blood flow and blood volume), v_e (fraction of extra-vascular, extra-cellular volume) and native T_1 and T_2. 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 and the absolute difference between pre-therapy and post-therapy measurements calculated.

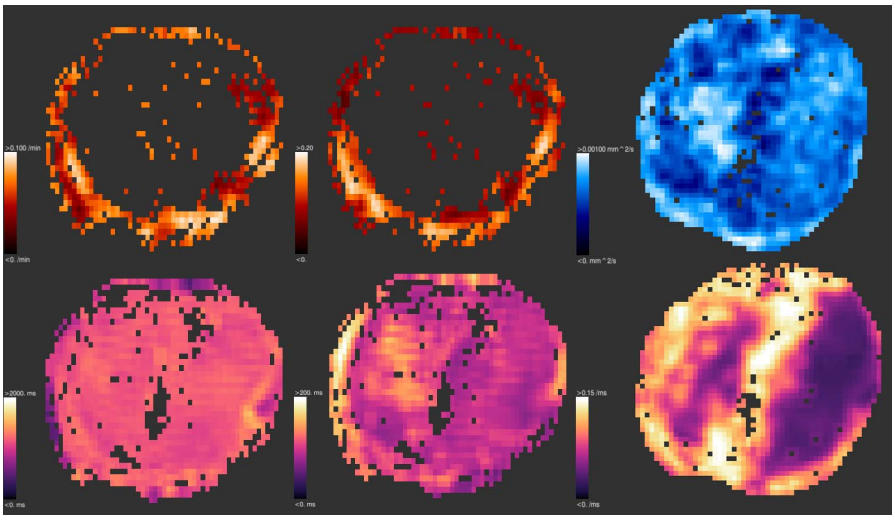

Figure 1: Example parameter maps of each MR parameter in a single tumour: (left to right, top to bottom): K^{trans}, v_e, ADC, native T_1, native T_2 and native R_2*.

Parameter (median)	Mean pre-therapy	Mean post-therapy	Mean percentage change due to treatment
K^{trans}	0.065 ± 0.008 /min	0.067 ± 0.013 /min	-5.7 ± 8.2 %
v_e	0.068 ± 0.009	0.068 ± 0.006	17.1 ± 15.7 %
ADC	648 ± 17 ×10^{-6} mm^2/s	630 ± 19 ×10^{-6} mm^2/s	-2.5 ± 2.6 %
Native R_2*	0.065 ± 0.004 /ms	0.080 ± 0.018 /ms	23.1 ± 26.5 %
Native T_1	1228 ± 16 ms	1180 ± 22 ms	-2.0 ± 2.1 %
Native T_2	88.2 ± 1.8 ms	89.8 ± 1.7 ms	1.5 ± 2.0 %

Table 1: MR parameter values pre- and post-treatment (24 hours) with putative HIF-1 inhibitor, NSC-134754.

Results & Discussion

Figure 1 shows examples of parametric maps of each of the MR biomarkers investigated, prior to therapy. K^{trans} and v_e maps revealed a perfused tumour perimeter surrounding a less-perfused core, as is typical in tumour xenograft models. Pre- and post-therapy MR parameter values, averaged across the cohort, are displayed in Table 1, along with the mean percentage change in each. Statistical analysis using paired t-tests revealed no significant difference with therapy in any parameter. This suggests that, to within the sensitivity of each measurement, treatment of orthotopic PC3 tumours with 75mg/kg NSC-134754 did not induce any significant physiological change at 24 hours following administration. Others have shown that DCE-MRI and diffusion MRI can be used to assess acute tumour response to a novel HIF inhibitor [3]. Further work is required to establish whether NSC-134754 exerts similar functional changes *in vivo* along with establishing the most appropriate dose and time window in which to measure such changes using MRI. Validation of the MRI biomarkers from this study is currently being undertaken using immunohistochemistry, fluorescence and ELISA techniques. It will be of interest to note whether HIF-1α, its downstream target VEGF, and other parameters including tumour hypoxia and perfusion have altered in response to acute treatment with NSC-134754.

Conclusion

Orthotopic PC3 prostate tumours treated with 75mg/kg of the putative HIF-1 inhibitor NSC-134754 induced no significant change in the MRI biomarkers K^{trans}, v_e, ADC, native R_2*, native T_1 and native T_2 at 24 hours following treatment. Further work is currently being undertaken to provide *ex vivo* qualification of these results.

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