

Response of Orthotopic PC3 Prostate Tumors to the HIF Pathway Inhibitor NSC-134754 Assessed by Diffusion Weighted MRI and Immunohistochemistry

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Introduction

The hypoxia-inducible factor (HIF) pathway is a key regulator in tumor cell adaptation to the hypoxic microenvironment. Therapeutic inhibition of the HIF pathway can be monitored using MRI-derived biomarkers to assess parameters such as reduced vascular permeability and cellularity [1]. NSC-134754 was identified from an *in vitro* screen of the NCI Diversity Set as a novel HIF pathway inhibitor [2]. The purpose of this study was to investigate acute response to the NSC-134754 using diffusion weighted magnetic resonance imaging (DW-MRI) in a murine orthotopic model of prostate cancer. *Ex vivo* qualification of histological markers of hypoxia, perfused vessels and necrosis were also obtained.

Materials and Methods

Animals and Tumors: Tumors were propagated by injecting 1×10^5 PC3 cells orthotopically into the ventral prostate gland of male NCr nude mice (n=22). Once tumors reached approximately 1cm in diameter (measured by palpation), MRI was performed.

Drug Preparation and Administration: NSC-134754 was obtained from the National Cancer Institute (USA), and dissolved in 0.9% saline on the day of study. Treated mice were administered with 100mg/kg i.p.

MRI Measurements: MRI was performed either 6h or 24h following administration of NSC-134754 on a 7T Bruker horizontal bore magnet. Anaesthetised mice were positioned supine in a 3cm birdcage coil in the magnet, and T₂-weighted, multi-slice RARE images acquired to provide tumor delineation. A diffusion-weighted spin-echo sequence (T_R=1500ms, b-value=150-750s/mm², 4 b-values, 1 average) was then used to determine the tumor apparent diffusion coefficient (ADC).

Data Analysis: Diffusion data was fitted using a 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 [3]. All data were fitted on a pixel-by-pixel basis using in-house software (ImageView), providing maps of tumor spatial heterogeneity and allowing the median ADC value in each tumor to be calculated.

Histology/Immunohistochemistry: At study end, pimonidazole hydrochloride (HPI Inc, USA) and Hoechst 33342 (Sigma, UK) were administered i.p. and i.v. respectively. Tumors were snap frozen on liquid N₂ and 10µm sections were processed and imaged on a fluorescence microscope for the following: Hoechst 33342 (perfused vessel marker), pimonidazole (hypoxia marker), haematoxylin and eosin (H&E) (necrosis) and HIF-1α.

For the parameters perfusion, hypoxia and necrosis, image analysis software was used to: i) define regions of interest over the whole tumor section and ii) calculate enhanced particles (parameter of interest) expressed as a % of each tumor section. Non-tumor regions were excluded from all analysis and necrotic areas from fluorescence analysis.

Results

Representative ADC maps calculated for control, 6 or 24 hours post treatment mice are shown in Figure 1a. An increased ADC was evident in the tumors 24 hours after treatment. Statistical analysis revealed a significant increase in tumor ADC 24h post treatment with NSC-134754 compared to control and 6h treatment (Figure 1b). *Ex vivo* histological qualification demonstrated a significant increase in pimonidazole staining 24h following treatment with NSC-134754 compared to controls. Increased necrosis was also apparent after treatment. There was no significant difference in the mean % of perfused vessels in any cohort (Figure 2). Representative snapshots (x40) and composite tiled images of triple fluorescence staining followed by H&E (on same section) for control and 24h treated tumors are shown in Figure 3.

Discussion and Conclusion

The results of this study suggest that ADC can provide a non-invasive imaging biomarker of tumor response 24h after administration of 100mg/kg of the HIF pathway inhibitor NSC-134754 in a murine orthotopic model of prostate cancer. Complimentary *ex vivo* histological analysis revealed physiological changes within the tumor microenvironment, with a significant increase in the hypoxic fraction of treated tumors and an associated increase in necrosis (24h post-NSC-134754) compared to controls. Interestingly, no change in the % of perfused vessels was detected. Further work will be required to understand the mechanism of action of NSC-134754 *in vivo* and therapeutic implications of HIF pathway inhibition. Of particular interest will be the determination of the optimum time point for qualifying increased ADC with necrosis, in addition to tumor growth

outcome and microenvironment alterations with chronic treatment.

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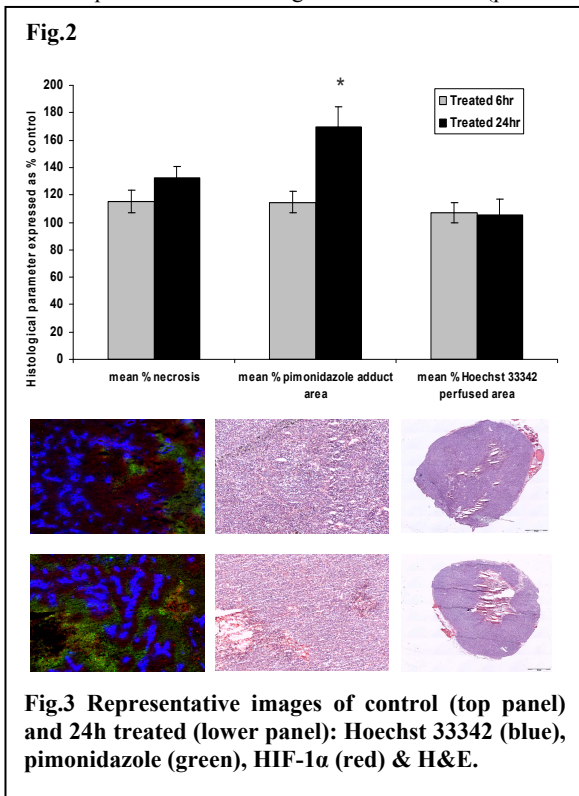
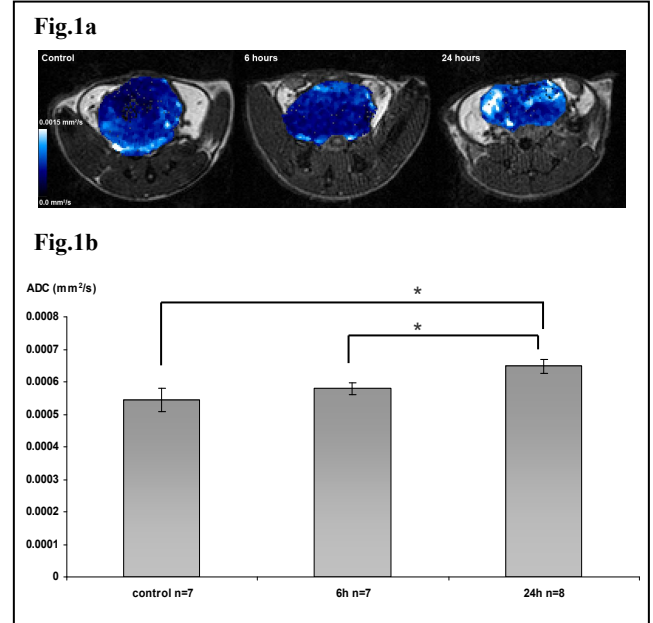


Fig.3 Representative images of control (top panel) and 24h treated (lower panel): Hoechst 33342 (blue), pimonidazole (green), HIF-1α (red) & H&E.