Intrinsic Susceptibility MRI Investigation of Acquired Resistance to EGFR Therapy in a Xenograft Model of Squamous Cell Carcinoma of the Head and Neck.

Lauren CJ Baker[1], Carol Box[2], Arti Sikka[1], Gary Box[2], Suzanne A Eccles[2], and Simon P Robinson[1]

[1]*Cancer Research UK and EPSRC Cancer Imaging Centre, Division of Radiotherapy and Imaging, The Institute of Cancer Research, Sutton, Surrey, United Kingdom,*
[2]*Tumour Biology and Metastasis Team, Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, Sutton, Surrey, United Kingdom*

Introduction: Overexpression of the epidermal growth factor receptor (EGFR) has been identified as a negative prognostic factor in squamous cell carcinoma of the head and neck (SCCHN). Despite the development of EGFR-targeted tyrosine kinase inhibitors (TKI's), patient response rates are variable, and in those that initially respond, acquired resistance frequently occurs [1]. Tumor hypoxia is known to adversely affect loco-regional tumor control and disease-free survival in patients with head and neck cancer [2]. Furthermore, colocalization of EGFR expression *and* tumor hypoxia in SCCHN were recently associated with poor outcome, suggesting a role for hypoxia in drug resistance [3]. Imaging strategies to identify and monitor patients with emerging resistance to EGFR-TKI therapy are urgently required. Quantitation of tumor R_2* and hyperoxia-induced ΔR_2* using intrinsic susceptibility MRI are being evaluated and qualified as non-invasive imaging biomarkers of tumor hypoxia [4,5]. In this study, intrinsic susceptibility MRI was used to investigate functional tumor vasculature and oxygenation *in vivo* in human xenograft models of SCCHN with either acquired resistance or sensitivity to EGFR-TKIs. Histopathological correlates of tumor vascular perfusion and hypoxia were also sought.

Methods:

Tumor model: Xenografts were established from human CAL27 SCCHN cell lines, with either acquired resistance or sensitivity to multiple EGFR TKIs (gefitinib, erlotinib and afatinib). Female NCr nude mice were injected subcutaneously with either $5x10^5$ resistant (R) cells (n=8) or $5x10^6$ sensitive (S) cells (n=7).

MRI: When tumor volumes reached approximately 250mm^3, the hypoxia marker pimonidazole (60mg/kg) was injected i.p. Mice were then imaged on a 7T Bruker horizontal bore Microimaging system, using a 3cm birdcage coil, with a nosepiece positioned for gas delivery. During air-breathing, multi gradient-echo (MGRE) images were acquired from three contiguous 1mm thick axial slices through the tumor, with T_R=200ms, T_E=6-28ms, 4ms echo spacing and 8 averages. After 45 minutes, to allow for full bioreduction of pimonidazole, 100% O_2 was delivered @ 1l/min and a second set of MGRE images were acquired. Following MRI, the perfusion marker Hoechst 33342 (15mg/kg) was injected via a lateral tail vein. After 1 minute, tumors were excised and snap frozen over liquid nitrogen. Apparent R_2* maps were calculated on a voxel-by-voxel basis [6]. Median R_2* for each slice was determined from a region of interest drawn over the whole tumor. Tumor ΔR_2* was derived from subtracting oxygen-breathing R_2* maps from air-breathing R_2* maps.

Histology: Whole tumor sections, cut approximately in the same plane as for MRI, were processed for Hoechst 33342 uptake and pimonidazole adducts using fluorescence microscopy. Hypoxic fraction and vessel perfusion were quantified as a % of the whole tumor section area, with 2 to 3 sections analysed per tumor.

Figure 1

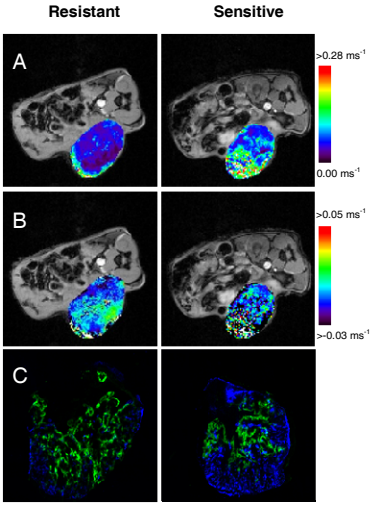
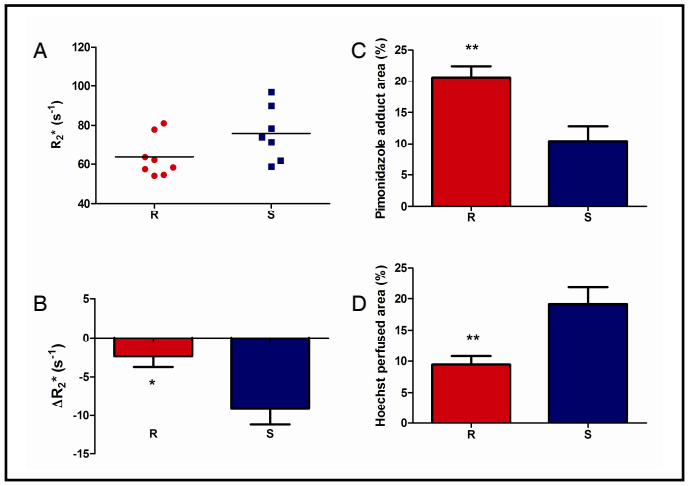


Figure 2



Results: Baseline R_2* maps of resistant tumors revealed regions of relatively fast R_2* primarily in the tumor periphery, while sensitive tumors exhibited a more heterogeneous distribution of R_2* across the whole tumor (**Fig.1A**). There was no significant difference in mean baseline R_2* between resistant (64 ± 4s^{-1}) and sensitive tumors (76 ± 5s^{-1}, p=0.07) (**Fig.2A**). Oxygen-breathing resulted in a reduction in R_2* for both resistant and sensitive tumors, consistent with an overall increase in tumor oxygenation. A significantly greater ΔR_2* was observed for sensitive tumors (-9.1 ± 2.0s^{-1}) compared to resistant tumors (-2.4 ± 1.4s^{-1}) (*p<0.05) (**Figs.1B & 2B**). Resistant tumors had significantly (**p<0.01) lower levels of Hoechst 33342 uptake which was restricted primarily to the tumor periphery (**Fig. 1C, blue**), and significantly (**p<0.01) increased pimonidazole adduct formation (**Fig. 1C, green**), compared to the sensitive tumors (**Figs. 2C & 2D**).

Conclusions: EGFR TKI-resistant tumors demonstrated peripheral regions of fast R_2* compared to a more heterogeneous distribution observed in size-matched sensitive tumors. Of significance, regions of relatively fast R_2* were spatially related to Hoechst 33342 perfusion, whilst slower R_2* corresponded to regions of hypoxia. There was no significant difference in baseline R_2* between resistant and sensitive tumors, however hyperoxic-induced ΔR_2* was significantly smaller with resistance. This attenuated hemodynamic response was associated with lower levels of Hoechst 33342 uptake and an increased hypoxic fraction. ΔR_2* therefore informs on both hemodynamic/functional response and tumor oxygenation. Overall, these findings suggest that resistance to EGFR-TKIs in the CAL27 model could result from a poorly perfused/hypoxic phenotype. Intrinsic susceptibility MRI revealed spatial and functional differences in tumor vasculature and oxygenation, providing a useful non-invasive imaging strategy for the investigation of EGFR-TKI drug resistance and tumor hypoxia.

References:

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