# The Response of RIF-1 Fibrosarcomas to the Vascular Targeting Drug ZD6126 Assessed by *In Vivo* $^1$H MRS

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## INTRODUCTION

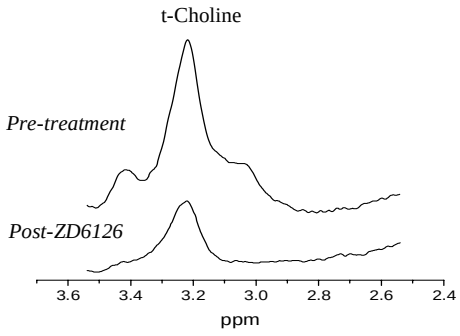
Novel anti-cancer therapies are being developed to exploit differences between normal and tumour endothelium to selectively target the destruction of tumour endothelium and induce massive haemorrhagic necrosis, whilst leaving normal blood vessels relatively unaffected. ZD6126 is a vascular targeting agent shown to have significant anti-tumour activity against a broad range of rodent model systems[1, 2]. The clinical development of anti-vascular therapies requires the development and validation of quantitative endpoints which are associated with tumour blood vasculature and its response, as the majority of these therapies are not cytostatic. Localised $^1$H MRS techniques have been developed to quantify tumour choline concentration, an important metabolic marker associated with membrane turnover, malignancy and tumour grade, and lactate, another significant tumour metabolite associated with glycolysis, acidosis and hypoxia[3,4]. We have used $^1$H MRS *in vivo* to assess RIF-1 fibrosarcomas prior to and 24 hours after treatment with 200mg/kg ZD6126, a dose and timing previously shown to induce massive haemorrhagic necrosis in this tumour model[2].

## METHODS

RIF-1 tumours were grown subcutaneously in female C3H nude mice. Tumours were placed into a 15 mm diameter two turn RF coil and $^1$H MRS performed using a 4.7T Varian Unity Inova MR spectrometer. Voxels in the range of 400–600 mm$^3$ were selected from scout gradient echo images and localised shimming yielded linewidths of the order of 25-30 Hz. The PRESS localisation method with water suppression was used to detect total choline (t-choline) (consists signals from Phosphocholine (PC), Glycerol-3- Phosphocholine (GPC) and choline compounds) with a TR=2s, 64 transients and TE=20, 68, 136, 272 and 408 ms. For unsuppressed water, 16 transients were acquired with same acquisition parameters as above except with a lower receiver gain. A modified Multiple Quantum Coherence edited sequence (SSel-MQC) with a single shot ISIS localisation[5] and a TR=3s was used to detect the lactate signal. After pre-treatment $^1$H MRS measurements, 200mg/kg ZD6126 or saline was administered i.p. and $^1$H MRS repeated 24 hours later. Peak areas of observed metabolites were quantified using the MRUI software[6]. Apparent choline concentration was calculated using tumour unsuppressed water as a reference[3].

## RESULTS

Figure 1 shows PRESS localised $^1$H MRS spectra (TE=272 ms) acquired from one RIF-1 tumour prior to and post treatment with ZD6126. All six tumours showed a decrease in t-choline in response to ZD6126. The data are summarised in Table 1 (mean ± 1 s.e.m., *p<0.03).




| **Table 1** | 200mg/kg ZD6126 (n=6) | | Saline (n=4) | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| t-Choline (mM) | 3.66 ± 0.44 | 1.94 ± 0.28* | 3.80 ± 0.51 | 5.30 ± 1.08 |
| Water $T_2$ (ms) | 50.8 ± 1.1 | 49.6 ± 7.0 | 52.6 ± 0.9 | 51.9 ± 0.8 |
| t-Choline $T_2$ (ms) | 200.2 ± 8.5 | 153.4 ± 11.9* | 204.5 ± 17.4 | 192.7 ± 10.0 |
| Lactate/water (x10$^{-3}$) | 1.85 ± 0.19 | 1.32 ± 0.39 | 2.64 ± 0.51 | 1.44 ± 0.31 |

## DISCUSSION

The significant decrease in the choline resonance following ZD6126 is consistent with reduced cell membrane turnover associated with necrosis following the disruption of the tumour vasculature. The significant decrease in choline $T_2$ after treatment also suggests that the remaining choline resonance is present in less mobile molecules. The lack of significant change in the lactate/water ratio is not consistent with lactate accumulation as a result of hypoxia/acidosis from ZD6126-induced vascular shutdown at this time point, nor is it consistent with macrophage infiltration. We surmise that any lactate produced following treatment with ZD6126 has been cleared from the tumour. The data suggest that a decrease in choline may provide a novel non-invasive biomarker of tumour response 24 hours after treatment with vascular targeting agents.

## REFERENCES

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