COMBINED LOOK-LOCKER ARTERIAL SPIN LABELING (ASL) AND INTRINSIC R2*SUSCEPTIBILITY RESPONSE TO VASCULAR DISRUPTION IN A CLINICALLY RELEVANT LIVER METASTATIC MODEL OF COLORECTAL CARCINOMA

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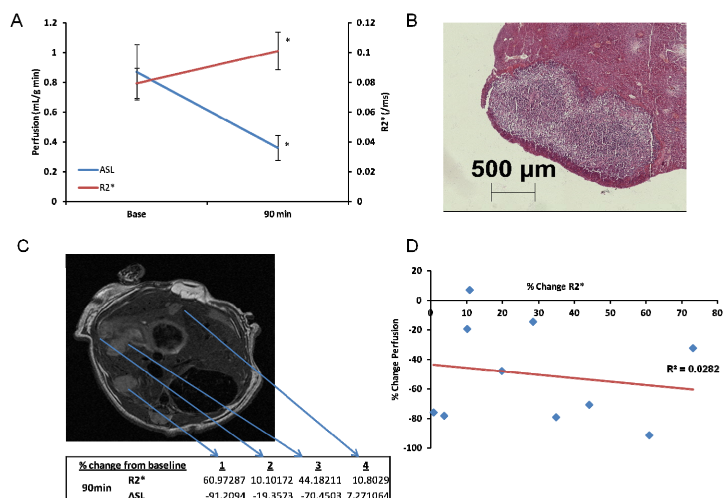
Introduction

The main cause of mortality for patients with colorectal carcinoma (CRC) is metastatic dissemination of cancer cells to the liver. Surgical resection of metastases leads to a 5-year survival rate of ≈40%, but is only possible in 10-20% of cases[1]. Other treatment approaches are therefore necessary, either to 'de-bulk' tumour mass and allow resection with curative intent or to directly prolong long-term survival. One such treatment is the vascular disrupting agent (VDA) OXi4503, which increases survival in murine models of liver metastases through destruction of tumour vasculature and resultant central necrosis[2], albeit with a surviving rim of viable cells[3]. A phase I clinical trial of OXi4503 in patients with liver metastases has recently been reported using R2* as a potential early biomarker of therapeutic efficacy[4], although the observed change in R2* was variable and the physiological mechanisms underpinning these changes difficult to interpret. Novel therapeutic approaches require effective non-invasive methods to assess response in patients and experimental models. Tumour perfusion (measured by arterial spin labelling [ASL]), which has recently been demonstrated in a preclinical liver metastatic model[5], may provide a further potential biomarker of efficacy of OXi4503. **Objective:** To acquire and compare measurements of R2* and perfusion in a preclinical orthotopic liver metastasis model of CRC before and after administration of OXi4503 and assess suitability to monitor tumour response.

Method

The colorectal carcinoma cell line SW1222 was grown *in vitro* by tissue culture techniques and prepared to a concentration of $1x10^7$ cells/mL in serum free media. To establish liver metastases MF1 *nu/nu* mice (n=3) were injected intrasplenically with 100µl cell suspension, cells were allowed to wash through to the liver for 1 minute, and splenectomy performed. Tumour development was initially monitored by IVIS bioluminescence, and later with T2 weighted MRI. MRI was performed using a 9.4T Agilent VNMRS 20cm horizontal bore system with a 39mm birdcage coil and a warm air blower to maintain animal temperature. To assess perfusion of metastases respiratory gated (SA instruments, New York, USA) fast spin echo images were used to define a suitable imaging slice across the liver followed by a segmented FAIR Look-Locker ASL sequence with a single slice spoiled gradient readout. R2* values, as a reflection of presence of deoxyhaemoglobin, were assessed by a multi-gradient echo (MGE) image sequence covering the entire liver. A total of n=10 distinct metastases were evaluable. Both ASL and R2* were acquired at baseline and 90 minutes following i.v. dosing via remote line of 40mg/kg OXi4503 without movement of the animal between scans. For histological analysis tumours were excised at 24hr post OXi4503 when central tumour necrosis is expected[6]. Perfusion and R2* maps were created using IDL and Amira 5.3.1 software, with median values tested for significance by paired t-test.



% change from baseline		1	2	3	4
90min	R2*	60.97287	10.10172	44.18211	10.8029
	ASL	-91.2094	-19.3573	-70.4503	7.271064

Figure 1 **(A)** Median values of ASL and R2* (n=10) at baseline and 90min following 40mg/kg OXi4503 i.v. * denotes significance ($p<0.01$). **(B)** H&E at 24h post-dose showing central tumour necrosis. **(C)** Table showing individual metastases ASL and R2* values. **(D)** Scatter plot of % change in ASL vs R2* showing an inverse correlation of R^2=-0.03

FAIR Look-Locker ASL sequence parameters: *30 x 30mm FOV, 128x128 matrix, TE: 1.18 ms, TI: 110 ms,* TR_{RF}*: 2.3 ms,* TR_I*: 13 s, 50 inversion recovery readouts. Localised inversion thickness: 6 mm, global inversion slice thickness: 200 mm; imaging readout slice thickness: 1 mm.*
MGE sequence parameters: *8 echoes,* TE_1*=2ms, echo spacing=2ms, TR=280ms; 128x128 matrix, 40x40mm FOV, 1mm slice thickness.*

Results

Figure 1A shows significant ($p<0.01$) changes in median values for both ASL and R2* across the n=10 metastases 90 minutes after OXi4503 administration. **1B** shows that central tumour necrosis has occurred within the metastases at 24 hours post dose. **1C** shows an example of an imaging slice from which ASL and R2* have been calculated. Values of percentage change from baseline for the four metastases present are depicted, and show variable response in ASL and R2*. **1D** shows a scatter plot of the change in these values across the n=10 metastases, with an R^2 value of 0.03 (no correlation).

Discussion

We were able to detect acute changes in tumour pathophysiology caused by OXi4503 with both ASL and R2*, with a significant decrease in mean perfusion and increase in R_2*. This is consistent with the mechanism of action of VDAs: cessation of blood flow leads to a reduction in tumour perfusion and an increase in paramagnetic deoxygenated haemoglobin. The central tumour necrosis at 24hr observed in H&E sections is expected at this dose and demonstrates successful i.v. administration via remote line. Individual tumour deposits displayed a variable response, both within and between mice, although all metastases showed an increase R_2* and all but one an increase in perfusion at 90 minutes post-dose. However, changes in R_2* and perfusion were not correlated, indicating a complex relationship between changes in flow and accumulation of deoxyhaemoglobin, which may be specific to individual tumours. The detection of a variable response, even in tumour deposits within the same liver (such as in Fig. **1C**), highlights the need for robust assessment of response within individual patients.

This study is the first to use ASL in this type of tumour model and to assess response to this class of agent, and we have demonstrated its utility in this context. Arguably, given the mechanism of action of vascular disrupting agents, ASL provides response biomarkers that afford a less ambiguous interpretation than intrinsic susceptibility (R_2*) measures. However, an approach combining the two may provide deeper insights in to the mechanics of tumour response *in vivo*, by relating flow changes to changes in blood oxygen saturation. We are currently collating data from later time points and it will be of interest to determine the relationship between such physiological changes and longer-term response.

References: **(1)** Penna C and Nordlinger B, *British Medical Bulletin* (2002) 64:127-140, **(2)** Chan LS et al, *Anticancer Drugs* (2008) 19(1):17-22, **(3)** Pedley RB et al, *Cancer Research* (2001) 61(12):4716-22, **(4)** Zweifel M et al, *Proc. Intl. Soc. Mag. Res. Med.* 19 (2011) 339, **(5)** Ramasawmy R et al, *Proc Brit Chap ISMRM* (2011) poster 62, **(6)** Chan LS et al *Anticancer Res* (2007) 27:2317-2324

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