

## Vessel Size Index (VSI) MRI in Solid Tumours - Validation with Microvascular Corrosion Casts

J. S. Burrell<sup>1</sup>, J. Halliday<sup>2</sup>, S. Walker-Samuel<sup>3</sup>, J. C. Waterton<sup>2</sup>, P. J. Withers<sup>4</sup>, R. S. Bradley<sup>4</sup>, J. Boul<sup>1</sup>, Y. Jamin<sup>1</sup>, L. C. Baker<sup>1</sup>, and S. P. Robinson<sup>1</sup>

<sup>1</sup>The Institute of Cancer Research, Sutton, Surrey, United Kingdom, <sup>2</sup>AstraZeneca, Manchester, United Kingdom, <sup>3</sup>UCL, London, United Kingdom, <sup>4</sup>School of Materials, University of Manchester, Manchester, United Kingdom

**Introduction:** Non-invasive imaging biomarkers for assessing tumour pathophysiology and response are continually being sought: such biomarkers require evaluation and qualification before they can be deployed in clinical trials. Susceptibility contrast MRI allows non-invasive determination of fractional blood volume (fBV, %) and vessel size index (R<sub>v</sub>, μm), biomarkers that are being exploited for the evaluation of anti-vascular therapies [1]. For determination of R<sub>v</sub>, tumour R<sub>2</sub>\* and R<sub>2</sub> are measured before and after the injection of a USPIO particle contrast agent, and the ratio of the resulting changes in relaxivities (ΔR<sub>2</sub>\*/ΔR<sub>2</sub>), when combined with apparent diffusion coefficient (ADC) and an estimated blood concentration of USPIO particles, is proportional to a weighted vessel size index. Vascular corrosion casting involves injecting a vascular network with a low viscosity monomer resin which completely fills the entire vascular tree. Shortly after injection, the resin polymerises and sets solid, allowing surrounding tissue to be corroded away with an alkali solution, leaving a replication of the vascular network [2]. Corrosion casts can subsequently be imaged with high resolution CT, from which information about the vascular network, including vessel sizes, can be calculated. **In this study, susceptibility contrast-derived MRI derived values of R<sub>v</sub> were compared with vessel sizes measured from corrosion casts of the same tumours.**

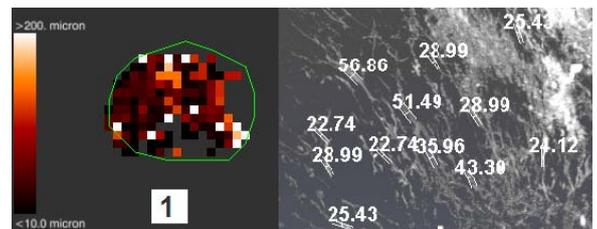
**Methods:** Six female NCr nude mice were injected subcutaneously on the right flank with 5×10<sup>6</sup> SW1222 colorectal tumour cells. Tumours were imaged two weeks later at an approximate diameter of 1cm. Mice were imaged 24 hours after an i.p. injection of 200mg/kg of the vascular disrupting agent ZD6126 (n=3) or vehicle (n=3). All images were acquired on a 7T horizontal bore Bruker system using a 3cm birdcage coil. Animals were anaesthetised and restrained using dental paste in order to limit motion artefacts [3]. TurboRARE images were acquired for tumour delineation, followed by a diffusion weighted MRI (b=1.7, 115, 200, 427, 664, 904 s/mm<sup>2</sup>), multiple gradient echo (MGE) and a multiple spin echo (MSE)

for determination of R<sub>2</sub>\* and R<sub>2</sub>. Images were acquired from three contiguous 1mm slices with identical geometry, a FOV of 3.3×3.3 cm<sup>2</sup> and a matrix size of 64×64. Two minutes after the injection of 200μmol/kg USPIO contrast agent ferumoxtran-10 (Sinerem®, Guerbet Research, France) via a cannulated tail vein, a second set of identical MGE, and MSME images were acquired. Immediately following the MRI acquisition each animal was killed by asphyxiation. Following midline laparotomy, the ascending carotid and femoral arteries were ligated, the heart exposed and the ascending aorta isolated, and a blunted cannula inserted through the bottom of the heart and into the aorta. Saline was first injected at 60ml/hr by a power injector and allowed to drain through a small incision in the right ventricle. Subsequently, monomer resin (Mercox 2) was mixed with a catalyst to begin the polymerising process, and injected via the same cannula at 60μl/hr until the vascular bed was completely filled. The tissue sample was then dissolved in 7.5% KOH, dissected and freeze dried. CT images of the cast were taken on a Metris X-tek XTH225 CT scanner.

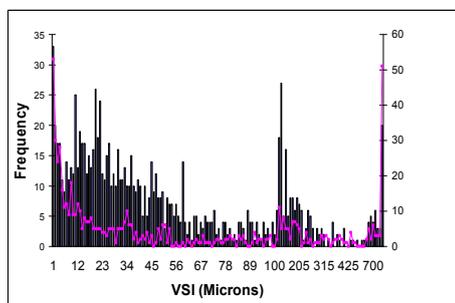
**Results and Discussion:** A calculated VSI map and CT image of a vascular cast from the same SW1222 tumour are shown in Figure 1. Histograms of MRI derived VSI values for ZD6126 and vehicle treated tumours are shown in Figure 2. The median R<sub>v</sub> values were 24 ± 6μm and 35 ± 5μm in the ZD6126 treated and vehicle cohorts respectively. Although there was no significant difference in R<sub>v</sub> between the ZD6126 treated and vehicle cohorts (p=0.1), visual inspection of the histograms shows an apparent difference in vessel size index distribution in the two groups. Treatment with ZD6126 significantly lowered fBV compared to the vehicle cohort (2 ± 1% and 13 ± 1% respectively), precluding delivery of the resin and successful casting at this dose. Vascular corrosion casts were successfully obtained for vehicle treated mice. A histogram of VSI values from these tumours is shown in Figure 3, with a median R<sub>v</sub> = 38 ± 6μm. The corresponding vessel size information derived from CT images of the vascular casts from the same tumours is shown in Figure 4, with a median vessel diameter of 39 ± 2μm. There was no significant difference between the VSI and vascular cast derived median vessel size (p=0.6). Both the VSI and vascular cast vessel size histograms showed two populations; one centred around 30μm and a second population of large diameter vessels centred around 150μm.

**Conclusion:** VSI was compared to vessel diameters measured from CT images of vascular casts of the same SW1222 colorectal tumours. Median vessel diameter values from the two methods were in good agreement. The distribution of vessel size values measured by the two different techniques also showed an apparent similarity. As a proof of principle, vascular corrosion casts can provide appropriate histological qualification of susceptibility contrast MRI vessel size imaging. In future work a lower dose of ZD6126 which induces more subtle anti-vascular effects will be used to test the sensitivity of VSI measurements, and validated with vascular casts. Vascular corrosion casting also offers spatial and structural vascular information, and therefore could be used in future work to validate MRI measurements of more complicated phenomena, such as vascular normalisation.

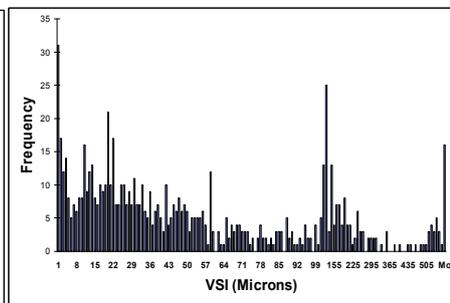
**Acknowledgements:** This work was supported by BBSRC, AstraZeneca, The Royal Society, Cancer Research UK grant number C1060/A5117 and also NHS funding to the NIHR Biomedical Research Centre. We acknowledge the support received from the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) grant C1060/A10334, also NHS funding to the NIHR Biomedical Research Centre.



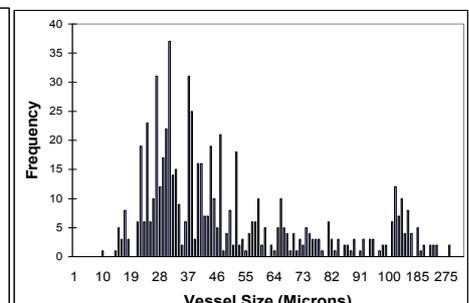
**Figure 1.** Representative VSI map and CT image of vascular cast from a control SW1222 colorectal tumour.



**Figure 2.** Histogram of MRI derived VSI values for ZD6126 (pink) and vehicle (black) treated SW1222 colorectal tumours.



**Figure 3.** Histogram of MRI derived VSI values from vehicle treated SW1222 colorectal tumours.



**Figure 4.** Histogram of vascular cast derived vessel size values for vehicle treated SW1222 colorectal tumours.

**References:** 1) Tropres I, *et al*, *Mag Res Med*, 2001. 2) Murakami, T *Arch.histol.* 1971 3) Landuyt, W. *et al*. *J Magn Reson Imaging*, 2002.