Investigating quantitative imaging biomarkers of response to cabozantinib in a VCaP model of prostate bone metastasis

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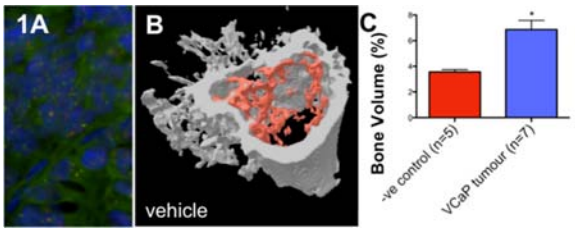
Introduction: Prostate cancer (PCa) growth is incurable once it has metastasized to the bone microenvironment (BM). The altered BM provides a permissive niche to support tumour growth, and therapeutic strategies that target tumour-bone interactions and/or restore bone homeostasis are being pursued. This demands preclinical models that faithfully replicate tumour-bone interactions, and non-invasive imaging methods to interrogate such orthotopic models *in vivo*, improving the accuracy of, and accelerating pre-clinical drug development. We have used a multi-modality imaging approach, to assess the radiology and response of an orthotopic VCaP PCa bone metastasis model to the c-Met/VEGFR2 inhibitor cabozantinib (Exelixis, Inc.).

Materials and Methods: *Animals and Tumours:* Male castrate SCID mice were injected with $2x10^6$ luciferase expressing VCaP PCa cells (VCaP-luc) through the proximal end of the right tibia. *Drug Administration:* After 27 days, mice bearing established intratibial VCaP-luc prostate tumours were treated daily with 30mg/kg p.o. cabozantinib (CABO, n=7) or vehicle alone (vehicle control, n=7) for 15 days.

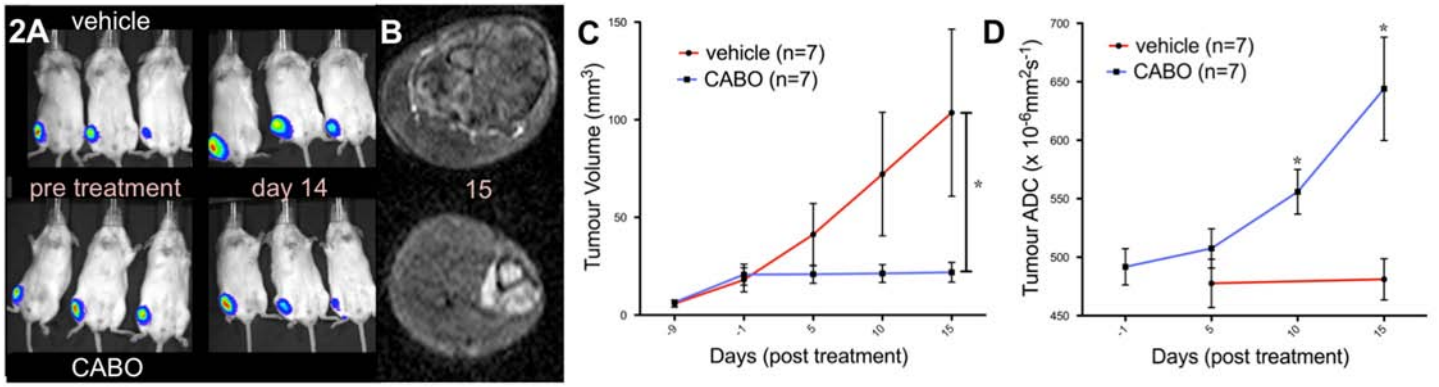
Longitudinal imaging: Bioluminescence imaging (BLI) confirmed tumour development and progression at day 18 and 25 after intratibial injection, and therapeutic response after 14 days of treatment. MRI studies were carried out on a Bruker 7T horizontal bore Microimaging system. Mice were anaesthetised, and tibias restrained in a single leg home-built RF saddle coil. T_2-weighted TurboRARE images were acquired using a slice thickness of 0.5mm for 40 slices from a 128x128 matrix over a 20x20mm field of view (FOV), using an echo time (TE) of 36ms and repetition time (TR) of 7000ms and 3 averages, giving a total acquisition time of ~3 minutes. On these T_2-weighted images, tumour was identified as a hyperintense signal enclosed within the cortical bone, and in the surrounding muscle. Tumour burden was quantified from ROIs drawn on the periphery of the hyperintense signal in OsiriX and followed through each slice of the tibia. EPI-diffusion weighted images were acquired from four 1mm thick axial tumour bearing slices, using T_E=32ms, T_R=3000ms, FOV 20x20 mm, matrix 128x128, 8 averages, b values of 0, 30, 60, 100, 150, 200, 300, 500, 750 and 1000, giving AQ ~8min. Data were fitted on a voxel-by-voxel basis using in-house software, providing maps of tumour spatial heterogeneity of ADC.

µCT imaging: After sacrifice, each tibia underwent an 18µm resolution µCT scan (Skyscan 1076). Images were binarized, allowing bone volume (BV) calculations to be made.

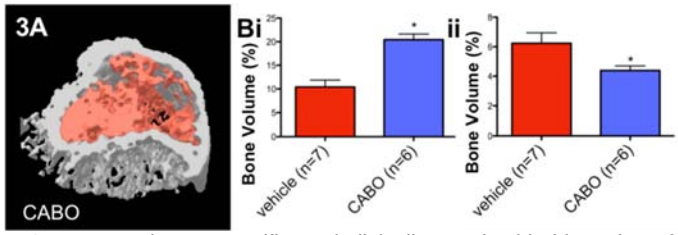
Results: *Model Characterisation:* BLI revealed successful tumour propagation within the injected limb. MRI showed initial malignant growth within the BM, before further invading into the surrounding muscle. Furthermore, fluorescent in-situ hybridization analysis on xenograft tissue, shown in Figure 1A, confirmed that VCaP-luc tumours retained the rearrangement of the ERG oncogene, the most common chromosomal abnormality found in human PCa (40-80% of cases). On µCT images, areas of osteosclerotic and osteolytic activity could be identified, yet the abnormal bone turnover induced, exhibited a predominantly sclerotic phenotype (Figure 1B). Tumour bearing tibias had 93.2% more bone volume (BV) compared to contralateral tibias (Figure 1C).



Therapeutic Response: Semi-quantitative photon flux measures from BLI showed a 51.9% regression photon flux after 14 days of treatment (p=0.016, Figure 2A). Representative images of a single axial slice through the tibia from vehicle control and CABO treated mice are shown in Figure 2B. Mean tumour volume was significantly (p=0.038) smaller in the CABO group after 15 days treatment (22 ± 5 mm^3) compared to the vehicle control cohort (104 ± 43 mm^3, Figure 1C). From baseline, ADC significantly increased after 10 (p=0.024) and 15 (p=0.010) days of treatment, from 492 ± 16 to 556 ± 19 and 644 ± 44 ($x10^{-6}$ mm^2/s) respectively (Figure 1D). There was no change in tumour ADC of the vehicle treated group. The increased ADC was associated with extensive necrosis determined histologically in the CABO group only.



Treatment had no significant effect on µCT derived gross BV. When trabecular bone was quantified separately (shown in Figures 1B and 3A in red), cabozantinib treated tumour bearing tibias showed a 95.2% increase in BV ratio (p=0.001, Figure Bi). Moreover, there was significantly less tumour-related bone growth outside the bone marrow cavity in cabozantinib treated mice (p=0.048, Figure Bii).



Conclusions. These data suggest that the intratibial VCaP-luc model is sensitive to the novel small-molecule kinase inhibitor cabozantinib and that MRI provides a non-invasive quantifiable measure of therapeutic efficacy, correlating with rapid BLI measurements. In addition, early increase in tumour ADC may constitute a specific, and clinically translatable biomarker of therapeutic response in this model. µCT has afforded information as to the tumour-stromal interaction, showing that the VCaP-luc tumours deregulate normal bone function to generate a predominately osteosclerotic phenotype. Cabozantinib treatment in this model resulted in increased ADC, consistent with decreased cellularity and increased necrosis. Cabozantinib also decreased tumour-related bone growth on tibial cortex, but established an increased trabecular BV. These results may reflect direct effects on tumor cells and vasculature, coupled with effects on the bone microenviroment.

We acknowledge the support received for The Institute of Cancer Research CR-UK and EPSRC Cancer Imaging Centre, in association with the MRC and Department of Health (England) grant #C1060/A10334, and NHS funding to the NIHR Biomedical Research Centre.