

The tumour microenvironment plays a significant role in regulating tumour growth, metabolism, DNA repair, metastasis and response to therapy including radiation treatment. It is well recognized that the oxygenation of a tumour greatly affects the response to ionizing radiation as hypoxic cells are substantially more resistant to radiation than normoxic cells. Hypoxia reduces the killing of cells by X-rays by 2-3 folds and the outcome of patients with more hypoxic tumours is significantly worse compared to those with less hypoxic tumours. Thus reduction of hypoxia has long been postulated as a means to improve the outcome of radiation therapy. Tumours are characteristically hypoxic in large part through inadequate oxygen delivery due to poor perfusion through aberrant tumour vasculature. Conversely radiation influences the microenvironment of the tumour.

Radiation can alter tumour angiogenesis and perfusion in a time and dose-dependent manner, which occurs through several factors including vasodilation, reduced interstitial fluid pressure, infiltration

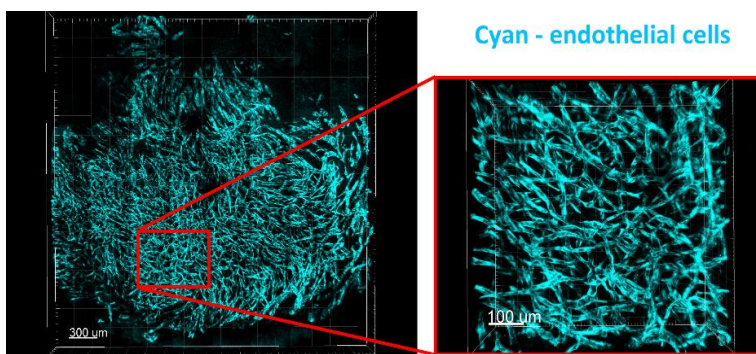


Fig 1. A 3D image of tumour vasculature from a MC-38 allograft in the VeCad-Tomato mouse. Image was acquired 9 days tumour inoculation with 2P microscope in a living mouse. Image on the left shows the tiles of the entire tumour with 3.5 mm diameter and 300 µm thickness after reassembled and reconstructed in 3D. The right shows a higher power view indicating the detail of vascular networks that can be imaged.

of immune cells, endothelial cell damage, HIF1 α stabilization, oxygen consumption and VEGF induction. Thus, there has been great interest in targeting these processes in combination with radiation to favourably modify the tumour microenvironment, and improve tumour control.

In this project we intended to develop strategies to enhance radiation therapy by delineating the vascular response to radiation. Direct observation of the vasculature during and after radiation with state of the art microscopic techniques could resolve many of these outstanding issues. We aimed to improve understanding of the development of tumour vasculature and its response to therapy.

We have developed a transgenic mouse model that we used to visualize fluorescently labelled tumour

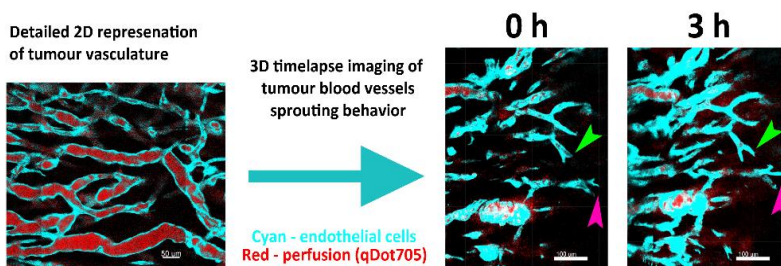


Fig 2. Images of tumour endothelial cells in the VeCad-Tomato mouse. Left side: a single Z plane of a 3D image where blood vessel wall (cyan) can be distinguished from blood vessel volume labelled with perfusion dye (red). Right side: 3D time-lapse image of developing sprouts (green and purple arrowheads) of tumour vasculature.

vasculature in the xenografted tumours generated in VE-cadherin-CreERT2, flox-STOP-flox-tdTomato mice (**Fig 1**). This provided new data about the progression of angiogenesis in the 3D tumour model. Using this model we observed sprouting (**Fig 2**) and intussusception of vessels in 3D tumour model and showed functional linkages by using perfusion dyes in combination with our model.

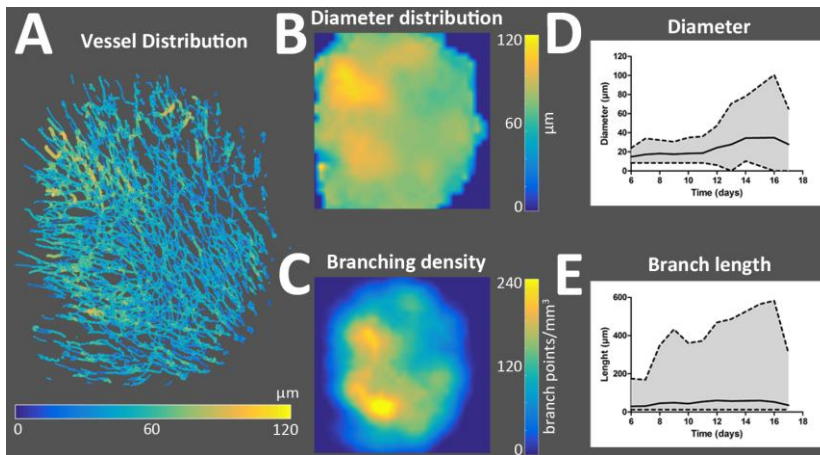


Fig 3. 3D reconstruction of MC38 tumour vascular network. 2D representation of blood vessel diameters (A). Distribution of diameters (B) and density of branches/bifurcations in mm^3 (C). Changes in time of diameter (D) and branch length (E). Mean value (solid line) and associated maximum and minimum values (dashed line) are presented.

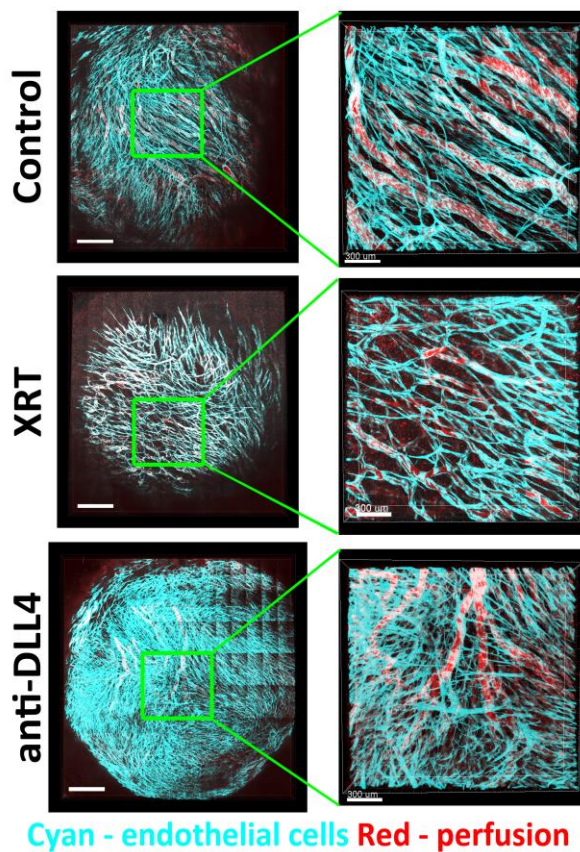


Fig 4. Representative images of control tumours, tumours receiving single dose 15 Gy XRT or anti-DLL4 antibodies. In the right column a magnified view of the indicated insert (green) is shown. Scale bar: 1 mm in the overview and 300 μm in the inserts.

In collaboration with Dr. Julia Schnabel and Prof. Sir Mike Brady we developed in-house software in order to fully visualize the tips and the formation of the sprouts and to quantify the developing vascular network (**Fig 3, Fig 4**). We used this approaches to follow the response of tumour vasculature to radiation therapy using newly developed biocompatible window chamber and Small Animal Radiation Research Platform (SARRP) and the involvement of apoptosis in the process. We have also determined the sprouting behaviour in the presence of different angiogenesis inhibitors (**Fig 4**).

Our work highlighted the importance of imaging approaches such as intravital microscopy in combination with fluorescent proteins expressed in specific population of cells in order to delineate the mechanisms of vascular response to therapies. Our observations provide the missing data in literature about the response of tumour vasculature to radiation therapy. This could have a profound implication in clinics when vascular targeted therapies are used in combination with radiation therapy.