

Edema control by anti-VEGF therapy prolongs survival despite persistent tumor growth in mice

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Introduction: Recent clinical trials of anti-vascular endothelial growth factor (VEGF) agents for glioblastoma showed promising progression-free and overall survival rates [1]. However, it is unclear whether this is due to anti-tumor or anti-edema effects of these agents. Thus the mechanisms leading to improved survival in patients remain unclear. Our goal was to determine whether alleviation of edema by anti-VEGF agents alone, without affecting tumor growth, could increase survival in mice. Here we examine in detail the impact of cediranib (AZD2171, RECENTIN®, AstraZeneca) treatment on MRI biomarkers of tumor growth, tumor blood volume, blood vessel caliber, edema, and permeability in a U87 mouse brain tumor model. In addition, we validate the MRI biomarkers of tumor angiogenesis with histology, optical microscopy, and wet-dry weight measurement methods.

Materials and Methods:

In vivo two-photon microscopy: U87GFP tumors were implanted in nude mice with previously implanted cranial windows. When tumors reached a diameter of 2-3 mm, animals were anaesthetized and 3-6 locations per animal were investigated using a multi-photon laser-scanning microscope. To visualize the vessels, 150 μ l of tetramethylrhodamine labeled dextran (MW 2 million, 10 mg/ml) was injected intravenously. Stacks of 250 μ m depth and 5 μ m Z-steps were acquired, and a virtual vascular cast was generated in 3D by custom image analysis software. Length-weighted average vessel diameter was calculated based on the virtual cast.

Magnetic Resonance Imaging: U87GFP tumors were implanted in nude mice and imaged on a 9.4 Tesla MRI scanner (Bruker Biospin, Billerica, MA) when tumor diameters reached 2-3 mm. Tumor volume was assessed from T2-weighted RARE images. Tumor edema was assessed from R2 and ADC maps, generated from multi-echo spin-echo and diffusion-weighted spin-echo images, respectively. Tumor blood vessel permeability (K_{trans}) was assessed from Dynamic Contrast Enhanced (DCE) MR images [2]. Steady-state R2 and R2* maps generated before and after injection of superparamagnetic iron-oxide nanoparticles (SPION) were used to generate $\Delta R2$ and $\Delta R2^*$ maps from which the tumor relative vessel caliber index (rVCI) was determined [3].

Water content analysis by dry/wet weight measurements: Anesthetized mice were euthanized by cervical dislocation and the brains were collected. Brains were dissected into several compartments: tumor, ipsilateral hemisphere, contralateral hemisphere and midbrain. Tissues were weighed immediately and dried in a vacuum for up to 2 weeks. Weights were collected throughout the drying period until the final dry weight was established.

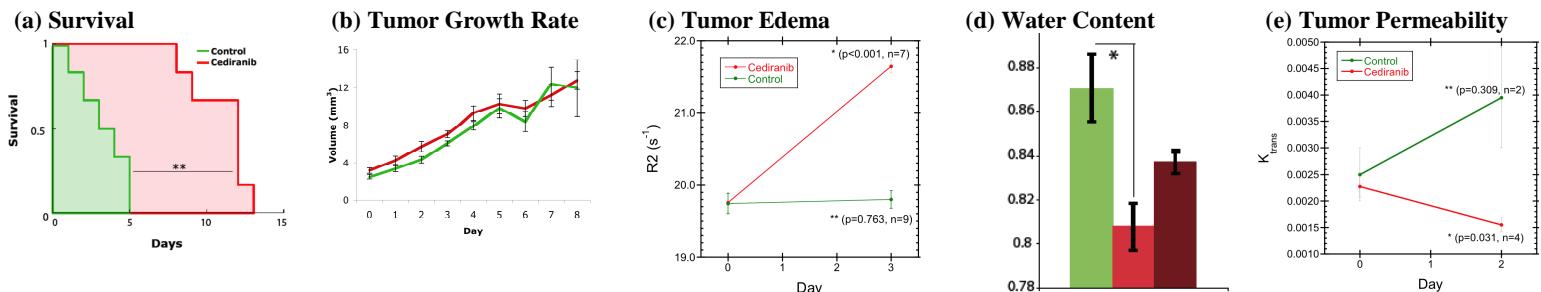


Figure 1: (a) : Cediranib (6 mg/kg bodyweight/day) treatment leads to a statistically significant survival benefit ($p<0.001$). (b) Cediranib does not delay tumor growth as measured by either 2-photon microscopy or MRI. (c) Cediranib treatment results in a significant increase ($p<0.001$) in tumor R2*. (d) Water content measured by dry/wet weight method in the tumor indicates that cediranib transiently but significantly (* $p<0.05$) decreases tumor water content. (e) Cediranib treatment results in a statistically significant ($p=0.031$) decrease in permeability (K_{trans}). In contrast, an increase in K_{trans} is observed for untreated control animals.

Conclusions: While cediranib treatment lead to a statistically significant increase in survival of U87 brain tumor mice, it had no effect on the tumor growth rate. Cediranib treated animals died with significantly larger tumors than control animals indicating that the survival benefit is independent of tumor size. While cediranib did decrease tumor blood volume it had no effect on the average vessel caliber. Cediranib lead to a statistically significant decrease in tumor edema and vascular permeability. The survival benefit of cediranib therefore appears to be primarily mediated by its anti-cerebral edema effects and normalization of vascular permeability. Finally, the MRI biomarkers of tumor angiogenesis were in good agreement with the histology and intravital microscopy measurements.

References:

1. Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, Kozak KR, Cahill DP, Chen PJ, Zhu M, Ancukiewicz M, Mrugala MM, Plotkin SR, Drappatz J, Louis DN, Ivy P, Scadden DT, Benner T, Loeffler JS, Wen PY, Jain RK. "AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients". *Cancer Cell* **11**:83-95 (2007).
2. Tofts P, Brix G, Buckley D, Evelhoch J, Henderson E, Knopp M, Larsson H, Lee T, Mayr N, Parker G, Port R, Taylor J, Weisskoff R. Estimating kinetic parameters from dynamic contrast-enhanced T1-weighted MRI of diffusible tracer: standardized quantities and symbols. *J Magn Reson Imaging* **1999**;10:223-232
3. Dennie J, Mandeville JB, Boxerman JL, Packard SD, Rosen BR, Weisskoff RM. "NMR imaging of changes in vascular morphology due to tumor angiogenesis". *Magn Reson Med* **40**:793-799 (1998).