

DCE/DSC MRI studies of intracranial mouse glioma: enhanced anti-vascular/anti-tumor efficacy of Cediranib via combination with the autophagy inhibitor Quinacrine

Merryl Lobo^{1,2}, Sarah Green³, Matthias Schabel², Yancey Gillespie⁴, Randall Woltjer³, and Martin Pike^{1,2}

¹Department of Biomedical Engineering, Oregon Health and Science University, Portland, Oregon, United States, ²Advanced Imaging Research Center, Oregon Health and Science University, Portland, Oregon, United States, ³Department of Pathology, Oregon Health and Science University, Portland, Oregon, United States,

⁴Departments of Surgery, Microbiology and Cell, Developmental & Integrative Biology, University of Alabama at Birmingham, Birmingham, Alabama, United States

Introduction: Malignant glioma, affecting 12-15,000 people per year in the U.S alone, allows only a 14 month average survival for the most common form, glioblastoma multiforme.¹ Despite the induction of robust angiogenesis, anti-angiogenic monotherapy has largely failed to induce durable responses, with tumors developing treatment resistance by adapting to hypoxic/nutrient stress or circumventing the direct cytotoxic cell signaling changes caused by treatment.² Recent studies have shown that autophagy, a cellular degradation pathway that provides energy and biosynthetic substrates, could be an important drug tolerance and resistance mechanism. Late stage inhibition of this process could cause an accumulation of autophagic vacuoles, which has been shown to trigger cell death.^{3,4} Quinacrine, an FDA-approved drug for malaria, is a potent late stage autophagic inhibitor.⁴ We hypothesized that the anti-angiogenic/anti-tumor efficacy of Cediranib, a pan VEGFR (vascular endothelial growth factor receptor) and PDGFR (platelet derived growth factor receptor) inhibitor, will be synergistically enhanced in intracranial glioma via combination with Quinacrine. For this study, we have employed the syngeneic intracranial mouse 4C8 glioma model, and have utilized a comprehensive, perfusion MRI approach by sequentially implementing dynamic contrast enhanced (DCE) MRI and dynamic susceptibility contrast (DSC-MRI) to noninvasively quantify key vascular biomarkers, including K^{trans} (contrast agent extravasation rate constant, a measure of vascular permeability), blood flow (CBF) and blood volume (CBV), while monitoring tumor growth.

Methods: Brain tumors were induced by intracerebral injection of 4C8 glioma cells in B6D2F1 mice (n=24). After MRI screens determined tumor growth had initiated, mice pretreatment perfusion scans were obtained and mice were randomized into untreated (U, vehicle), Cediranib (C, 6 mg/kg daily), Quinacrine (Q, 50mg/kg daily), and Cediranib plus Quinacrine (C+Q) groups, after which additional perfusion scans were obtained biweekly, using an ultra-high field Bruker-Biospin 11.75T MRI instrument. DCE-MRI was implemented using a spoiled GE-FLASH fully relaxed (M_0) precontrast image (TR 6000 ms, TE 1.2 ms, FA 90°, 1 slice (1 mm thick), 195 μ m in-plane resolution) followed by the DCE T_1 weighted image series (450 images) using the same parameters except with TR 15.6 ms and FA 20° (2.0s/image) during which Gd-DTPA was injected (Magnevist, Berlex Inc, i.v., 10X diluted, 3.0 μ l/g, 0.15 mmol/kg). The arterial input function was determined by blind estimation^{5,6} from the tumor tissue response and K^{trans} calculations followed the general approach described in Tofts PS *et al.*, modified to include a blood plasma volume term (v_p).^{7,8} DSC-MRI was then implemented (150 T_2^* weighted images, Paravision FLASH, TR 8, TE 4.2 ms, FA 5°, 195 μ m in-plane resolution, 1 s/image) employing Feridex, an SPIO agent (Berlex Inc, 4X diluted, 2.4 μ l/g, 26.9ug iron/g). CBF, CBV calculations followed the model-independent method described in Ostergaard L. *et al.*⁹ A multislice T_2 weighted image set (spin echo RARE, 98 μ m in-plane resolution) was obtained to assess tumor volume. The two-tailed Unpaired Student's t test was employed to test for statistical differences for the MRI data.

Results: T_2 weighted imaging revealed that C+Q tumor size was reduced by 60% in comparison to U at 11 days of tumor growth ($p < 0.05$), while

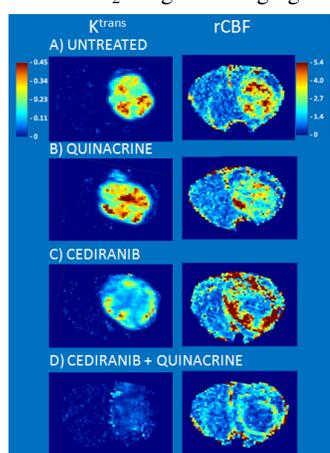


Figure 2. K^{trans} (min^{-1}) and $rCBF$ (relative to contralateral brain) parametric maps during latter stages of tumor growth.

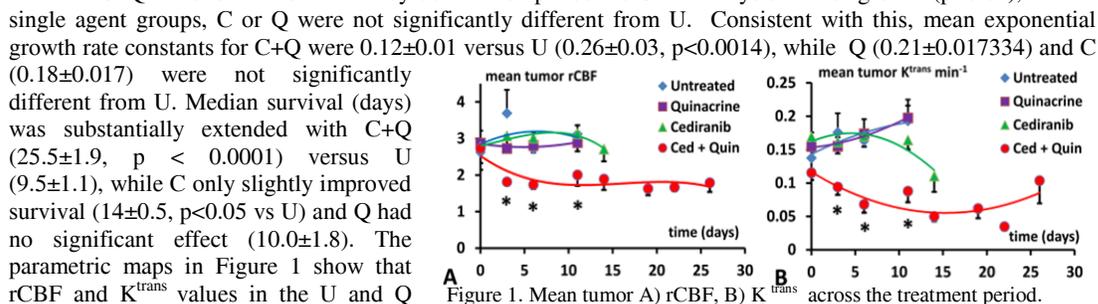


Figure 1. Mean tumor A) $rCBF$, B) K^{trans} across the treatment period.

single agent groups, C or Q were not significantly different from U. Consistent with this, mean exponential growth rate constants for C+Q were 0.12 ± 0.01 versus U (0.26 ± 0.03 , $p < 0.0014$), while Q (0.21 ± 0.017334) and C (0.18 ± 0.017) were not significantly different from U. Median survival (days) was substantially extended with C+Q (25.5 ± 1.9 , $p < 0.0001$) versus U (9.5 ± 1.1), while C only slightly improved survival (14 ± 0.5 , $p < 0.05$ vs U) and Q had no significant effect (10.0 ± 1.8). The parametric maps in Figure 1 show that $rCBF$ and K^{trans} values in the U and Q groups indicated generally high values with a heterogeneous distribution typical of glioma. Cediranib effectively curbed vasculature development in the tumor core but a persistent, well vascularized rim remained resistant to treatment. In contrast, C+Q tumors showed dramatic reductions in K^{trans} and $rCBF$ across the tumor, with a much thinner angiogenic rim. Figure 2 indicates mean tumor values of these parameters over time, and shows that these reductions occurred rapidly with treatment and persisted throughout the treatment period. Substantial increases in $rCBV$ occurred with tumor growth in U and Q, which indicated values of (5.11 ± 0.38), and Q (4.07 ± 0.5) respectively, at 11 days after tumor growth initiation. In comparison, $rCBV$ was slightly attenuated in C (3.82 ± 0.26 , $p < 0.05$ versus U), and substantially attenuated in C+Q (2.43 ± 0.37 , $p < 0.05$ versus U). Taken together, the perfusion MRI results indicate that C+Q treatment had a profound effect on the tumor vascular network, effectively slowed tumor growth and extended survival in 4C8 mouse glioma. Histological approaches

further corroborated these results, showing a 2-fold reduction in mean vessel density ($p < 0.01$ vs U, with CD31 staining) and 3-fold increase in tumor necrosis ($p < 0.05$ vs U, with Hematoxylin & Eosin staining) with C+Q treatment.

Conclusion: In summary, using a novel treatment combination with the intracranial 4C8 mouse glioma model, we documented a synergistic increase in anti-vascular/anti-tumor efficacy of anti-angiogenic inhibitor Cediranib in combination with the autophagy inhibitor Quinacrine, using a comprehensive DCE/DSC perfusion MRI approach and immunohistology. We showed a markedly decreased tumor perfusion and tumor growth, increased tumor necrosis and improved survival, suggesting a new and promising treatment avenue for malignant glioma.

References: 1. Gladson CL, *et al.*, 5:33-50(2010). 2. Rahman R, *et al.*, 251231(2010). 3. Levine B, *et al.*, 115: 2679-2688(2005). 4. Gupta A, *et al.*, 107: 14333-14338(2010) 5. Schabel MC, *et al.*, 55(16):4807-4823(2008) 6. Schabel MC, *et al.*, 55(16): 4783-4806(2010). 7. Tofts PS, *et al.*, 7:91-101(1997) 8. Tofts PS, *et al.*, 10: 223-232(1999). 9. Ostergaard L, *et al.*, 36:715-725(1996)