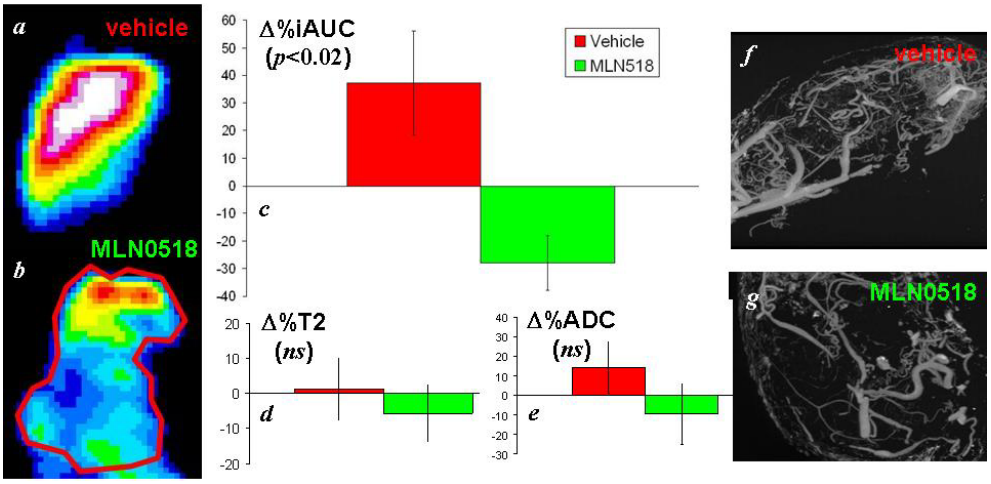
Acute vascular and non-vascular enhanced MRI measurements made in C6 tumour xenografts before and after MLN0518, a potent PDGFRβ inhibitor, treatment.

D. P. Bradley[1], J. Terkelsen[2], D. Cvet[2], B. Hibner[2], K. Burke[2], and M. D. Silva[2]

[1]Imaging Sciences, Millennium: The Takeda Oncology Company, Cambridge, MA, United States, [2]Millennium: The Takeda Oncology Company

Introduction PDGFR inhibition is a promising target for anti-cancer therapy due to several functional outcomes, including perturbation of both tumor vasculature and stromal infiltration, decrease of tumoral interstitial fluid pressure, 'normalisation'of tumor microenvironmental parameters, and enhancement of adjuvant therapy delivery for improving antitumor efficacy in preclinical models[1, 2]. MLN0518 is a potent, ATP-competitive and reversible inhibitor of Type III receptor tyrosine kinases that crosses the BBB; in vitro inhibition is FLT3, cKIT and PDGFRß with a median IC_{50} of approximately 30 nM[3]. Acute anti-tumor activity of MLN0518 manifesting both as a decrease in orthotopic C6 tumor volume and in a relative K^{trans} measure has previously been demonstrated [*unpublished data*]. The current investigation explored multi-contrast MRI (*mc*MRI), µCT and IHC methods during MLN0518 treatment. T_1, T_2, ADC and DCE MRI measurements at 7T (Varian, CA USA) through the tumor were performed in one imaging session[4] before and 72 hours after initiation of treatment. CD31 and PGDFRβ IHC data was also terminally collected. In a separate complimentary experiment, animals were s.c. implanted and therapeutically treated in the same manner as the MRI component, and vessel casting using µCT was performed to extract detailed vascular architecture.

Methods All in vivo procedures were conducted and approved under the Millennium IACUC protocols. NCR mice were inoculated with C6 glial cells (1^{e5} cells in 100 µL). When tumors reached ~100mm[3], animals were randomized into vehicle (n=12, 5% dextrose) and MLN0518 (n=12, 20 mg/kg s.c. in 200 µL B.I.D.). Animals were scanned before and after 72hrs BID dosing of vehicle or MLN0518. For the MRI procedure; anaesthesia was induced at 3-4% isoflurane in air and this was reduced to 1-1.5% during the imaging session, at which point body temperature and respiration were monitored. RF excitation and detection was performed using a 63mm quadrature transmit-receive coil. Fast spin-echo T_2-weighted coronal pilot scans were acquired to locate tumor. Single sagittal slice images were acquired using a set of preparation pulses followed by a fast imaging FLASH module in separate experiments. T_1, T_2 and ADC measurements were acquired using an inversion-recovery (5-1500ms), CPMG (8 – 100ms, in ~12 ms increments), and PFGSTE (b0-1100mm/s[2]) pulse preparation in front of a FLASH imaging sequence (TR/TE, 0.01/0.003ms, PExRO 128/64, FoV 60x60cm, SlThk 2mm, nt=2, acqn. time ~ 2.2s/image). DCE-MRI was the FLASH imaging module with no preparation pulses. Magnevist™ (Schering, NJ USA) was manually injected after 10 baseline scans were acquired, the DCE acquisition continued for a further 70 scans. Parametric T_1, T_2, ADC and iAU[Gd]C maps were generated for the whole image. Whole tumor ROIs were drawn and no regional segmentation performed. After the second imaging session animals were euthanized and tumors excised. Tumors were halved; ½ into formalin for CD31 and *p*PDGFRβ analysis and ½ snap frozen for Western analysis. For µCT, after the final dose of MLN0518 (n=2) or vehicle (n=2), animals were flushed with saline, followed by 4% Formalin, then perfused with MicroFil (Flow Tech Inc., MA USA). After the 24 hour cure time, the tumors were excised and submitted for µCT scanning. **Results** For the MRI study, animals were excluded on the basis of lobulated growth of s.c. tumor, chronic necrosis (as determined by T_2), or failed i.v. injection of Magnevist™. Vehicle and MLN0518 treatment: a-b/ iAUC maps, c-e/ percentage change from baseline in iAUC, T_2 and ADC f-g/ uCT vascular cast images after different treatment regimens. 2 ½ days of BID MLN0518 dosing significantly altered iAUC values and inhibited tumor growth. CD31 area revealed no difference between groups. **Conclusion** DCE-MRI using a clinically approved contrast agent and a series of clinically translatable acquisition routines demonstrates MLN0518 antivascular effects. Neither T2 nor ADC demonstrated any significant change, possibly indicating cellular integrity was maintained during MLN0518's anti-tumor effect. CD31 concentration in the MLN0518 treated tumors was not different to the vehicle treated animals, and this could be due to the functional component of the vasculature changing but not the vascular endothelial cell concentration. µCT reveals an exquisite vascular phenotype difference between vehicle and MLN0518 treated tumors, the density of cast perfused vessels appears to have greatly decreased. DCE-MRI is currently being deployed in PhI and PhII MLN0518 glioblastoma investigations and the present *in vivo* results support a haemodynamic change during acute dosing of MLN0518 in this preclinical C6 glial tumor model.



References 1. Pietras,K. Increasing tumor uptake of anticancer drugs with imatinib. *Semin. Oncol.* **31**, 18-23 (2004). 2. Pietras,K. *et al.* Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. *Cancer Res.* **62**, 5476-5484 (2002). Kelly,L.M. *et al.* CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML). *Cancer Cell* **1**, 421-432 (2002). 4. Bradley,D.P. *et al.* Examining the acute effects of cediranib (RECENTIN, AZD2171) treatment in tumor models: a dynamic contrast-enhanced MRI study using gadopentate. *Magn Reson. Imaging* (2008).