Chronic dosing with MLN0518 (tandutinib), a small molecule PDGFRα/β inhibitor, reduces tumour growth, hypoxia, and perfusion in C6 glioma xenografts: An investigation using susceptibility contrast enhanced MRI and immunohistochemical methods

J. K. Boult[1], S. Walker-Samuel[1], D. P. Bradley[2], and S. P. Robinson[1]
[1]CRUK and EPSRC Cancer Imaging Centre, The Institute of Cancer Research and Royal Marsden NHS Trust, Sutton, Surrey, United Kingdom, [2]Imaging Sciences Group, Millennium: The Takeda Oncology Company, Cambridge, MA, United States

Introduction

Glioblastoma multiforme (GBM) is heavily reliant upon angiogenesis for growth and represents one of the most vascularised human tumours. MLN0518 (tandutinib) is a potent ATP-competitive and reversible type III receptor tyrosine kinase inhibitor (RTKi) that crosses the blood brain barrier. *In vitro* it demonstrates activity against PDGFRα/β, FLT3 and c-KIT in the submicromolar range but exhibits no significant inhibition of a broad range of other kinases. MLN0518 has shown efficacy against C6 xenografts both subcutaneously and intracerebrally [1]. MLN0518 is currently in phase II trials as a monotherapy and in combination with bevacizumab for recurrent GBM.

In this study we aimed to investigate 1) vascular haemodynamic and structural response, and 2) hypoxic changes of C6 glioma xenografts to MLN0518 using MRI and immunohistochemical methods.

Methods

Tumour model and drug treatment: $2x10^6$ rat C6 glioma cells were inoculated subcutaneously on the flank of female NCr nude mice (n=17). Once tumours reached 100-200mm^3 they were randomised to receive either 20mg/kg MLN518 or vehicle (5% dextrose) subcutaneously twice daily for 10 days.

MRI measurements: Following 10 days of treatment mice underwent MRI on a 7T Bruker horizontal bore system using a 3cm birdcage coil. The change in R_2* and R_2 induced by USPIO (ferumoxtran-10, Sinerem, Guerbet), and the apparent diffusion coefficient (ADC), were used to estimate vessel size index (VSI) and fractional blood volume (fBV), according to the approach described by Troprès et al [2]. R_2* and R_2 were estimated from data acquired using a multi-gradient echo sequence (T_R=200ms, 8 echoes ranging from 6 to 28ms) and a spin echo sequence (T_R=3000ms, T_E=8 and 80ms), respectively, acquired pre- and post-administration of USPIO. ADC values were estimated from a diffusion-weighted spin-echo sequence (6 b-values from 0 to 900s/mm^2, T_E=32ms, T_R=2000ms).

Data analysis: Parameter estimation was undertaken using a Bayesian maximum *a posteriori* algorithm, which took into account the Rician distribution of noise in magnitude MR data in order to provide unbiased parameter estimates [3]. All data were fitted on a pixel-by-pixel basis using in-house software (ImageView). The median value of each parameter in each tumour was measured.

Histological analysis: Mice were administered with 60mg/kg pimonidazole (a hypoxia marker) i.p., followed by the perfusion marker Hoechst 33342 (15mg/kg) i.v.. Total endothelial cell concentration (CD31), degree of perfused vasculature (Hoechst 33342), and hypoxia (pimonidazole adduct formation) were quantified by immunohistochemistry and fluorescence microscopy (n=31). Hoechst 33342 images were also used to obtain vessel diameters. Haematoxylin and eosin staining was performed to quantify necrosis.

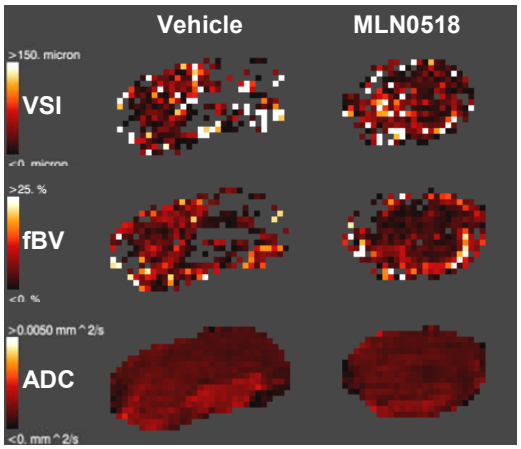

Figure 1. Representative VSI, fBV and ADC maps of C6 xenografts treated with vehicle or 20mg/kg MLN0518.

Imaging Biomarker	Vehicle	20mg/kg MLN0518
Vessel size index (μm)	23.8 ± 2.7	21.9 ± 4.7
Fractional blood volume (%)	3.74 ± 0.5	4.51 ± 0.6
ADC (x10^{6}mm^2/sec)	917 ± 2.5	921 ± 4.3
Hoechst vessel size (μm)	29.1 ± 0.4	28.1 ± 0.3
% vessels perfused	42.5 ± 2.3	31.7 ± 2.0 *
Perfused vessel area (%)	1.87 ± 0.2	1.16 ± 0.1 *
Pimonidazole (%)	10.9 ± 1.0	7.84 ± 0.6 *
Necrosis (%)	41.2 ± 2.2	41.1 ± 2.4

Table 1. Summary of quantitative MRI and histological biomarkers. Mean of median MR values and mean histology values ± 1sem. * p<0.05.

Results and Discussion

Tumours in mice treated with 20mg/kg MLN0518 grew significantly slower than vehicle treated tumours (volume doubling time 5.7±0.3 days vs. 4.5±0.2 days, p<0.01), as previously demonstrated [1].

Representative parametric maps of VSI, fractional blood volume and ADC are shown in Figure 1. In both vehicle and MLN0518 treated tumours the larger vessels and greater percentage of blood volume were around the tumour perimeter, suggesting a less perfused core. Histological analysis of Hoechst 33342 uptake in perfused vessels confirms this pattern of perfusion (Figure 2, blue).

MR parameter values, averaged across each cohort, are displayed in Table 1 alongside histological readouts. Statistical analysis revealed that treatment of C6 xenografts with 20mg/kg MLN0518 did not induce any significant difference in MRI vascular biomarkers following 10 days treatment compared to control tumours. It has been previously shown that DCE-MRI can be used to assess a more acute tumour response to this drug [4]. Further work is currently underway to establish the most appropriate timepoint at which to interrogate tumour response. Vessel size measurements from Hoechst 33342 stained tumour sections also showed no alteration in vessel size with MLN0518 treatment (Table 1).

ADC is a surrogate biomarker for cellularity; the observation of no change in percentage necrosis from H&E stained sections therefore correlates with this ADC data, and also previous data with MLN0518 at a more acute timepoint [4].

The distribution of Hoechst 33342 uptake (perfused vessels) and CD31 staining (total blood vessels, Figure 2, red) revealed that the percentage of the total vessels perfused at the time of Hoechst injection was 17.1% lower in MLN0518 than vehicle-treated tumours (p<0.01) and equated to a 32.5% reduction in an overall perfused vessel area in treated tumours (p<0.01).

Despite having a lower perfused vessel area MLN0518 treated tumours were 25.3% less hypoxic than control tumours as assessed by pimonidazole adduct formation (p<0.05). Tumour hypoxia is well known to promote a more metastatic, malignant, angiogenic and chemo-/radio-resistant lesion.

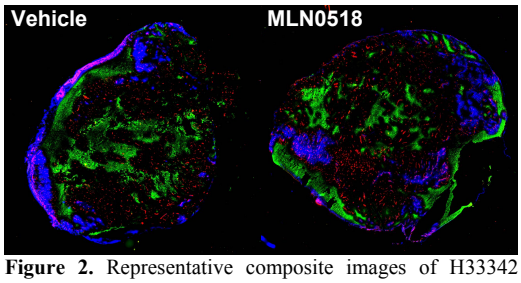

Figure 2. Representative composite images of H33342 uptake (blue), CD31 expression (red) and pimonidazole adduct formation (green).

Conclusions

MLN0518 chronically limits the growth of C6 glioma xenografts, and reduces both the mean perfused vessel fraction and hypoxic area, however vessel size and blood volume are unaffected at this time point.

References. [1] Hibner B *et al*, (2008) *Neuro Onc*ol 10:759-915. [2] Troprès I *et al*, (2001) *Magn Reson Med* 45(3):397-408. [3] Walker-Samuel S *et al*, (2008) ISMRM Cancer Workshop, Nice. [4] Bradley DP *et al*, (2009) *Proc Intl Soc Mag Reson Med* 17.

Acknowledgments. We acknowledge the support received for the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) (grants C1060/A10334 and C16412/A6269), the Biotechnology and Biological Sciences Research Council (grant S20430), NHS funding to the NIHR Biomedical Research Centre, The Royal Society and Millennium Pharmaceuticals, Inc..