Efficacy of soluble panobinostat (MTX110) in preclinical models of adult glioblastoma

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Introduction

- Panobinostat is a histone deacetylase (HDAC) inhibitor with antineoplastic and anti-angiogenic effects in glioma[1].
- Trials using systemic administration of chemotherapeutics have failed in GBM despite compelling pre-clinical evidence.
- Penetrating the blood-brain barrier to achieve therapeutic brain exposures is the biggest challenge for any new GBM therapy.
- MTX110 (Midatech Pharma plc) is a water-soluble form of panobinostat currently in clinical development for DIPG and medulloblastoma using direct tumour delivery.
- We explored the therapeutic potential of MTX110 in GBM in our pre-clinical *in vivo* adult brain tumor models using subcutaneous tumour placement of either IDH1 wild type (WT) or IDH1 mutated patient derived cell lines.

Materials and Methods

Six-week-old male and female athymic mice (BALB/c (nu/nu) were injected subcutaneously at an injection volume of 50 µL with either D08-0537MG cells (IDH1 R132H heterozygous mutation cell line) or IDH1 WT cell line D08-0308MG. Tumour volume was measured twice weekly using hand held Vernier calipers (Scientific Products, McGraw, IL). When tumour volume reached 100-300mm^3, mice (8-10/group) were stratified to either vehicle (control) or MTX110 15/mg/kg I.P. 5 days weekly for 3 weeks. Measurements of tumour size were taken twice weekly until the animal reached the prescribed humane survival endpoint of tumour volume 5X volume at the start of treatment. The median growth delay response of the xenografts was calculated as T-C, which is defined as the difference in days in the median time required for tumours in treated (T) animals and control (C) to reach a volume five times greater than that measured at the start of treatment. Statistical analysis was performed using SAS statistical analysis program and the Wilcoxon rank order test.

Western Blot analysis was run on tumour samples taken at 2, 4, 8, 24 and 48h taken after the last dose of MTX110. Snap-frozen tumour tissue samples were pulverised in liquid nitrogen and lysed in RIPA buffer containing protease inhibitors, PMSF and benzonase (10ul RIPA buffer per mg of tissue). Lysates were incubated on ice for 15 mins, homogenized using Qiashredders and then subjected to immunoblotting for acetyl-His-H3 and acetyl-His-H4, with B-actin as loading control.

Results
MTX110 Delays GBM Tumour Growth

Figure 1 MTX110 slows tumour growth in an IDH1 mutated GBM tumour model

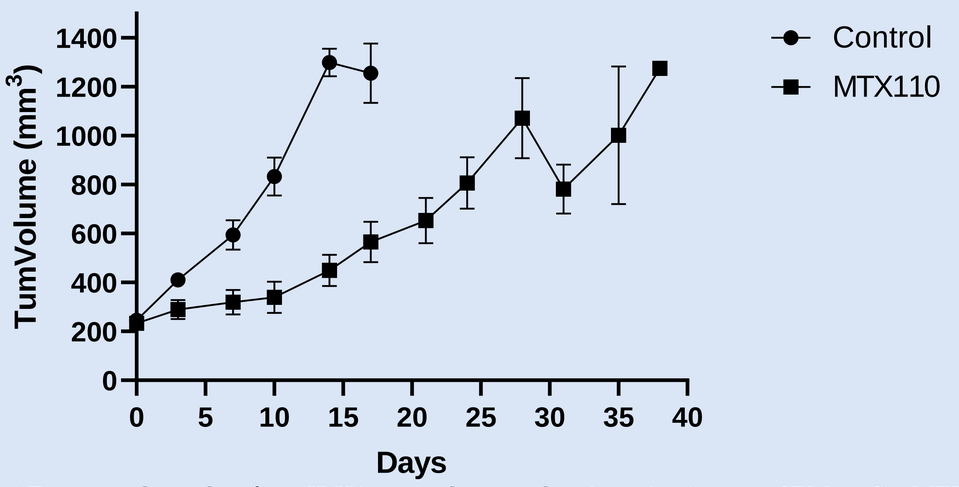

Figure 1 Tumour volume data from IDH1 mutated tumour bearing mice (mean ± SEM, n=8). MTX110 resulted in a significant reduction in tumour growth compared to control (T-C 23.35 days p=0.018).

Figure 2 MTX110 slows tumour growth in an IDH1 WT GBM tumour model

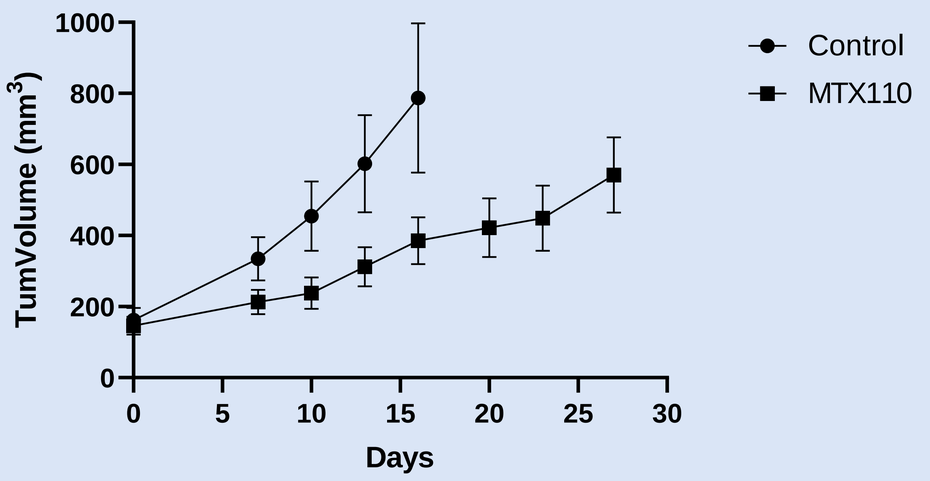

Figure 2 Tumour volume data from IDH1 WT tumour bearing mice (mean ± SEM, n=8). MTX110 resulted in a non-significant reduction in tumour growth compared to control (T-C 10.75 days p> 0.05).

Results
MTX110 increases tumour H3 and H4 Acetylation

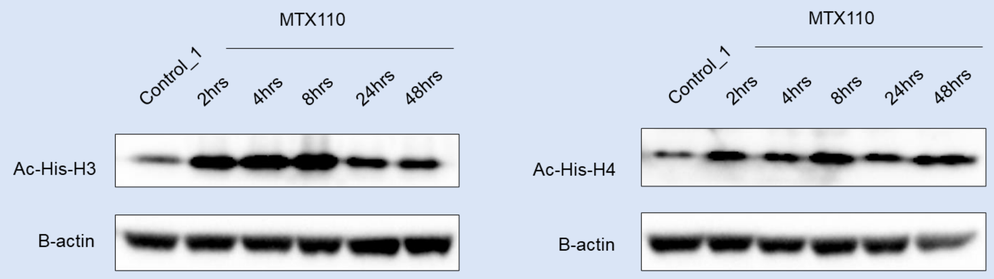

Fig 3 MTX110 results in H3 and H4 acetylation in tumour. H3 and H4 acetylation measured in tumour samples after last treatment of MTX110 in IDH1 WT tumours. MTX110 resulted in histone acetylation as early as 2h after dosing, with acetylation decreasing from 8h onwards but still evident at 48h, which is consistent with published panobinostat data[2].

Conclusions

This is the 1st pre-clinical *in vivo* assessment of the potential of MTX110 in GBM. The results are particularly encouraging because only a low dose of MTX110 could be administered in this model due to systemic panobinostat toxicity[3]. Clinically, MTX110 is administered directly into the tumour using convection enhanced delivery (CED), which generates substantially higher local doses in the tumour environment. The results support further investigation of the potential of direct brain delivery of MTX110 for the treatment of GBM. Further preclinical research is ongoing to investigate MTX110 in combination with radiation therapy in both IDH1 wild type and IDH1 mutated xenograft lines.

1. Nature Medicine 2015 21(6):555-559 2. nda/2015/205353Orig1s000PharmR 3. PLoS ONE (2015) 12(1): e0169485