DMSO-based contrast as a potential intermediate endpoint biomarker of GBM response to therapy.

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INTRODUCTION: Dimethyl Sulfoxide (DMSO) is a common administration vehicle for some drugs, such as temozolomide (TMZ) [1]. Moreover, it can be easily detected by ^{1}H MRS/MRSI in both control and tumour-bearing mice [2,3]. DMSO has also been reported to cross the blood-brain-barrier and to produce contrast between GL261 mouse glioblastoma (GBM) and nearby/peritumoral brain parenchyma [3].
PURPOSE: To evaluate the effect of TMZ in mouse GBM progression by MRI and to image early tumour response by perturbation of the basal brain MRSI pattern with DMSO.

MATERIALS AND METHODS: Twenty-one C57BL/6 female mice were used in this study; all of them inoculated with GL261 mouse glioma cells, as described in [4]. MR studies were carried out at 7T in a horizontal magnet, anesthetizing the animals with isoflurane (1.5-2.0 %), monitoring their breathing pattern, and controlling their rectal temperature (37ºC).
The therapy protocol started when the tumours were big enough to fit a (2.5 mm)^3 voxel. The treatment consisted in three TMZ cycles (Fig. 1) distributed along 25 days post-inoculation as follows: cycle 1, of five consecutive days (days 11 to 15); cycle 2, of two consecutive days (days 19-20); and cycle 3, identical in length to cycle 2 (days 24-25).TMZ was diluted in the administration vehicle, 10% DMSO in saline, and administered once a day intragastrically (60mg/Kg) to 12 mice. Six GL261 mice were used as control and did not receive either treatment or administration vehicle.
T_{2W} MRI scout images (RARE factor 8; TR/TEeff 4200/36ms; matrix 256 x 256; FOV 19.2 x 19.2mm) were acquired before and during treatment at the time points shown in Fig.1, for tumour volume measurements.
After detecting tumour volume changes in response to treatment, 3 additional mice were treated with TMZ cycles 1 and 2 and studied by "perturbation enhanced" MRSI during tumour response to therapy (days 22-28 post-inoculation) to follow possible MRS pattern changes. A reference T_{2W} image and a 12 ms TE control MRSI scan were initially acquired. DMSO (10% in saline) was then injected intraperitoneally (0min_PI_DMSO), followed by 7 repeated 12ms TE MRSI acquisitions (22min each), interleaved with one 136ms TE MRSI at 110min_PI_DMSO. Parameters for PRESS-MRSI were the same as in [4]. MRSI grids were post-processed with 3DiCSI [5] and exported in ASCII format to MatLab (home-written scripts) to generate time-course maps of brain MR-detectable DMSO changes based on peak heights.
Statistical analysis used were U Mann-Withney and ANOVA, setting significance at p<0.05.

RESULTS: Mice in the control tumour group survived 19 days in average while survival of the treated animals was significantly higher (29 days). The tumour growth rate significantly slowed down after the second cycle of TMZ in treated mice (p<0.05) (Fig. 1). Tumour volume remained stable for 4 days (24-28 post-inoculation) and finally recurred after the third cycle, killing the animals. The three animals studied by MRSI perturbation protocol during the constant volume response period showed significantly different DMSO accumulation compared to control tumour-bearing mice (Fig 2). DMSO accumulation inside the tumour mass was 4-fold lower in responding as compared to untreated GL261 tumours, which displayed the same apparent intensity seen in normal brain parenchyma. Hot-spots of high DMSO accumulation at the tumour periphery were of varying intensity position and shape and their origin is under investigation.

CONCLUSIONS: TMZ produces a transient response in GL261 tumours which can be monitored by T_{2W} MRI as volume growth arrest. MRSI-detection of differential DMSO accumulation in GL261 tumours provides a biomarker of this response: DMSO strongly accumulates inside the tumour in untreated animals highlighting these lesions as "hot-spots". On the other hand, DMSO accumulates much less inside the tumour in TMZ-treated mice. In summary, DMSO-based perturbation enhanced MRSI has the potential to become an intermediate endpoint biomarker of GBM response to therapy.

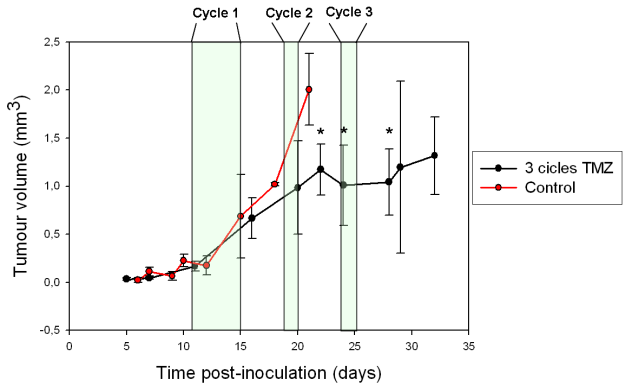

FIGURE 1: Tumour volume change (mean±SD) in two groups of GL261 tumour-bearing mice: The control group (n=6) is in red (all animals were dead at day 22) and the treated group (n=12) is in black. Rectangles in light green highlight the 3 TMZ cycles. (*) Labels significant volume differences (p<0.05) between control (day 21) and treated groups (days 22-32).

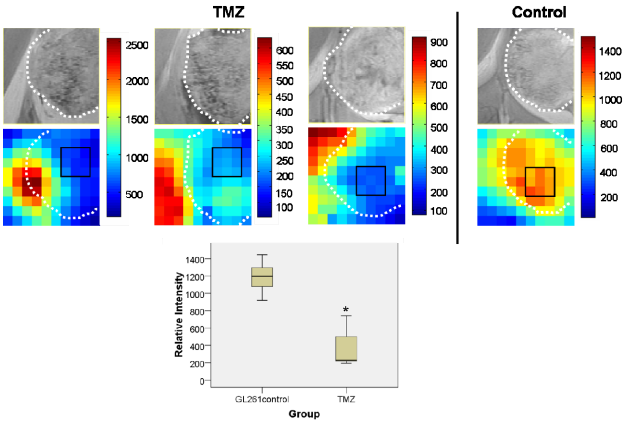

FIGURE 2: Top rows show T_{2W} reference images (5.5x5.5mm) and DMSO enhanced MRSI maps based on peak heights (A.U. scale). From left to right, three GL261 mice explored after the second TMZ cycle (days 22, 22 and 23 post-inoculation, respectively) and one example of an untreated GL261 tumour, at day 14 post-inoculation. The bottom row shows a box-plot comparing DMSO relative signal intensity inside the three tumours under treatment (black squares at the top of the image) with the intensity detected inside 3 control tumours. (*) Labels significant differences (p<0.05).

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[5] http://mrs.cpmc.columbia.edu/3dicsi.html