Characterization of an orthotopic mouse model developed from human glioblastoma spheres

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Introduction: In preclinical studies, it is important to dispose of tumor models that are close to the human pathology in terms of disease development, histological end transcriptomic specification, drug failure as well as diagnostic features. These models must have a sufficient survival rate allowing therapeutic studies. In this context, we developed a new orthotopic mouse model derived from human glioblastoma (GBM) spheres [1], which mimics clinical features of human GBM.

Materials and methods: Human glioblastoma neurospheres (5×10^5 in 5 µl phosphate buffered saline) were injected into the right caudate nucleus (Bregma level, 2 mm lateral, 2.5 mm depth) in female Nude mice (n=50, 5-6 weeks old; 20-25g weight, Harlan France). Once per month for three months after tumor implantation, mice were imaged in a 47/40 Bruker Biospec USR AV III scanner acquiring 12 contiguous slices of 0.7 mm thickness with a field of view of 15×15 mm^2 and a matrix of 128×128 using T_2 weighted (Rapid Acquisition Relaxation Enhanced (RARE) imaging, $TR/TE_{effective}$ = 3500/33 ms, NA = 6) and T_1 weighted (Multi Slice Multi Echo, TR/TE = 300/6.3 ms, NA = 8) sequences. The mice were equipped with an intraperitoneal catheter and injected with 6 mmol/kg of Gd-DOTA (diethylen triaminepenta-acetic acid gadolinium) inside the scanner. T_1 weighted images were acquired prior to, as well as 60 minutes after Gd-DOTA injection. After MRI at day 45 (D45), animals with similar mean tumor volume (0.038 cm3 $\pm$ 0.04) were randomized into a placebo (n=25) and a temozolomide (TMZ, Temodal from Schering-Plough; Kenilworth, NJ) treatment group (n=25). Treatment consisted in 262.5 Kg/Kg body weight per day [2] of TMZ by gavage during 5 days. Ten animals in each group were imaged at D60 and D90 and euthanized when they became moribund. Remaining animals were imaged and sacrificed for histological examination or transcriptomic studies at different time points (D30/D60/D90). T_1 weighted contrast enhancement was quantified as $S_{CE} = (S_{post} - S_{pre})/S_{pre}$, S_{post} and S_{pre} being the pre and 60min post contrast signal, respectively. The MR tumor volumes were evaluated using Image J 1.43u [3] by manually delineating the signal enhancement on the normalized images S_{CE}, on all adjacent slices containing the lesion and by summing the pixels (pixel volume = 0.0096 mm^3) within all tumor regions of interest (ROIs). Haematoxylin and eosin examination was performed to confirm and/or complete the MR findings.

Results and discussion: In most mice, clinical signs appeared 75 days after tumor implantation in this slowly developing tumor model. TMZ treated mice had significant better survival than untreated ones (Fig. 2, p-value=0.035). During the first month, tumor growth shows similar contrast enhancement in the needle trajectory in all mice including sham mice (n = 3) due to the surgical sequels (Fig.1:arrows in A-a2, B-a2, C-a2). Similar to human GBM, this model presents an infiltrative growth pattern with an irregular outline, heterogeneous contrast enhancement, expansion of the tumor bearing hemisphere, invasion of the ventricles (Fig.1: B-bc1, B-bc2, C-c1, C-c2) and the appearance of a large necrotic zone with low signal enhancement in the tumor center 45 days after TMZ treatment (Fig1: arrow in C-c2). To mimic the treatment response in clinics, this TMZ dose is in therapeutic failure. Tumor growth is significantly slower in the TMZ treated group than in the untreated group (Fig.3: * p-value= 0.03, ** p-value= 0.01). It improved overall survival by stabilizing the tumor volume but healing is exceptional.

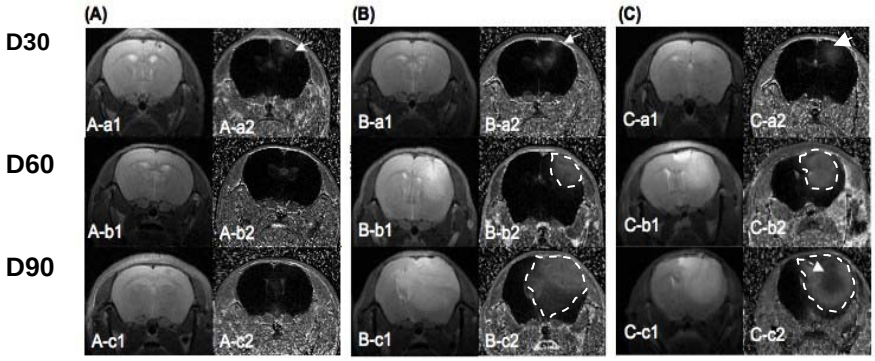

Fig.1 - *In vivo* longitudinal MRI features at 30 days (first row), 60 days (second row) and 90 days (third row) after tumor implantation for three representative mice: sham mouse (A), placebo mouse (B) and temozolomide treated mouse (C)

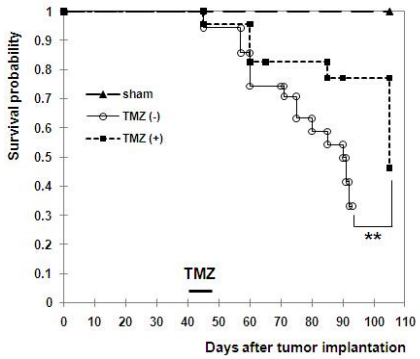

Fig.2 – Kaplan-Meier analysis

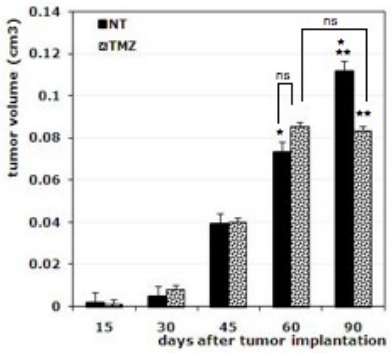

Fig 3 – Evolution of tumor volume as assessed by MRI for untreated (NT) and TMZ treated mice.

Conclusion: Similar to clinically encountered tumors, this new orthotopic glioblastoma mouse model develops slowly, has an infiltrating growth pattern and it mimics the cellular heterogeneity encountered in clinical tumors. This model also presents a temozolomide failure. It will be useful for the preclinical evaluation of new treatment strategies.

[1] Platet and al., Cancer Lett. 2007. [2] Cheng and al., Mol Cancer Ther. 2005. [3] Abramoff and al., Bioph. Inter. 2004