

Evaluation of Gemcitabine as an alternative treatment to Temozolomide for high grade gliomas.

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Introduction: Glioblastoma multiforme (GBM) remains a challenging malignancy due to intrinsic resistance to therapy. Presently, Temozolomide (TMZ) combined with radiation (IR) is the standard of care of patient bearing GBM [1]. But this treatment has showed limited effectiveness in patients with low methylated MGMT status which is considered a predictive biomarker for GBM treated with TMZ+IR [2]. Gemcitabine (GEM), a deoxycytidine analog, is a potent radiosensitizer that demonstrates cytotoxic, anti-clonogenic, and radiosensitizing effects in GBM cell lines [3]. In this study, we evaluate the efficacy of GEM + IR to standard of care and TMZ+IR using a genetically engineered GBM model [Ink4a/Arf^{-/-} Pten^{loxP/loxP}/ Ntv-a RCAS/PDGF(+)/Cre(+)] [4]. We also tested the sensitivity of DW-MRI as a surrogate imaging biomarker of tumor response to cytotoxic, radiation and combination therapies.

Methods and Materials:

Transgenic mice [Ink4a/Arf^{-/-} Pten^{loxP/loxP}/ Ntv-a] (n=48) were orthotopically injected with RCAS/PDGF(+)/Cre(+) cells (4.10⁴ for each cell line). MRI was performed at 9.4T using a quadrature mouse head coil. Tumor bearing animals were introduced to the study when the tumors reach a volume of ~30mm³. Mice were randomized in 6 groups (n=8 per group): no therapy, GEM (10 mg.kg⁻¹; every 3rd day; 4 doses total), TMZ (50 mg.kg⁻¹; 5 days a week; 2 weeks), IR (1Gy; 5 days a week; 2 weeks), GEM+IR and TMZ+IR. Therapeutic efficacy was evaluated by monitoring tumor volume and changes in apparent diffusion coefficient (ADC) values. Tumor volume was determined by delineating the tumor from healthy tissue by contrast-enhanced MRI. Brief, 50μl of Gd-DTPA was administered IP and ~5 minutes post-injection a T1-weighted image was acquired using a SE sequence with the following parameters: TR/TE=500/15ms, 128x129, and 30x30. DW-MRI was acquired using a SE sequence (TR/TE=4000/32 ms, 128x64, 30x30 and b-values of 120 and 1200 s/mm²). Images were acquired daily for a week and every two days following until the animals became moribund. Group comparisons in percent change in tumor volume and ADC were assessed using univariate ANOVA and Bonferroni post-hoc test to account for multiple comparisons. Overall survival was determined using a Kaplan-Meier curves and log-rank test. Statistical significance was assessed at p<0.05.

Results: Mice treated with GEM, TMZ or radiation alone had significant reductions in tumor volume compared to control mice as early as day 3 (Fig. 1a; p<0.001). Both combined treatment (GEM+IR and TMZ+IR) resulted in improved effectiveness over lone therapies. Indeed, we observed a significant reduction in tumor volume compared to GEM, TMZ or IR groups (Fig. 1a; p<0.001). Furthermore, both combined treatments had significant reductions in tumor volume as early as day 7 and prolonged survival compared to the other groups (Fig. 1a p<0.01 and Fig. 1b p<0.01; respectively). There was no significant difference between both combined treatment either in tumor growth or in survival (Fig. 1a-b). ADC values were found to increase immediately upon administration of GEM in both alone (Fig. 2) and combination therapies (data not shown). In contrast, a gradual increase in ADC was observed in tumors treated with IR and TMZ either alone and in combination.

Discussion: GEM concurrent with radiation results in significant reductions in tumor volume and prolongs survival in this clinically-relevant GBM mouse model. GEM combined with radiation had the same effect on mouseGBM's as compared to TMZ combined with radiation. We also demonstrated the successful implementation of an imaging biomarker surrogate (ADC) which correlated with efficacy. Ink4a/Arf^{-/-} Pten^{loxP/loxP}/ Ntv-a RCAS/PDGF(+)/Cre(+) mice serve as a valuable pre-clinical GBM model with the potential to identify novel therapeutics for early phase I/II testing for GBM patients. Based on these results, GEM combined with IR appears to be a suitable alternative to treat tumors that have a poor response to traditional therapies (eg. unmethylated MGMT).

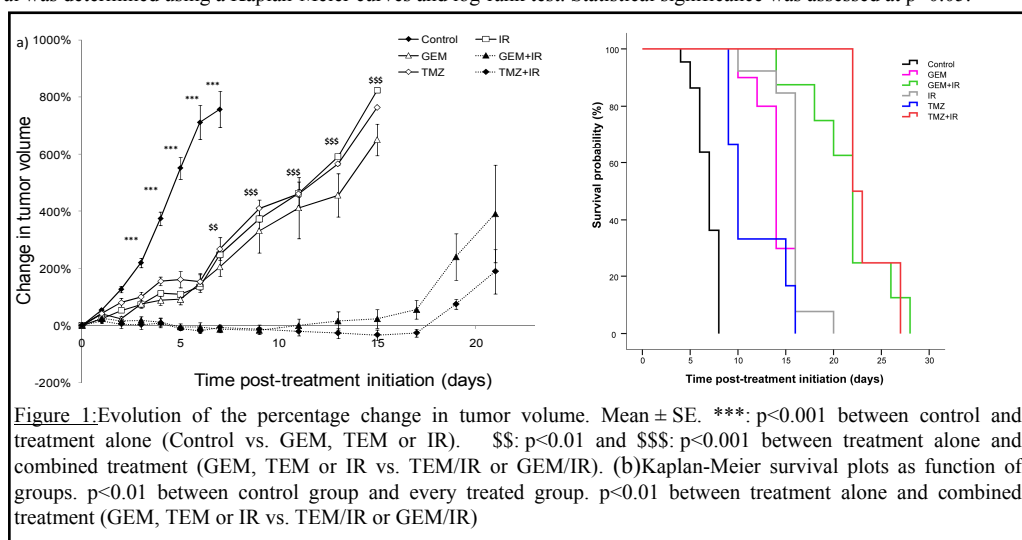


Figure 1: Evolution of the percentage change in tumor volume. Mean \pm SE. ***: p<0.001 between control and treatment alone (Control vs. GEM, TEM or IR). \$\$\$: p<0.01 and \$\$\$: p<0.001 between treatment alone and combined treatment (GEM, TEM or IR vs. TEM/IR or GEM/IR). (b) Kaplan-Meier survival plots as function of groups. p<0.01 between control group and every treated group. p<0.01 between treatment alone and combined treatment (GEM, TEM or IR vs. TEM/IR or GEM/IR)

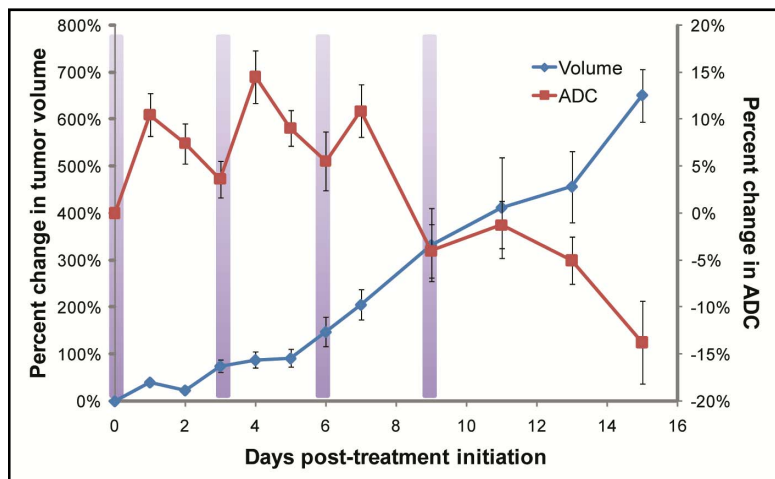


Figure 2: Evolution of the percentage change in tumor volume and the percentage change in ADC in the GEM group as function of time. Mean \pm SE.

References:

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