# Gelatin Methacryloyl Model of Glioblastoma to Benchmark Patterns of Invasion and Chemotherapeutic Resistance

Victoria Kriuchkovskaia[1], Rebecca Riggins[2], Brendan A.C. Harley[1]
[1]University of Illinois Urbana-Champaign, USA and [2]Georgetown University, Washington DC, USA

**Statement of Purpose:** Glioblastoma (GBM) is the most common and lethal form of brain cancer with a median survival of only 12 months. Unlike most forms of cancer that metastasize into secondary organs, GBM primarily invades diffusely throughout the brain. Due to challenges associated with surgical removal of the entire tumor and resistance to chemo- and radiotherapy, GBM typically recurs rapidly near the primary tumor site, and the only widely-used therapeutic against GBM, temozolomide (TMZ), is merely effective. TMZ acts as a DNA alkylating agent that induces cell cycle arrest at G2/M, leading to apoptosis. TMZ modifies specific DNA or RNA sites by addition of methyl groups; the methylated sites can remain mutated or be reversed by several mechanisms, including dealkylation by a demethylating enzyme, O6-methylguanine methyltransferase (MGMT). When MGMT is expressed (MGMT+ tumor), GBM cells are resistant to TMZ; patients with MGMT+ tumors have a two-year survival rate of only 15%. Over 60% of newly diagnosed GBM cases are MGMT+, to which TMZ provides no benefit. Previously, we have shown that a methacrylamide-functionalized gelatin (GelMA) hydrogel platform can support addition of brain-mimetic molecules and formation of vascular networks to study GBM behavior[1]. In this study, we assess GBM response to TMZ using a unique set of isogenically matched GBM cell lines (8MGBA vs. 8MGBA-TMZres; 42MGBA vs. 42MBGA-TMZres) that exhibit TMZ responsiveness or resistance[2] along with disparate proliferative and invasive patterns in two-dimensional culture. This study seeks to define changes in invasion and proliferation in response to TMZ within three-dimensional gelatin hydrogels.

**Methods:** 8MGBA, 8MGBA-TMZres, 42MGBA, and 42MBGA-TMZres cell lines[2] were cultured in DMEM with 10% FBS (100 µM TMZ continuously applied to 8MGBA-TMZres). Cells were encapsulated in GelMA hydrogels (5 wt% GelMA, 1x10$^6$ cells/mL) and cultured for 8 days with daily media change. After 24 hours, TMZ doses between 0 – 500 µM were applied then maintained for 7 days with daily media change. Growth rate inhibition (GR)[3] statistics were determined after 48 hours and 7 days of culture. Relative cell count was assessed 48 h and 7 d after treatment with the alamarBlue assay. Metabolic activity was assessed using an MTT assay at days 0, 3, and 7 of culture. GBM cell spheroids incapsulated in GelMA hydrogels were generated to measure relative invasion of wild type vs. TMZres lines. Spheroid outgrowth area was quantified with image by measuring radial distributions of r25, r50, and r75 metrics—the radii at which 25%, 50%, and 75% of the maximum fluorescent intensity are observed, respectively.

**Results:** We observed changes in proliferative phenotypes between the cell lines consistent with 2D cell culture. Notably, 42MGBA-TMZres cells exhibited significantly increased metabolic activity compared to wild-type 42MBGA cells (Fig 1B). 8MGBA-TMZres cells did not show significant changes in metabolic activity relative to 8MGBA (Fig 1A). GR analysis revealed apparent differences between WT and TMZres lines (Fig 1C). Ongoing experiments are quantifying the relative advantage of GBM cell proliferation versus invasion (hypothesized[2]: invasion advantage for 8MGBA-TMZres) in GelMA hydrogels in response to 0 – 500 µM TMZ to quantify the degree of acquired TMZ resistance by isogenically matched GBM cell lines. These results provide insight into potential common pathways between the "go" or "grow" phenotypes that could be used as therapeutic targets for GBM.

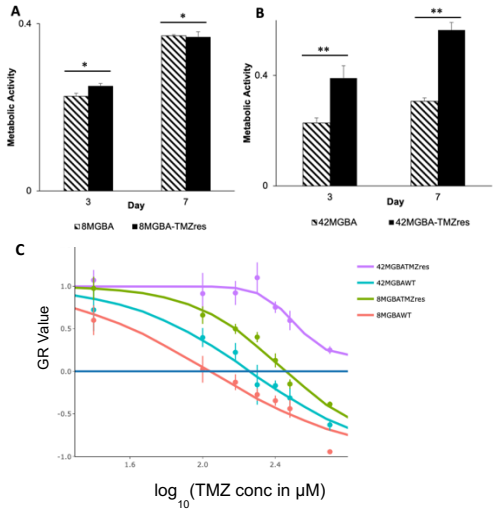


**Figure 1. A.-B.** Metabolic activity of the cell lines assessed with MTT assay. **A.** 8MGBA-TMZres did not show significant changes in metabolic activity relative to 8MGBA. **B.** 42MGBA-TMZres exhibited significantly increased metabolic relative to 42MBGA. Data presented as mean ± SD. N = 9. t-test. *n.s.; **p<0.05. **C.** TMZ dose response, 7-day TMZ treatment: Growth rate inhibition (GR) curves of isogenically matched GBM cell lines (8MGBA vs. 8MGBA-TMZres; 42MGBA vs. 42MBGA-TMZres). Cells were treated with 0 – 500 µM TMZ. Relative cell viability was assessed 7 days post TMZ treatment. Data presented as mean ± SD. N = 9.

**Conclusions:** We have shown that a GelMA hydrogel platform can be used to assess GBM response to TMZ in 3D culture. This model provides insight into rapid tumor cell proliferation and diffusive local invasion. Further, this platform will be advanced to include a previously-developed model of GBM perivascular niche[1]; this next-generation model will be used to benchmark the effect of perivascular niche on GBM therapeutic resistance and invasion. Hence, ongoing experiments aim to develop a vascularized hydrogel model of GBM to benchmark new TMZ variants hypothesized to overcome perivascular-induced resistance.

**References:**

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3. Hafner M. Nat Methods. 2016; 13:521-527.