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Effective sensitization of temozolomide by ABT-888 is lost with development of TMZ resistance in glioblastoma xenograft lines

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Abstract

Resistance to temozolomide (TMZ) and radiotherapy (RT) is a major problem for patients with GBM but may be overcome using the PARP-inhibitor ABT-888. Using two primary GBM xenografts, the efficacy of ABT-888 combined with RT and/or TMZ was evaluated. Treatment with ABT-888 combined with TMZ resulted in significant survival prolongation (GBM12: 55.1%, p=0.005; GBM22: 54.4%, p=0.043). ABT-888 had no effect with RT alone, but significantly enhanced survival in GBM12 when combined with concurrent RT/TMZ. With multi-cycle therapy, ABT-888 further extended the survival benefit of TMZ in the inherently sensitive GBM12 and GBM22 xenograft lines. However, after *in vivo* selection for TMZ resistance, the derivative GBM12TMZ and GBM22TMZ lines were no longer sensitized by ABT-888 in combination with TMZ, and a similar lack of efficacy was observed in two other TMZ resistant tumor lines. Thus, the sensitizing effects of ABT-888 were limited to tumor lines that had not been previously exposed to TMZ, and these results suggest that patients with newly diagnosed GBM may be more likely to respond to combined TMZ/PARP inhibitor therapy than patients with recurrent disease.

Keywords

PARP; ABT-888; temozolomide; radiotherapy; glioblastoma

Introduction

Temozolomide chemotherapy is an integral component of therapy for malignant gliomas. A recent landmark randomized clinical trial demonstrated that TMZ chemotherapy given both during and after definitive radiation resulted in an unprecedented 16% absolute gain in two year overall survival as compared to RT alone (1,2). These results changed the standard of care such that nearly all patients with newly diagnosed GBM are treated with RT and TMZ followed by TMZ alone. TMZ monotherapy also has moderate efficacy as salvage therapy for TMZ-

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naïve, recurrent, high-grade gliomas (3), and several trials are evaluating the efficacy of TMZ-based chemotherapy regimens in patients who fail first-line TMZ/RT therapy.

TMZ is a monofunctional DNA methylating agent that induces a variety of methyl adducts, and failure to repair key methylation lesions results in significantly enhanced tumor cell death. For example, removal of cytotoxic O6-methylguanine lesions is performed by O6-methylguanine DNA-methyltransferase (MGMT), and silencing of MGMT expression through MGMT promoter hypermethylation is associated with a significantly greater two year survival for patients treated with RT and TMZ (4). Other DNA methylation lesions are repaired in the multi-enzyme process of base excision repair (BER). While BER is robust in essentially all tumors, several strategies have been devised to suppress BER and thereby sensitize tumors to TMZ and other alkylating agents (5). Poly(ADP-ribose) polymerase (PARP) modulates the efficiency of BER and numerous small molecule inhibitors of PARP activity have been developed as potential chemo-sensitizing agents (6). Previous pre-clinical studies suggest that PARP inhibitors enhance the efficacy of TMZ in both sensitive and resistant tumors and enhance the efficacy of radiation therapy (7–12). In anticipation of developing a clinical trial evaluating PARP inhibitors in combination with TMZ in patients with GBM, we tested the *in vivo* efficacy of a clinical PARP inhibitor (ABT-888) in combination with TMZ and/or RT using a unique panel of GBM xenografts initially derived from patient tumors.

The Mayo panel of primary GBM xenograft lines was developed by implanting patient tumor specimens into the flank of mice. These lines are maintained exclusively by serial heterotopic transplantation, and this method effectively preserves key molecular features of the original patient tumor samples, such as EGFR amplification and MGMT methylation status that otherwise are commonly lost in cell culture systems (data not shown and (13)). Using these xenograft lines, the efficacy of multiple agents, including radiation and temozolomide, have been evaluated in an orthotopic therapy evaluation model (14–16), and consistent with clinical results, sensitivity to TMZ is correlated with MGMT promoter hyper-methylation status. In addition to the primary xenograft lines, we also have developed TMZ-resistant tumor lines through serial cycles of TMZ treatment *in vivo*. Using these models, we tested ABT-888 combined with RT and TMZ to model upfront therapy, in combination with multiple cycles of TMZ to model adjuvant therapy, and in combination with TMZ in the TMZ-resistant lines to model therapy for tumors progressing on TMZ therapy.

Methods

Intracranial Xenograft Model

All xenograft therapy evaluations were performed using an orthotopic tumor model for GBM (13). Prior institutional review board authorization was obtained for the use of human tissue to establish the xenograft lines and institutional animal care and use committee approval was obtained prior to any animal experimentation. Each of the xenografts used in this study were derived from primary tumors of different patients and were maintained exclusively by serial passage in mice. As described previously, flank tumor xenografts were harvested, mechanically disaggregated, and grown in short-term cell culture (5 to 14 days) in DMEM media supplemented with 2.5% fetal bovine serum, 1% penicillin, and 1% streptomycin (15). Cells were harvested by trypsinization and injected (3×10^5 cells per mouse, suspended in 10 μl) into the right basal ganglia of anesthetized athymic nude mice (Athymic Ncr-nu/nu: NCI Frederick) using a small animal stereotactic frame (ASI Instruments, Houston, Tex.).

Therapy Evaluation

Mice with established intracranial xenografts were randomized to treatment groups of 10 mice each. Radiation was delivered to the entire head of unanesthetized mice, immobilized in a

plastic restraint, through a single right lateral beam from a ^{137}Cs source. The remainder of the body was shielded with a lead block. The radiation schedule used during the course of this study was 2 Gy Monday through Friday for 2 weeks (20 Gy total administered over 11 days). TMZ was purchased from the Mayo Clinic Pharmacy, suspended in Ora-plus (Paddock Laboratories, Minneapolis), and administered by oral gavage. Two dosing schedules were used: for the RT/TMZ/ABT-888 study, TMZ was dosed at 33 mg/kg/day Monday–Friday for 2 weeks. Otherwise, TMZ was dosed at 66 mg/kg/day for 5 days. In the indicated experiments, TMZ dosing was repeated in 28 day cycles. ABT-888 (obtained from the Cancer Therapy Evaluation Program of the National Cancer Institute) was suspended in double distilled water and administered by oral gavage at 7.5 mg/kg twice daily Monday–Saturday coinciding with TMZ therapy, and given 1 hour prior to TMZ dosing. All mice used for therapy response evaluations were killed at the time of reaching a moribund condition.

Acquired TMZ resistance model

To develop models of acquired TMZ resistance, inherently TMZ sensitive tumor lines (GBM12, GBM22, and GBM39) were maintained as flank tumors and treated with successively higher doses of TMZ until the tumor growth was unaffected by dosing with TMZ at 120 mg/kg/day for 5 days. The resulting TMZ-resistant tumor lines are denoted as GBM12TMZ, GBM14TMZ, GBM22TMZ and GBM39TMZ. A detailed evaluation of mechanisms of resistance for these tumor lines will be reported elsewhere. These tumor lines were used to establish intracranial tumors as described above.

PARP activity analysis

PARP activity was determined in tumor homogenates using a validated assay as described previously (17). Briefly, tumor homogenates were incubated *in vitro* in a reaction buffer containing NAD+, and following termination of the reaction, replicate samples ($n \geq 3$) were blotted onto nitrocellulose membranes along with purified PAR standards. Membranes were blotted with a PAR-specific antibody, and chemiluminescence detected during a 5-minute exposure was measured using a Fuji LAS3000 UV Illuminator (Raytek, Sheffield, United Kingdom) and digitized using the imaging software (Fuji LAS Image version 1.1, Raytek). The acquired image was analyzed using Aida Image Analyzer (version 3.28.001), and results were expressed in LAU/mm^2. Three background areas on the exposed blot were measured and the mean of the background signal from the membrane was subtracted from all results. The protein concentration of the homogenate was measured using the BCA protein assay (Thermo Fisher Inc, UK) and Titertek Multiscan MCC/340 plate reader. Results were expressed in terms of pmol PAR formed/μg protein.

PAR western blotting

Flank tumor specimens were processed for western blotting as described previously using a Triton X-100-containing lysis buffer (15). Antibodies used in this study were specific for poly-ADP-ribose polymer (PAR) (Cat#4336-BPC-100, Trevigen, Gaithersburg, MD), β-actin (Cat#A5441, Sigma, St. Louis, MO), horseradish peroxidase-conjugated rabbit anti-goat and goat anti-mouse (Pierce, Rockford, IL) secondary antibodies. Blots were developed with Super Signal Chemiluminescence reagent (Pierce).

Statistical Analysis

Cumulative survival probabilities were estimated using the Kaplan-Meier method (18). The log rank test was used to compare survival of groups (19). Two-way categorical comparisons were performed using Fisher's exact test. All tests were two-sided and a p-value < 0.05 was considered to be statistically significant. Weight change over time between treatment groups

was compared using repeated measures of analysis of variance. A two-sample rank sum test was used to determine differences at specific time points.

Results

ABT-888 combined with RT and TMZ

Two MGMT hypermethylated xenograft lines (GBM12 and GBM22) were selected for our initial studies with ABT-888 in combination with RT and TMZ. For each xenograft line, mice with established intracranial xenografts were randomized into 8 treatment groups to evaluate all possible combinations of RT (2 Gy daily, 5 of 7 days × 2 weeks), TMZ (33 mg/kg/day, 5 of 7 days × 2 weeks), and ABT-888 (7.5 mg/kg twice daily, 6 of 7 days × 2 weeks). During and after therapy, mice were monitored until reaching a moribund state, at which time they were euthanized. Treatment with ABT-888 alone had no impact on survival relative to placebo therapy in either tumor line, while similar to previous results, TMZ therapy significantly extended survival in both tumor lines as compared to placebo: relative median survival benefit (100^{*}(median survival treatment group – median survival placebo group) / median survival placebo group) in GBM12 tumors treated with TMZ was 143% (Figure 1A, $p<0.001$) and in GBM22 median survival benefit was 421% (Figure 1B, $p<0.001$). In both tumor lines, the addition of ABT-888 to TMZ therapy significantly extended median survival relative to TMZ alone (GBM12 – 56% ($p=0.005$); GBM22 – 54% ($p=0.043$)). In contrast, the addition of ABT-888 to RT had no effect on survival relative to RT alone ($p=0.10$ for GBM12 and $p=0.51$ for GBM22). TMZ combined with RT was significantly more effective than either treatment alone (survival prolongation for GBM12 – RT/TMZ vs. TMZ alone – 124% ($p=0.003$) or vs. RT alone – 245% ($p<0.001$); GBM 22 RT/TMZ vs. TMZ alone – 23% ($p=0.51$) or vs. RT alone – 542% ($p<0.001$)). Finally, the addition of ABT-888 to concurrent RT and TMZ provided additional survival benefit for GBM12 (112%, $p=0.11$ by log rank test). The lack of statistical significance ($p<0.05$) likely is due to the limited sample sizes in these groups and the termination of the experiment at 365 days before all mice had reached a moribund state. No additional survival benefit was observed for the combination of ABT-888 to RT/TMZ in GBM22 (2.5%, $p=0.38$). As a crude measure of tolerability for the regimens tested, body weight was monitored serially in all mice. In the GBM12 study (Figure 1C), the lowest point for body weight was observed on Day 12, at which point, mice treated with RT/TMZ had lost 8% body weight ($p<0.001$), and RT/TMZ/ABT-888 had lost 14% ($p<0.003$) compared to placebo treated mice. By 20 days following completion of therapy, mice had recovered to their mean starting body weight regardless of treatment group (placebo vs. RT/TMZ $p=0.05$; placebo vs. RT/TMZ/ABT-888 $p=0.28$). Similar results were seen with GBM22 (data not shown). Thus, ABT-888 combined with TMZ was well tolerated and enhanced the efficacy of TMZ-containing regimens.

Cyclical TMZ therapy combined with ABT-888

The clinical standard of care following completion of RT/TMZ is 6 to 12 months of adjuvant TMZ therapy (TMZ 150–200 mg/m^2 on days 1–5 of a 28 day cycle). Therefore, a similar regimen was evaluated in our xenograft model with 5 days of TMZ with or without ABT-888 given in up to 3 – 28 day cycles. For each line, mice with established orthotopic xenografts were randomized into 8 treatment groups of 10 mice each: placebo, ABT-888 alone, or 1 to 3 cycles of TMZ without or with ABT-888. Both GBM12 (Figure 2A) and GBM22 (Figure 2B) were highly sensitive to TMZ with a single cycle of TMZ resulting in a 94% and 190% increase in median survival relative to placebo ($p=0.030$ and <0.001), respectively. In GBM12, a second cycle resulted in an additional 45% prolongation in survival relative to cycle 1 ($p=0.002$), while a second cycle provided no significant benefit in GBM22 (16% prolongation; $p=0.24$). A third cycle of TMZ produced no benefit in either xenograft line. Thus, while both tumor lines were

significantly sensitive to TMZ in the first cycle, subsequent cycles of TMZ were significantly less effective.

Combined therapy with ABT-888 and TMZ prolonged survival across multiple cycles of TMZ. For GBM12, treatment with TMZ and ABT-888 prolonged survival relative to TMZ alone in all 3 cycles: cycle 1: 28% median survival prolongation (p=0.064), cycle 2: 28% (p=0.053), and cycle 3: 95% (p=0.010). In GBM22, significant survival benefit was observed only in the second and third cycle; cycle 1: −10% (p=0.72), cycle 2: 46% (p<0.001), and cycle 3: 32% (p=0.031). Thus, ABT-888 significantly enhanced survival when combined with TMZ in both GBM12 and GBM22, which are inherently sensitive to TMZ.

Resistant Xenograft Lines

The development of TMZ resistance during adjuvant therapy occurs in over 30% of patients, and therefore, the combination of ABT-888 with TMZ was evaluated in tumor lines derived from GBM12 and GBM22 that had been selected *in vivo* for resistance to TMZ (GBM12TMZ and GBM22TMZ). As these lines are models for tumors that are progressing on therapy, each tumor line was treated with a single cycle of TMZ (66 mg/kg/day × 5 days) to mimic the setting of recurrent disease in which further disease progression after the first cycle would warrant a change in therapy. TMZ resistance was evident as compared to the previously tested parental lines used in the upfront therapy experiments; survival benefit with TMZ alone (66 mg/kg/day × 5 days) was 94% for parental GBM12 as compared to 30% for resistant GBM12TMZ, and 190% for parental GBM22 versus 63% for resistant GBM22TMZ. The addition of ABT-888 did not provide a clinically significant survival benefit in either tumor line. Prolongation in median survival following treatment with ABT-888/TMZ as compared to TMZ alone was 2.3% for GBM12TMZ (Figure 3A; p=0.044) and 0% for GBM22TMZ (Figure 3B; p=0.74). A third TMZ resistant tumor line (GBM39TMZ) also was tested in this model. Similar to the other 2 resistant lines, the combination of ABT-888 with TMZ was no more effective than TMZ alone; treatment with ABT-888/TMZ as compared to TMZ alone prolonged median survival by 4.9% for GBM39TMZ (Figure 3C; p=0.74). Thus, ABT-888 did not provide any survival benefit in combination with TMZ in 3 xenograft lines previously selected for TMZ resistance.

Evaluation of PARP activity

The levels of PARP activity were evaluated in both the parental and corresponding TMZ-resistant tumor lines to evaluate whether differential levels of endogenous PARP activity might account for the lack of ABT-888 sensitizing effects in some lines. Using an *in vitro* PARP activity assay on tumor homogenates, no significant differences in PARP activity levels were detected between the parental tumors and the corresponding TMZ-resistant tumor lines (Figure 4A), although these levels were significantly elevated in comparison to normal brain.

Differential pharmacodynamic effects of ABT-888 in the TMZ resistant versus TMZ sensitive tumor lines may also account for the lack of efficacy in the resistant tumor lines. Therefore, the effects of ABT-888 treatment on PARP activity were assessed in mice with established tumors. Previous studies with GBM12 intracranial xenografts demonstrated an open blood-brain barrier (unpublished data), and ABT-888 freely crosses the blood-brain barrier. Thus, the efficacy of ABT-888-mediated PARP inhibition was compared between GBM12 and GBM12TMZ using flank tumors in order to facilitate PAR western blotting. Mice were randomized to treatment with or without ABT-888 using the same 5 day dosing schedule described above. Two hours after the 10^{th} dose of ABT-888 or placebo, mice were killed, tumors were flash-frozen, and subsequently were processed for analysis of PAR levels. As seen in Figure 4B, ABT-888 (15 mg/kg/day) was highly effective at suppressing PARP activity, as reflected by the reduced level of PAR modifications, in both the parental GBM12 and the

TMZ resistant GBM12TMZ xenograft lines. Thus, the lack of TMZ-sensitizing effects is not due to failure to effectively inhibit PARP activity in the resistant lines.

High-dose ABT-888 therapy

A recently published study evaluating the pharmacokinetics of ABT-888 in nude mice suggests that doses of 20 mg/kg/day ABT-888 will provide serum drug levels that are clinically achievable in humans (10). Therefore, we tested a high-dose regimen of ABT-888 (40 mg/kg/day in 2 divided doses), to ensure maximal drug exposure in another TMZ resistant tumor line. In the GBM14TMZ xenograft line that also had been subjected to *in vivo* TMZ selection, TMZ therapy alone was associated with a 66% survival benefit ($p<0.001$), but combinations of TMZ with ABT-888 (15 mg/kg/day) or ABT-888 (40 mg/kg/day) were not associated with any additional benefit as compared to TMZ alone (Figure 5; $p=0.90$ and $p=0.63$, respectively). Thus, resistance to the sensitizing effects of ABT-888 could not be overcome with supra-therapeutic dosing of ABT-888.

Discussion

The pre-clinical animal model studies presented demonstrate that not all GBM tumors will benefit equally from combined therapy with TMZ and the PARP inhibitor ABT-888. Specifically, ABT-888 combined with TMZ only enhanced survival in the two TMZ-naive xenograft lines (GBM12 and 22), while derivative tumor lines, which had been selected *in vivo* for TMZ resistance (GBM12TMZ and GBM22TMZ), were unaffected by the addition of ABT-888 to TMZ therapy. Along with the lack of survival benefit with combined therapy for two other TMZ-resistant lines (GBM14TMZ, and GBM39TMZ), these results suggest that combined therapy with ABT-888 and TMZ may not be effective in GBM tumors that already have developed resistance to TMZ. These data are in contrast to several *in vitro* and *in vivo* studies that demonstrate improved efficacy of TMZ when combined with various PARP inhibitors including ABT-888 (8–12). One of the key differences between these previous studies and the current study is the exclusive *in vivo* evaluation of therapies using the unique Mayo GBM xenograft panel. In this model, primary patient tumor samples are implanted directly into mice, serially passaged as heterotopic xenografts, and used for therapy evaluations exclusively in the intracranial location. In contrast to typical cell culture models, propagation of tumors in the flank preserves key features of the primary patient tumor samples including MGMT promoter methylation status and inherent TMZ responsiveness (manuscript in preparation). In contrast, many of the previous studies were performed in non-GBM models, and all studies have been performed using tumor cell models which have been subjected to prolonged culture on plastic, which selects for characteristics that may be far removed from primary tumors. From these observations, we believe that the Mayo xenograft panel provides a robust platform for testing novel TMZ-sensitizing strategies for GBM therapy.

The data presented demonstrate that the lack of a TMZ-sensitizing effect of ABT-888 in certain tumor lines is not due to a failure to effectively inhibit PARP activity. PAR formation was effectively suppressed in flank tumor from both GBM12 and GBM12TMZ with the ABT-888 dosing regimen used for the majority of the studies (15 mg/kg/day; Figure 4B). While the blood-brain barrier potentially could limit access of the drug to the intracranial tumors, the parental GBM12 line lacks an intact blood-brain barrier (J. Poduslo and J. Sarkaria, unpublished data), and ABT-888 effectively penetrates an intact blood-brain barrier and shows demonstrable accumulation in the CNS (20). Consistent with effective inhibition of PARP activity in intracranial tumors, ABT-888 effectively sensitized the GBM12 and GBM22 xenograft lines (Figure 1 and Figure 2). Moreover, a higher dose ABT-888 regimen (40 mg/kg/day), which would provide dose levels in mice that would be supra-therapeutic in humans (21), was equally ineffective in the GBM14TMZ resistant tumor line. Thus, ABT-888 was

ineffective in a subset of tumor lines despite effective suppression of PARP activity in the resistant tumors.

Resistance to TMZ therapy requires integrity of both short-patch BER pathway and the MGMT repair protein in order to repair cytotoxic N3-methyladenine and O6-methylguanine lesions, respectively, and abrogation of either pathway leads to significant increased cell killing after TMZ treatment (reviewed in (5)). TMZ resistance in the GBM12TMZ and GBM14TMZ lines can be reversed with the MGMT inhibitor O6-benzylguanine and both lines demonstrate a marked up-regulation of MGMT protein and mRNA levels (unpublished data). In conjunction with the lack of TMZ sensitization by ABT-888, these data would be consistent with incomplete disruption of BER in these tumor lines by PARP inhibition. In support of this possibility, several cell culture models of PARP deficiency demonstrate slowed kinetics of BER without complete abrogation of BER activity (22–24). The key cytotoxic lesion induced by TMZ and processed by BER is N3-methyladenine, which can lead to cytotoxicity only when encountered by a replication fork during S-phase (11). Since cell cultures grown *in vitro* typically have a much higher S-phase fraction than tumors grown *in vivo*, we speculate that any delayed kinetics of BER following ABT-888 may not be manifest as increased cell killing in our TMZ-resistant models because of the much longer average time available to a cell prior to replication. Differential effects of PARP inhibition on BER between the TMZ sensitive and resistant tumor lines also could explain the results observed. Future studies will address the mechanisms of PARP-mediated sensitization in our xenograft model and will specifically measure rates of various DNA repair processes involved in processing TMZ-induced damage.

The current set of studies was designed to guide clinical development of ABT-888 in GBM. While these results need to be validated with other clinically used PARP inhibitors, there are several important observations that may guide the general development of PARP inhibitor based TMZ-sensitizing strategies in GBM. First, of the 6 xenograft lines tested, only the two that were inherently sensitive to TMZ were effectively sensitized by ABT-888, while ABT-888 combined with TMZ was ineffective in TMZ-resistant lines. These data suggest that combined therapy with TMZ and a PARP inhibitor likely will be more effective in newly diagnosed GBM patients, and that PARP inhibition combined with TMZ in patients who have progressed on TMZ is less likely to provide significant benefit. Second, for the two tumor lines in which robust sensitization to TMZ were observed, there were no observed radiosensitizing effects of ABT-888. Although this is a limited data set, these observations reduce our enthusiasm for studies integrating PARP inhibitors with radiation monotherapy in patients who are not suitable candidates for combined TMZ/RT therapy. Third, the efficacy of TMZ was reduced with latter cycles of therapy in TMZ-naïve tumors. This observation is similar to clinical experiences in which over 30% of newly diagnosed patients progress while receiving TMZ therapy (2), and this may reflect relatively early development of TMZ resistance in these tumors. Given the lack of efficacy of combined therapy in TMZ-resistant tumors, these data suggest that PARP inhibitors may be most effective when integrated early during therapy before resistance develops. While these observations remain to be confirmed in clinical trials, we believe the studies performed in the Mayo GBM xenograft model have helped delineate a potential strategy for optimizing the integration of PARP inhibitors with TMZ for therapy of GBM patients.

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Figure 1A

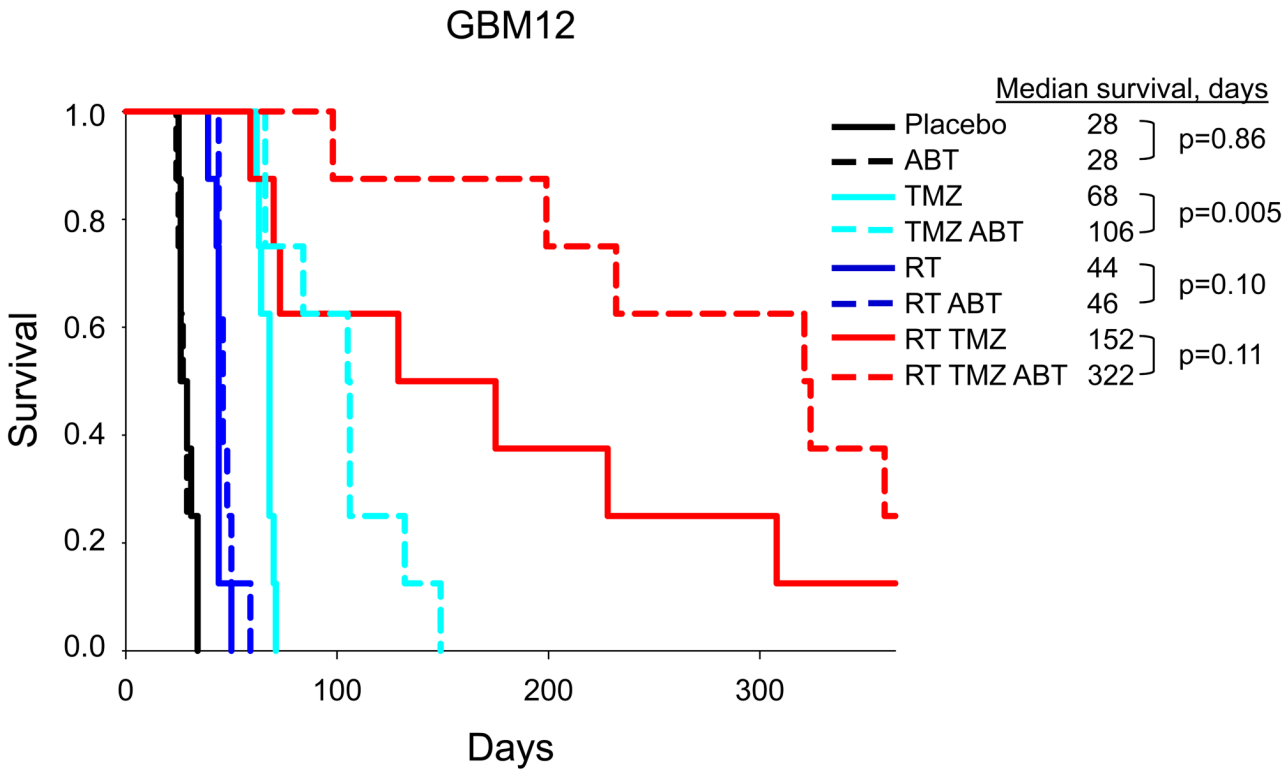

Figure 1B

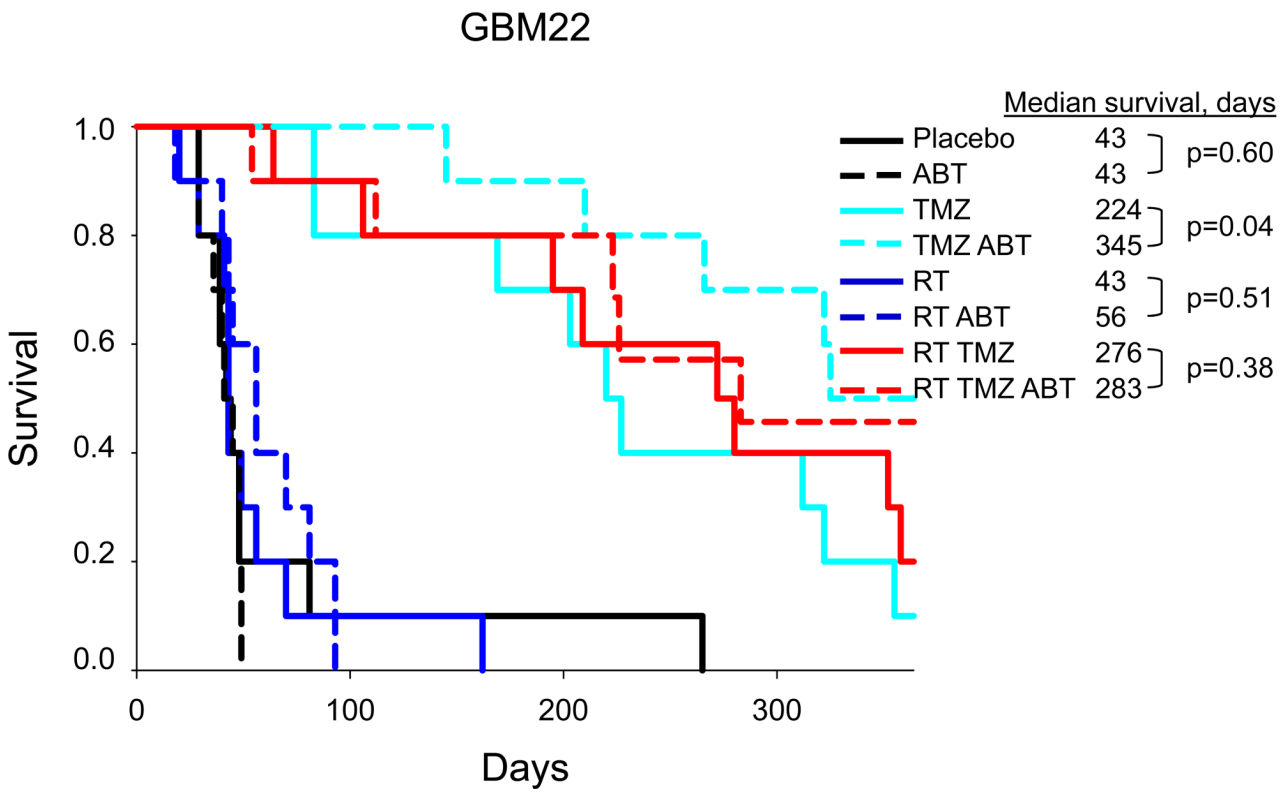

Figure 1C

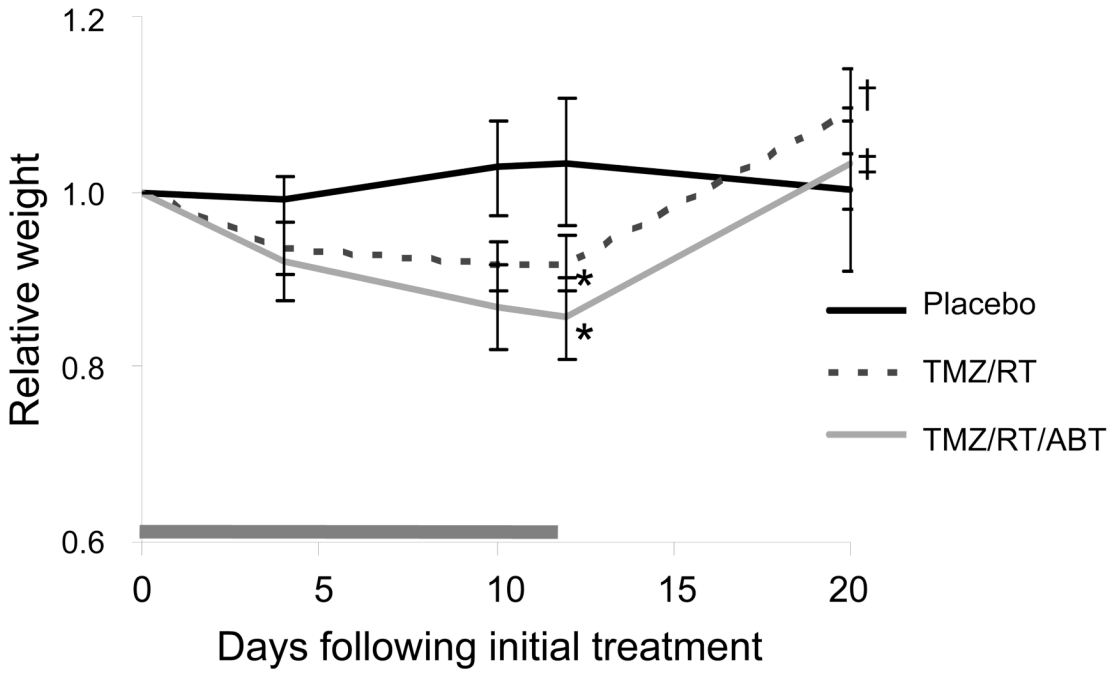

Figure 1.

ABT-888 combined with chemo-radiation in GBM orthotopic xenografts. Mice with established orthotopic xenografts from (A) GBM12 and (B) GBM22 were randomized to therapy with the indicated combinations of temozolomide (TMZ – 33 mg/kg/day, 5 of 7 days × 2 weeks), radiation (RT – 2 Gy/day, 5 of 7 days × 2 weeks) and ABT-888 (15 mg/kg/day, 6 of 7 days × 2 weeks). Mice were followed until reaching a moribund state, and survival results are shown. Indicated p-values are comparing an indicated treatment with or without ABT-888. C) The change in relative body weight for mice from the GBM12 experiment treated with placebo, RT/TMZ, or ABT-888/RT/TMZ is shown. The gray bar indicates the duration of treatment. * - $p < 0.005$; † - $p = 0.05$; ‡ - $p = 0.28$.

Figure 2A

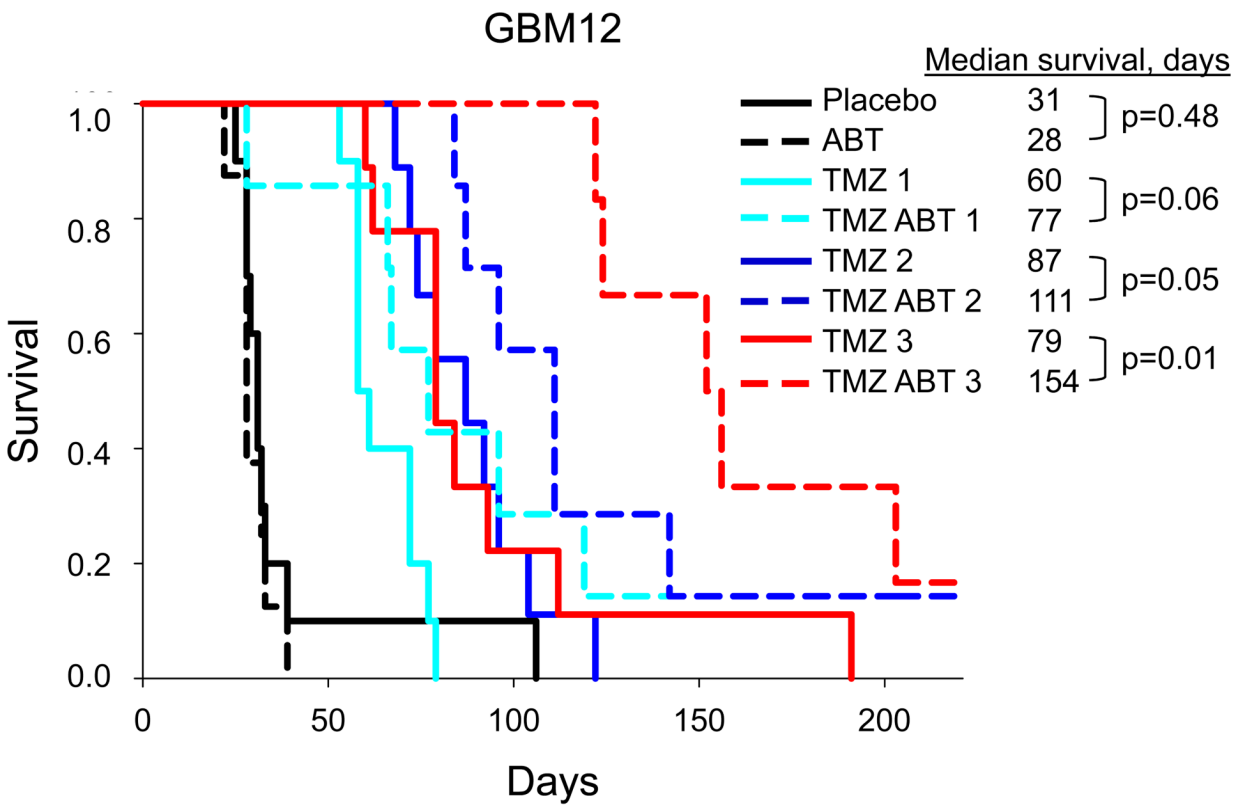

Figure 2B

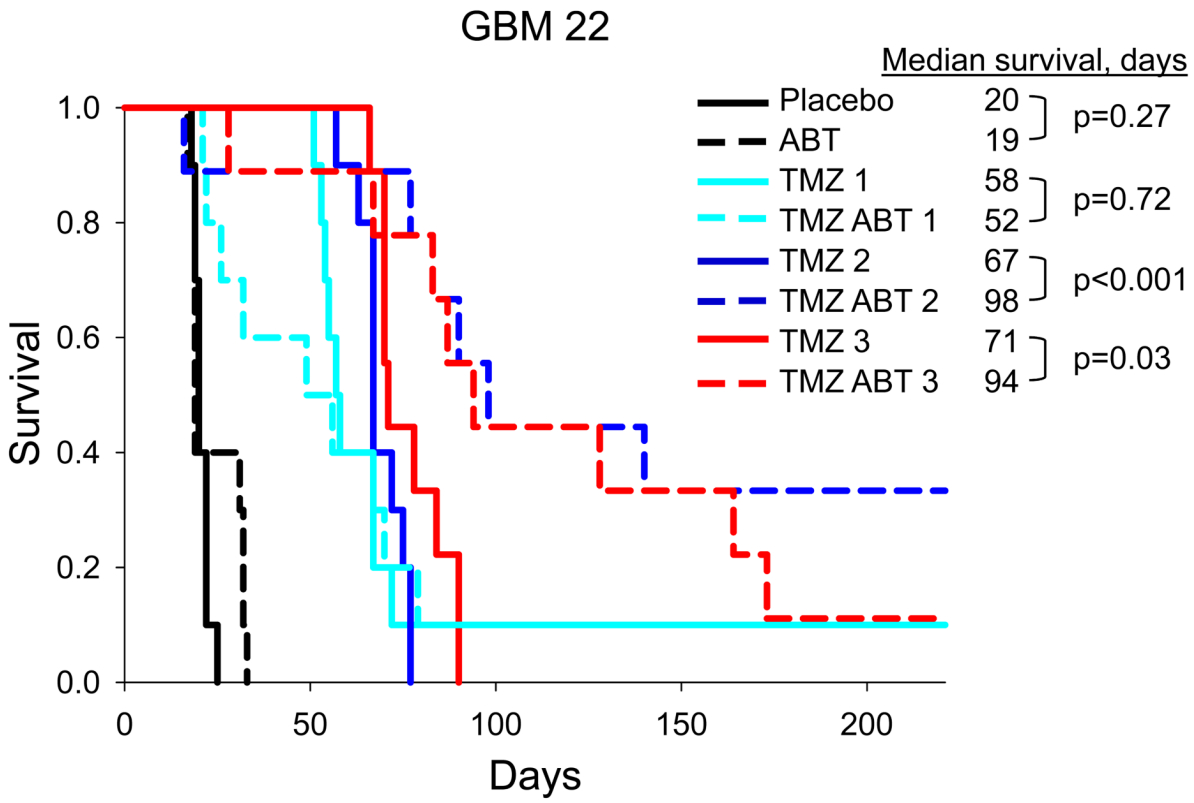

Figure 2.
ABT-888 combined with adjuvant temozolomide in GBM orthotopic xenografts. Mice with established orthotopic xenografts from (A) GBM12 and (B) GBM22 were randomized to therapy with TMZ (66 mg/kg/day, day 1–5) administered in 1, 2 or 3 – 28 day cycles (TMZ 1, TMZ 2, and TMZ 3, respectively), or combined therapy with ABT-888 (15 mg/kg/day, day 1–6) and TMZ for 1, 2 or 3 – 28 day cycles (TMZ ABT 1, TMZ ABT 2, and TMZ ABT 3, respectively), or placebo or ABT-888 alone for 3 cycles. Indicated p-values are comparing TMZ alone to TMZ + ABT-888 for each cycle.

Figure 3A

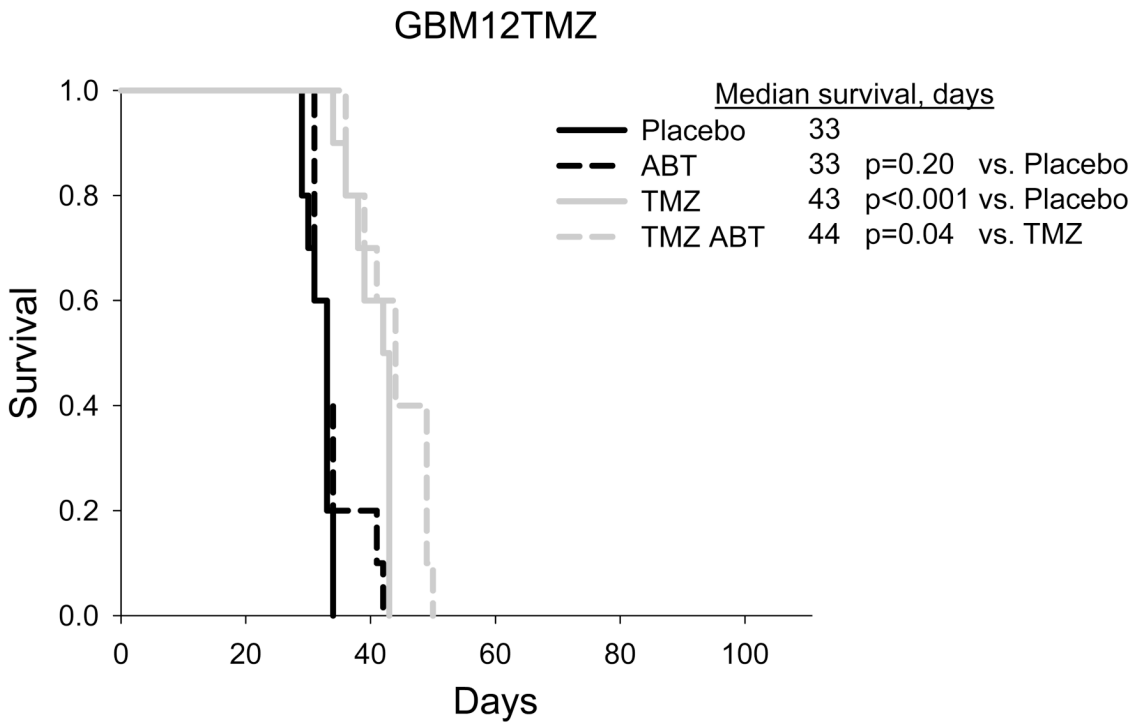

Figure 3B

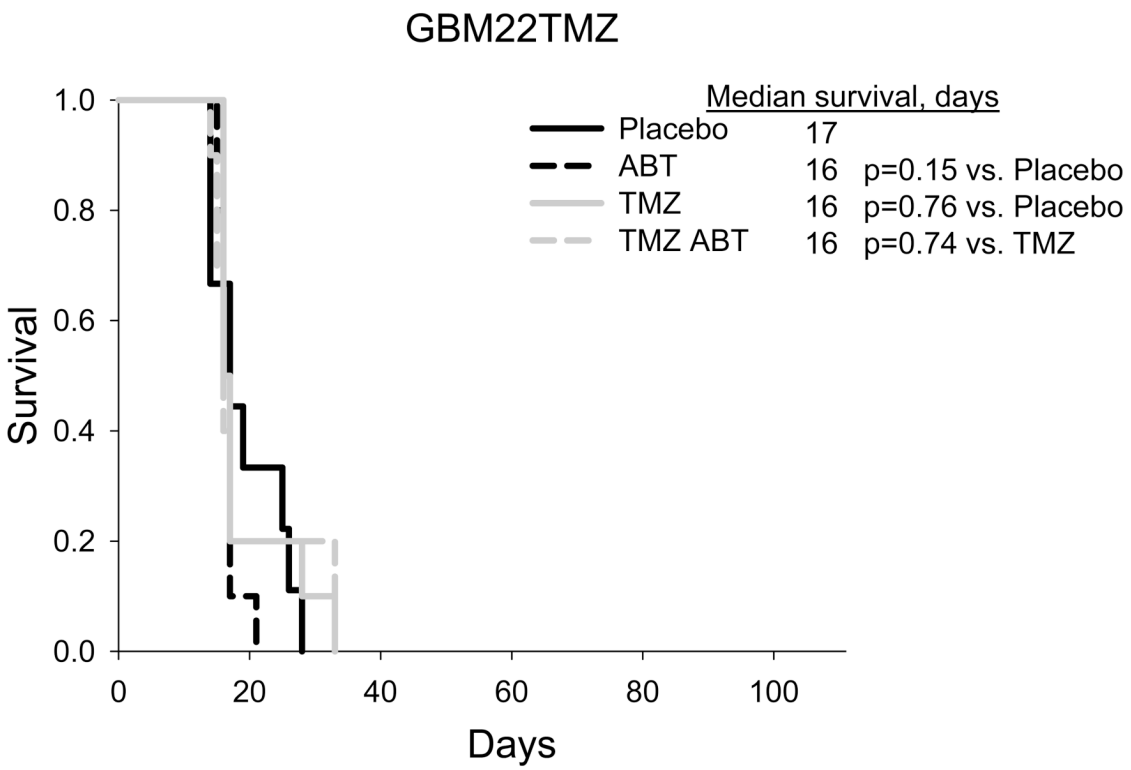

Figure 3C

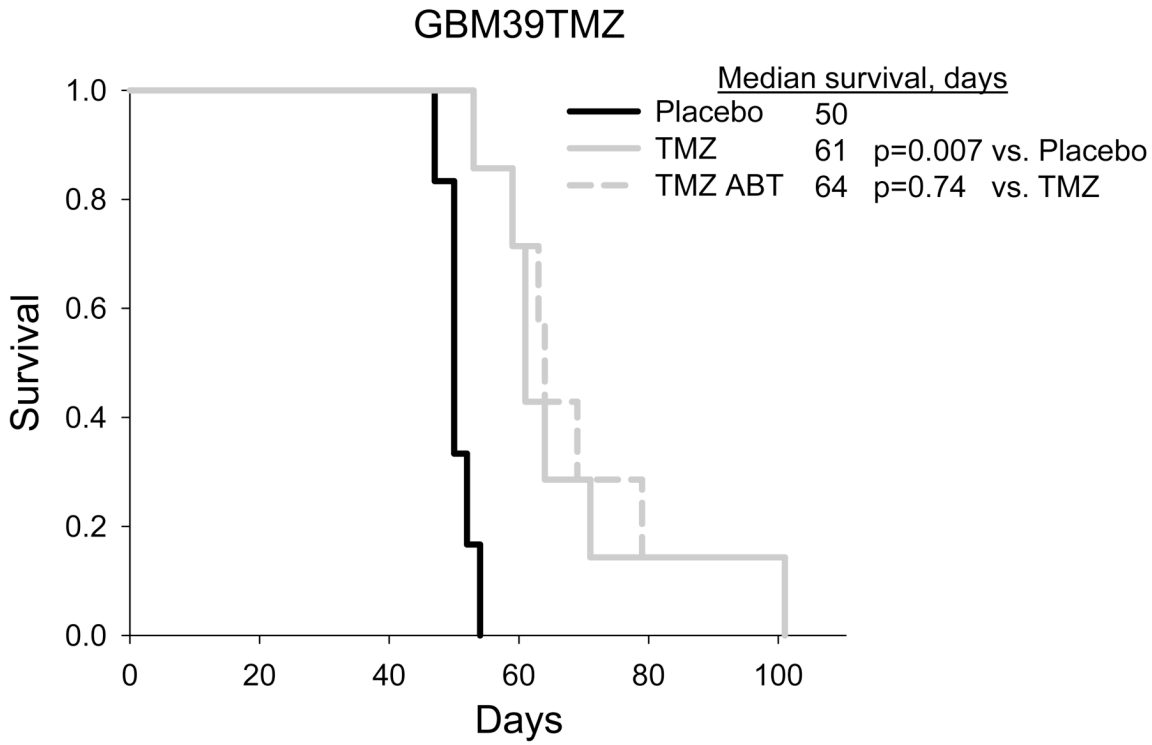

Figure 3.

ABT-888 combined with temozolomide in xenograft lines with acquired TMZ resistance. Mice with established orthotopic xenograft from (A) GBM12TMZ, (B) GBM22TMZ, and (C) GBM39TMZ were randomized to therapy with placebo, ABT-888 alone, TMZ alone and TMZ + ABT-888, except for GBM39 in which the ABT-888 alone arm was omitted. The p-values shown correspond to the comparison between TMZ alone and TMZ + ABT-888.

Figure 4A

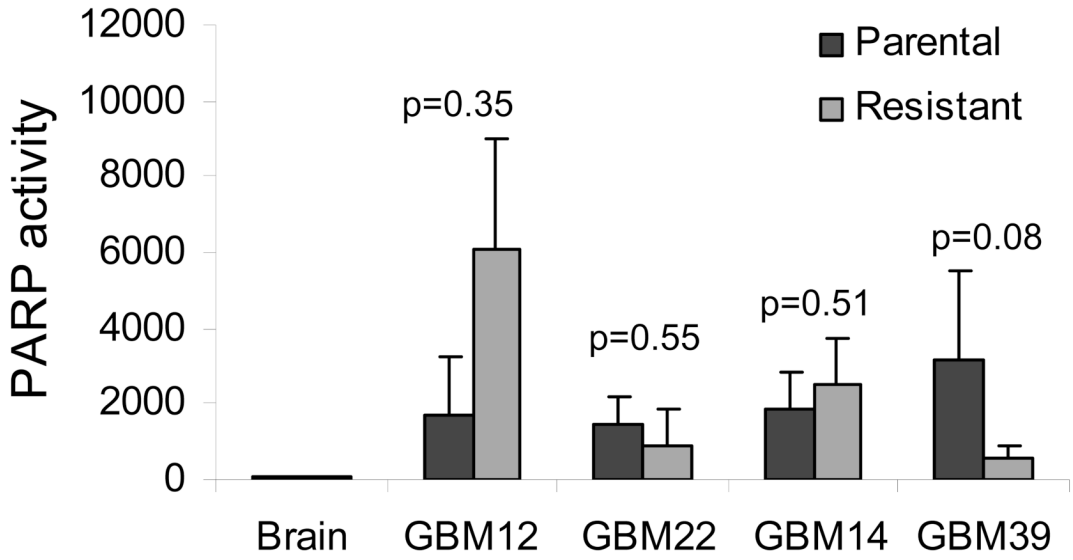

Figure 4B

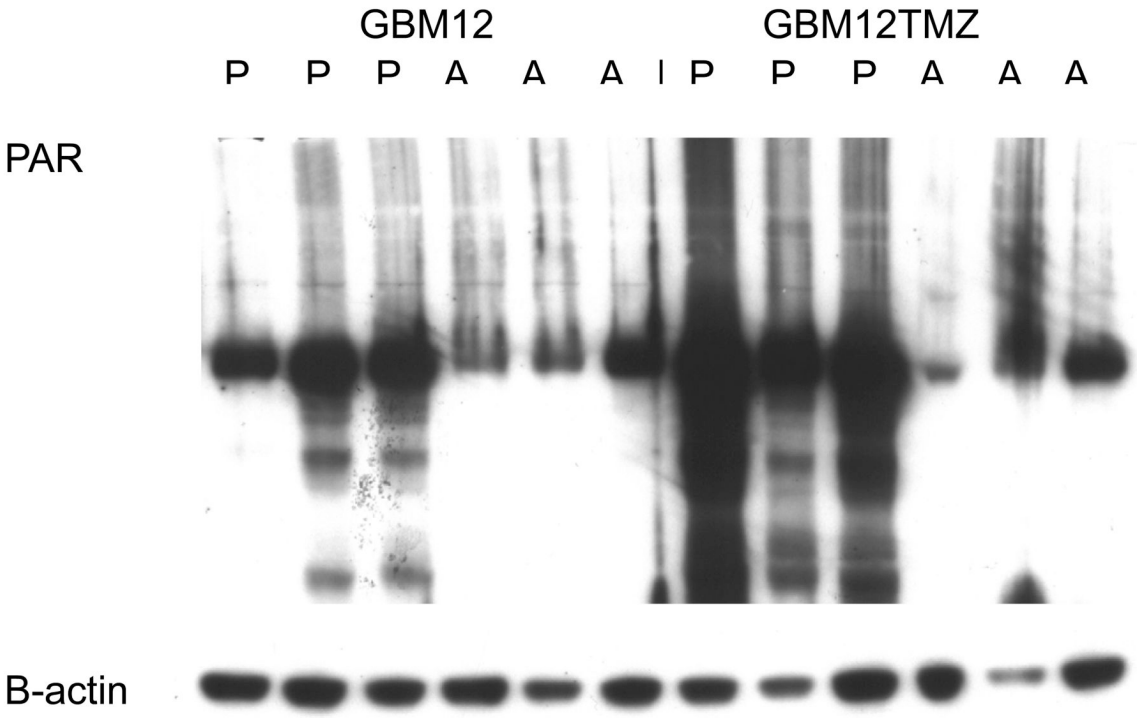

Figure 4.
PARP activity in xenograft lines: A) Endogenous PARP activity levels were evaluated in the indicated tumor lines and compared to those in normal brain. B) Inhibition of PARP activity was evaluated by western blotting for PAR. Mice with established flank tumors from GBM12 (n=6) or GBM12TMZ (n=6) were treated with Placebo (P) or ABT-888 15 mg/kg/day (A) in divided doses and killed after the final dose. Individual tumor lysates were resolved by SDS-PAGE and immunoblotted with an antibody specific for PAR polymer and subsequently for actin.

Figure 5

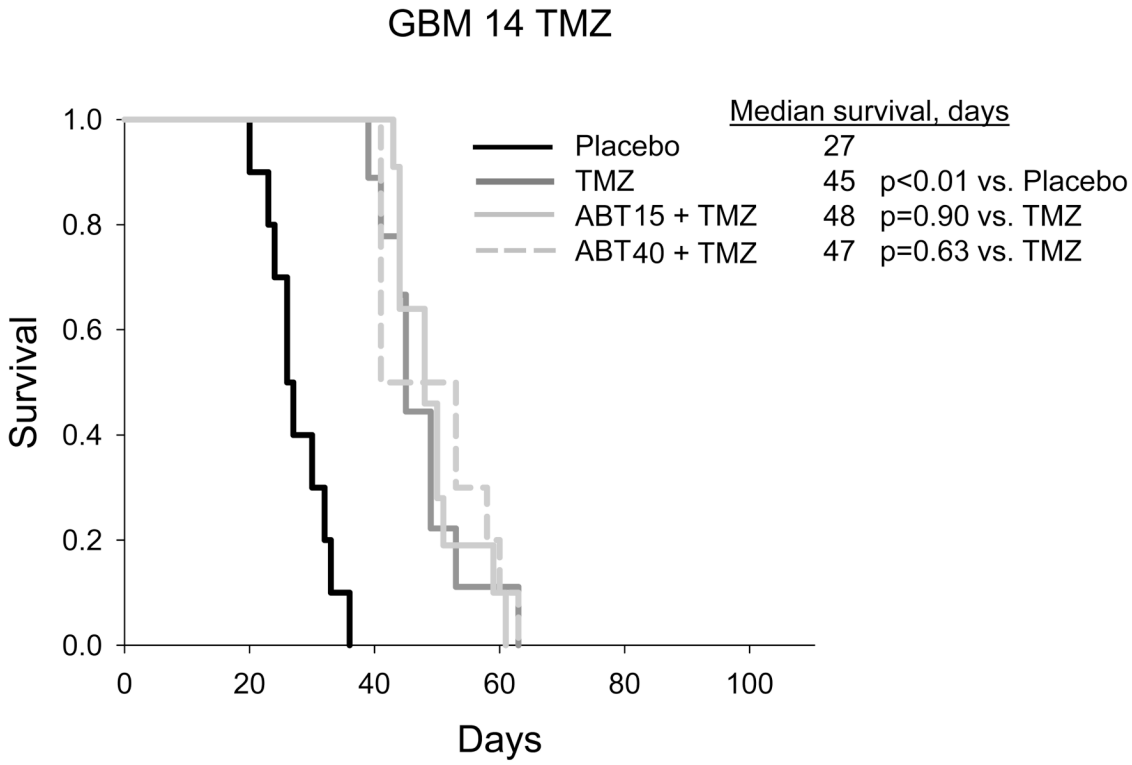

Figure 5.
High-dose ABT-888 therapy. Mice with established intracranial xenografts derived from the secondary TMZ resistant line, GBM14TMZ, were randomized to therapy with placebo, TMZ alone (66 mg/kg/day × 5 days), ABT-888 (15 mg/kg/day, divided dose) + TMZ or ABT-888 (40 mg/kg/day, divided dose) + TMZ. Survival curves are shown for each arm. P-values are shown for TMZ relative to placebo and for the two ABT-888 treatment arms relative to TMZ alone.