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

Michelle J. Clarke,[1] Evan A. Mulligan,[5] Patrick T. Grogan,[2] Ann C. Mladek,[2] Brett L. Carlson,[2] Mark A. Schroeder,[2] Nicola J. Curtin,[5] Zhenkun Lou,[3] Paul A. Decker,[4] Wenting Wu,[4] E. Ruth Plummer,[5] and Jann N. Sarkaria[2]

Departments of [1]Neurologic Surgery, [2]Radiation Oncology, [3]Pharmacology, and [4]Biostatistics, Mayo Clinic, Rochester, Minnesota and [5]Department of Medical Oncology, Northern Institute for Cancer Research, Newcastle, United Kingdom

Abstract

Resistance to temozolomide and radiotherapy is a major problem for patients with glioblastoma but may be overcome using the poly(ADP-ribose) polymerase inhibitor ABT-888. Using two primary glioblastoma xenografts, the efficacy of ABT-888 combined with radiotherapy and/or temozolomide was evaluated. Treatment with ABT-888 combined with temozolomide resulted in significant survival prolongation (GBM12: 55.1%, $P = 0.005$; GBM22: 54.4%, $P = 0.043$). ABT-888 had no effect with radiotherapy alone but significantly enhanced survival in GBM12 when combined with concurrent radiotherapy/temozolomide. With multicycle therapy, ABT-888 further extended the survival benefit of temozolomide in the inherently sensitive GBM12 and GBM22 xenograft lines. However, after *in vivo* selection for temozolomide resistance, the derivative GBM12TMZ and GBM22TMZ lines were no longer sensitized by ABT-888 in combination with temozolomide, and a similar lack of efficacy was observed in two other temozolomide-resistant tumor lines. Thus, the sensitizing effects of ABT-888 were limited to tumor lines that have not been previously exposed to temozolomide, and these results suggest that patients with newly diagnosed glioblastoma may be more likely to respond to combined temozolomide/poly(ADP-ribose) polymerase inhibitor therapy than patients with recurrent disease. [Mol Cancer Ther 2009;8(2):407–14]

Introduction

Temozolomide chemotherapy is an integral component of therapy for malignant gliomas. A recent landmark randomized clinical trial showed that temozolomide chemotherapy given both during and after definitive radiation resulted in an unprecedented 16% absolute gain in 2-year overall survival compared with radiotherapy alone (1, 2). These results changed the standard of care such that nearly all patients with newly diagnosed glioblastoma are treated with radiotherapy and temozolomide followed by temozolomide alone. Temozolomide monotherapy also has moderate efficacy as salvage therapy for temozolomide-naive, recurrent, high-grade gliomas (3), and several trials are evaluating the efficacy of temozolomide-based chemotherapy regimens in patients who failed first-line temozolomide/radiotherapy therapy.

Temozolomide 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 O^6-methylguanine lesions is done by O^6-methylguanine DNA-methyltransferase (MGMT), and silencing of MGMT expression through MGMT promoter hypermethylation is associated with a significantly greater 2-year survival for patients treated with radiotherapy and temozolomide (4). Other DNA methylation lesions are repaired in the multienzyme process of base excision repair (BER). Although BER is robust in essentially all tumors, several strategies have been devised to suppress BER and thereby sensitize tumors to temozolomide 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 chemosensitizing agents (6). Previous preclinical studies suggest that PARP inhibitors enhance the efficacy of temozolomide 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 temozolomide in patients with glioblastoma, we tested the *in vivo* efficacy of a clinical PARP inhibitor (ABT-888) in combination with temozolomide and/or radiotherapy using a unique panel of glioblastoma xenografts initially derived from patient tumors.

The Mayo Clinic panel of primary glioblastoma xenograft lines was developed by implanting patient tumor

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Requests for reprints: Jann N. Sarkaria, Department of Radiation Oncology, Mayo Clinic, 200 First Street Southwest, Rochester, MN 55905. Phone: 507-266-3877; Fax: 507-284-0079. E-mail: sarkaria.jann@mayo.edu

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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 epidermal growth factor receptor amplification and MGMT methylation status that otherwise are commonly lost in cell culture systems (data not shown; ref. 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 temozolomide is correlated with MGMT promoter hypermethylation status. In addition to the primary xenograft lines, we also have developed temozolomide-resistant tumor lines through serial cycles of temozolomide treatment *in vivo*. Using these models, we tested ABT-888 combined with radiotherapy and temozolomide to model upfront therapy, in combination with multiple cycles of temozolomide to model adjuvant therapy, and in combination with temozolomide in the temozolomide-resistant lines to model therapy for tumors progressing on temozolomide therapy.

Materials and Methods

Intracranial Xenograft Model

All xenograft therapy evaluations were done using an orthotopic tumor model for glioblastoma (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 before 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 (5-14 days) in DMEM supplemented with 2.5% fetal bovine serum, 1% penicillin, and 1% streptomycin (15). Cells were harvested by trypsinization and injected (3×10^5 per mouse, suspended in 10 μL) into the right basal ganglia of anesthetized athymic nude mice (athymic Ncr-*nu/nu*: National Cancer Institute-Frederick) using a small animal stereotactic frame (ASI Instruments).

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 from Monday to Friday for 2 weeks (20 Gy total administered over 11 days). Temozolomide was purchased from the Mayo Clinic Pharmacy, suspended in Ora-plus (Paddock Laboratories), and administered by oral gavage. Two dosing schedules were used. For the radiotherapy/temozolomide/ABT-888 study, temozolomide was dosed at 33 mg/kg/d from Monday to Friday for 2 weeks. Otherwise, temozolomide was dosed at 66 mg/kg/d for 5 days. In the indicated experiments, temozolomide 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 from Monday to Saturday coinciding with temozolomide therapy and given 1 h before temozolomide dosing. All mice used for therapy response evaluations were killed at the time of reaching a moribund condition.

Acquired Temozolomide Resistance Model

To develop models of acquired temozolomide resistance, inherently temozolomide-sensitive tumor lines (GBM12, GBM22, and GBM39) were maintained as flank tumors and treated with successively higher doses of temozolomide until the tumor growth was unaffected by dosing with temozolomide at 120 mg/kg/d for 5 days. The resulting temozolomide-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-min exposure was measured using a Fuji LAS3000 UV Illuminator (Raytek) 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) 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 PAR (Trevigen), β-actin (Sigma), and horseradish peroxidase-conjugated rabbit anti-goat and goat anti-mouse (Pierce) 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 done using Fisher's exact test. All tests

were two-sided and $P < 0.05$ was considered to be statistically significant. Weight change over time between treatment groups was compared using repeated-measures ANOVA. A two-sample rank-sum test was used to determine differences at specific time points.

Results

ABT-888 Combined with Radiotherapy and Temozolomide

Two MGMT hypermethylated xenograft lines (GBM12 and GBM22) were selected for our initial studies with ABT-888 in combination with radiotherapy and temozolomide. For each xenograft line, mice with established intracranial xenografts were randomized into 8 treatment groups to evaluate all possible combinations of radiotherapy (2 Gy/d, 5 of 7 days × 2 weeks), temozolomide (33 mg/kg/d, 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 effect on survival relative to placebo therapy in either tumor line; although similar to previous results, temozolomide therapy significantly extended survival in both tumor lines compared with placebo: relative median survival benefit [100 × (median survival treatment group - median survival placebo group) / median survival placebo group] in GBM12 tumors treated with temozolomide was 143% ($P < 0.001$; Fig. 1A), and in GBM22, median survival benefit was 421% ($P < 0.001$; Fig. 1B). In both tumor lines, the addition of ABT-888 to temozolomide therapy significantly extended median survival relative to temozolomide alone (GBM12: 56%, $P = 0.005$; GBM22: 54%, $P = 0.043$). In contrast, the addition of ABT-888 to radiotherapy had no effect on survival relative to radiotherapy alone ($P = 0.10$ for GBM12 and $P = 0.51$ for GBM22). Temozolomide combined with radiotherapy was significantly more effective than either treatment alone [survival prolongation for GBM12 radiotherapy/temozolomide versus temozolomide alone: 124% ($P = 0.003$) versus radiotherapy alone: 245% ($P < 0.001$); GBM22 radiotherapy/temozolomide versus temozolomide alone: 23% ($P = 0.51$) versus radiotherapy alone: 542% ($P < 0.001$)]. Finally, the addition of ABT-888 to concurrent radiotherapy and temozolomide provided additional survival benefit for GBM12 (112%; $P = 0.11$, 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 radiotherapy/temozolomide 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 (Fig. 1C), the lowest point for body weight was observed on day 12, at which point mice treated with radiotherapy/temozolomide had lost 8% body weight ($P < 0.001$) and radiotherapy/temozolomide/ABT-888 had lost 14% ($P < 0.003$) compared with placebo-treated mice. By 20 days following completion of therapy, mice had recovered to their mean starting body weight regardless of treatment group (placebo versus radiotherapy/temozolomide, $P = 0.05$; placebo versus radiotherapy/temozolomide/ABT-888, $P = 0.28$). Similar results were seen with GBM22 (data not shown). Thus, ABT-888 combined with temozolomide was well tolerated and enhanced the efficacy of temozolomide-containing regimens.

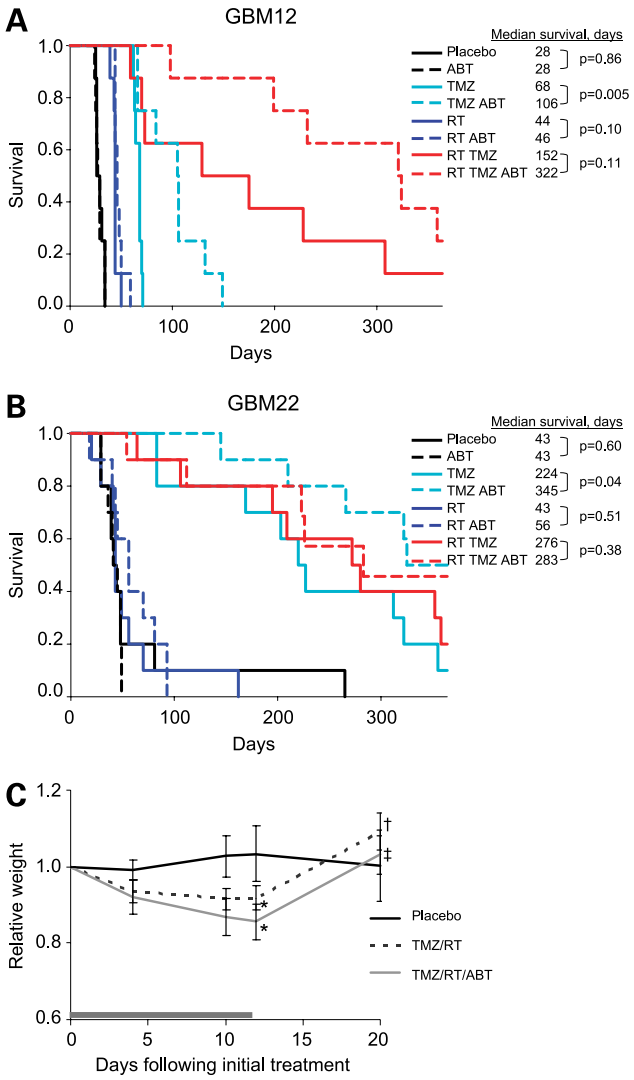

Figure 1. ABT-888 combined with chemoradiation in glioblastoma orthotopic xenografts. Mice with established orthotopic xenografts from (**A**) GBM12 and (**B**) GBM22 were randomized to therapy with the indicated combinations of temozolomide (*TMZ*; 33 mg/kg/d, 5 of 7 d × 2 wk), radiation (*RT*; 2 Gy/d, 5 of 7 d × 2 wk), and ABT-888 (15 mg/kg/d, 6 of 7 d × 2 wk). Mice were followed until reaching a moribund state, and survival results are shown. *P* values are comparing an indicated treatment with or without ABT-888. **C,** change in relative body weight for mice from the GBM12 experiment treated with placebo, radiotherapy/temozolomide, or ABT-888/radiotherapy/temozolomide. *Gray columns,* duration of treatment. *, $P < 0.005$; †, $P = 0.05$; ‡, $P = 0.28$.

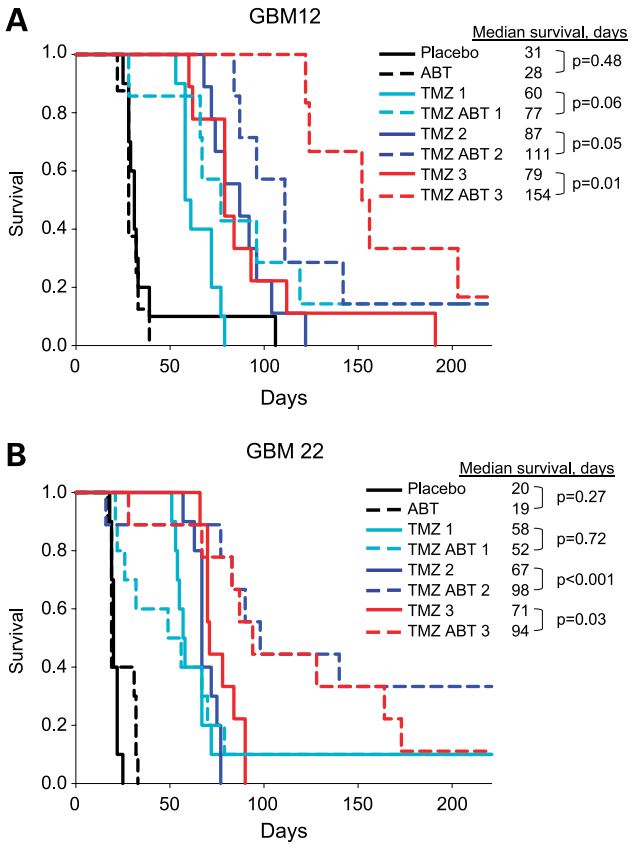

Figure 2. ABT-888 combined with adjuvant temozolomide in glioblastoma orthotopic xenografts. Mice with established orthotopic xenografts from (**A**) GBM12 and (**B**) GBM22 were randomized to therapy with temozolomide (66 mg/kg/d, day 1-5) administered in one, two, or three 28-day cycles (TMZ 1, TMZ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

Cyclical Temozolomide Therapy Combined with ABT-888

The clinical standard of care following completion of radiotherapy/temozolomide is 6 to 12 months of adjuvant temozolomide therapy (150-200 mg/m^2 temozolomide on days 1-5 of a 28-day cycle). Therefore, a similar regimen was evaluated in our xenograft model with 5 days of temozolomide with or without ABT-888 given in up to three 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 one to three cycles of temozolomide without or with ABT-888. Both GBM12 (Fig. 2A) and GBM22 (Fig. 2B) were highly sensitive to temozolomide with a single cycle of temozolomide resulting in 94% and 190% increase in median survival relative to placebo ($P = 0.030$ and $P < 0.001$), respectively. In GBM12, a second cycle resulted in an additional 45% prolongation in survival relative to cycle 1 ($P = 0.002$), whereas a second cycle provided no significant benefit in GBM22 (16% prolongation; $P = 0.24$). A third cycle of temozolomide produced no benefit in either xenograft line. Thus, although both tumor lines were significantly sensitive to temozolomide in the first cycle, subsequent cycles of temozolomide were significantly less effective.

Combined therapy with ABT-888 and temozolomide prolonged survival across multiple cycles of temozolomide. For GBM12, treatment with temozolomide and ABT-888 prolonged survival relative to temozolomide alone in all three 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 cycles: 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 temozolomide in both GBM12 and GBM22, which are inherently sensitive to temozolomide.

Resistant Xenograft Lines

The development of temozolomide resistance during adjuvant therapy occurs in >30% of patients; therefore, the combination of ABT-888 with temozolomide was evaluated in tumor lines derived from GBM12 and GBM22 that had been selected *in vivo* for resistance to temozolomide (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 temozolomide (66 mg/kg/d × 5 days) to mimic the setting of recurrent disease in which further disease progression after the first cycle would warrant a change in therapy. Temozolomide resistance was evident compared with the previously tested parental lines used in the upfront therapy experiments; survival benefit with temozolomide alone (66 mg/kg/d × 5 days) was 94% for parental GBM12 compared with 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/temozolomide compared with temozolomide alone was 2.3% for GBM12TMZ ($P = 0.044$; Fig. 3A) and 0% for GBM22TMZ ($P = 0.74$; Fig. 3B). A third temozolomide-resistant tumor line (GBM39TMZ) also was tested in this model. Similar to the other two resistant lines, the combination of ABT-888 with temozolomide was no more effective than temozolomide alone; treatment with ABT-888/temozolomide compared with temozolomide alone prolonged median survival by 4.9% for GBM39TMZ ($P = 0.74$; Fig. 3C). Thus, ABT-888 did not provide any survival benefit in combination with temozolomide in three xenograft lines previously selected for temozolomide resistance.

Evaluation of PARP Activity

The levels of PARP activity were evaluated in both parental and corresponding temozolomide-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 temozolomide-resistant tumor lines (Fig. 4A), although these levels were significantly elevated in comparison with normal brain.

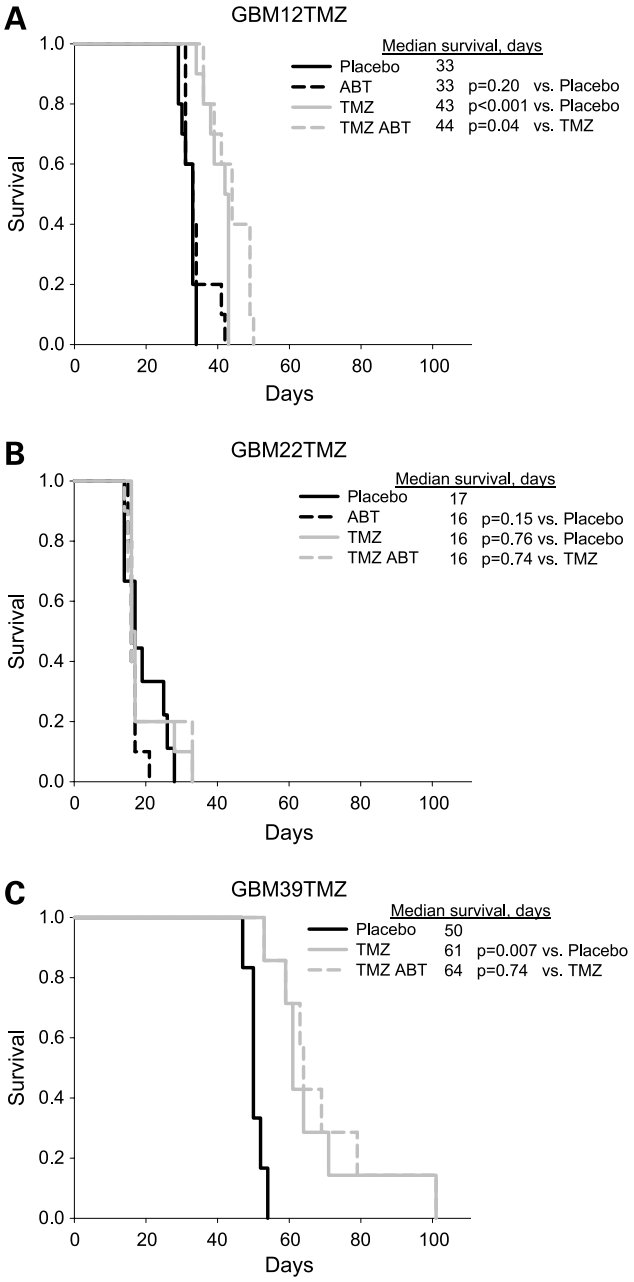

Figure 3. ABT-888 combined with temozolomide in xenograft lines with acquired temozolomide resistance. Mice with established orthotopic xenograft from (**A**) GBM12TMZ, (**B**) GBM22TMZ, and (**C**) GBM39TMZ were randomized to therapy with placebo, ABT-888 alone, temozolomide alone, and temozolomide + ABT-888, except for GBM39 in which the ABT-888 alone arm was omitted. *P* values correspond to the comparison between temozolomide alone and temozolomide + ABT-888.

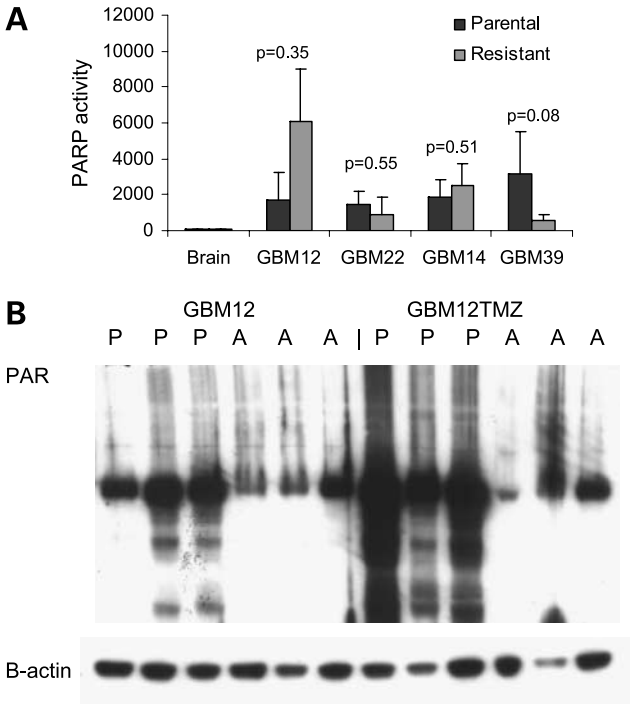

Figure 4. PARP activity in xenograft lines. **A,** endogenous PARP activity levels were evaluated in the indicated tumor lines and compared with those in normal brain. **B,** inhibition of PARP activity was evaluated by Western blotting for PAR. Mice with established flank tumors from GBM12 ($n = 6$) or GBM12TMZ ($n = 6$) were treated with placebo (*P*) or ABT-888 15 mg/kg/d (*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 and subsequently for actin.

Differential pharmacodynamic effects of ABT-888 in the temozolomide-resistant versus temozolomide-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 showed an open blood-brain barrier,[6] 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 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 10th dose of ABT-888 or placebo, mice were killed and tumors were flash-frozen and subsequently were processed for analysis of PAR levels. As seen in Fig. 4B, ABT-888 (15 mg/kg/d) was highly effective at suppressing PARP activity, as reflected by the reduced level of PAR modifications, in both parental GBM12 and temozolomide-resistant GBM12TMZ xenograft lines. Thus, the lack of temozolomide-sensitizing effects is not due to failure to effectively inhibit PARP activity in the resistant lines.

[6] J. Sarkaria and J. Poduslo, unpublished data.

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/d 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/d in two divided doses) to ensure maximal drug exposure in another temozolomide-resistant tumor line. In the GBM14TMZ xenograft line that also had been subjected to *in vivo* temozolomide selection, temozolomide therapy alone was associated with a 66% survival benefit ($P < 0.001$), but combinations of temozolomide with ABT-888 (15 mg/kg/d) or ABT-888 (40 mg/kg/d) were not associated with any additional benefit compared with temozolomide alone ($P = 0.90$ and 0.63, respectively; Fig. 5). Thus, resistance to the sensitizing effects of ABT-888 could not be overcome with supratherapeutic dosing of ABT-888.

Discussion

The preclinical animal model studies presented show that not all glioblastoma tumors will benefit equally from combined therapy with temozolomide and the PARP inhibitor ABT-888. Specifically, ABT-888 combined with temozolomide only enhanced survival in the two temozolomide-naive xenograft lines (GBM12 and GBM22), whereas derivative tumor lines, which have been selected *in vivo* for temozolomide resistance (GBM12TMZ and GBM22TMZ), were unaffected by the addition of ABT-888 to temozolomide therapy. Along with the lack of survival benefit with combined therapy for two other temozolomide-resistant lines (GBM14TMZ and GBM39TMZ), these results suggest that combined therapy with ABT-888 and temozolomide may not be effective in glioblastoma tumors that already have developed resistance to temozolomide. These data are in contrast to several *in vitro* and *in vivo* studies that show improved efficacy of temozolomide 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 Clinic glioblastoma 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 temozolomide responsiveness.[7] In contrast, many of the previous studies were done in nonglioblastoma models, and all studies have been done 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 Clinic xenograft panel provides a robust platform for testing novel temozolomide-sensitizing strategies for glioblastoma therapy.

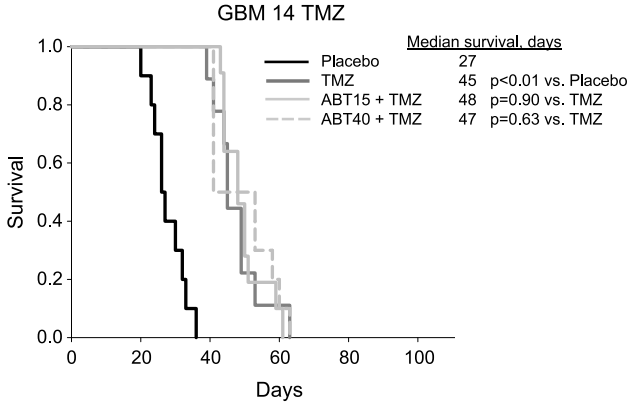

Figure 5. High-dose ABT-888 therapy. Mice with established intracranial xenografts derived from the secondary temozolomide-resistant line, GBM14TMZ, were randomized to therapy with placebo, temozolomide alone (66 mg/kg/d × 5 d), ABT-888 (15 mg/kg/d, divided dose) + temozolomide, or ABT-888 (40 mg/kg/d,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

The data presented show that the lack of a temozolomide-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/d; Fig. 4B). Although 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,[8] and ABT-888 effectively penetrates an intact blood-brain barrier and shows demonstrable accumulation in the central nervous system (20). Consistent with effective inhibition of PARP activity in intracranial tumors, ABT-888 effectively sensitized the GBM12 and GBM22 xenograft lines (Figs. 1 and 2). Moreover, a higher-dose ABT-888 regimen (40 mg/kg/d), which would provide dose levels in mice that would be supratherapeutic 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 temozolomide therapy requires integrity of both short-patch BER pathway and the MGMT repair protein to repair cytotoxic N^3-methyladenine and O^6-methylguanine lesions, respectively, and abrogation of either pathway leads to significant increased cell killing after temozolomide treatment (reviewed in ref. 5). Temozolomide resistance in the GBM12TMZ and GBM14TMZ lines can be reversed with the MGMT inhibitor O^6-benzylguanine and both lines show a marked up-regulation of MGMT

[7] In preparation.
[8] J. Poduslo and J. Sarkaria, unpublished data.

protein and mRNA levels.[6] In conjunction with the lack of temozolomide 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 show slowed kinetics of BER without complete abrogation of BER activity (22–24). The key cytotoxic lesion induced by temozolomide and processed by BER is N^3-methyladenine, which can lead to cytotoxicity only when encountered by a replication fork during S phase (11). Because 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 temozolomide-resistant models because of the much longer average time available to a cell before replication. Differential effects of PARP inhibition on BER between temozolomide-sensitive and temozolomide-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 temozolomide-induced damage.

The current set of studies was designed to guide clinical development of ABT-888 in glioblastoma. Although 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 temozolomide-sensitizing strategies in glioblastoma. First, of the six xenograft lines tested, only the two that were inherently sensitive to temozolomide were effectively sensitized by ABT-888, whereas ABT-888 combined with temozolomide was ineffective in temozolomide-resistant lines. These data suggest that combined therapy with temozolomide and a PARP inhibitor likely will be more effective in newly diagnosed glioblastoma patients and that PARP inhibition combined with temozolomide in patients who have progressed on temozolomide is less likely to provide significant benefit. Second, for the two tumor lines in which robust sensitization to temozolomide was 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 temozolomide/radiotherapy therapy. Third, the efficacy of temozolomide was reduced with latter cycles of therapy in temozolomide-naive tumors. This observation is similar to clinical experiences in which >30% of newly diagnosed patients progress while receiving temozolomide therapy (2), and this may reflect relatively early development of temozolomide resistance in these tumors. Given the lack of efficacy of combined therapy in temozolomide-resistant tumors, these data suggest that PARP inhibitors may be most effective when integrated early during therapy before resistance develops. Although these observations remain to be confirmed in clinical trials, we believe that the studies done in the Mayo Clinic glioblastoma xenograft model have helped delineate a potential strategy for optimizing the integration of PARP inhibitors with temozolomide for therapy of glioblastoma patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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