ABCB1, ABCG2, and PTEN Determine the Response of Glioblastoma to Temozolomide and ABT-888 Therapy

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Abstract

Purpose: Little is known about the optimal clinical use of ABT-888 (veliparib) for treatment of glioblastoma. ABT-888 is a PARP inhibitor undergoing extensive clinical evaluation in glioblastoma, because it may synergize with the standard-of-care temozolomide (TMZ). We have elucidated important factors controlling ABT-888 efficacy in glioblastoma.

Experimental Design: We used genetically engineered spontaneous glioblastoma mouse models and allograft models that were orthotopically transplanted into wild-type (WT) and Abcb1/Abcg2-deficient (KO) recipients.

Results: ABT-888/TMZ is not efficacious against p53; p16ink4a/p19arf; K-Ras12; LucR allografts in wild-type recipients, indicating inherent resistance. Abcb1/Abcg2 mediate efflux of ABT-888 at the blood–brain barrier (BBB), causing a 5-fold reduction of ABT-888 brain penetration (P<0.0001) that was fully reversible by elacridar. Efficacy studies in WT and KO recipients and/or concomitant elacridar demonstrate that Abcb1/Abcg2 at the BBB and in tumor cells impair TMZ/ABT-888 combination treatment efficacy. Elacridar also markedly improved TMZ/ABT-888 combination treatment in the spontaneous p53; p16ink4a/p19arf; K-Ras12; LucR glioblastoma model. Importantly, ABT-888 does enhance TMZ efficacy in Pten-deficient glioblastoma allografts and spontaneous tumors, even in Abcb1/Abcg2 proficient wild-type mice. Loss of PTEN occurs frequently in glioblastoma (36%) and in silico analysis on patient with glioblastoma samples revealed that it is associated with a worse overall survival (310 days vs. 620 days, n = 117).

Conclusions: The potential of ABT-888 in glioblastoma can best be demonstrated in patients with PTEN null tumors. Therefore, clinical trials with ABT-888 should evaluate these patients as a separate group. Importantly, inhibition of ABCB1 and ABCG2 (by elacridar) may improve the efficacy of TMZ/ABT-888 therapy in all glioblastoma patients. Clin Cancer Res; 20(10); 2703–13. ©2014 AACR.

Introduction

Glioblastoma (GBM) is the most common and aggressive primary brain tumor and only very few chemotherapeutic agents are available that exert a meaningful response. The current standard-of-care is surgical resection followed by chemo-radiation therapy consisting of the DNA-alkylating agent temozolomide (TMZ) and radiotherapy. This DNA damaging treatment modality significantly increases the overall median survival to 14.6 months after diagnosis (1). However, even with this aggressive treatment regimen the prognosis of patients with glioblastoma remains dismal and novel therapeutics are urgently needed.

PARP inhibitors enhance the activity of DNA-damaging therapies, because of the critical function of PARP-1 and PARP-2 in base excision repair (2, 3). Several preclinical studies suggest that PARP inhibitors enhance the efficacy of TMZ in both sensitive and resistant tumors (4, 5). Moreover, their capacity to sensitize glioblastoma cells to TMZ treatment and reverse TMZ resistance has been reported (6–8). ABT-888 (veliparib) is a potent PARP-1/2 inhibitor and the clinically most advanced candidate for glioblastoma, with several ongoing clinical trials. Obviously, the combination
Translational Relevance

Glioblastoma is a uniformly lethal disease and there is a great but yet unmet need for better therapies. There is a clear rationale for the clinical evaluation of PARP inhibitors such as ABT-888 (veliparib) in combination with the standard therapy in patients with high-grade glioma. PARP inhibitors can augment the cytotoxic effects of DNA damage by interfering in DNA repair. Of all PARP inhibitors, ABT-888 is the clinically most advanced candidate agent for glioblastoma. Failure or success of these trials may not just determine the fate of ABT-888, but may set the stage for the whole concept of using PARP inhibitors in this disease. We here show that ABC transporters at the blood–brain barrier and tumor cells and PTEN status of tumor cells are important determinants of efficacy of ABT-888 and temozolomide combination therapy. This information needs to be taken into account during the evaluation of ongoing clinical trials.

of ABT-888 with chemo-radiation therapy is receiving considerable interest, because it has shown promise in preclinical models, including intracranial models (9, 10). The outcome of these clinical trials may not only determine the fate of ABT-888, but may set the stage for the whole concept of using PARP inhibitors in the treatment of glioblastoma. To maximize the chances for successful implementation of this drug, it is important to have a thorough understanding of the factors that may pose threats to this objective. Because we felt that critical information was lacking, we have performed comprehensive in vivo studies in a set of clinically relevant glioma models to interrogate the potential of ABT-888 in high-grade glioma and identified critical factors that may determine its success in the clinic.

A pitfall of many intracranial tumor models, including those that were used to demonstrate efficacy of ABT-888 (9, 10), is the very leaky tumor vasculature, resulting in excellent drug penetration throughout the whole tumor area. However, because of the invasive nature of gliomas, the permeability of the blood–brain barrier (BBB) in such brain tumors (blood brain tumor barrier, BBTB) is much more heterogeneous in patients with glioblastoma and may therefore compromise adequate drug exposure to a substantial fraction of glioblastoma cells (11–13). Especially agents that are recognized by ATP-binding cassette (ABC) drug efflux transporters expressed at the BBB are at risk. Of all efflux transporters present in the BBB, ABCB1 (also known as P-glycoprotein; P-gp or MDR1) and ABCG2 (breast cancer resistance protein, BCRP) are dominant (14). Together, they are responsible for the efflux of a wide range of therapeutic agents, including many of the small molecule inhibitors that are currently under clinical investigation for brain cancer (15, 16). Moreover, ABCB1 and ABCG2 have established roles in conferring multidrug resistance by limiting intracellular drug accumulation in tumor cells (17, 18). Besides this issue of adequate drug exposure, many intracranial tumor models also lack many important characteristics, including those involved in drug resistance, as a consequence of culturing cells in serum-containing medium (19, 20). By ignoring these intrinsic and extrinsic drug resistance mechanisms, these models may not be predictive for the clinical efficacy of potential glioblastoma therapeutics, such as ABT-888.

We have previously developed a range of Cre-LoxP conditional transgenic mouse models of glioblastoma for chemotherapy intervention studies (21). These models allow interrogation of the influence of frequently altered genes in glioblastoma (e.g., P53 or PTEN status) on tumor sensitivity (22). In this study, we found that a serum-free cultured cell line derived from a spontaneous p53; p16Ink4a/p19Arf; K-RasV12; LucR glioblastoma and re-injected into nude mice did not respond to ABT-888 and temozolomide combination therapy. By using Abcb1a1b- and/or Abcg2-deficient recipient mice and the dual ABC-transporter inhibitor elacridar, we demonstrate that these drug efflux transporters are causing resistance in this clinically relevant model. Moreover, we found that ABT-888 is more active in PTEN-deficient tumors in our in vivo models.

Materials and Methods

Drugs

ABT-888 was obtained from Selleck Chemicals, TMZ for in vitro experiments from Sigma-Aldrich, and TMZ for in vivo studies from TEVA Pharma. Elacridar was kindly provided by GlaxoSmithKline (Research Triangle Park) and zosuquidar by Eli Lilly.

Animals

Mice were housed and handled according to institutional guidelines complying with Dutch legislation. All experiments with animals were approved by the animal experiment committee of the institute. The animals used for pharmacokinetics studies were female wild-type (WT), Abcb1a1b−/−, Abcg2−/−, and Abcb1a1b−/−; Abcg2−/− mice of FVB genetic background, between 9 and 14 weeks of age. Pten; p16Ink4a/p19Arf; K-RasV12; LucR and p53; p16Ink4a/p19Arf; K-RasV12; LucR conditional mice were generated as described previously (21). Athymic (nude) wild-type and Abcb1a1b−/−; Abcg2−/− mice of FVB background were used as recipient animals for orthotopic injection of neurosphere cultured glioblastoma cells generated from the above-mentioned conditional mouse models.

ABT-888 brain penetration

ABT-888 (10 mg/kg) was administered orally (p.o.) or intravenously and elacridar (100 mg/kg, p.o.) was given 15 minutes before ABT-888. Blood was collected by cardiac puncture or tail vein (for serial sampling). Brains were homogenized in 3 mL 1% (w/v) bovine serum albumin. ABT-888 plasma and brain samples were analyzed by liquid chromatography/tandem mass spectrometry (LC/MS-MS) as described (23) or for more sensitive quantitation over a...
concentration range of 0.3 to 100 ng/mL, a modified LC/MS-MS system utilizing an ABI4000 mass detector (m/z 245.2–84.2 for ABT-888, and 248.2–87.2 for [D$_3$]-ABT-888). Pharmacokinetic parameters were calculated using PKSolver (24).

**Pharmacokinetic calculations and statistical analysis**

Brain and plasma concentrations among multiple strains were compared by one-way ANOVA with the Bonferroni post hoc test. Survival fractions were calculated according to the Kaplan–Meier method using GraphPad Prism v6 (GraphPad Software, Inc.). The log-rank test was used to compare survival of groups.

### Intracranial tumor models and bioluminescence imaging

The procedures of stereotactic intracranial injection of lentivirus or tumor cells and bioluminescence imaging (BLI) have been described in detail (21). In short, Pten; p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR and p53; p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR mice were injected intracranially with 2 μL of CMV-Cre lentivirus suspension 2 mm lateral and 1 mm anterior to the bregma, 3 mm below the skull. For orthotopic transplantation models, 2 μL of cell suspension containing 5,000 cells was injected in athymic wild-type and conditional mice after sacrifice by cervical dislocation. Tumor development was monitored by bioluminescence using the IVIS 200 (Perkin Elmer Inc.). BLI values were log-converted. Mean and SE BLI values of each cohort were calculated for each time point, until most animals within the cohort had to be sacrificed because of disease progression. Mice were sacrificed when clear neurologic symptoms occurred or weight loss (≥20%) was observed.

### Drug formulation and treatment regimen

ABT-888 [in dimethyl sulfoxide (DMSO):saline; 1:10] was administered orally at a dose of 10 mg/kg/twice a day for 5 days. TMZ (100 mg capsule) was dissolved just before administration in 2 mL of ethanol plus 18 mL saline, filtered, and administered orally at a dose of 100 mg/kg every day 5× within 30 minutes after preparation. Elacridar was administered orally at a dose of 100 mg/kg 15 minutes before ABT-888 or TMZ administration.

### Glioblastoma cell cultures and proliferation assays

Glioblastoma cell lines have been derived from tumors generated in p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR, Pten; p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR, p53; p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR, and p53;Pten; p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR conditional mice after lentiviral infection. Small amounts of tumor tissue were triturated mechanically in ice-cold Ca$^{2+}$ and Mg$^{2+}$-free HBSS (Gibco). Cell suspensions were cultured in ultra low binding 6-well plates (Corning) in serum-free Medium Hormone-Mix (MHM) medium supplemented with 10 ng/mL EGF and bFGF (Sigma) as described previously (25).

Proliferation assays were carried out with glioblastoma cells (2,000/well) seeded on laminin coated 96-well black-well/clear-bottom plates (Greiner Bio-One) in MHM medium supplemented with 10 ng/mL EGF and bFGF. Treatment was started after 1 day with MHM medium containing 0.1% DMSO (control), 100 μmol/L TMZ alone, or in combination with increasing concentrations of ABT-888 (0.3–30 μmol/L). Cell density/viability was determined on day 0 (treatment start) and day 5 using bioluminescence imaging on an IVIS Lumina II Imaging System (Perkin Elmer Inc.) with 150 μg/mL of beetle luciferin (Promega) in each well.

### Histology and immunohistochemistry

Brain tissue was fixed in 4% formaldehyde; paraffin embedded and cut into 4 μm coronal sections that were stained for hematoxylin and eosin and for Abcg2/ABCG2 using the BXP-53 antibody (Abcam).

### Western blotting

For PARP inhibition analyses, glioblastoma cells were cultured on laminin in 6-well plates until 80% to 90% cell confluence. Cells were incubated with drugs for 4 hours and subsequently lysed with complete IPA buffer containing phosphatase inhibitors. Lysates were processed for Western blotting. Primary antibodies used in this study are rabbit anti-PAR (1:1,000; Trevigen), mouse anti-β-tubulin isotype III (1:1,000; Sigma), and Mdr (H-19) rabbit polyclonal (1:200; Santa Cruz Biotechnology) and BXP-53 rat monoclonal (1:400; Abcam) for Abcb1 and Abcg2, respectively. Enhanced chemiluminescence was used for detection using Molecular imaged ChemiDoc XRS+ system (Bio-Rad). Data were analyzed using ImageLab software (version 2.0.1) from Bio-Rad laboratories.

### Results

p53; p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR glioblastoma allografts are resistant to TMZ or ABT-888 and TMZ treatment

GBM652457 cells were isolated from a lentivirally induced tumor in a p53; p16$^{ink4a}$/p19$^{Arf}$;K-Ras$^{v12}$;LucR mouse. Tumors that developed after intracranial injection into nude mice resemble many features of human high-grade gliomas (21). Mice received either vehicle, single treatment with 100 mg/kg TMZ every day or combined with 10 mg/kg ABT-888 twice a day for 5 days. Although a slight delay in tumor growth was observed, no significant difference in median survival was found between treated and control (Fig. 1A; 11 vs. 11 days; $P = 0.37$) or the 2 treatment groups (12 vs. 11 days; $P = 0.22$).

### Brain penetration of ABT-888 is restricted by Abcb1 and Abcg2 and can be enhanced by elacridar

We found that ABT-888 is transported by Abcb1, Abcg2, and ABCB1 in vitro (Supplementary Fig. S1). Consequently, we evaluated the impact of Abcb1a/1b and/or Abcg2 on the plasma and brain pharmacokinetics of ABT-888 using wild-type, Abcb1a/1b$^{-/-}$, Abcg2$^{-/-}$, and Abcb1a/1b$^{-/-}$/Abcg2$^{-/-}$ mice. Following intravenous administration of 10 mg/kg of ABT-888, the AUCplasma differed just 1.3-fold between the
various strains (Table 1 and Fig. 2A). Consequently, Abcb1 and Abcg2 do not play a critical role in the systemic clearance of ABT-888. In contrast, the AUC\textsubscript{brain} of both Abcb1\textsuperscript{a/b} and Abcg2\textsuperscript{-/-} mice was significantly higher than in wild-type mice (3.7- and 4.9-fold, respectively; both P < 0.0001), demonstrating that Abcb1 and—to a lesser extent—Abcg2 at the BBB severely impair the brain entry of ABT-888. Interestingly, a profound continuous increase in the ABT-888 brain-to-plasma ratio was observed in Abcb1\textsuperscript{a/b} and Abcg2\textsuperscript{-/-} mice in a time course of 4 hours. This implies that the brain clearance does not keep up with the systemic clearance of ABT-888, demonstrating the importance of Abcb1 and Abcg2 in clearance of ABT-888 from the brain.

Similarly, inhibition of Abcb1 and Abcg2 by elacridar also increased ABT-888 brain accumulation. Concomitant elacridar slightly increased the AUC\textsubscript{plasma} by 1.2-fold (Table 1; P = 0.14). This was also seen in Abcb1\textsuperscript{a/b} and Abcg2\textsuperscript{-/-} mice (1.3-fold; P = 0.0099) and thus most likely because of inhibition of other drug elimination pathways. Importantly, co-administration of elacridar in wild-type mice caused an 11-fold increased ABT-888 brain concentration (P < 0.0001) at 4 hours after drug administration and a brain concentration-to-AUC\textsubscript{plasma} ratio similar to that of Abcb1/
1b/b;Abcg2−/− mice (with or without elacridar (P = 0.25 and P > 0.99, respectively)) and 9-fold higher than wild-type controls (P < 0.0001; Fig. 2B). Thus, elacridar significantly enhances the brain accumulation of ABT-888 by inhibition of Abcb1 and Abcg2 at the BBB.

Co-administration of elacridar enhances the efficacy of TMZ and ABT-888 against glioblastoma
To investigate whether absence of Abcb1 and Abcg2 at the BBB/BBTB would improve TMZ/ABT-888 treatment, we repeated the in vivo efficacy experiment with p53;p16Ink4a/p19Arf;K-Rasv12;LucR GBM652457 cells in Abcb1a/b−/−;Abcg2−/− nude mice. We observed a better overall survival with TMZ/ABT-888 treatment in Abcb1a/b−/−;Abcg2−/− mice (Fig. 1B) than in wild-type mice (Fig. 1A). Mice receiving ABT-888+TMZ survived significantly longer than untreated controls (15 vs. 9 days; P = 0.004) whereas mice receiving TMZ alone did not (11 vs. 9 days; P = 0.30).

Besides the presence of Abcb1 and Abcg2 at the BBB/BBTB, their expression in tumor cells may also impair the intracellular accumulation and efficacy of ABT-888. Many of the murine-derived glioblastoma cell lines, including GBM652457, express Abcg2 and also some Abcb1 and immunochemistry of Abcg2 revealed its presence in blood

### Table 1. Plasma and brain AUC of ABT-888

<table>
<thead>
<tr>
<th>AUC0-4h</th>
<th>Route</th>
<th>WT</th>
<th>Abcb1a/b−/−</th>
<th>Abcg2−/−</th>
<th>Abcb1a/b;Abcg2−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>i.v.</td>
<td>1,004 ± 31</td>
<td>1,157 ± 70</td>
<td>1,191 ± 47</td>
<td>1,297 ± 59b</td>
</tr>
<tr>
<td>Brain</td>
<td>i.v.</td>
<td>856 ± 32</td>
<td>3,145 ± 95b</td>
<td>906 ± 43</td>
<td>4,155 ± 202b/b/c</td>
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</table>

WT WT + elacridar Abcb1a/b;Abcg2−/− Abcb1a/b;Abcg2−/− + elacridar

Plasma p.o. 1,468 ± 85 1,788 ± 85d 1,380 ± 74 1,843 ± 123b

NOTE: WT, Abcb1a/b−/−, Abcg2−/−, and Abcb1a/b−/−;Abcg2−/− mice received i.v. or p.o. ABT-888 (10 mg/kg). AUC0-4h in ng/mL·h or ng/g·h (mean ± SEM).a,bP < 0.01, cP < 0.0001 compared with WT mice. dP < 0.0001 compared with Abcb1a/b−/−.eP < 0.05, fP < 0.01, compared with Abcb1a/b;Abcg2−/− mice not receiving elacridar. One-way ANOVA with the Bonferroni post hoc test.

Figure 2. The brain penetration of ABT-888 is limited by Abcb1 and Abcg2. A, ABT-888 plasma concentrations, brain concentrations, and brain-to-plasma ratios following intravenous administration of 10 mg/kg of ABT-888 (n = 5/time point/strain). B, ABT-888 levels following 10 mg/kg p.o. administered to wild-type and Abcb1a/b−/−;Abcg2−/− mice with/without co-administration of 100 mg/kg elacridar p.o. Blood samples were collected from the tail at 15 minutes, 1, 2, and 4 hours (n = 8/strain). Brain samples were harvested at 4 hours after drug administration. Data, mean ± SEM; ****, P < 0.0001 compared with wild-type. ANOVA followed by Bonferroni post hoc analysis.
vessels and tumor cells (Fig. 3). As assessed by Western blotting, ABT-888 has PARP inhibitory activity in the submicromolar range in our glioblastoma cell lines in vitro, which was modestly enhanced by elacridar (Supplementary Fig. S2). To investigate whether elacridar can improve the efficacy of TMZ and ABT-888 in vivo, we administered TMZ and ABT-888 with or without elacridar to both wild-type and Abcb1a/b−/−;Abcg2−/− mice bearing orthotopic p53; p16−/−;p19−/−;K-Ras12;LucR GBM652457, Abcb1a/b−/−; Abcg2−/− mice receiving elacridar survived significantly longer than Abcb1a/b−/−; Abcg2−/− mice not receiving elacridar (Fig. 1C, median survival 26.5 vs. 22 days; P = 0.025). Wild-type mice receiving elacridar in combination with TMZ and ABT-888 responded similarly as Abcb1a/b−/−; Abcg2−/− mice receiving the combination. Elacridar significantly prolonged the survival of wild-type mice receiving TMZ and ABT-888 treatment (median survival 25 vs. 18 days for wild-type mice receiving TMZ + ABT-888 vs. TMZ + ABT-888, respectively; P = 0.039). Together, these results support the idea that elacridar inhibited Abcb1 and Abcg2 both at the BBB and in tumor cells.

As a second model, we used our spontaneous high-grade glioma model induced by lentivirus in LoxP conditional p53;p16Ink4a/p19Arf;K-Rasv12;LucR GBM652457 tumors in WT (A) and Abcb1a/b−/−;Abcg2−/− nude mice (B). In wild-type mice, Abcg2 is expressed at the BBB, in blood vessels throughout the tumor (BBTB) as well as on tumor cells. The presence of Abcg2 in tumor cells is even better visualized in the Abcg2-deficient Abcb1a/b−/−;Abcg2−/− recipients. Notably, the expression in tumor cells is not uniform throughout the tumor. C, similar ABCG2 staining was observed in vessels and tumor cells in human glioblastoma samples as depicted in these 2 examples. Bar size: 2 mm (A, B, left) and 200 μm (all other panels). D, Western blot analysis demonstrates the presence of Abcb1 and Abcg2 in glioblastoma cell lines of different origins: p53;p16Ink4a/p19Arf/K-Ras12;LucR (GBM652457, GBM910300), Pten;p16Ink4a/p19Arf/K-Ras12;LucR (GBM696677), P53;Pten;p16Ink4a/p19Arf/K-Ras12;LucR (GBM707263, GBM707251), and p16Ink4a/p19Arf; K-Ras12;LucR (GM763663). Control cell lines: CT26 is a nondrug-selected murine colon cancer and K1735 is a nondrug-selected murine melanoma cell line, both with endogenous expression of Abcb1, K1735-Taxol was drug-selected by stepwise increasing paclitaxel concentrations up to 0.5 μmol/L, MDCK-Abcb1 (murine Abcg2 transduced) and MDCK-parent (nontransduced) Madine–Darby Canine Kidney cells.
PTEN deficiency renders glioblastoma sensitive to TMZ and ABT-888 treatment in vitro and in vivo

Deletion or inactivation of PTEN is a frequent event in glioblastoma. By performing an in silico analysis on 117 glioblastoma patient samples, we found that patients whose tumor is deficient in PTEN have a significantly worse overall survival than patients whose tumors are proficient in PTEN, viz. 310 days vs. 620 days; $P = 5.16 \times 10^{-6}$ (Fig. 4A). However, it was recently shown by in vitro experiments that PTEN-null astrocytes have a compromised homologous recombination DNA repair pathway, rendering these more sensitive to treatment with TMZ and the PARP inhibitor ABT-888 because of synthetic lethality (27). We have now used our glioblastoma models to interrogate the role of PTEN in the in vivo response to TMZ and ABT-888 treatment. Glioblastoma cell lines from spontaneous tumors including genotypes with additional Pten deletion demonstrate similar PARP inhibition by ABT-888 (Supplementary Fig. S2).
However, PTEN;P16\textsubscript{ink4a}/P19\textsubscript{Arf};KRas\textsubscript{v12};LucR glioblastoma cells were much more sensitive to TMZ+ABT-888 than P53;P16\textsubscript{ink4a}/P19\textsubscript{Arf};KRas\textsubscript{v12};LucR cells (Fig. 4B). Similarly, additional deletion of Pten sensitized P53-deficient cells to ABT-888, albeit not to the level as of P53 proficient cells.

We treated intracranial PTEN;p16\textsubscript{ink4a}/p19\textsubscript{Arf};KRas\textsubscript{v12};LucR GBM696677 glioblastoma established in wild-type and Abcb1a/b\textsuperscript{−/−};Abcg2\textsuperscript{−/−} mice with TMZ or TMZ+ABT-888 (Fig. 4C). The survival is short, because of the aggressive growth of these cells in vivo. In wild-type mice TMZ+ABT-888 treatment improved median survival relative to control group or TMZ-treated animals (10, 8, and 7 days for TMZ+ABT-888, TMZ, and controls, respectively; TMZ+ABT-888 vs. TMZ; \( P = 0.044 \); TMZ+ABT-888 vs. control; \( P = 0.0004 \)).

Note that such a therapeutic benefit of ABT-888 was not found in wild-type mice bearing P53;P16\textsubscript{ink4a}/P19\textsubscript{Arf};KRas\textsubscript{v12};LucR glioblastoma (Fig. 1A). Importantly, Abcb1a/b\textsuperscript{−/−}; Abcg2\textsuperscript{−/−} mice benefitted even more from treatment (median survival: 12 days vs. 9 days for KO mice receiving TMZ+ABT-888 vs. TMZ, respectively. \( P = 0.0006 \)). Subsequently, we evaluated the effect of PTEN deficiency in our lentivirally induced spontaneous PTEN;p16\textsubscript{ink4a}/p19\textsubscript{Arf};KRas\textsubscript{v12};LucR glioblastoma model. Whereas the treatment effect in the P53; P16\textsubscript{ink4a}/P19\textsubscript{Arf};KRas\textsubscript{v12};LucR glioblastoma model generally becomes apparent not before some days after completion of the 5-day treatment, a marked effect in the spontaneous PTEN-deficient tumors was already observed between days 0 and 7 (Fig. 4D). Although there seems to be a trend toward a more accelerated growth later on, the median survival of mice receiving TMZ and ABT-888 combination treatment relative to TMZ alone increased significantly (19 days vs. 15 days; \( P = 0.0045 \)) whereas TMZ treatment alone did not affect median survival compared with vehicle control (15 days vs. 14 days; \( P = 0.15 \)).

**Discussion**

This study, using clinically relevant in vivo high-grade glioma mouse models, shows that the PARP inhibitor ABT-888 can improve the efficacy of TMZ chemotherapy against high-grade gliomas, but also identified two important factors that need to be considered when analyzing the clinical trials with ABT-888 in glioblastoma. First, ABCB1 and ABCG2 restrict both BBB penetration and tumor cell entry of ABT-888. Second, therapeutic benefit of ABT-888 added to TMZ in ABC-transporter proficient wild-type mice was only significant in PTEN-deficient tumors. Importantly, responses were observed at a dose level of ABT-888 yielding clinically relevant systemic exposure. Based on these results, we propose that the PTEN status of the tumor should be taken into account during the analysis of the clinical trials. Moreover, concomitant inhibition of ABCB1 and ABCG2 by elacridar may further improve the efficacy of ABT-888+TMZ combination treatment, so that PTEN proficient tumors may also become responsive and PTEN-deficient tumors are sensitized even further.

P53, PTEN, and P16\textsubscript{ink4a}/P19\textsubscript{Arf} are among the most frequently mutated or deleted genes in glioblastoma (22). We have previously developed high-grade glioma models using loxp\textsuperscript{+} conditional transgenic mice that are deleted in brain cells following intracranial injection of Lenti-Cre virus. These spontaneous tumors, as well as tumors from neurosphere cultured cell lines derived of these and re-infected into recipient mice, resemble many features that are characteristic for human glioblastoma (21). In this study, we have used these models to evaluate the efficacy of ABT-888 to potentiate the activity of TMZ against high-grade glioma. Notably, the first study using wild-type mice injected with P53;Ink4a/Arf;KRas\textsuperscript{v12} GBM652457 cells demonstrated only a very minor response to this combination therapy. We demonstrate that ABT-888 is a good substrate of the drug efflux transporters Abcb1 and Abcg2 and observed a more favorable response against GBM652457 cells injected into Abcb1a/b\textsuperscript{−/−}; Abcg2\textsuperscript{−/−} mice. The expression of ABCB1 in the tumor vessels of gliomas in patients is well documented (28–30). Similarly, we were able to demonstrate the presence of ABCG2 in tumor vessels of patient specimens. Importantly, both in humans and in mice, ABCG2/Abcg2 was also found in tumor cells. Just recently, expression of ABCG2 in glioblastoma was also demonstrated by Bahita and colleagues (31), who reported nuclear localization of ABCG2 in a subpopulation of glioblastoma cells. A similar pattern was also seen in some of our patient specimens (Fig. 3C). Abcg2 staining was not uniform throughout the tumor. Considering that ABCG2 is a marker of early progenitor or stem cells, these Abcg2 positive regions may reflect the areas of the tumor enriched for tumor initiating cells. The presence of ABCB1 in glioblastoma tumor cells is still debatable (32). However, we successfully used elacridar in combination with TMZ and ABT-888 to further increase the efficacy in tumors grown in Abcb1a/b\textsuperscript{−/−}; Abcg2\textsuperscript{−/−} recipient mice, which indicates that drug transporters in these cells form a secondary barrier, as schematically depicted in Fig. 5.
systemic exposure of ABT-888. Recent clinical trials report a peak plasma level of 500 ng/mL with an AUC of 3,500 ng/mL/h (35). Previous preclinical studies have been using much higher doses of up to 50 mg/kg. Further increasing the dose in patients to improve efficacy, may result in unacceptable toxicity. Interestingly, co-administration of elacridar hardly affected the systemic exposure of ABT-888 (Fig. 2), but enhanced the brain concentration of ABT-888 by 11-fold at 4 hours. Consequently, this combination may enable improved local drug delivery to the brain, without increasing systemic exposure and possibly toxicity of ABT-888.

When exploring the efficacy of ABT-888 in our panel of spontaneous high-grade glioma derived cell lines, we also found that cell lines that are deficient in PTEN were much more sensitive to TMZ and ABT-888 treatment. Our results are in line with the in vitro data showing that PTEN-null astrocytes have a disturbance in the homologous recombination DNA repair pathway causing synthetic lethality when base excision repair activity is inhibited by a PARP inhibitor (27). Next, we took advantage of the fact that we could further explore the clinical relevance of this finding using our in vivo high-grade glioma models. Both wild-type and Abcb1a/1b−/−;Abcg2−/− mice bearing tumors from Pten;Ink4a/Arf;K-Rasv12 GBM696677 responded better to the TMZ and ABT-888 treatment than the mice bearing P53-null; PTEN proficient tumors. Notably, however, although the PTEN-deficient GBM696677 cell line is more sensitive to TMZ and ABT-888 treatment, the effect of this combination on the tumor growth in vivo was not as dramatic as might have been hoped for based on the results obtained in vitro. This finding on one hand reminds us that the tumor micro-environment of glioblastoma cells proliferating in vivo can help these cells to escape from lethal drug effects. On the other hand, it highlights the importance of using the appropriate preclinical models to evaluate drug efficacy.

We expect that these preclinical findings will be useful to interpret the outcome of the currently ongoing clinical trials with ABT-888 in patients with glioma (ClinicalTrials.gov identifiers: NCT01026493, NCT01514201, and...
NCIT00770471). In particular, our data call for a subgroup analysis in patients with glioblastoma with PTEN deletion. PTEN loss is a frequent event in glioblastoma (36% of cases). Notably, as assessed by an in silico analysis performed on 117 patients with glioblastoma, PTEN loss is associated with a more dismal prognosis but our study provides reasonable evidence that this subgroup of patients may benefit most from ABT-888. This benefit, however, may get lost when analyzing all glioblastomas as one group. Furthermore, we have shown that the efficacy of ABT-888 and TMZ combination therapy is attenuated by ABCB1 and ABCG2, supporting the initiation of clinical trials investigating the potential of ABC-transporter inhibitors (e.g., elacridar) in glioma therapy.

**Disclosure of Potential Conflicts of Interest**

J.H. Beumer reports receiving a commercial research grant from Abbvie. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

Conception and design: F. Lin, M.C. de Gooijer, E.M. Roig, J.H. Beijnen, O. van Tellingen

Development of methodology: F. Lin, E.M. Roig, J.H. Beumer, O. van Tellingen

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Determinants of Glioblastoma Response to ABT-888


# Clinical Cancer Research

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