



Published in final edited form as:

*Pediatr Blood Cancer*. 2013 May ; 60(5): 783–790. doi:10.1002/pbc.24368.

## Initial Testing (Stage 1) of Temozolomide by the Pediatric Preclinical Testing Program

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### Abstract

**Background**—The DNA methylating agent temozolomide was developed primarily for treatment of glioblastoma. However, preclinical data have suggested a broader application for treatment of childhood cancer. Temozolomide was tested against the PPTP solid tumor and ALL models.

**Procedures**—Temozolomide was tested against the PPTP *in vitro* panel at concentrations ranging from 0.1 to 1,000  $\mu$ M and was tested against the PPTP *in vivo* panels at doses from 22 to 100 mg/kg administered orally daily for 5 days, repeated at day 21.

**Results**—*In vitro* temozolomide showed cytotoxicity with a median relative IC<sub>50</sub> (rIC<sub>50</sub>) value of 380  $\mu$ M against the PPTP cell lines (range 1 to > 1,000  $\mu$ M). The three lines with rIC<sub>50</sub> values lesser than 10  $\mu$ M had low MGMT expression compared to the remaining cell lines. *In vivo* temozolomide demonstrated significant toxicity at 100 mg/kg, but induced tumor regressions in 15 of 23 evaluable solid tumor models (13 maintained CR [MCR], 2 CR) and 5 of 8 ALL models (3 MCR, 2 CR). There was a steep dose response curve, with lower activity at 66 mg/kg temozolomide and with tumor regressions at 22 and 44 mg/kg restricted to models with low MGMT expression.

**Conclusions**—Temozolomide demonstrated high level antitumor activity against both solid tumor and leukemia models, but also elicited significant toxicity at the highest dose level.

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Conflict of interest: Nothing to declare.

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Lowering the dose of TMZ to more closely match clinical exposures markedly reduced the antitumor activity for many xenograft lines with responsiveness at lower doses closely related to low MGMT expression.

### Keywords

developmental therapeutics; preclinical testing; temodar

## INTRODUCTION

Temozolomide (TMZ) is an oral DNA methylating agent with proven antitumor activity in both preclinical and clinical studies. Originally synthesized in 1987 as a treatment for melanoma, TMZ is an imidazotetrazine derivative of the alkylating agent dacarbazine [1–3]. Unlike other chemotherapeutic agents in this class that are metabolized in the liver, TMZ undergoes spontaneous breakdown to the short-lived monomethyl triazeno imidazole carboximide (MITC) at all sites. TMZ readily crosses the blood–brain barrier (BBB), and it is currently approved for the treatment of newly diagnosed glioblastoma multiforme (GBM) and is also approved for the treatment of refractory anaplastic astrocytoma.

The main cytotoxic action of TMZ is through the methylation of DNA at the O6 position of guanine. The active metabolite of TMZ is MITC that decomposes to the reactive methyl diazonium cation that methylates DNA at the O6 position of guanine as well as at the N7 and N3 positions of guanine and adenine, respectively [4]. The cellular toxicity of temozolomide-induced DNA damage at clinically relevant concentrations is not immediate and requires at least two cycles of DNA replication. The cytotoxic effect of temozolomide results from the inability of O6-methyl guanine (O6-meG) to correctly pair with cytosine during DNA replication, resulting in a single nucleotide mismatch. Futile cycles of mismatch repair with removal and reinsertion of thymine opposite O6-meG eventually result in an apoptosis-inducing double strand DNA break at the next cycle of DNA replication [5–7]. Documented resistance mechanisms to TMZ include expression of O6-methylguanine-DNA methyltransferase (MGMT) [8] and loss of DNA mismatch repair activity [5,9].

Preclinical studies have demonstrated the broad activity of TMZ against cancer xenografts [lung cancer [10], neoplastic meningitis [11], adenocarcinoma [12], pediatric solid tumors [13], and melanoma [14,15]]. *In vivo* studies have documented that the efficacy of TMZ is dependent on the schedule of delivery, with an improved response when the drug is given at equal doses for a period of five consecutive days [14,16]. The antitumor activity of TMZ has been demonstrated in orthotopic intracranial tumors, further demonstrating its ability to cross the BBB [17]. In addition, preclinical studies have also demonstrated that TMZ works synergistically with O6-benzylguanine [14] and can improve efficacy of other chemotherapeutics [18].

Clinical trials have investigated the efficacy of TMZ treatment against many types of cancer such as lymphomas [19,20]—including T-cell lymphomas [21] and low-grade non-Hodgkin's lymphoma [22]—leukemia [23–25], pancreatic cancer [26], renal cell carcinoma [27], nasopharyngeal carcinoma [28], sarcoma [29], breast cancer [30,31], melanoma [32–34], brain metastases in metastatic malignant melanoma [35], and brain tumors [36]. TMZ

was granted FDA approval in the treatment of recurrent anaplastic astrocytoma in 1999, with subsequent approval for the first-line therapy of glioblastoma multiforme [37].

TMZ was selected for systematic testing by the NCI-supported Pediatric Preclinical Testing Program's (PPTP) because of its substantial preclinical and clinical activity against a wide range of cancer types. Its evaluation also provides an opportunity to compare clinical activity for temozolomide to activity observed against preclinical models. This report describes the testing of TMZ against the PPTP's *in vitro* panel and also describes testing against its *in vivo* panel at a range of dose levels.

## MATERIALS AND METHODS

***In vitro* testing**—Testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates [38]. Cells were incubated in the presence of drug for 96 hours at concentrations from 0.1 to 1,000  $\mu\text{M}$  with replicates of 6–12 for each concentration evaluated. Mean fluorescence values were determined for each concentration tested and then normalized to the mean control fluorescence for the line to determine relative mean fluorescence values. For analysis of *in vitro* testing results, a non-linear regression, sigmoidal dose-response model was fitted using GraphPad Prism 5.03 to the relative mean fluorescence values vs. the log-transformed concentration (X) for the *in vitro* PPTP study data:

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\text{LogrIC}_{50} - X) \times \text{HillSlope}}}$$

The terms are defined as follows:  $r\text{IC}_{50}$  (relative  $\text{IC}_{50}$ ) is the concentration of agent that gives a response half way between Bottom and Top; HillSlope describes the steepness of the dose-response curve; and Top and Bottom are the plateaus in the T/C% values at low and high concentrations, respectively. The *F*-test was used to determine whether there is statistical evidence for a plateau at higher concentrations. The null hypothesis is that the simpler model (bottom T/C% = 0) is correct and the alternative is that not constraining the bottom T/C% value is correct. If the *F*-test *P*-value is <0.05, then the conclusion is that the more complicated model with the bottom unconstrained fits significantly better and that the agent therefore has a non-zero plateau effect at higher concentrations. Absolute  $\text{IC}_{50}$  values represent the concentration at which the agent reduces cell survival to 50% of the control value [39]. To compare activity between cell lines, the ratio of the median  $r\text{IC}_{50}$  to individual cell line's  $r\text{IC}_{50}$  value is used (larger values connote greater sensitivity).

***In vivo* tumor growth inhibition studies**—CB17SC *scid*<sup>-/-</sup> female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [40]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*<sup>-/-</sup> mice as described previously [41].

Female mice were used irrespective of the patient gender from which the original tumor was derived. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Ten mice (solid tumors) or eight mice (leukemias) were used in each control or treatment group. Tumor volumes ( $\text{cm}^3$ ) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [42] and responses were determined using three activity measures as previously described [42]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

### Analysis for MGMT Expression

Western blot analysis was as described previously [13]. Antibodies against MGMT and GAPDH (loading control) were from Cell Signaling (Danvers, MA). Immunoreactive bands were visualized by using Western Lightning Plus ECL (Perkin Elmer, Inc., Waltham, MA) and HyBlot CL film (Denville Scientific, Inc., Metuchen, NJ). Image Quant (GE Healthcare Life Sciences, Piscataway, NJ) was used to integrate immunoreactive bands for quantitation of MGMT:GAPDH.

### Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS<sup>®</sup>, was used to compare event-free survival distributions between treatment and control groups. *P*-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies. The Mann–Whitney test was used to test the difference of medians of  $\text{rIC}_{50}$  values between the groups of lines with similar tumor types to the remaining lines of the panel and to compare differences in MGMT expression levels between different histology- or response-defined groups.

### Drugs and Formulation

TMZ was provided to the PPTP by the Drug Repository at NIH, through the Cancer Therapy Evaluation Program (NCI). TMZ was dissolved in sterile water, and was administered at 100 mg/kg P.O. daily for 5 days with the cycle repeated at day 21. For repeat testing TMZ was administered at 66, 44, or 22 mg/kg on the same schedule. Drug was provided to each consortium investigator in coded vials for blinded testing.

## RESULTS

### Temozolomide *In Vitro* Testing

TMZ was evaluated against the 23 cell lines in the PPTP *in vitro* panel using 96-hour exposures to concentrations ranging from 0.1–1,000  $\mu\text{M}$ . The median  $\text{rIC}_{50}$  for the *in vitro* panel was 380  $\mu\text{M}$  (Table I). There was an approximately 150-fold range in  $\text{rIC}_{50}$  values, with the most sensitive cell lines being the rhabdomyosarcoma cell line Rh30, the neuroblastoma cell line NB-1643, and the glioblastoma cell line GBM2, which all had  $\text{rIC}_{50}$  values < 10  $\mu\text{M}$  (Fig. 1A). The least sensitive cell line (Rh18) had a  $\text{rIC}_{50}$  of >1,000  $\mu\text{M}$ . Concentration response curves for the three sensitive cell lines and a typical non-sensitive cell line (CHLA-266,  $\text{rIC}_{50}$  of 405  $\mu\text{M}$ ) are shown in Figure 1B. Each of the sensitive cell

lines shows a non-zero plateau at concentrations up to 300  $\mu\text{M}$ , consistent with prior results showing that the primary response to temozolomide in the first 48–72 hours of exposure for MGMT negative lines is G2-M cell cycle arrest with apoptosis initiating between 72 and 120 hours [43].

### Temozolomide *In Vivo* Testing

TMZ was initially evaluated at 100 mg/kg daily  $\times 5$  q 21 days in 41 xenograft models. Eighty-five of 77 mice died during the study (10.9%), with 4 of 388 in the control arms (1.0%) and 81 of 389 in the TMZ treatment arms (20.8%). Toxicity was greatest in the panels of soft tissue sarcoma and kidney tumors, and toxicity was distributed evenly between cycle 1 and cycle 2 of treatment. Ten solid tumor xenografts were inevaluable because of toxicity while no toxicity was observed in the ALL models. A complete summary of results is provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Antitumor effects were evaluated using the PPTP activity measures for time to event (EFS T/C), tumor growth delay (tumor volume T/C), and objective response. TMZ induced significant growth delay in 21 of 23 solid tumor lines (91.3%) at the 100 mg/kg dose, and demonstrated high or intermediate activity in most tumors, Table II. The *in vivo* testing results for the objective response measure of activity are presented in Figure 2 in a “heat-map” format as well as a “COMPARE”-like format, based on the scoring criteria described in the Material and Methods and the Supplemental Response Definitions Section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of five (stable disease). Objective responses were seen in 15 of 23 solid tumor models (maintained complete regressions (MCR) in 13 lines and CR in 2 lines) with examples of typical solid tumor response shown in Supplemental Figure 1. Complete responses were seen in five of eight ALL models with three models maintaining their response throughout the observation period.

Because of the excessive toxicity in some tumor panels when TMZ was administered at 100 mg/kg, TMZ was re-evaluated against eight tumor lines for which there was excessive toxicity, at a dose of 66 mg/kg on the same schedule of administration. Among these eight models, the toxicity rate for treated animals was reduced to 8.0%, with two lines excluded because of excessive toxicity. TMZ induced significant growth delay in four of six evaluable models, and induced MCR in two lines (Table II and Supplemental Table II). Tumors in surviving mice from the excluded groups were also MCR (Supplemental Table II). However, these data suggested a steep dose-response relationship, as several tumors (BT-29, SKNEP1, Rh30R, Rh41) exhibiting MCR at 100 mg/kg exhibited progressive disease at the 66 mg/kg dose level. To further define the dose response relationship, we examined responses of five models to TMZ at 66, 44, and 22 mg/kg using the same schedule of administration (Table III and Supplemental Table III). The Wilms tumor, KT-10 demonstrated CR, SD, and PD2 responses at 66, 44, and 22 mg/kg, respectively. For the ALL-3 model, reduction in temozolomide dose from 100–66 mg/kg reduced the response from CR to PD1. Similarly CHLA-258 did not respond at any dose level below 100 mg/kg.

The MGMT-deficient glioblastoma (GBM2) and rhabdomyosarcoma (Rh28) lines were highly responsive with MCR and CR, respectively, at the lowest dose tested. Dose-response tumor growth curves and relative tumor volume curves are shown in Figure 3.

### MGMT and Sensitivity to Temozolomide

MGMT expression at the RNA level was strongly correlated with expression at the protein level (Supplemental Figure 2A). The low values for MGMT expression at the RNA level correspond to undetectable MGMT expression at the protein level. For the *in vitro* panel, MGMT expression at the RNA level was significantly lower ( $P = .007$ ) for the three cell lines with  $rIC_{50}$  values  $<10 \mu\text{M}$  compared to the remaining PPTP cell lines (Supplemental Figure 2B). For the *in vivo* panel, MGMT RNA expression levels were significantly higher in the ALL xenografts compared to the solid tumors ( $P < 0.001$ ). For the solid tumor panel MGMT expression was comparable for xenografts achieving CR/MCR to 100 mg/kg temozolomide compared to those with PD1/PD2 responses to the same dose, Supplemental Figure 3. However, when testing was done at reduced doses among four xenografts with MCRs to 100 mg/kg, sensitivity to temozolomide as assessed by objective response was maintained down to 22 mg/kg in two xenografts with low MGMT expression (Rh28 and GBM2), but was lost at 44 mg/kg in two xenografts with high MGMT expression (KT-10 and CHLA258). For the ALL xenografts the efficacy at the 100 mg/kg dose stratified according to MGMT expression, with models attaining CR/MCR having lower MGMT expression than lines with PD1/PD2 response ( $P = 0.036$ ). However, the MGMT-expressing ALL-3 xenograft lost responsiveness to temozolomide at the more clinically relevant doses below 100 mg/kg.

## DISCUSSION

TMZ has been shown to be efficacious in preclinical models of various tumor xenografts, including malignant glioma [44]. The drug is particularly useful in adult patients with brain tumors due to its excellent penetration into the CNS and almost 100% bioavailability and linear pharmacokinetics after oral administration [45], and it is now a component of standard frontline therapy for adults with high grade glioma [37]. Phase I studies in adults and children have shown that the drug has an excellent safety profile with myelosuppression being the dose-limiting toxicity [46–48], however there are reports of therapy-related leukemia after temozolomide treatment (recently reviewed by Ogura et al. [49]).

TMZ showed limited activity in a phase 2 study against recurrent pediatric brain tumors, including objective responses for high-grade glioma (1 of 23), low grade glioma (1 of 21), and medulloblastoma (3 of 25) [50]. Another phase 2 study in children with recurrent high grade gliomas produced an objective response rate of 20% (4 of 20) [51]. However, temozolomide failed to improve outcome in children with high-grade astrocytomas when given in combination with radiation therapy and then used as maintenance therapy [52]. The same TMZ regimen was no more effective than previously reported regimens for the treatment of children with diffuse intrinsic pontine gliomas [53]. Temozolomide induced occasional objective responses in a phase 2 study that included children with non-CNS solid tumors [54], and it induced a 20% objective response rate (5 of 25) in a phase 2 study for

children with recurrent neuroblastoma [55]. The current focus of pediatric clinical research for TMZ is its use in combination with other cytotoxic agents with an emphasis on topoisomerase I inhibitors based on preclinical data for the irinotecan plus TMZ combination [56]. Phase 1 and 2 studies of the irinotecan plus TMZ combination have shown objective responses for both neuroblastoma [57–59] and for Ewing sarcoma [60,61].

A key question in interpreting TMZ preclinical results is the relative systemic exposures in mice at the tested TMZ doses compared to the exposures achievable in humans. Stevens et al. [16] administered TMZ orally to mice at a dose of 20 mg/kg, and the TMZ area-under-the-curve (as interpolated by Middlemas et al. [13]) was approximately 38  $\mu\text{g}/\text{ml}$  hour. Murine systemic exposure for TMZ and MTIC at a TMZ dose of 66 mg/kg administered orally and given with irinotecan were 47 and 1.3  $\mu\text{g}/\text{ml}$  hour, respectively [56]. These results were similar to previous results for TMZ administered to mice as a single agent at 66 mg/kg 40 and 1.9  $\mu\text{g}/\text{ml}$  hour for TMZ and MTIC, respectively [13]. In children with leukemia receiving TMZ at 200 mg/m<sup>2</sup>, the TMZ systemic exposure was 30  $\mu\text{g}/\text{ml}$  hour [25]. Phase 1 studies in children with solid tumors support a TMZ systemic exposure of approximately 40  $\mu\text{g}/\text{ml}$  hour at the 200 mg/m<sup>2</sup> dose [47,62,63]. In adults, a systemic exposure of ~30  $\mu\text{g}/\text{ml}$  hour was observed in a phase 1 study at the 200 mg/m<sup>2</sup> dose [64], while a second study observed systemic exposures in the 30–35  $\mu\text{g}/\text{ml}$  hour range at this dose [65], with similar results observed for TMZ given in combination with cisplatin [66]. The totality of these results are consistent with a clinically relevant dose of TMZ in mice being lower than 66 mg/kg.

TMZ produced CRs or MCRs across 15 of 23 evaluable solid tumor xenografts in the PPTP *in vivo* testing panel at the 100 mg/kg dose (Table II). Nonresponders represented four of six osteosarcoma lines and two of four Ewing sarcoma lines. Due to toxicity issues at the 100 mg/kg dose level, 8 xenograft lines that were highly responsive at 100 mg/kg (MCR) despite toxicity were again tested at 66 mg/kg. Of the six evaluable studies, two (33%) produced the same median group response (MCR) while the remaining four xenografts had progressive disease (PD) when treated at the lower dose, indicating a steep dose response relationship. To further define the relationship between dose and tumor response, four solid tumor models and one ALL model were treated with TMZ at doses between 66 and 22 mg/kg. Only the two xenografts known to be deficient in MGMT demonstrated regressions at the two lower doses tested (22 and 44 mg/kg).

The most sensitive cell lines *in vitro* (NB-1643, Rh30, and GBM2) all had rIC<sub>50</sub> values <10  $\mu\text{M}$ , while the median IC<sub>50</sub> value was 380  $\mu\text{M}$  and the least sensitive cell line (Rh18) had a rIC<sub>50</sub> > 1,000  $\mu\text{M}$ . The three hypersensitive cell lines had low MGMT expression, supporting MGMT expression status as a primary mechanism defining high level sensitivity to temozolomide. The *in vivo* results are similar, with high TMZ doses producing MCRs in most models tested, but with responsiveness at lower dose levels tracking with low MGMT expression. One of the alternative mechanisms of resistance to temozolomide, loss of mismatch repair, was not observed within the MGMT low expressing PPTP xenografts. The significantly higher levels MGMT expression in the ALL versus the solid tumor xenografts point to potential differences between regulation of MGMT expression between pediatric ALL and pediatric solid tumors.

In conclusion, TMZ showed high-level activity across the PPTP panel at high doses that produce systemic exposures not achievable in humans. There was a steep dose-response curve, and lowering the dose of TMZ to more closely match clinical exposures markedly reduced the antitumor activity for many xenograft lines with responsiveness closely related to low MGMT expression. These results suggest that targeting patients whose tumors have low MGMT expression may be an effective strategy for optimizing use of temozolomide in the pediatric setting. Additional work is needed to further address the potential for enhancing efficacy by combination with other agents.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

Grant sponsor: National Cancer Institute; Grant numbers: NO1-CM-42216, CA21765, CA108786.

This work was supported by NO1-CM-42216, CA21765, and CA108786 from the National Cancer Institute and used TMZ supplied by the NIH Drug Repository. In addition to the authors this paper represents work contributed by the following: Denise Alexander, Sherry Ansher, Catherine A. Billups, Ingrid Boehm, Joshua Courtright, Mila Dolotin, Kathryn Evans, Edward Favours, Danuta Gasinski, Henry S. Friedman, Debbie Payne-Turner, Doris A. Phelps, Jennifer Richmond, Chandra Tucker, Amy E. Watkins, Joe Zeidner, Ellen Zhang, and Jian Zhang. Children's Cancer Institute Australia is affiliated with the University of New South Wales and Sydney Children's Hospitals Network.

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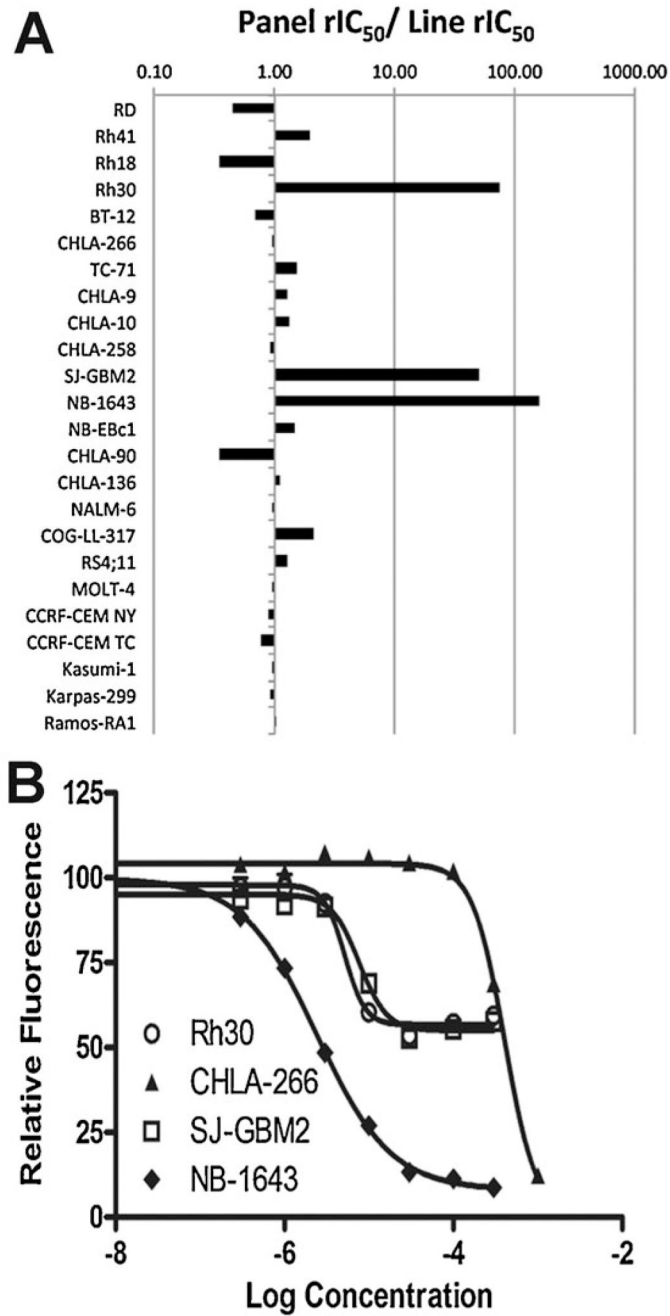
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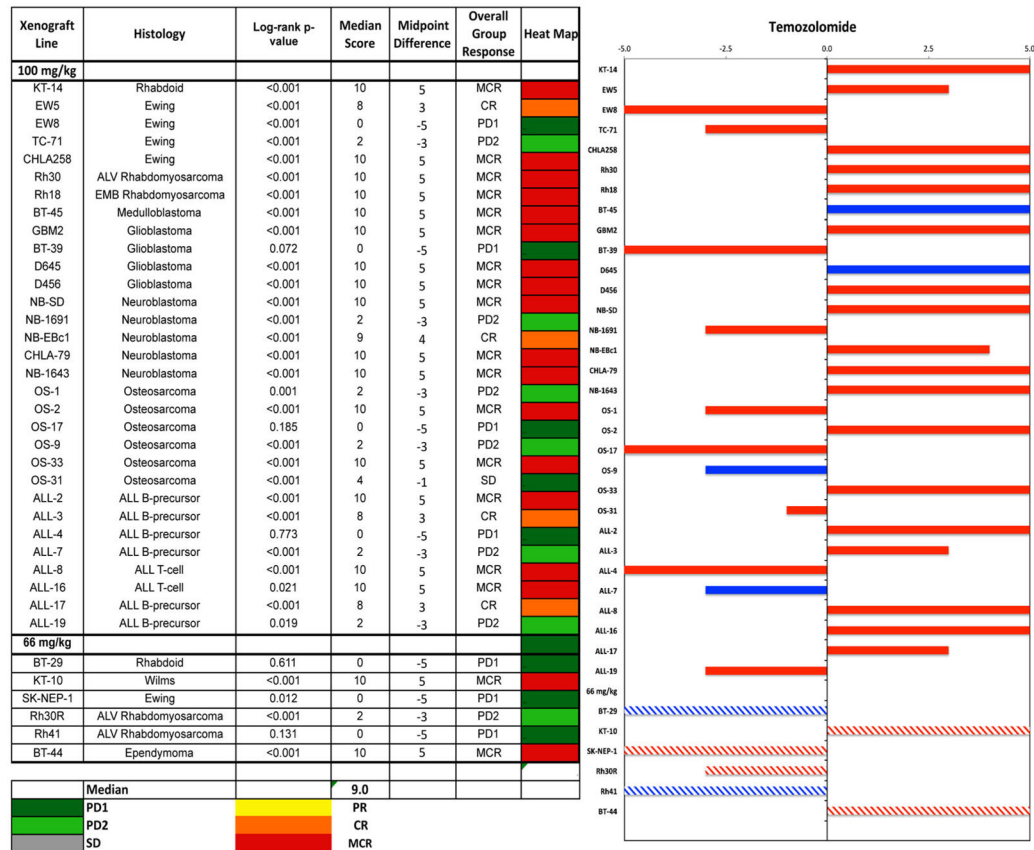
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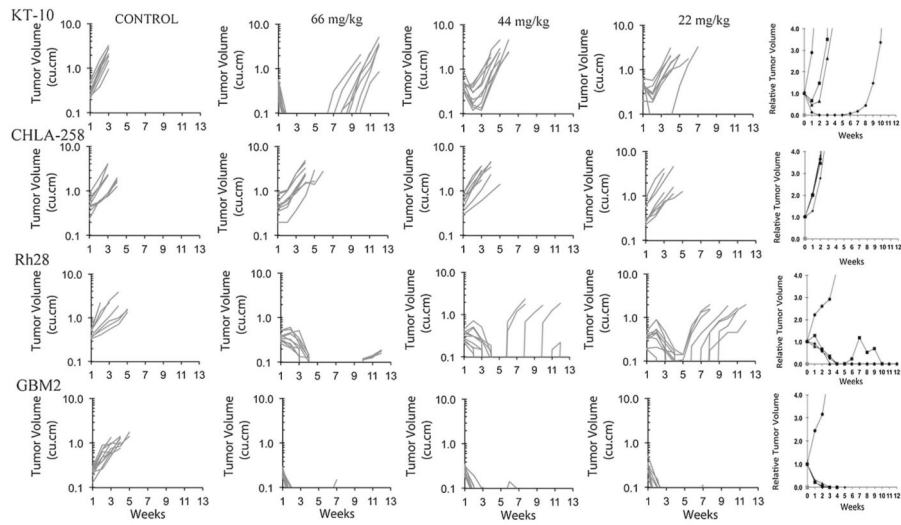
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**Fig. 1.**  
**A:** Temozolomide *in vitro* activity. The figure shows the ratio of the median rIC<sub>50</sub> of the entire panel to that of each cell line. Higher ratios are indicative of greater sensitivity to temozolomide and are shown in the figure by bars to the right of the midpoint line (ratio = 1.0). **B:** Concentration-response curves for sensitive cell lines and a typical non-sensitive cell line.



**Fig. 2.** Temozolomide *in vivo* objective response activity. **Left:** The colored heat map depicts group response scores. A high level of activity is indicated by a score of six or more, intermediate activity by a score of 2 but <6, and low activity by a score of <2. **Right:** Representation of tumor sensitivity based on the difference of individual tumor lines from the midpoint response (stable disease). Bars to the right of the median represent lines that are more sensitive, and to the left are tumor models that are less sensitive. Red bars indicate lines with a significant difference in EFS distribution between treatment and control groups, while blue bars indicate lines for which the EFS distributions were not significantly different. For results from re-testing at 66 mg/kg (hatched bars) significant and not significant are color coded as pink and light blue, respectively.



**Fig. 3.** Temozolomide dose-response activity against solid tumor xenografts. Individual tumor volume graphs are shown for KT-10, CHLA-258, Rh28, and GBM2 for control (untreated) and treated tumor groups. Relative tumor volume graphs are shown in the right panels: (③) Control; (⑤) 22 mg/kg; (⑩) 44 mg/kg; (①) 66 mg/kg.

TABLE I

*In Vitro* Sensitivity of Cell Lines to Temozolomide

Cell line	Histotype	rIC <sub>50</sub> (μM)	Panel rIC <sub>50</sub> /line rIC <sub>50</sub>	MGMT relative expression
RD	Rhabdomyosarcoma	858	0.44	1.15
Rh41	Rhabdomyosarcoma	191	1.99	0.79
Rh18	Rhabdomyosarcoma	>1,000	0.35	1.04
Rh30	Rhabdomyosarcoma	5	74.91	0.37
BT-12	Rhabdoid	558	0.68	1.00
CHLA-266	Rhabdoid	405	0.94	1.22
TC-71	Ewing sarcoma	245	1.55	0.82
CHLA-9	Ewing sarcoma	294	1.30	1.19
CHLA-10	Ewing sarcoma	290	1.31	0.67
CHLA-258	Ewing sarcoma	406	0.94	1.76
SJ-GBM2	Glioblastoma	8	49.71	0.34
NB-1643	Neuroblastoma	2	157.7	0.44
NB-EBc1	Neuroblastoma	256	1.49	0.80
CHLA-90	Neuroblastoma	1,098	0.35	1.06
CHLA-136	Neuroblastoma	345	1.10	0.92
NALM-6	ALL	392	0.97	1.37
COG-LL-317	ALL	181	2.11	1.39
RS4;11	ALL	300	1.27	1.92
MOLT-4	ALL	399	0.95	1.12
CCRF-CEM (1)	ALL	424	0.90	1.10
CCRF-CEM (2)	ALL	502	0.76	1.10
Kasumi-1	AML	389	0.98	1.86
Karpas-299	ALCL	407	0.93	0.79
Ramos-RA1	NHL	372	1.02	1.37
Median		380	1.00	1.08
Minimum		2	0.35	0.34
Maximum		>1,000	150.20	1.92

TABLE II

*In Vivo* Sensitivity of PPTP Xenografts to Temozolomide (100 and 66 mg/kg)

Line description	Tumor type	Median time to event	P-value	EFS T/C	Median RTV at end of study	Tumor volume T/C <sup>a</sup>	Median group response	EFS activity <sup>b</sup>	Relative MGMT expression
KT-14	Rhabdoid	>EP	<0.001	>4.2	0.0	0.12	MCR	High	0.99
EW5	Ewing	>EP	<0.001	>3.9	2.3	0.04	CR	Int	1.21
EW8	Ewing	12.7	<0.001	1.5	>4	0.37	PD1	Low	1.33
TC-71	Ewing	25.6	<0.001	2.5	>4	0.30	PD2	Int	1.02
CHLA258	Ewing	>EP	<0.001	>3.8	0.0	0.01	MCR	High	1.64
Rh30	ALV RMS	>EP	<0.001	>3.3	0.0	0.00	MCR	Hfgh	0.99
Rh18	EMB RMS	>EP	<0.001	>3.5	0.0	0.11	MCR	High	1.12
BT-45	Medulloblastoma	>EP	<0.001	>2.7	0.0	0.24	MCR	High	1.20
GBM2	Glioblastoma	>EP	<0.001	>3.4	0.0	0.11	MCR	Hfgh	0.37
BT-39	Glioblastoma	11.0	0.072	1.1	>4	0.83	PD1	Low	0.77
D645	Glioblastoma	>EP	<0.001	>4.7	0.0	0.21	MCR	High	0.37
D456	Glioblastoma	>EP	<0.001	>5.8	0.0	0.12	MCR	Hfgh	0.37
NB-SD	Neuroblastoma	>EP	<0.001	>3.5	0.0	0.06	MCR	High	0.74
NB-1691	Neuroblastoma	41.0	<0.001	6.5	>4	0.42	PD2	Int	0.98
NB-EBc1	Neuroblastoma	>EP	<0.001	>2.4	0.4	0.52	CR	Hfgh	1.07
CHLA-79	Neuroblastoma	>EP	<0.001	>4.2	0.2	0.08	MCR	High	0.38
NB-1643	Neuroblastoma	>EP	<0.001	>5.7	0.1	0.11	MCR	High	0.45
OS-1	Osteosarcoma	>EP	0.001	>1.4	3.4	0.65	PD2	NE	0.83
OS-2	Osteosarcoma	>EP	<0.001	>2.0	0.2	0.46	MCR	High	0.52
OS-17	Osteosarcoma	16.1	0.185	1.2	>4	0.87	PD1	Low	0.93
OS-9	Osteosarcoma	41.4	<0.001	1.6	>4	0.80	PD2	Low	0.84
OS-33	Osteosarcoma	>EP	<0.001	>3.8	0.1	0.33	MCR	High	0.35
OS-31	Osteosarcoma	>EP	<0.001	>2.5	1.0	0.61	SD	Int	0.68
ALL-2	ALL B-precursor	>EP	<0.001	>2.5	0.1		MCR	Hfgh	1.66
ALL-3	ALL B-precursor	>EP	<0.001	>4.4	1.7		CR	Int	1.64
ALL-4	ALL B-precursor	5.8	0.773	1.2	>25		PD1	Low	2.30



Line description	Tumor type	Median time to event	P-value	EFS T/C	Median RTV at end of study	Tumor volume T/C <sup>a</sup>	Median group response	EFS activity <sup>b</sup>	Relative MGMT expression
ALL-7	ALL B-precursor	12.6	<0.001	1.9	>25		PD2	Low	2.04
ALL-8	ALL T-cell	>EP	<0.001	>4.6	0.3		MCR	High	1.29
ALL-16	ALL T-cell	>EP	0.021	>2.7	0.2		MCR	High	1.42
ALL-17	ALL B-precursor	38.5	<0.001	4.9	>25		CR	Int	1.69
ALL-19	ALL B-precursor	9.6	0.019	3.2	>25		PD2	Int	3.27
66 mg/kg									
BT-29	Rhabdoid	14.0	0.611	1.0	>4	0.83	PD1	Low	0.98
KT-10	Wilms	>EP	<0.001	>3.9	0.0	0.01	MCR	High	1.74
SK-NEP-1	Ewing	18.3	0.012	1.4	>4	0.70	PD1	Low	1.08
Rh30R	ALV RMS	23.9	<0.001	2.1	>4	0.32	PD2	Int	0.86
Rh41	ALV RMS	12.5	0.131	1.4	>4	0.72	PD1	Low	0.92
BT-44	Ependymoma	>EP	<0.001	>1.7	0.0	0.44	MCR	NE	1.01

NE, not evaluable.

<sup>a</sup> *Tumor volume T/C value*: Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = T/C > 0.15; Intermediate activity = T/C > 0.45 but < 0.15; and Low activity = T/C > 0.45.

<sup>b</sup> Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = Progressive disease with EFS T/C > 1.5, and PD2 = Progressive disease with EFS T/C > 1.5.

TABLE III

*In Vivo* Dose Response of PPTP Xenografts to Temozolomide

Line description	Tumor type	Dose (mg/kg)	Median time to event	P-value	EFS T/C	Median RTV at end of study	Tumor volume T/C <sup>a</sup>	Median group response	EFS activity <sup>b</sup>
KT-10	Wilms	66	70.9	<0.001	7.6	>4	0.00	CR	Int
		44	25.5	<0.001	2.7	>4	0.13	SD	Int
		22	23.1	<0.001	2.5	>4	0.22	PD2	Int
CHLA258	Ewing	66	18.1	0.156	1.2	>4	0.72	PD1	Low
		44	14.6	0.871	0.9	>4	1.03	PD1	Low
		22	15.6	0.551	1.0	>4	1.00	PD1	Low
Rh28	ALV RMS	66	>EP	<0.001	>6.8	0.0	0.36	MCR	High
		44	>EP	<0.001	>6.8	1.2	0.36	CR	Int
		22	69.9	<0.001	5.6	>4	0.50	CR	Int
GBM2	Glioblastoma	66	>EP	0.008	>2.4	0.2	0.12	MCR	High
		44	>EP	0.034	>2.4	0.3	0.14	MCR	High
		22	>EP	0.046	>2.4	0.1	0.11	MCR	High

<sup>a</sup> *Tumor volume T/C value*: Relative tumor volumes (RTV) for control (C) and treatment (T) mice were calculated at day 21 or when all mice in the control and treated groups still had measurable tumor volumes (if less than 21 days). The T/C value is the mean RTV for the treatment group divided by the mean RTV for the control group. High activity = T/C > 0.15; Intermediate activity = T/C > 0.45 but >0.15; and Low activity = T/C > 45.

<sup>b</sup> Objective response measures are described in detail in the Supplemental Response Definitions. PD1 = Progressive disease with EFS T/C > 1.5, and PD2 = Progressive disease with EFS T/C > 1.5.