

#5356 PEDIATRIC PRECLINICAL TESTING PROGRAM (PPTP) EVALUATION OF THE DNA METHYLATING AGENT TEMOZOLOMIDE



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ABSTRACT

Background: Temozolomide is DNA methylating agent that has been approved in the United States for treatment of astrocytoma. This drug in many ways resembles more established compounds, such as dacarbazine and procarbazine, in that it gives rise to a methyl diazonium ion that attacks nucleophilic sites including the O⁶-guanine position in DNA. Temozolomide, however, differs from these drugs, which have to be activated by enzymatic oxidation, in that it degrades spontaneously via base-catalyzed hydrolysis to the final active methylating species.

Methods: The PPTP includes a molecularly characterized *in vitro* panel of cell lines (n=27) and *in vivo* panel of xenograft models representing most of the common types of childhood solid tumors and childhood acute lymphoblastic leukemia (ALL). Temozolomide (provided by the NCI Drug Repository) was tested *in vitro* at concentrations from 100 nM to 1 mM. Temozolomide was administered PO using a daily x 5 schedule repeated at 21 days at a dose of 100 mg/kg, or 66 mg/kg. Three measures of antitumor activity were used: 1) an objective response measure modeled after the clinical setting; 2) a treated to control (T/C) tumor volume measure; and 3) a time to event (4-fold increase in tumor volume) measure based on the median event-free survival (EFS) of treated and control animals for each xenograft. Biomarkers of temozolomide sensitivity, MGMT, MLH1, MSH2 and p53 genotype were determined.

Results: The median temozolomide IC₅₀ value for the PPTP cell lines was 390 μM (range 2 to > 1000 μM), with the neuroblastoma cell line NB-1643 having the lowest IC₅₀ value. There were no significant differences in IC₅₀ values between the rhabdomyosarcoma, neuroblastoma, Ewing sarcoma, and ALL cell lines. *In vivo* temozolomide induced significant toxicity at 100 mg/kg resulting in exclusion of 10/42 lines from analysis. For the 32 lines evaluable there were 13 maintained complete responses (MCR) and 2 CR in the solid tumor panels and 3 MCR and 2 CR in the ALL panel. Retesting the excluded lines at 66 mg/kg resulted in excessive toxicity leading to exclusion of 2/9 lines. At this dose there were 2 MCR and progressive disease in the remaining 5 lines. Overall 17/30 tumor models demonstrated objective responses (≥PR). Of these 7 are MGMT-negative, 3 are MLH1-negative, and 11 have wild type p53. All 7/7 MGMT-negative tumors responded, irrespective of p53 genotype (5/7 wild type).

Conclusions: *In vitro* temozolomide induced cytotoxicity with no apparent cell type specificity. *In vivo* temozolomide demonstrated high activity, but with a steep dose response curve, consistent with other DNA damaging agents. Responses poorly correlated with MGMT, MLH1, MSH2 levels, or p53 genotype. However, all MGMT-negative tumors were responsive, irrespective of MLH1 or p53 status. The results suggest that temozolomide may have potential for treatment of a range of pediatric malignancies. (Supported by NCI-1CM1002-02)

TEMOZOLOMIDE IN VITRO ACTIVITY

- Temozolomide demonstrated cytotoxic activity with the median relative IC₅₀ for all cell lines being 390 μM, well above clinically achievable drug levels (30-50 μM).
- GBM2, Rh30 and NB-1643 cell lines demonstrated high sensitivity and had the lowest MGMT expression levels among all of the PPTP cell lines.

PPTP IN VITRO & IN VIVO TESTING MODELS

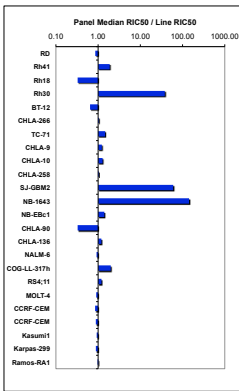
In vitro: *In vitro* testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable (using fluorescein diacetate [FDA]) cell numbers in tissue culture multiwell plates (Keshelava, et al. Methods Mol.Med., 110: 139-153, 2005). Testing was for 96 hours at concentrations from 1.0 nM to 10.0 μM with replicates of 6-12 per data point. Data were analyzed by fitting a non-linear regression model-sigmoidal dose-response model to the response-relative fluorescence values vs. the concentration.

In vivo: Standard PPTP methods for *in vivo* testing were employed (see <http://pptp.nci.nih.gov/documents/detailedAnalysisMethods.pdf>). Temozolomide 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.

Solid tumor testing: For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2-0.5 cm³. Two perpendicular tumor diameters were measured at either once or twice weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula (π/6)×d³, where d represents the mean diameter.

Acute lymphoblastic leukemia testing: For each xenograft line, 8 mice were inoculated with 3-5 x 10⁶ mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.

Cell Line	Histology	Relative IC ₅₀ (micromolar)
RD	RMS	420
RH41	RMS	191
Rh18	RMS	>1000
Rh30	RMS	9
BT-12	Rhabdoid	559
CHLA-266	Rhabdoid	358
TC-71	Ewing	245
CHLA-9	Ewing	294
CHLA-10	Ewing	283
CHLA-258	Ewing	363
SJ-GBM2	GBM	6
NB-1643	NB	3
NB-EB-1	NB	256
CHLA-90	NB	>1000
CHLA-136	ALL	302
NALM-6	ALL	392
COG-LL-317	ALL	181
RS4;11	ALL	300
MOLT-4	ALL	399
CCRF-CEM	ALL	424
CCRF-CEM	ALL	406
Kasumi-1	AML	389
Karpas-299	ALCL	407
Ramos-RA1	NHL	372
Median		360



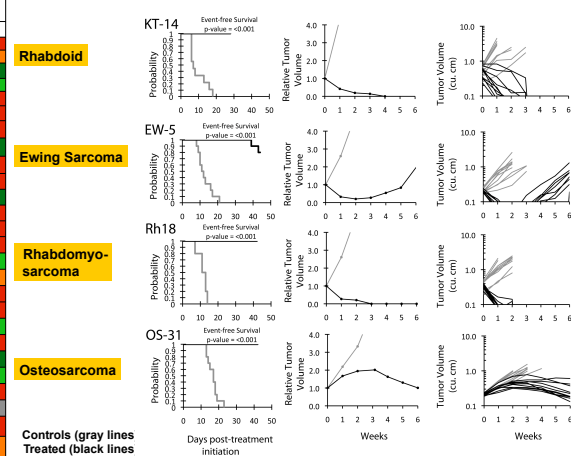
Temozolomide was provided for testing by NCI Drug Repository

TEMOZOLOMIDE IN VIVO ACTIVITY

Xenograft Line	Histology	P-value	EFS T/C	Median final RTV	Heat map
100mg/kg					
KT-14	Rhabdoid	<0.001	> 4.2	0	MCR
EW5	Ewing	<0.001	> 3.9	2.3	CR
EW8	Ewing	<0.001	1.5	>4	PD1
TC-71	Ewing	<0.001	2.5	>4	PD2
CHLA258	Ewing	<0.001	> 3.8	0	MCR
Rh30	ALV RMS	<0.001	> 3.5	0	MCR
Rh18	EMB RMS	<0.001	> 3.5	0	MCR
BT-28	Medulloblastoma	0.111	1.1	>4	PD1
BT-45	Medulloblastoma	<0.001	> 2.7	0	MCR
GBM2	Glioblastoma	<0.001	> 3.4	0	MCR
BT-39	Glioblastoma	0.072	1.1	>4	PD1
D645	Glioblastoma	<0.001	> 4.7	0	MCR
D456	Glioblastoma	<0.001	> 5.8	0	MCR
NB-SD	Neuroblastoma	<0.001	> 3.5	0	MCR
NB-1691	Neuroblastoma	<0.001	6.5	>4	PD2
NB-EB-1	Neuroblastoma	<0.001	> 2.4	0.4	CR
CHLA-79	Neuroblastoma	<0.001	> 4.2	0.2	MCR
NB-1643	Neuroblastoma	<0.001	> 5.7	0.1	MCR
OS-1	Osteosarcoma	0.001	> 1.4	3.4	PD2
OS-2	Osteosarcoma	<0.001	> 2.0	0.2	MCR
OS-17	Osteosarcoma	0.185	1.2	>4	PD1
OS-9	Osteosarcoma	<0.001	1.6	>4	PD2
OS-33	Osteosarcoma	<0.001	> 3.8	0.1	MCR
OS-31	Osteosarcoma	<0.001	> 2.5	1	SD
ALL-2	ALL B-precursor	<0.001	> 2.5	0.1	MCR
ALL-3	ALL B-precursor	<0.001	> 4.4	1.7	CR
ALL-4	ALL B-precursor	0.773	1.2	>25	PD1
ALL-7	ALL B-precursor	<0.001	1.9	>25	PD2
ALL-8	ALL T-cell	<0.001	> 4.6	0.3	MCR
ALL-16	ALL T-cell	0.021	> 2.7	0.2	MCR
ALL-17	ALL B-precursor	<0.001	4.9	>25	CR
ALL-19	ALL B-precursor	0.019	3.2	>25	PD2
66mg/kg					
BT-29	Rhabdoid	0.611	1.0	>4	PD1
KT-10	Wilms	<0.001	> 3.9	0	MCR
SK-NEP-1	Ewing	0.012	1.4	>4	PD1
RH-30R	ALV RMS	<0.001	2.1	>4	PD2
RH-41	ALV RMS	0.131	1.4	>4	PD1
BT-28	Medulloblastoma	0.111	1.1	>4	PD1
BT-44	Ependymoma	<0.001	> 1.7	0	MCR

- Red shading in the p-value columns indicates a significant difference in EFS distribution or Tumor Volume T/C between treated and control groups.
- Shading in the EFS columns indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (gray) activity.
- PD1 (Progressive Disease 1): >25% ↑ in tumor volume, TGD value ≤1.5; PD2 (Progressive Disease 2): >25% ↑ in tumor volume, TGD value >1.5; SD (Stable Disease): <25% ↑ in tumor volume, <50% regression

Testing was supported by NCI N01CM42216



IN VIVO RESULTS AND CONCLUSIONS

- Temozolomide (100 mg/kg daily x 5) caused regressions in 15/20 solid tumors and 5/8 evaluable ALL models, but with excessive toxicity.
- Retesting at 66 mg/kg (daily x 5) resulted in exclusion of 2/9 models due to toxicity. Only 2/7 evaluable models responded.
- Dosing temozolomide at 66 mg/kg may more closely approximate temozolomide plasma exposure in patients.
- Of 17/30 responding tumor models 7 are MGMT-negative, 3 MLH1-negative, and 11 have wild type p53.
- All 7/7 MGMT-negative tumor models responded irrespective of p53 genotype.
- At higher temozolomide concentrations that exceed those tolerated in humans, the relationship between temozolomide activity and MGMT expression is lost in both the *in vitro* and the *in vivo* settings.