Systemic Administration of GPI 15427, a Novel Poly(ADP-Ribose) Polymerase-1 Inhibitor, Increases the Antitumor Activity of Temozolomide against Intracranial Melanoma, Glioma, Lymphoma

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ABSTRACT

Purpose: Temozolomide (TMZ) is a DNA methylating agent that has shown promising antitumor activity in recent clinical trials against high grade gliomas, metastatic melanoma, and brain lymphoma. In this study, we tested whether systemic administration of GPI 15427, a novel poly(ADP-ribose) polymerase (PARP-1) inhibitor capable of crossing the blood-brain barrier, could enhance the efficacy of TMZ against metastatic melanoma, glioblastoma multiforme, and lymphoma growing in the brain.

Experimental Design: Murine B16 melanoma or L5178Y lymphoma cells were injected intracranially in syngeneic mice. An orthotopic xenograft of the human SJGBM2 L5178Y lymphoma cells were injected intracranially in syngeneic mice. An orthotopic xenograft of the human SJGBM2 lymphoma growing in the brain.

Results: In all models, systemic administration of GPI 15427 shortly before TMZ significantly increased life span of tumor-bearing mice with respect to untreated controls or to groups treated with either GPI 15427 or TMZ only. Moreover, GPI 15427 increased the antimitotic effect of TMZ.

Conclusions: These data indicate that systemic administration of the poly(ADP-ribose) polymerase-1 inhibitor GPI 15427 significantly enhances TMZ antitumor efficacy against solid or hematological neoplasias even when located at the central nervous system site.

INTRODUCTION

TMZ is a well-tolerated, oral anticancer drug with high CNS penetration. It has shown promising activity against a number of incurable forms of cancer such as metastatic melanoma (1–5), high-grade gliomas (6–17), and brain lymphoma (18–20). Moreover, TMZ has been reported to reduce the incidence of CNS relapses of malignant melanoma in the brain and is currently evaluated for the treatment of leptomeningeal metastases from leukemia and lymphoma (4, 5, 21, 22).

Despite the substantial improvement in health-related quality-of-life, when compared with standard regimens, TMZ treatment did not result in significant survival benefit in metastatic melanoma or recurrent glioblastoma multiforme (3, 9). Thus, clinical studies on the combination of TMZ with other chemotherapeutic agents or radiotherapy are under way (22–27).

As a methylating agent, TMZ produces a wide spectrum of methyl adducts mostly represented by N-methylpurines, which are promptly repaired by the BER. The cytotoxic activity of TMZ has been mainly attributed to methylation of the O6 position of guanine, although this lesion accounts for only a small percentage of total DNA adducts (28). During DNA synthesis, O6-methylguanine mispairs with thymine rather than cytosine. The non-Watson/Crick pairings activate the MR system that, in turn, excises thymine, only to reinsert this base again during repair DNA synthesis. The futile cycles of MR intervention lead to growth arrest and/or induction of apoptosis (29, 30).

The two main mechanisms of resistance to TMZ come from high levels of AGT, a DNA repair protein that selectively removes the methyl adduct from the O6 position of guanine, and functional defects of MR (28). Therefore, one strategy to increase the efficacy of TMZ relies on using an adjunct drug to suppress the resistance mechanisms to TMZ. In AGT-proficient
cells, depletion of AGT activity restores sensitivity to O6-alkylating agents (31) and O6-benzylguanine, a specific inhibitor of the enzyme, is currently evaluated in clinical trials (32). However, in cancers with MR defects, which are frequently detected in human malignancies, even AGT-deficient cells are tolerant to cytotoxicity induced by TMZ (29). At present, no therapeutic strategies are being investigated in clinical trials to overcome drug resistance because of MR deficiency.

A novel approach to abrogate resistance to TMZ relies on the pharmacological inhibition of PARP-1, a component of BER (33–39). When activated by strand breaks, PARP-1 binds to DNA and synthesizes homopolymers of ADP-ribose from NAD⁺ to modify itself and other nuclear acceptor proteins, e.g., histones (40). These negatively charged polymers cause the electrostatic repulsion of histones from DNA and favor the recruitment of the other BER components to complete the repair process.

The enhancement of TMZ cytotoxicity by PARP-1 inhibition is likely attributable to increased DNA damage consequent to disruption of the repair process of N-methylpurines after the initial removal of the methylated base by the N-methylpurine-DNA glycosylase. Noteworthy, TMZ + PARP-1 inhibitor combination is effective not only in AGT-proficient but also in MR-deficient tumors and against tumor cells with low proliferation rate (41, 42).

In this study, we have investigated whether systemic administration of GPI 15427 (Guilford Pharmaceuticals, Inc., Baltimore, MD), a novel PARP-1 inhibitor capable of crossing the blood-brain barrier, could enhance the antitumor efficacy of TMZ against melanoma, glioblastoma multiforme, and lymphoma growing in the brain. The results indicate that in all tumor models systemic administration of GPI 15427 + TMZ significantly increased survival of tumor-bearing mice with respect to treatment with TMZ as a single agent.

MATERIALS AND METHODS

Cell Lines. The murine melanoma cell line B16 of C57BL/6 (H-2b/H-2b) origin (American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 containing 10% FCS (Invitrogen, Milan, Italy), 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin (Flow Laboratories, McLean, VA), at 37°C in a 5% CO₂ humidified atmosphere.

The human glioblastoma multiforme cell line SJGBM2 was cultured in DMEM (Invitrogen) supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics. SJGBM2 cell line was a kind gift from Dr. Peter J. Houghton (St. Jude Children’s Research Hospital, Memphis, TN).

Drugs. TMZ was provided by Schering-Plough Research Institute (Kenilworth, NJ), and GPI 15427 was synthesized in Guilford Laboratories (Guilford Pharmaceuticals, Inc.; Ref. 43). Drug stock solutions were prepared by dissolving TMZ in DMSO and GPI 15427 in 70% PBS without potassium.

PARP-1 Activity Assay. PARP-1 activity was assessed using a commercially available kit (Trevigen, Gaithersburg, MD), based on the measure of radiolabeled NAD⁺ incorporation. The assay was performed using purified PARP-1 protein (Trevigen), untreated or in the presence of graded concentrations of GPI 15427. The enzymatic reaction was carried out by incubating purified PARP-1 with 2 µCi 32P-NAD⁺ (Amersham, Milan, Italy), 10 µM NAD⁺, 1× PARP-1 buffer, 10 µg of nuclease-treated salmon testes DNA, 10 µg of histones, according to the manufacturer’s instructions. The experiments were repeated three times, and the mean IC₅₀ ± SE was calculated.

In Vitro Studies. Cells were treated with GPI 15427 (0.1–25 µM) or with TMZ (1–250 µM). For the experiments to assess the enhancing effect of GPI 15427 on TMZ-induced tumor growth inhibition, the methylating agent was added to cell cultures 15 min after PARP-1 inhibitor. GPI 15427 was used at concentrations nontoxic and capable of abrogating PARP-1 activity. The final concentration of DMSO was always <0.1% (v/v) and did not contribute to toxicity (data not shown).

Cells were cultured for 3 days, and apoptosis or cell cycle analysis was evaluated daily by flow cytometry analysis of DNA content as described previously (34).

Long-term survival was analyzed by colony formation assay (38, 39, 42). Cell line chemosensitivity to TMZ, GPI 15427, or to the drug combination was evaluated in terms of IC₅₀, i.e., the concentration of the drug expressed in µM, capable of inhibiting colony-forming ability by 50%. The IC₅₀ was calculated on the regression line in which the number of colonies was plotted versus the drug concentrations.

In Vivo Studies. The i.c. transplantation procedure was performed as described previously (38). Cells (10⁶ in 0.03 ml of RPMI 1640) were injected i.c. through the center-middle area of the frontal bone to a 2-mm depth, using a 0.1-ml glass microsyringe and a 27-gauge disposable needle.

Murine melanoma B16 or lymphoma L5178Y cells (10⁴) were injected i.c. into male B6D2F1 (C57BL/6 × DBA/2) mice. Human glioblastoma multiforme SJGBM2 cells (10⁴) were injected i.c. into male athymic BALB/c mice (nu/nu genotype). Before tumor challenge, animals were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) in 0.9% NaCl solution (10 ml/kg/i.p.).

Histological evaluation of tumor growth in the brain was performed 1–5 days after tumor challenge to determine the timing of treatment. Brains were fixed in 10% phosphate-buffered formaldehyde, cut along the axial plane, and embedded in paraffin. Histological sections (5-µm thick) were stained with H&E and analyzed by light microscopy.

Drug toxicity was evaluated by treating intact mice (five/group) with the compounds under study, used as single agents or in combination. Control groups were treated with vehicles only. Body weight was measured three times weekly, and survivals were recorded for 3 weeks after the last treatment. Toxicity was assessed on the basis of apparent drug-related deaths and net body weight loss [i.e., (initial weight − lowest weight)/initial weight × 100%]. Death was considered drug related when it occurred within 7 days after the last treatment.

GPI 15427 was dissolved in 70 mM PBS without potassium and injected i.v. at different doses (40–200 mg/kg).

TMZ was dissolved in DMSO (40 mg/ml), diluted in saline (5 mg/ml), and administered i.p. at doses commonly used for in vivo preclinical studies (44–46). Experiments were performed using different doses and schedules of TMZ + GPI 15427 to determine the maximal-tolerated dose of the drug combination.
PARP Inhibitor and Temozolomide for CNS Tumors

GPI 15427 was administered 15 min before TMZ administration. Control mice were always injected with drug vehicles.

In tumor-bearing mice, treatment started on day 2 after challenge, when tumor infiltration in the surrounding brain tissue was histologically evident. Mice were treated daily with 40 mg/kg/i.v. GPI 15427 + 100 mg/kg/i.p. TMZ for 3 days and monitored for mortality for 90 days. MSTs were determined, and the percentage of increase in life span was calculated as: [(MST (days) of treated mice/MST (days) of control mice) − 1] × 100. Efficacy of treatments was evaluated by comparing survival curves between treated and control groups.

To assess the ability of different treatments to reduce tumor growth, histological examination of the brains was performed using additional animals that were not considered for the analysis of survival. Mice were sacrificed at different time points after tumor challenge, selected within the MST range of untreated tumor-bearing animals.

The efficacy of TMZ + GPI 15427 treatment was also evaluated on melanoma growing s.c. For this purpose, B16 cells (2.5 × 105) were inoculated s.c. in the flank of the animal. Tumors were measured with calipers and volume calculated according to the formula: [(width)2 × length]/2. Treatment started 6 days after challenge, when the volume of tumor nodules reached 100–150 mm3. Melanoma growth was monitored by measuring tumor nodules every 3 days for 3 weeks.

To evaluate the influence of the drugs under study on generation of artificial metastases, B16 cells (2.5 × 105) were injected into the tail vein of B6D2F1 mice. Animals were treated with the drugs under study using the 3-day schedule 24 h after tumor challenge (see above). Two weeks after tumor challenge, animals were sacrificed and lungs removed and fixed in Bouin’s solution to distinguish tumor nodules from tissue. The number of metastases was determined using a dissecting microscope.

All procedures involving mice and care were performed in compliance with national and international guidelines (European Economic Community Directive 86/109, OLJ318 and “NIH Guide for Care and Use of Laboratory Animals”).

Statistical Analysis. Survival curves were generated by Kaplan-Meier product-limit estimate, and statistical differences between the various groups (eight animals/group) were evaluated by log-rank analysis with Yates correction (software Primer of Biostatistics; McGraw-Hill, New York, NY). Statistical significance was determined at an α = 0.05 level. Differences were considered statistically significant when \( P < 0.05 \).

For statistical analysis of the growth of melanoma nodules (s.c.) or of metastasis number in lungs, the significance of the differences between experimental groups (eight animals/group) was evaluated by \( t \) test. \( P \) s are two-sided (software Microsoft Excel).

RESULTS

PARP-1 Inhibitor Enhances in Vitro Sensitivity of Melanoma, Lymphoma, and Glioblastoma Multiforme Cell Lines to TMZ

Initially, B16, SJGBM2, and L5178Y cells were exposed to 0.1–25 \( \mu M \) GPI 15427 as a single agent. Cell growth was analyzed by colony formation assay, and the results indicated

| Table 1 In vitro chemosensitivity to GPI 15427 or to TMZ + GPI 15427 of melanoma, lymphoma, and glioblastoma multiforme cells*
<table>
<thead>
<tr>
<th>Treatment</th>
<th>B16</th>
<th>SJGBM2</th>
<th>L5178Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI 15427 IC50 (( \mu M ))</td>
<td>123 ± 20</td>
<td>38 ± 2</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>TMZ IC50 (( \mu M ))</td>
<td>80 ± 10</td>
<td>45 ± 5</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>TMZ IC50 (( \mu M )) + 0.3 ( \mu M ) GPI 15427</td>
<td>60 ± 5</td>
<td>30 ± 5</td>
<td>60 ± 5</td>
</tr>
<tr>
<td>TMZ IC50 (( \mu M )) + 0.6 ( \mu M ) GPI 15427</td>
<td>45 ± 5</td>
<td>25 ± 5</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>TMZ IC50 (( \mu M )) + 1.2 ( \mu M ) GPI 15427</td>
<td>ND</td>
<td>22 ± 4</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

*Data represent the mean values of three independent experiments ± SE.

that GPI 15427 exhibited some intrinsic growth inhibition at higher concentration and that B16 melanoma was more susceptible to the antiproliferative effect induced by GPI 15427 with respect to SJGBM2 and L5178Y cell lines (Table 1).

For each cell line, GPI 15427 concentrations devoid of toxic effects (0.3–1.2 \( \mu M \)) were tested for their ability to enhance growth inhibition induced by TMZ. These concentrations were able to abrogate PARP-1 activity (>99% inhibition), with an IC50 of 31 nM for GPI 15427 on the activity of purified PARP-1 protein (SE ±0.5). In all tumor cell lines, the PARP-1 inhibitor increased growth inhibition induced by TMZ (Table 1). In the case of B16 cell line, the maximal enhancement of TMZ-induced growth inhibition was achieved at 0.6 \( \mu M \) concentration (8-fold). In B16 cells, GPI 15427 alone, at the concentration of 1.2 \( \mu M \), induced 18 ± 3% growth inhibition with respect to control and was not considered for additional combination studies with TMZ. For SJGBM2 and L5178Y cell lines, the maximal increase of TMZ growth inhibitory effect was observed at 1.2 \( \mu M \) GPI 15427 (~4-fold for SJGBM2 and ~10-fold for L5178Y, respectively). In SJGBM2 and L5178Y cell lines, 2.5 \( \mu M \) GPI 15427 showed intrinsic growth inhibitory effect, therefore, this concentration was not tested in association with TMZ.

In B16 and SJGBM2 cells, TMZ + GPI 15427 mainly provoked cytostasis without induction of apoptosis, whereas in L5178Y lymphoma cells, the drug combination induced also apoptosis (data not shown).

In Vivo Studies

Toxicity

Animals were treated with graded doses of GPI 15427 in combination with TMZ. Three administrations of GPI 15427 (40 mg/kg/day/i.v.) + TMZ (100 mg/kg/day/i.p.) were well tolerated with a maximal weight loss of 12%. All mice recovered the initial body weight 2 weeks after treatment. This schedule was used for the studies on antitumor activity.

Systemic Administration of PARP-1 Inhibitor + TMZ Enhances Survival of Mice-Bearing Malignancies at the CNS Site

Melanoma. The antitumor activity of the drug combination, (GPI 15427 40 mg/kg/day/i.v. + TMZ 100 mg/kg/day/i.p. for 3 days), was initially tested in B16 melanoma growing s.c. in B6D2F1 mice and compared with the effects induced by TMZ or GPI 15427, used as single agents. The results show that
GPI 15427 significantly enhanced \( P < 0.0001 \) the antitumor effect of TMZ, whereas treatment with GPI 15427 only did not affect tumor growth (Fig. 1).

It was then investigated whether systemic administration of GPI 15427 might increase the efficacy of TMZ also against B16 melanoma growing at the CNS site. The ability of GPI 15427 to penetrate into brain was assessed by measuring the concentrations of the compound achieved in target tissues after a single i.v. administration. As shown in Fig. 2, GPI 15427 consistently achieved brain to plasma concentration ratios greater than unity (2.3 \( \pm \) 0.7, mean \( \pm \) SD) during the 6 h after dosing. These results suggest that GPI 15427 crosses the blood-brain barrier effectively and can achieve high concentrations in the target organ.

Drug treatment (GPI 15427 40 mg/kg/day/i.v. + TMZ 100 mg/kg/day/i.p. for 3 days) started 2 days after tumor challenge when neoplastic infiltration of the brain tissue was evidenced in histological sections. The results, illustrated in Fig. 3, show that the increase in survival time achieved by GPI 15427 + TMZ combination was significantly higher \( P < 0.0001 \) than that provoked by TMZ as a single agent. No significant differences in survival times were observed between control and GPI 15427-treated groups (Fig. 3 and Table 2).

The increase in survival detected in the group treated with the drug combination was accompanied by a marked reduction of tumor growth. Histological studies revealed a pronounced and diffuse tumor infiltration in the brain of control or GPI 15427-treated mice and a limited but multifocal infiltration in TMZ-treated mice. Only few infiltrating melanoma cells were, instead, present in GPI 15427-treated groups (Fig. 3 and Table 2).

The PARP-1 inhibitor GPI 15427 also increased the anti-metastatic activity of TMZ against B16 melanoma. In fact, the number of pulmonary metastases observed after treatment with GPI 15427 + TMZ was significantly lower \( P = 0.004 \) than that detected in mice treated with TMZ used as single agent (Fig. 5). Treatment with GPI 15427 did not significantly reduce metastases with respect to control animals.

Lymphoma. Systemic administration of GPI 15427 + TMZ significantly increased survival of B6D2F1 mice-bearing L5178Y lymphoma growing in the brain. The increase in median survival time induced by the drug combination was significantly higher \( P < 0.0001 \) than that provoked by TMZ used as a single agent (Fig. 6 and Table 2).

Glioblastoma Multiforme. The efficacy of drug treatment was then investigated using an orthotopic model of a human glioblastoma multiforme xenograft in nude mice. The response of SJGBM2 to TMZ, used as a single agent or in combination with GPI 15427, is shown in Fig. 7 and Table 2. The results indicate that systemic administration of GPI 15427 + TMZ significantly prolonged \( P < 0.0001 \) survival of tumor-bearing mice with respect to controls or to animals treated with the single agents. It should be noted that in this tumor model, TMZ was completely ineffective.

Microscopic examination of control animals injected with SJGBM2 revealed multifocal brain involvement. Similar results were obtained in animals treated with GPI 15427 or TMZ only (data not shown). Treatment with GPI 15427 + TMZ resulted, instead, in a decreased number of sites of neoplastic infiltration. In the control group, all animals (seven of seven) presented tumor infiltration in at least two brain regions distant from the site of injection, whereas in the group treated with the drug combination, only two of seven mice showed this pattern (total brain regions infiltrated by tumor cells: control, 24; GPI 15427 + TMZ, 13; \( P = 0.0007 \)). Moreover, brains of control mice showed large tumor masses both at the site of injection and in the parenchyma surrounding the ventricles; in contrast, animals treated with GPI 15427 + TMZ showed minimal tumor infiltration of ventricles (Fig. 8).
DISCUSSION

Malignant melanoma, glioblastoma multiforme, and brain lymphoma are tumor types highly refractory to chemotherapy. The poor prognosis of malignant melanoma is largely attributable to metastases into CNS, which occur in two-thirds of patients with metastatic melanoma. No effective treatment for the advanced malignancy has been presently defined. Even after aggressive first-line therapies, glioblastoma multiforme and brain lymphoma invariably recur with a median survival of few months. In the present study, we demonstrate, for the first time, that systemic administration of GPI 15427, a potent PARP-1 inhibitor capable of crossing the blood-brain barrier, significantly increased the antitumor activity of the methylating agent TMZ against malignant melanoma, glioblastoma multiforme, or lymphoma growing at the CNS site. In fact, combined treatment with tolerable doses of TMZ and GPI 15427 significantly prolonged survival of tumor-bearing mice and reduced tumor infiltration of the brain with respect to TMZ used as a single agent.

Table 2 Influence of GPI 15427 on survival of animals treated with TMZ

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>MST (range)</th>
<th>ILS(^a) (versus CTR)</th>
<th>P(^b) (versus CTR)</th>
<th>ILS(^c) (versus TMZ)</th>
<th>P(^b) (versus TMZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16 melanoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>15 (13–17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPI 15427</td>
<td>15 (13–18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMZ</td>
<td>18 (13–20)</td>
<td>20%</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPI 15427+TMZ</td>
<td>23 (21 to &gt;90(^d))</td>
<td>53%</td>
<td>&lt;0.0001</td>
<td>27%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L5178Y lymphoma</td>
<td></td>
<td></td>
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<tr>
<td>CTR</td>
<td>16.5 (15–18)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>GPI 15427</td>
<td>17.5 (16–19)</td>
<td>6%</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMZ</td>
<td>21.5 (16–22)</td>
<td>30%</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPI 15427+TMZ</td>
<td>25.5 (24–27)</td>
<td>54%</td>
<td>&lt;0.0001</td>
<td>18%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SJGBM2 glioblastoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>20 (19–22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPI 15427</td>
<td>20 (19–22)</td>
<td>0.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMZ</td>
<td>20 (20–23)</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPI 15427+TMZ</td>
<td>26.5 (25–29)</td>
<td>32%</td>
<td>&lt;0.0001</td>
<td>32%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\) ILS of drug-treated mice was calculated comparing their MST with those of control animals injected with drug vehicles only (CTR).

\(^b\) P was calculated comparing survival curves of drug-treated groups versus CTR or comparing survival curves of mice treated with the drug combination versus mice treated with TMZ.

\(^c\) ILS of mice treated with GPI 15427 + TMZ was calculated comparing their MST with those of mice treated with TMZ only.

\(^d\) In the group treated with GPI 15427 and TMZ, one animal survived >90 days.
of the enzyme from damaged DNA, and completion of the repair process mediated by BER (40).

The PARP-1 inhibitor GPI 15427 acts as a potent inhibitor of the enzyme, being capable of inhibiting the activity of purified PARP-1 at nanomolar concentrations. In all tumor models, at concentrations devoid of growth inhibitory effects, GPI 15427 was capable of significantly increasing the antiproliferative activity of TMZ.

The enhancing effect exerted by GPI 15427 on TMZ antitumor activity is likely the consequence of increased DNA damage that eventually results in apoptosis and/or growth arrest. Indeed, we previously demonstrated that interruption of BER process induced by PARP-1 inhibitors after the removal of methylpurines generates strand breaks (55). Moreover, repeated treatments with TMZ and PARP-1 inhibitors down-regulate transcription and delay recovery of BER components in tumor cells (42). This might additionally contribute to sensitize cancer cells to TMZ + GPI 15427.

It should be noted that all tumor cell lines tested here are AGT deficient (38, 56, 57). Therefore, in all these cases, treatment with O6-benzylguanine would most likely be ineffective in potentiating the antitumor activity of TMZ. In comparison, in these tumor lines, combination of PARP-1 inhibitor with TMZ markedly lowered TMZ IC50 to levels below the concentrations of the methylating agent detected in the plasma or in the brain of treated patients. In fact, even if TMZ permeates the blood-brain barrier, the concentrations of the drug reached in the brain are ~30–40% those present in the plasma (28).

We previously demonstrated that the antitumor activity of the methylating agent TMZ against lymphoma cells can be enhanced at the CNS site by intracerebral injection of the PARP-1 inhibitor NU 1025 (38). However, the intranasal route could be an inadequate strategy against tumors located in the brain, especially considering that the growth pattern of these malignancies can often be diffuse and multifocal. When NU 1025 was delivered systemically, this compound did not improve the efficacy of TMZ. In contrast, the PARP-1 inhibitor used in this study easily penetrates the blood-brain barrier; in fact, after i.v. administration GPI 15427 reached at the tumor site concentrations capable of increasing the antitumor activity of TMZ not only against lymphoma but also against melanoma and glioblastoma multiforme.

In the melanoma and lymphoma models, TMZ was able to significantly increase median survival time with respect to untreated or GPI 15427-treated controls. Noteworthy, the increase in survival time observed in tumor-bearing animals treated with the drug combination was higher than that achieved with TMZ only.

The increase in survival time observed in the melanoma-bearing animals treated with the drug combination was associated with a marked reduction of brain infiltration with respect either to controls or to groups treated with GPI or TMZ only, as indicated by the results of histological studies.

The human glioblastoma multiforme SJGBM2, characterized by a defective expression of the MLH-1 component of MR system (57, 58), was the least responsive tumor to TMZ in vivo.
with respect to melanoma or lymphoma. In fact, treatment with TMZ did not prolong survival and affect tumor growth pattern in the brain of tumor-bearing mice. In this regard, it should be noted that PARP-1 inhibitors are capable of sensitizing MR-deficient cells, which are tolerant to \(O^6\)-methylguanine damage, to TMZ (37). Interestingly, histological examination of brain sections from mice treated with the drug combination revealed a reduction of the tumor mass and a decrease in the number of metastases.

Fig. 5  Effect of TMZ ± GPI 15427 on B16 pulmonary metastases in B6D2F1 mice. Animals were injected i.v. with B16 melanoma cells and treated with the 3-day schedule (eight mice/group). After 14 days, mice were sacrificed, and the number of artificial metastases recorded by counting tumor nodules on lung surface. A, representative photographs of lung metastases of B16 melanoma cells in untreated (CTR, control) or drug-treated mice (GPI 15427, TMZ, or GPI 15427 + TMZ). B, histograms represent the mean values of the number of metastases detected in the different treatment groups. Bars: ±SE. Statistical significant differences (\(P\)) between groups are indicated on top of columns (i.e., TMZ versus CTR or GPI 15427 + TMZ versus CTR or TMZ). GPI 15427 versus CTR, \(P = 0.37\).

Fig. 6  Systemic administration of PARP-1 inhibitor + TMZ enhances survival of mice bearing L5178Y lymphoma at the CNS site. Survival of tumor bearing mice (eight mice/group) after treatment with TMZ ± GPI 15427. A significant increase (\(P < 0.0001\)) in survival was observed when mice treated with GPI 15427 + TMZ were compared with controls or to animals treated with GPI 15427 or TMZ administered as single agents. TMZ significantly increased survival with respect to control mice (\(P = 0.03\)). GPI 15427 versus CTR, \(P = 0.13\).
sites of brain infiltration, which were mostly limited to the parenchyma surrounding the site of injection.

In poorly responsive tumors, the methylation agent has been shown to synergize with topoisomerase I inhibitors, and these drugs are currently evaluated in clinical trials in association with TZM for the treatment of high-grade gliomas (23, 57). Interestingly, PARP-1 inhibitors would be potentially useful as resistance modifiers also in combination with topoisomerase I inhibitors (54). Thus, additional studies are warranted to investigate the use of PARP-1 inhibitors for enhancing the antitumor activity of both classes of chemotherapeutic compounds against resistant glioblastoma multiforme.

Systemic administration of both PARP-1 inhibitor and TMZ could also limit metastatic spreading of the tumor. In fact, treatment with GPI 15427 + TMZ achieved a more pronounced reduction of lung metastases than single TMZ treatment. This can be particularly effective for the treatment of malignant melanoma, which can spread to almost any organ sites. Actually, besides the brain, the lungs represent one of the most frequent sites of metastatic penetration by melanoma.

Overall, this study demonstrates that PARP-1 is indeed a responsive target for pharmacological approaches aimed at increasing chemosensitivity of tumor cells to TMZ. This is the first study on a PARP-1 inhibitor that, upon systemic administration, reaches in the brain tissue concentrations capable of enhancing TMZ antitumor activity at the CNS site. This finding is of particular interest considering that the effective therapeutic options for patients with primary brain tumors or neoplastic spread of solid tumors to CNS are very limited.

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Systemic Administration of GPI 15427, a Novel Poly(ADP-Ribose) Polymerase-1 Inhibitor, Increases the Antitumor Activity of Temozolomide against Intracranial Melanoma, Glioma, Lymphoma

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