Activity of Temozolomide against Human Tumor Colony-Forming Units

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ABSTRACT

8-Carbamoyl-3-methylimidazo(5,1-d)-1,2,3,5-tetrazin-4(3H)-one (temozolomide) is a new imidazotetrazine compound with promising preclinical and clinical activity in nitrosourea-sensitive and -resistant models and manageable toxicity in Phase I and II clinical trials. In this study, we investigated the antiproliferative activity of temozolomide against a large variety of human tumors taken directly from patients in an in vitro soft agar tumor cloning system. Temozolomide was studied using a continuous exposure at final concentrations from 0.1 to 10.0 μM against a total of 222 tumor specimens, of which 101 (45%) were evaluable. A decrease in tumor colony formation was considered significant if survival of colonies treated with temozolomide was ≤50% of that in controls. In vitro responses were seen in 9 of 101 (9%; 95% confidence interval (CI), 3–14), 16 of 100 (16%; 95% CI, 8.5–23), and 35 of 101 (35%; 95% CI, 26–45) tumor specimens at concentrations of 0.1, 1.0, and 10.0 μM, respectively (P < 0.001). The level of O6-guanine-alkyltransferase was evaluable in 19 specimens before treatment but did not correlate with a response to temozolomide. At a concentration of 10 μM, a good cytotoxic activity was seen in breast (42%; 95% CI, 15–72), ovarian (36%; 95% CI, 11–69), and non-small cell lung cancers (27%; 95% CI, 6–61). Interestingly, activity was also seen in renal cell carcinomas (50%; 95% CI, 19–81), colon cancers (42%; 95% CI, 15–72), melanomas (33%; 95% CI, 13–59), and some other tumors, including sarcomas and both prostatic and pancreatic carcinomas that are usually considered very resistant to several conventional chemotherapy agents. Moreover, we observed that a subset of tumors that were not sensitive to dacarbazine, carmustine, cisplatin, doxorubicin, 5-fluorouracil, etoposide, and vinblastine were sensitive to temozolomide. These data indicate both that temozolomide is an active drug in vitro against a large variety of human tumors, including some tumors usually resistant to conventional chemotherapy, and that further clinical evaluation is warranted.

INTRODUCTION

Temozolomide1 (SCH52365, CCRG 81045, NSC 362856, and M&B 39831) is a new antineoplastic imidazotetrazinone (1, 2) that has shown promising antitumor activity against high-grade glioma (3–6), melanoma (7, 8), and mycosis fungoides (8, 9) with acceptable toxicity in Phase I clinical trials (10–13). Temozolomide has been shown to be a produg that exerts its antitumor effects through the formation of MTIC, the putative active chemical metabolite of dacarbazine (DTIC, deticine; Refs. 1, 2, 14, and 15). Several studies have shown that the cytotoxicity of imidazotetrazinone correlates mainly with alkylation by MTIC of the O6-position of guanine in DNA (16–22). Recent reports have also indicated that temozolomide may induce programmed cell death (23). Unlike dacarbazine, which needs an enzymatic oxidative N-demethylation, temozolomide forms MTIC through a nonenzymatic chemical decomposition (2, 14). Therefore, conversion of temozolomide into MTIC is not supposed to vary with metabolism in humans as opposed to the limited extent of demethylation observed with dacarbazine in previously published clinical trials (1, 2).

In vitro studies using several human cancer cell lines have shown that temozolomide exerts a significant antiproliferative activity at concentrations ranging from 2.7 to 257 μM (19, 24). Preclinical studies have demonstrated the schedule dependency of the cytotoxicity of temozolomide (25). Recent Phase I studies have shown that the pharmacokinetics of temozolomide are linear with doses and that good oral bioavailability will allow protracted oral treatment (10–12, 15, 26). The peak plasma level after oral administration and 1-h i.v. injection of 200 mg/m2 temozolomide was about 10 mg/l (about 50 μM). In this study, the half-life of temozolomide was 1.81 h, and residual total plasma concentrations ranging from 0.1 to 10 μM were maintained for about 12 h (12).

To define human cancers that warrant Phase II clinical trials, we selected 222 human tumor specimens from patients with breast, ovarian, and non-small cell lung cancers, and we compared the activity of temozolomide with that of dacarbazine (DTIC, deticine).

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3 The abbreviations used are: temozolomide, 8-carbamoyl-3-methylimidazo(5,1-d)-1,2,3,5-tetrazin-4(3H)-one; MTIC, 5-(3-methyl)-1-triazene-1-ylimidazole-4-carboxamide; MeG, methylguanine; dacarbazine, 5-(3,3-dimethyltriazen-1-yl)imidazole-4-carboxamide; carmustine, 1,3-bis(2-chloroethyl)-1-nitrosourea; cisplatin, cis-diamminodichloroplatinum; 5-FU, 5-fluorouracil; CI, confidence interval.
trials, we investigated the antiproliferative activity of temozolomide against a large variety of human tumor colony-forming units taken directly from patients using an in vitro soft agar cloning system in glass capillary tubes (27). On the basis of previous preclinical studies showing the schedule dependency of temozolomide and the relatively short half-life of this drug and considering its typical schedule of administration (daily for 5 days by mouth), we set up an assay to optimize the cytotoxic activity of this agent by using continuous exposure of 0.1, 1.0, and 10.0 μM temozolomide.

**MATERIALS AND METHODS**

**Drugs.** Temozolomide was provided by Schering-Plough Research Institute (Kenilworth, NJ). Dacarbazine, carmustine (BCNU), cisplatin, doxorubicin, 5-FU, etoposide, and vinblastine were obtained from individual companies.

**Collection of Tumor Cells.** After written informed consent was obtained according to institutional guidelines, tumor specimens (biopsies, bone marrow, pleural effusions, and ascites) were collected by sterile standard techniques as part of routine clinical procedures. Samples were collected and delivered within an average time of 4 h and were processed immediately upon arrival in the laboratory. Internal controls indicated that the viability of cells was maintained when samples were processed within 3 days for solid specimens and 5 days for fluid ones. Biopsies of solid tumors were stored in McCoy’s 5A medium (Life Technologies, Inc., Grand Island, NY) containing 10% newborn calf serum, 10 mM HEPES, 100 μg/ml penicillin, and 90 μg/ml streptomycin (all Life Technologies) for transport to the laboratory. Preservative-free heparin (10 units/ml; O’Neill, Johns, and Feldman, St. Louis, MO) was added immediately after collection of fluids to prevent coagulation. Solid specimens were minced and passed repeatedly through metal sieves with 40-μm mesh (EC Apparatus, St. Petersburg, FL) to obtain a single-cell suspension. When necessary, effusions were centrifuged at 150 × g for 5–7 min and passed through 25-gauge needles to obtain single-cell suspensions (28). All specimens were washed twice in McCoy’s 5A medium containing 5% horse serum (Sigma Chemical Co.), 10% heat-inactivated FCS (Hyclone, Logan, UT), 2 mM sodium pyruvate, 2 mM glutamine, 90 units/ml penicillin, 90 μg/ml streptomycin, and 35 μg/ml L-serine (all Life Technologies). The viability of cells (from 40 to 100%) was determined on a hemocytometer with trypan blue. Only viable cells determined the final concentration of plated cells.

**O6-Alkylguanine-DNA Alkyltransferase Assay.** Tissue samples were homogenized in a Ten Broeck cell disruptor (Fisher Scientific, Pittsburgh, PA) at 4°C in 4 volumes/cell extract buffer, which consisted of 1 mM EDTA, 70 mM HEPES, 5% glycerol, and 1 mM DTT (pH 7.8). Cell extracts were prepared by sonication three times for 5 s at 4°C to complete cell disruption using a microsonicator equipped with a 3/32-inch diameter probe (Kontes, Vineland, NJ) followed by centrifugation at 10,000 × g for 2 min to remove cellular debris. The DNA and protein content of each cell extract was determined prior to removal of cellular debris. The protein content was assayed by the method of Bradford using Bio-Rad standard I (Bio-Rad, Richmond, CA). The activity of O6-alkylguanine-DNA alkyltransferase (alkyltransferase) in cell extract was measured by the amount of [3H]methyl group removed from [3H]O6-MeG present in calf thymus DNA alkylated with [3H]N-methylnitrosourea (specific activity, 0.039 fmol of O6-MeG/μg DNA). To assay the alkyltransferase, a sample of cell extract containing 250 or 500 μg of protein was combined with 7.2 μg [3H]methyl DNA (containing 160 fmol of O6-MeG) in assay buffer consisting of 70 mM HEPES (pH 7.8), 0.1 mM EDTA, 50% glyceral, 1 mM DTT, and 25 μM spermidine in a total volume of 200 μl and incubated for 60 min at 37°C. The reaction was stopped with 40 μl of 50% trichloroacetic acid and incubated at 4°C for 30 min. The precipitate was collected by centrifugation at 10,000 × g for 2 min and washed with 400 μl of 95% ethanol. The pellet was hydrolyzed with 80 μl of 0.1 N HCl at 80°C for 60 min and then neutralized with 400 μl of 0.02 M Tris base (pH 10.6). Hydrolyzed purines present in the supernatant were separated by HPLC using a Varian 5000 liquid chromatograph equipped with an MCH-5 15 cm × 5 μm silica-bonded C18 column (Varian Associates, Sunnyvale, CA), by elution at 1.0 ml/min with a gradient of 100% 0.01 M KH2PO4 (pH 6.2) to 75% 0.01 M KH2PO4-25% methanol run over 25 min. N2-MeG was used as an internal standard. Alkyltransferase activity was expressed as fmol of O6-MeG removed/mg of protein. All assays were repeated two to five times/sample.

**In Vitro Exposure of Tumor Cells to Drugs.** Stock solutions of temozolomide and standard agents were prepared in sterile, enriched Connaught Medical Research Laboratories Medium 1066 (Irvine Scientific, Irvine, CA) and stored at −80°C in sufficient aliquots for individual assays. Tumor cells were exposed continuously for 14 days to temozolomide at final concentrations of 0.1, 1.0, and 10.0 μM in the same medium at 37°C. Those concentrations of temozolomide were selected because they corresponded to achievable plasma levels in humans. Temozolomide activity was compared to that of a large panel of cytotoxic drugs. The tumor cells were exposed to standard anticancer agents for 1 h at 37°C at the following concentrations (μg/ml): dacarbazine, 0.10; carmustine, 0.10; cisplatin, 0.20; doxorubicin, 0.04; 5-FU, 6.0; etoposide, 3.0; and vinblastine, 0.05. The single concentration of each drug corresponded to approximately 1/10 of the peak plasma concentration for each drug in humans (29). Those concentrations were predictive of clinical response in previously published studies (28). To ensure in vitro activation of dacarbazine, the drug was exposed to white light for 1 h.

**Culture of Cells in Capillary Tubes.** Tumor cells to be cloned were suspended in 0.3% agar in enriched Connaught Medical Research Laboratories Medium 1066 (Life Technologies) supplemented with 15% heat-inactivated horse serum, 2% fetal bovine serum, 0.3 mM ascorbic acid, 100 μg/ml penicillin, 100 μg/ml streptomycin, 4 mM glutamine, 2 μg/ml insulin, 3 μg/ml transferrin, 4.8 ng/ml hydrocortisone, 1.2% minimal essential medium, nonessential amino acids, 3.75 μg/ml catalase, and 0.022% sodium pyruvate (Sigma). The tumor cells were transferred to capillary tubes and adjusted to a final concentration of 5 × 10⁵ cells/ml in the presence of the appropriate temozolomide dilution or control medium. Prior to plating, asparagine (100 μg/ml) and HEPES buffer (10 mM; Research Organics, Inc., Cleveland, OH) were added to the cells. One hundred μl of the resultant mixture containing 20,000 cells were
placed into 100-μl capillary tubes (micropipette tips; Borosilicate glass; Fisher Scientific Co., Houston, TX) by capillary action. The ends were sealed with S/P Miniseal Clay (American Scientific, Grand Prairie, TX). A 1-mm gap was left between the clay and the agar to prevent contamination from the clay. Six capillary tubes were prepared for each data point. The six tubes were placed in a 12 × 75-mm test tube, which was placed in a microtube rack (Interex Corp., Natick, MA) at a 30-degree angle in a 7% CO2 incubator at 37°C. Colonies (>50 cells) developed usually by day 14 of culture. To determine the number of colonies in the tubes, the agar was extracted from the tube (breaking the ends and applying positive pressure on the end of the tube). The number of colonies on each slide was then counted under a microscope at X30 magnification. However, this study was not conducted to obtain blinded comparison of cytotoxic effects of drugs by observers.

Quality Control. To ensure the presence of an excellent single-cell suspension, a positive control consisting of the cell toxin orthosodium vanadate at a concentration of 200 μM was used. For an experiment to be considered acceptable, the orthosodium vanadate had to produce less than 30% survival of colony-forming units. When survival was ≥30%, then the single-cell suspension on day 0 was poor and the tumor sample test was considered nonevaluable. The use of a positive control has been shown to greatly increase the reproducibility of the human tumor cloning assay (27).

Data Analysis and Statistics. The results were expressed as the percentage of survival of tumor colony-forming units for a particular drug relative to its control. This quantity was calculated as the ratio of the mean number of colonies surviving in the six drug-treated capillary tubes: the mean number of colonies growing in the six control capillary tubes. Data were expressed as means ± SD. A significant inhibition of colony-forming unit formations was defined as colony formation ≤0.5 × control. Statistical comparisons were performed using the McNemar Test with two-sided Ps (GraphPad, San Diego, CA). P < 0.05 was considered to indicate statistical significance.

RESULTS

In Vitro Responses. As can be seen in Table 1, a total of 222 freshly explanted tumor specimens were exposed to temozolomide for 14 days at concentrations of 0.1, 1.0, and 10.0 μM. Of these specimens, 101 (45%) showed adequate growth in diluent controls. The major subgroups of tumors tested, followed by the number of evaluable specimens, were: melanomas, 18; breast carcinomas, 12; colon carcinomas, 12; ovarian carcinomas, 11; non-small cell lung carcinomas, 11; and renal cell carcinomas, 10. Details about the other types of tumors tested are summarized in Table 1.

Temozolomide showed potent antiproliferative effects against human tumors in the cloning assay. In vitro activity against human tumor colony-forming units was noted in 9 of 101 (9%; 95% CI, 3–14) evaluable specimens at 1 μM, 16 of 100 (16%; 95% CI, 8.5–23) evaluable specimens at 1.0 μM, and 35 of 101 (35%; 95% CI, 26–45) evaluable specimens at 10.0 μM (Table 1). There was a significant dose-response relationship with temozolomide (P < 0.001). Specifically, at a concentration of 10.0 μM, a tumor response rate was noted in renal cell carcinoma (50%; 95% CI, 19–81), breast cancer (42%; 95% CI,
Evaluation of Antiproliferative Activity. The overall analysis of tumor cell survival (Fig. 1) at a concentration of 10.0 μM temozolomide shows that the inhibition of cancer cell proliferation is 50 ± 4.8% in breast (n = 12), 48 ± 4.7% in ovarian (n = 11), 46.5 ± 6.2% in colon (n = 12), and 43.3 ± 2.9% in non-small cell lung (n = 11) cancers. Surprisingly, at this concentration of 10.0 μM, inhibition of cancer cell growth is also observed in tumors that usually show little sensitivity to conventional chemotherapy, with an inhibition of cell growth of 41.2 ± 4.5% in malignant melanoma (n = 18), 44.5 ± 9.6% in renal cell carcinoma (n = 10), 46.7 ± 16.5% in prostate cancer (n = 3), and 39.7 ± 2.3% in soft tissue sarcomas (n = 3).

O\(^6\)-Alkyltransferase Activity. Only 19 specimens contained a sufficient number of tumor cells to perform both the human tumor-cloning assay and the measurement of the O\(^6\)-alkyltransferase activity. O\(^6\)-alkyltransferase activity was detected in 18 (95%) samples. However, large differences in the biochemical transferase activity were observed (mean, 101.8; range, 0–502.4 fmol/mg protein) from one sample to another (Table 2). No significant correlation between initial levels of O\(^6\)-alkyltransferase and in vitro responses to temozolomide was observed.

Effects in Tumors Resistant to Conventional Chemotherapy. Subsequently, we tested the in vitro responses to temozolomide in 66 evaluable tumor specimens that concurrently did not show significant inhibition of colony-forming units (>0.5 × control) after exposure to several clinically relevant antitumor agents. At a concentration of 10.0 μM, temozolomide had activity in 4 of 12 (33%; 95% CI, 10–65) tumor specimens that were resistant to dacarbazine (2 melanomas, 1 colon cancer, and 1 ovarian cancer), 4 of 13 (31%; 95% CI, 9–61) specimens resistant to carmustine (3 melanomas and 1 breast cancer), 6 of 24 (25%; 95% CI, 10–47) specimens resistant to cisplatin (2 ovarian cancers, 2 lung cancers, and 2 melanomas); 4 of 14 (28%; 95% CI, 8–58) specimens resistant to doxorubicin (2 breast cancers, 1 lung cancer, and 1 ovarian cancer); 5 of 11 (45%; 95% CI, 17–77) specimens resistant to 5-FU (2 colon cancers, 2 breast cancers, and 1 melanoma); 4 of 11 (36%; 95% CI, 11–69) specimens resistant to vinblastine (2 lung cancers and 2 breast cancers); and 2 of 8 (25%; 95% CI, 3–61) specimens resistant to etoposide (1 lung cancer and 1 breast cancer).

**DISCUSSION**

Our data show that temozolomide exerts potent cytotoxic activity against a large variety of human tumor colony-forming units taken directly from patients. In this study, we measured the cytotoxic activity of temozolomide with a human tumor-cloning assay in which capillary tubes were used instead of the two-layer agar system (27). This method improves significantly the plating efficiency, reduces the number of tumor cells as compared to the conventional two-layer agar system, and, therefore, enables a greater number of drugs to be tested at multiple concentrations with small human tumor biopsies. Unfortunately, this study was not designed initially to address the question whether continuous exposure to temozolomide induces direct cytotoxic effects or programmed cell death. Given recent reports indicating that apoptosis may play an important role in the
Table 2: Biochemical determination of \(O^6\)-alkyltransferase activity in human tumor specimens treated with temozolomide

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Tumor type</th>
<th>(O^6)-Alkyltransferase (fmol/mg protein) ± SD</th>
<th>(%) survival at 1 μM Temozolomide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Breast cancer</td>
<td>5.1 ± 2.5</td>
<td>49 ± 74 (%95)</td>
</tr>
<tr>
<td>2</td>
<td>Breast cancer</td>
<td>502.4 ± 9.9</td>
<td>97 ± 82 (%49)</td>
</tr>
<tr>
<td>3</td>
<td>Colon cancer</td>
<td>0.0 ± 0.0</td>
<td>41 ± 60 (%56)</td>
</tr>
<tr>
<td>4</td>
<td>Colon cancer</td>
<td>1.2 ± 1.2</td>
<td>132 ± 103 (%97)</td>
</tr>
<tr>
<td>5</td>
<td>Colon cancer</td>
<td>69.3 ± 16.2</td>
<td>105 ± 70 (%55)</td>
</tr>
<tr>
<td>6</td>
<td>Colon cancer</td>
<td>112.3 ± 4.4</td>
<td>62 ± 65 (%73)</td>
</tr>
<tr>
<td>7</td>
<td>Colon cancer</td>
<td>65.0 ± 1.7</td>
<td>82 ± 76 (%97)</td>
</tr>
<tr>
<td>8</td>
<td>Melanoma</td>
<td>11.3 ± 7.0</td>
<td>92 ± 92 (%83)</td>
</tr>
<tr>
<td>9</td>
<td>Melanoma</td>
<td>25.3 ± 8.9</td>
<td>54 ± 69 (%40)</td>
</tr>
<tr>
<td>10</td>
<td>Melanoma</td>
<td>43.8 ± 12.7</td>
<td>77 ± 74 (%58)</td>
</tr>
<tr>
<td>11</td>
<td>Ovarian carcinoma</td>
<td>117.9 ± 2.4</td>
<td>88 ± 84 (%70)</td>
</tr>
<tr>
<td>12</td>
<td>Ovarian carcinoma</td>
<td>445.6 ± 107.9</td>
<td>42 ± 51 (%52)</td>
</tr>
<tr>
<td>13</td>
<td>Ovarian carcinoma</td>
<td>28.1 ± 0.6</td>
<td>111 ± 104 (%105)</td>
</tr>
<tr>
<td>14</td>
<td>Ovarian carcinoma</td>
<td>111.5 ± 3.6</td>
<td>83 ± 69 (%60)</td>
</tr>
<tr>
<td>15</td>
<td>Ovarian carcinoma</td>
<td>51.5 ± 5.7</td>
<td>67 ± 56 (%43)</td>
</tr>
<tr>
<td>16</td>
<td>Ovarian carcinoma</td>
<td>107.5 ± 11.4</td>
<td>66 ± 49 (%31)</td>
</tr>
<tr>
<td>17</td>
<td>Ovarian carcinoma</td>
<td>131.9 ± 11.1</td>
<td>78 ± 71 (%65)</td>
</tr>
<tr>
<td>18</td>
<td>Ovarian carcinoma</td>
<td>40.0 ± 8.8</td>
<td>77 ± 40 (%34)</td>
</tr>
<tr>
<td>19</td>
<td>Ovarian carcinoma</td>
<td>64.1 ± 2.4</td>
<td>61 ± 42 (%37)</td>
</tr>
</tbody>
</table>

cytotoxicity of temozolomide in cultured cancer cells (23), additional prospective evaluation of temozolomide-induced DNA fragmentation in human tumor cells taken directly from patients is warranted.

Our results indicated that continuous exposure to temozolomide at a clinically relevant concentration of 10.0 μM induced an overall 36% in vitro response in human tumors. As indicated previously, this schedule was selected based on some preclinical and clinical studies suggesting a relationship between the duration of exposure to temozolomide and the cytotoxic activity (11, 25). This in vitro study does not pretend to provide any schedule design for a clinical trial, and it is important to remember that high concentrations of temozolomide may not be sustainable for 14 days in patients. However, our results suggest that the duration of exposure may be an important parameter in the optimization of the cytotoxicity of temozolomide against human cancer cells. Previous reports using human tumor cell lines and xenografts in athymic mice have also indicated that temozolomide has remarkable antiproliferative and antitumor effects against human cancers (3, 4, 14, 22, 24, 30). In Phase I and II clinical trials using oral temozolomide for 5 consecutive days, objective responses have been observed in malignant melanoma and primary brain tumors (7, 8, 31). This has been particularly important, considering that those tumors are known to be resistant to most conventional anticancer agents. Unfortunately, most of the brain tumor specimens have not been fully evaluable in our assay. However, in our study, we have observed an in vitro activity in 33% of evaluable melanoma specimens, which is consistent with a recent Phase II study showing that temozolomide induces 21% objective responses in advanced melanomas (7). Interestingly, in our study, temozolomide also shows a potent cytotoxic activity against breast (42%), renal (50%), non-small cell lung (27%), colon (42%), ovarian (36%) and renal (50%) cancers. Therefore, additional clinical evaluation of temozolomide may be recommended in those indications, for which new anticancer therapy is urgently needed.

Previous studies have reported that resistance to MTIC, the active metabolite of temozolomide, correlates with the presence of a high level of \(O^6\)-guanine-alkyl-transferase (19, 23, 25, 32, 33). This enzyme induces resistance to dacarbazine and several nitrosoureas by repairing \(O^6\)-guanine defects in DNA. In our study, only 19 specimens were evaluable for the in vitro response in the tumor-cloning assay contained enough material to measure the level of \(O^6\)-alkyltransferase. We observed that the enzyme activity varied markedly from one sample to another, but no correlation between the initial enzyme activity and in vitro responses to several concentrations of temozolomide was observed. Surprisingly, of the eight specimens in which in vitro responses were evaluated, seven had high levels of \(O^6\)-alkyltransferase activity. Conversely, only one specimen with a low level of \(O^6\)-alkyltransferase activity was sensitive to temozolomide. Although the number of specimens tested in this study remains limited, our data suggest that sensitivity and resistance to temozolomide in human tumors in vitro cannot be predicted by the initial biochemical measurement of \(O^6\)-alkyltransferase activity. Nevertheless, a recent study has demonstrated that continuous exposure to temozolomide induces a depletion of \(O^6\)-guanine-alkyl-transferase in peripheral blood lymphocytes of patients, suggesting that a cumulative depletion of this enzyme plays a role in the cytotoxicity of temozolomide (34).

Interestingly, we have tested temozolomide in some tumor samples that have been shown to be resistant to classical anticancer drugs. The effects of continuous-exposure temozolomide in tumors that showed resistance to other anticancer agents may reflect specific effects of temozolomide and/or the use of an optimal schedule of administration. Because the schedule of temozolomide was different from that of other anticancer agents, this study does not provide direct evidence on the cross-resistance between classical anticancer drugs and temozolomide. Nevertheless, the observed activity of continuous-exposure temozolomide in some tumors resistant to dacarbazine, various nonclassical alkylating agents (such as carmustine and cisplatin) and nonalkylating agents (such as 5-FU, doxorubicin, vinblastine, and etoposide) encourages additional testing of combinations and warrants testing the effects of this drug in clinical trials, when conventional chemotherapy has failed.

In summary, temozolomide shows a potent in vitro cytotoxic activity against a large variety of human tumors in the human tumor cloning assay. Those data encourage additional clinical trials of temozolomide, including Phase II studies in melanoma and in breast, non-small cell lung, colon, ovarian, and renal cancers.

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