Phase II Trial of Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer, with Assessment of Methylguanine-DNA Methyltransferase as a Potential Biomarker

M. Catherine Pietanza¹, Kyuichi Kadota², Kety Huberman³, Camelia S. Sima⁴, John J. Fiore¹, Dyana K. Sumner¹, William D. Travis², Adriana Heguy³, Michelle S. Ginsberg⁵, Andrei I. Holodny⁵, Timothy A. Chan⁶, Naiyer A. Rizvi³, Christopher G. Azzoli¹, Gregory J. Riely¹, Mark G. Kris¹, and Lee M. Krug¹

Abstract

Purpose: This phase II study was conducted to assess the efficacy of temozolomide in patients with relapsed small cell lung cancer (SCLC).

Experimental Design: Patients with disease progression after one or two prior chemotherapy regimens received temozolomide at 75 mg/m²/d for 21 days of a 28-day cycle. The primary endpoint was the overall response rate [ORR; complete response (CR) plus partial response (PR)], which was evaluated separately in sensitive and refractory cohorts. In the available tissue, we assessed O⁶-methylguanine-DNA methyltransferase (MGMT) promoter methylation status by PCR and MGMT expression by immunohistochemistry.

Results: Sixty-four patients were accrued: 48 patients in the sensitive cohort and 16 in the refractory group. One CR and 10 PRs were noted in sensitive patients [ORR, 23%; 95% confidence interval (CI), 12%–37%]. Two PRs were seen in the refractory cohort (ORR, 13%; 95% CI, 2%–38%). As second- and third-line treatment, the ORR was 22% (95% CI, 9%–40%) and 19% (95% CI, 7%–36%), respectively. Among patients with target brain lesions, 38% had a CR or PR (95% CI, 14%–68%). Grade ≥3 thrombocytopenia and neutropenia were observed in nine patients (14%). A greater number of cases with methylated MGMT had a response compared to those with unmethylated MGMT (38% vs. 7%; P = 0.08).

Conclusion: Temozolomide has activity in relapsed SCLC, particularly for brain metastases. Response to temozolomide may correlate with MGMT methylation in SCLC. Clin Cancer Res; 18(4); 1138–45. ©2012 AACR.

Introduction

Temozolomide is a nonclassic oral alkylating agent, which produces O⁶-alkylguanine (O⁶-AG) lesions on DNA. The DNA repair protein O⁶-AG DNA alkyltransferase, which is encoded by the O⁶-methylguanine-DNA methyltransferase (MGMT) gene, removes alkyl groups from the O⁶ position of guanine. Left unrepaired, chemotherapy-induced lesions trigger cytotoxicity and apoptosis. High levels of MGMT activity in cancer cells blunt the therapeutic effects of alkylating agents and thus can be an important determinant of treatment failure (1, 2). Epigenetic silencing of MGMT via hypermethylation of specific CpG islands of its promoter leads to loss of MGMT activity and improved sensitivity to alkylating agents (1, 2). Temozolomide is used in patients with glioblastoma multiforme and in refractory astrocytoma. In the phase III study of temozolomide in glioma, MGMT promoter methylation status was analyzed retrospectively and found to be an independent favorable prognostic factor (3). A recent large Radiation Thoracic Oncology Group study has confirmed these findings and has shown that MGMT promoter methylation is associated with response to temozolomide in glioma (4).

There is strong rationale to study temozolomide in patients with small cell lung cancer (SCLC). Alkylating agents have single-agent efficacy in SCLC (5). Temozolomide crosses the blood–brain barrier and brain metastases are common in this disease (6). SCLC has aberrantly methylated MGMT (2, 7). Finally, anecdotal responses to temozolomide have
initial therapy or within 60 days after completing first-line treatment. Patients who had received 1 or 2 prior chemotherapeutic regimens were eligible. Those with asymptomatic progression of disease in the brain were eligible. Inclusion required age ≥18, Karnofsky performance status of ≥60%, and measurable disease as per Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 (10). Patients were required to have leukocytes >3,000/mm³, platelets >100,000/mm³, bilirubin <1.5 mg/dL, serum creatinine <2.0 mg/dL, and alanine aminotransferase and aspartate aminotransferase <2.5 times the upper limit of normal.

Patients were excluded if they had leptomeningeal involvement or had received chemotherapy or radiation treatment within 21 days.

Treatment
Patients were treated with temozolomide (75 mg/m²/d) orally on days 1 to 21 of a 28-day cycle. Patients were instructed to fast at least 2 hours before and 1 hour after temozolomide administration. Ondansetron (8 mg orally) was given before temozolomide as needed. Temozolomide was continued until progression of disease, development of unacceptable toxicity, or withdrawal of consent. Initially, the dose of temozolomide was escalated to 100 mg/m²/d after cycle one if during the first 28 days, both white blood cells (WBC) and platelets remained above 3,000 and 100,000/µL, respectively, and no nonhematologic grade ≥3 toxicity occurred. However, because of myelosuppression observed among the first 26 patients, the protocol was amended, eliminating the dose escalation. Dosing was interrupted if a patient developed hematologic toxicities (i.e., WBC <3,000/µL and/or platelets <100,000/µL) or grade 3 nonhematologic toxicities (except for alopecia, nausea, and vomiting) until resolution. Upon resuming temozolomide, the dose was lowered. Two dose reductions were permitted (50 and 35 mg/m²/d). Removal from study occurred if patients’ toxicities did not resolve within 21 days (including grade ≥3 neutropenia and thrombocytopenia) for any recurrent grade 3 nonhematologic adverse event or for any grade 4 nonhematologic toxicity. Patients who developed grade ≥3 lymphopenia received prophylaxis for Pneumocystis carinii pneumonia.

Study evaluation
During the first 2 cycles of therapy, patients were assessed every 2 weeks. Thereafter, they were evaluated every 4 weeks. At each visit, a history, physical examination, toxicity assessment, complete blood count, and comprehensive metabolic panel were conducted. At cycle 3 and beyond, patients were required to have a complete blood count on day 15. All toxicities were graded using National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Tumor assessments at baseline included a computed tomography (CT) of the chest as well as of other relevant sites of the disease and a contrast-enhanced MRI or CT of the brain. Follow-up scans to assess response were obtained after cycles 1 and 2 and every 2 cycles thereafter. Responses were determined using RECIST 1.0 (10) and confirmation
was required on repeat imaging at least 4 weeks later. All imaging studies were reviewed by independent radiologists (A.I. Holodny and M.S. Ginsberg). An internal radiological review of randomly selected patients was conducted by the Therapeutic Response Committee at Memorial Sloan-Kettering Cancer Center (MSKCC) to verify response assessments. This review confirmed that tumor response was valid and complied with RECIST 1.0 (10).

MGMT analyses

Patients were requested to provide 10 unstained slides for MGMT analyses which were all conducted at MSKCC.

For the methylation status of the promoter region of MGMT, paraffin-embedded tumor tissue was manually dissected and DNA was extracted. Methylation-specific PCR was used to analyze the first 8 samples; primers were designed using MSPPrimer software (http://www.mspprimer.org/cgi-mspprimer/design.cgi; refs. 11–13). For the remainder of the samples, we used the EpiTyper system (Sequenom; refs. 14, 15), which is automated, requires a small amount of DNA, and allows interrogation of multiple CpGs within the CpG island within one amplicon, in contrast to MSPprimer that examines only CpGs in the primer region.

The EpiTyper assay uses base-specific cleavage of bisulfite-treated DNA and matrix-assisted laser desorption/ionization–time-of-flight/mass spectrometry (MALDI-TOF/MS). Specific PCR primers for bisulfite-converted DNA were designed using the EpiDesigner software (http://www.epidesigner.com; ref. 16) for the entire CpG island of the MGMT gene. T7 polymerase tags are added to the reverse primer to obtain a product that can be in vitro transcribed, and a 10-mer tag is added to the forward primer to balance the PCR conditions. For primer sequences, target chromosomal sequence, and EpiTyper-specific tags, see Supplementary Table S1. One microgram of tumor DNA was subjected to bisulfite treatment using the EZ-96 DNA methylation kit, which resulted in the conversion of unmethylated cytosines into uracil, following the manufacturer’s instructions (Zymo Research). PCR reactions were carried out in duplicate for each of the 2 selected primer pairs, for a total of 4 replicates per sample. For each replicate, 1 μL of bisulfite-treated DNA was used as template for a 5 μL PCR reaction in a 384-well microtiter PCR plate, using 0.2 units of KAPA2G Fast HotStart DNA polymerase (Kapa Biosystems), 200 μmol/L deoxynucleotide triphosphate (dNTP), and 400 nmol/L of each primer. Cycling conditions were 94°C for 2 minutes, 45 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 1 minute, and one final cycle at 72°C for 5 minutes. Unincorporated dNTPs were deactivated using 0.3 U of shrimp alkaline phosphatase (SAP) in 2 μL at 37°C for 20 minutes, followed by heat inactivation at 85°C for 5 minutes. Two microliters of SAP-treated reaction was transferred into a fresh 384-well PCR plate and in vitro transcription and T cleavage were carried out in a single 5 μL reaction mix using the MassCleave Kit (Sequenom) containing 1 × T7 polymerase buffer, 3 mmol/L dithiothreitol, 0.24 μL of T Cleavage mix, 22 units of T7 RNA and DNA polymerase, and 0.09 mg/mL of RNase A. The reaction was incubated at 37°C for 3 hours. After the addition of a cation exchange resin to remove residual salt from the reactions, 10 nL of EpiTyper reaction product was loaded onto a 384-element SpectroCHIP II array (Sequenom). SpectroCHIPs were analyzed using a Bruker Biflex III MALDI-TOF mass spectrometer (SpectroREADER, Sequenom). Results were analyzed using the EpiTyper Analyzer software and manually inspected for spectra quality and peak quantification.

For MGMT expression by immunohistochemistry, monoclonal mouse anti-MGMT antibody was used (Invi trogen, clone MT23.2). Formalin-fixed, paraffin-embedded tissue was cut in 4-μm sections and sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched with 3% H2O2 and the slides were incubated in 2% bovine serum albumin and then with primary anti-MGMT antibody (1:2,000 dilution). The second antibody (1:500 dilution) was applied and incubated; peroxidase-conjugated streptavidin was used, and antibody binding was visualized with diaminobenzidine and counterstained with Harris-modified hematoxylin. A single pathologist (K. Kadota) evaluated and scored the slides as negative when there was no MGMT expression and positive when there was any definite expression of MGMT. A scoring system was not applied because of the small amount of tissue in many of the biopsy specimens.

Statistical analysis

The primary endpoint was the overall response rate (ORR; complete response (CR) plus partial response (PR)) according to RECIST 1.0 (10), assessed separately for sensitive and refractory diseases. Secondary endpoints included overall survival (OS) and time to progression (TTP), response rates in patient groups stratified by second- or third-line treatment and presence or absence of brain metastases, and presence of MGMT promoter hypermethylation and MGMT expression by immunohistochemistry in available tumor samples, which were correlated with response, OS, and TTP.

In both sensitive and refractory cohorts, sample sizes were chosen to yield 80% power with type I error of 0.05. For the sensitive group, a Minimax Simon 2-stage design was used. The null and desired response rates were chosen to be 15% and 30%, respectively. If at least 4 responses were noted among the 23 patients accrued in stage I, enrollment would be extended to 48. At the end of the trial, if 12 or more patients were found to have a response, the agent would be considered effective and worthy of further testing. For the refractory group, a single-stage binomial design was used and 16 patients were enrolled. The null and desired response rates were chosen to be 5% and 25%, respectively. If 3 or more of the 16 patients achieved response, efficacy of the drug would be considered promising for refractory disease.

Response rates, along with exact 2-sided 95% confidence intervals (CI) were calculated and reported. OS and TTP were defined as the time from the date on which the patient first received any temozolomide to the date of death due to any cause and to the date of documented progression,
respectively, and were estimated using the Kaplan–Meier method.

Fisher’s exact test was used to correlate MGMT promoter methylation status and MGMT expression by immunohistochemistry in tissue with response, whereas log-rank test was used to compare the strata defined by the 2 variables with respect to OS of the patient. All MGMT analyses were combined across the sensitive and refractory cohorts because of the reduced sample size and given the exploratory nature of these investigations.

Results

Patient characteristics

We enrolled 64 patients between September 2008 and October 2010. Baseline characteristics are listed in Table 1. There were 48 and 16 patients with sensitive and refractory diseases, respectively. The groups were evenly distributed between patients who had received 1 or 2 lines of prior therapy. Twenty-four patients had progressive brain metastases; of these, 12 had received prior cranial irradiation: 8 prophylactically (prophylactic cranial irradiation, PCI) and 4 for treatment.

All patients received at least one cycle of temozolomide (median cycles, 1; range, 0.25–14). Reasons for discontinuation of temozolomide were disease progression (N = 50), intercurrent illness/symptomatic deterioration (N = 8), and unacceptable toxicity (N = 5).

Efficacy

In the sensitive group, one CR and 10 PRs were noted for a 23% ORR (95% CI, 12%–37%; Fig. 2). An additional patient had a PR that was not confirmed on follow-up imaging and therefore, was not included in the ORR. Two PRs were seen in the refractory cohort for a 13% ORR (95% CI, 2%–38%). Among the entire cohort of 64 patients, there were one CR and 12 PRs for a 20% ORR (95% CI, 11%–32%). Six patients (9%), 3 in each cohort, had stable disease for at least 3 cycles. There was no difference in ORR between patients receiving temozolomide as second-line (ORR = 22%; 95% CI, 9%–40%) and third-line therapy (ORR = 13%; 95% CI, 2%–38%).

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Sensitive SCLC (N = 48)</th>
<th>Refractory SCLC (N = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: male/female</td>
<td>24/24</td>
<td>5/11</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>68 (48–87)</td>
<td>64.5 (46–79)</td>
</tr>
<tr>
<td>Karnofsky performance status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥90%</td>
<td>12 (25%)</td>
<td>5 (31%)</td>
</tr>
<tr>
<td>80%</td>
<td>18 (38%)</td>
<td>6 (38%)</td>
</tr>
<tr>
<td>70%</td>
<td>14 (29%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>60%</td>
<td>4 (8%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Previous lines of therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onea</td>
<td>25 (52%)</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>Twoa</td>
<td>23 (48%)</td>
<td>9 (56%)</td>
</tr>
<tr>
<td>Median time from diagnosis to treatment, mo (range)</td>
<td>12 (6–52 mo)</td>
<td>7 (3–18 mo)</td>
</tr>
<tr>
<td>New brain metastasesa</td>
<td>18 (38%)</td>
<td>6 (38%)</td>
</tr>
</tbody>
</table>

With the exception of one patient, first-line treatment was a platinum/etoposide doublet; that individual had received carboplatin and paclitaxel as neoadjuvant therapy followed by cisplatin and etoposide adjuvantly when surgical resection revealed SCLC.

Second-line treatment included rechallenge with platinum/etoposide doublet (N = 12); topotecan (N = 8); obatoclax/topotecan (N = 3); cyclophosphamide/doxorubicin/vincristine (N = 3); taxanes (N = 2); SNS-595 (N = 1); and sunitinib (N = 1).

Brain metastases found at time of enrollment.

Figure 2. Waterfall plot. The best calculated responses on the basis of measurable lesions for 63 evaluable patients. One refractory patient did not have diagnostic imaging as she progressed clinically and therefore is not included. The patients with platinum-refractory disease are marked. †, progression of disease based on appearance of new nontarget lesions; ‡, progression of disease based on symptomatic deterioration; ††, PR was not confirmed.
and third-line treatment (ORR = 19%; 95% CI, 7%–36%), \( P = 0.99 \).

Before starting temozolomide, 24 patients had progressive brain metastases, 13 of which included target lesions according to RECIST 1.0 (10) that were assessable for response (Supplementary Table S2). Progressive brain metastases were defined as new lesions at the time of enrollment in the trial; these did not include residual lesions that had been previously irradiated. One patient had a brain-only recurrence; the remainder relapsed at systemic sites as well. Of the 13 patients with target lesions in the brain, treatment with temozolomide led to complete resolution of these metastases in 4 patients, including the patient with brain-only disease, and a PR in one patient for a response rate of 38% in the brain (95% CI, 14%–68%). Responses in the brain correlated with systemic responses to temozolomide (systemic CR = 1, PR = 3, and unconfirmed PR = 1). All of these patients had sensitive relapse and 3 had received prior PCI.

The median duration of response to temozolomide was 3.5 months (range, 1.4–14.7 months; mean, 4.1 months). Median TTP and OS for all treated patients were 1.6 (95% CI, 0.9–3.0 months) and 5.8 months (95% CI, 4.2–7.0 months), respectively. For the 48 sensitive patients, median TTP and OS were 1.6 (95% CI, 0.9–3.5 months) and 6.0 months (95% CI, 4.2–7.2 months), respectively (Fig. 3A and B). One-year survival rate for those with sensitive relapse was 21% (95% CI, 11%–39%). The median TTP and OS were 1 (95% CI, 0.8–3.4 months) and 5.6 months (95% CI, 2.5–7.7 months), respectively, for the 16 refractory patients (Fig. 3C and D). No patients with refractory disease were alive after 1 year.

Toxicity

Table 2 lists the most common treatment-related toxicities. Prior to the first amendment, a dose escalation to 100 mg/m^2/d was required at the start of the second cycle. However, this was stopped because of prolonged thrombocytopenia (grade ≥2) for ≥4 weeks in 3 of the first 26 patients. Less myelosuppression was noted after the trial was amended. Further therapy could not be administered in 7 patients because of prolonged thrombocytopenia and neutropenia. Four of these patients underwent bone marrow biopsies. The bone marrow in 2 patients was normocellular with trilineage hematopoesis; megakaryocytes were normal in number and microlobulated in morphology. In 2 patients, the bone marrow biopsy revealed myelodysplastic syndrome (MDS). They each had been treated with etoposide/platinum first-line and, subsequently, one received temozolomide for 1 month and the other for almost 15 months. A fifth patient underwent a bone marrow aspirate only that was hypocellular and revealed...
Three patients had grade normal trilineage hematopoiesis without extrinsic cells. Dose reduction for toxicity: thrombocytopenia (developed febrile neutropenia. Four patients required a MGMT analyses were no treatment-related deaths. There patient died because of disease progression on study. There were no treatment-related deaths.

**Table 2. Adverse events**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1, Grade 2, Grade 3, Grade 4, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
</tr>
<tr>
<td>Hematologic</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>17 (27)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td></td>
</tr>
<tr>
<td>MDSa</td>
<td></td>
</tr>
<tr>
<td>Nonhematologic</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>18 (28)</td>
</tr>
<tr>
<td>Nausea</td>
<td>22 (34)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>17 (27)</td>
</tr>
<tr>
<td>Constipation</td>
<td>14 (22)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Rash/desquamation</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Transaminitis</td>
<td>4 (6)</td>
</tr>
</tbody>
</table>

*Cytogenetics are as follows: deletion 5q and loss of p53 in 87% of cells and deletion 7q in 88% of cells in patient who received temozolomide for 1 month; deletion 5q in 74% of cells in patient who received temozolomide for 15 months.

MGMT analyses

We obtained 38 tumor samples for MGMT promoter methylation analysis (N = 36), MGMT expression by immunohistochemistry (N = 31), or both (N = 29). MGMT promoter methylation status was ascertained in 27 patients (42%); an additional 9 samples were indeterminate because of inadequate amounts of DNA. In patients for whom MGMT methylation could be determined, the overall promoter methylation rate was 48% (95% CI, 29%–68%). Patients with MGMT promoter methylation in their tumors had a better response to treatment than those with unmethylated MGMT, although the comparison did not reach statistical significance (38% vs. 7%; P = 0.08). However, the favorable response rate did not translate into an improved TTP for patients with MGMT promoter methylation (P = 0.29). In contrast, negative MGMT expression by immunohistochemistry did not correlate with response and showed a weak trend toward improved TTP (3.6 vs. 1.3 months; P = 0.1; Table 3).

**Table 3. MGMT analyses**

<table>
<thead>
<tr>
<th>Response</th>
<th>Median TTP, mo (95% CI)</th>
<th>Median survival, mo (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PR</td>
<td>SD + POD</td>
</tr>
<tr>
<td>MGMT methylation (n = 27)a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylated (n = 13)</td>
<td>1.7 (0.8–3.6)</td>
<td>5.6 (3.4–7.6)</td>
</tr>
<tr>
<td>Unmethylated (n = 14)</td>
<td>0.8 (0.7–4.3)</td>
<td>6.1 (1.9–15.5)</td>
</tr>
<tr>
<td>MGMT expression (n = 31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n = 13)</td>
<td>3.6 (0.8–NR)</td>
<td>5.2 (3.6–NR)</td>
</tr>
<tr>
<td>Positive (n = 18)</td>
<td>1.3 (0.8–3.5)</td>
<td>6.1 (4.0–7.6)</td>
</tr>
</tbody>
</table>

Abbreviations: NR, not reached; POD, progression of disease.

*aThe first 8 samples were carried out using methylation-specific PCR.

Discussion

This phase II study assessed the efficacy of temozolomide in patients with relapsed SCLC. We found a 20% ORR (95% CI, 11%–32%) for temozolomide in this patient population, which did not meet the prespecified criteria for sufficient activity of this agent. However, this should not result in temozolomide being abandoned as a potential therapy in this disease. In particular, we observed activity in several challenging subgroups of patients. Two PRs were noted in patients with refractory SCLC [13% (95% CI, 0%–29%)]. We observed a 19% response rate in patients receiving third-line treatment, which comprised 50% of the study population and for which no standard therapeutic options exist currently. In patients with brain metastases, regressions were detected even in some who had received prior radiation therapy.

The toxicities observed with temozolomide were mild. Only 6% of patients experienced grade ≥3 nonhematologic toxicities. Likewise, grade ≥3 anemia, neutropenia, and thrombocytopenia occurred in only 3%, 5%, and 9%, respectively. Generally, cytopenias with temozolomide developed in patients treated with ≥4 cycles. Although lymphopenia was noted, no patient developed P. carinii pneumonia. Two patients developed MDS, an adverse effect reported...
previously (17–19). It is difficult to establish with certainty whether this complication was due to temozolomide, prior etoposide, or the combination. The cytogenetic abnormalities noted in these patients were deletion 5q and deletion 7q in one and deletion 5q in the other, which commonly are observed after treatment with alkylating agents including temozolomide. Etoposide-related MDS commonly show balanced chromosomal aberrations such as 11q23 and 21q22 (20), which were not found in our cases.

This study raises the possibility that the activity of temozolomide may be associated with the MGMT status. In patients with MGMT promoter methylation, we noted a 31% higher response rate as compared with those with unmethylated MGMT \( (P = 0.08) \). MGMT expression by immunochemistry was evaluated as well and a higher response rate was noted in patients without detectable MGMT expression, compared with those with increased MGMT expression; the association between response and MGMT expression was less strong than that seen with MGMT promoter methylation. A larger number of cases will need to be studied to prove that this level of improved activity of temozolomide is associated with MGMT promoter methylation or MGMT expression.

We chose the regimen of temozolomide for 21 days of a 28-day cycle to enhance treatment response in SCLC on the basis of the phase II data available. Loss of MGMT activity leads to improved sensitivity to alkylating agents. In pancreatic neuroendocrine tumors, lack of MGMT expression correlates with treatment response to temozolomide (21). Epigenetic silencing of the MGMT gene via hypermethylation of specific Cpg islands of its promoter leads to loss of MGMT activity (1, 2). MGMT deletion in the tumor also can be achieved by alternative dosing schedules of temozolomide, delivering more prolonged exposure and higher cumulative doses, thus reducing the cell’s ability for DNA repair and sensitizing tumor cells to the toxic effects of the drug (22). Phase II studies evaluating dose-dense/intense temozolomide schedules \([75 \text{ mg/m}^2/\text{d} \text{ for 21 days every 28 days (ref. 23), 150 mg/m}^2/\text{d} \text{ on days 1 to 7 and 15 to 21 every 28 days (ref. 24), and 50 mg/m}^2/\text{d} \text{ (ref. 25)})\] in patients with recurrent glioblastoma had been found to be tolerable and efficacious. Importantly, these dose-dense/intense regimens had not shown differences in outcomes between patients with tumors with methylated and unmethylated MGMT promoters (23–25). This suggested that MGMT inactivation with protracted temozolomide administration increased the sensitivity of unmethylated tumors to the agent (23). However, the more recent phase III study of temozolomide in newly diagnosed glioblastoma comparing the standard regimen \([150–200 \text{ mg/m}^2/\text{d for 5 days})\] with the dose-dense schedule \([75 \text{ mg/m}^2/\text{d for 21 days})\] did not reveal improved outcomes for protracted dosing of temozolomide regardless of methylation status and confirmed the prognostic significance of MGMT methylation (4).

Additional studies with temozolomide are ongoing here. Because the protracted dosing schedule of temozolomide for 21 days of a 28-day cycle used in this study led to prolonged thrombocytopenia in some patients, we are currently evaluating the standard dosing schedule of 200 mg/m\(^2\)/d for 5 of 28 days in a similar patient population (ClinicalTrials.gov Identifier: NCT00740636). We are also interested in exploring mechanisms to enhance temozolomide activity and possibly overcome its resistance. DNA repair proteins are upregulated in SCLC, including PARP-1 (26). PARP targets proteins that facilitate DNA repair of single-stranded or double-stranded DNA breaks (27). If PARP-1 is inhibited, single-strand breaks become double-strand breaks and cell apoptosis occurs during DNA replication (28, 29). Therefore, we plan to study temozolomide in combination with the PARP inhibitor ABT-888.

Despite not achieving a CR and PR rate of more than 20% in patients with relapsed SCLC, these data are sufficient to suggest several areas of study for temozolomide. Careful patient selection, exploitation of MGMT as a biomarker, optimization of the dosing schedule, and use in combination with a PARP inhibitor have the potential to further improve outcomes with temozolomide in SCLC.

Disclosure of Potential Conflicts of Interest

M.C. Pietanza is a consultant/advisory board member for Bristol-Myers Squibb, G.J. Riely has commercial research grant from Merck, Bristol-Myers Squibb, and Pfizer, is a consultant/advisory board member for Chugai, Tragara, Ariad, Merck, and Boehringer Ingelheim, and has consulted for Novartis and Daiichi. No potential conflicts of interests were disclosed by other authors.

Acknowledgments

The authors thank Isabella Bergagnini and Valerie Baez for the collection and organization of data.

Grant Support

The clinical trial for this study was also supported in part by Merck & Co., Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must, therefore, be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 10, 2011; revised December 5, 2011; accepted December 15, 2011; published OnlineFirst January 6, 2012.

References


Clinical Cancer Research

Phase II Trial of Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer, with Assessment of Methylguanine-DNA Methyltransferase as a Potential Biomarker

M. Catherine Pietanza, Kyuichi Kadota, Kety Huberman, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-11-2059

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2012/01/06/1078-0432.CCR-11-2059.DC1

Cited articles
This article cites 25 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/18/4/1138.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/18/4/1138.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.