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

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Supported, in part, by Merck & Co., Inc.

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Running Title: Temozolomide in Relapsed SCLC and MGMT Assessment

Key Words: Temozolomide, SCLC, MGMT

**Translational Relevance**

The development of new therapeutic strategies in small cell lung cancer (SCLC) hinges on exploiting its molecular and cellular abnormalities. Here, we report the results of a phase II trial evaluating temozolomide, a non-classic oral alkylating agent, in the second- and third-line treatment of relapsed SCLC. We demonstrate that the agent has activity, with responses in third-line treatment and in those with progressive brain metastases. Available diagnostic tissue was evaluated for *MGMT* promoter methylation, MGMT expression by immunohistochemistry, or both. We show that patients with *MGMT* promoter methylation in their tumors had a better response to treatment compared to those with unmethylated *MGMT*, although the comparison did not reach statistical significance. Based on these results, we could consider devising future clinical trials in SCLC using MGMT to enrich for patients.
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 75mg/m²/day for 21 days of a 28-day cycle. The primary endpoint was overall response rate (ORR; complete plus partial response), which was evaluated separately in sensitive and refractory cohorts. In available tissue, we assessed 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 complete response (CR) and 10 partial responses (PR) were noted in sensitive patients, ORR 23% [95% CI, 12% to 37%]. Two PRs were seen in the refractory cohort, ORR 13% [95% CI, 2% to 38%]. As second- and third-line treatment, the ORR was 22% [95% CI, 9% to 40%] and 19% [95% CI, 7% to 36%], respectively. Among patients with target brain lesions, 38% had a CR or PR [95% CI, 14% to 68%]. Grade ≥3 thrombocytopenia and neutropenia were observed in 9 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.
Introduction

Temozolomide is a non-classic oral alkylating agent, which produces $O^6$-alkyl-guanine ($O^6$-AG) lesions on DNA. The DNA-repair protein $O^6$-AG DNA alkyltransferase, which is encoded by the $O^6$-methyl-guanine-DNA methyltransferase ($MGMT$) gene, removes alkyl groups from the $O^6$ 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 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 been noted in SCLC (8). We undertook this phase II study to assess the efficacy and safety
of temozolomide in patients sensitive and refractory to first-line platinum-based chemotherapy.

**Materials and Methods**

This was a single arm, open label, single-institution phase II study, which was reviewed and approved by the Institutional Review Board. Written informed consent was provided by all patients.

**Eligibility Criteria**

All patients had SCLC that was sensitive or refractory to first-line platinum-based chemotherapy. Sensitive disease was defined as progression or relapse 60 days or more after the completion of first-line chemotherapy. Refractory disease was defined as progression during initial therapy or within 60 days after completing first-line treatment. Patients that had received one or two prior chemotherapeutic regimens were eligible. Those with asymptomatic progression of disease in the brain were eligible. Inclusion required age \( \geq 18 \), Karnofsky performance status of \( \geq 60\% \), and measurable disease as per Response Evaluation in Solid Tumors (RECIST) 1.0. Patients were required to have: leukocytes \( >3000/mm^3 \), platelets \( >100,000/mm^3 \), 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 received chemotherapy or radiation treatment within 21 days.
Treatment

Patients were treated with temozolomide 75mg/m$^2$/day 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 8mg 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 100mg/m$^2$/day after cycle one if during the first 28 days, both white blood cells and platelets remained above 3,000/µl and 100,000/µl, respectively, and no non-hematologic grade ≥ 3 toxicity occurred. However, due to 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 non-hematologic toxicities (except for alopecia, nausea and vomiting) until resolution. Upon resuming temozolomide, the dose was lowered. Two dose reductions were permitted (50mg/m$^2$/day and 35mg/m$^2$/day). Removal from study occurred if patients’ toxicities did not resolve within 21 days (including grade ≥3 neutropenia and thrombocytopenia), for any recurrent grade 3 non-hematologic adverse event, or for any grade 4 non-hematologic toxicity. Patients who developed grade ≥3 lymphopenia received prophylaxis for pneumocystis carinii pneumonia.

Study Evaluation

During the first two cycles of therapy, patients were assessed every two weeks. Thereafter, they were evaluated every four weeks. At each visit, a history, physical
examination, toxicity assessment, complete blood count, and comprehensive metabolic panel were performed. 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 other relevant sites of 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 two cycles thereafter. Responses were determined using RECIST 1.0 (10) and confirmation was required on repeat imaging at least four weeks later. All imaging studies were reviewed by independent radiologists (AIH and MSG). An internal radiological review of randomly selected patients was conducted by the Therapeutic Response Committee at 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 ten unstained slides for MGMT analyses, which were all performed 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 ([http://www.mspprimer.org](http://www.mspprimer.org)) (11-13). For the remainder of the samples, we used the
EpiTyper system (Sequenom, San Diego, CA) (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) (16), for the entire CpG island of the MGMT gene. T7-promoter tags are added to the reverse primer to obtain a product that can be in vitro transcribed, and a 10-mere tag is added to the forward primer to balance the PCR conditions. For primer sequences, target chromosomal sequence, and EpiTyper specific tags see Supplemental Table 1. One μg of tumor DNA was subjected to bisulfite treatment using the EZ-96 DNA methylation Kit, which results in the conversion of unmethylated cytosines into uracil, following the manufacturer’s instructions (Zymo Research, Orange, CA). 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, Cape Town, South Africa), 200 μM dNTPs, and 400 nM 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 1 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 μl of SAP-treated reaction were 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 X T7 polymerase buffer, 3 mM DTT, 0.24 μl of T Cleavage mix, 22 units of T7 RNA and DNA polymerase, and 0.09 mg/ml of RNAseA. The reaction was incubated at 37 °C for 3 h. After the addition of a cation exchange resin to remove residual salt from the reactions, 10 nl of EpiTyper reaction product were loaded onto a 384-element SpectroCHIP II array (Sequenom). SpectroCHIPS were analyzed using a Bruker Biflex III matrix-assisted laser desorption/ionization–time of flight (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 (Invitrogen, clone MT23.2). Formalin-fixed paraffin-embedded tissue was cut in 4μm sections, and sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched with 3% H₂O₂, the slides were incubated in 2% bovine serum albumin and then with primary anti-MGMT antibody 1:2000. 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 (KK) evaluated and scored the slides as negative if there was no MGMT expression and positive if
there was any definite expression of MGMT. A scoring system was not applied due to the small amount of tissue in many of the biopsy specimens.

Statistical Analysis

The primary endpoint was overall response rate (ORR; complete response (CR) plus partial response (PR)) according to RECIST 1.0 (10), assessed separately for sensitive and refractory disease. 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 the 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 two-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 1, 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 two-sided 95% confidence intervals (CI) were calculated and reported. Overall survival and time to progression were defined as the time from the date the patient first received any temozolomide to the date of death due to any cause and to the date of documented progression, respectively, and 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, while log-rank test was used to compare the strata defined by the two variables with respect to overall survival of the patient. All MGMT analyses were combined across the sensitive and refractory cohorts due to 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 disease, respectively. The groups were evenly distributed between patients that had received one or two lines of prior therapy. Twenty-four patients had progressive brain metastases; of these, 12 had received prior cranial irradiation: eight prophylactically (PCI) and four for treatment.
All patients received at least one cycle of temozolomide (median cycles 1, range 0.25 to 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% to 37%] (Figure 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% to 38%]. Among the entire cohort of 64 patients, there was one CR and 12 PRs, for a 20% ORR [95% CI: 11% to 32%]. Six patients (9%), three in each cohort, had stable disease for at least three cycles. There was no difference in ORR between patients receiving temozolomide as second-line [ORR = 22%; 95% CI: 9% to 40%] and as third-line treatment [ORR = 19%; 95% CI: 7% to 36%], p = 0.99.

Prior to 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 (Supplemental Table 2). Progressive brain metastases were defined as new lesions at the time of enrollment in the trial; these did not include residual lesions that previously had been 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 four patients, including the patient with brain-only disease, and a partial response in one
patient, for a response rate of 38% in the brain [95% CI: 14% to 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 three had received prior PCI.

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

Toxicity

Table 2 lists the most common treatment-related toxicities. Prior to the first amendment, a dose escalation to 100mg/m²/day was required at the start of the second cycle. However, this was stopped due to prolonged thrombocytopenia (grade ≥2) for ≥4 weeks in three of the first 26 patients. Less myelosuppression was noted after the trial was amended. Further therapy could not be administered in seven patients due to prolonged thrombocytopenia and neutropenia. Four of these patients underwent bone research.
The bone marrow in two patients was normocellular with trilineage hematopoiesis; megakaryocytes were normal in number and micro-lobulated in morphology. In two 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 one month, the other for almost 15 months. A fifth patient underwent a bone marrow aspirate only that was hypocellular and revealed normal trilineage hematopoiesis without extrinsic cells. Three patients had grade ≥3 neutropenia and one patient developed febrile neutropenia. Four patients required a dose reduction for toxicity: thrombocytopenia (N = 2), grade 3 fatigue (N = 1) and grade 3 pruritus (N = 1). One patient died due to disease progression on study. There were no treatment-related deaths.

*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 due to inadequate amounts of DNA. In patients for whom *MGMT* methylation could be determined, the overall promoter methylation rate was 48% (95% CI: 29% to 68%). Patients with *MGMT* promoter methylation in their tumors had a better response to treatment compared to 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 towards improved TTP (3.6 months vs. 1.3 months, \( p = 0.1 \)) (Table 3).

**Discussion**

This phase II study assessed the efficacy of temozolomide in patients with relapsed SCLC. We found a 20% ORR [95% CI: 11% to 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 partial responses were noted in patients with refractory SCLC (13% [95% CI, 0% to 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 that had received prior radiation therapy.

The toxicities observed with temozolomide were mild. Only 6% of patients experienced grade \( \geq 3 \) non-hematologic toxicities. Likewise, grade \( \geq 3 \) anemia, neutropenia and thrombocytopenia occurred in only 3%, 5% and 9%, respectively. Generally, cytopenias with temozolomide developed in patients treated with \( \geq 4 \) cycles. Although lymphopenia was noted, no patient developed *pneumocystis 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 myelodysplastic syndromes commonly demonstrate 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 MGMT status. In patients with MGMT promoter methylation, we noted a 31% higher response rate compared to those with unmethylated MGMT \( p = 0.08 \). MGMT expression by immunohistochemistry was evaluated as well, and a higher response rate was noted in patients without detectable MGMT expression compared to 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 based upon 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 depletion 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 (75mg/m²/day for 21 days every 28 days (23); 150mg/m²/day on days 1-7 and 15-21 every 28 days (24); and 50mg/m²/day (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 to 200mg/m²/day for 5 days) to the dose-dense schedule (75mg/m²/day 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. Since 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 currently are evaluating the standard dosing schedule of 200mg/m²/day for 5 out of 28 days in a similar patient population (ClinicalTrials.gov Identifier: NCT00740636). We also are interested in exploring mechanisms to enhance temozolomide activity and possibly overcome its resistance. DNA repair proteins are upregulated in SCLC, including poly (ADP-ribose) polymerase (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 complete response and partial response 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.

**Acknowledgments:** We would like to thank Isabella Bergagnini and Valerie Baez for the collection and organization of data.

**Grant Support:** Supported, in part, by Merck & Co., Inc.
References

Figure Legend

Figure 1. Study Schema.

Figure 2. Waterfall Plot. The best calculated responses based on measurable lesions for 63 evaluable patients. One refractory patient did not have diagnostic imaging as she progressed clinically, therefore is not included. The patients with platinum-refractory disease are marked. *Progression of disease based on appearance of new non-target lesions. ‡Progression of disease based on symptomatic deterioration. †Partial response was not confirmed.

Figure 3. Kaplan Meier Curves for Outcomes. Time to progression (A) and overall survival (B) for the 48 patients with platinum sensitive SCLC. Time to progression (C) and overall survival (D) for the 16 patients with platinum refractory SCLC.
<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Sensitive-SCLC (N = 48)</th>
<th>Refractory-SCLC (N = 16)</th>
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</thead>
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<tr>
<td><strong>Sex: Male/Female</strong></td>
<td>24/24</td>
<td>5/11</td>
</tr>
<tr>
<td><strong>Median Age (Range)</strong></td>
<td>68 (48 – 87)</td>
<td>64.5 (46 – 79)</td>
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<tr>
<td><strong>Karnofsky Performance Status</strong></td>
<td></td>
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<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><strong>Previous Lines of Therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One*</td>
<td>25 (52%)</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>Two‡</td>
<td>23 (48%)</td>
<td>9 (56%)</td>
</tr>
<tr>
<td><strong>Median time from Diagnosis to Treatment, months (Range)</strong></td>
<td>12 (6 – 52 months)</td>
<td>7 (3 – 18 months)</td>
</tr>
<tr>
<td><strong>New Brain Metastases§</strong></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); sunitinib (N=1).

§Brain metastases found at time of enrollment.
Table 2. Adverse Events

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1</th>
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<th>Grade 3</th>
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<tr>
<td></td>
<td>N</td>
<td>N</td>
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<td>N</td>
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<tr>
<td><strong>Hematologic</strong></td>
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<td></td>
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<td>6 (9%)</td>
<td>9 (14%)</td>
<td>2 (3%)</td>
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<tr>
<td>Thrombocytopenia</td>
<td>5 (8%)</td>
<td>2 (3%)</td>
<td>5 (8%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>6 (9%)</td>
<td>2 (3%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
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<tr>
<td>Lymphopenia</td>
<td></td>
<td>17 (27%)</td>
<td>2 (3%)</td>
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<tr>
<td>Neutropenia</td>
<td>1 (2%)</td>
<td>2 (3%)</td>
<td>1 (2%)</td>
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<tr>
<td>Febrile Neutropenia</td>
<td>1 (2%)</td>
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<td></td>
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<tr>
<td>Myelodysplastic Syndrome§</td>
<td></td>
<td></td>
<td></td>
<td>2 (3%)</td>
</tr>
<tr>
<td><strong>Non-Hematologic</strong></td>
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<tr>
<td>Fatigue</td>
<td>18 (28%)</td>
<td>23 (36%)</td>
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<tr>
<td>Nausea</td>
<td>22 (34%)</td>
<td>9 (14%)</td>
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</tr>
<tr>
<td>Vomiting</td>
<td>17 (27%)</td>
<td>4 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>14 (22%)</td>
<td>4 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6 (9%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>5 (8%)</td>
<td>2 (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash/Desquamation</td>
<td>4 (6%)</td>
<td>1 (2%)</td>
<td>2 (3%)</td>
<td></td>
</tr>
<tr>
<td>Transaminitis</td>
<td>4 (6%)</td>
<td>2 (3%)</td>
<td></td>
<td></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 that received temozolomide for one month; deletion 5q in 74% of cells in patient that received temozolomide for 15 months.
<table>
<thead>
<tr>
<th>Response</th>
<th>Median Time to Progression, Months (95%CI)</th>
<th>Median Survival, Months (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PR</td>
<td>SD + POD</td>
</tr>
<tr>
<td><strong>MGMT Methylation (n = 27)</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylated (n = 13)</td>
<td>5  (38%)</td>
<td>8  (62%)</td>
</tr>
<tr>
<td>Unmethylated (n = 14)</td>
<td>1  (7%)</td>
<td>13 (93%)</td>
</tr>
<tr>
<td><strong>MGMT Expression (n = 31)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n = 13)</td>
<td>5  (38%)</td>
<td>8  (62%)</td>
</tr>
<tr>
<td>Positive (n = 18)</td>
<td>3  (17%)</td>
<td>15 (83%)</td>
</tr>
</tbody>
</table>

*The first eight samples were performed using methylation-specific PCR. † NR, not reached.
Figure 1

Recurrent SCLC after 1 or 2 prior regimens
No chemotherapy or radiotherapy in prior 3 weeks
KPS ≥ 60%

Cohort 1: Sensitive Disease
Relapse > 2 months after first-line therapy
N = 48
Temozolomide 75 mg/m² PO
21 out of 28 days
Evaluable for Response
N = 48

Cohort 2: Refractory Disease
Progression during initial treatment or ≤ 2 months after first-line therapy
N = 16
Temozolomide 75 mg/m² PO
21 out of 28 days
Evaluable for Response
N = 16
Figure 2
Figure 3

A

Median TTP = 1.6 months
95%CI : 0.9 – 3.5 months

B

Median OS = 6.0 months
95%CI : 4.2 – 7.2 months
Figure 3

C. Median TTP = 1.0 months
95% CI: 0.8 – 3.4 months

D. Median OS = 5.6 months
95% CI: 2.5 – 7.7 months
Phase II Trial of Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer, with Assessment of Methyl-Guanine-DNA Methyltransferase as a Potential Biomarker

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

Clin Cancer Res  Published OnlineFirst January 6, 2012.

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

Supplementary Material
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