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**A Randomized, Double-Blinded, Phase II Study of Temozolomide in Combination With Either Veliparib or Placebo in Patients With Relapsed-Sensitive or Refractory Small-Cell Lung Cancer**

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**SCHEMA**

Although first-line treatment for SCLC with cisplatin or carboplatin plus etoposide (with the addition of radiation therapy in limited stage) results in a 60-80% overall response rate, the majority of patients suffer relapse within months of completing initial therapy. The strongest predictor of outcome for patients with relapsed disease is the duration of remission. Patients who maintain an appropriate response to initial therapy are deemed “sensitive”, while those without response to first-line treatment or with early progression are considered “refractory”. Those with “sensitive” disease have a higher likelihood of response to second-line chemotherapy, though at best it is half that expected in the first-line setting, and their survival averages around 6 months. The chance of obtaining a response to second-line therapy for patients with “refractory” disease is less than 10%, and survival is 3 to 4 months.<sup>1</sup> Currently, the only approved second-line agent in SCLC is topotecan, which was shown to have a response rate of 24%, and median survival of 6 months in patients with chemosensitive recurrent disease.<sup>2</sup> Novel treatment strategies based on the unique biology of SCLC are necessary.

We have shown that temozolomide has single agent activity in patients with relapsed sensitive and refractory SCLC. However, after a median of 3.5 months (range, 1.4 to 14.7 months) [mean, 4.1 months] of treatment with single-agent temozolomide, the patients develop progression of disease. Data indicate that the combination of poly (ADP-ribose) polymerase (PARP)-1 inhibitors and temozolomide result in dramatic tumor growth delay or regression where temozolomide by itself is not efficacious.<sup>3</sup> Therefore, we believe that the addition of ABT-888 will overcome resistance to temozolomide and lead to improved progression-free survival and outcomes in this patient population, and merits further study.

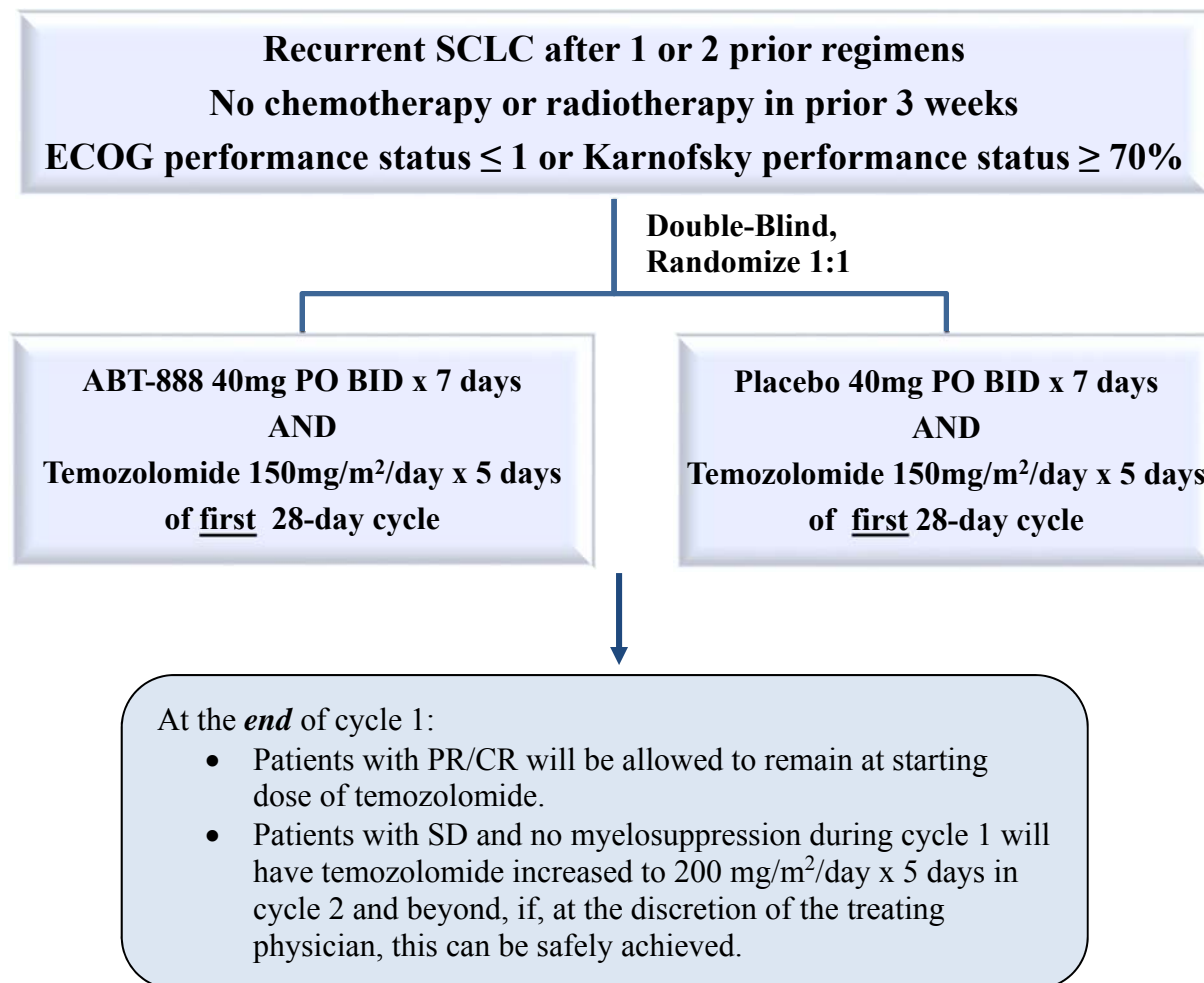
This is a multi-institutional, double-blind, placebo-controlled, randomized phase II study of ABT-888, a PARP-1 inhibitor, and temozolomide compared to placebo and temozolomide in patients with relapsed sensitive or refractory SCLC ([Figure 1](#)), as defined below:

**“Sensitive” Disease:** Relapse 60 days after the completion of first-line platinum-based chemotherapy.

**“Refractory” Disease:** No response to initial platinum-based therapy, or progression within 60 days after completing treatment; and any patient in need of third-line therapy.

The primary endpoint is progression-free survival (PFS) at four months. We hypothesize that PFS at four months will improve by 20% in patients receiving ABT-888 and temozolomide compared to placebo and temozolomide.

Patients with relapsed sensitive disease or refractory disease after one or two prior chemotherapy regimens will be enrolled from MSKCC and other high-volume SCLC centers. Eligibility allows for patients with brain metastases.

**Figure 1. Study Schema**

ABT-888 and placebo will be supplied by the NCI Division of CTEP. Temozolomide will be obtained by prescription. All treatment will be performed in the outpatient setting. Tumor response will be measured radiographically during week 4, week 8 and every 8 weeks thereafter using RECIST 1.1 criteria.<sup>4</sup>

The primary endpoint is to demonstrate an improvement in progression free survival (PFS) at four months in patients with relapsed sensitive or refractory SCLC receiving ABT-888 and temozolomide compared to placebo and temozolomide. Patients will be stratified according to sensitive disease versus refractory disease. An interim evaluation for early evidence futility will be performed after half of the patients have their 4 months evaluation.

Secondary objectives include the evaluation of RECIST response (ORR), overall survival (OS), and tolerability of ABT-888 with temozolomide in this patient population.

Available tumor samples will be analyzed for methylated *MGMT* promoter by PCR, *MGMT* expression by immunohistochemistry (IHC), PARP-1 expression by IHC, mRNA BRCA-1



expression, PTEN expression by IHC, RAD51 expression by IHC and BRCA-1 expression by IHC. Priority will be given to MGMT and PARP-1 analyses. Each of these will be correlated to clinical outcomes.

Additionally, peripheral blood will be collected at the time of radiographic imaging to identify and enumerate circulating tumor cells (CTCs) using the Veridex CellSearch™ System in this cohort of patients with SCLC. We will correlate CTCs with PFS, ORR, and OS at each time point, as well as with patient characteristics at baseline. Using the Veridex CellSearch™ System, we also will measure  $\gamma$ H2AX-positive CTCs.

Similarly, at baseline and at the time of radiographic imaging, peripheral blood samples will be collected to assess plasma markers for apoptosis and angiogenesis, including M30, M65, pro-GRP, sVEGF, sVEGFR2, and sKIT, and correlate these markers with outcome in the two treatment arms.

This study design requires the accrual of 100 treated patients with SCLC. This study will be the front-line study for this patient population at MSKCC and at each of the participating centers. Thus, once the study is open at all participating centers, we conservatively estimate an accrual of 6 patients per month. We anticipate full patient enrollment within 20 months.

**Rationale for MSKCC Amendment 10:**

After the first 24 patients were accrued and evaluated for at least one cycle, it was noted that 14 of these patients incurred the following adverse events: grade 3/4 neutropenia (n = 10 patients); grade 3/4 thrombocytopenia (n = 13 patients); and grade 4 febrile neutropenia (n = 1 patient) leading to sepsis and death. Other than the patient with febrile neutropenia, the noted grade 3/4 neutropenic or thrombocytopenic episodes did not impose any safety issues for the patients.

Of these 24 patients, 8 patients in total have had a partial response (n = 3) or unconfirmed partial response (n = 5). Interestingly, all patients that have had a partial response or unconfirmed partial response have had myelosuppression. Due to myelosuppression, four of the patients had cycle #2 of temozolomide held for a period of time, and at the end of cycle #2 had progression of disease.

Therefore, in this amendment we are initiating temozolomide at a lower dose, in an attempt to avoid myelosuppression and dose delays, leading to more durable responses in patients.

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## 1.0 OBJECTIVES

### 1.1 Primary Objectives

The primary objective of this study is to demonstrate an improvement in progression free survival (PFS) at four months in patients with relapsed sensitive or refractory SCLC receiving ABT-888 and temozolomide compared to placebo and temozolomide.

### 1.2 Secondary Objectives

- 1.2.1 Determine the objective response rate (ORR) (based on RECIST 1.1 criteria)<sup>4</sup> in both arms of the study: ABT-888 and temozolomide and placebo and temozolomide.
- 1.2.2 Determine the overall survival (OS) of patients in both arms of the study.
- 1.2.3 Determine the ORR, PFS and OS of ABT-888 and temozolomide and placebo and temozolomide, in the following patient groups:
  - Sensitive disease vs. Refractory disease
  - Second-line treatment vs. Third-line treatment
  - Brain metastases vs. No brain metastases
- 1.2.4 Determine the safety and tolerability of ABT-888 and temozolomide in patients with SCLC.

### 1.3 Exploratory Objectives

- 1.3.1 Evaluate available tumor samples for methylated *MGMT* promoter by the EpiTyper assay, as well as *MGMT* expression by immunohistochemistry and determine if these correlate with PFS, ORR, and OS.
- 1.3.2 Evaluate available tumor samples for PARP-1, BRCA-1 and RAD51 expression by immunohistochemistry and determine if they correlate with PFS, ORR, and OS.
- 1.3.3 Evaluate available tumor samples for mRNA BRCA-1 expression and determine if it correlates PFS, ORR, and OS.
- 1.3.4 Evaluate available tumor samples for PTEN expression by immunohistochemistry and determine if it correlates PFS, ORR, and OS.
- 1.3.5 Identify and enumerate circulating tumor cells (CTCs) using the Cell Search<sup>TM</sup> System in these patients with SCLC at baseline and at the time of repeat imaging.
  - a. Correlate the number of CTCs with PFS and OS at each time point.
  - b. Correlate the change in CTCs with radiographic response.

- c. Correlate the number of CTCs at baseline with patient characteristics (disease burden, location of metastases, progression at existing sites or new sites of disease).
- 1.3.6 Evaluate  $\gamma$ H2AX-positive CTCs using the CellSearch™.
    - a. Assess the percentage increase in DNA fragments during treatment and correlate with outcome in each of the treatment groups.
  - 1.3.7 Evaluate plasma markers for apoptosis and angiogenesis.
    - a. Assess changes in plasma markers for apoptosis and angiogenesis, including M30, M65, pro-GRP, sVEGF, sVEGFR2, and sKIT, and correlate these markers with outcome in the two treatment arms.

## 2.0 BACKGROUND

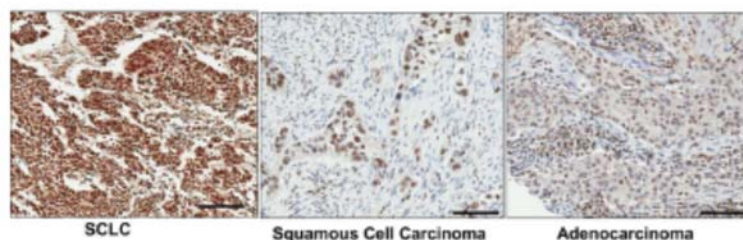
### 2.1 ABT-888

ABT-888 is an orally available, small molecule inhibitor of poly(ADP-ribose) polymerase (PARP). PARP is an essential nuclear enzyme that plays a role in recognition of DNA damage and facilitation of DNA repair. Therefore, inhibition of PARP is expected to enhance the effects of DNA damage. Expression of PARP is higher in tumor cells as compared to normal cells. This overexpression has been linked to drug resistance and the ability of tumor cells to withstand genotoxic stress. Hence, it is anticipated that PARP inhibitors will function as sensitizing agents for chemotherapy and radiation therapy that are designed to cause DNA damage.

#### Mechanism of Action

Poly (ADP-ribosylation) (PAR) occurs after single or double-stranded DNA damage and represents the posttranslational modification of histones and other nuclear proteins by PARP. Based on conserved genetic sequences, encoded for by 18 different genes, 18 nuclear proteins have been classified as members of the PARP superfamily. The superfamily is further subdivided into three branches, the PARP-1 group, the tankyrase group, and other PARP enzymes. The PARP-1 group of NAD<sup>+</sup>-dependent enzymes has been extensively studied, and its members PARP-1 and PARP-2 are generally considered as the primary enzymes involved in DNA repair.<sup>5</sup>

PAR has been implicated in many cellular processes including replication, transcription, differentiation, gene regulation, protein degradation, and spindle maintenance. Enhanced PARP-1 expression and/or activity in tumor cells, as compared to normal cells, has been demonstrated in malignant lymphomas<sup>6</sup>, hepatocellular carcinoma<sup>7</sup>, cervical carcinoma<sup>8</sup>, colorectal carcinoma<sup>9</sup>, non-Hodgkin's lymphoma<sup>10</sup>, leukemic lymphocytes<sup>11</sup>, and colon adenomatous polyps.<sup>12</sup> In SCLC, we identified high PARP-1 expression in a panel of 35 SCLC cell lines by proteomic profiling.<sup>13</sup> This observation was confirmed in archival SCLC tumors by immunohistochemistry (IHC). The mean IHC score in SCLC tumors was 262/300 (n=12), as compared to 104/300 (n=24) and 120/300 (n=15) in lung adenocarcinoma and squamous cell carcinoma, respectively (see [Figure 2](#)).



**Fig. 2.** PARP1 highly expressed in SCLC relative to other lung cancers.

PARP-1 and PARP-2 are nuclear proteins and are the only members of the PARP family with zinc-finger DNA binding domains. These domains localize PARP-1 and PARP-2 to the site of DNA damage. PARP-1 is highly conserved and has three structural domains (N-terminal DNA-binding domain; automodification domain, and the NAD<sup>+</sup>-binding domain). The catalytic domain is located at the C-terminus end of the protein. In knockout mouse models, deletion of PARP-1 is sufficient to impair DNA repair.<sup>14-16</sup> The residual PARP-dependent repair activity (~ 10%) is due to PARP-2. This suggests that only PARP-1 and PARP-2 need to be inhibited to impair DNA repair.<sup>17-19</sup>

The zinc finger domain of PARP binds to both single- and double-stranded DNA breaks, resulting in increased catalytic activity.<sup>17,19,20</sup> Once activated, PARP cleaves NAD<sup>+</sup> and attaches multiple ADP-ribose units to the target nuclear protein. This results in a highly negative charge on the target protein and affects its function. Overactivation of PARP can be induced by DNA damage, leading to the depletion of NAD<sup>+</sup> and energy stores and, thus, cellular demise by necrosis. An alternate mechanism has been identified where PARP overactivation can induce cell death through apoptosis by releasing the Apoptosis Inducing Factor (AIF) from mitochondria.<sup>21</sup> Consequently, multiple mechanisms to prevent overactivation of PARP exist. First, auto-PAR negatively regulates PARP activity.<sup>22</sup> In addition, the cleavage of PARP by caspases yields a peptide fragment that acts as a trans-dominant negative inhibitor for uncleaved PARP. PAR of proteins is a dynamic process with a short half-life ( $t_{1/2}$ ) of <1 min. The enzymes responsible for degrading these polymers are poly(ADP-ribose) glycohydrolase (PARG), which cleaves ribose-ribose bonds, and ADP-ribosyl protein lyase, which removes the protein proximal to the ADP-ribose monomer.

Increased PARP activity is one of the mechanisms by which tumor cells avoid apoptosis caused by DNA damaging agents. PARP activity is essential for the repair of single-stranded DNA breaks through the base excision repair (BER) pathways.<sup>19,23</sup> Therefore, inhibition of PARP sensitizes tumor cells to cytotoxic agents (*e.g.* alkylators [temozolomide, cyclophosphamide, BCNU] and topoisomerase I inhibitors [irinotecan, camptothecin, topotecan]) which induce DNA damage that would normally be repaired through the BER system. A significant therapeutic window appears to exist between a PARP inhibitor's ability to potentiate therapeutic benefit *versus* potentiation of undesirable side effects. As expected, PARP inhibitors do not potentiate agents that do not cause DNA damage.

Ionizing radiation induces both double- and single-stranded DNA breaks. While part of the radiosensitization caused by PARP inhibition is through the inhibition of the single-stranded break repair pathways, it appears likely that repair of double-stranded



breaks, which are thought to be more cytotoxic, is also affected. Double-stranded breaks are strong activators of PARP-1, resulting in PARP-1 mediated activation of DNA-PK and Ku80, important components of the non-homologous end-joining (NHEJ) double-stranded break repair pathway.<sup>24,25</sup> Also, small molecule inhibitors of PARP can directly inhibit the repair of double-stranded breaks.<sup>14,26</sup> Thus, it is likely that PARP activity is important for repair of both the single- and double-stranded stranded DNA breaks caused by ionizing radiation.

PARP activity is also known to be important for endothelial function and, in preclinical models, PARP inhibition abrogated endothelial migration and vessel sprouting, and reduced angiogenesis *in vivo*.<sup>27-29</sup> Consistent with these findings, PARP-1 knockout mice demonstrated reduced levels of growth factor-induced angiogenesis.<sup>27</sup> These studies suggest that PARP inhibition may also exert antiangiogenic effects in addition to its anti-tumor cell effects.

### Nonclinical Activity

*In vitro*, ABT-888 inhibited PARP-1 and PARP-2 with  $K_i$  values of 3.6 nM and 2.9 nM, respectively. These values were observed in enzyme assays measuring the incorporation of [<sup>3</sup>H]-NAD<sup>+</sup> into histone H1, an important physiological substrate of PARP. In assays measuring inhibition of H<sub>2</sub>O<sub>2</sub>-induced poly(ADP-ribosyl)ation in C-41 cervical carcinoma cells, ABT-888 inhibited PARP with an EC<sub>50</sub> value of 2.4 nM. The extent of DNA damage in cells was indicated by  $\gamma$ -H2AX levels. To determine the effect of ABT-888 in combination with cytotoxic agents on DNA damage, the cellular content of  $\gamma$ -H2AX in C-41 cells was assayed by flow cytometry using an anti- $\gamma$ -H2AX antibody. Addition of 1 mM of temozolomide alone resulted in increased numbers of  $\gamma$ -H2AX foci, a result which was further potentiated by ABT-888 in a dose-dependent manner. When cell survival was measured by an AlamarBlue assay, ABT-888 potentiated cytotoxicity in the same concentration range as used in the  $\gamma$ -H2AX assay, demonstrating that ABT-888 potentiates cytotoxicity of temozolomide by delaying DNA repair. ABT-888 achieved a maximal potentiation of approximately 15-fold. ABT-888 also potentiates the DNA damage caused by irinotecan.

The combination of PARP inhibitors with different classes of chemotherapeutics was examined. Cisplatin-induced potentiation was observed in a long-term clonogenic assay, but not in the short-term cytotoxicity assay. The potentiation of cisplatin by ABT-888 *in vitro* is consistent with the potent enhancement of the efficacy of platinum agents (cisplatin and carboplatin) observed *in vivo*. PARP inhibition was shown to sensitize cells that are mismatch repair (MMR)-deficient to a greater extent than cells that are MMR competent.<sup>30</sup> Alkylating agents such as temozolomide form methyl adducts in DNA and resistance is frequently encountered in the clinic with either the overexpression of O<sup>6</sup>-alkylguanine DNA alkyltransferase (AGT) or functional defects in the MMR system. However, when PARP was inhibited, cells were sensitized to methylpurine formation, regardless of their resistance factors.<sup>31</sup>

Recently, the interaction between ABT-888 and cytotoxic chemotherapy in a panel of SCLC cell lines (H146, H69, H187, H209, and H182) was evaluated.<sup>32</sup> Exponentially

growing cells were treated by continuous exposure for 24-48 hours to vehicle, ABT-888 and cytotoxic agents (cisplatin, carboplatin, etoposide) in the presence or absence of ABT-888. As a single agent, ABT-888 showed negligible cytotoxicity. Co-administration of ABT-888 and chemotherapeutic agents demonstrated enhanced cytotoxicity of cisplatin, carboplatin and to a lesser extent, etoposide, in a cell-specific and dose dependent manner. However, there was no sequence dependence interaction between ABT-888 and the cytotoxic agents. These results support clinical evaluation of ABT-888 with chemotherapy in SCLC.<sup>32</sup>

There are data to suggest that PARP inhibitors have activity against some BRCA-deficient cells in the absence of any DNA damaging agent.<sup>33,34</sup> These inhibitors did not demonstrate single agent activity in BRCA-competent cells, and restoring functional BRCA to deficient cells abrogated single agent cytotoxicity. It is possible that, in BRCA-deficient cells, PARP inhibition stops the BER pathway, and thus single-stranded breaks are carried through DNA synthesis, resulting in double-stranded breaks. The increase in double-stranded breaks cannot be repaired by homologous recombination (HR), due to the lack of BRCA1 or 2, resulting in increased cell death. However, since not all BRCA deficient cells are sensitive to the PARP inhibitors, it is unclear why single agent cytotoxicity is observed in some BRCA-deficient cells.

Consistent with PARP-1 being a radiosensitization target, PARP-1 knockout mice showed enhanced sensitivity to  $\gamma$ -radiation.<sup>35,36</sup> There is evidence to suggest that PARP inhibitors sensitize cancer cells to radiation, both *in vitro* and *in vivo*.<sup>37-39</sup> Furthermore, a PARP inhibitor in the same class as ABT-888 potentiated radiation in the HCT116 colon carcinoma model. ABT-888 was tested, in combination with cytotoxic agents, in several tumor models and demonstrated a similar profile of antitumor activity to that seen in the literature (See [Table 1](#) below). ABT-888 substantially increased the efficacy of cytotoxic therapies, when measured by either treated/control tumor volumes (%T/C) or by increased time for tumors to grow to a particular size (%ILS).

**Table 1. Preclinical data for ABT-888 mediated potentiation of cytotoxic agents**

	<b>Breast carcinoma (human MX-1)</b>	<b>Glioblastoma multiforme (rat 9L)</b>	<b>B cell lymphoma (human DOHH2)</b>	<b>Melanoma (murine B16F10)</b>	<b>Small Cell Lung Cancer</b>
Carboplatin	Yes				Yes
Cisplatin	Yes		No		Yes
Cyclophosphamide	Yes				
Irinotecan				Yes	
Temozolomide		Yes		Yes	Yes

ABT-888 potentiated cytotoxic therapy when administered either parenterally or orally (PO). When administered parenterally, significant efficacy was observed at doses as low as 1 mg/kg/day, and maximal efficacy was achieved at approximately 12.5 mg/kg/day. 3.1 mg/kg/day PO (divided, twice daily) provided significant potentiation, with maximal potentiation achieved at approximately 25 mg/kg/day. No increased

toxicity was observed at any of these ABT-888 doses, either parenteral or PO. Supratherapeutic doses of ABT-888 (50 mg/kg/day), administered via osmotic minipump (OMP), resulted in skin toxicity at the pump implantation site. The observation that supratherapeutic doses of PARP inhibitors may potentiate toxicity is consistent with preclinical and clinical observations. It is also consistent with the results from a two-week ABT-888/cisplatin combination study. When administered as a continuous infusion, an ABT-888  $C_{ss}$  (plasma concentration at steady-state) of 70 ng/mL was maximally efficacious (area under the curve [AUC]=1.7  $\mu\text{g}\cdot\text{hr}/\text{mL}$ ). Comparable efficacy was seen in oral studies at a 25 mg/kg/day (divided, twice daily) dose that yielded AUCs between 1.6 and 3.0  $\mu\text{g}\cdot\text{hr}/\text{mL}$ . At this dose, the plasma concentrations were above 70 ng/mL for only 2-4 hours per dose, demonstrating that 24 hour/day coverage above 70 ng/mL was not required for efficacy.

An enzyme-linked immunosorbent assay (ELISA) that can measure PAR formation was used to demonstrate PARP inhibition in murine tumors *in vivo* and human peripheral blood mononuclear cells (PBMCs) *ex vivo* at clinically relevant doses. This ELISA was used as the primary assay for PARP biomarker analysis. The degree of PARP inhibition was assessed in B16F10 syngeneic flank tumors from mice treated *in vivo* using tumor efficacy schedules. In this study, PAR formation was measured in tumors treated with ABT-888 alone. Two hours after administration, ABT-888 inhibited PAR formation in B16F10 tumors in a dose-dependent manner. The same response was reflected in a parallel efficacy experiment, where temozolomide (50 mg/kg/day, PO, daily  $\times$  5) was administered with ABT-888. In another study, PAR formation was measured in tumors treated simultaneously with temozolomide and ABT-888. As in the ABT-888 only study, tumor PAR levels in the combination study were also inhibited. Inhibition of PARP activity was significant at 12.5, 5 and 1 mg/kg/day in both the vehicle and temozolomide treated groups. Overall, these results indicate the ability of ABT-888 to inhibit both baseline and cytotoxic-induced PARP activity in tumors treated *in vivo* and provide evidence of the ability of ABT-888 to target PARP *in vivo*.

Inhibition of PAR was similarly analyzed with *ex vivo* treatment of human PBMCs from eight healthy volunteers. The cells from one of the eight volunteers showed no detectable PARP activity, while in another patient, PARP activity was not assessable by the assay. In the remaining six individuals, not only were baseline levels of PAR detected, but more importantly, a dose-dependent inhibition of PAR was observed with *ex vivo* treatment with ABT-888. Inhibition occurred at 10 nM (2.4 ng/mL), and PAR formation was almost eliminated at 300 nM (71 ng/mL).

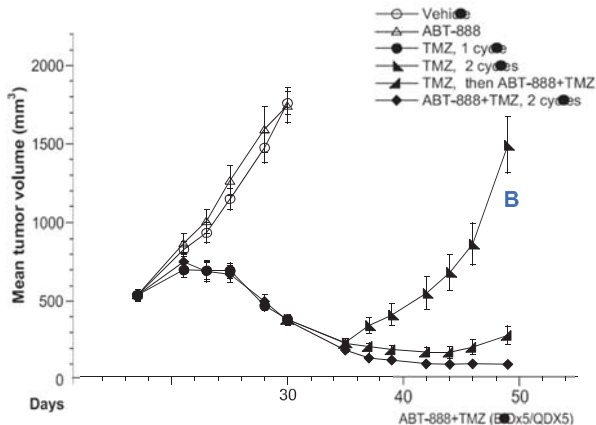
#### *ABT-888 and Temozolomide*

PARP inhibition has been shown to sensitize tumors to temozolomide. The B16 syngeneic melanoma model is moderately sensitive to temozolomide. Yet, when ABT-888 was administered orally in this model, there was significant potentiation of temozolomide (62.5mg/kg/day for five days) in a dose-dependent manner. Maximum potentiation was seen at 19 days with % T/C values (verses temozolomide) of 10 ( $P = 0.0003$ ), 16 ( $P < 0.0001$ ), and 23 ( $P < 0.0001$ ), for the 25, 12.5 and 3.1 mg/kg/day ABT-888 combination groups, respectively. The combination was found to be well

tolerated with maximum body weight loss of 11% for the ABT-888 25mg/kg/day and temozolomide combination, compared with 7% for temozolomide and 2% for ABT-888. Once the dosing period ended, the mice rapidly regained weight.<sup>3,40</sup>

*In vivo* efficacy of ABT-888 in combination with temozolomide was shown in a syngeneic orthotopic 9L glioma model. Tumor growth inhibition with ABT-888 was dose dependent, with ABT-888 at 50 mg/kg/day in combination with temozolomide at 17mg/kg providing the most significant reduction in tumor volume compared to temozolomide alone ( $P < 0.005$ ). ABT-888 as a single agent was not efficacious in this model. Animal survival was significantly prolonged in the group receiving the combination of ABT-888 at 50 mg/kg/day with temozolomide versus temozolomide alone (median survival 22 versus 19 days, respectively;  $P < 0.0132$ ). The combination led to a weight loss of 9% compared to 8% for temozolomide alone. Further, drug concentration of ABT-888 was measured in the central nervous system two hours after dosing.<sup>40</sup>

The combination of ABT-888 (25 mg/kg/day, orally, twice daily for 5 to 6 days) with temozolomide (50mg/kg/day, orally, once daily for 5 days) was shown to have varying levels of sensitivity across a broad spectrum of models (55 to 100% tumor growth inhibition) in B-cell lymphoma, small cell lung carcinoma, non-small cell lung carcinoma, pancreatic, ovarian, breast and prostate xenografts.<sup>3</sup> In tumors where temozolomide monotherapy was minimal (B-cell lymphoma, BRCA-2 deficient pancreas, small cell carcinoma), the combination of ABT-888 and temozolomide showed marked *in vivo* efficacy; suggesting that temozolomide resistance may be overcome by PARP-inhibition. In experimental metastases models of prostate and breast cancers to the bone, brain and lung, that acquired resistance to temozolomide, profound efficacy was seen with ABT-888 and temozolomide combination treatment. Further, temozolomide resistance was overcome in crossover treatments, indicating that combination therapy may overcome temozolomide resistance. For example, in the Calu-6 NSCLC model (see [Figure 3](#) below), significant tumor growth inhibition was observed following initial treatment with temozolomide at 50 mg/kg/day, but the tumor was unresponsive to a second cycle of single agent temozolomide treatment. When the combination of temozolomide 50 mg/kg/day and ABT-888 25 mg/kg/day was administered, similar efficacy results were noted to that of temozolomide alone for the first cycle of treatment. However, in contrast to temozolomide alone, ABT-888 and temozolomide resulted in profound, sustained anti-tumor response through multiple cycles of treatment. This suggests that the addition of ABT-888 prevents resistance that rapidly develops towards temozolomide alone. Tumor MGMT, mismatch repair or PAR polymer did not correlate with the degree of sensitivity to ABT-888 and temozolomide.<sup>3</sup>



**Figure 3. Efficacy of ABT-888 in combination with temozolomide in Calu-6 NSCLC xenograft model.**

ABT-888 was administered at 25mg/kg/day, orally, twice daily (BID) for 5 days concurrently with temozolomide at 50mg/kg/d, orally once (QD) daily for 5 days. Here, significant temozolomide monotherapy activity was observed at early time point (time point A); and a second cycle of temozolomide proved ineffective (time point B). Yet, the combination produced sustained activity and led to tumor responses (time point B). Adapted from Palma, *et al.* 2008.

### Nonclinical Pharmacology and Toxicology

The pharmacokinetics (PK) of ABT-888 was evaluated in CD-1 mice, Sprague-Dawley rats, beagle dogs and cynomolgus monkeys. The non-clinical PK profile of ABT-888 was characterized by high plasma clearance (CL) values, ranging from a high of 4.1 L/hr•kg in the mouse to a low of 0.57 L/hr•kg in the dog. ABT-888 exhibits moderate volumes of distribution ( $V_{ss}$ ) in all species ( $V_{ss} > 2.0$  L/kg), with terminal elimination  $t_{1/2}$  in the 1.2-2.7 hr range. In rats and dogs, [ $^3$ H]ABT-888 was rapidly absorbed and cleared primarily in the urine as intact parent drug. A-925088 (M8), a lactam derivative and the major product of ABT-888 metabolism, was also cleared primarily in the urine. In both rats and dogs, parent drug was the major component in systemic circulation, followed by M8. Elimination of total radioactivity was rapid, with most (>80%) of the dose recovered within 24 hours post-dose, indicating that parent drug and the major metabolites are not likely to accumulate. Bioavailability following an oral dose was high ( $F > 50\%$ ) in all species, with values ranging from a low of 56.1% in the monkey to a high of 92.0% in the mouse, and low animal-to-animal variability across all species.

The bioavailability from a non-formulated capsule was only slightly lower than from the solution formulation with values of 59.7% and 65.5% in fasted and non-fasted dogs, respectively. This suggests that there are no major food effects. The compound has high solubility at physiological pH and high permeability. Protein binding values in plasma (assessed *in vitro* as % bound at 5  $\mu$ M) for ABT-888 were moderate in all species averaging 42% in dog, 41% in monkey, 43% in mouse, 49% in rat and 51% in human. The stability of ABT-888 was evaluated in rat, dog, monkey and human plasma and the drug was found to be very stable, with minimal degradation over the 8-hour incubation interval. *In vitro* metabolism studies indicated that several CYPs (1A1, 1A2, 2C9 and 2C19) have the potential to mediate the formation of M8. However, ABT-888 is not a potent inhibitor of the major human CYPs *in vitro*, indicating a low risk for drug-drug interactions at the anticipated therapeutic concentrations. ABT-888 partitioned slowly into and out of the brain, in both mouse and rat, with high plasma to

brain ratios (~3:1) during the first 3-6 hours after dosing. The plasma to brain ratios approached 1:1 in samples obtained 12 hours after dosing.

PK parameters in humans were estimated by a variety of methods. The oral clearance (CL/F) of ABT-888 was estimated as a function of the projected clearance after IV administration (CL) and the fraction of the dose systemically available after oral administration (F). Clearance predictions were based on allometric scaling. Bioavailability was estimated by simulations with sensitivity analyses using software which took into account human gastrointestinal physiology and the drug's physicochemical characteristics.  $V_{ss}$  was estimated either from an average of values observed in animal species, a method averaging the fraction unbound in animal tissues, or by allometric scaling. Terminal phase  $t_{1/2}$  values were estimated either by regression relationships between animal and human  $t_{1/2}$  values<sup>41</sup>, or from the estimates of CL and  $V_{ss}$ . The human PK profile is projected to have CL=26 L/hr, with oral bioavailability of ~70%. The predicted human  $t_{1/2}$  of ABT-888 is ~4 hrs. Simulations of 50 mg twice daily dosing in humans mimic a maximally efficacious dosing regimen in mouse (12.5 mg/kg, twice daily), with concentrations above 71 ng/mL for 8 of 24 hours and an AUC<sub>24</sub> of 3  $\mu\text{g}\cdot\text{hr}/\text{mL}$  at steady state.

ABT-888 was tested in receptor-binding, CNS/neurobehavioral, cardiovascular, cardiac electrophysiological and gastrointestinal assays. In 74 receptor-binding assays at a concentration of 10  $\mu\text{M}$  (2.4  $\mu\text{g}/\text{mL}$ ), ABT-888 displaced control-specific binding at the human H<sub>1</sub> (61%), the human 5-HT<sub>1A</sub> (91%), and the human 5-HT<sub>7</sub> (84%) sites only, with IC<sub>50</sub> values of 1.2-5.3  $\mu\text{M}$ .

ABT-888 did not display clear adverse CNS effects in the rat and mouse between 3-30 mg/kg PO. At 100 mg/kg PO, mild sedation-like effects were observed, followed in time by mild excitation. At 300 mg/kg PO, more moderate to marked CNS effects were observed, including abnormal gait and sedation. Further, at 100 mg/kg, PO, there was an increased incidence of death after electrically-induced tonic convulsions in mice. Death was also noted in a second convulsant model (audiogenic seizures in mice). In a repeated dosing mini-Irwin observational test, in which rats were dosed with ABT-888 at 30, 100, and 300 mg/kg intraperitoneally (IP) every day for 5 days, tonic-clonic seizures/death were observed in approximately 50% of the animals treated at the highest dose on day 1. A similar incidence of seizures was observed after dosing the remaining animals at the same dose on each of the subsequent days. In an acute follow-up study with rats dosed with ABT-888 300 mg/kg IP, protection against seizures was not provided by pretreatment with either valproic acid (300 mg/kg IP, 15 min prior to ABT-888) or diphenylhydantoin (75 mg/kg IP, 100 min prior to ABT-888). In a 2-week toxicology study, seizures were also noted in dogs treated with ABT-888 at either 60 mg/kg/day, 30 mg/kg twice daily, or 30 mg/kg every day. Plasma concentrations in dogs with seizures were in excess of 5.4  $\mu\text{g}/\text{mL}$  (26-fold the predicted clinical  $C_{max}$  of 0.21  $\mu\text{g}/\text{mL}$ ).

In the anesthetized dog, ABT-888 produced no physiologically relevant changes in mean arterial pressure, heart rate,  $dP/dt_{max}$ , pulmonary arterial pressure, or systemic or pulmonary vascular resistance compared to vehicle controls at mean plasma

concentrations as high as  $4.45 \pm 0.13 \mu\text{g/mL}$  (21-fold the predicted clinical  $C_{\text{max}}$  of  $0.21 \mu\text{g/mL}$ ). As mean plasma concentrations increased to  $12.96 \pm 0.92 \mu\text{g/mL}$  (62-fold), ABT-888 produced a modest reduction in mean arterial pressure ( $-16 \pm 5\%$  below baseline) and systemic vascular resistance ( $-10 \pm 7\%$  below baseline).

ABT-888 blocked hERG current with an  $\text{IC}_{50}$  value of  $57.6 \pm 1.7 \mu\text{g/mL}$  ( $236 \pm 7 \mu\text{M}$ ), a value 278-fold higher than the predicted clinical  $C_{\text{max}}$ . The M8 metabolite of ABT-888 (A-925088) minimally affected hERG at the highest concentration tested ( $81.5 \mu\text{g/mL}$ ). While no effect on repolarization (*in vitro* action potential duration measures) was noted at the lowest measured concentration of ABT-888 ( $0.42 \mu\text{g/mL}$ , 2-fold higher than the predicted clinical  $C_{\text{max}}$ ), ABT-888 prolonged the action potential duration at the intermediate and highest measured concentrations (4.8% and 18.6% prolongation at  $4.22 \pm 0.02$  and  $39.49 \pm 0.70 \mu\text{g/mL}$  respectively), suggesting delayed repolarization risk between 20- and 190-times the  $C_{\text{max}}$ . There was a trend (7%) towards delayed repolarization in the anesthetized dog model (QTc intervals) at plasma concentrations 21-fold higher than the predicted clinical  $C_{\text{max}}$ ; greater concentrations elicited prolongation ( $15 \pm 3\%$  above baseline [QTcV] at  $12.96 \pm 0.92 \mu\text{g/mL}$ ). In humans, QTc prolongation is predicted to be less than 3 msec at the anticipated dose of 50 mg twice daily. These cardiac effects need to be monitored during clinical trials.

Gavage administration of ABT-888 up to 10 mg/kg was generally well tolerated in the ferret emesis model. No emesis was noted at this dose (resulting in mean plasma concentrations of  $3.80 \pm 0.11 \mu\text{g/mL}$ , a value 18-fold greater than the predicted  $C_{\text{max}}$ ), with significant emesis noted in response to the 20 mg/kg dose (resulting in mean plasma concentrations of  $6.61 \pm 0.26 \mu\text{g/mL}$ , a value 31-fold greater than predicted  $C_{\text{max}}$ ). Parenteral (subcutaneous) dosing of ABT-888 at doses and plasma concentrations similar to those used in the gavage study revealed a similar emetic dose-response relationship, suggesting a centrally-mediated emetic response. ABT-888 had no significant effect on gastrointestinal transit up to 100 mg/kg (resulting in a mean plasma concentration of  $1.63 \pm 0.14 \mu\text{g/mL}$ , a value 7-fold greater than the predicted clinical  $C_{\text{max}}$ ).

ABT-888 dihydrochloride was evaluated in repeated dose toxicity studies in rats and dogs. When administered as a sole agent to rats, the compound did not result in adverse effects at  $C_{\text{max}}$  values that were greater than 19-fold the estimated therapeutic peak plasma drug concentration (highest dose tested). When rats were administered ABT-888 dihydrochloride in conjunction with a cytotoxic agent (cisplatin), no clinically meaningful exacerbations of cisplatin-associated toxicity were apparent at  $C_{\text{max}}$  values that were up to 8-fold greater for ABT-888 than the estimated therapeutic value. Exacerbation of cisplatin-associated toxicity was limited to rats that received ABT-888 dihydrochloride in conjunction with cisplatin at the highest dose that yielded  $C_{\text{max}}$  values 22-fold greater than the estimated therapeutic peak plasma drug concentration. In dogs, emesis, body weight losses related to anorexia, and convulsions were observed at doses of 30 mg base/kg/day with  $C_{\text{max}}$  values 26-fold greater than the estimated therapeutic peak plasma concentration. ABT-888 dihydrochloride was found to be negative *in vitro* for both mutagenicity and clastogenicity.

The non-toxic dose observed in the most sensitive mammalian species (beagle dogs) was 300 mg/m<sup>2</sup>. Emesis and QT prolongation were observed in animal models, at 31-fold and 21-fold higher concentrations than the predicted clinical C<sub>max</sub> (0.21 µg/mL), respectively. Based on different sensitivities to seizures between rodents and dogs, the plasma concentration that would be associated clinically with pro-convulsant activity will be difficult to define.

### **Clinical Investigations**

A single-dose pharmacokinetic and pharmacodynamic endpoint study in cancer patients was initiated under an exploratory IND by the National Cancer Institute as the initial study in their phase 0 program.<sup>42</sup> In this study, participants had baseline assessments of PAR in peripheral blood mononuclear cells (PBMCs) and at higher dose levels, in tumor from needle biopsies, assessed by a validated immunoassay. Participants received a single dose of ABT-888 at 10mg (n = 3), 25mg (n = 3), or 50 mg (n = 7). PBMCs were collected over a 24 hour period at all dose levels, and tumor biopsies were obtained at the 25 mg dose level, approximately 3 to 6 hours after administration of ABT-888. The pharmacokinetic results from this study demonstrated that ABT-888 is orally bioavailable and primarily cleared through renal excretion, with a half-life of 4 to 5 hours. Additionally, this study provided proof of mechanism, as tumor PARP inhibition (> 90%) was demonstrated in 5 out of 6 human tumor biopsies that were obtained 3 to 6 hours after dosing with either 25 mg or 50 mg ABT-888. No serious adverse events, dose limiting toxicities (DLTs), or deaths were reported for this study.<sup>42</sup>

Multiple Phase I studies of ABT-888 in combination with various chemotherapeutic agents are ongoing, as are Phase II clinical trials.

Study M06-862 is a Phase I dose-escalation study designed to assess the safety, tolerability, and pharmacokinetic profile of ABT-888 in combination with temozolomide in subjects with non-hematologic malignancies, including metastatic melanoma, *BRCA*-deficient breast, ovarian, primary peritoneal or fallopian tube cancer, and hepatocellular carcinoma (HCC). A dosing cycle consists of 7 days on ABT-888 (Days 1 through 7) and 5 days on temozolomide (Days 1 through 5), with a subsequent 21-day rest period. As of 24 March 2009, 30 subjects were enrolled into the trial. For the dose-escalation portion of the study, cohorts of 3 subjects each were treated with the following doses of ABT-888: 10 mg BID in combination with 150 mg/m<sup>2</sup> temozolomide QD, or 20, 40, 60, or 80 mg BID in combination with 200 mg/m<sup>2</sup> temozolomide QD. No DLTs were reported for the first 3 dose cohorts (10, 20, and 40 mg BID). In cohorts receiving 60 mg and 80 mg, events of Grade 4 thrombocytopenia and neutropenia were reported. The MTD combination dose was defined as ABT-888 40 mg BID and temozolomide 200 mg/m<sup>2</sup> QD (Clinical Brochure for ABT-888, Abbott, 2010).



Although in the M06-862 Phase I study no DLTs were reported in subjects receiving 40 mg ABT-888 BID/200 mg/m<sup>2</sup> temozolomide QD, 8 of all 12 subjects receiving this dose in the expanded safety cohort experienced Grade 3 or 4 neutropenia and/or thrombocytopenia. In general, subjects with these events recovered after dose reduction and remained on study. In order to minimize these hematologic toxicities, the protocol was modified to treat new subjects with 40 mg ABT-888 BID in combination with 150 mg/m<sup>2</sup> temozolomide QD in the first cycle. If a subject does not experience grade 3 or 4 hematologic toxicities or other clinically significant NCI Common Terminology Criteria for Adverse Events (CTCAE) toxicities greater than grade 3, the temozolomide dose may be escalated to 200 mg/m<sup>2</sup> QD (Clinical Brochure for ABT-888, Abbott, 2010).

Study M10-128 is an ongoing Phase I study designed to assess the safety, tolerability, and pharmacokinetic profile of ABT-888 continuous dosing administered concurrently with conventional whole brain radiation therapy (WBRT) in patients with brain metastases for up to 15 consecutive days. The initial dose of ABT-888 was 10 mg BID, and was escalated in subsequent cohorts. As of 24 March 2009, 17 subjects were enrolled and preliminary safety data are available for 12 subjects. All 12 subjects (100%) experienced at least 1 adverse event. The most common adverse events, reported in > 20% of all subjects, were fatigue (8 subjects, 66.7%), headache (7 subjects, 58.3%), alopecia (6 subjects, 50.0%), radiation skin injury (5 subjects, 41.7%), nausea and decreased appetite (4 subjects, 33.3% each), and dizziness and insomnia (3 subjects, 25%).

Study A10-153 is an ongoing NCI sponsored Phase I study designed to assess the safety and tolerability of ABT-888 in combination with carboplatin/paclitaxel. The trial is currently ongoing and has accrued subjects up to the dose level of 50 mg ABT-888 plus carboplatin (AUC = 6) and paclitaxel (200 mg/m<sup>2</sup>). The MTD has not been reached.

Study M10-440, is a randomized, double-blinded, placebo-controlled Phase II study evaluating ABT-888 in combination with temozolomide versus temozolomide alone in subjects with metastatic melanoma. The primary objective of the study is to assess whether ABT-888 administered in combination with temozolomide can prolong PFS compared with temozolomide alone in subjects with metastatic melanoma. Subjects are randomized into 1 of 3 arms in a 1:1:1 ratio. One cohort of subjects receives temozolomide with placebo. Subjects in the second and third arms both receive temozolomide, and 40 or 20 mg BID of ABT-888, respectively. Temozolomide is administered at 150 mg/m<sup>2</sup> per day in the first cycle; the dose may be escalated to 200 mg/m<sup>2</sup> in subsequent cycles if the subject does not experience grade 3 or 4 neutropenia or thrombocytopenia, or other clinically significant CTCAE toxicities greater than grade 3. Temozolomide is administered orally on Days 1 through 5 of each 28-day cycle, and oral ABT-888 or oral placebo is administered on Days 1 through 7 of each cycle. This study is ongoing, but not recruiting further patients.

Preliminary results of a Phase II trial of ABT-888 and temozolomide in metastatic breast cancer patients were recently reported.<sup>43</sup> Forty-one previously-treated patients were enrolled and received temozolomide 150mg/m<sup>2</sup>/day on days 1 to 5 with ABT-888 40mg twice daily on days 1 to 7 of a 28-day cycle. Due to increased episodes of grade 4 thrombocytopenia (n = 4), ABT-888 was decreased to 30mg twice daily. Of the 24 patients evaluable for response in early 2010, best response was noted as follows: complete response (n = 1), partial response (n = 3), stable disease (n = 7) and progression of disease (n= 14). The authors concluded that the combination of ABT-888 and temozolomide was active in metastatic breast cancer.<sup>43</sup>

## 2.2 Temozolomide

Temozolomide is a non-classic oral alkylating agent, which produces O<sup>6</sup>-alkyl-guanine (O<sup>6</sup>-AG) lesions on DNA. The DNA-repair protein O<sup>6</sup>-AG DNA alkyltransferase, which is encoded by the O<sup>6</sup>-methyl-guanine-DNA methyltransferase (*MGMT*) gene, removes alkyl groups from the O<sup>6</sup> 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.<sup>44,45</sup> 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.<sup>44,45</sup> Temozolomide has good penetration into the central nervous system and has been approved for use in patients with newly diagnosed glioblastoma multiforme concomitantly with radiotherapy and then as maintenance treatment, as well as for use 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.<sup>46</sup>

Temozolomide has also been found to induce endothelial apoptosis and inhibit tumor vasculature in preclinical models, suggesting that its effects may be due at least in part to antiangiogenic effects.<sup>47,48</sup>

Temozolomide has been studied at multiple different dosing schedules, as well as in melanoma and in other solid tumors. Several phase II trials have been reported using temozolomide in patients with brain metastases from solid tumors, including small cell lung cancer (SCLC). In one of the studies, two out of five patients with SCLC pretreated with WBRT, showed disease stabilization with temozolomide.<sup>49-51</sup>

We are conducting a Phase II study of single-agent temozolomide in patients with relapsed or refractory SCLC, given as second- or third-line therapy (Pietanza *et al.*, 2011, in preparation). Patients with brain metastases are eligible. The protocol originally was designed to evaluate temozolomide at 75mg/m<sup>2</sup>/day for 21 out of 28 days. Sixty-four patients were accrued and there was one complete response and 12 partial responses, for a 20% overall response rate (ORR) [95% CI: 11% to 32%]. Six patients (9%) 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%; one-sided 95% CI: 7% to 365%], *p* = 0.99. Prior to starting temozolomide, 24 patients had progressive brain metastases,

13 of which included target lesions according to RECIST<sup>52</sup> that were assessable for response. Four patients obtained a complete response and one a partial response in the brain, for a 38% ORR [95% CI: 14% to 68%]. Responses in the brain correlated with systemic responses to temozolomide. The median duration of response to temozolomide was 3.5 months (range, 1.4 to 14.7 months; mean 4.1 months). Median progression-free survival (PFS) 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 (Pietanza *et al.*, 2011, in preparation). For the 48 sensitive patients, median PFS 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. One-year survival for those with sensitive relapse was 21% [95% CI: 11% to 39%]. The median PFS 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. No patients with refractory disease were alive at 1 year.

The protocol has been amended so that an additional 25 patients are accrued to evaluate temozolomide at the standard dosing regimen of 200mg/m<sup>2</sup>/day for 5 days of a 28-day cycle (ClinicalTrials.gov Identifier: NCT00740636). Thus far, 15 patients have been enrolled since January 2011, nine patients with sensitive disease and six patients with refractory disease. Of the 14 evaluable patients, we have observed: confirmed partial response (n = 1); unconfirmed partial response (n = 2), and stable disease (n = 1).

Temozolomide is well-tolerated. The primary dose-limiting toxicity is myelosuppression. In general, the incidence of grade 3 and 4 neutropenia and thrombocytopenia is less than 10%, and this is reversible and non-cumulative. The nadir occurs in the fourth week. Non-hematologic toxicity includes mild to moderate nausea, vomiting, constipation and fatigue. Nausea and vomiting are controlled by anti-emetic medication.

In our study, we found that the protracted dosing schedule (75mg/m<sup>2</sup>/day for 21 days of a 28-day cycle) led to prolonged thrombocytopenia (grade  $\geq 2$ ) for  $\geq 4$  weeks in a subgroup of our patients. Further therapy could not be administered in seven patients due to prolonged thrombocytopenia and neutropenia. Four of these patients underwent bone marrow biopsies. 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. Of the 64 patients, grade  $\geq 3$  thrombocytopenia, neutropenia and neutropenic fevers were noted in 6 patients (9%), 3 patients (5%) and 1 patient (2%), respectively. Non-hematologic toxicities included grade  $\leq 2$  fatigue (64%), nausea (48%), vomiting (33%), and constipation (28%). There were no treatment-related deaths.

In an attempt to decrease the amount of prolonged thrombocytopenia in our patients with SCLC, we have amended the protocol to evaluate an additional 25 patients at the standard dosing regimen of 200mg/m<sup>2</sup>/day for 5 days of a 28-day cycle, as noted above

(ClinicalTrials.gov Identifier: NCT00740636). The protocol does allow for a dose reduction to 150mg/m<sup>2</sup>/day if thrombocytopenia or neutropenia are noted. Thus far, 14 patients have been enrolled since January 2011. Thrombocytopenia has been noted in three patients (one patient each grade 1, 2 and 3), yet for less than 2 weeks.

As noted above, a Phase II trial of ABT-888 and temozolomide in metastatic breast cancer patients was recently reported.<sup>43</sup> Forty-one previously-treated patients were enrolled and received temozolomide 150mg/m<sup>2</sup>/day on days 1 to 5 with ABT-888 40mg twice daily on days 1 to 7 of a 28-day cycle. Here, increased episodes of grade 4 thrombocytopenia (n = 4) were noted and ABT-888 was decreased to 30mg twice daily.<sup>43</sup>

### 2.3 Small Cell Lung Cancer

In 2010, there were an estimated 29,000 new cases of small cell lung cancer in the United States.<sup>53</sup> Compared to non-small cell lung cancer (NSCLC), SCLC has a unique natural history with a shorter doubling time, higher growth fraction, earlier development of widespread metastases, and uniform initial response to chemotherapy and radiation.

First line treatment for SCLC involves combination chemotherapy with cisplatin or carboplatin plus etoposide (with the addition of radiation therapy in limited stage), which results in a 60-80% overall response rate. However, all patients with extensive stage SCLC, and the majority of patients with limited SCLC, suffer relapse within months of completing initial therapy. The strongest predictor of outcome for patients with relapsed disease is the duration of remission. Patients who maintain an appropriate response to initial therapy for 3 months or more are deemed “sensitive”. These patients have a higher likelihood of response to second-line chemotherapy, though at best it is half that expected in the first-line setting, and their survival averages around 6 months. Patients with “refractory” disease either had no response to initial therapy, or progressed within 3 months after completing treatment. Their chance of response to second-line therapy is less than 10%, and survival is 3 to 4 months.<sup>1</sup>

Single agent topotecan or CAV (cyclophosphamide, doxorubicin and vincristine) are appropriate treatment regimens for patients with sensitive relapse. A randomized trial comparing topotecan to CAV showed a response rate of 24% for topotecan and 18% for CAV, which was not statistically significant, with median survival of 6 months for both.<sup>2</sup> In this trial, sensitive disease was defined as relapse 60 days (or 2 months) after the completion of first line chemotherapy. This study led to the U.S. Food and Drug Administration’s approval of intravenous topotecan with an indication for sensitive relapsed SCLC (> 60 days from completion of first line treatment).

In three single arm phase II studies, topotecan was studied in patients with recurrent or progressive SCLC after treatment with first line chemotherapy. The three studies stratified patients for sensitive disease (response to initial therapy for  $\geq$  90 days) or refractory disease (no response to initial therapy, or progression within 90 days after completing treatment) and included patients with stable brain metastases.<sup>54-56</sup> The

European Organization for Research and Treatment of Cancer study reported a 21.7% overall response rate (37.8% among sensitive patients, 6.4% among refractory patients) and a 33-week median duration of response.<sup>54</sup> Depierre et al. showed a 14% response rate (median survival, 25.7 weeks) in sensitive patients and a 2% response rate (median survival, 16.3 weeks) in refractory patients.<sup>55</sup> In the third study, the response rates were similar - 19% (median survival, 26.6 weeks) and 3% (median survival, 20.4 weeks) for patients with sensitive and refractory disease, respectively.<sup>56</sup> A pooled analysis of the sensitive patients treated with second-line topotecan in these three SCLC studies reported an 18% response rate.<sup>57</sup>

Oral topotecan was evaluated in a randomized phase III trial and was beneficial in both sensitive and refractory disease patients, with a partial response of 7% and prolonged overall survival at 26 weeks compared to 14 weeks with best supportive care.<sup>58</sup> Currently, intravenous and oral topotecan remain the only drug approved for second-line treatment of SCLC.

Brain metastases are common in this disease. At the time of presentation, at least 18% of patients with SCLC have brain metastases.<sup>59</sup> During the course of the disease, the incidence of metastases increases, and has been reported as high as 80% at 2 years.<sup>60</sup> A recent study showed that the response rate of asymptomatic brain metastases is much lower than the systemic response in patients receiving first line treatment for SCLC.<sup>59</sup> Prophylactic cranial irradiation reduces the risk of developing brain metastases by about 50% and has been shown to improve survival, even in patients with extensive stage disease.<sup>61,62</sup>

The prognosis for patients with recurrent SCLC is poor, especially for those with refractory disease. Furthermore, there are no accepted regimens for patients who have failed first- and second-line treatments for SCLC. As few options exist, there is a clear need for new therapeutic agents.

## 2.4 Rationale

### Temozolomide:

There is strong rationale to study temozolomide in patients with SCLC. Alkylating agents have single agent efficacy in SCLC.<sup>63</sup> Temozolomide crosses the blood-brain barrier, and brain metastases are common in this disease.<sup>60</sup> SCLC has aberrantly methylated *MGMT*.<sup>45,64</sup> Anecdotal responses to temozolomide have been noted in SCLC.<sup>65</sup> Further, our Phase II study assessing the efficacy and safety of temozolomide in SCLC patients sensitive and refractory to first-line platinum-based chemotherapy has shown promising results and the agent merits further evaluation (Pietanza *et al.*, 2011, in preparation).

The Phase II study originally was designed to evaluate temozolomide at 75mg/m<sup>2</sup>/day for 21 out of 28 days, given as second- or third-line therapy. Sixty-four patients were accrued and there was one complete response and 12 partial responses, for a 20% overall response rate (ORR) [95% CI: 11% to 32%]. In the sensitive group, defined as progression or relapse 60 days after the completion of first-line chemotherapy<sup>2</sup>, one CR

and 10 PRs were noted for a 23% ORR [95% CI: 12% to 37%]. An additional patient had a PR that was not confirmed on follow-up imaging and therefore, was not included in the ORR. In the refractory cohort, defined as progression during initial therapy or within 60 days after completing first-line treatment<sup>2</sup>, two PRs were seen for a 13% ORR [95% CI: 2% to 38%]. 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%],  $p = 0.99$ . Responses also were noted in patients with progressive brain metastases [38% ORR, 95% CI: 14% to 68%]. The median duration of response to temozolomide was 3.5 months (range, 1.4 to 14.7 months; mean 4.1 months). Median progression-free survival (PFS) and median 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 patients with sensitive disease, median PFS and median 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. The median PFS and median 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 patients with refractory disease (Pietanza *et al.*, 2011, in preparation). Overall, single agent temozolomide was well tolerated in this patient population, with myelosuppression being the most common grade  $\geq 3$  toxicity.

The protocol has been amended so that an additional 25 patients are accrued to evaluate the safety of temozolomide at the standard dosing regimen of 200mg/m<sup>2</sup>/day for 5 days of a 28-day cycle (ClinicalTrials.gov Identifier: NCT00740636). This standard regimen will be the dosing schedule used here.

#### Temozolomide and ABT-888:

*MGMT* encodes the DNA-repair protein O<sup>6</sup> alkyl-guanine DNA alkyltransferase (MGMT).<sup>45</sup> Tumors with loss of MGMT activity, accomplished via hypermethylation of specific CpG islands of the *MGMT* promoter, have improved disease response to alkylating agents.<sup>44,45</sup> Temozolomide is an oral alkylating agent that produces O<sup>6</sup> alkyl guanine lesions. Repair of these alkylated bases in DNA is performed by MGMT and a set of enzymes of the base excision repair (BER) pathway.<sup>66</sup> Ultimately, ineffective mismatch repair, removal of the methyl adducts to O<sup>6</sup> guanine by MGMT and interruption of the BER pathway can lead to temozolomide resistance.<sup>3,67,68</sup> The BER pathway is initiated by PARP-1, a member of the PARP family of enzymes, when it recognizes single strand breaks.<sup>69</sup> If PARP-1 is inhibited, single-strand breaks become double strand breaks and cell apoptosis occurs during DNA replication.<sup>68,70</sup> DNA repair inhibitors, such as ABT-888 may enhance temozolomide activity and overcome resistance.

Temozolomide is one of the most studied alkylating agents in combination with PARP-1 inhibitors, specifically ABT-888. The efficacy of the combination of ABT-888 and temozolomide has been observed pre-clinically in SCLC.<sup>3</sup> Further, preclinical models in non-SCLC have shown that ABT-888 with temozolomide results in profound, sustained anti-tumor response through multiple cycles of treatment; suggesting that the addition of the PARP-1 inhibitor prevents resistance that develops towards

temozolomide alone.<sup>3</sup> Multiple phase I and II studies have documented the safety and efficacy of PARP-1 inhibitors and temozolomide in a variety of solid tumors.

In our current phase II trial of temozolomide in SCLC, we have demonstrated that this agent is effective and tolerable in patients that have progressed after standard first-line platinum-based therapy. However, after a median of 3.5 months (range, 1.4 to 14.7 months) [mean, 4.1 months] of treatment with single-agent temozolomide, the patients develop progression of disease. As stated above, many preclinical models demonstrated that the combination of PARP-1 inhibitors and temozolomide result in dramatic tumor growth delay or regression where temozolomide by itself is not efficacious.<sup>3</sup> Therefore, we believe that the addition of ABT-888 will overcome resistance to temozolomide and lead to improved response rates and outcomes in this patient population, and merits further study.

## 2.5 Correlative Studies Background

### 2.5.1 O<sup>6</sup> Methyl-Guanine-DNA Methyltransferase (MGMT)

The *MGMT* (O<sup>6</sup> methyl-guanine-DNA methyltransferase) gene is located on chromosome 10q26 and encodes the DNA-repair protein O<sup>6</sup> alkyl-guanine (O<sup>6</sup>-AG) DNA alkyltransferase (MGMT). This protein removes alkyl groups from the O<sup>6</sup> position of guanine, an important site of DNA alkylation. If O<sup>6</sup>-AG lesions are left unrepaired, carcinogenesis results. Therefore, AGT/MGMT expression is necessary for maintaining normal cell physiology and genomic stability leads. A broad range of expression of AGT/MGMT is noted across normal tissues and tumors.<sup>44</sup>

Alkylating agents such as temozolomide, dacarbazine, streptozotocin, and BCNU produce O<sup>6</sup>-AG lesions. Left unrepaired, chemotherapy induced lesions, especially O<sup>6</sup>-methylguanine, trigger cytotoxicity and apoptosis. Yet high levels of MGMT activity in cancer cells blunt the therapeutic effects of alkylating agents. Therefore, the presence of the MGMT protein may be an important determinant of treatment failure.<sup>44,45</sup>

Loss of MGMT activity is accomplished via hypermethylation of specific CpG islands of the *MGMT* promoter in tumor cells. Although such epigenetic silencing of the *MGMT* gene is associated with increased carcinogenic risk, it leads to improved sensitivity to alkylating agents.<sup>44</sup> Therefore, while tumors with a loss of *MGMT* expression may have a worse prognosis due to increased oncogene mutations, they have an improved disease response to chemotherapy, specifically alkylating agents such as temozolomide.<sup>44,45</sup> Aberrantly methylated *MGMT* is found in SCLC.<sup>45,64</sup>

### *MGMT and Temozolomide*

As part of the Phase III study performed by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC), which led to the approval of temozolomide in newly diagnosed glioma, a retrospective analysis evaluated *MGMT* promoter methylation status.<sup>46</sup> Irrespective of treatment, *MGMT* promoter methylation

was an independent favorable prognostic factor. Patients with tumors demonstrating methylation of the *MGMT* gene had a significant improvement in median survival as well as in 2-year survival rate (46% vs 14%). In the absence of methylation of the *MGMT* promoter, there was a smaller and statistically insignificant difference in survival between treatment groups. These findings strongly supported the impact of methylated *MGMT* promoter expression on response to temozolomide in patients with glioblastoma.<sup>46</sup>

In a retrospective study, archival neuroendocrine tumor specimens were assessed for the prevalence of MGMT deficiency by immunohistochemistry.<sup>71</sup> A cohort of these patients received treatment with a temozolomide-based regimen. Lack of MGMT expression correlated with treatment response to temozolomide in pancreatic neuroendocrine tumors.<sup>71</sup>

In our phase II temozolomide study in patients with SCLC, we obtained 38 tumor samples (59%) for *MGMT* promoter methylation analysis (N = 36), MGMT expression by immunohistochemistry (N = 31), or both (N = 29) (Pietanza *et al.*, 2011, in preparation). *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 time to progression 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 time to progression (3.6 months vs. 1.3 months,  $p = 0.1$ ) (Pietanza *et al.*, 2011, in preparation).

*MGMT* promoter methylation or MGMT expression may be important biomarkers in SCLC patients treated with temozolomide. However, the number of samples evaluated in the phase II study of temozolomide in SCLC was small. Larger number of samples will need to be analyzed to draw conclusions regarding *MGMT* promoter methylation or MGMT expression and temozolomide treatment in SCLC. If strong correlations can be made, MGMT may be used for the enrichment of patients in future trials using temozolomide.

### 2.5.2 Poly (ADP-ribose) Polymerase (PARP), BRCA-1 and RAD51

As stated previously, enhanced PARP-1 expression and/or activity in tumor cells, as compared to normal cells, has been demonstrated in multiple malignancies, including, lymphomas, hepatocellular carcinoma, cervical carcinoma, colorectal carcinoma, and non-Hodgkin's lymphoma.<sup>6-10</sup> Recent proteomic studies comparing NSCLC and SCLC cell lines, found that PARP-1 was highly expressed in SCLC compared to NSCLC.<sup>13</sup> In addition, PARP-1 is



overexpressed in SCLC and other neuroendocrine lung tumors and predicts response to PARP-1 inhibitors.<sup>13,72</sup>

Additional upregulation of DNA repair proteins have been noted in SCLC when compared to NSCLC, such as BRCA-1 and RAD51.<sup>13</sup> RAD51 is known to mediate DNA-double strand break repair, and its expression is linked to PTEN.<sup>73</sup>

### 2.5.3 Breast Cancer Gene (BRCA) and Phosphatase and Tensin Homolog (PTEN)

Synthetic lethality occurs when combining two non-lethal mutations, which individually have no effect, leads to a lethal phenotype.<sup>74</sup> Such an approach allows for the delivery of a drug having significant anti-tumor efficacy, with low toxicity.

As stated, PARP-1 plays a key role in the repair of single-stranded DNA breaks through the base excision repair (BER) pathways.<sup>19,23</sup> By inhibiting PARP, there is an accumulation of DNA single strand breaks, leading to DNA double strand breaks at replication forks. These breaks generally are repaired by the homologous-recombination double-stranded DNA repair pathway.<sup>75</sup> The tumor suppressor proteins, BRCA-1 and BRCA-2 are key components of the homologous-recombination pathway<sup>76</sup> and thus, cells mutant for *BRCA1* or *BRCA2* are deficient in double strand break repair. It has been shown that tumor cells with *BRCA* deficiencies are exquisitely sensitive to PARP inhibition even in the absence of any other insults<sup>33,34</sup>, which is described as “synthetic lethality”. PARP inhibitors administered as a single agent have been found to be efficacious and well-tolerated in breast, ovarian and prostate cancers associated with *BRCA1* or *BRCA2* mutation in several Phase I and II clinical trials.<sup>77-79</sup>

Overexpression of BRCA1 has been found in patients with SCLC<sup>80</sup> and is associated with decreased sensitivity to cisplatin<sup>81</sup> and poor survival in patients with NSCLC.<sup>82</sup>

Similarly, the tumor suppressor gene, *phosphatase and tensin homolog (PTEN)* has been shown to be involved in homologous-recombination. PTEN deficiency causes a homologous recombination defect in human tumor cells, which causes these cells to be exquisitely sensitive to PARP inhibitors.<sup>83</sup> In SCLC, there is approximately a 10-15% incidence of mutation of *PTEN*, as well as a lack of expression of PTEN.<sup>84-86</sup>

Thus, in a study evaluating PARP inhibitors in SCLC, the role of BRCA1 and PTEN should be explored.

### 2.5.4 Circulating Tumor Cells (CTCs)

Circulating tumor cells (CTCs), tumor-derived epithelial cells shed into the peripheral blood and likely represent the mechanism by which metastases are established<sup>87</sup>, may prove to be a useful tool in SCLC. The limited availability of tissue in SCLC has been a barrier to understanding the biology of this malignancy. New diagnostic tests are needed to aid in monitoring treatment response, assess risk status for patients, serially analyze tumor genotypes in a non-invasive manner, and eventually characterize the tumor progenitor cell or “cancer stem cell”, which may be the mechanism in which this disease circumvents treatment. CTCs may represent a source of tissue in SCLC that would enable us to accomplish these tasks.

CTC detection and enumeration correlate with the aggressiveness of tumors. In cancers of the breast, colon and prostate, CTCs have been found to be independent predictors of survival.<sup>88-91,92</sup> Patients with metastatic breast cancer with  $\geq 5$  CTCs per 7.5 mL of blood before treatment and at the first follow-up visit were found to have a statistically significant shorter progression-free survival and overall survival.<sup>88</sup> Danila and colleagues showed that the baseline CTC number in patients with progressive castration-resistant metastatic prostate cancer was strongly associated with survival, without a threshold effect.<sup>91</sup> Further, the authors demonstrated that the shedding of tumor cells into the circulation of patients with prostate cancer represents an intrinsic biology of the tumor, more closely related to osseous metastases and line of therapy, as opposed to burden of disease.<sup>91</sup>

Recently, CTCs have been measured in patients with Stage IV NSCLC using CellSearch.<sup>93</sup> Patients were divided into favorable ( $< 5$  CTCs) and unfavorable ( $\geq 5$  CTCs) prognostic groups according to baseline CTC number. In univariate analysis, progression-free survival was 6.8 vs 2.4 months ( $p < 0.001$ ) and overall-survival was 8.1 vs 4.3 months ( $p < 0.001$ ) for patients with fewer than five CTCs compared with five or more CTCs at baseline, respectively. CTC number was the strongest predictor of overall survival (hazard ratio, 7.92; 95% confidence interval, 2.85 to 22.01;  $p < 0.001$ ) in multivariate analysis. The authors concluded that CTCs may have prognostic utility in NSCLC, as well.<sup>93</sup>

The same research group explored the use of CTCs in patients with limited and extensive stage SCLC using the Veridex CellSearch System<sup>TM</sup>. The authors noted that 86% of patients had detectable CTCs at diagnosis and found that the number of CTCs decreased in response to chemotherapy.<sup>94</sup> Further, in univariate analysis, CTC number was found to be prognostic for patient survival.

In our ongoing phase II trial of temozolomide for patients with relapsed sensitive or refractory SCLC, CTCs are being enumerated before initiating temozolomide and at the time of repeat imaging (at four weeks, eight weeks, and every eight weeks thereafter) using the Veridex CellSearch System<sup>TM</sup>. To date, 39 patients have had blood evaluated for CTCs before and after temozolomide. Prior to starting therapy, the number of CTCs have ranged from

zero (0) to 929: 7 patients had 0 CTCs detected (18%); 13 had between 1 and 4 CTCs (33%); and 19 had  $\geq 5$  CTCs detected (49%). The number of CTCs is higher in patients with liver metastases (21%  $< 5$  CTCs vs 79%  $\geq 5$  CTCs,  $p < 0.001$ ). Patients with new sites of metastases have significantly higher numbers of CTCs (60%  $\geq 5$  CTCs), compared to those that progress at pre-existing sites of disease (100%  $< 5$  CTCs),  $p = 0.008$ . At the end of cycle 1, the change in number of CTCs correlates to RECIST response ( $p = 0.041$ ). Increased CTCs at the end of cycle 1 are associated with worse overall survival, compared to stable or decreased CTCs (median: 5 vs 8 months,  $p = 0.008$ ). At disease progression with temozolomide, high CTC numbers ( $\geq 5$ ) are associated with worse overall survival (median: 3 months), compared to low CTC numbers (median survival, not reached),  $p = 0.003$ . In this cohort of patients we have found that at baseline, the number of CTCs is not predictive of response to treatment, nor associated with progression-free and overall survival. No significant correlations have been found between patient characteristics (gender, age, Karnofsky performance status, response to first line therapy, previous lines of treatment) and baseline CTCs. For patients with brain and bone metastases, numbers of CTCs are similar to those without metastases at these sites. By incorporating CTCs in ongoing studies, we can continue to develop these as potential biomarker in this disease.

The Veridex CellSearch System™, which has been approved by the US Food and Drug Administration for clinical use in breast, colorectal, and prostate cancers, is one of the most widely used methods to identify and quantify CTCs.

#### 2.5.4.1 $\gamma$ H2AX in Individual Circulating Tumor Cells

Phosphorylated H2AX ( $\gamma$ H2AX) is a marker of DNA double strand damage, which may be an effective pharmacodynamic biomarker following treatment with DNA-damaging drugs such as temozolomide, topotecan, and PARP-inhibitors. Following double strand break damage, ataxia telangiectasia mutated protein becomes activated, phosphorylates H2AX at Ser<sup>139</sup> and mobilizes DNA damage repair proteins. In cells treated with DNA-damaging agents,  $\gamma$ H2AX foci appear in the nucleus within minutes in a dose dependent manner and  $\gamma$ H2AX loss or dephosphorylation correlates time-wise with DNA repair. It has been shown that with continued exposure to biologically effective drug doses, double strand breaks increase with time until the entire nucleus appears  $\gamma$ H2AX positive and nuclear disintegration is observed.<sup>95</sup>

An assay has been developed using the Veridex CellSearch System™ in conjunction with  $\gamma$ H2AX-AF488 antibody staining to identify  $\gamma$ H2AX-positive CTCs.<sup>95</sup> In patients treated with topotecan alone, a combination of topotecan and a PARP inhibitor, or a combination of cyclophosphamide and a PARP inhibitor, the percentage of  $\gamma$ H2AX-positive CTCs increased post-treatment. The

increase of  $\gamma$ H2AX-positive cells was noted irrespective of increases or decreases in the total CTC count. Importantly, this method makes use of only one sample to report CTC count and measure drug activity by monitoring percentage of  $\gamma$ H2AX-positive CTCs.<sup>95</sup>

#### 2.5.5 Plasma Biomarkers

**Plasma biomarkers for apoptosis, angiogenesis, and SCLC.** Plasma biomarkers have the important advantage that they can be used for monitoring changes during treatment non-invasively, including those related to induction of apoptosis<sup>96,97</sup> or response to therapy.<sup>98,99</sup> Furthermore, baseline levels of plasma markers may be markers of prognosis, tumor bulk, or response to treatment.<sup>98</sup> In patients with SCLC, it has been demonstrated that the apoptosis marker M30 changes in a dose-dependent manner in patients treated with the BCL-2 inhibitor ABT-263. Furthermore, circulating levels of pro-GRP, (which is derived from a gene next to BCL-2) were found to correlate with BCL-2 copy number and correlate with percent tumor change.<sup>97</sup>

As noted above, both temozolomide and PARP have been shown to exert antiangiogenic effects in preclinical testing. It is not known if these effects are an important component of their clinical activity. To evaluate this issue, plasma markers of angiogenesis will be assessed. Because tumor vasculature may be impacted by an array of circulating cytokines and angiogenic factors, we have used both individual assays (e.g. ELISA) and multiplex technologies (e.g. multiplex arrays) to assess biologically relevant proteins, such as cytokines, angiogenic factors, and receptors, and soluble markers of hypoxia and endothelial damage, using small amounts (i.e., less than one milliliter) of plasma. We refer to this approach as CAF (cytokine and angiogenic factor) profiling. We and other investigators have studied a number of these circulating biomarkers in peripheral blood and observed that baseline levels, or changes in these factors, may be markers of drug response or the emergence of therapeutic resistance.<sup>96,98,100-102</sup>

### 3.0 PATIENT SELECTION

#### 3.1 Eligibility Criteria

- 3.1.1 Patients must have histologically or cytologically confirmed small cell lung cancer. Confirmation will be done at MSKCC or locally for participating sites.
- 3.1.2 Patients' disease has relapsed or progressed after one or two prior chemotherapy regimens, one of which must have been an etoposide-platinum doublet. Eligible patients will be defined as follows:

**“Sensitive” Disease:** Patients who had one previous line of chemotherapy and maintained an appropriate response for > 60 days.

**“Refractory” Disease:** Those patients with either (a) no response to first-line chemotherapy or progression  $\leq 60$  days after completing treatment, or (b) “sensitive” or “refractory” disease in need of third-line therapy (i.e. completed or failed two previous lines of chemotherapy).

- 3.1.3 Age  $\geq 18$  years. Children currently are excluded from this study as there are no dosing or adverse event data available on the use of ABT-888 in patients  $<18$  years of age. They may be eligible for future pediatric phase 1 combination trials.
- 3.1.4 ECOG performance status  $\leq 1$  (Karnofsky  $\geq 70\%$ , see [Appendix A](#)).
- 3.1.5 Patients with asymptomatic brain metastases that do not require immediate whole brain radiation therapy and are on stable doses of steroids are allowed.
- 3.1.6 Patients must have measurable disease, which is defined as at least one lesion that can be accurately measured in at least one dimension on a CT scan as per RECIST version 1.1.<sup>4</sup> Brain metastases can be considered measurable disease if they meet this criterion.
- 3.1.7 Patients must have normal organ and marrow function as defined below:
- |                             |  |
|-----------------------------|--|
| - absolute neutrophil count | $\geq 1,500/\text{mcL}$  |
| - platelets                 | $\geq 100,000/\text{mcL}$  |
| - hemoglobin                | $\geq 8.5\text{g/dL}^*$  |
| - total bilirubin           | $\leq 1.5 \text{ mg/dL X institutional upper limit of normal}$   |
| - AST(SGOT)/ALT(SGPT)       | $\leq 3.0 \text{ X institutional upper limit of normal}$   |
| - creatinine                | $\leq 1.5 \text{ X institutional upper limit of normal}$   |
|                             | OR   |
| - creatinine clearance      | $\geq 50 \text{ mL/min/1.73 m}^2$ for patients with creatinine levels $\geq 1.5 \text{ X upper limit of institutional normal}$ . |
- \*The use of transfusion to achieve this criterion should be at the discretion of the investigators.
- 3.1.8 The effects of ABT-888 on the developing human fetus are unknown. For this reason and because other therapeutic agents or modalities used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.
- 3.1.9 For women of child-bearing potential, negative pregnancy test within 14 days prior to starting temozolomide and ABT-888.

- 3.1.10 Ability to understand and the willingness to sign a written informed consent document.
- 3.1.11 Able to swallow pills.
- 3.1.12. Patients will not be excluded based on the diagnosis of Acquired Immune Deficiency Syndrome (AIDS); given the increased risk of infection, these patients should have CD4 counts above 200 cells/mm<sup>3</sup>. Patients with AIDS or HIV not receiving agents with the potential for pharmacokinetic interactions with ABT-888 may be eligible.

### **3.2 Exclusion Criteria**

- 3.2.1 Patients who have had chemotherapy or radiotherapy within 3 weeks prior to entering the study.
- 3.2.2 Patients who have not recovered from adverse events due to agents administered more than 3 weeks earlier. Toxicities should have resolved to baseline or to within one grade level of their baseline (not to exceed grade 2).
- 3.2.3 Patients who have been administered ABT-888, any other PARP-inhibitor, or temozolomide.
- 3.2.4 Patients may not be receiving any other investigational agents.
- 3.2.5 Patients with leptomeningeal involvement.
- 3.2.6 Patients with active seizures or a history of seizures.
- 3.2.7 History of allergic reactions attributed to compounds of similar chemical or biologic composition to ABT-888 or temozolomide.
- 3.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- 3.2.9 Pregnant women are excluded from this study because ABT-888 is PARP inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ABT-888, breastfeeding should be discontinued if the mother is treated with ABT-888. These potential risks also apply to temozolomide.
- 3.2.10 Patients with either AIDS or HIV on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ABT-

888. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

3.2.11 Patients with a synchronous active malignancy requiring treatment.

### **3.3 Inclusion of Women and Minorities**

Both men and women of all races and ethnic groups are eligible for this trial.

## **4.0 REGISTRATION PROCEDURES**

### **4.1 General Guidelines**

Eligible patients will be entered on study centrally at Memorial Sloan Kettering Cancer Center (MSKCC) by the Study Coordinator/Multi-Center Trial Core Representative. All sites should call the Study Coordinator/Multi-Center Trial Core Representative to verify availability.

Following registration, patients should begin protocol treatment within 5 business days. Issues that would cause treatment delays should be discussed with the Principal Investigator. The Study Coordinator should be notified of cancellations as soon as possible.

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Patients may be randomized by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO ([PIO@ctep.nci.nih.gov](mailto:PIO@ctep.nci.nih.gov)) except for Group studies.

### **4.2 Registration Process for Participating Sites**

Central registration for this study will take place at Memorial Sloan Kettering Cancer Center (MSKCC).

To complete registration and enroll a participant from another institution, the study staff at that site must contact the designated research staff at MSKCC to notify him/her of the participant registration. The site staff then needs to e-mail registration/eligibility documents to the Multi-Center Trial Core Representative at [medmctcore@mskcc.org](mailto:medmctcore@mskcc.org).

The following documents must be sent for each enrollment within 24 hours of the informed consent form being signed:

- The completed or partially completed MSKCC eligibility checklist
- The signed informed consent
- The signed HIPAA Authorization form (Research Authorization)

- Supporting source documentation for eligibility questions (laboratory results, pathology report, radiology reports, MD notes, physical exam sheets, medical history, prior treatment records, and EKG report).

Upon receipt, the research staff at Memorial Sloan Kettering Cancer Center will conduct an interim review of all documents. If the eligibility checklist is not complete, the patient will be registered PENDING and the site is responsible for sending a completed form within 30 days of the consent.

If the eligibility checklist is complete, participant meets all criteria, all source documentation is received, the participating site IRB has granted approval for the protocol, and the site is in good standing with MSKCC, the MSKCC research staff will send the completed registration documents to the MSKCC Protocol Participant Registration (PPR) Office to be enrolled as stated in [section 4.3](#). The participant will be registered.

Once eligibility has been established and the participant is registered, the participant will be assigned an MSKCC Clinical Research Database (CRDB) number (protocol participant number). The patient will be assigned to treatment Arm A or Arm B. This number is unique to the participant and must be written on all data and correspondence for the participant. This protocol participant number will be relayed back to study staff at the registering site via e-mail and will serve as the enrollment confirmation.

#### **4.3 MSKCC Research Participant Registration**

Confirm eligibility as defined in the section entitled [Patient Selection](#).

Obtain written informed consent by following procedures defined in section entitled [Informed Consent Procedures](#).

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm (ET) at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (<http://ppr/>). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

#### **4.4 Randomization**

Patients will be randomized in a double-blinded, 1:1 fashion to receive either ABT-888 and temozolomide as the investigational arm, or placebo and temozolomide as the control arm. After eligibility is established and immediately after consent is obtained, patients will be registered in the Protocol Participant Registration (PPR) system and



randomized using the Clinical Research Database (CRDB). The participating sites will receive the protocol participant number via e-mail.

Randomization will be accomplished by the method of random permuted block, and patients will be stratified by center and by type of relapse (sensitive vs. refractory, as described in [Section 13.3](#)).

Patients who are registered and randomized and do not start treatment due to no longer being eligible at the time of starting drug, will be replaced, after a discussion with the Principal Investigator at MSKCC. Up to a maximum of 10 patients will be replaced for a sample size of 110 patients. These additional patients will be randomized in a double-blinded 1:1 fashion, as described above.

## 5.0 TREATMENT PLAN

### 5.1 Agent Administration

This is a Phase II multi-institutional, double-blind, placebo-controlled, randomized study of ABT-888 in combination with temozolomide (TMZ) versus placebo with temozolomide (TMZ) in patients with SCLC whose disease has progressed after one or two prior chemotherapy regimens.

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for ABT-888/placebo and TMZ are described in [Section 7](#). Appropriate dose modifications for ABT-888/placebo and TMZ are described in [Section 6](#). No investigational or commercial agents or therapies other than those described [below](#) may be administered with the intent to treat the patient's malignancy.

Subjects will be randomized in a double-blind, 1:1 ratio to receive ABT-888 OR Placebo with temozolomide:

<i>Agent</i>	<i>Premedications; Precautions</i>	<i>Dose</i>	<i>Route</i>	<i>Schedule</i>	<i>Cycle Length</i>
ABT-888	N/A	40 mg	PO in a.m. (at the same time as TMZ, under fasting conditions) and in p.m.	Days 1-7, week 1	28 days (4 weeks)

Placebo	N/A	Placebo for 40 mg	PO in a.m. (at the same time as TMZ, under fasting conditions ) and in p.m.	Days 1-7, week 1	
<b>Cycle 1 TMZ</b>	Patients should fast 2 hours before and 1 hour after TMZ. Administer ondansetron 8mg PO before TMZ.	150mg/m <sup>2</sup> /day *	PO in a.m.	Days 1-5, week 1	
<b>Cycle 2 and beyond TMZ</b>	Patients should fast 2 hours before and 1 hour after TMZ. Administer ondansetron 8mg PO before TMZ.	Patients with PR/CR at the end of cycle 1 will be allowed to remain at starting dose of temozolomide (150mg/m <sup>2</sup> /day)* Patients with SD and no myelosuppression during cycle 1 will have temozolomide increased to 200 mg/m <sup>2</sup> /day, if, at the discretion of the treating physician, this can be safely achieved*			
* Dosing of temozolomide will be based on actual body weight <b>at study initiation</b> . The daily dose will be rounded up to the nearest 5 mg.					

#### 5.1.1 ABT-888/Placebo

Subjects will self-administer the morning dose of ABT-888/ placebo and TMZ at the same time under fasting conditions (no food at least 2 hours before and 1 hour after these agents). The evening dose of ABT-888/ placebo should be taken approximately 12 hours after the morning dose in the same calendar day. Fasting is not required for the evening dose of ABT-888/placebo.

Efforts should be made to take the ABT-888/placebo at the same time points each day. Missed or vomited doses of ABT-888/placebo will not be replaced. Patients will be instructed to keep a pill diary to record the time and dose each day, and will bring this diary to each clinic visit for verification.

Because there is a potential for interaction of ABT-888/placebo with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

ABT-888/placebo is not known to be a potent inhibitor of the major human CYPs *in vitro*, indicating a low risk for drug-drug interactions at the proposed dosing concentrations.

#### 5.1.2 Temozolomide

Subjects will self-administer the morning dose of ABT-888/placebo and TMZ at the same time under fasting conditions (no food at least 2 hours before and 1 hour after these agents). Water is allowed during the fasting period. Patients will be instructed to swallow the capsules whole, in rapid succession, without chewing them with approximately 250ml of water. Antiemetic prophylaxis (preferably with ondansetron) will be given before each temozolomide administration.

Efforts should be made to take the TMZ at the same time points each day. Missed or vomited doses of TMZ will not be replaced. Patients will be instructed to keep a pill diary to record the time and dose each day, and will bring this diary to each clinic visit for verification.

The TMZ dose will be determined using the body surface area (BSA) calculated from the height and weight obtained at the **screening** evaluation. Dose delays and reductions should be managed as in [Section 6.0](#).

Because there is a potential for interaction of TMZ with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The dose will not be recalculated each cycle unless the patient's weight has increased or decreased by >10%.

During cycle 1 of treatment, all patients will initiate temozolomide at 150mg/m<sup>2</sup>/day. At the *end* of cycle 1, those patients who achieve a PR/CR, will be allowed to remain at the starting dose of temozolomide. However, those patients with SD and no myelosuppression during cycle 1 will have temozolomide increased to 200 mg/m<sup>2</sup>/day in cycle 2, if, at the discretion of the treating physician, this could be safely achieved.

TMZ is not known to be a potent inhibitor of the major human CYPs *in vitro*, indicating a low risk for drug-drug interactions at the proposed dosing concentrations.

## 5.2 General Concomitant Medication and Supportive Care Guidelines

- Anti-emetics:** Prophylactic antiemetics should be prescribed for all patients taking temozolomide as per Clinical Center and ASCO guidelines, although ondansetron is recommended.
- Supportive Care:** Best supportive care and treatment will be given as appropriate to each subject as per Clinical Center and ASCO guidelines (antiemetics, antibiotics, packed red blood cell and platelet transfusions, nutritional support, non-radiation palliative treatment for pain).
- Growth Factors:** Colony stimulating factors, such as filgrastim or peg-filgrastim can be administered during the first cycle of therapy according to the Clinical Center and/or ASCO guidelines. Filgrastim or peg-filgrastim growth factor support should be administered in subsequent cycles for patients who have experienced Grade 3 or 4 neutropenia or neutropenic fevers in previous cycles.
- PCP prophylaxis:** If lymphopenia  $\geq$  Grade 3 ( $\leq 500/\mu\text{l}$ ) occurs, one of the following agents is to be administered as per the physician's choice: trimethoprim-sulfamethoxazole (Bactrim DS) 1 tablet orally 3 times per week, dapsone 100mg orally each day (except in patients with G6-PD deficiency), or atovaquone 750mg orally twice daily.
- Seizures:** Seizures were seen in some animal toxicology studies, although at doses much higher than those anticipated for this study. Seizures in animals were successfully treated with lorazepam; thus, lorazepam should be administered.
- Anti-cancer Agents:** No anti-cancer agents, investigational agents or anti-cancer hormonal therapy may be taken concurrently with ABT-888/placebo and TMZ. Hormonal contraceptives are allowed.

**Radiation:** Radiation therapy is not allowed during the study. If radiation therapy is needed to treat the symptoms due to underlying SCLC, it will be considered clinical progression.

**Surgery:** If the subject requires surgery during the study, then this needs to be discussed with the Investigator on a case-by-case system.

### **5.3 Duration of Therapy**

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

### **5.4 Duration of Follow Up**

When a subject discontinues the study, a Final Visit will be conducted (preferably prior to the initiation of another anticancer therapy). However, these procedures should not interfere with the initiation of any new treatments or therapeutic modalities that the Investigator feels are necessary to treat the subject's condition.

Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients who are taken off of study for any reason other than death or disease progression will continue to undergo re-imaging studies every 8 weeks (+/- 1 week) until disease progression, start of new anticancer therapy, or death. Patients will be followed in clinic or by telephone on an approximately every 12 week schedule (+/- 2 weeks) until death for the purposes of survival followup.

### **5.5 Criteria for Removal from Study**

Patients will be removed from study when any of the criteria listed in [Section 5.3](#) applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

## 6.0 DOSE MODIFICATIONS/DOSING DELAYS

### 6.1 Required Laboratory Parameters for Initiation of a Treatment Cycle

A cycle of therapy may be initiated provided that the patient meets the following criteria on Day 1 of each cycle:

- ANC  $\geq$  1,500/ $\mu$ L
- Platelets  $\geq$  100,000/ $\mu$ L
- All grade  $\geq$  2 non-hematologic adverse events (except for alopecia, nausea and vomiting) must have resolved to grade  $\leq$  1 (or tolerable grade 2 or baseline)
- No evidence of progressive disease

A CBC will be obtained on day 1 of each cycle (or within 48 hours if same-day blood work is not available).

### 6.2 Dose Reductions or Delays

If a subject experiences an adverse event that results in ABT-888/placebo and/or TMZ being interrupted during a cycle, the subject will complete the planned activities of the cycle as scheduled (i.e., CT scans and lab tests will be performed as in [Section 10](#)).

Study drug interruptions for events that are clearly not related to the study drug, e.g., underlying cancer, planned surgical procedures or acute viral illnesses, should not necessitate a dose reduction. The timing of dose resumption should be at the discretion of the Investigator.

For hematologic toxicity that has not resolved (i.e. ANC  $\leq$  1,500/ $\mu$ l **and/or** platelets  $\leq$  100,000/ $\mu$ l) **for up to and including 3 weeks**, further treatment should be stopped and the patient removed from study.

For non-hematologic adverse events that have not resolved at the start of a cycle, treatment should be delayed by 1 week for up to 3 consecutive weeks. If **after 3 weeks of delays**, adverse events have not resolved, then further treatment should be stopped and the patient removed from study.

If a subject experiences an adverse event that results in ABT-888/placebo and/or TMZ being delayed for up to 3 weeks, the planned activities of the cycle that include the history and physical exam, routine blood work (CBC and CMP) can be delayed as well at the discretion of the treating physician and/or Investigator. However, diagnostic imaging for assessment of response and blood collections for circulating tumor cells and plasma biomarkers should be performed as scheduled in [Section 10](#).

### 6.3 Dose Modification Schedules

Dosing is based on adverse events during the prior treatment cycle (see text [below](#)). If multiple adverse events are seen, the dose administered should be based on the dose reduction required for the most severe grade of any single adverse event.

**Table 3. Dose Modification for ABT-888/Placebo**

Dose Level	Dose	Remarks
0	40 mg, twice daily (Total: 80mg daily)	Starting dose.
-1	40 mg, am daily 20 mg, pm daily (Total: 60mg daily)	Reduction if prior adverse event* (except for alopecia, nausea and vomiting) while receiving total of 80 mg daily.
-2	20 mg, twice daily (Total: 40mg daily)	Reduction if prior adverse event* (except for alopecia, nausea and vomiting) while receiving total of 60 mg daily. Lowest possible dose.

\*Definition of prior adverse event is stated in text [below](#).

**Table 4a. Dose Modification for Temozolomide at 150mg/m<sup>2</sup>/day**

Dose Level	Dose	Remarks
0	150 mg/m <sup>2</sup> /day	Starting Dose.
-1	125 mg/m <sup>2</sup> /day	Reduction if prior adverse event* (except for alopecia, or nausea and vomiting not maximally managed with antiemetic therapy) while receiving 150 mg/m <sup>2</sup> /day.
-2	100 mg/m <sup>2</sup> /day	Reduction if prior adverse event* (except for alopecia, or nausea and vomiting not maximally managed with antiemetic therapy) while receiving 125 mg/m <sup>2</sup> /day. Lowest possible dose.

\*Definition of prior adverse event is stated in text [below](#).

**Table 4b. Dose Modification for Temozolomide at 200mg/m<sup>2</sup>/day**

Dose Level	Dose	Remarks
0	200 mg/m <sup>2</sup> /day	Starting Dose.
-1	150 mg/m <sup>2</sup> /day	Reduction if prior adverse event* (except for alopecia, or nausea and vomiting not maximally managed with antiemetic therapy) while receiving 200 mg/m <sup>2</sup> /day.
-2	100 mg/m <sup>2</sup> /day	Reduction if prior adverse event* (except for alopecia, or nausea and vomiting not maximally managed with antiemetic therapy) while receiving 150 mg/m <sup>2</sup> /day. Lowest possible dose.

\*Definition of prior adverse event is stated in text [below](#)

### 6.3.1 ABT-888/Placebo Dose Reduction and Delays

The following are guidelines for dose reduction, delay and discontinuation of ABT-888/placebo (see [Table 5](#)).

- ABT-888/placebo should be discontinued at the same time of discontinuation of TMZ.
- For any subject who experiences Grade 3/4 toxicity that is not attributable to TMZ (see [Table 5](#)) or the underlying disease, the ABT- 888/placebo dose will be discontinued until the toxicity resolves to  $\leq$  grade 1 or to baseline if grade 2 at the time of study entry.
- ABT-888/placebo will be reduced to one lower dose level in the next cycle; two dose reductions are allowed. The dose of ABT-888/placebo will be reduced to a total of 60 mg daily (40 mg in am and 20 mg in pm) and may be reduced again to a total of 40 mg daily (20 mg BID) due to toxicity. Any dose reduction beyond a total of 40 mg daily (20 mg BID) will result in ABT-888/placebo discontinuation.
- When ABT-888/placebo is discontinued, TMZ should be discontinued.
- A **three week** delay in beginning the next cycle is allowed for toxicities to recover. If after 3 consecutive weeks of delays the adverse events have not resolved, then treatment will be discontinued.
- Any  $\geq$  grade 2 event of seizure attributed to ABT-888/placebo requires interruption of ABT-888/placebo and discussion with the Primary Investigator regarding the decision to resume treatment.

### 6.3.2 Temozolomide Dose Reduction and Delays

The following are guidelines for dose reduction, delay and discontinuation of temozolomide (see [Table 5](#)).

- Dosing is based on adverse events during the prior treatment cycle, according to the worst non-hematologic toxicities and lowest blood counts observed. If multiple adverse events are seen, the dose administered should be based on the dose reduction required for the most severe grade of any single adverse event.
- If at any time during the previous cycle, the patient experiences  $\geq$  Grade 3 neutropenia (ANC < 1000/ $\mu$ L) and/or thrombocytopenia (< 50,000/ $\mu$ L) TMZ should be dose reduced as per [Table 5](#).
- Use of peg-filgrastim is at the discretion of the treating physician.
- If any hematologic adverse event recurs after a reduction was already made for that event, then one further dose reduction of TMZ will be allowed. If this toxicity recurs despite this dose reduction, then the patient will be removed from the TMZ treatment arm.
- For any  $\geq$  Grade 3 toxicities observed during the previous cycle or at the start of a cycle attributable to TMZ, dose reduction and/or delay guidelines should be followed per [Table 5](#).



- If any Grade 3 non-hematologic adverse event recurs (except alopecia) after a reduction was already made for that event, then one further dose reduction of TMZ will be allowed. If this toxicity recurs despite this dose reduction, then the patient will be removed from the TMZ treatment arm.
- If no further Grade 3 or 4 non-hematologic toxicity occurs on subsequent repeat dosing, then the total dose administered for the next cycle is to be the same as the dose administration during the previous cycle.
- For any grade 4 non-hematologic toxicity (except for alopecia or nausea and vomiting despite adequate antiemetic therapy) that a patient experiences, TMZ treatment should be discontinued.
- 100 mg/m<sup>2</sup>/day is the lowest dose of TMZ that is allowed.
- Subjects must stop TMZ and ABT-888/placebo if they are at the 100 mg/m<sup>2</sup>/day dose level of TMZ and require an additional dose reduction ([Table 5](#)) due to toxicities. Subjects will be removed from protocol directed therapy if this occurs.
- A **three week** delay in beginning the next cycle is allowed for toxicities to recover. If after 3 consecutive weeks of delays the adverse events have not resolved, then treatment will be discontinued.
- When ABT-888/placebo is discontinued, TMZ should be discontinued.

**Table 5. ABT-888/Placebo and TMZ Dose Reduction or Delay**

	<b>Adverse Event</b>	<b>Dose Reduction</b>
<b>Hematologic Toxicity Attributable to TMZ<sup>§</sup></b>	ANC < 1000/ $\mu$ L* and/or Platelets < 50,000/ $\mu$ L*	1. Hold TMZ and ABT-888/placebo. Check CBC weekly until recovery to: ANC > 1500/ $\mu$ L <b>and</b> platelets > 100,000/ $\mu$ L <b>and</b> 2. Reduce TMZ dose at the the next cycle as indicated in Tables <a href="#">4a</a> & <a href="#">4b</a> .***
	1000/ $\mu$ L* $\leq$ ANC < 1500/ $\mu$ L* and/or 50,000/ $\mu$ L* $\leq$ Platelets < 100,000/ $\mu$ L*	1. Hold TMZ and ABT-888/placebo. Check CBC weekly until recovery to: ANC $\geq$ 1500/ $\mu$ L <b>and</b> platelets > 100,000/ $\mu$ L <b>and</b> 2. Maintain Current TMZ Dose at the next cycle
	Grade 3 or 4 neutropenia associated with single oral temperature $\geq$ 38.1°C requiring parenteral antibiotics at any time during the previous cycle.	1. Hold TMZ and ABT-888/placebo. Check CBC weekly until recovery to: ANC > 1500/ $\mu$ L <b>and</b> platelets > 100,000/ $\mu$ L <b>and</b> 2. Reduce TMZ dose at the next cycle as indicated in Tables <a href="#">4a</a> & <a href="#">4b</a> .*** 3. Use of peg-filgrastim is at the discretion of the treating physician.
	Any other CTC $\geq$ Grade 3 deemed to be clinically significant by treating physician (e.g., Hgb < 8 g/dL)	1. Delay cycle until Grade 1 (or Grade 2 if present at baseline) <b>and</b> 2. Reduce TMZ dose at the next cycle as indicated in Tables <a href="#">4a</a> & <a href="#">4b</a> .***
	<b>Adverse Event</b>	<b>Dose Reduction</b>
<b>Non-hematologic Toxicity Attributable to TMZ<sup>‡</sup></b>	Intolerable Grade 2	1. Delay cycle until Grade 1 <b>and</b> 2. Maintain Current TMZ Dose at the next cycle
	Nausea/Vomiting CTC $\geq$ Grade 3 despite optimal antiemetic therapy	1. Delay cycle until Grade 1 (or Grade 2 if present at baseline) <b>and</b> 2. Reduce TMZ dose for the next cycle as indicated in Tables <a href="#">4a</a> & <a href="#">4b</a> .***
	Fatigue, constipation, anorexia and headaches CTC $\geq$ Grade 3	
	Any other CTC Grade 3 deemed to be clinically significant by treating physician	1. Delay cycle until Grade 1 (or Grade 2 if present at baseline) <b>and</b> 2. Reduce TMZ dose for the next cycle as indicated in Tables <a href="#">4a</a> & <a href="#">4b</a> .***
	Any Grade 4 (except for alopecia, or nausea and vomiting not maximally managed with antiemetic therapy)	1. TMZ should be stopped.
	<b>Adverse Event</b>	<b>Dose Reduction**</b>
<b>Attributable to ABT-888/Placebo</b>	Any Grade 3 or 4 toxicity which is not attributable to TMZ or underlying disease	1. Delay cycle until Grade 1 (or Grade 2 if present at baseline) <b>and</b> 2. Reduce ABT-888/placebo by one dose level** <b>and</b> 3. Reduce TMZ dose for the next cycle as indicated in Tables <a href="#">4a</a> & <a href="#">4b</a> .***

- § If any hematologic adverse event recurs after a reduction was already made for that event, then one further dose reduction of TMZ will be allowed. If this toxicity recurs despite this dose reduction, then TMZ will be discontinued.
- ‡ If any Grade 3 non-hematologic adverse event recurs (except alopecia) after a reduction was already made for that event, then one further dose reduction of TMZ will be allowed. If this toxicity recurs despite this dose reduction, then TMZ will be discontinued.
- \* Regardless of attribution.
- \*\* Two dose reductions of ABT-888/placebo will be allowed. Subjects must discontinue ABT-888/placebo if they are at 20 mg BID dose level and require an additional dose reduction due to toxicities. The subject will be removed from protocol directed therapy if this occurs. If ABT-888/placebo is discontinued then TMZ also will be discontinued.
- \*\*\* TMZ dose cannot be reduced below 100 mg/m<sup>2</sup>/day. TMZ will be discontinued if further dose reduction is required below the 100 mg/m<sup>2</sup>/day. ABT-888/placebo will also be discontinued and the subject will be removed from protocol directed therapy.

## 7.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs ([Section 7.1](#)) and the characteristics of an observed AE ([Section 7.2](#)) will determine whether the event requires expedited (via CTEP-AERS) **in addition** to routine reporting.

### 7.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

#### 7.1.1 CAEPR for ABT-888 (NSC 737664)

#### **Comprehensive Adverse Events and Potential Risks list (CAEPR) for ABT-888 (Veliparib, NSC 737664)**

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted [below](#)). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' [http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/aeguidelines.pdf](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf) for further clarification. *Frequency is provided based on 565 patients.* [Below](#) is the CAEPR for ABT-888 (veliparib).

**NOTE:** Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Adverse Events with Possible Relationship to for ABT-888 (Veliparib) (CTCAE 4.0 Term) [n= 565]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
<b>BLOOD AND LYMPHATIC SYSTEM DISORDERS</b>			
	Anemia		<i>Anemia (Gr 3)</i>
	Febrile neutropenia		<i>Febrile neutropenia (Gr 3)</i>
<b>GASTROINTESTINAL DISORDERS</b>			
	Abdominal pain		
	Constipation		<i>Constipation (Gr 2)</i>
	Diarrhea		<i>Diarrhea (Gr 2)</i>
Nausea			<i>Nausea (Gr 2)</i>
	Vomiting		<i>Vomiting (Gr 2)</i>
<b>GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS</b>			
Fatigue			<i>Fatigue (Gr 2)</i>
<b>INVESTIGATIONS</b>			
	Lymphocyte count decreased		<i>Lymphocyte count decreased (Gr 3)</i>
Neutrophil count decreased			<i>Neutrophil count decreased (Gr 4)</i>
Platelet count decreased			<i>Platelet count decreased (Gr 4)</i>
	Weight loss		<i>Weight loss (Gr 2)</i>
	White blood cell decreased		<i>White blood cell decreased (Gr 4)</i>
<b>METABOLISM AND NUTRITION DISORDERS</b>			
	Anorexia		<i>Anorexia (Gr 2)</i>
	Dehydration		<i>Dehydration (Gr 2)</i>
	Hypophosphatemia		<i>Hypophosphatemia (Gr 2)</i>
<b>NERVOUS SYSTEM DISORDERS</b>			
	Dizziness		
	Dysgeusia		<i>Dysgeusia (Gr 2)</i>
	Headache		<i>Headache (Gr 2)</i>
		Seizure	
<b>SKIN AND SUBCUTANEOUS TISSUE DISORDERS</b>			
	Rash maculo-papular		
<b>VASCULAR DISORDERS</b>			
		Thromboembolic event <sup>2</sup>	

<sup>1</sup>This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting [PIO@CTEP.NCI.NIH.GOV](mailto:PIO@CTEP.NCI.NIH.GOV). Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

<sup>2</sup>Thromboembolic events, including deep vein thrombosis and pulmonary embolism, have been observed at a higher frequency compared to control arm when administered in combination with temozolomide.

**Also reported on ABT-888 (veliparib) trials but with the relationship to ABT-888 (veliparib) still undetermined:**

**CARDIAC DISORDERS** - Left ventricular systolic dysfunction

**EAR AND LABYRINTH DISORDERS** - Vertigo

**EYE DISORDERS** - Blurred vision

**GASTROINTESTINAL DISORDERS** - Abdominal distension; Colitis; Dry mouth; Dyspepsia; Dysphagia; Enterocolitis; Flatulence; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (mouth ulceration); Lower gastrointestinal hemorrhage; Mucositis oral; Small intestinal obstruction

**GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS** - Chills; Edema limbs; Fever; Non-cardiac chest pain; Pain

**HEPATOBIILIARY DISORDERS** - Hepatic failure

**INFECTIONS AND INFESTATIONS** - Lymph gland infection; Skin infection; Upper respiratory infection

**INJURY, POISONING AND PROCEDURAL COMPLICATIONS** - Bruising

**INVESTIGATIONS** - Alkaline phosphatase increased; Blood bilirubin increased; Creatinine increased; Electrocardiogram QT corrected interval prolonged

**METABOLISM AND NUTRITION DISORDERS** - Hyperglycemia; Hyponatremia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia

**MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS** - Arthralgia; Back pain; Bone pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (muscle spasms); Myalgia; Pain in extremity

**NERVOUS SYSTEM DISORDERS** - Ataxia; Depressed level of consciousness; Lethargy; Paresthesia; Peripheral sensory neuropathy; Syncope

**PSYCHIATRIC DISORDERS** - Anxiety; Confusion; Depression; Insomnia; Psychosis

**RENAL AND URINARY DISORDERS** - Hematuria

**RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS** - Cough; Dyspnea; Epistaxis; Hypoxia; Pharyngolaryngeal pain; Pleural effusion; Respiratory failure

**SKIN AND SUBCUTANEOUS TISSUE DISORDERS** - Alopecia; Dry skin; Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Purpura

**VASCULAR DISORDERS** - Hot flashes; Hypotension; Vascular disorders - Other (brainstem infarction)

**Note:** ABT-888 (veliparib) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

#### 7.1.2 Adverse Event List for Temozolomide (Temodar®)

Temozolomide (Temodar®) dosing likely will produce side effects, and not all side effects may be known. Based on previous investigations in humans, many side effects disappear shortly after temozolomide is stopped. Please see the temozolomide package insert for the comprehensive list of adverse events.

- **Common:**  
*Cardiovascular:* Peripheral edema  
*Central nervous system:* Fatigue, headache, seizure, dizziness, coordination abnormality

*Dermatologic:* Alopecia, rash

*Gastrointestinal:* Nausea, vomiting, constipation, anorexia, diarrhea

*Hematologic:* Lymphopenia, thrombocytopenia, neutropenia, leukopenia

*Neuromuscular & skeletal:* Weakness

*Miscellaneous:* Fever, Viral infection

- **Less Likely:**

*Central nervous system:* Memory impairment, insomnia, somnolence, ataxia, paresis, anxiety, depression, confusion, paresthesia

*Dermatologic:* Pruritus, dry skin

*Gastrointestinal:* Abdominal pain, dysphagia, taste perversion, weight gain

*Genitourinary:* Urinary frequency

*Hematologic:* Anemia

*Musculoskeletal:* Arthralgia, myalgia

*Ocular:* Vision changes

*Respiratory:* Upper respiratory tract infection, cough, dyspnea

*Miscellaneous:* Allergic reaction

- **Very Rare:**

Opportunistic infections, especially *pneumocystis carinii* pneumonia. Myelodysplastic syndrome.

Hepatic failure.

## 7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (<http://ctep.cancer.gov>).
- **‘Expectedness’:** AEs can be ‘Unexpected’ or ‘Expected’ (see [Section 7.1](#) above) for expedited reporting purposes only. ‘Expected’ AEs (the ASAEL) are **bold and italicized** in the CAEPR ([Section 7.1.1](#)).
- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

## 7.3 Expedited Adverse Event Reporting

- 7.3.1** Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP home page (<http://ctep.cancer.gov>). The reporting procedures to be followed are presented in the “CTEP, NCI Guidelines: Adverse Event Reporting Requirements” which can be downloaded from the CTEP home page (<http://ctep.cancer.gov>). These requirements are briefly outlined in the table below ([Section 7.3.3](#)).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

- 7.3.2** CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

In addition to reporting to CTEP-AERS:

- Participating sites are responsible for submitting all SAEs to their local IRB per institutional guidelines
- Participating sites are responsible for reporting all SAEs to the MSKCC PI via e-mail within 3 calendar days of learning of the event. Participating sites should use the SAE Report Template ([Appendix K](#)) to report SAEs to MSKCC.
- Participating sites should notify the MSKCC PI of any grade 5 event immediately.

**SAE contact information for the Coordinating Center is listed below:**

M. Catherine Pietanza, M.D.  
Memorial Sloan Kettering Cancer Center  
300 East 66<sup>th</sup> Street  
New York, New York 10065  
Telephone: 646-888-4203  
E-mail: [pietanzm@mskcc.org](mailto:pietanzm@mskcc.org)

Multi-Center Trial Core Representative  
Email: [medmctcore@mskcc.org](mailto:medmctcore@mskcc.org)

The MSKCC Research Staff is responsible for submitting all SAEs to the MSKCC IRB/PB within 5 days of learning of the event as specified in [section 7.6](#).

**Safety Reports:**

- MSKCC will distribute outside safety reports to the participating sites immediately upon receipt.
- MSKCC must submit safety reports to the MSKCC IRB/PB according to institutional guidelines.
- Participating sites must submit safety reports to their institution’s IRBs within 30 days of receipt from MSKCC or per their own institutional guidelines, however

all safety reports should be submitted within 90 days of the date of the report, per CTEP guidelines.

### 7.3.3 Expedited Reporting Guidelines: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention<sup>1,2</sup>

<b>FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)</b>				
<b>NOTE:</b> Investigators <b>MUST</b> immediately report to the sponsor (NCI) <b>ANY</b> Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)				
An adverse event is considered serious if it results in <b>ANY</b> of the following outcomes:				
<ol style="list-style-type: none"> <li>1) Death</li> <li>2) A life-threatening adverse event</li> <li>3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for <math>\geq</math> 24 hours</li> <li>4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions</li> <li>5) A congenital anomaly/birth defect.</li> <li>6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).</li> </ol>				
<b>ALL SERIOUS</b> adverse events that meet the above criteria <b>MUST</b> be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table <a href="#">below</a> .				
Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization $\geq$ 24 hrs	10 Calendar Days			24-Hour 5 Calendar Days
Not resulting in Hospitalization $\geq$ 24 hrs	Not required		10 Calendar Days	
<b>NOTE:</b> Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR				
<b>Expedited AE reporting timelines are defined as:</b>				
<ul style="list-style-type: none"> <li>o "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.</li> <li>o "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.</li> </ul>				
<p><sup>1</sup>Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:  <b>Expedited 24-hour notification followed by complete report within 5 calendar days for:</b></p> <ul style="list-style-type: none"> <li>• All Grade 4, and Grade 5 AEs</li> </ul> <p><b>Expedited 10 calendar day reports for:</b></p> <ul style="list-style-type: none"> <li>• Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization</li> <li>• Grade 3 adverse events</li> </ul> <p><sup>2</sup> For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. <a href="#">Footnote "1"</a> above applies after this reporting period.</p>				
Effective Date: May 5, 2011				



**Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.**

- Expedited AE reporting timelines defined:
  - “24 hours; 5 calendar days” – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  - “10 calendar days” - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

#### **7.4 Routine Adverse Event Reporting**

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

#### **7.5 Secondary AML/MDS**

AML/MDS events must be reported via CTEP-AERS (in addition to your routine AE reporting mechanisms). In CTCAE v4.0, the event(s) may be reported as either: 1) Leukemia secondary to oncology chemotherapy, 2) Myelodysplastic syndrome, or 3) Treatment-related secondary malignancy.

#### **7.6 Serious Adverse Event (SAE) Reporting for MSKCC Patients Only**

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at [sae@mskcc.org](mailto:sae@mskcc.org). The report should contain the following information:

Fields populated from CRDB:

- Subject’s name (generate the report with only initials if it will be sent outside of MSKCC)

- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
  - An explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

## 8.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in [Section 7.1](#).

### 8.1 CTEP IND Agent ABT-888 (NSC 737664) and Matching Placebo

**Clinical Supplies:** ABT-888(NSC 737664/ IND 77840) and matching Placebo will be provided free of charge by Abbott Laboratories and distributed by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI).

ABT-888 20 mg and 0 mg matching placebo for ABT-888 will be supplied as immediate release capsules. The ABT-888 capsule contains ABT-888, microcrystalline cellulose, colloidal silicon dioxide, magnesium stearate, gelatin, sodium lauryl sulfate, and titanium dioxide. May contain FD&C blue #1, FD&C yellow #6, or FD&C yellow #5. The matching placebo capsule contains microcrystalline cellulose, colloidal silicon dioxide, magnesium stearate, gelatin, sodium lauryl sulfate, and titanium dioxide. May contain FD&C blue #1, FD&C yellow #6, or FD&C yellow #5.

ABT-888 and matching Placebo will be supplied in bottles containing 64 – 20 mg capsules (ABT-888) or 64 – 0 mg capsules (Placebo for ABT-888) with a child-

resistant cap and a tamper-evident seal.

In order to dispense a single cycle at a time, ABT-888(veliparib)/placebo capsules may be repackaged from the supplied HDPE bottles into amber (or other low-actinic) child resistant pharmacy dispensing bottles. The bottles should be labeled according to State regulations and also include the information from the original patient specific bottle sent from the PMB. Expiration will be 30 days from the repackaging date (or the original retest date, whichever is earlier) when stored at 15°C to 25°C (59°F to 77°F).

Each blinded, patient-specific bottle will be labeled with ...

- the protocol number (i.e., "9026")
- the bottle number (i.e., "Bottle 1 of 2" and "Bottle 2 of 2")
- the number of capsules (i.e., "64 tablets")
- the patient ID number (e.g., "9026-XXX", where "9026-XXX" represents the protocol number and sequence number which is the unique patient identifier assigned at registration)
- the patient initials (i.e., first initial, last initial [e.g., "FML"])
- the agent identification (i.e., "ABT-888 20 mg or Placebo")
- a blank line for the pharmacist to enter the patient's name
- administration instructions (i.e., "Take \_\_ capsules daily.")
- storage instructions (i.e., "Store at room temperature (15°C to 25°C; 59°F to 77°F).")
- emergency contact instructions
- a Julian date

The Julian date indicates the day the bottle was labeled and shipped and is composed of the last two digits of the calendar year (e.g., 2009 = 09, 2010 = 10) and a day count (e.g., January 1 = 001, December 31 = 365). For example, a bottle labeled and shipped on January 1, 2009 would have a Julian date of '09001' and a bottle labeled and shipped on December 31, 2009 would have a Julian date of '09365'. The Julian date will be used by PMB for recalls. When a lot expires, PMB will determine the last date the expired lot was shipped and will recall all bottles (i.e., both ABT-888 and Placebo) shipped on or before that date thus eliminating any chance of breaking the blind.

Questions about drug orders, transfers, returns, or accountability should be addressed to the PMB by calling 240-276-6575 Monday through Friday between 8:30am and 4:30pm Eastern Time. You may also contact the PMB via e-mail at [PMBAfterHours@mail.nih.gov](mailto:PMBAfterHours@mail.nih.gov).

#### 8.1.1 Blinded Agent Ordering, Transfers and Returns

**Blinded Agent Orders: No blinded starter supplies will be available for this study.**

Blinded, patient-specific supplies will be sent to the registering investigator at

the time of randomization and should arrive within 7 to 10 business days. Patients will be randomized by the Protocol Participant Registration Office (PPR) at Memorial Sloan Kettering Cancer Center. The assigned patient ID number must be recorded by the registering institution at the time of randomization for proper clinical supply dispersion. Once a patient has been randomized, the PPR will electronically transmit a clinical drug request for that patient to the PMB. This request will be entered and transmitted by the PPR the day the patient is randomized and will be processed by PMB the next business day and shipped the following business day. Shipments within the United States will be sent by US Priority Mail (generally two to three day delivery). Thus, if a patient is registered on Monday, the PPR would enter a clinical drug request for that patient on Monday and PMB would process that request on Tuesday and ship the drug on Wednesday. Sites could expect to receive their order approximately Friday or Monday. Shipments to United States sites can be expedited (i.e., receipt on Thursday in example above) by the provision of an express courier account name and number to the PPR at the time the patient is registered/randomized.

The initial request will be for one 64 capsule bottle of ABT-888/placebo (a 2-Cycle / 8-week supply at a dose of 40 mg BID given orally on days 1 through 7 of a 28 day schedule). Three weeks before the next bottle is needed, sites may reorder another 64 capsule bottles (a 2-cycle / 8-week supply at a dose of 40 mg BID given orally on days 1 through 7 of a 28 day schedule) by using the On-line Agent Order Processing (OAOP) program. All drug orders should be shipped directly to the physician responsible for treating the patient.

#### **Agent Transfers:**

Bottles **MAY NOT** be transferred from one patient to another patient or from one protocol to another protocol. All other transfers (e.g., a patient moves from one participating clinical site to another participating clinical site, the principal investigator at a given clinical site changes) must be approved in advance by the PMB. To obtain an approval for transfer, investigators should complete and submit to the PMB (fax number 240-276-7893) a Transfer Investigational Agent Form available on the CTEP home page (<http://ctep.cancer.gov>). The patient ID number (e.g., "9026-XXX") and the patient initials (e.g., "FML") should be entered in the "Received on NCI Protocol No." and the "Transferred to NCI Protocol No." fields in addition to the protocol number (i.e., "9026").

#### **Agent Returns:**

**Only undispensed clinical supplies should be returned to the PMB.** When it is necessary to return study drug (e.g., sealed bottles remaining when a patient permanently discontinues protocol treatment, expired bottles recalled by the PMB), investigators should return the study drug to the PMB using the NCI Return Drug List available on the CTEP home page (<http://ctep.cancer.gov>) or by calling the PMB at 240-276-6575. The patient ID number (e.g., "9026-

XXX") and the patient initials (e.g., "FML") should be entered in the "Lot Number" field. Opened bottles with remaining capsules should be documented in the patient-specific NCI Investigational Agent Accountability Record (i.e., logged in as "returned by patient" and logged out as "destroyed on site") and destroyed on-site in accordance with institutional policy.

#### 8.1.2 Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of all drugs received from the PMB using the NCI Investigational Agent Accountability Record available on the CTEP home page (<http://ctep.cancer.gov>) or by calling the PMB at 240-276-6575. A separate NCI Investigational Agent Accountability Record must be maintained for each patient ID number (e.g., "9026-XXX") on this protocol.

#### 8.1.3 Emergency Unblinding

In the event of an emergency or severe adverse reaction necessitating identification of the medication for the welfare of the patient, please contact **Dr. M. Catherine Pietanza at 646-888-4203** first to ensure the reason for unblinding is valid. If deemed valid, **Dr. M. Catherine Pietanza** will then send an email to the coordinating center which includes (1) study chair, (2) statistician, and (3) study coordinator. The coordinating center will then require the protocol number, the patient ID number and the patient initials to unblind the patient. Note that if a patient is unblinded, he/she must discontinue protocol treatment but should continue to be followed for outcome evaluations.

## 8.2 Temozolomide (Temodar®)

**Product description:** Temozolomide is supplied in white opaque, preservative-free, two-piece, hard gelatin capsules of the following dosage strengths: 5mg, 20mg, 100mg, 140mg, 180mg and 250 mg. Each capsule contains drug substance in combination with lactose, anhydrous NF, colloidal silicon dioxide NF, sodium starch glycolate NF, tartaric acid NF, and stearic acid NF. The capsule shells contain gelatin NF, titanium dioxide USP, and sodium lauryl sulfate NF.

Temozolomide will be provided by prescription.

**Solution preparation:** Capsules of temozolomide are available in 5mg, 20mg, 100mg, 140mg, 180mg and 250 mg. The daily dose will be rounded up to the nearest 5 mg. Each daily dose should be given with the least number of capsules.

The capsules are packaged in 30 cc, 28 mm, 48 Type I amber glass bottles and should be stored at 25°C, but temperatures between 15°C and 30°C are permissible. Capsules are stable for at least 30 months when stored in amber glass bottles at this temperature.

**Route of administration:** Temozolomide should be administered in the fasting state. Patients will be instructed to fast at least 2 hours before and 1 hour after temozolomide administration. Water is allowed during the fast period. Patients will be instructed to swallow the capsules whole, in rapid succession, without chewing them with approximately 250ml of water.

If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose.

Antiemetic prophylaxis will be given before each temozolomide administration. Prophylactic antiemetics should be prescribed as per Clinical Center and ASCO guidelines, although ondansetron is recommended.

For additional pharmaceutical information, please refer to the drug package insert.

## 9.0 CORRELATIVE/SPECIAL STUDIES

### 9.1 Baseline Tumor - Evaluations

We will request patients to submit *available*, pre-treatment tumor biopsies, including diagnostic samples (either from the lung primary or a metastatic site), once informed consent is obtained. Patients will not be required to undergo an additional biopsy. Formalin fixed paraffin embedded (FFPE) tumor samples will be used for these studies. Diagnostic tumor specimens can be submitted as a tumor block or on 15 to 20 unstained slides. The tumor block should be a minimum of 0.5 to 1 cubic cm. For needle biopsies, more material will be requested. For exact handling of the baseline tumor biopsy samples please refer to the separate lab manual and [Appendix L](#).

The studies that will be performed utilizing this baseline tissue from all patients enrolled onto this study, include, *but are not limited to*, *MGMT* promoter methylation analysis, IHC for *MGMT*, IHC for PARP-1, mRNA BRCA-1 expression, IHC for PTEN expression, IHC for RAD51, and IHC for BRCA-1. Given that there are often small quantities of tissue from SCLC cases, priority will be given to *MGMT* promoter methylation analysis, IHC for *MGMT*, and IHC for PARP-1.

#### 9.1.1 *MGMT* Promoter Methylation Analysis

Epigenetic silencing of the *MGMT* gene by promoter hypermethylation leads to improved sensitivity to alkylating agents, such as temozolomide.<sup>44,45</sup> *MGMT* promoter methylation status was analyzed retrospectively in the Phase III study of temozolomide in newly diagnosed glioma.<sup>46</sup> Irrespective of treatment, *MGMT* promoter methylation was an independent favorable prognostic factor. Patients with tumors demonstrating methylation of the *MGMT* gene had a significant improvement in median survival as well as in 2-year survival rate.<sup>46</sup>

Aberrantly methylated *MGMT* is found in SCLC<sup>45,64</sup> and may be an important biomarker in SCLC patients treated with temozolomide. In our Phase II study of temozolomide in patients with SCLC, we obtained tumor samples for *MGMT*

promoter methylation analysis and found that 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 time to progression for patients with *MGMT* promoter methylation ( $p = 0.29$ ). (Pietanza *et al.*, 2011, in preparation).

Approximately five to ten unstained slides obtained from formalin fixed paraffin-embedded tumor tissue will be required for this testing. DNA will be extracted from paraffin embedded tissue sections (see [Appendix C](#)). DNA will be extracted from the tumor area contained in at least 10x5 $\mu$ m-thick sections placed on uncharged glass slides. A hematoxylin-eosin stained slide will be used to distinguish tumor cells. DNA methylation analysis will be carried out using the EpiTyper system from Sequenom (San Diego, CA). The EpiTyper assay is a tool for the detection and quantitative analysis of DNA methylation using base-specific cleavage of bisulfite-treated DNA and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). We have used this technique in our ongoing Phase II study of temozolomide in SCLC patients and have optimized the assay for smaller amounts of DNA. See [Appendix D](#) for details on this procedure.

#### 9.1.1.1 Site Performing Analysis:

Memorial Sloan Kettering Cancer Center

#### 9.1.2 *MGMT* Analysis by Immunohistochemistry

The presence of the *MGMT* protein in tumor cells may be an important determinant of treatment failure with alkylating agents<sup>44,45</sup>. Loss of *MGMT* activity is accomplished via hypermethylation of specific CpG islands of the *MGMT* promoter in tumor cells. Lack of *MGMT* expression has been correlated with treatment response to temozolomide in pancreatic neuroendocrine tumors.<sup>71</sup>

In our Phase II study of temozolomide in SCLC patients, *MGMT* expression by immunohistochemistry was evaluated. (Pietanza *et al.*, 2011, in preparation). Although negative *MGMT* expression by immunohistochemistry did not correlate with response, it did show a weak trend towards improved time to progression (3.6 months vs. 1.3 months,  $p = 0.1$ ) (Pietanza *et al.*, 2011, in preparation). *MGMT* expression may be important biomarkers in SCLC patients treated with temozolomide.

For *MGMT* expression by immunohistochemistry, one to two unstained slides obtained from formalin fixed paraffin-embedded tumor tissue will be required. Immunohistochemistry will be performed for *MGMT* using validated and

quantitative methods developed by the MSKCC Pathology Core. Monoclonal mouse anti-MGMT antibody will be used (Invitrogen, clone MT23.2). The pathologist will score the slides as negative if there is no MGMT expression and positive if there is any definite expression of MGMT. See [Appendix E](#) for details on this procedure.

9.1.2.1 Site Performing Analysis:

Memorial Sloan Kettering Cancer Center

9.1.3 PARP-1, BRCA-1 and RAD51 Analyses by Immunohistochemistry

PARP-1 has been shown to be highly expressed in SCLC compared to NSCLC in proteomic studies comparing NSCLC and SCLC cell lines<sup>13</sup>. In addition, PARP-1 is overexpressed in SCLC and other neuroendocrine lung tumors and predicts response to PARP-1 inhibitors.<sup>13,72</sup> Thus, PARP-1 expression in baseline tumors should be evaluated in the setting of this clinical trial and correlated with clinical outcomes. In addition, because PARP1 is a co-activator of E2F1<sup>103</sup>, we predict that tumors with high PARP1 expression will have high expression of E2F1-targets, including E2F1-regulated DNA repair proteins such as RAD51 and BRCA1. To test this hypothesis, we will measure these markers by IHC in the clinical specimens.

Antibodies for E2F1 targets will be validated using formalin-fixed paraffin embedded (FFPE) cell lines with high or low protein levels for each marker (based on previous RPPA data) as positive and negative controls. This is the same approach used for the validation of PARP1 antibody for IHC application. IHC will then be performed on the tumor samples to assess the level and localization of the markers. E2F1-targets will include HR proteins RAD51 and BRCA1 which were shown in cell lines to correlate with PARP expression. Additional E2F1 targets may be added based on the results of ongoing in vitro studies.

Four to six unstained slides obtained from formalin fixed paraffin-embedded tumor tissue will be required for PARP-1, RAD51, and BRCA1 expression by immunohistochemistry.

9.1.3.1 Site Performing Analysis:

M.D. Anderson Cancer Center

9.1.4 Breast Cancer Gene (BRCA)-1 Analysis by mRNA expression

Tumor cells with *BRCA* deficiencies have been found to be very sensitive to PARP inhibition even in the absence of any other insults.<sup>33,34</sup> Overexpression



of BRCA1 has been found in patients with SCLC<sup>80</sup> and is associated with decreased sensitivity to cisplatin<sup>81</sup> and poor survival in patients with NSCLC.<sup>82</sup>

In this study evaluating the PARP-1 inhibitor, ABT-888, in SCLC, the role of BRCA1 should be explored.

Approximately five to ten unstained slides obtained from formalin fixed paraffin-embedded tumor tissue will be required for this testing. RNA will be extracted from paraffin embedded tissue sections (see [Appendix C](#)). RNA will be extracted from the tumor area contained in at least 10x5µm-thick sections placed on uncharged glass slides. A hematoxylin-eosin stained slide will be used to distinguish tumor cells. RNA analysis will be carried out. See [Appendix F](#) for details on this procedure.

#### 9.1.4.1 Site Performing Analysis:

Memorial Sloan Kettering Cancer Center

#### 9.1.5 PTEN Analysis by Immunohistochemistry

PTEN deficiency causes human tumor cells to be exquisitely sensitive to PARP inhibitors through a defect in homologous recombination.<sup>83</sup> In SCLC, there is approximately a 10-15% incidence of mutation of *PTEN*, as well as a lack of expression of PTEN.<sup>84-86</sup>

Thus, in a study evaluating a PARP inhibitor in SCLC, the role of PTEN should be explored. We anticipate that SCLC patients whose tumors lack PTEN expression will have improved clinical outcomes in those receiving ABT-888.

PTEN status will be assessed by immunohistochemistry on tumor samples obtained at diagnosis. One to two unstained slides obtained from formalin fixed paraffin-embedded tumor tissue will be required for this analysis. Immunohistochemistry will be performed for PTEN using validated and quantitative methods developed by the MSKCC Pathology Core. See [Appendix G](#) or details on this procedure.

#### 9.1.5.1 Site Performing Analysis:

Memorial Sloan Kettering Cancer Center

#### 9.1.6 Shipping of Tumor Specimens

Necessary slides will be cut from paraffin embedded pre-treatment tumor. This material will be packaged in a way to ensure they do not break. All slides (and/or blocks) must be shipped with appropriate identifiers including a pathology report from the collaborating institutions. The slides can be shipped any day of the week. The PI of the study (Dr. M. Catherine Pietanza,

(██████████) and the Multi-Center Trial Core Representative (Lakeisha Lubin, ██████████) will be notified by email the day that the slides are sent. This email will contain the following information:

- Subject name
- Subject number
- Shipment date
- Contents of shipment

Approximately twenty unstained slides from paraffin embedded tumor or the tumor block will be sent via messenger (if at MSKCC) or shipped via overnight carrier (if not at MSKCC) to:

M. Catherine Pietanza, MD  
Memorial Sloan Kettering Cancer Center  
300 East 66<sup>th</sup> Street, BAIC 1259  
New York, NY 10065  
Telephone: ██████████  
Email: ██████████

Detailed processing and shipment instructions can be found in the separate lab manual and [Appendix L](#).

Test	Laboratory	Material Needed
<i>MGMT</i> promoter methylation analysis <sup>§</sup>	Adriana Heguy, Ph.D. Geoffrey Beene Translational Oncology Core Facility MSKCC	5 to 8 unstained slides from paraffin embedded tumors
IHC for PARP-1, RAD51, and BRCA1 <sup>§</sup>	Lauren Byers, M.D. M.D. Anderson	4 to 6 unstained slides from paraffin embedded tumors
IHC for <i>MGMT</i> <sup>§</sup> and PTEN	William D. Travis, M.D. Pathology Core MSKCC	2 to 4 unstained slides from paraffin embedded tumors
mRNA BRCA-1	Agnes Viale, Ph.D. Genomic Core Laboratory MSKCC	5 to 8 unstained slides from paraffin embedded tumors

<sup>§</sup> *MGMT* promoter methylation analysis and immunohistochemistry for PARP-1 and *MGMT* will be given priority.

## 9.2 Circulating Tumor Cells (CTCs)

CTCs may represent a source of tissue in SCLC and may aid in monitoring treatment response, assess risk status for patients, and serially analyze tumor genotypes in a non-invasive manner. In our Phase II study of temozolomide in patients with SCLC, CTCs were measured using the Veridex CellSearch System™ at baseline and at the time of repeat imaging (at four weeks, eight weeks, and every eight weeks thereafter). CTCs

were detected in 82% of patients. We found a significantly higher number of CTCs in patients with liver metastases and those that progress at new sites. Increased CTCs at the end of cycle 1 and upon disease progression were associated with a poorer prognosis. By incorporating CTCs in ongoing studies, we can continue to develop these as potential biomarker in this disease.

## 9.2.1 Collection of Specimens

### 9.2.1.1 Veridex CellSearch System™

For all patients on the study, peripheral blood will be collected on day 1 of cycle 1 (prior to the first dose of ABT-888/placebo and temozolomide) and each time a CT scan is obtained to evaluate disease status (during week 4, week 8, and every 8 weeks thereafter) for CTC identification and enumeration using the Veridex CellSearch System™. Blood will be collected in two Cell Save® Tubes, which is a 10mL evacuated blood collection tube, containing a preservative for CTC enumeration. In each tube, at least 7.5ml of peripheral blood will be collected.

## 9.2.2 Handling of Specimens

### 9.2.2.1 Veridex CellSearch System™

After collection, the Cell Save® Tube must be inverted eight times to prevent clotting and then can be stored at room temperature. On the specific form (see [Appendix H](#)) the following must be recorded: the study number, the patient's unique identifier, date and time.

The Cell Save® Tubes will be sent via messenger (if at MSKCC) or shipped via overnight carrier (if not at MSKCC), see [section 9.2.3](#). See [Appendices I and J](#) for additional details regarding this system and standard operating procedures.

With the aid of the Cell Search™ Epithelial Cell Kit, CTCs are immunomagnetic selected and stained on the Cell Track® Auto Prep® System. Once the Cell Track® Auto Prep® System dispenses the final sample into a cartridge, the CTCs are identified and enumerated on the Cell Tracks® Analyzer II, a semi-automated fluorescence microscope.

The operator of the Cell Track® Auto Prep® System will mix 7.5 mL of blood from the Cell Save® Tube with 6.5 mL of the Dilution Buffer into a labeled conical tube and centrifuge it at 800 x g for 10 minutes with the brake in the off position. The conical tube is then placed on the instrument that utilizes a software protocol for the immunomagnetic selection and staining of CTCs using the Cell Search™ Epithelial Cell Kit.

The captured and stained cells are dispensed into a sample cartridge

which sits inside a MagNest®; here the cells migrate to the analytical surface of the cartridge due to the magnetic field of the MagNest®. The Cell Tracks® Analyzer II will scan the entire surface of the cartridge with a series of fluorescence filters that are defined for the Cell Search™ Epithelial Cell Kit. The cell images from each filter are compiled and presented in a gallery format for cell identification and enumeration by the user.

There is an additional channel on the Cell Tracks® Analyzer II that may be used for a user-defined marker. In the present study, the CTCs will be phenotyped using anti-phospho-histone  $\gamma$ H2AX (Ser<sup>139</sup>, clone JBW301) conjugated to FITC. The marker will be added by the CellTracks AutoPrep during sample preparation. The  $\gamma$ H2AX to be used will be purchased from Millipore (catalog # 16-202A) and diluted to 5.6 ug/ml using PBS/0.1%BSA before being placed on the AutoPrep. The final concentration of the conjugate during staining will be 1ug/ml. The marker may be optimized with cell lines known to be positive or negative for  $\gamma$ H2AX expression before testing it with patient samples. The samples stained with  $\gamma$ H2AX-FITC will be analyzed on Cell Tracks® Analyzer II using a user defined research protocol.

### 9.2.3 Shipping of Specimens

#### 9.2.3.1 Veridex CellSearch System™

Samples at MSKCC will be sent via messenger.

#### **Non-MSKCC Specimen Shipping Procedures:**

Shipment of human blood samples must comply with appropriate regulations as specified by the carrier. At a minimum, all samples must be packaged within two containers with absorbent material between containers to control any spill or leakage. The outer container must be puncture-resistant (e.g. cardboard mailing tube, corrugated cardboard box). A biohazard sticker must be affixed to both the inner and outer containers.

All samples should be shipped via overnight courier at room temperature. A copy of each of the specific forms for the respective patients should be included with each shipment.

#### **All specimens are to be shipped to:**

**MSKCC Clinical Chemistry Laboratory  
Attn. Rashmi Kamath or Iris Rodriguez, Schwartz 359**

**CTCs For SCLC ABT-888 and Temozolomide**  
**411 East 67<sup>th</sup> Street**  
**New York, NY 10065**  
**Phone: [REDACTED]**

Please notify either Rashmi Kamath by email at [REDACTED] or telephone at [REDACTED] or Iris Rodriguez by email at [REDACTED] or telephone at [REDACTED]. An e-mail should be sent with the tracking number to Rashmi Kamath ([REDACTED]), Iris Rodriguez ([REDACTED]), and the MSKCC Multi-Center Trial Core Representative, Lakeisha Lubin ([REDACTED]). Samples should be shipped on the same day of collection to arrive Mondays through Fridays. Special arrangements can be made for Saturday arrival. A name, phone number, fax number, or emails address should be included with samples so that receipt can be acknowledged.

Detailed processing and shipment instructions can be found in the separate lab manual and [Appendix L](#).

#### 9.2.4 Sites Performing Analyses

##### 9.2.4.1 Veridex CellSearch System™

Memorial Sloan Kettering Cancer Center

### 9.3 Plasma biomarkers

Plasma biomarkers will be examined at baseline and during treatment to further investigate the mechanisms of action of the study drugs and identify potentially useful markers for non-invasively monitoring response during treatment. The analyses will include markers of apoptosis (e.g. M30 and M65)<sup>97</sup> and tumor bulk (pro-GRP). Furthermore, as noted previously, both temozolomide and PARP inhibitors have been shown to exert antiangiogenic effects in preclinical testing. It is not known if these effects are an important component of their clinical activity. To evaluate this issue, plasma markers of angiogenesis will also be assessed.

#### 9.3.1 Collection of Specimens

##### 9.3.1.1 Plasma

For all patients on the study, peripheral blood will be collected on day 1 of cycle 1 (prior to the first dose of ABT-888/placebo and temozolomide) and each time a CT scan is obtained to evaluate disease status (during week 4, week 8, and every 8 weeks thereafter) for analysis of plasma biomarkers.

### 9.3.1.2 Plasma Analyses

The analysis of plasma markers will be conducted at MD Anderson Cancer Center.

Plasma assays will be assessed using established methods as we and others have previously described<sup>96-98,100-102</sup>. Pro-GRP will be measured using ARCHITECT ELISA kits (Abbott Diagnostics, Abbott Park, IL). M30 and M65 will be assessed by ELISA (Peviva, Bromma, Sweden). Markers of angiogenesis-related pathways, including sVEGFR2, sKIT, VEGF, OPN, CA-9, and other related biomarkers will be assessed using a combination of multiplex technology (e.g. mainly Searchlight platforms) and validated, enzyme-linked immunosorbent assays (ELISA) assays. Other analytical platforms will be considered if they are established to have advantages (e.g. lower volume requirements or greater sensitivity). Plasma assays for tumor markers relevant described above (e.g. circulating MGMT promoter methylation) will also be considered. For each plate, the standard curves will be assessed to ensure that the expected assay range was achieved. For each individual sample, the mean concentration is calculated for duplicate samples, and the coefficient of variance % (CV%) is calculated for each of the analytes. If the median CV% is greater than 25%, analysis of the sample will be repeated. In our experience, less than 10% of samples require repeat analysis.

### 9.3.2 Handling and Processing of Plasma Specimens

1. Label four (4) 1.8 ml cryovials with the study number, patient study number, and procedure date, and clearly mark cryovials “plasma.”
2. Collect 8 mL of blood into EDTA Vacutainer® blood collection tube (BD Sciences, Lavender top)
3. Process: Spin EDTA Vacutainer® blood collection tube (BD Sciences, Lavender top) in a standard clinical centrifuge at ~2500 RPM at 4° Celsius for 10 minutes.
4. **Centrifuge within 30 minutes of collection.** If the interval between specimen collection and processing is anticipated to be greater than one hour, keep specimen on ice until centrifuging is done.
5. Aliquot 1 mL of plasma into cryovials labeled with the study number, patient number and procedure date and marked “plasma.”
6. Place cryovials into divider box. Store at a minimum -70° Celsius until ready for shipment/analysis.

### 9.3.3 Shipping of Specimens

Samples should be shipped in batches to the address below. Shipment of human blood samples must comply with appropriate regulations as specified by the

carrier. A biohazard sticker must be affixed to both the inner and outer containers.

All samples should be shipped via overnight courier on dry ice.

**All specimens are to be shipped to:**

**Thoracic Blood Biomarker laboratory  
Attn. Dr. John Heymach or Dr. Uma Giri  
Room #: T8.3970, UT MD Anderson Cancer Center,  
1515 Holcombe Blvd  
Houston, Texas- 77030  
Phone - [REDACTED]**

Please notify either Uma Giri by email at [REDACTED] or telephone at [REDACTED] or Dr. Hai Tran by email at [REDACTED] or telephone at [REDACTED]. Samples should be shipped on the same day of collection to arrive Mondays through Fridays. Special arrangements can be made for Saturday arrival. A name, phone number, fax number, or emails address should be included with samples so that receipt can be acknowledged. Please also include the Multi-Center Trial Core Representative, Lakeisha Lubin ([REDACTED]) on e-mail notifications.

9.3.4 Site Performing Plasma Analyses

MD Anderson Cancer Center

**10.0 STUDY CALENDAR**

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Study	Cycle 1 (each cycle is 28 days)				Cycle 2 (each cycle is 28 days)				Cycle 3 and Beyond (each cycle is 28 days)				End of Study Visit <sup>d</sup>	Post-Treatment Follow-up <sup>f</sup>
		Wk 1 Day 1	Wk 2 Day 8	Wk 3 Day 15	Wk 4 Day 22	Wk 1 Day 1	Wk 2 Day 8	Wk 3 Day 15	Wk 4 Day 22	Wk 1 Day 1	Wk 2 Day 8	Wk 3 Day 15	Wk 4 Day 22		
ABT-888/Placebo <sup>a</sup> Temozolomide <sup>b</sup>		d1-7 d1-5				d1-7 d1-5				d1-7 d1-5					
Informed consent <sup>g</sup>	X														
Medical history	X														
Concurrent meds	X	X-----X													
Physical exam	X	X <sup>i</sup>		X <sup>j</sup>		X <sup>ij</sup>		X <sup>j</sup>		X <sup>ij</sup>				X	
Review Pill Diary		X <sup>i</sup>				X <sup>ij</sup>				X <sup>ij</sup>				X	
Vital signs	X	X <sup>i</sup>		X <sup>j</sup>		X <sup>ij</sup>		X <sup>j</sup>		X <sup>ij</sup>				X	
Height	X														
Weight <sup>c</sup>	X	X <sup>i</sup>		X <sup>j</sup>		X <sup>ij</sup>		X <sup>j</sup>		X <sup>ij</sup>				X	
Performance status	X	X <sup>i</sup>		X <sup>j</sup>		X <sup>ij</sup>		X <sup>j</sup>		X <sup>ij</sup>				X	
CBC w/diff, plts	X	X <sup>k</sup>	X <sup>l</sup>	X <sup>k</sup>	X <sup>l</sup>	X <sup>k</sup>	X <sup>l</sup>	X <sup>k</sup>	X <sup>l</sup>	X <sup>k</sup>		X <sup>l</sup>		X	
Chemistry <sup>d</sup>	X <sup>t</sup>	X <sup>k</sup>		X <sup>k</sup>		X <sup>ku</sup>		X <sup>k</sup>		X <sup>ku</sup>				X	
EKG (as indicated) <sup>e</sup>	X														
Adverse event evaluation		X-----X <sup>s</sup>													
CT chest <i>and</i> other relevant sites	X <sup>m</sup>				X <sup>n</sup>				X <sup>n</sup>				X <sup>n</sup>		X
MRI brain with gad <i>or</i> CT head with contrast	X <sup>m</sup>				X <sup>n,o</sup>				X <sup>n,o</sup>				X <sup>n,o</sup>		X
Pregnancy Test <sup>f</sup>	X														
Paraffin block or unstained slides (if available) <sup>g</sup>	X														
Plasma for biomarkers <sup>h</sup>		X <sup>p</sup>			X <sup>p</sup>				X <sup>p</sup>					X	X
Peripheral blood collection of CTC <sup>h</sup>		X <sup>p</sup>			X <sup>p</sup>				X <sup>p</sup>					X	X



- § The informed consent must be signed within 3 weeks of starting the study.
- a. ABT-888/Placebo: Dose as assigned; given orally twice daily on days 1 to 7 of each cycle. Dosing window of +/- 2 days at the start of each cycle.
  - b. Temozolomide: Dose as assigned for all patients; given orally once daily in the fasting state on days 1 to 5 of each cycle. Dosing window of +/- 2 days at the start of each cycle.
  - c. Dosing of temozolomide will be based on actual body weight **at study screening**. The daily dose will be rounded up to the nearest 5 mg.
  - d. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
  - e. An EKG will be performed at baseline for all patients. Additional EKGs will be performed as clinically indicated.
  - f. Pregnancy test (women of childbearing potential) must be done within 14 days prior to starting treatment.
  - g. For correlative studies: Pre-treatment tumor biopsies (including diagnostic samples) will be obtained from patients if available. The studies that will be performed include, but are not limited to, *MGMT* promoter methylation analysis, IHC for *MGMT*, IHC for *PARP-1*, mRNA *BRCA-1* expression, IHC for *PTEN* expression, and others. Given that there are often small quantities of tissue from SCLC cases, priority will be given to *MGMT* promoter methylation analysis, IHC for *MGMT*, and IHC for *PARP-1*.
  - h. Blood will be collected from all patients at time of re-staging scans to be assessed by the Veridex CellSearch® System and sent to MSKCC.
  - i. Pre-treatment physical exam, review of pill diary, vital signs, weight, and performance status can take place up to 2 days prior to commencement of each cycle, as long as the patient does not report any significant changes in the intervening period.
  - j. May be performed within +/- 2 days of scheduled time.
  - k. May be obtained within 48 hours if same-day blood work is not available.
  - l. CBC for cycles 1 and 2, days 8 and 22, as well as for day 15 of cycle 3 and beyond can be obtained at a local laboratory. May be obtained within 48 hours if same-day blood work is not available.
  - m. CT scan of the chest and other relevant sites, as well as MRI brain with gadolinium (or CT scan brain with contrast if MRI contraindicated) will be performed ≤ 2 weeks prior to the start of therapy.
  - n. Restaging scans to assess response must be obtained anytime during week 4, week 8, and every 8 weeks thereafter, regardless of dosing delays. However, scans may be moved up if clinically indicated.
  - o. Only if brain metastases are present at baseline or if deemed medically appropriate.
  - p. Obtain during pre-treatment evaluation visit of cycle 1, and during week 4, week 8 and every 8 weeks thereafter, to correspond with timing of diagnostic imaging.
  - q. Should a patient come off study during an unscheduled visit, a full evaluation is expected to occur, including: physical exam, review of pill diary, vital signs, weight, performance status, adverse event evaluation; in addition peripheral blood should be obtained for CBC, CMP, and CTCs.
  - r. If a patient is taken off study for any reason other than disease progression, an off-study CT scan is not necessary; however, the patient should undergo a CT scan 8 weeks (+/- 1 week) from the time of the last scan and continue having CT scans every 8 weeks (+/- 1 week) until progression is documented, the start of new anticancer therapy, or death. Overall survival data will be collected every 12 weeks on all patients after study discontinuation until death.
  - s. Adverse event evaluation as per Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.
  - t. Liver function tests should be performed prior to treatment initiation. If abnormal, the decision to initiate temozolomide treatment should carefully consider the benefits and risks for the individual patient.
  - u. Liver function tests should be performed after each treatment cycle. For patients with significant liver function abnormalities, the benefits and risks of continuing treatment should be carefully considered.

## 11.0 MEASUREMENT OF EFFECT

### 11.1 Antitumor Effect – Solid Tumors

Although response is not the primary endpoint of this trial, patients will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated for response with a CT scan of the chest and other clinically relevant sites during week 4, week 8 and every 8 weeks thereafter. Brain metastases will be included with other indicator lesions in the determination of response. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response. Patients will be re-evaluated for response until progression is documented, the start of new anticancer therapy, or death.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)<sup>4</sup>. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

### 11.1.1 Definitions

Evaluable for toxicity. All patients who receive at least one dose of treatment will be evaluable for toxicity from the time of their first treatment with ABT-888/placebo and temozolomide.

Evaluable for objective response. All patients randomized will be included in the intent-to-treat analysis of efficacy. An additional ‘as-treated’ analysis will be performed including only those patients who have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable and will be declared non-responders.)

### 11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter for non-nodal lesions and short axis for nodal lesions to be recorded) as  $\geq 10$  mm with CT scan or  $\geq 10$  mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Lesions that are previously irradiated must show clear evidence of progression over a minimum of 3 months to include as measurable. This period of time is used to discount tumor edema in an irradiated field as a false sign of disease progression. Should the period of time be less than 3 months, lesions in a previously irradiated field cannot be included as measurable disease.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be  $\geq 15$  mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter  $< 10$  mm or pathological lymph nodes with  $\geq 10$  to  $< 15$  mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are

preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

### 11.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and  $\geq 10$  mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, size will be measured using calipers.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is preferred for brain lesions (although CT brain with contrast can be used for patients that cannot tolerate or are unable to undergo MRI). MRI is acceptable in certain

situations (e.g. for body scans), but not the lung.

The modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

#### 11.1.4 Response Criteria

##### 11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm, to minimize the possibility that small changes in a small number of target lesions is falsely interpreted as progression. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify

#### 11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances. In this setting, to achieve *'unequivocal progression'* on the basis of non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

New Lesions: The appearance of new malignant lesions denotes disease progression.

While there are no specific criteria for the identification of new radiographic lesions, the findings of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial

or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

#### **11.1.4.3 Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

<b>Table 7. Evaluation of Best Overall Response</b>				
<b>Target Lesions</b>	<b>Non-Target Lesions</b>	<b>New Lesions</b>	<b>Overall Response</b>	<b>Best Overall Response for This Category also Requires:</b>
CR	CR	No	CR	≥ 4 wks. Confirmation
CR	Non-CR/ Non-PD	No	PR	≥ 4 wks. Confirmation
PR	Non-CR/ Non-PD	No	PR	
SD	Non-CR/ Non-PD	No	SD	documented at least once ≥ 4 wks. from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	
* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.				
<u>Note:</u> Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “ <i>symptomatic deterioration</i> .” Every effort should be made to document the objective progression even after discontinuation of treatment.				

### 11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

### 11.1.6 Progression-Free Survival



Progression-free survival (PFS) is defined as the duration of time from randomization to time of progression or death, whichever occurs first.

#### **11.1.7 Overall Survival**

Overall survival (OS) is defined as the duration of time from randomization to time of death.

#### **11.1.8 Response Review**

The investigator of each site will review radiologic studies prospectively with an independent radiologist at his/her respective site.

### **12.0 DATA REPORTING / REGULATORY REQUIREMENTS**

Adverse event lists, guidelines, and instructions for AE reporting can be found in [Section 7](#) (Adverse Events: List and Reporting Requirements).

#### **12.1 Data Reporting**

##### **12.1.1 Method**

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site ([http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/cdus.htm](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/cdus.htm)).

MSKCC will assign a RSA and RPC to manage data entry for this study. The RSA and RPC will enter data into a secure database (CRDB) for MSKCC and outside patients respectively.

MSKCC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator review.

**Note:** All adverse events that have occurred on the study, including those reported through CTEP-AERS, must be reported via the monitoring method identified above.

Weekly meetings will occur to monitor patient accrual. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of once per year, more frequently if indicated.

### **12.1.2 Responsibilities for Data Submission at Participating Sites**

Standardized Case Report Forms (CRFs) and directions for use as well as sign off requirements have been generated for this study. A schedule of required forms is shown in [Table 8](#). Participating sites will be responsible for filling out these CRFs and submitting them to MSKCC per the designated timelines. MSKCC staff will enter the data into a secure database (Clinical Research Database – CRDB) to facilitate CDUS transfer.

Research staff at MSKCC will review data as it is submitted. In the event of inconsistent or missing data, queries will be sent by MSKCC Research staff to the participating sites. Queries will be sent by MSKCC Research staff twice a month. Participating sites should respond to data queries within 14 days of receipt.

Participating sites should e-mail CRFs to MSKCC to the contact provided below. Submissions should include a cover page listing all CRFs enclosed per participant.

Email: [REDACTED] to the attention of Multi-Center Trial Core Representative

### **12.1.3 Data Submission Timelines for Participating Sites**

Data and source should be submitted to MSKCC according to [Table 8](#) Data Submission Timelines for Therapeutic Studies:

<b>Table 8. Data and Source Submission Requirements and Timelines for Therapeutic Studies</b>								
	Baseline	Cycle #1		Cycle #2		Cycle #3/ D#1 and beyond	SAE	Off Study
		D#1	D#15	D#1	D#15			
<b>SUBMISSION SCHEDULE</b>								
Source Documentation	<b>Within 24 hours</b> (see <a href="#">section 4</a> )	<b>within 14 days of visit</b>					<b>Within 3 days of event</b> (see <a href="#">section 7</a> ); updates to be submitted as available	<b>Within 14 days of visit</b>
CRFs	<b>Within 7 days of visit</b>							
<b>Required Forms</b>								
<i>Demographics Form</i>	<b>X</b>							
<i>Medical History Form</i>	<b>X</b>							
<i>Concomitant Medications Form</i>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Physical Exam Form</i>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>		<b>X</b>
<i>Treatment Form</i>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>		<b>X</b>
<i>Laboratory Form</i>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>		<b>X</b>
<i>Lesion/EOD Form</i>	<b>X</b>		<b>X</b> <sup>1</sup>		<b>X</b> <sup>1</sup>	<b>X</b> <sup>1</sup>		<b>X</b>
<i>Adverse Event Form</i>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
<i>Serious Adverse Event Form</i>							<b>X</b>	
<i>Off Study Form</i>								<b>X</b>
1. CT scans of the chest and other relevant sites of disease, as well as brain imaging if applicable, will be performed during week 4, week 8, and every 8 weeks thereafter.								

**Data**

Standardized Case Report Forms (CRFs), directions for use and sign off requirements have been generated for this study. Blank case report forms will be sent to the study staff at each participating site for use. The participating Site PI is responsible for ensuring these forms are completed accurately, legibly and in a timely manner.

**Source Documentation**

Source documentation refers to original records of observations, clinical findings and evaluations that are subsequently recorded as data. Source documentation should be consistent with data entered into CRFs. The participating site is responsible for maintaining adequate and correct source documentation for all submitted data.

Relevant source documentation to be submitted throughout the study includes:

- Baseline measures to assess pre-protocol disease status (ex. CT, brain imaging with MRI or CT scan)
- Treatment records, including prior therapy administered with dates
- Toxicities/adverse events not previously submitted with SAE Reports
- Response designation

Source documentation should include a minimum of two identifiers to allow for data verification. MSK will maintain the confidentiality of any subject-identifiable information it may encounter.

### **Regulatory Documentation**

Prior to implementing this protocol at MSKCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSKCC Institutional Review Board/Privacy Board (IRB/PB) and CTEP. Prior to implementing this protocol at the participating sites, approval for the MSKCC IRB/PB approved protocol must be obtained from the participating site's IRB and submitted to the CTEP PIO.

The following documents must be provided to MSKCC before the participating site can be initiated and begin enrolling participants:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form

Upon receipt of the required documents, MSKCC will formally contact the site and grant permission to proceed with enrollment.

### **Amendments**

Each change to the protocol document must be organized and documented by MSKCC and first approved by the MSKCC IRB/PB. Upon receipt of MSKCC IRB/PB approval, MSKCC will immediately distribute all non expedited amendments to the participating sites, for submission to their local IRBs.

Participating sites must obtain approval for all non expedited amendments from their IRB within 90 calendar days of MSKCC IRB/PB approval. If the amendment is the result of a safety issue or makes eligibility criteria more restrictive, sites will not be permitted to continuing enrolling new participants until the participating site IRB approval has been granted.

The following documents must be provided to MSKCC for each amendment within the stated timelines:

- Participating Site IRB approval

- Participating Site IRB approved informed consent form and HIPAA authorization

### **Additional IRB Correspondence**

#### ***Continuing Review Approval***

The Continuing Review Approval letter from the participating site's IRB and the most current approved version of the informed consent form should be submitted to MSKCC within 7 days of expiration. Failure to submit the re-approval in the stated timeline will result in suspension of study activities.

#### ***Violations***

A protocol violation is anything that occurs with a participant, which deviated from the protocol without prior approval from the MSKCC IRB/PB. For protocol violations that are identified after they occur, the participating site should report to MSKCC as soon as possible. The MSKCC PI will in turn report the violation to the MSKCC IRB/PB and CTEP.

Participating sites should report violations to their institution's IRBs as soon as possible per that site's institutional guidelines. Approvals/acknowledgments from the participating site IRB for protocol violations should be submitted to MSKCC as received.

#### ***Other correspondence***

Participating sites should submit other correspondence to their institution's IRB according to local guidelines, and submit copies of that correspondence to MSKCC.

### **Document maintenance**

The MSKCC PI and the Participating Site PI will maintain adequate and accurate records to enable the implementation of the protocol to be fully documented and the data to be subsequently verified.

The participating sites will ensure that all participating site IRB correspondence (IRB approval letters referencing protocol version date and amendment number, IRB approved protocol, appendices, informed consent forms, deviations, violations, and approval of continuing reviews) is maintained in the regulatory binder on site and sent to MSKCC.

A regulatory binder for each site will also be maintained at MSKCC; this binder may be paper or electronic.

After study closure, the participating site will maintain all source documents, study related documents and CRFs for 7 years.

### **Noncompliance**

If a participating site is noncompliant with the protocol document, accrual privileges may be suspended and/or contract payments may be withheld (if applicable), until the outstanding issues have been resolved.

### **Quality Assurance for Participating Sites**

Each site participating in the accrual of participants to this protocol may be audited by the staff of the MSKCC study team for protocol and regulatory compliance, data verification and source documentation. Audits may be accomplished in one of two ways: (1) selected participant records can be audited on-site at participating sites or (2) source documents for selected participants will be sent to MSKCC for audit. Audits may be determined by participant accrual numbers and rate of accrual, but can also be prompted by reported SAEs or request of MSKCC PI.

Audits will be conducted at least annually during the study (or more frequently if indicated). The number of participants audited will be determined by available time and the complexity of the protocol.

The audit will include a review of source documentation to evaluate compliance for:

- Informed consent documents and procedures
- Adherence to eligibility criteria
- Protocol defined treatment
- Required baseline, on study and follow-up protocol testing
- IRB documents (submitted amendments, annual continuing review reports, SAEs)
- Required specimen submission
- Case Report Form submissions to MSKCC: time lines and accuracy

### **Response Review**

Since therapeutic efficacy is a stated primary objective, all sites participant's responses are subject to review by MSKCC's Therapeutic Response Review Committee (TRRC). Radiology, additional lab reports and possibly bone marrow biopsies and/or aspirates will need to be obtained from the participating sites for MSKCC TRRC review and confirmation of response assessment. These materials must be sent to MSKCC promptly upon request.

## **12.2 CTEP Multicenter Guidelines**

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in [Appendix B](#)

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.
- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO ([PIO@ctep.nci.nih.gov](mailto:PIO@ctep.nci.nih.gov)) except for Group studies.

### 12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as “Collaborator(s)”) and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the “Intellectual Property Option to Collaborator”

([http://ctep.cancer.gov/industryCollaborations2/intellectual\\_property.htm](http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm)) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient’s family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: <http://ctep.cancer.gov/>.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):
  - a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
  - b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

- c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator ([http://ctep.cancer.gov/industryCollaborations2/intellectual\\_property.htm](http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm)). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: [ncicteppubs@mail.nih.gov](mailto:ncicteppubs@mail.nih.gov)

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

## 12.4 Data and Safety Monitoring



The Data and Safety Monitoring (DSM) Plans at Memorial Sloan Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: <http://www.cancer.gov/clinicaltrials/conducting/dsm-guidelines/page1>. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: <http://inside2/clinresearch/Documents/MSKCC%20Data%20and%20Safety%20Monitoring%20Plans.pdf>

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center’s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

## 13.0 STATISTICAL CONSIDERATIONS

### 13.1 Study Design/Endpoints

The study will be performed as a double-blind, placebo controlled, randomized Phase II in patients with relapsed sensitive or refractory SCLC. Eligible patients will be randomized 1:1 to one of two treatment arms: ABT-888 and temozolomide as the investigational arm, versus placebo and temozolomide as the control arm. The randomization will be stratified by center and by type of relapse (sensitive vs. refractory, as described in [Section 13.3](#)).

The primary objective is to compare the two treatment regimens with respect to efficacy, expressed as progression-free survival (PFS) at 4 months post-randomization. PFS will be calculated as proportion of patients alive and without evidence of disease at 4 months after randomization. PFS at 4 months will be compared across the two arms using a Fisher exact test.

In our current Phase II study of temozolomide in SCLC, PFS at four months was 18%

for the combined sensitive and refractory groups (22% in the sensitive group and 7% in the refractory group). This trial enrolled sensitive and refractory patients in a proportion 4:1, which is the expected proportion in the current study. Based on this, the expected PFS at 4 months in the control group is 15%. With a randomization of 50 patients per arm, there will be 85% power to detect an improvement in PFS at 4 months from 15% to 35% (one-sided type I error = 0.15).

One interim analysis for early assessment of inefficacy is planned after 50 patients (half of the total planned sample size) have had their 4 months PFS evaluation. The interim analysis will be done as “intent to treat”. The trial will be terminated early if at the interim analysis more events (progressions or deaths) are observed in the treatment arms than in the control arm; this is equivalent to a one-sided p-value of 0.50 or larger at the interim analysis. This approach offers a 50% chance of stopping accrual if the experimental regimen is inefficient and leads to minimal loss of power compared to an analysis without intermediate look.<sup>104,105</sup> If, based on these criteria, the interim analysis indicates that the null hypothesis is unlikely to be rejected at the end of the trial, accrual to both arms will be stopped and the trial will be terminated for inefficacy.

Accrual to the study will be halted after the first 50 patients (half of the total planned sample size) are enrolled and have had their 4 months PFS evaluation to allow for the interim analysis. Results at that time will determine whether enrollment will continue to 100 treated patients.

All patients randomized will be included in the ‘intent to treat’ analysis, regardless of the medication actually received. Therefore, untreated, randomized patients will also be included in the ‘intent to treat’ analysis. Patients who are taken off study early and have no evidence of progression by 4 months will undergo an imaging study at 4 months (+/- 1 week) for determination of the primary endpoint. In addition, we will perform an ‘as treated’ analysis, which will include only patients who received at least one cycle of the assigned treatment.

### **13.2 Sample Size/Accrual Rate**

The study design requires an accrual of 100 treated patients in total, randomized in proportion 1:1 in the two treatment arms (50 patients per arm). Patients registered and randomized who are not treated, due to no longer being eligible at the time of starting drug, will be replaced. Up to a maximum of 10 patients will be replaced for a sample size of 110 patients. Untreated, randomized patients will be included in the ‘intent to treat’ analysis, but will not be counted toward the total accrued or the ‘as treated’ analysis.

This study will be a multi-institution phase II study led by MSKCC. In our current phase II study of temozolomide we have been able to accrue 2 to 3 patients per month; thus we anticipate the same monthly accrual from our site alone. Once the study is open at all participating centers that include, M.D. Anderson Cancer Center, Johns Hopkins Kimmel Cancer Center, Johns Hopkins Bayview Medical Center and University Hospitals Case Medical Center. We expect to enroll 5 to 6 patients per month. Therefore, we anticipate full patient enrollment within 20 months.

At the time of this 14th amendment (May 2014), additional sites have been added to the protocol, which include Washington University School of Medicine, Emory University Winship Cancer Institute, Virginia Commonwealth University, UNC Lineberger Comprehensive Cancer Center and Moffitt Cancer Center. As such, we plan to complete patient enrollment within an additional 10 months.

### **13.3 Stratification Factors**

Patients who are randomized will be stratified by center and by relapse type (“sensitive,” defined as relapse greater than 60 days after the completion of first-line platinum-based chemotherapy versus “refractory,” defined as no response to initial platinum-based therapy, or progression within 60 days after completing treatment; and any patient in need of third-line therapy).

### **13.4 Analysis of Secondary and Exploratory Endpoints**

Objective response rates (ORR, by RECIST 1.1 criteria) and the corresponding exact two-sided 95% confidence intervals will be calculated and reported in both arms of the study. Comparisons between treatment arms will be performed using Fisher -exact test.

Overall survival (OS) will be estimated in each treatment group using Kaplan-Meier method, with the time origin at the date of randomization. Patients alive at the time of the last follow-up will be censored. Group comparisons will be performed using log-rank test.

In addition, subgroup analyses will be performed for all efficacy endpoints. PFS at 4 months, ORR and OS will be reported separately and compared as described above for patients with: sensitive vs. refractory disease, brain metastases or no brain metastases at time of study entry, and second-line or third-line treatment.

Safety and tolerability will be determined separately for the treatment arms: ABT-888 with temozolomide and placebo with temozolomide. Toxicity data (AEs, laboratory data and vital sign data) will be collected and tabulated according to CTCAE version 4.0 and summarized using descriptive statistics. Adverse events will be listed individually per patient.

#### **13.4.1 Toxicity Stopping Rules**

In order to reduce patient risk, the study design includes early termination of the trial in the event of excessive toxicity. Events counting towards the evaluation of excessive toxicity are grade 3 and 4 neutropenic fevers and grade 3 and 4 hemorrhage events (such as epistaxis, oral hemorrhage, gastrointestinal hemorrhage, pleural hemorrhage, pharyngeal hemorrhage, retroperitoneal hemorrhage, intracranial hemorrhage, vaginal hemorrhage) that occur in the setting of grade 3 and 4 neutropenia and thrombocytopenia, respectively, and grade 4 non-hematologic toxicities thought to be at least possibly related to treatment, such as life threatening pulmonary emboli, cerebrovascular events, acute myocardial infarction, left ventricular systolic dysfunction, hemorrhagic events, and seizures. The excessive toxicities will be evaluated separately in each arm. This analysis will be done in the first 15 patients and after the first 30 patients in the respective arm have completed the first two cycles of treatment, prior to the administration of the combination therapy to subsequent patients in that arm. The accrual will be stopped for excessive toxicity if: more than 6 patients experience excessive toxicities among the first 15 patients enrolled, or more than 10 patients experience excessive toxicities among the first 30 patients enrolled. In addition, the regimen will be deemed too toxic if more than 15 patients experience excessive toxicities among the 50 patients who receive the respective treatment. Under this stopping rule, the probability of stopping the trial early is <5% if the true toxicity rate is 20%, and >95% if the true toxicity rate is 50%. If either of the two arms will be deemed too toxic, the accrual will be stopped in both arms and the trial will be terminated.

Toxicities also will be reviewed across all patients enrolled (without unblinding) after 20 patients have completed cycle 1 or earlier if the investigators notice an increased rate of treatment delay due to toxicities.

#### 13.4.2 Correlative Endpoints

Correlative endpoints will be based on biopsy specimens available at the time of study enrollment. No additional biopsies will be performed as part of this protocol. The availability of a biopsy is not a condition for enrollment in the study; based on our prior experience, we anticipate that approximately 60% of the patients enrolled will have material available for the analysis of the correlative endpoints. This analysis will be exploratory and hypothesis-generating.

For all patients in both study arms, the presence of *MGMT* promoter methylation will be assessed by the EpiTyper assay, while *MGMT* expression will be assessed by immunohistochemistry. For both tests, results will be expressed as binary variables. Associations with objective response, with progression free survival and with overall survival will be tested using Fisher's exact test and log-rank test, respectively.

We will assess PARP-1 expression in available tumor specimens from patients in both study arms, and use Fisher's exact test to correlate response and log-

rank test to correlate with progression free survival and overall survival. Similar analyses will be conducted for RAD51 IHC, BRCA1 IHC, mRNA BRCA-1 and PTEN IHC.

Circulating tumor cells (CTCs) will be identified and enumerated using the Cell Search™ System in these patients with SCLC at baseline and at the time of repeat imaging. We will correlate the (a) number of CTCs at baseline and at each follow-up time with PFS and OS, using Cox proportional hazards model (landmark analyses and time-dependent analyses); (b) the change in CTCs with radiographic response; and (c) the number of CTCs at baseline with patient characteristics (disease burden, location of metastases, and progression at existing sites or new sites of disease). The number of CTC will be explored as a continuous variable and the presence of a threshold predictive of the outcome will be investigated; we will avoid an arbitrary grouping of the variable that is not supported by the data.

From the same sample that we will identify and enumerate CTCs at baseline and at the time of repeat imaging, we will measure nuclear  $\gamma$ H2AX levels. We will use Wilcoxon test to compare the percentage increase of  $\gamma$ H2AX positive cells between the two treatment groups.

Plasma assays for pro-GRP, M30, M65, angiogenesis-related pathways (including sVEGFR-2, s-KIT, sVEGF, sKIT, as well as relevant markers), will be evaluated in these patients with SCLC at baseline and at the time of repeat imaging. Changes in these plasma markers will be correlated with outcome in the two treatment arms.

### **13.5 Reporting and Exclusions**

All patients randomized will be included in the evaluation of the primary objective. Patients randomized who received at least one dose of the assigned treatment will be included in the evaluation of toxicity. Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the primary objective. If a patient interrupts the treatment regimen early and there is no evidence of disease progression before 4 months, all efforts will be made for the patients to be evaluated for efficacy at 4 months. If this is not possible, the patient will be considered as having progressed before 4 months. Given the terminal nature of the disease and our recent experience (in the recently completed phase II trial, only 1 of the 64 patients enrolled was lost to follow-up), we anticipate minimal loss to follow-up.

### **13.6 Randomization**

Patients will be randomized 1:1 to one of two treatment arms: ABT-888 and temozolomide as the investigational arm, versus placebo with temozolomide as the control arm. After eligibility is established and immediately after consent is obtained, patients will be registered in the Protocol Participant Registration (PPR) system and

randomized using the Clinical Research Database (CRDB), by calling the MSKCC PPR Office at 646-735-8000 between the hours of 8:30 am and 5:30 pm, Monday - Friday. Randomization will be accomplished by the method of random permuted block, and patients will be stratified by center and by type of relapse (sensitive vs. refractory, as described in [Section 13.3](#)).

## 14.0 PROTECTION OF HUMAN SUBJECTS

Risks: The standard of care for patients with sensitive or refractory relapsed small cell lung cancer and an adequate performance status is chemotherapy. Topotecan is the only FDA approved regimen for this indication. Other options include various other chemotherapy regimens (such as CAV or paclitaxel) or best supportive care. It is unknown whether ABT-888 and temozolomide, as well as placebo with temozolomide, will be more or less effective in this group of patients.

Benefits: Given the low level of toxicity observed in past trials of ABT888 and temozolomide, this drug combination has the potential to be a better tolerated therapeutic option if effective. Patients who progress on either arm of this study, can still receive standard chemotherapy after removal from the study. Additionally, the protocol allows for patients receiving third line treatment for their SCLC, of which there is no standard of care.

Possible toxicities/side effects: See [section 7](#).

Consent process: Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent which must conform to MSKCC IRB guidelines, as well as the IRB of their respective institutions.

Costs: Patients will be charged for physician visits, routine laboratory and radiologic studies required for monitoring their condition. Patients will be charged for temozolomide. Patients will not be billed for the study drug. Patients will not be charged for correlative tests.

Alternatives: Alternative treatment options include standard chemotherapy, best supportive care, or participation in other investigational studies.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential. Patient's names or any other personally identifying information will not be used in reports or publications resulting from this study.

### 14.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

## **15.0 INFORMED CONSENT PROCEDURES**

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

### **15.1 For Participating Centers**

The investigators listed on the cover page and their qualified designees at each participating institution may obtain consent and care for the participants according to good clinical practice and protocol guidelines.

Triplicate originals or copies of the signed informed consent should be distributed as follows: One copy will be given to the participant to be retained for their personal records. One copy will be maintained on file at the MSKCC. The third copy will be confidentially maintained by the participating institution.

A note will be placed in the medical record documenting that informed consent was obtained for this study, and that the participant acknowledges the risk of participation.

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**APPENDIX A****PERFORMANCE STATUS CHART**

<b>ECOG Performance Status Scale</b>		<b>Karnofsky Performance Scale</b>	
<b>Grade</b>	<b>Descriptions</b>	<b>Percent</b>	<b>Description</b>
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

## APPENDIX B

### CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

#### Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

#### Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is

responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

#### Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

#### Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.



## **APPENDIX C**

### **DNA and RNA EXTRACTION TECHNIQUES**

Genomic DNA and RNA are extracted from 10 micron slices (“curls”) of formalin-fixed, paraffin embedded tissue using the SPRI-TE FFPE kit (Beckman Coulter), in an automated fashion, using the SPRI-TE Nucleic Acid Extractor and the SPRI-TE FFPE kit.

The extraction is based on the Solid Phase Reversible Immobilization (SPRI) technology, which utilizes paramagnetic beads to bind nucleic acids and remove contaminants in a fully automated set-up. Briefly, the samples are incubated off-line in a lysis buffer to melt the paraffin. Proteinase K is added to digest the tissue and inactivate nucleases. The samples are then loaded onto the Extractor and the procedure is fully automated.

RNA extraction consists of an additional off-line step, digestion with DNA-free (Ambion), a kit for DNA and divalent cations removal.

**APPENDIX D****QUANTITATIVE DNA METHYLATION ANALYSIS**

DNA methylation analysis will be carried out using the EpiTyper system from Sequenom (San Diego, CA). The EpiTyper assay is a tool for the detection and quantitative analysis of DNA methylation using 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 are designed using the EpiDesigner software ([www.epidesigner.com](http://www.epidesigner.com)), for the entire CpG island of the genes of interest. T7-promoter 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 [Table below](#).

One µg of tumor DNA is 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 are 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 is 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 are: 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 are 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 are transferred into a fresh 384-well PCR plate, and *in vitro* transcription and T cleavage are 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 is 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 are loaded onto a 384-element SpectroCHIP II array (Sequenom). SpectroCHIPS are analyzed using a Bruker Biflex III matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometer (SpectroREADER, Sequenom). Results are analyzed using the EpiTyper Analyzer software, and manually inspected for spectra quality and peak quantification.

**MGMT EpiTyper Primers**

<b>Amplicon Name</b>	<b>Target Genomic Sequence</b>	<b>Left Primer Plus Tag</b>	<b>Right Target Plus Tag</b>	<b>Target Length</b>	<b>Target CpG</b>
MGMT_001	gctggtctgggggtccctgactaggggagcggcaccaggagggg agagactcgcgctccgggctcagcgtagccgccccgagcagga ccgggattctactaagcgggcgccgtcctacgacccccgcgcg ttcaggaccactcgggcacgtggcaggtcgcttgacgccccg gactatccctgtgacaggaaaagg	aggaagagagGTTGGTT TGGGGGTTTTTGATT AG	cagtaatcgcactcactatagg gagaaggctCCTTTTCCT ATCACAAAATAATCC	201	21
MGMT_002	actatccctgtgacaggaaaaggtacgggcccatttggcaactaa ggcacagagcctcaggcgggaagctgggaaggcggccccggct tgtaccggccgaaggccatccgggtcaggcgcacagggcagc ggcgtgccggaggaccagggccggcgtgccggcgtccagcga ggatgcgacagactgcctcaggccccggcggccggcacagggca tgcggcaccgggtcgggagggaacacccccgcccctcccgggct ccgccccagctccgcccccgcgccccggccccgcccccgcg cgctctctgtctttctcaggtcct	aggaagagagATTATTTT TGTGATAGGAAAAGG TA	cagtaatcgcactcactatagg gagaaggctAAAACCTA AAAAAAACAAAAAA C	329	39
MGMT_003	gctctctgtctttctcaggtcctcggtccgccccgctctagacccc ccccacgcccattccccgtgcccctcggccccgccccgcgcc ccggatatgctgggacagcccgcgcccctagaacgctttgctccc gacgcccgcaggtcctcgcggtgcgcaccgcttgcgacttggtgag tgtctgggtgcctcgctcccgaagagtgcggagctctccctcgg gacggtggcagcctcgagtggctcctgcaggcgccctcactcggc gtcgggtgtggggccgcccctgacccccacccatcccgggagct ccaggtgcgcccccaagtgcctcccaggtgttgcagcctttcccc gggctgggggtcctggactaggctgcgctgcagtgactgtggact gg	aggaagagagGTTTTTTT GTTTTTTTLAGGTTTT	cagtaatcgcactcactatagg gagaaggctCCAATCCA CAATCACTACAAC	415	41
MGMT_004	ctgaggctctgtgccttagttgccaatggcccgtacctttctgtca cagggatagtcgcgggcgtgcaagcgacctgccagtgcccga gtggctctgaaagcgcgccccgggtcgtaggacggcggcccgtta	aggaagagagTTGAGGT TTTGTGTTTTAGTTTG TT	cagtaatcgcactcactatagg gagaaggctACAAAATCT CTACTAATCTAAAAAT	249	22

Version Date: 11/05/2015

NCI Protocol Number: 9026

IRB#:12-021 A(21)

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gtgagaatcccggctctgctcggggcggctacgctgagcccggag  
cgcgagtctctcccctctggtgccgctcccctagtcagggaccccc  
agaccagcagagaccctgt

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CCCTA

**APPENDIX E**

**AVIDIN BIOTIN IMMUNOPEROXIDASE PROTOCOL for PARAFFIN SECTIONS**

**Antibody:** MGMT (clone MT23.2) Mouse Monoclonal Antibody

1. 5  $\mu$ m sections heated at 65 C for 30 minutes to melt paraffin
2. Deparaffinize sections through xylene and graded alcohols (100%, 95%)
3. Wash in d H<sub>2</sub>O and transfer to PBS
4. Quench in PBS with 0,1 % H<sub>2</sub>O<sub>2</sub> to reduce endogenous peroxidase
5. Wash in PBS
6. Boil in citric acid (pH 6.0) in steamer for 40 minutes
7. Cool for 20 minutes at room temperature
8. Wash in d H<sub>2</sub>O
9. Apply 10% NHS, blocking serum for 30 minutes incubation
10. Suction off blocking serum and apply primary antibody at 1:2000 dilution, incubate overnight at 4 °C
11. Wash in PBS
12. Apply secondary antibody, anti mouse IgG made in horse, 1:500 dilution, incubate 30 minutes at room temperature
13. Wash in PBS
14. Apply Avidin Biotin complex, 1:25 dilution, incubate 30 minutes at room temperature
15. Wash in PBS
16. DAB with H<sub>2</sub>O<sub>2</sub> to develop brown color
17. Wash in tap water
18. Counterstain lightly with hematoxylin
19. Dehydrate and coverslip

## **APPENDIX F**

### **mRNA BRCA-1 ANALYSIS**

#### **Real-Time PCR**

One µg of total RNA will be reverse-transcribed using the Thermoscript RT-PCR system (Invitrogen) at 52 C for 1 hour.

20ng of resultant cDNA will be used in a Q-PCR reaction using an 7500 Real-Time PCR System (Applied Biosystems) and pre-designed BRCA1 TaqMan ABI Gene expression Assays. Primers will be chosen based on their ability to span the most 3' exon-exon junction.

Amplification will be carried for 40 cycles (95C for 15sec, 60C for 1min). To calculate the efficiency of the PCR reaction, and to assess the sensitivity of each assay, we will also perform a 6 point standard curve (5, 1.7,0.56,0.19,0.062, and 0.021ng). Triplicates CT values will be averaged, amounts of target will be interpolated from the standard curves and normalized to HPRT (hypoxanthine guanine phosphoribosyl transferase, assay Hs99999909m1).

**APPENDIX G**

**AVIDIN BIOTIN IMMUNOPEROXIDASE PROTOCOL for PARAFFIN SECTIONS**

**Antibody:** PTEN (clone 6H2.1) Mouse Monoclonal Dako

1. 5 µm sections heated at 65 C for 30 minutes to melt paraffin
2. Deparaffinize sections through xylene and graded alcohols (100%, 95%)
3. Wash in d H<sub>2</sub>O and transfer to PBS
4. Quench in PBS with 0,1 % H<sub>2</sub>O<sub>2</sub> to reduce endogenous peroxidase
5. Wash in PBS
6. Boil in EDTA (pH 8) in steamer for 40 minutes
7. Cool for 20 minutes at room temperature
8. Wash in d H<sub>2</sub>O
9. Apply 10% NHS, blocking serum for 30 minutes incubation
10. Suction off blocking serum and apply primary antibody at 1:75 dilution, incubate overnight at 4 °C
11. Wash in PBS
12. Apply secondary antibody, anti mouse IgG made in horse, 1:500 dilution, incubate 30 minutes at room temperature
13. Wash in PBS
14. Apply Avidin Biotin complex, 1:25 dilution, incubate 30 minutes at room temperature
15. Wash in PBS
16. DAB with H<sub>2</sub>O<sub>2</sub> to develop brown color
17. Wash in tap water
18. Counterstain lightly with hematoxylin
19. Dehydrate and coverslip

**APPENDIX H**

**CIRCULATING TUMOR CELLS REQUISITION FORM**

**Ship to:**

**MSKCC Clinical Chemistry Laboratory  
Memorial Hospital, Schwartz 359**

**Attn: Rashmi Kamath or Iris Rodriguez (CTCs For SCLC ABT-888 and Temozolomide)  
411 East 67<sup>th</sup> Street  
New York, NY 10065**

For additional questions contact the following personnel:

Rashmi Kamath or Iris Rodriguez ( [REDACTED] or [REDACTED] ).

**Investigator:** M. Catherine Pietanza

**Site:** \_\_\_\_\_

**Subject ID:** \_\_\_\_\_

**Subject Initials:** \_ \_ \_

**Subject DOB:** \_ \_ / \_ \_ \_ \_ / \_ \_ \_ \_

**Date Drawn:** \_ \_ / \_ \_ \_ \_ / \_ \_ \_ \_

**Time Drawn:** \_ \_ : \_ \_

**Contact who completed requisition form:**

**Name:** \_\_\_\_\_

**Phone No. (with area code):** \_\_\_\_\_

**Please Mark:**

**Visit**

	<b>Cycle #1 Day # (Baseline)</b>
	<b>Cycle #2 Day #1</b>
	<b>Cycle #3 Day #1</b>
	<b>Cycle #5 Day #1</b>
	<b>Cycle #7 Day #1</b>
	<b>Cycle # _ Day #1</b>
	<b>At time of Progression</b>

**Platform**

<b>Veridex CellSearch™ System</b>	<b>No. of CellSave™ Tubes Enclosed:</b>
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## APPENDIX I

## CELL SEARCH™ CIRCULATING TUMOR CELL ENUMERATION

<i>Cell Search™ Circulating Tumor Cell Enumeration</i>									
<b>Lab Section:</b> Clinical Chemistry Laboratory					<b>Doc.#:</b> CTC-01-08				
<b>Related Documents:</b> None					<b>Effective Date:</b> 01/01/09				
SUBJECT: Standard Operating Procedures	<b>Review ed</b>								
PREPARED BY: Rita Gonzalez-Espinoza	<b>Review ed</b>								
APPROVED BY:	<b>Review ed</b>								
DATE OF THIS VERSION:	<b>Review ed</b>								
REPLACING VERSION: N/A	<b>Review ed</b>								

**Purpose:** This procedure describes the materials and steps needed to perform the enumeration and identification of circulating tumor cells (CTC) of epithelial origin (EpCAM+, cytokeratins 8+, 18+, and/or 19+, and CD45-) in whole blood.

**Specimen:** 7.5 mL of whole blood from the CellSave® Preservative Tube.

- Blood samples may be stored or transported in CellSave® Tubes for up to 96 hours at Room Temperature (15 - 30°C) prior to processing.
- Do not refrigerate samples.

**Materials:** The following materials are required to perform the CTC enumeration.

<b>Supplies</b>	<b>Controls</b>
Cell Search™ Circulating Tumor Cell Reagent Kit that contains: <ul style="list-style-type: none"> <li>•Anti-EpCAM Ferrofluid</li> <li>•Staining Reagent (anti-CK-PE/anti-CD45-APC)</li> <li>•Nucleic Acid Dye</li> <li>•Capture Enhancement Reagent</li> <li>•Permeabilization Reagent</li> <li>•Cell Fixative</li> <li>•Dilution Buffer</li> <li>•15 mL conical centrifuge tubes and caps</li> <li>•Disposable cartridges and plugs</li> </ul>	CellSearch™ Circulating Tumor Cell Control Kit This control checks the overall system performance, including instrument, reagents and operator technique. The controls are single-use 3.0 mL bottles of fixed SK-BR-3 cells (a human breast carcinoma cell line). The Control Cell population concentration approximates 1000 cells/test for the High Control Cell and 50 cells/test for the Low Control Cell, but varies from lot to lot.
Cell Save® Preservative Tubes	
CellTracks® AutoPrep® Instrument Buffer	

CellTracks® AutoPrep® System
CellTracks® Analyzer II
Swing bucket centrifuge capable of 800 x g
Test tube racks
Calibrated micro-pipettes and tips
Vortex mixer

**Quality:** Circulating Tumor Cell Controls are run once per day of the instrument use or when using a new lot of CellSearch™ Circulating Tumor Cell Reagent Kit.

**Control:** Each single use bottle contains two populations of SK-BR-3 cells at different concentrations (low and high). The two cell populations are distinguished from each other by use of fluorescent dyes that are specific for each population.

- Remove one CellSearch™ CTC Control bottle from the refrigerator 30 minutes before use and allow warming to room temperature.
- Affix an orange CellSearch™ CTC Control bar code label vertically to a 15 mL Cell Tracks® AutoPrep tube contained in the Cell Search™ CEC Kit.
- Vortex the Cell Search™ CTC Control bottle gently for 5 seconds and mix by inverting the bottle 5 times.
- Pour the contents out of the control bottle into the pre-labeled Cell Tracks® AutoPrep sample tube. Use a pipette to transfer any residual liquid in the bottle and bottle cap to the sample tube.
- Place the tube on the Cell Tracks® AutoPrep System when prompted by the software.

**Procedure:** Enumeration of cells for both controls and patient samples are performed using the following steps.

Step	Action						
1	Bring to room temperature (15 - 30°C) the Circulating Tumor Cell Reagent Kit before use.						
2	<table border="1"> <thead> <tr> <th>If...</th> <th>Then...</th> </tr> </thead> <tbody> <tr> <td>the daily QC has not been done,</td> <td>run the CellSearch™ CTC Cell Control</td> </tr> <tr> <td>The daily QC has been done,</td> <td>proceed to step 3</td> </tr> </tbody> </table>	If...	Then...	the daily QC has not been done,	run the CellSearch™ CTC Cell Control	The daily QC has been done,	proceed to step 3
If...	Then...						
the daily QC has not been done,	run the CellSearch™ CTC Cell Control						
The daily QC has been done,	proceed to step 3						
3	Transfer 7.5 mL of whole blood from the CellSave® Preservative Tube into a corresponding labeled 15mL conical tube.						
4	Add 6.5 mL of dilution buffer and mix by inverting the capped tube five times.						
5	Centrifuge the sample(s) at 800 x g for 10 minutes with the brakes off.						
6	Process the samples on the CellTracks® AutoPrep® System within 1 hour of the above preparation. Refer to the Cell Tracks® AutoPrep® System User's Guide for full instructions.						
7	Once the sample has been processed allow the filled cartridge within the						

	MagNest® device incubate in the dark for a minimum of 20 minutes and analyze within 24 hours.
8	Scan each cartridge within the MagNest® on the CellTracks® Analyzer II.
9	Review all of the images that have been taken from the cartridge and select the cell that are considered CTCs (CK-PE+, DAPI+, and CD45-).
10	Once 20 cartridges have been reviewed the images need to be archived onto DVDs.

**Interpretation:** Results are reported as the number of CTC / 7.5 mL of blood. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression free survival and overall survival in certain cancers (breast, prostate and colon).

**Limitations:**

- If the patient is on doxorubicin therapy, allow at least 7 days following administration of a dose of therapy before blood draws. The results of a Cell Search® test should be interpreted with caution if samples are drawn within 7 days of administration of doxorubicin therapy.
- CTC that does not express EpCAM will not be detected by the Cell Search™ test.
- CTC that express EpCAM but not cytokeratins 8, 18, and 19 will not be detected by the Cell Search™ test.
- Refrigerating samples prior to processing could adversely affect sample integrity.

**Results Reporting:** The results are to be entered into the Laboratory Information System after the PhD has reviewed and signed the Control and Patient Report Forms.

**References:**

1. Cell Search™ Circulating Tumor Cell Kit (Product Insert) Veridex LLC, Warren, NJ
2. Cell Search Circulating Cell Control Kit (Product Insert) Veridex LLC, Warren, NJ

**Cell save tube:**



It is a 10ml evacuated blood collection tube, containing a preservative for Circulating Tumor Cells enumeration.

## **COLLECTION OF BLOOD SAMPLES**

### **Cell Save Tube (for CTC analysis) Collection**

In each tube, at least 7.5 ml of peripheral blood will be collected. It is essential that the tubes are filled completely in order to be processed for CTC. After collection, the tube must be **inverted eight times** to prevent clotting and **then stored and transport the sample at room temperature.**

## APPENDIX J

### STANDARD OPERATING PROCEDURE FOR CTC COLLECTION

#### RSA

- Each RSA schedules the consented patients involved in CTC (Circulating Tumor Cells) study according to their respective therapeutic protocols for CTC blood draw.

*Note: In case of an unscheduled accrual and Consent, the RSA/SA orders the CTC draw through OMS at MSKCC (other institutions will use their ordering systems) on the same day while patient is consented for the protocol.*

- The Order of the blood draw for scheduled patient is put through the OMS at MSKCC (other institutions will use their ordering systems) a week prior to the patient's visit. The schedule file prepared by each RSA can be forwarded to the designated RSA for CTC.
- Each RSA should communicate with the designated CTC RSA, to identify and confirm the scheduled patient for the CTC draw a week prior to the patient's visit.
- The designated CTC RSA is responsible for preparing the CTC "kits" for patients a week prior to their scheduled visit in the clinic.

*Note: RSA should also maintain backup of prepared CTC kit, In case of an unscheduled accrual and CTC draw.*

- The patient is drawn for the blood and the tubes are ready to be delivered at Clinical Chemistry Laboratory -Research Area.
- Blood from outside center is delivered at room temperature within 24 hours to Clinical Chemistry Laboratory research area.
- It is the responsibility of the outside centers to make sure the blood drawn is sufficient in volume before delivery. Specific instructions are provided to the outside Center in a lab manual provided to them at the start of the Trial.

**STANDARD OPERATING PROCEDURE FOR CTC COLLECTION**

**CLINICAL CHEMISTRY**

The delivery is made to Clinical Chemistry Laboratory (Research Area) Room: S359.

- The Clinical Research Area clerk receives the sample throughout the day and with each delivery the clerk makes sure that all the tubes have sufficient volume of blood drawn.
- In case of insufficient blood the clerk has to immediately notify a QNS (quantity not sufficient) to the technologist scheduled for CTC run on the particular date and to Rita Gonzalez-Espinoza (Senior Tech/Supervisor at Clinical Chemistry: 212-639-5969).
- QNS should be reported immediately to designate RSA for CTC.
- If the blood volume is sufficient then at the end of the day, the clerk accessions these test in the Clinical Laboratory Information System (LIS).
- The Cell Save® tube used to determine the circulating Cell number has been validated to sit at room temperature for 96 hours. CTC run must take place within these 96 hours.

**APPENDIX K**

**SERIOUS ADVERSE EVENT REPORT FORM FOR NON-MSKCC SITES**

**INSTRUCTIONS: Please submit this form within 3 calendar days.**

**GENERAL INFORMATION**

Site Name: \_\_\_\_\_ Site Principal Investigator: \_\_\_\_\_

Date of Report: d \_\_\_ d \_\_\_ / m \_\_\_ m \_\_\_ / y \_\_\_ y \_\_\_ y \_\_\_

Check one:

Initial Report

Follow-up Report → Date of original Report d \_\_\_ d \_\_\_ / m \_\_\_ m \_\_\_ / y \_\_\_ y \_\_\_ y \_\_\_

Corresponding CTEP-AERS Ticket Number: \_\_\_\_\_

**PARTICIPANT INFORMATION**

Subject Initials: \_\_\_ \_\_\_ \_\_\_

Subject ID: \_\_\_\_\_

Participant Status:  Alive  Deceased

**PROTOCOL THERAPY**

Type of Protocol Therapy: Drug Therapy

Drug Therapy Agent:  ABT-888/Placebo  Temozolomide

Treatment Start Date: d \_\_\_ d \_\_\_ / m \_\_\_ m \_\_\_ / y \_\_\_ y \_\_\_ y \_\_\_

Last Protocol Treatment Date: d \_\_\_ d \_\_\_ / m \_\_\_ m \_\_\_ / y \_\_\_ y \_\_\_ y \_\_\_



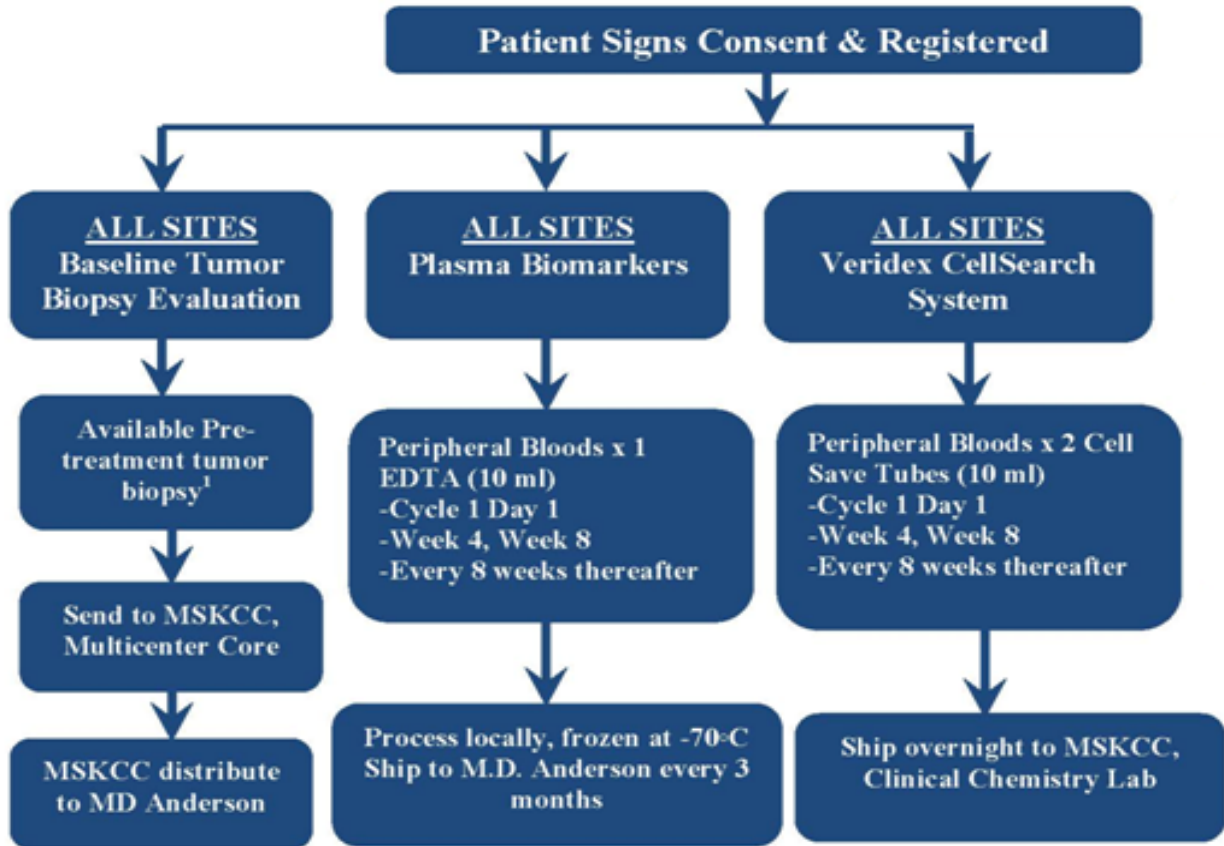


<b>SAE DETAILED INFORMATION</b>
Brief description of event, including relevant findings: _____ _____ _____ _____ _____
Complications and sequelae (including death): _____ _____ _____ _____ _____
Patient's past medical history related to this event: _____ _____ _____ _____ _____

<b>Reporting Information</b>
SAE Reported By (print): _____
Reported by Signature: _____ Date: <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u>
Site PI Name (print): _____ Site PI Signature: _____ Date: <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u> / <u>  </u>

APPENDIX L

CORRELATIVE STUDIES INSTRUCTIONAL FLOW CHART



<sup>1</sup> Patients will not be required to undergo an additional biopsy

**APPENDIX M PHARMACOKINETIC COLLECTION SHEET FOR PLASMA BIOMARKERS  
MSK USE ONLY****Protocol 12-021****A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP  
Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory  
Small Cell Lung Cancer  
(NCI# 9026)**

Principal Investigator: M. Catherine Pietanza, MD										THOR				
Patient Study ID:					Patient MRN:					Patient Initials:				
Rx	REQUIRED SAMPLES						PK ID	Time Points	Expected Time (HH:MM)	Actual Time (HH:MM)	Comments	MD/RN	Processed by (DTU Tech)	
Cycle 1 Day 1: Date ___ / ___ / ___														
VS Pre-dose	BP	HR	RR	TEMP	WT		00:00							
Plasma biomarkers						Pre-dose		Pre-dose						
RX	Temozolomide +ABT-888/Placebo							00:00						
IRB #	Specimen Type	Required Specimen				Final Storage				Centrifuge Settings			Storage	
		Tube	Additive	Amt	Temp	YLD	Amt	Tube	Media	Speed	Time	Temp	Temp	
12-021	Plasma**	1 EDTA		10 mL	ROOM	4	1.8 mL/aliquot	cryovial	plasma	2500 RPM	10 min	4C	-70C	

\*\*Note: Centrifuge plasma biomarkers within 30 minutes of collection.

Cycle 1 Day 1 kit set up by: Initials: \_\_\_\_\_ Date: \_\_\_\_\_

**Protocol 12-021**  
**A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer (NCI# 9026)**

Principal Investigator: M. Catherine Pietanza, MD										THOR				
Patient Study ID:				Patient MRN:						Patient Initials:				
Rx	REQUIRED SAMPLES						PK ID	Time Points	Expected Time (HH:MM)	Actual Time (HH:MM)	Comments	MD/RN	Processed by (DTU Tech)	
Cycle 2 Day 1: Date ____/____/____														
VS Pre-dose	BP	HR	RR	TEMP	WT		00:00							
Plasma biomarkers						Pre-dose		Pre-dose						
RX	Temozolomide +ABT-888/Placebo						00:00							
IRB #	Specimen Type	Required Specimen				Final Storage				Centrifuge Settings			Storage	
		Tube	Additive	Amt	Temp	YLD	Amt	Tube	Media	Speed	Time	Temp	Temp	
12-021	Plasma**	1 EDTA		10 mL	ROOM	4	1.8 mL/aliquot	cryovial	plasma	2500 RPM	10 min	4C	-70C	

\*\*Note: Centrifuge plasma biomarkers within 30 minutes of collection.

*Cycle 2 Day 1 kit set up by: Initials: \_\_\_\_\_ Date: \_\_\_\_\_*

**Protocol 12-021**  
**A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer (NCI# 9026)**

Principal Investigator: M. Catherine Pietanza, MD										THOR			
Patient Study ID:				Patient MRN:				Patient Initials:					
Rx	REQUIRED SAMPLES					PK ID	Time Points	Expected Time (HH:MM)	Actual Time (HH:MM)	Comments	MD/RN	Processed by (DTU Tech)	
Cycle 3 Day 1: Date ____ / ____ / ____													
VS Pre-dose	BP	HR	RR	TEMP	WT		00:00						
Plasma biomarkers						Pre-dose		Pre-dose					
RX	Temozolomide +ABT-888/Placebo						00:00						
IRB #	Specimen Type	Required Specimen				Final Storage				Centrifuge Settings			Storage
		Tube	Additive	Amt	Temp	YLD	Amt	Tube	Media	Speed	Time	Temp	Temp
12-021	Plasma**	1 EDTA		10 mL	ROOM	4	1.8 mL/aliquot	cryovial	plasma	2500 RPM	10 min	4C	-70C

\*\*Note: Centrifuge plasma biomarkers within 30 minutes of collection.

*Cycle 3 Day 1 kit set up by: Initials: \_\_\_\_\_ Date: \_\_\_\_\_*

**Protocol 12-021**  
**A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer (NCI# 9026)**

Principal Investigator: M. Catherine Pietanza, MD										THOR				
Patient Study ID:				Patient MRN:						Patient Initials:				
Rx	REQUIRED SAMPLES						PK ID	Time Points	Expected Time (HH:MM)	Actual Time (HH:MM)	Comments	MD/RN	Processed by (DTU Tech)	
Cycle ____ (Odd #) Day 1: Date ____/____/____														
VS Pre-dose	BP	HR	RR	TEMP	WT		00:00							
Plasma biomarkers						Pre-dose		Pre-dose						
RX	Temozolomide +ABT-888/Placebo							00:00						
IRB #	Specimen Type	Required Specimen				Final Storage				Centrifuge Settings			Storage	
		Tube	Additive	Amt	Temp	YLD	Amt	Tube	Media	Speed	Time	Temp	Temp	
12-021	Plasma**	1 EDTA		10 mL	ROOM	4	1.8 mL/aliquot	cryovial	plasma	2500 RPM	10 min	4C	-70C	

\*\*Note: Centrifuge plasma biomarkers within 30 minutes of collection.

**Cycle \_\_\_\_ (Odd #) Day 1 kit set up by:**  
**Initials: \_\_\_\_\_ Date: \_\_\_\_\_**

**Protocol 12-021**  
**A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer (NCI# 9026)**

Principal Investigator: M. Catherine Pietanza, MD										THOR			
Patient Study ID:				Patient MRN:				Patient Initials:					
Rx	REQUIRED SAMPLES					PK ID	Time Points	Expected Time (HH:MM)	Actual Time (HH:MM)	Comments	MD/RN	Processed by (DTU Tech)	
End of Study Visit: Date ____/____/____													
VS Pre-dose	BP	HR	RR	TEMP	WT		00:00						
Plasma biomarkers						Pre-dose		Pre-dose					
RX	Temozolomide +ABT-888/Placebo						00:00						
IRB #	Specimen Type	Required Specimen				Final Storage				Centrifuge Settings			Storage
		Tube	Additive	Amt	Temp	YLD	Amt	Tube	Media	Speed	Time	Temp	Temp
12-021	Plasma**	1 EDTA		10 mL	ROOM	4	1.8 mL/aliquot	cryovial	plasma	2500 RPM	10 min	4C	-70C

\*\*Note: Centrifuge plasma biomarkers within 30 minutes of collection.

*End of Study kit set up by:*  
*Initials: \_\_\_\_\_ Date: \_\_\_\_\_*

**Protocol 12-021**  
**A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer (NCI# 9026)**

Principal Investigator: M. Catherine Pietanza, MD										THOR			
Patient Study ID:				Patient MRN:						Patient Initials:			
Rx	REQUIRED SAMPLES					PK ID	Time Points	Expected Time (HH:MM)	Actual Time (HH:MM)	Comments	MD/RN	Processed by (DTU Tech)	
Follow Up Visit: Date ____/____/____													
VS Pre-dose	BP	HR	RR	TEMP	WT		00:00						
Plasma biomarkers						Pre-dose		Pre-dose					
RX	Temozolomide +ABT-888/Placebo						00:00						
IRB #	Specimen Type	Required Specimen				Final Storage				Centrifuge Settings			Storage
		Tube	Additive	Amt	Temp	YLD	Amt	Tube	Media	Speed	Time	Temp	Temp
12-021	Plasma**	1 EDTA		10 mL	ROOM	4	1.8 mL/aliquot	cryovial	plasma	2500 RPM	10 min	4C	-70C

\*\*Note: Centrifuge plasma biomarkers within 30 minutes of collection.

*Follow Up Visit kit set up by:*  
*Initials: \_\_\_\_\_ Date: \_\_\_\_\_*



**Protocol 12-021**  
**A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP**  
**Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory**  
**Small Cell Lung Cancer**  
**(NCI# 9026)**

Principal Investigator: M. Catherine Pietanza, MD											THOR		
Patient Study ID:				Patient MRN:				Patient Initials:					
Rx	REQUIRED SAMPLES						PK ID	Time Points	Expected Time (HH:MM)	Actual Time (HH:MM)	Comments	MD/RN	Processed by (DTU Tech)
Unscheduled Visit _____ : Date ____ / ____ / ____													
VS Pre-dose	BP	HR	RR	TEMP	WT		00:00						
Plasma biomarkers						Pre-dose		Pre-dose					
RX	Temozolomide +ABT-888/Placebo						00:00						
IRB #	Specimen Type	Required Specimen				Final Storage				Centrifuge Settings			Storage
		Tube	Additive	Amt	Temp	YLD	Amt	Tube	Media	Speed	Time	Temp	Temp
12-021	Plasma**	1 EDTA		10 mL	ROOM	4	1.8 mL/aliquot	cryovial	plasma	2500 RPM	10 min	4C	-70C

\*\*Note: Centrifuge plasma biomarkers within 30 minutes of collection.

*Unscheduled Visit \_\_\_\_\_ kit set up by:*  
*Initials: \_\_\_\_\_ Date: \_\_\_\_\_*

Version Date: 11/05/2015

NCI Protocol Number: 9026  
IRB#:12-021 A(21)

**APPENDIX N LAB REQUISITION FORM FOR CIRCULATING TUMOR CELLS  
MSK USE ONLY**

MEMORIAL SLOAN KETTERING CANCER CENTER  
RESEARCH LABORATORY REQUISITION

DR. M. Catherine Pietanza, MD

REFRIGERATE \_\_\_\_\_  
C \_\_\_\_\_  
H \_\_\_\_\_  
K \_\_\_\_\_

**Protocol 12-021**  
*Circulating Tumor Cells*

Cycle 1 Day 1     Cycle 2 Day 1  
 Cycle 3 Day 1     Cycle 5 Day 1  
 Cycle \_\_\_\_ (Odd # cycle) Day 1  
 End of Study     Follow up  
 Unscheduled \_\_\_\_\_

Attn: RSA. Please call RSA at 0596 once sample is drawn. RSA will deliver labs to appropriate destination.

Attn: Rashmi Kamath, ext. 122-5969 (Schwartz 359) (Final destination)

**REQUEST:**

**2 Cell Save® Tubes (10mL)** (evacuated blood collection tube containing a preservative—yellow/purple-top tube )

**DRAWN BY:** \_\_\_\_\_

**DATE:** \_\_\_\_\_ **TIME:** \_\_\_\_\_

Cut here ↓  
12-021

Objectives of the Study

To identify and enumerate circulating tumor cells using the Cell Search TM System in this cohort of patients with SCLC; and, to determine if the number of circulating tumor cells after each cycle of treatment correlates with response.

Chemotherapy Unit / Phlebotomy Area Collection Instructions  
Blood sample will be collected in 2 Cell Save® Tubes (10mL Purple & Yellow Top Tube).

**Notes:**

1. In each tube, at least 7.5ml of peripheral blood will be collected.
2. After collection, the tube must be inverted eight times to prevent clotting and then can be stored at **room temperature**.
3. Tubes should then be delivered at **room temperature immediately** to Rashmi Kamath in Schwartz 359.

Version Date: 11/05/2015

NCI Protocol Number: 9026

IRB#:12-021 A(21)

**APPENDIX O LABORATORY MANUAL**

**MSK USE ONLY**

**A Multi-Center, Randomized, Double-Blind Phase II Study  
Comparing ABT-888, a PARP Inhibitor, versus Placebo with  
Temozolomide in Patients with Relapsed Sensitive or Refractory  
Small Cell Lung Cancer**

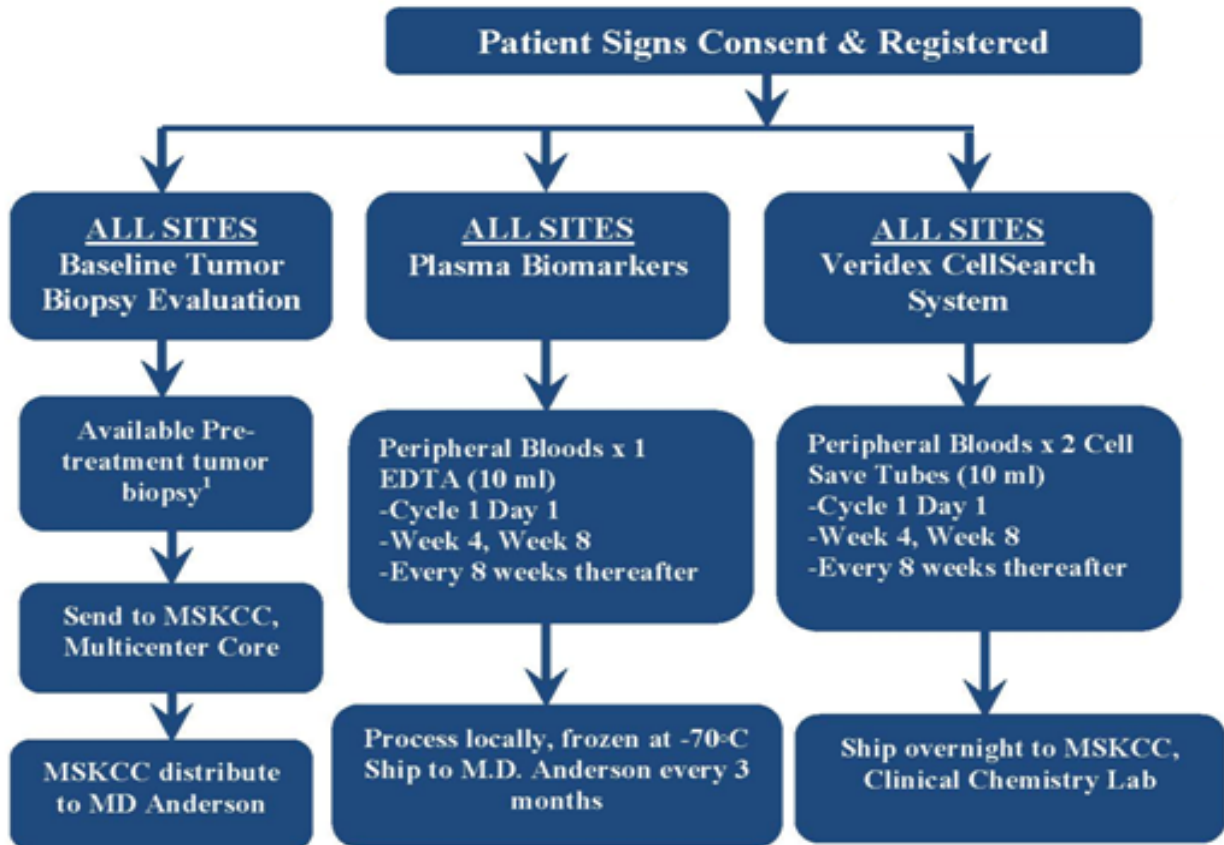
**NCI Study # 9026**

**Version 3.0  
December 2014**

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**CORRELATIVE STUDIES INSTRUCTIONAL FLOW CHART**



<sup>1</sup> Patients will not be required to undergo an additional biopsy

## 1. Baseline Tumor Biopsy

### 1.1 Overview

Pre-treatment tumor biopsy specimen will be collected for the following correlative studies that are outlined in detail in protocol section 9.1 Baseline Tumor Biopsy Evaluations:

- MGMT promoter methylation analysis
- IHC for PARP-1, RAD51, BRCA
- IHC for MGMT and PTEN
- mRNA BRCA-1

### 1.2 Specimen Collection

At the baseline visit, available pre-treatment tumor biopsies, including diagnostic samples should be obtained. The sample can be either from the lung primary or a metastatic site. Additional biopsy is not required. Baseline tumor specimen can be paraffin embedded tumor block (minimum of 0.5 to 1 cubic cm) or 15-20 unstained slides cut from paraffin embedded tumor block.

### 1.3 Sample Handling and Shipment

Please complete Appendix 1 Tumor Biopsy Requisition Form for each participant specimen and include with the shipment. Specimens should be carefully packaged with suitable packing material to ensure they do not break. Pre-printed shipping labels will be provided by MSKCC. All baseline tumor biopsy samples and the associated requisition form will be shipped to MSKCC for analysis at baseline when the samples are obtained. Samples should be sent on Monday through Thursday to the following address:

**M. Catherine Pietanza, MD**  
**Memorial Sloan-Kettering Cancer Center**  
**300 East 66<sup>th</sup> Street, BAIC 1259**  
**New York, NY 10065**

## 1.4 Notification and Personnel

Prior to or on the day that specimen are shipped, please notify by e-mail the MSKCC PI and the study team.

**Study PI:** Dr. M. Catherine Pietanza

**E-mail:** [REDACTED]

**Telephone:** [REDACTED]

**Research Study Assistant:** Patrick Nolan

**E-mail:** [REDACTED]

**Telephone:** [REDACTED]

In the e-mail notification, please include the following information:

- MSKCC assigned subject ID
- Date of shipment
- Shipment tracking number
- Copy of Appendix 1 Tumor Biopsy Requisition Form

## 2. Circulating Tumor Cells (CTCs)

### Overview

Circulating Tumor Cells will be measured using the Veridex CellSearch System (**all participating sites**) and CTC-Chip (**MSKCC and M.D. Anderson Cancer Center ONLY**).

### Description of Collection Tubes

#### CellSave Preservative Tube



**Stabilizes CTC for up to 96 hours at room temperature**

**CellSave tube:** 10ml Evacuated blood collection tube containing a proprietary fixative for the preservation of CTCs.

**EDTA tube:** Evacuated blood collection tube for plasma collection and genotyping analysis





**Section I: Veridex CellSearch****2.1 Specimen Collection**

Peripheral blood will be collected in two CellSave® Tubes (10mL evacuated blood collection tube containing preservative for CTC enumeration). At least 7.5mL of peripheral blood should be collected for each tube.

Veridex CellSearch peripheral blood samples will be collected pre-treatment for the time points listed in Table 1.

<b>Table 1 Veridex CellSearch peripheral blood sampling schedule</b>
<b>Collection Sample Sequence</b>
No. 1 Pre-treatment Cycle 1
No. 2 Week 4
No. 3 Week 8
No. 4 Week 16
Subsequent collection will occur every 8 weeks at the time of re-staging scans. The last sample will be collected at the time of progression.

**2.2 Sample Handling and Shipment**

Immediately after sample collection, the CellSave® Tubes must be inverted eight times to prevent clotting. The samples should be maintained and shipped at ambient temperature (DO NOT REFRIGERATE).

Please complete Appendix 2 Circulating Tumor Cells Requisition Form for each patient and include with the shipment. Specimens should be carefully packaged with suitable packing material to ensure they do not break. Pre-printed shipping labels and shipping supplies will be provided by MSKCC. All Veridex CellSearch CTC samples and the associated requisition forms will be shipped to MSKCC for analysis at the time of sample collection. Samples should be sent on Monday through Friday to the following address:

**MSKCC Clinical Chemistry Research Lab  
ATTN: Rashmi Kamath  
411 East 67<sup>th</sup> Street, Schwartz 359  
New York, NY 10065**

For Friday shipments only, please include instructions on the package that UPS deliveries must be made directly to the 3<sup>rd</sup> floor of the Schwartz Building.

**2.3 Notification and Personnel**

Prior to or on the day that specimen are shipped, please notify by e-mail the MSKCC PI and the study team.

**Study PI:**  
**E-mail:**  
**Telephone:**

**Dr. M. Catherine Pietanza**



**Lab Contact:** Rashmi Kamath**E-mail:** [REDACTED]**Telephone:** [REDACTED]**Research Study Assistant:** Patrick Nolan**E-mail:** [REDACTED]**Telephone:** [REDACTED]

In the e-mail notification, please include the following information:

- MSKCC assigned subject ID
- Shipment date
- Shipment tracking number
- Appendix 2 Circulating Tumor Cells Requisition Form

### 3. Plasma Biomarkers

#### 3.1 Overview

Plasma biomarkers are collected from all protocol patients at baseline and during treatment for analyses including markers of apoptosis (e.g. M30 and M65) and tumor bulk (pro-GRP).

#### 3.2 Specimen Collection

Peripheral blood will be collected in one EDTA Tube (lavender top). Approximately 8ml of peripheral blood will be collected.

Peripheral blood samples for plasma biomarker will be collected pre-treatment for the time points listed in Table 3.

<b>Table 3 Plasma biomarker sampling schedule</b>
<b>Collection Sample Sequence</b>
No. 1 Pre-treatment Cycle 1
No. 2 Week 4
No. 3 Week 8
No. 4 Week 16
Subsequent collection will occur every 8 weeks at the time of re-staging scans. The last sample will be collected at the time of progression.

#### 3.3 Sample Handling

Within 30 minutes of sample collection, the EDTA Tube should be processed as follows:

7. Label four (4) 1.8 ml cryovials with the study number, MSKCC assigned patient study number, and procedure date, and clearly mark cryovials “plasma.”
8. Collect 8 mL of blood into EDTA Vacutainer® blood collection tube (BD Sciences, Lavender top)

9. Process: Spin EDTA Vacutainer® blood collection tube (BD Sciences, Lavender top) in a standard clinical centrifuge at ~2500 RPM at 4° Celsius for 10 minutes.
10. Centrifuge within 30 minutes of collection. If the interval between specimen collection and processing is anticipated to be greater than one hour, keep specimen on ice until centrifuging is done.
11. Aliquot 1 mL of plasma into cryovials labeled with the study number, patient number and procedure date and marked “plasma.”
12. Place cryovials into divider box. Store at a minimum –70<sup>0</sup> Celsius until ready for shipment/analysis.

### 3.4 Sample Shipment

Please complete Appendix 3 Plasma Biomarker Requisition Form for each patient and include with the shipment. Specimens should be carefully packaged on dry ice and suitable packing material to ensure they do not break. Shipping labels will be provided by M.D. Anderson, and distributed to each site from MSKCC.

All plasma samples and the associated requisition forms will be batched and shipped to M.D. Anderson for analysis every 3 months. Samples should be sent on Monday through Wednesday to the following address:

**Thoracic Blood Biomarker laboratory  
Attn. Dr. John Heymach or Dr. Uma Giri  
Room #: T8.3970, UT MD Anderson Cancer Center,  
1515 Holcombe Blvd  
Houston, Texas- 77030  
Phone - [REDACTED]**

### 3.5 Notification and Personnel

Prior to or on the day of shipment, please notify by e-mail the study team and attach a copy of specimen inventory with your shipment.

**M.D. Anderson Contact:**

**Dr. Uma Giri**

**E-mail:**

**Telephone:**

**M.D. Anderson Contact:**

**Dr. Hai Tran**

**E-mail:**

**Telephone:**

**Research Study Assistant:**

**Patrick Nolan**

**E-mail:**

Version Date: 11/05/2015

NCI Protocol Number: 9026  
IRB#:12-021 A(21)

Telephone: [REDACTED]

**APPENDICES**

Appendix 1  
**Tumor Biopsy Requisition form**

**Study:** NCI 9026 (MSKCC 12-021) A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung Cancer

**Investigator:** M. Catherine Pietanza, MD

<b>Participating Site:</b>																					
<b>MSKCC Assigned Subject ID:</b>																					
<b>Subject Initials:</b>																					
<b>Type of Sample: (please circle)</b>	Unstained Slides / Tumor Block																				
<b>If slides, number of slides included:</b>																					
<b>Date of Biopsy:</b>	<table style="margin-left: auto; margin-right: auto;"> <tr> <td>__</td><td>__</td><td>/</td><td>__</td><td>__</td><td>/</td><td>__</td><td>__</td><td>__</td><td>__</td> </tr> <tr> <td>M</td><td>M</td><td></td><td>D</td><td>D</td><td></td><td>Y</td><td>Y</td><td>Y</td><td>Y</td> </tr> </table>	__	__	/	__	__	/	__	__	__	__	M	M		D	D		Y	Y	Y	Y
__	__	/	__	__	/	__	__	__	__												
M	M		D	D		Y	Y	Y	Y												
<b>Date Sample Obtained:</b>	<table style="margin-left: auto; margin-right: auto;"> <tr> <td>__</td><td>__</td><td>/</td><td>__</td><td>__</td><td>/</td><td>__</td><td>__</td><td>__</td><td>__</td> </tr> <tr> <td>M</td><td>M</td><td></td><td>D</td><td>D</td><td></td><td>Y</td><td>Y</td><td>Y</td><td>Y</td> </tr> </table>	__	__	/	__	__	/	__	__	__	__	M	M		D	D		Y	Y	Y	Y
__	__	/	__	__	/	__	__	__	__												
M	M		D	D		Y	Y	Y	Y												
<b>Person Completing Requisition Form:</b>																					
<b>Phone Number:</b>																					

All baseline tumor baseline specimens will be sent to Memorial Sloan-Kettering Cancer Center (MSKCC) via overnight carrier to:

**M. Catherine Pietanza, MD**  
Memorial Sloan-Kettering Cancer Center  
300 East 66<sup>th</sup> Street, BAIC 1259  
New York, NY 10065

Appendix 2

**Circulating Tumor Cells Requisition Form**

**Study:** NCI 9026 (MSKCC 12-021) A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung

**Investigator:** M. Catherine Pietanza, MD

**Site:** \_\_\_\_\_ **Subject ID:** \_\_\_\_\_

**Subject Initials:** \_\_\_\_\_ **Subject DOB:** \_\_\_/\_\_\_/\_\_\_\_\_

**Date Drawn:** \_\_\_/\_\_\_/\_\_\_\_\_ **Time Drawn:** \_\_\_:\_\_\_

**Contact who completed requisition form:**

**Name:** \_\_\_\_\_ **Phone No. (with area code):** \_\_\_\_\_

**Please Mark:**

<b>Visit</b>	<input type="checkbox"/> <b>Cycle #1 Day # (Baseline)</b>
	<input type="checkbox"/> <b>Cycle #2 Day #1</b>
	<input type="checkbox"/> <b>Cycle #3 Day #1</b>
	<input type="checkbox"/> <b>Cycle #5 Day #1</b>
	<input type="checkbox"/> <b>Cycle #7 Day #1</b>
	<input type="checkbox"/> <b>Cycle #__ Day #1</b>
	<input type="checkbox"/> <b>At time of Progression</b>
<b>No. of CellSave™ Tubes Enclosed:</b>	

All CTC samples will be sent to Memorial Sloan Kettering Cancer Center (MSKCC) via overnight carrier to: **MSKCC Clinical Chemistry Research Lab**  
**ATTN: Rashmi Kamath or Amrita Herkal**  
**411 East 67<sup>th</sup> Street Schwartz 359**  
**New York, NY 10065**

Appendix 3

**Plasma Biomarker Requisition Form**

**Study:** NCI 9026 (MSKCC 12-021) A Multi-Center, Randomized, Double-Blind Phase II Study Comparing ABT-888, a PARP Inhibitor, versus Placebo with Temozolomide in Patients with Relapsed Sensitive or Refractory Small Cell Lung

**Investigators:** John Heymach, MD PhD / Lauren Byers, MD / Uma Giri, MD

**Site:** \_\_\_\_\_ **Subject ID:** \_\_\_\_\_

**Subject Initials:** \_\_\_\_\_ **Subject DOB:** \_\_\_\_/\_\_\_\_/\_\_\_\_\_

**Date Drawn:** \_\_\_\_/\_\_\_\_/\_\_\_\_\_ **Time Drawn:** \_\_\_\_:\_\_\_\_

**Contact who completed requisition form:**

**Name:** \_\_\_\_\_

**Phone No. (with area code):** \_\_\_\_\_

**Please Mark:**

<input type="checkbox"/>	<b>Cycle #1 Day # (Baseline)</b>	<b># of Cryovials:</b> _____
<input type="checkbox"/>	<b>Cycle # _____</b>	<b># of Cryovials:</b> _____
<input type="checkbox"/>	<b>Cycle # _____</b>	<b># of Cryovials:</b> _____
<input type="checkbox"/>	<b>Cycle # _____</b>	<b># of Cryovials:</b> _____
<input type="checkbox"/>	<b>Cycle # _____</b>	<b># of Cryovials:</b> _____
<input type="checkbox"/>	<b>Cycle # _____</b>	<b># of Cryovials:</b> _____
<input type="checkbox"/>	<b>Cycle # _____</b>	<b># of Cryovials:</b> _____

All plasma biomarker samples will be sent to MD Anderson via overnight carrier to:

**Thoracic Blood Biomarker laboratory  
Attn. Dr. John Heymach or Dr. Uma Giri  
Room #: T8.3970, UT MD Anderson Cancer Center,  
1515 Holcombe Blvd  
Houston, Texas- 7703**

MSKC PROTOCOL # <u>12-021</u>	<b>INSTITUTION:</b> _____	<b>PATIENT ID (MSKCC Assigned):</b> _____	<b>PATIENT INITIALS:</b> _____
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Please refer to MSKCC lab manual for specific directions and guidance on sample collection and shipment.

**CYCLE:** \_\_\_\_ **Day:** \_\_\_\_

**Circulating Tumor Cells: Veridex CellSearch System**

<b>Date of Collection:</b> ____ / ____ / ____ _____ mm dd y y y y	<b>Date of Shipment to MSKCC:</b> ____ / ____ / ____ _____ mm dd yyyy
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Not Done, please notify MSKCC

**Reason:** \_\_\_\_\_  
 \_\_\_\_\_

**Circulating Tumor Cells: CTC-Chip [M.D. Anderson Participants ONLY]**

<b>Date of Collection:</b> ____ / ____ / ____ mm dd y y y y	<b>CTC Results:</b> _____	<b>Date Report Sent to MSKCC:</b> ____ / ____ / ____ mm dd yyyy
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Not Done, please notify MSKCC

**Reason:** \_\_\_\_\_  
 \_\_\_\_\_

**Plasma Biomarkers**

<b>Date of Collection:</b> ____ / ____ / ____ mm dd yyyy	
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Not Done, please notify MD Anderson and MSKCC

**Reason:** \_\_\_\_\_