Randomized, Double-Blind, 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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Supplemental Methods

Randomization

Once eligibility was established, patients were registered in the Protocol Participant Registration (PPR) system and randomized using the Clinical Research Database (CRDB) at MSKCC. Randomization was accomplished by the method of random permuted block. Each patient was assigned a unique protocol participant number.

Veliparib and matching placebo capsules were provided by Abbott Laboratories and distributed by the Pharmaceutical Management Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute. The Pharmaceutical Management Branch labeled each blinded, patient specific bottle with specific information, including protocol number, participant number determined at randomization, agent identification, and administration instructions. This was a double blinded-study, and physicians, nurses, pharmacists, data managers, statisticians, and participants all remained blinded after assignment to the treatment intervention. Notably, during the course of the study, there were no incidences of unblinding, including for emergency reasons.

Treatment

Patients were instructed to fast at least 2 hours before and 1 hour after temozolomide administration. Ondansetron 8mg orally was given before temozolomide as needed. Veliparib or placebo with temozolomide was continued until progression of disease, development of unacceptable toxicity, or withdrawal of consent. Due to grade 3/4 hematologic toxicities observed among the first 24 patients, the protocol was amended, changing the initial dose of temozolomide to 150mg/m^2/day and allowing for a dose escalation to 200mg/m^2/day in the

absence of grade 3/4 adverse events during the first cycle. Dosing was interrupted if a patient developed hematologic toxicities (i.e., ANC <1,500/µl and/or platelets <100,000/µl) or grade 3 non-hematologic toxicities (except for alopecia, nausea and vomiting) until resolution. Upon resuming temozolomide and veliparib/placebo, the dose of either was lowered depending on the attribution of the toxicity. For hematologic toxicity, temozolomide generally was reduced first. Two dose reductions for each agent were permitted: 150mg/m^2/day (125g/m2/day if the starting dose was 150mg/m^2/day) and 100mg/m^2/day for temozolomide; and 40mg am with 20mg pm and 20mg twice daily for veliparib/placebo. Removal from study occurred if patients' toxicities did not resolve within 21 days (including grade ≥3 neutropenia and thrombocytopenia) for any recurrent grade 3 non-hematologic adverse event or for any grade 4 non-hematologic toxicity. Patients who developed grade ≥3 lymphopenia received prophylaxis for *pneumocystis carinii* pneumonia.

Study Evaluation – Tumor Assessments:

Tumor assessments at baseline included computed tomography (CT) of the chest, other relevant sites of disease, and contrast-enhanced MRI or CT of the brain. Follow-up scans to assess response were obtained at week four and eight of treatment, and every eight weeks thereafter. Responses were determined using RECIST 1.1[1]. Imaging studies were reviewed by radiologists at the respective institutions. Radiological review of randomly selected patients was conducted by the Therapeutic Response Committee at Memorial Sloan Kettering Cancer Center to verify that response assessments complied with RECIST 1.1.

Immunohistochemical Analysis

Patients were requested to submit *available*, pre-treatment tumor material, including diagnostic samples, upon signing informed consent.

PARP1 and SLFN11 immunohistochemical analysis was performed on unstained formalin-fixed paraffin-embedded (FFPE) sections from patients' original diagnostic biopsies. Briefly, FFPE sections were rehydrated, antigen retrieval was performed using a steamer (pH=9), intrinsic peroxidase activity was quenched with 3% hydrogen peroxide and 5% goat serum solution was used to block non-specific binding before incubating with a primary antibody against PARP1 (RB1516P1, Fisher Scientific) and SLFN11 (HPA023030, Sigma-Aldrich), respectively. After three washes, slides were incubated with DAKO Envisual + Dual Link. After three additional washes, slides were incubated with DAKO chromagen substrate and counterstained with hematoxylin. Sections were scored by a pathologist (J.F.) for intensity (0-3+) and extent (0-100%) of staining by light microscopy. By multiplying intensity and extent of staining, each tumor was assigned an H-score (range 0-300). For SLFN11, an immunohistochemistry (IHC) score of 1 or greater was considered positive. As reported elsewhere, SLFN11 IHC scoring was validated using a panel of 12 SCLC PDX models [2]. SLFN11 expression assayed by IHC and by reverse-phase protein array (RPPA) were highly correlated (Rho=0.82, $p<0.001$). Similarly SLFN11 protein expression (RPPA) was highly correlated to *SLFN11* gene expression (Rho=0.81, $p<0.001$) [2]. For PARP1, patients were categorized as low or high using a median cut-off as using an IHC score of 0 vs ≥1 comparison resulted in only one patient in the PARP1 negative group in the placebo arm resulting in a significantly under-powered analysis.

MGMT Promoter Methylation

DNA was extracted from FFPE sections using the MasterPure Complete DNA Purification Kit (Epicentre) and quantified by Qbit. The MGMT assay was performed as initially described by Esteller et al 1999 and as modified to for real time PCR product detection using SYBR green (qMS-PCR) [3,4]. Bisulfite conversion of 500 ng of DNA was performed (Zymo EZ96 DNA methylation kit, Zymo Research) and amplification of bisulfite-modified DNA encompassed the standard enhancer region within the first intron of *MGMT* as previously described [5]. An

amplicon derived from the COL2A1 gene (a region of the promotor with no methylated CpG sites) served as a loading control reference. Amplification was performed using real-time PCR in an Applied Biosystems 7900HT Fast Real-Time PCR system. A ΔCT was defined as the difference in the cycle in which the detected amplification curve (on a log-linear plot) crosses an empirically determined threshold (set for each batch based within the geometric region of amplification) between the *MGMT* amplicon and the reference *ACTB* amplicon. Control methylated and unmethylated DNAs were used with each sample tested. A ΔΔCT, defined as the difference in ΔCT between the u*MGMT* and m*MGMT* amplicons of greater than 8 cycles (>256 fold difference) indicated the presence of promotor methylation. A ΔΔCT >8 cycles was coded as unmethylated. All reactions were carried out in triplicate from replicate bisulfite converted DNA preparations. Discordant results were repeated (including bisulfite conversion when sufficient DNA was available). Cases in which discordant results persisted after repeated assays or for which amplification failed secondary to poor quality DNA (or other technical reasons) were coded as failures/no data.

Tumor Mutational Analysis of Actionable Cancer Genes Using Next Generation Sequencing (NGS)

At MSKCC, DNA was extracted from available biopsied tissue (N = 8) and cytology specimens (and patient-matched normal tissue) using Qiagen nucleic acid extraction kits. Using the MSK-IMPACT assay (Integrated Mutation Profiling of Actionable Cancer Targets), which has been validated for clinical use [6,7], bar-coded sequence libraries were prepared (Kapa Biosystems), and exon capture was performed on bar-coded pools by hybridization (Nimblegen SeqCap) using custom oligonucleotides to capture all protein-coding exons and select introns of 341 cancer associated genes. DNA was sequenced on an Illumina HiSeq 2500 as paired-end 100-base pair reads. Sequence data were analyzed to identify single nucleotide variants, indels, and copy number alterations involving these 341 genes. At MDACC, DNA was extracted from

available biopsied tissue (N = 9) and sequenced using an in-house targeted sequencing platform [8].

Circulating Tumor Cells (CTCs)

Peripheral blood was collected from all patients for CTC enumeration utilizing the Veridex CellSearch System™. CTCs were quantified at the following times: (i) prior to initiating the study drugs; (ii) during week 4, week 8 and every eight weeks thereafter at the time of tumor re-imaging; and (iii) at disease progression.

Statistical Analysis

Secondary objectives included: ORR (complete response (CR) plus partial response (PR)) according to RECIST 1.1 [1], OS; and safety and tolerability of veliparib with temozolomide in this patient population. Exploratory objectives included: PARP1 and SLFN11 IHC expression, *MGMT* promoter methylation, and CTC quantification, all of which were correlated with PFS, ORR and OS.

ORR and the corresponding exact two-sided 95% confidence intervals were calculated and reported for both arms of the study. The chi-squared test was used for performing comparisons between treatment arms. Comparisons between treatment arms also were done in subgroups using Fisher's exact test. OS was estimated in each treatment group using the Kaplan-Meier method, with the time origin at the date of randomization; patients alive at the time of the last follow-up were censored. Group comparisons for OS were performed using the log-rank test and the Cox proportional hazards model.

An interim analysis for early assessment of inefficacy was planned after 50 patients had had their 4 month PFS evaluation. The interim analysis was performed as "intent to treat". The trial

was to be terminated early if at the interim analysis more events (progressions or deaths) were observed in the treatment arms than in the control arm; which was equivalent to a one-sided p-value of 0.50 or larger at the interim analysis. This approach offered a 50% chance of stopping accrual if the experimental regimen was inefficient and led to minimal loss of power compared to an analysis without intermediate look[9,10]. Enrollment was halted during this analysis, and restarted once it was determined that the prespecified boundary for inefficacy had not been met.

Fisher's exact test was used to correlate PARP1 and SLFN11 IHC expression and *MGMT* promoter methylation in tumor samples with response, while the log-rank test was used to compare the strata defined by the three variables with respect to PFS and OS. Cox regression model was used to check the interaction of SLFN11 IHC groups and treatment arms.

We analyzed the number of CTCs at baseline and after one cycle with response using summary statistics and the Kruskal-Wallis test. We dichotomized CTCs at 5 to analyze with OS using the log-rank test (with time origin being the end of cycle 1 for the analysis of CTCs after one cycle). We also calculated the change in CTCs from baseline to after one cycle of treatment, and compared the change by response using the Wilcoxon rank sum test. The survival from the end of cycle 1 of the patients who had no change or a decrease in CTC was compared to those who had an increase in CTC using the log-rank test. All statistical tests were two-sided and 5% was set as the level of significance. Statistical analyses were done using R version 3.2.0 (R Development Core; Vienna, Austria), including the "survival" and "Hmisc" packages.

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