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ORIGINAL ARTICLE

Randomized Phase II Study of Paclitaxel plus Alisertib versus Paclitaxel plus Placebo as Second-Line Therapy for SCLC: Primary and Correlative Biomarker Analyses

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ABSTRACT

Introduction: We assessed the Aurora A kinase inhibitor, alisertib, plus paclitaxel (henceforth referred to as alisertib/paclitaxel) as second-line treatment for SCLC.

Methods: In this double-blind study, patients with relapsed or refractory SCLC were stratified by relapse type (sensitive versus resistant or refractory) and brain metastases and randomized 1:1 to alisertib/paclitaxel or placebo plus paclitaxel (henceforth referred to as placebo/paclitaxel) in 28-day cycles. The primary end point was progression-free survival (PFS). Associations of c-Myc expression in tumor tissue (prespecified) and genetic alterations in circulating tumor DNA (retrospective) with clinical outcome were evaluated.

Results: A total of 178 patients were enrolled (89 in each arm). The median PFS was 3.32 months with alisertib/paclitaxel versus 2.17 months with placebo/paclitaxel (hazard ratio [HR] = 0.77, 95% confidence limit [CI]: 0.557–1.067, $p = 0.113$ in the intent-to-treat population versus HR = 0.71, 95% CI: 0.509–0.985, $p = 0.038$ with corrected analysis applied). Among 140 patients with genetic alternations, patients with cell cycle regulator mutations (cyclin-dependent kinase 6 gene [*CDK6*], retinoblastoma-like 1 gene [*RBL1*], retinoblastoma-like 2 gene [*RBL2*], and retinoblastoma 1 gene [*RB1*]) had significantly improved PFS with alisertib/paclitaxel versus with placebo/paclitaxel (3.68 versus 1.80 months, respectively [HR = 0.395, 95% CI: 0.239–0.654, $p = 0.0003$]), and overall survival (7.20 versus 4.47 months, respectively [HR = 0.427, 95% CI: 0.259–0.704, $p = 0.00085$]). A subset of patients with c-Myc expression showed significantly improved PFS with alisertib/paclitaxel. The incidence of grade 3 or higher drug-related adverse events was 67% (58 patients) with alisertib/paclitaxel versus 22% (25 patients) with placebo/paclitaxel. Twelve patients (14%) versus 11 (12%) died on study, including four versus zero treatment-related deaths.

Conclusions: Efficacy signals were seen with alisertib/paclitaxel in relapsed or refractory SCLC. c-Myc expression and mutations in cell cycle regulators may be potential predictive biomarkers of alisertib efficacy; further prospective validations are warranted.

Keywords: Phase II; Aurora A kinase; Alisertib; Paclitaxel; SCLC

Introduction

SCLC is an aggressive malignancy, accounting for 13% to 18% of all lung cancer diagnoses.[1,2] For many

patients with SCLC, the outlook is bleak. In treated extensive-stage SCLC, the median overall survival (OS) is just 7 to 12 months.[3,4] The prognosis for relapsed or refractory SCLC is more dismal, with a median OS of 4 to 5 months.[5]

There is a strong correlation between the efficacy of second-line therapy and the quality and duration of response (DOR) to initial treatment (sensitive versus resistant or refractory relapse).[6] Response rates are particularly poor ($\leq$10%) in patients with resistant or refractory disease who relapse 3 months or less from the end of initial therapy.[5] Patients with platinum-sensitive disease (relapse $>$3 months from end of initial therapy) have a relatively better outcome (response rate $\sim$25%).[5]

Because of poor responses to current treatments, there is a critical unmet medical need in patients with SCLC, thereby justifying efforts to evaluate novel targeted agents (with validated predictive biomarkers).[7,8] One potential target is Aurora A kinase (AAK), a key regulator of mitosis.[9] AAK is amplified or overexpressed in several solid tumors, including SCLC, and may play a role in tumorigenesis.[10,11] Inhibition of AAK leads to disrupted mitosis and cell death, reduced proliferation, and induction of apoptosis in SCLC cells.[10,12]

Amplification of v-myc avian myelocytomatosis viral oncogene homolog gene (*MYC*) in SCLC cell lines is associated with improved sensitivity to Aurora kinase inhibitors.[13,14] Amplification and overexpression of the *MYC* gene family occurs in 18% to 31% of SCLCs and is more common in chemorefractory disease.[15] The gene product, c-Myc (a transcription factor), binds directly to AAK, and inhibition of this interaction by AAK inhibitors results in c-Myc degradation and cell death.[16,17] Thus, the Myc–AAK protein complex represents an actionable drug target for AAK inhibitors.

Alisertib (MLN8237) is an investigational, selective, oral, small molecule AAK inhibitor that has been studied in various solid tumors and hematologic malignancies.[18-23] Single-agent activity was demonstrated in a phase II study of patients with relapsed or refractory solid tumors, including SCLC (n = 48);[23] in patients with SCLC, the objective response rate (ORR) was 21% and the median progression-free survival (PFS) was 2.1 months.[23] On the basis of preclinical evidence of synergy, alisertib plus paclitaxel (henceforth referred to as alisertib/paclitaxel) was evaluated in patients with breast and ovarian cancer[19] and showed promise over paclitaxel alone, with PFS and ORR trending in favor of the combination.[19] Preclinical data have also shown alisertib/paclitaxel synergy in SCLC; increased antitumor activity with the combination versus with the single agents was demonstrated in xenograft tumor models derived from human SCLC cell lines and human SCLC primary tumors (Takeda, data on file). The preclinical and clinical data thus provided justification for this phase II study of alisertib/paclitaxel as second-line therapy for relapsed or refractory SCLC. As part of this study, analyses were undertaken to assess the impact of c-Myc protein expression and genetic alterations on clinical outcomes.

Patients and Methods

Study Design

This multicenter, randomized, double-blind, placebo-controlled phase II trial (NCT02038647) enrolled patients across 54 sites in the United States (19 sites), Canada (three sites), and Europe (32 sites) from May 7, 2014, to October 26, 2015. The trial was conducted in accordance with applicable regulatory requirements, International Conference on Harmonization Good Clinical Practice guidelines, and the ethical principles founded in the Declaration of Helsinki. Study documentation was approved by the institutional review board and/or independent ethics committee at each site. Patients provided written informed consent.

Patients were randomized 1:1 to receive either alisertib (40 mg by mouth twice daily for 3 weeks on days 1–3, 8–10, and 15–17) plus paclitaxel (60 mg/m^2 intravenously on days 1, 8, and 15) or placebo (by mouth twice daily as per alisertib) plus paclitaxel (80 mg/m^2 intravenously on days 1, 8, and 15) in 28-day cycles. The dosing schedule permitted maximal overlap of systemic exposures between alisertib and paclitaxel while providing sufficient treatment-free periods to allow recovery from toxicities associated with both agents. Randomization was stratified by type of relapse after primary treatment, based on the common definition for each type[5,6] (with sensitive defined as relapsed $>$90 but $<$180 days after primary treatment and resistant or refractory defined as relapsed $\leq$90 days after primary treatment) and presence of brain metastases (yes versus no) at study entry. Patients received treatment until progressive disease, discontinuation because of toxicity, loss to follow-up, study termination, protocol violation, or patient withdrawal. The study team and site staff responsible for assessing patients were blinded to treatment assignment; participating sites were required to designate a non-blinded study pharmacist for dose preparations.

Patients

The study enrolled patients at least 18 years old with a pathologically (histologically or cytologically) confirmed diagnosis of SCLC. Patients were required to have progressed within 180 days of last platinum dose, after receiving a standard platinum-based chemotherapy

regimen as first-line treatment, and to have measurable disease per the Response Evaluation Criteria in Solid Tumors version 1.1 within 2 weeks before randomization. Other key inclusion and exclusion criteria can be found in the Supplementary Methods.

End Points and Assessments

The primary end point was PFS (time from randomization to progressive disease or death), with patients stratified by type of relapse after primary treatment (sensitive versus resistant or refractory disease) and presence of brain metastases. PFS was also analyzed in patient subgroups according to baseline characteristics. Secondary end points were safety and/or tolerability, OS, ORR including complete response, disease control rate (DCR: complete response, partial response, or stable disease $\geq$8 weeks), and DOR. Exploratory end points included correlative biomarker studies to evaluate the impact of c-Myc expression and genetic alterations on clinical outcomes (PFS and OS).

Extent of disease was evaluated according to the Response Evaluation Criteria in Solid Tumors version 1.1 at screening, after every cycle for the first 6 months, and subsequently every two cycles (between days 21 and 28) to assess disease response and progression; because of the aggressiveness of SCLC, disease assessments were performed more frequently than in common clinical practice. Radiographic images (by contrast-enhanced computed tomography or magnetic resonance imaging) were assessed locally and submitted for central review if the results were positive. For biomarker evaluations, c-Myc expression was assessed by immunohistochemical analysis of c-Myc expression in tumor tissue, and genetic alterations were assessed retrospectively by next-generation sequencing (NGS) of circulating tumor DNA (ctDNA) from peripheral blood by using a custom PlasmaSelect-R (Personal Genome Diagnostics, Inc., Baltimore, MD) targeted gene NGS panel (Supplementary Table 1).

Details of follow-up for PFS and OS, and the methods used for the biomarker assessments, are provided in the Supplementary Methods. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. The use of myeloid growth factors for the management of neutropenia was not mandated but was permitted at the discretion of the investigator if clinically indicated, according to local guidelines and the product label.

Statistical Analysis

The intent-to-treat (ITT) population included all randomized patients and was used for the primary analysis of PFS and all secondary efficacy end points. The safety population included all patients who received at least one dose of any study drug.

Full statistical methods are provided in the Supplementary Methods. Assuming a median PFS of 3 months for the placebo plus paclitaxel (henceforth referred to as placebo/paclitaxel) arm and also assuming that alisertib/paclitaxel could improve median PFS to 5 months (40% reduction of hazard), a minimum of 138 PFS events were required for the primary analysis (two-sided alpha 0.05, power 85%). PFS was tested by using a two-sided stratified log-rank test to compare arms. Hazard ratios (HRs) and 95% confidence intervals (CI) were estimated by using a Cox proportional hazard regression model stratified by type of relapse and presence of brain metastases, with treatment arm included as a factor in the model. Kaplan-Meier survival curves were provided for each arm. OS and other time-to-event end points were analyzed by using similar methods.

Assessment of c-Myc expression by immunohistochemistry was analyzed as (1) percentage of positive cells or (2) dichotomized readout converted to a binary variable (positive versus negative) based on modal intensity. For both methods, a Cox proportional hazard model was used to analyze PFS, with treatment and c-Myc expression as the main effects. Interactions between NGS-identified genetic alterations and treatments (alisertib versus placebo) were tested with a Cox proportional hazards model for PFS and OS. The genetic alterations tested included single-gene mutations (mutant allele frequency $\geq$0.01), pathway gene mutations, and mutation load.

A protocol amendment (January 2015) corrected the stratification definition of relapse type after primary treatment so that relapses were recorded "from last administration of platinum-based chemotherapy" rather than "from initial response." To maintain balance, the primary end point was analyzed by using the original stratification definition of relapse type. However, a sensitivity analysis with use of the corrected stratification definition was also included.

Results

Patients

A total of 178 patients were enrolled and randomized to receive alisertib/paclitaxel (n = 89) or placebo/paclitaxel (n = 89 [Supplementary Fig. 1]). Patient characteristics at baseline are shown in Table 1. Two patients in the alisertib/paclitaxel arm were randomized but did not receive any study drug and were included in the ITT population analysis only. When the corrected definition of relapse type (sensitive versus resistant or refractory disease) was used, 53 (30%) patients had

Table 1. Demographics and Baseline Disease Characteristics (Intent-to-Treat Population)

Characteristic	Alisertib/Paclitaxel (n = 89)	Placebo/Paclitaxel (n = 89)
Median age, y (range)	62 (37-81)	62 (46-86)
Male sex, n (%)	51 (57)	50 (56)
Median time since initial diagnosis, mo (range)	7.4 (3-12)	7.8 (3-26)
VALG stage at initial diagnosis, n (%)		
Limited	18 (20)	8 (9)
Extensive	61 (69)	62 (70)
Unknown	10 (11)	19 (21)
ECOG PS, n (%)		
0	27 (30)	18 (20)
1	62 (70)	71 (80)
Smoking history, n (%)		
Never	4 (4)	7 (8)
Former/current	52 (58)/33 (37)	52 (59)/29 (33)
Missing[a]	0	1 (1)
No prior prophylactic cranial irradiation, n (%)	59 (66)	63 (71)
No brain involvement at baseline, n (%)	62 (70)	63 (71)
Type of relapse after primary treatment (by IVRS), n (%)		
Sensitive[b]	36 (40)	40 (45)
Resistant/refractory[b]	36 (40)/17 (19)	34 (38)/15 (17)
Type of relapse after primary treatment (corrected),[c] n (%)		
Sensitive	29 (33)	35 (42)
Resistant/refractory	41 (46)/19 (21)	33 (39)/16 (19)
Not classifiable or missing[a]	0	5 (6)

[a]Missing data were treated as missing and no data imputation was applied.
[b]Sensitive disease is defined as progression of disease observed >90 days from the last dose of first-line platinum-based chemotherapy, resistant disease is defined as progression of disease observed ≤90 days after first-line platinum-based chemotherapy, and refractory disease is defined as no objective response and progression either during or immediately after first-line platinum-based chemotherapy.
[c]Protocol Amendment 2 (January 2015) corrected the stratification definition for time to relapse to follow standard guidance by counting from date of last dose of frontline chemotherapy as opposed to by counting from initial response to first-line platinum-based chemotherapy, as originally set out in the protocol; the stratification factors for the study were corrected accordingly. When the corrected definition of time to relapse for type of relapse after primary treatment (sensitive versus resistant or refractory disease) was used, 53 patients (30%) had their relapse stratification factor changed after initial classification. In all, 15 patients in the alisertib plus paclitaxel arm and 14 in the placebo plus paclitaxel arm were reassigned from "sensitive" to "resistant or refractory"' relapse, and 8 and 11 patients, respectively, were reassigned from "resistant or refractory" to "sensitive" relapse.
ECOG PS, Eastern Cooperative Oncology Group performance status; IVRS, Interactive Voice Response System; VALG, Veterans Administration Lung Study Group.

their relapse stratification factor changed after initial classification, and there was a higher ratio of percentage of sensitive patients in the placebo/paclitaxel arm to that in the alisertib/paclitaxel arm (42:33) compared with when the original definition (45:40) was used (see Table 1). In addition, for five patients in the placebo/paclitaxel arm who had been enrolled under the original definition, sufficient data had not been provided to allow classification under the corrected definition, so they were excluded from the corrected stratification analysis.

Efficacy

The median PFS was 3.32 months in the alisertib/paclitaxel arm versus 2.17 months in the placebo/paclitaxel arm (HR = 0.77, 95% CI: 0.557–1.067, p = 0.113). In the prespecified sensitivity analysis, when the corrected definition for the stratification factor of relapse type was used, the HR was 0.71 (95% CI: 0.509–0.985, p = 0.038; [Fig. 1*A*]). There were no differences in PFS between arms based on the presence of metastases, enrollment region, Eastern Cooperative Oncology Group performance status, age category, race, or disease stage (data not shown). Although there was no difference in PFS for male patients (HR = 1.03, 95% CI: 0.677–1.578, p = 0.876), there was a marginal difference for female patients in favor of alisertib/paclitaxel compared with placebo/paclitaxel (median PFS 4.4 versus 2.6 months [HR = 0.60, 95% CI: 0.367–0.992, p = 0.043]). For patients with resistant or refractory relapse (corrected definition), the median PFS was 2.86 months with alisertib/paclitaxel versus 1.68 months with placebo/paclitaxel (HR = 0.66, p = 0.037 [Fig. 1*B*]). For patients with sensitive relapse (corrected definition), the median PFS was 3.72 months with alisertib/paclitaxel versus 3.34 months with placebo/paclitaxel, respectively (HR = 0.86, 95% CI: 0.493–1.497, p = 0.590).

At data cutoff, there was no significant difference in OS between arms (Fig. 1*C*). The median OS was 6.11 months with alisertib/paclitaxel versus 5.42 months with placebo/paclitaxel. Per protocol, a further OS

analysis, including data from at least 80% of patients, was conducted on October 15, 2016. Overall, 151 patients had died at this time, which was an increase of 23 deaths from the previous analysis. The updated median OS was 6.86 months with alisertib/paclitaxel versus 5.58 months with placebo/paclitaxel (HR = 0.93, 95% CI: 0.652–1.341, $p = 0.714$; HR with use of the corrected definition for relapse type = 0.73, 95% CI: 0.520–1.021, $p = 0.064$).

The ORR was 22% with alisertib/paclitaxel versus 18% with placebo/paclitaxel (Table 2). The DCR was 58% versus 46%, respectively. For the subgroup of resistant or refractory patients when the corrected definition for relapse type was used, the DCR was significantly higher with alisertib/paclitaxel than with placebo/paclitaxel (55% versus 33% [odds ratio = 0.40 (range 0.18–0.87), $p = 0.020$]). The median DOR among responders was 3.16 months in the alisertib/paclitaxel arm and 2.79 months in the placebo/paclitaxel arm (see Table 2). The median time to progression was 3.58 months with alisertib/paclitaxel versus 2.60 months with placebo/paclitaxel (HR = 0.67, $p = 0.038$) (see Table 2).

Exploratory Correlative Biomarker Studies

In all, 46 tumor tissue samples were evaluable for the c-Myc expression by immunohistochemistry analysis; 33 (72%) were positive (a modal intensity of 1+, 2+, or 3+) for c-Myc expression and 13 (28%) were negative (modal intensity = 0). PFS by c-Myc expression is shown in Fig. 1*D* and 1*E*. In c-Myc–positive patients, the median PFS was 4.64 months with alisertib/paclitaxel (n = 17) versus 2.27 months with placebo/paclitaxel (n = 16) (HR = 0.29, 95% CI: 0.12–0.72). In c-Myc–negative patients, the median PFS was 3.32 months with alisertib/paclitaxel (n = 6) versus 5.16 months with placebo/paclitaxel (n = 7) (HR = 11.8, 95% CI: 1.52–91.2). c-Myc expression was strongly associated with improved PFS when c-Myc was evaluated as a continuous variable of percentage of cells staining positive ($p_{\text{continuous}} = 0.0045$) or a binary (positive versus negative) mode ($p_{\text{binary}} = 0.0006$).

Out of 176 patients, 155 (88%) provided plasma samples that were processed for NGS analysis of ctDNA. In all, 142 patient samples (81%) were successfully sequenced and genetic alterations were identified from 140 patients (80%) (Supplementary Fig. 1). The full

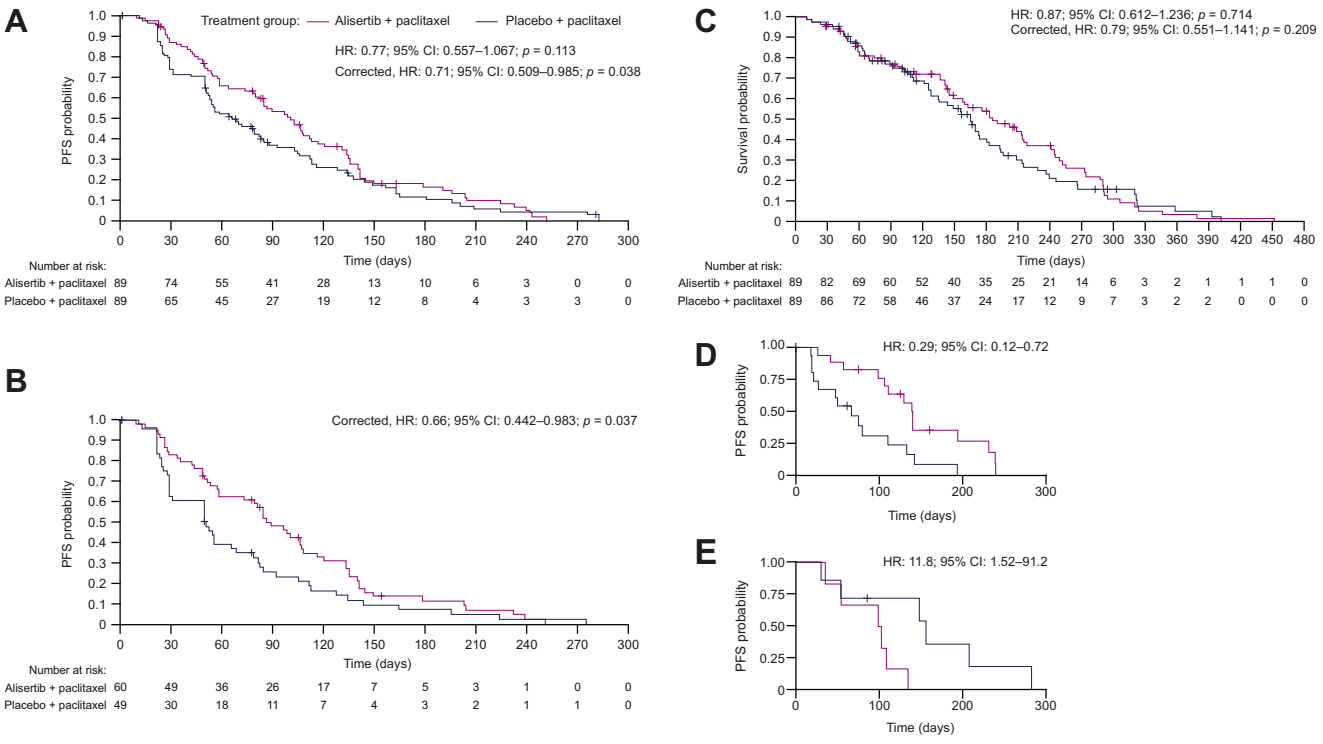


Figure 1. Clinical outcomes for the intent-to-treat population and according to resistant or refractory relapse and c-Myc expression. (*A*) Progression-free survival (PFS) in all patients. (*B*) PFS in patients with resistant or refractory relapse. (*C*) Overall survival in all patients. *(D)* PFS in patients positive for c-Myc expression. *(E)* PFS in patients negative for c-Myc expression. (*A-C*) Hazard ratios (HRs), 95% confidence intervals (CIs), and *p* values for the comparison of alisertib plus paclitaxel versus placebo plus paclitaxel are shown per protocol and with use of the corrected definition for the stratification factor of relapse type.

Table 2. Best Overall Response Rate (ITT Population), Duration of Response, and Time to Progression

Variable	Alisertib/Paclitaxel (n = 89)	Placebo/Paclitaxel (n = 89)	OR/HR (95% CI); *p* Value
Response, n (%)			
ORR (CR + PR)	20 (22)	16 (18)	0.74 (0.35-1.55); 0.406
CR	1 (1)	0	NE
PR	19 (21)	16 (18)	NE
Stable disease	49 (55)	44 (49)	NE
DCR (CR + PR + stable disease ≥8 weeks)	52 (58)	41 (46)	0.59 (0.32-1.08); 0.077
PD	13 (15)	23 (26)	NE
No on-study imaging[a]	7 (8)[b]	6 (7)[c]	NE
Median DOR (responders), mo (95% CI)	3.16 (2.76-4.64)	2.79 (1.91-5.82)	NE
Median TTP (ITT), mo (95% CI)	3.58 (2.86-4.41)	2.60 (1.74-3.45)	0.67 (0.462-0.982); 0.038

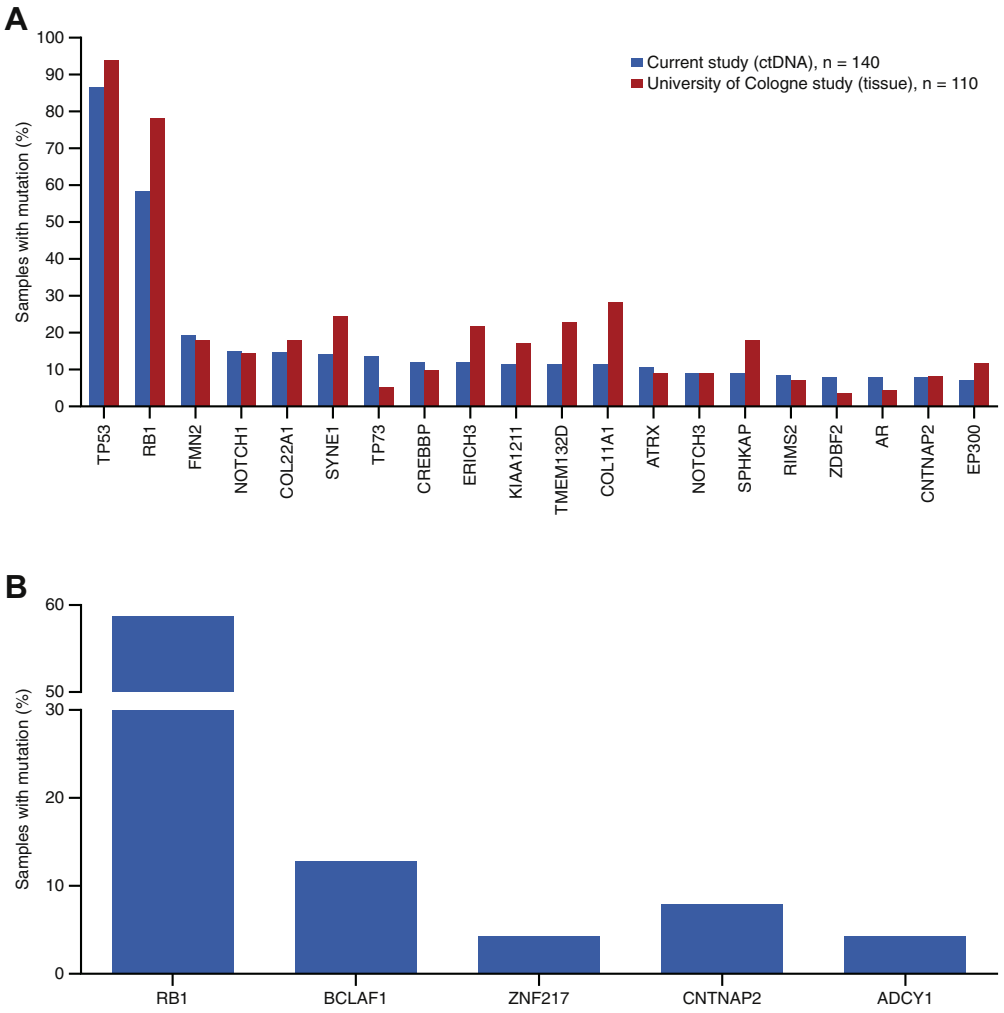
[a]Missing data were treated as missing and no data imputation was applied.
[b]Three patients died before on-study imaging, two patients were not assessed, and two patients were not dosed.
[c]Four patients died before to on-study imaging, one patient was not assessed, and one patient was too ill for imaging.
CI, confidence interval; CR, complete response; DCR, disease control rate; DOR, duration of response; HR, hazard ratio; ITT, intent-to-treat population; NE, not evaluated; OR, odds ratio; ORR, objective response rate; PD, disease progression; PR, partial response; TTP, time to progression.

results from this genetic profiling are provided in the Supplementary Results (Supplementary Figs. 2–6 and see also Supplementary Fig. 1). The genetic mutation profiles revealed from the SCLC plasma ctDNA samples were concordant with those previously identified in SCLC primary tumor tissue,[24] matching the mutational frequency trends in characterized genes, such as tumor protein p53 gene (*TP53*), retinoblastoma 1 gene (*RB1*), formin 2 gene (*FMN2*), notch receptor 1 gene (*NOTCH1*), collagen type XXII alpha 1 chain gene (*COL22A1*), spectrin repeat containing nuclear envelope protein 1 gene (*SYNE1*), CREB binding protein gene (*CREBBP*), ATRX chromatin remodeler gene (*ATRX*), notch receptor 3 gene (*NOTCH3*), regulating synaptic membrane exocytosis 2 gene (*RIMS2*), and contactin associated protein-like 2 gene (*CNTNAP2*) (Fig. 2*A*). The mutation spectrum of the key genes also showed high concordance (see Supplementary Fig. 3). The correlative analysis of clinical outcomes (PFS and OS) was assessed in relation to (1) individual gene mutations, (2) biological pathway gene mutations, and (3) overall mutation load.

At the individual gene mutation level, five of the mutated genes were found to have marginal significance to PFS or OS in the alisertib/paclitaxel arm: *RB1*, adenylate cyclase 1 gene (*ADCY1*), *CNTNAP2*, zinc finger protein 217 gene (*ZNF217*), and BCL associated transcription factor 1 gene (*BCLAF1*) (Fig. 2*B*). When grouped by biological pathway (see Supplementary Figs. 4 and 5), mutations in genes involved in cell cycle regulation (cyclin-dependent kinase 6 gene [*CDK6*], retinoblastoma-like 1 gene [*RBL1*], retinoblastoma-like 2 gene [*RBL2*], and retinoblastoma 1 gene [*RB1*]) were significantly associated with improved PFS ($p = 0.0011$) and OS ($p = 0.00096$) after alisertib/paclitaxel treatment. No such association was shown for mutated genes involved in NOTCH (*NOTCH1, NOTCH2,* and *NOTCH3*) or phosphoinositide 3-kinase (KIT proto-oncogene receptor tyrosine kinase gene [*KIT*], phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene [*PIK3CA*], phosphatase and tensin homolog gene [*PTEN*], tumor protein p73 gene [*TP73*]) signaling, or histone modifications (*CREBBP* and E1A binding protein p300 gene [*EP300*]) (see Supplementary Fig. 5). Patients with mutations in cell cycle regulators ("mutant") had improved median PFS (3.68 versus 1.80 months [HR = 0.395, 95% CI: 0.239–0.654, $p = 0.0003$]) (Fig. 3*A* and 3*B*) and OS (7.20 versus 4.47 months [HR = 0.427, 95% CI: 0.259–0.704, $p = 0.00085$]) with alisertib/paclitaxel (n = 40) compared with placebo/paclitaxel (n = 47) (Fig. 3*C* and 3*D*). Conversely, patients without mutations in cell cycle regulators ("wild type") had no improvement in median PFS (2.63 versus 2.60 months [HR = 1.31, 95% CI: 0.736–2.33, $p = 0.359$]) (see Fig. 3*A* and 3*B*) or OS (4.47 versus 5.95 months [HR = 1.70, 95% CI: 0.865–3.33, $p = 0.124$]) with alisertib/paclitaxel (n = 28) versus with placebo/paclitaxel (n = 25) (see Fig. 3*C* and 3*D*). Across the 140 samples sequenced, 2151 mutations were identified, with a mean mutational load of 12.93 mutations per megabase pair of panel sequenced and a median value of 8.52 mutations per megabase pair (see Supplementary Fig. 6). Correlation between mutational load and PFS or OS in the alisertib arm was marginally significant for OS ($p = 0.025$) but not for PFS ($p = 0.103$). Notably, patient samples with mutations in cell cycle regulation genes had a significantly higher mutational load (a mean of 19.03) compared with those without cell cycle regulation gene mutations (a mean of 9.06) ($p < 0.0001$) (see Supplementary Fig. 6).

Safety and Tolerability

The median numbers of cycles received in the alisertib/paclitaxel and placebo/paclitaxel arms were 3



	p-value for association with outcome (FDR)	
Gene	**PFS**	**OS**
RB1	0.0028 (FDR = 0.093)	0.0069 (FDR = 0.22)
ADCY1	0.020 (FDR = 0.22)	0.088 (FDR = 0.61)
ZNF217	0.029 (FDR = 0.24)	0.042 (FDR = 0.46)
CNTNAP2	0.020 (FDR = 0.22)	0.092 (FDR = 0.61)
BCLAF1	0.074 (FDR = 0.47)	0.019 (FDR = 0.31)

Figure 2. Association between gene mutations and clinical outcomes. *(A)* Gene mutation frequencies identified in this study by using circulating tumor DNA (ctDNA) were compared with those reported previously in primary tumor tissue samples from patients with SCLC.[24] Mutation frequencies for the 20 most commonly mutated genes are shown. *(B)* The interaction between genetic alterations and clinical outcomes (progression-free survival [PFS] and overall survival [OS]) was tested by using a Cox proportional hazards model. The *p* values and false discovery rates (FDRs) for the association with outcome are shown. Mutations in retinoblastoma 1 gene (*RB1*), BCL associated transcription factor 1 gene (*BCLAF*), contactin associated protein-like 2 gene (*CNTNAP2*), adenylate cyclase 1 gene (*ADCY1*), and zinc finger protein 217 gene (*ZNF217*) were independently associated with alisertib plus paclitaxel versus with placebo plus paclitaxel efficacy, as defined by hazard ratios for PFS and OS. Abbreviations: *TP53*, tumor protein p53 gene; *FMN2*, formin 2 gene; *NOTCH1*, notch receptor 1 gene; *COL22A1*, collagen type XXII alpha 1 chain gene; *SYNE1*, spectrin repeat containing nuclear envelope protein 1 gene; *TP73*, tumor protein p73 gene; *CREBBP*, CREB binding protein gene; *ERICH3*, glutamate rich 3 gene; *KIAA1211*, KIAA1211 gene (now known by the gene symbol *CRACD*); *TMEM132D*, transmembrane protein 132D gene; *COL11A1*, collagen type XI alpha 1 chain gene; *ATRX*, ATRX chromatin remodeler gene; *NOTCH3*, notch receptor 3 gene; *SPHKAP*, SPHK1 interactor, AKAP domain containing gene; *RIMS2*, regulating synaptic membrane exocytosis 2 gene; *ZDBF2*, zinc finger DBF-type containing 2 gene; *AR*, androgen receptor gene; *CNTNAP2*, contactin associated protein 2 gene; *EP300*, E1A binding protein p300 gene.

(range 1–9) and 2 (range 1–11), respectively. Diarrhea and fatigue were the most common adverse events (AEs); both were reported more frequently with alisertib/paclitaxel than with placebo/paclitaxel. Grade 3 or higher AEs were more common with alisertib/paclitaxel (76%) than with placebo/paclitaxel (51%), including the most common individual grade 3 or higher AE, neutropenia (Table 3). Drug-related AEs were also more common with alisertib/paclitaxel than with placebo/paclitaxel (see Table 3); the most common drug-related grade 3 or higher AEs were neutropenia, febrile neutropenia, leukopenia, anemia, diarrhea, and

A

PFS with or without cell cycle gene mutations

	Mutant		Wild type	
	n	Median, months	n	Median, months
Alisertib + paclitaxel	40	3.68	28	2.63
Placebo + paclitaxel	47	1.80	25	2.60
HR (95% CI)	0.395 (0.239–0.654)		1.31 (0.736–2.33)	
p-value	0.0003		0.359	

C

OS with or without cell cycle gene mutations

	Mutant		Wild type	
	n	Median, months	n	Median, months
Alisertib + paclitaxel	40	7.20	28	4.47
Placebo + paclitaxel	47	4.47	25	5.95
HR (95% CI)	0.427 (0.259–0.704)		1.70 (0.865–3.33)	
p-value	0.00085		0.124	

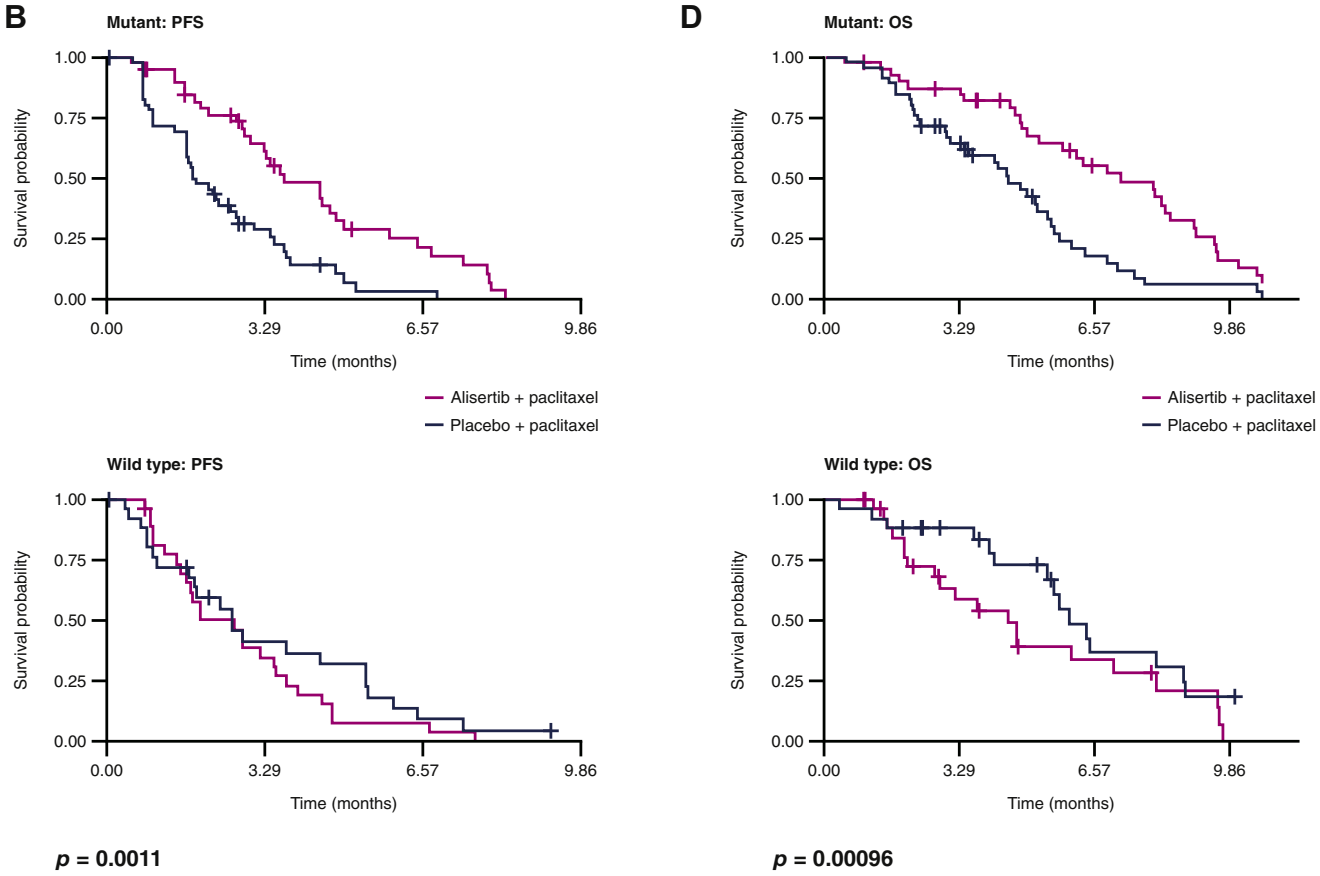


Figure 3. Clinical outcomes according to treatment and mutations in cell cycle regulation pathway genes. (*A*) Cox proportional hazard regression analysis of progression-free survival (PFS) in patients with (mutant) and without (wild-type) mutations in cell cycle pathway genes, respectively. In each subgroup, the hazard ratio (HR) between the two treatment arms (alisertib plus paclitaxel versus placebo plus paclitaxel) was estimated, along with the 95% confidence interval (CI) and *p* value. (*B*) Kaplan-Meier plots of PFS in mutant and wild-type patients, respectively. The *p* value in this panel is the interaction effect between treatment group and mutation status and is calculated by testing the difference between the HRs for the two subgroups. (*C*) Cox proportional hazard regression analysis of overall survival (OS) in mutant and wild-type patients, respectively. In each subgroup, the HR between the two treatment arms (alisertib plus paclitaxel versus placebo plus paclitaxel) was estimated, along with the 95% CI and *p* value. (*D*) Kaplan-Meier plots of OS in mutant and wild-type patients, respectively. The *p* value in this panel is the interaction effect between treatment group and mutation status and is calculated by testing the difference between the HRs for the two subgroups.

stomatitis, which were all reported more frequently with alisertib/paclitaxel than with placebo/paclitaxel. Similarly, in the alisertib/paclitaxel arm, 38 patients (44%) reported a serious AE (SAE) and 28 (32%) reported a drug-related SAE, compared with 28 patients (31%) and six (7%), respectively, in the placebo/paclitaxel arm. The most common (≥5% of patients in either treatment arm) all-cause SAEs with alisertib/paclitaxel were febrile neutropenia (10%), neutropenia (6%), diarrhea (6%), and stomatitis (5%). There were no SAEs of febrile neutropenia, neutropenia, or diarrhea with placebo/paclitaxel; one patient (1%) reported an SAE of stomatitis.

In the alisertib/paclitaxel and placebo/paclitaxel arms, 14 patients (16%) and five (6%) discontinued study treatment because of an AE, 33 patients (38%) and nine (10%) had a dose reduction because of an AE, and 12 patients (14%) and 11 (12%) died during the study (within 30 days of last dose). Four of these deaths, all in the alisertib/paclitaxel arm, were assessed as drug related, including one each due to neutropenic sepsis, sepsis, febrile neutropenia, and septic shock (see Table 3).

Table 3. Most Frequently Reported All-Cause and Drug-Related Treatment-Emergent AEs, Occurring in at Least 15% (All-Cause) or at Least 10% (Drug-Related) of Patients Overall (Any Grade) in Either Arm, Respectively, with the Corresponding Grade 3 or higher AEs (Safety Population), and All Drug-Related Fatal AEs

	Alisertib/Paclitaxel (n = 87)		Placebo/Paclitaxel (n = 89)	
AE	Any Grade	Grade ≥3	Any Grade	Grade ≥3
All-cause AE, n (%)	86 (99)	66 (76)	85 (96)	45 (51)
Diarrhea	51 (59)	14 (16)	18 (20)	1 (1)
Fatigue	38 (44)	9 (10)	29 (33)	5 (6)
Nausea	29 (33)	2 (2)	30 (34)	4 (4)
Anemia	38 (44)	12 (14)	18 (20)	3 (3)
Neutropenia	43 (49)	35 (40)	7 (8)	5 (6)
Vomiting	28 (32)	2 (2)	21 (24)	3 (3)
Decreased appetite	29 (33)	3 (3)	19 (21)	3 (3)
Dyspnea	21 (24)	4 (5)	19 (21)	2 (2)
Stomatitis	29 (33)	12 (14)	6 (7)	2 (2)
Cough	17 (20)	0	17 (19)	0
Constipation	8 (9)	1 (1)	21 (24)	0
Asthenia	14 (16)	3 (3)	11 (12)	0
Dizziness	14 (16)	0	8 (9)	0
Alopecia	14 (16)	0	5 (6)	0
Leukopenia	13 (15)	7 (8)	5 (6)	2 (2)
Decreased neutrophil count	14 (16)	11 (13)	4 (4)	1 (1)
Weight decreased	13 (15)	0	5 (6)	0
Drug-related AE, n (%)	81 (93)	58 (67)	72 (81)	22 (25)
Diarrhea	44 (51)	13 (15)	11 (12)	1 (1)
Fatigue	32 (37)	7 (8)	23 (26)	3 (3)
Nausea	24 (28)	2 (2)	26 (29)	4 (4)
Neutropenia	41 (47)	33 (38)	7 (8)	5 (6)
Anemia	31 (36)	9 (10)	14 (16)	1 (1)
Vomiting	23 (26)	2 (2)	14 (16)	2 (2)
Stomatitis	28 (32)	11 (13)	6 (7)	2 (2)
Decreased appetite	21 (24)	3 (3)	13 (15)	1 (1)
Leukopenia	13 (15)	7 (8)	5 (6)	2 (2)
Alopecia	12 (14)	0	5 (6)	0
Neutrophil count decreased	13 (15)	10 (11)	4 (4)	1 (1)
Febrile neutropenia	11 (13)	11 (13)	0	0
WBC count decreased	11 (13)	11 (13)	1 (1)	1 (1)
Asthenia	9 (10)	3 (3)	6 (7)	0
Drug-related fatal AE, n (%)				
Neutropenic sepsis	—	1 (1)	—	0
Sepsis	—	1 (1)	—	0
Febrile neutropenia	—	1 (1)	—	0
Septic shock	—	1 (1)	—	0

AE, adverse event; WBC, white blood cell.

Discussion

In this study of alisertib/paclitaxel versus placebo/paclitaxel as second-line therapy in patients with advanced SCLC, the primary end point of PFS in the ITT population was not met, but PFS was significantly improved when analyzed on the basis of the corrected definition of relapse type after first-line therapy (as intended by the protocol), with a positive trend favoring alisertib/paclitaxel ($p = 0.038$). However, this PFS benefit was not clinically meaningful. Similarly, the subgroup analysis of type of relapse after first-line therapy (corrected definition) demonstrated a statistically significant improvement in PFS with alisertib/paclitaxel over that with placebo/paclitaxel in patients with resistant or refractory relapsed SCLC ($p = 0.037$). However, the primary analysis of PFS conducted by using the original stratification definition of relapse type was not significant on statistical testing. Despite a trend in favor of alisertib/paclitaxel, there was no significant difference in OS between arms; however, the study was not powered to show an OS advantage. Notably, the correlative biomarker studies indicated that both c-Myc expression and mutations in cell cycle regulators (*CDK6, RBL1, RBL2, and RB1*) showed strong correlation with improved clinical outcomes (PFS and OS) in patients with SCLC who were receiving alisertib/paclitaxel. These findings are consistent with the mechanism of action of alisertib as a mitotic inhibitor disrupting cell cycle progression through mitosis. Thus, c-Myc expression or cell cycle gene mutations may serve as predictive biomarkers for alisertib.

When the results are interpreted, there are points to consider. Although not the primary end point, the sensitivity analysis of PFS using the corrected definition of relapse type may represent a more clinically relevant, scientifically valid approach. In the PFS sensitivity analysis with use of the corrected definition of relapse type, patients without a stratification factor were excluded, improving internal validity. There was also a higher ratio of sensitive patients in the placebo/paclitaxel arm to sensitive patients in the alisertib/paclitaxel arm (42:33), as compared with when the original definition was used (45:40); therefore, the original analysis favored placebo/paclitaxel. Finally, doses of paclitaxel were different in each arm to account for the pharmacodynamic synergy of paclitaxel with alisertib.

The median PFS with placebo/paclitaxel was slightly shorter than in a previous study of second-line paclitaxel monotherapy in patients with SCLC (4.76 months),[25] whereas the median OS was in line with values in previous reports (3.3–5.8 months).[25-27] A longer interval between restaging scans could lead to overestimation of PFS, and the shorter PFS duration in our study potentially reflects the more rigorous restaging scan schedule (every 4 weeks for the first six cycles), which enabled more accurate estimation of PFS than in previous studies.

Although cross-trial comparison should be approached with caution, the response rate of 22% observed with the alisertib/paclitaxel combination in the study is comparable to the 21% response rate associated with single-agent alisertib in 48 patients with SCLC in a phase 1/2 study,[23] which may suggest that synergy of the combination is limited in this treatment setting.

Because of the overlapping toxicity profile for alisertib and paclitaxel, there was a higher incidence of grade 3 or higher AEs and drug-related AEs with this combination versus with paclitaxel alone. Increased toxicity is a concern in this treatment setting, in which many patients are not clinically robust. Clinically manageable hematologic events were among the most frequent drug-related grade 3 or higher treatment-emergent AEs in patients who received alisertib/paclitaxel; of particular note, a high rate of grade 3 or higher neutropenia (38%) was observed. Overall, 44 patients in the study received myeloid growth factor support to manage neutropenia (34 patients in the alisertib/paclitaxel arm and 10 patients in the placebo/paclitaxel arm). Rates of on-study deaths were similar in both arms, but four AE-related deaths (due to febrile neutropenia, neutropenic sepsis, sepsis, and septic shock) occurred only with alisertib/paclitaxel.

AAK binds to c-Myc and prevents its degradation, thereby enhancing its growth-promoting effect in cancer cells.[16,17] Conversely, pharmacologic inhibition of AAK in preclinical studies results in greater growth inhibition in cell lines harboring *MYC* amplification.[13,14] We therefore anticipated that patients whose tumors harbored high c-Myc expression would be susceptible to an AAK inhibitor (as seen in preclinical studies).[28] Moreover, as c-Myc alteration may be correlated with poor response to chemotherapy, we anticipated that patients with resistant or refractory relapse would derive greater benefit. Consistent with this hypothesis, alisertib/paclitaxel showed significant benefit over placebo/paclitaxel in patients with resistant or refractory relapse and in patients with c-Myc expression. Although the numbers were low and the results need to be reproduced in a larger independent study, we expect that enrichment strategies for these patient subsets could aid further development of alisertib in SCLC.

With respect to genetic biomarkers, patients treated with alisertib/paclitaxel who had mutations in cell cycle regulator genes, including *CDK6, RBL1, RBL2,* and *RB1*, had significantly improved PFS and OS compared with those who received placebo/paclitaxel. Previous genomic landscape studies have implicated cell cycle

regulation genes as being commonly mutated in SCLC.[24,29] Our findings suggest a predictive value of these mutations with alisertib/paclitaxel treatment for SCLC. Interestingly, patients with mutated cell cycle regulators had worse outcomes in response to placebo/paclitaxel treatment; the implications of these findings warrant further investigation. Our study results are consistent with, and provide validation, of the findings from two preclinical reports in *Cancer Discovery,* which describe a synthetic lethal relationship between *RB1* mutations and inhibition of AAK or Aurora B kinase.[30,31] The predictive value of cell cycle mutations for alisertib treatment in other diseases is also of interest and worth further exploration. Importantly, our data highlight the emerging role of NGS profiling of plasma ctDNA for identifying novel predictive biomarkers.

No predictive value was demonstrated in genetic alterations in other cell cycle progression genes implicated in SCLC, such as amplification of Aurora kinase A gene (*AURKA*) and *MYC*[10,17,32,33] (data not shown). Similarly, pathway mutations in genes implicated in NOTCH and phosphoinositide 3-kinase signaling, and histone modifications, showed no association with PFS or OS in response to alisertib treatment. As these parameters were established retrospectively, the study was not optimized for this metric.

A key limitation of our study was the failure to use a validated biomarker for prospective patient selection. Although post hoc biomarker interrogation supported c-Myc expression and mutations in cell cycle regulator genes as promising biomarkers, these observations require more rigorous prospective testing and validation. Additionally, the study failed to demonstrate survival benefit of alisertib/paclitaxel compared with placebo/paclitaxel. Patients entering this study were stratified by platinum sensitivity status based on data available at the time, which suggested that platinum sensitivity status was correlated with efficacy outcomes. However, data from studies in relapsed SCLC published during the conduct of this trial suggest that platinum sensitivity may not be strongly associated with efficacy outcomes and that other prognostic subgroups could be more relevant in study designs.[34] Another limitation was that the comparator arm in this study was placebo/paclitaxel. At the time of this study's design, topotecan was the only agent approved in the relapsed setting by the U.S. Food and Drug Administration (FDA),[6,35] having demonstrated symptom reduction and less hematologic toxicity than with cyclophosphamide, doxorubicin, and vincristine,[36] and survival benefit over best supportive care.[37] Single-agent paclitaxel is frequently used off-label as a standard treatment for relapsed SCLC, and paclitaxel was chosen as the comparator in this study, rather than topotecan, because of the higher toxicity associated with topotecan, particularly the overlapping toxicity of neutropenia with alisertib, and the existence of preclinical and clinical data for the alisertib/paclitaxel combination.[19] Recently, other agents have shown similar or improved efficacy compared with topotecan, including amrubicin[8] and cisplatin, etoposide, and irinotecan,[38] and in August 2018, nivolumab received accelerated approval by the FDA for patients with metastatic SCLC with progression after platinum-based chemotherapy and at least one other line of therapy.[39] Approval was based on findings from the open-label, multicohort CHECKMATE-032 study (NCT01928394), which reported an ORR of 12% (95% CI: 6.5–19.5) in 109 patients (~65% with platinum-sensitive SCLC, defined as progression ≥90 days after the last dose of platinum-containing therapy), with 77% having a DOR of at least 6 months when treated with nivolumab with or without ipilimumab.[39] Further treatment options continue to emerge for SCLC, including immunotherapy; in 2019, FDA approvals for have been granted for pembrolizumab in relapsed or refractory SCLC, based on the results of the KEYNOTE-028 (NCT02054806) and KEYNOTE-158 (NCT02628067) studies,[40,41] and for atezolizumab in combination with chemotherapy in the frontline treatment of extensive-stage SCLC, based on the IMpower133 trial (NCT02763579).[42] There were seven patients who had received treatment with immunotherapy before starting our study (two in the alisertib/paclitaxel arm and five in the placebo/paclitaxel arm); postprogression treatment information was not collected. Despite the emerging range of treatment options, topotecan remains the standard therapy in the second-line setting. Whether the efficacy signal observed in the present study is sufficient to support a definitive superiority trial in comparison with topotecan in unselected patients is difficult to ascertain, particularly in light of the negative result of the phase II trial of cabazitaxel versus topotecan in a similar population of patients with relapsed SCLC.[7] However, a biomarker-enrichment strategy using c-Myc expression or mutations in cell cycle regulator genes could enhance the likelihood of success of such a comparative study.

In conclusion, alisertib/paclitaxel showed a modest efficacy signal as second-line therapy for SCLC. Because of the overlapping safety profile for alisertib and paclitaxel, grade 3 or higher AEs and drug-related AEs were more frequent with the combination than with paclitaxel alone. The predictive value of c-Myc expression and cell cycle gene mutations for AAK inhibitor susceptibility is promising, but prospective testing and validation are required. These results, along with those from a previous phase II study demonstrating activity of alisertib

monotherapy in patients with relapsed or refractory SCLC,[23] may warrant further testing in a larger study with predictive biomarker enrichment strategy.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2019.10.013.

References

1. Alvarado-Luna G, Morales-Espinosa D. Treatment for small cell lung cancer, where are we now?-a review. *Transl Lung Cancer Res*. 2016;5:26–38.
2. Sorensen M, Pijls-Johannesma M, Felip E. Small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2010;21(suppl 5):v120–v125.
3. Arcaro A. Targeted therapies for small cell lung cancer: where do we stand? *Crit Rev Oncol Hematol*. 2015;95:154–164.
4. Hamilton G, Rath B, Holzer S, et al. Second-line therapy for small cell lung cancer: exploring the potential role of circulating tumor cells. *Transl Lung Cancer Res*. 2016;5:71–77.
5. Jett JR, Schild SE, Kesler KA, et al. Treatment of small cell lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2013;143:e400S–e419S.
6. Owonikoko TK, Behera M, Chen Z, et al. A systematic analysis of efficacy of second-line chemotherapy in sensitive and refractory small-cell lung cancer. *J Thorac Oncol*. 2012;7:866–872.
7. Evans TL, Cho BC, Udud K, et al. Cabazitaxel versus topotecan in patients with small-cell lung cancer with progressive disease during or after first-line platinum-based chemotherapy. *J Thorac Oncol*. 2015;10:1221–1228.
8. von Pawel J, Jotte R, Spigel DR, et al. Randomized phase III trial of amrubicin versus topotecan as second-line treatment for patients with small-cell lung cancer. *J Clin Oncol*. 2014;32:4012–4019.
9. Barr AR, Gergely F. Aurora-A: the maker and breaker of spindle poles. *J Cell Sci*. 2007;120:2987–2996.
10. Lu Y, Liu Y, Jiang J, et al. Knocking down the expression of Aurora-A gene inhibits cell proliferation and induces G2/M phase arrest in human small cell lung cancer cells. *Oncol Rep*. 2014;32:243–249.
11. Marumoto T, Honda S, Hara T, et al. Aurora-A kinase maintains the fidelity of early and late mitotic events in HeLa cells. *J Biol Chem*. 2003;278:51786–51795.
12. Cowley DO, Rivera-Perez JA, Schliekelman M, et al. Aurora-A kinase is essential for bipolar spindle formation and early development. *Mol Cell Biol*. 2009;29:1059–1071.
13. Hook KE, Garza SJ, Lira ME, et al. An integrated genomic approach to identify predictive biomarkers of response to the aurora kinase inhibitor PF-03814735. *Mol Cancer Ther*. 2012;11:710–719.
14. Sos ML, Dietlein F, Peifer M, et al. A framework for identification of actionable cancer genome dependencies in small cell lung cancer. *Proc Natl Acad Sci U S A*. 2012;109:17034–17039.
15. Kim YH, Girard L, Giacomini CP, et al. Combined microarray analysis of small cell lung cancer reveals altered apoptotic balance and distinct expression signatures of MYC family gene amplification. *Oncogene*. 2006;25:130–138.
16. Brockmann M, Poon E, Berry T, et al. Small molecule inhibitors of aurora-a induce proteasomal degradation of

N-myc in childhood neuroblastoma. *Cancer Cell*. 2013;24:75–89.
17. Dauch D, Rudalska R, Cossa G, et al. A MYC-aurora kinase A protein complex represents an actionable drug target in p53-altered liver cancer. *Nat Med*. 2016;22:744–753.
18. Barr PM, Li H, Spier C, et al. Phase II intergroup trial of alisertib in relapsed and refractory peripheral T-cell lymphoma and transformed mycosis fungoides: SWOG 1108. *J Clin Oncol*. 2015;33:2399–2404.
19. Falchook G, Coleman RL, Roszak A, et al. Alisertib in combination with weekly paclitaxel in patients with advanced breast cancer or recurrent ovarian cancer: a randomized clinical trial. *JAMA Oncol*. 2019;5:e183773.
20. Falchook GS, Zhou X, Venkatakrishnan K, et al. Effect of food on the pharmacokinetics of the investigational Aurora A kinase inhibitor alisertib (MLN8237) in patients with advanced solid tumors. *Drugs R D*. 2016;16:45–52.
21. Goldberg SL, Fenaux P, Craig MD, et al. An exploratory phase 2 study of investigational Aurora A kinase inhibitor alisertib (MLN8237) in acute myelogenous leukemia and myelodysplastic syndromes. *Leuk Res Rep*. 2014;3:58–61.
22. Matulonis UA, Sharma S, Ghamande S, et al. Phase II study of MLN8237 (alisertib), an investigational Aurora A kinase inhibitor, in patients with platinum-resistant or -refractory epithelial ovarian, fallopian tube, or primary peritoneal carcinoma. *Gynecol Oncol*. 2012;127:63–69.
23. Melichar B, Adenis A, Lockhart AC, et al. Safety and activity of alisertib, an investigational aurora kinase A inhibitor, in patients with breast cancer, small-cell lung cancer, non-small-cell lung cancer, head and neck squamous-cell carcinoma, and gastro-oesophageal adenocarcinoma: a five-arm phase 2 study. *Lancet Oncol*. 2015;16:395–405.
24. George J, Lim JS, Jang SJ, et al. Comprehensive genomic profiles of small cell lung cancer. *Nature*. 2015;524:47–53.
25. Noronha V, Sahu A, Patil VM, et al. Weekly paclitaxel as metronomic palliative chemotherapy in small cell lung cancer. *South Asian J Cancer*. 2016;5:67–69.
26. Yamamoto N, Tsurutani J, Yoshimura N, et al. Phase II study of weekly paclitaxel for relapsed and refractory small cell lung cancer. *Anticancer Res*. 2006;26:777–781.
27. Smit EF, Fokkema E, Biesma B, et al. A phase II study of paclitaxel in heavily pretreated patients with small-cell lung cancer. *Br J Cancer*. 1998;77:347–351.
28. Mollaoglu G, Guthrie MR, Bohm S, et al. MYC drives progression of small cell lung cancer to a variant neuroendocrine subtype with vulnerability to Aurora kinase inhibition. *Cancer Cell*. 2017;31:270–285.
29. Peifer M, Fernandez-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nat Genet*. 2012;44:1104–1110.
30. Gong X, Du J, Parsons SH, et al. Aurora A kinase inhibition is synthetic lethal with loss of the RB1 tumor suppressor gene. *Cancer Discov*. 2019;9:248–263.
31. Oser MG, Fonseca R, Chakraborty AA, et al. Cells lacking the RB1 tumor suppressor gene are hyperdependent on Aurora B kinase for survival. *Cancer Discov*. 2019;9:230–247.
32. Dominguez-Brauer C, Thu KL, Mason JM, et al. Targeting mitosis in cancer: emerging strategies. *Mol Cell*. 2015;60:524–536.
33. Eymin B, Gazzeri S. Role of cell cycle regulators in lung carcinogenesis. *Cell Adh Migr*. 2010;4:114–123.
34. Lara PN Jr, Moon J, Redman MW, et al. Relevance of platinum-sensitivity status in relapsed/refractory extensive-stage small-cell lung cancer in the modern era: a patient-level analysis of southwest oncology group trials. *J Thorac Oncol*. 2015;10:110–115.
35. Teva Pharmaceuticals. Topotecan prescribing information. 2014. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022453s002lbl.pdf. Accessed February 22, 2019.
36. von Pawel J, Schiller JH, Shepherd FA, et al. Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. *J Clin Oncol*. 1999;17:658–667.
37. O'Brien ME, Ciuleanu TE, Tsekov H, et al. Phase III trial comparing supportive care alone with supportive care with oral topotecan in patients with relapsed small-cell lung cancer. *J Clin Oncol*. 2006;24:5441–5447.
38. Goto K, Ohe Y, Shibata T, et al. Combined chemotherapy with cisplatin, etoposide, and irinotecan versus topotecan alone as second-line treatment for patients with sensitive relapsed small-cell lung cancer (JCOG0605): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2016;17:1147–1157.
39. Bristol-Myers Squibb Company. OPDIVO (nivolumab) prescribing information. 2018. https://packageinserts.bms.com/pi/pi_opdivo.pdf. Accessed February 22, 2019.
40. Chung HC, Lopez-Martin JA, Kao SCH, et al. Phase 2 study of pembrolizumab in advanced small-cell lung cancer (SCLC): KEYNOTE-158 [abstract]. *J Clin Oncol*. 2018;36(suppl 15):8506.
41. Ott PA, Elez E, Hiret S, et al. Pembrolizumab in patients with extensive-stage small-cell lung cancer: results from the phase Ib KEYNOTE-028 study. *J Clin Oncol*. 2017;35:3823–3829.
42. Horn L, Mansfield AS, Szczesna A, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. *N Engl J Med*. 2018;379:2220–2229.