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Selumetinib with and without erlotinib in KRAS mutant and KRAS wild-type advanced nonsmall-cell lung cancer†

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Background: KRAS mutations in NSCLC are associated with a lack of response to epidermal growth factor receptor inhibitors. Selumetinib (AZD6244; ARRY-142886) is an oral selective MEK kinase inhibitor of the Ras/Raf/MEK/ERK pathway.

Patients and methods: Advanced nonsmall-cell lung cancer (NSCLC) patients failing one to two prior regimens underwent KRAS profiling. KRAS wild-type patients were randomized to erlotinib (150 mg daily) or a combination of selumetinib (150 mg daily) with erlotinib (100 mg daily). KRAS mutant patients were randomized to selumetinib (75 mg b.i.d.) or the combination. The primary end points were progression-free survival (PFS) for the KRAS wild-type cohort and objective response rate (ORR) for the KRAS mutant cohort. Biomarker studies of ERK phosphorylation and immune subsets were carried out.

Results: From March 2010 to May 2013, 89 patients were screened; 41 KRAS mutant and 38 KRAS wild-type patients were enrolled. Median PFS in the KRAS wild-type arm was 2.4 months [95% confidence interval (CI) 1.3–3.7] for erlotinib alone and 2.1 months (95% CI 1.8–5.1) for the combination. The ORR in the KRAS mutant group was 0% (95% CI 0.0% to 33.6%) for selumetinib alone and 10% (95% CI 2.1% to 26.3%) for the combination. Combination therapy resulted in increased toxicities, requiring dose reductions (56%) and discontinuation (8%). Programmed cell death-1 expression on regulatory T cells (Tregs), Tim-3 on CD8+ T cells and Th17 levels were associated with PFS and overall survival in patients receiving selumetinib.

Conclusions: This study failed to show improvement in ORR or PFS with combination therapy of selumetinib and erlotinib over monotherapy in KRAS mutant and KRAS wild-type advanced NSCLC. The association of immune subsets and immune checkpoint receptor expression with selumetinib may warrant further studies.

Key words: nonsmall-cell lung cancer, MEK, EGFR, KRAS, selumetinib, erlotinib

introduction

Lung cancer is the leading cause of cancer-related death in North America [1]. Current first-line therapy for advanced nonsmall-cell lung cancer (NSCLC) patients with wild-type epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase is platinum-based chemotherapy. The median overall survival (OS) for nonselected patients is only 10–12 months [2]. Approved second-line treatments include docetaxel, pemetrexed, nivolumab and erlotinib [3]. Erlotinib was shown to be inferior to docetaxel in wild-type EGFR NSCLC patients in second-line [4] therapy. Erlotinib remains the only approved third-line therapy regardless of EGFR and KRAS status with objective response rate (ORR) of 8.9% and median OS of 6.7 months [3]. Although erlotinib is approved for wild-type EGFR, its activity is mainly seen in patients with EGFR sensitizing mutations.

The Ras/Raf/MEK/MAPK pathway regulates processes involved in the proliferation and survival of normal cells. KRAS mutations

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lead to constitutive activation of this pathway in cancer cells, resulting in dysregulation of cell proliferation [5]. KRAS mutations occur mutually exclusive of EGFR mutations and are found in ~20% of NSCLC [6] and patients with KRAS mutations have poor prognosis [7, 8]. KRAS mutations are associated with intrinsic resistance to EGFR inhibitors [tyrosine kinase inhibitors (TKIs)] [9].

Preclinical models demonstrated that tumors with mutations in the RAS/RAF/MEK/ERK pathway are sensitive to inhibition of MEK/ERK signaling [10]. Selumetinib (AZD6244; ARRY-142886) is a potent, selective, orally-available and non-adenosine triphosphate competitive small molecule inhibitor of the mitogen-activated protein (MAP) kinase, MEK-1/2 [11]. Selumetinib inhibits MEK with an IC_{50} of 10–14 nM [12]. Activated MEK phosphorylates its only known substrates, ERK1 and ERK2. Phosphorylated ERK dimerizes and translocates to the nucleus [13], resulting in increased cell proliferation [14].

In a previous phase II trial in unselected NSCLC patients, selumetinib (mix and drink formulation) was compared with pemetrexed in second and third line therapies and found to have similar progression-free survival (PFS) [15]. Preclinical studies demonstrated increased growth inhibition in NSCLC cell lines and xenografts, using the combination of selumetinib with erlotinib regardless of KRAS status in EGFR wild-type tumors [14]. Therefore, we hypothesized that the addition of selumetinib to erlotinib would have a clinical benefit in advanced NSCLC in both second and third line, regardless of KRAS status and that the addition of selumetinib would have the greatest effect on KRAS-mutated patients.

An increasing number of anticancer drugs, including TKIs, have been shown to have immunomodulatory activity [16, 17]. A recent study demonstrated selumetinib can increase MHC-class I expression in papillary thyroid cancer cells [18].

patients and methods

patient population and study design

This was a randomized phase II trial in five US institutions sponsored by the Cancer Therapy Evaluation Program (CTEP), National Cancer Institute (NCI), reviewed and approved by each institutional review board (IRB). All patients provided written informed consent before enrollment and all procedures were carried out in accordance with the Helsinki Declaration.

Eligible patients had histologically proven advanced NSCLC, were greater than 18 years of age, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, adequate organ function and were treated or had refused treatment with a platinum-containing doublet chemotherapy regimen. Treated brain metastases were allowed if not requiring steroid or antiepileptic medications. Exclusion criteria included any uncontrolled disease unrelated to the primary malignancy, greater than two prior systemic treatments and a history of prior EGFR TKI (erlotinib) or an MEK inhibitor. Eligible candidates were verified and registered centrally with the NCI Central Registration Office within 24 h of signing consent. All registered patients underwent computer randomization to a treatment arm based on KRAS status (supplementary Figure S1, available at *Annals of Oncology* online). Patients with wild-type KRAS were randomized to single-agent erlotinib or the combination of erlotinib plus selumetinib. Patients with mutant KRAS were randomized to single-agent selumetinib or the combination of selumetinib plus erlotinib and began treatment within 5 days of randomization. Selumetinib and erlotinib were supplied by CTEP.

molecular analysis

KRAS status was initially carried out at a local Clinical Laboratory Improvement Amendments-certified laboratory. Confirmation testing was required and carried out centrally at the Laboratory of Pathology, NCI on paraffin-embedded tumors using pyrosequencing (PyroMark Q24, Qiagen, Valencia, CA) to detect the most common point mutations in codons 12, 13 and 61. All patients additionally underwent testing for mutations in EGFR in exons 18–21.

treatment

KRAS wild-type patients were randomized to receive either single-agent erlotinib at 150 mg orally o.d. or the combination of erlotinib 100 mg orally o.d. (in the morning) plus selumetinib 150 mg orally o.d. (in the evening), based on results from phase I testing. Patients with KRAS mutations were randomized to receive either single agent selumetinib 75 mg orally twice per day, based on single-agent phase I results [19] or a combination of erlotinib 100 mg orally o.d. plus selumetinib 150 mg orally o.d. Once daily selumetinib would potentially reduce the overlapping side-effects. One cycle of therapy was 28 days, and patients underwent imaging every two cycles.

end points and toxicity assessment

Different end points were chosen in the two cohorts to contain the total number of patients required. The primary end point of the KRAS mutant cohort was ORR and the primary end point of the KRAS wild-type cohort was PFS. Tumor assessment was done according to RECIST 1.1 [20]. Patients were evaluated at week 2, week 4 and then every 28 days afterward. Toxicities were defined by the NCI-Common Terminology Criteria of Adverse Events (CTCAE) version 4.0.

statistical considerations

Two separate statistical considerations were used for the KRAS mutant and the KRAS wild-type cohorts. Details of the statistical considerations are reported in supplementary Materials, available at *Annals of Oncology* online.

correlative studies

Correlative studies were carried out in patients enrolled at the NCI and were optional for the other sites. Details are given in supplementary Materials, available at *Annals of Oncology* online.

results

patient characteristics

From March 2010 to May 2013, 89 patients underwent screening for the study with 79 patients enrolling. Forty-one KRAS mutant patients and 38 KRAS wild-type patients were enrolled and underwent central randomization (supplementary Figure S1, available at *Annals of Oncology* online). Two patients decided to discontinue study medications before their day 28 clinical assessment, secondary to intolerable toxicities and therefore were not included in the clinical assessment of PFS or ORR but are included in OS analysis. The remaining 77 patients were able to complete at least 1 month of therapy. Of the 77 patients, only 70 patients were able to complete two cycles of therapy and able to undergo radiographic assessment. As of November 2013, all patients had come off study and final analysis was conducted in October 2014. The two cohorts had relatively similar patient characteristics (Table 1).

Table 1. Patient demographics and molecular/clinical characteristics

Characteristic	KRAS wild type ($N = 38$)		KRAS mutated ($N = 41$)	
	Erlotinib ($n = 19$)	Erlotinib + selumetinib ($n = 19$)	Selumetinib ($n = 11$)	Erlotinib + selumetinib ($n = 30$)
Sex				
Male	13 (68%)	10 (53%)	4 (36%)	14 (47%)
Female	6 (32%)	9 (47%)	7 (64%)	16 (53%)
Histology				
Lung cancer				
Adenocarcinoma	15 (79%)	15 (79%)	11 (100%)	30 (100%)
Squamous cell	4 (21%)	4 (21%)		
Mutational status				
EGFR				
Mutated (L858R)	1 (5%)	1 (5%)	11 (100%)	30 (100%)
Wild type	18 (95%)	18 (95%)		
KRAS	0 (0%)	0 (0%)		
G12A			0 (0%)	6 (20%)
G12C			4 (36%)	8 (27%)
G12D			1 (9%)	1 (3%)
G12K			1 (9%)	1 (3%)
G12R			1 (9%)	0 (0%)
G12S			0 (0%)	1 (3%)
G12V			4 (36%)	9 (30%)
Q61H			0 (0%)	2 (7%)
Q61L			0 (0%)	2 (7%)
Ethnicity				
White	13 (69%)	14 (74%)	8 (73%)	26 (90%)
African American	4 (21%)	4 (21%)	2 (18%)	3 (5%)
Asian	1 (5%)	0 (0%)	1 (9%)	1 (0%)
Unknown	1 (5%)	1 (5%)	0 (0%)	0 (0%)
Age				
Median age	64	64	64	66
Range	33–84	50–79	50–83	58–82
ECOG performance status				
0	2 (10%)	2 (10%)	1 (10%)	3 (10%)
1	11 (58%)	7 (37%)	5 (45%)	14 (47%)
2	6 (32%)	10 (53%)	5 (45%)	13 (37%)
No. of prior regimens				
1	10 (52%)	8 (42%)	6 (55%)	12 (40%)
2	9 (48%)	11 (58%)	5 (45%)	18 (60%)
Smoking				
Current	0 (0%)	0 (0%)	0 (0%)	9 (30%)
Former	13 (68%)	16 (84%)	11 (100%)	21 (70%)
Never-smoker	6 (32%)	3 (16%)	0 (0%)	0 (0%)

n, number of patients enrolled; EGFR, epidermal growth factor receptor; KRAS, Kirsten Rat Sarcoma Viral Oncogene homolog; ECOG, Eastern Cooperative Oncology Group.

treatment toxicity

The most common treatment-related toxicities in both cohorts (Table 2) were diarrhea and rash, with increased toxicity seen in combination therapy in 87% of patients. Grade 3 and 4 toxicities were also increased in combination therapy, with diarrhea, dehydration and fatigue all occurring in >20% of patients. Three deaths occurred within 30 days of last dose of study drugs, with the cause of death identified as unlikely related to selumetinib (1-pulmonary fibrosis, 1-disease progression and 1-myocardial infarction). Pulmonary fibrosis was felt to be associated with erlotinib.

treatment efficacy in mutant KRAS cohort

Of the 41 patients with mutant KRAS analyzed for efficacy, 39 were assessable for response and completed at least two cycles of therapy and underwent restaging CT post cycle 2 (Table 3 and supplementary Figure S2, available at *Annals of Oncology* online).

Eleven patients received selumetinib monotherapy with nine patients being evaluable for response. No responses were observed [95% confidence interval (CI) 0.0% to 33.6%] and this arm closed per the trial design. Eight of the nine patients (89%) [95% CI 51.8% to 99.7%] achieved disease stabilization. The selumetinib

Table 2. Common toxicities occurring >10% of the time ($N = 79$)

Grade	Selumetinib ($n = 11$)			Erlotinib ($n = 19$)			Selumetinib + erlotinib ($n = 49$)		
	All (%)	3 (%)	4 (%)	All (%)	3 (%)	4 (%)	All (%)	3 (%)	4 (%)
Diarrhea	36	11	0	42	11	0	87	37	0
Dehydration	0	0	0	0	0	0	32	22	0
Rash	36	11	0	42	0	0	67	13	0
Fatigue	22	11	0	16	5	0	51	20	0
Dizziness	0	0	0	0	0	0	26	0	0
Blurred vision	0	0	0	0	0	0	20	0	0
Headache	0	0	0	16	0	0	34	0	0
CPK (elevations)	45	11	0	5	0	0	31	8	2
Creatinine (increase)	11	0	0	18	0	0	27	0	0
Myalgia/myositis	0	0	0	11	0	0	10	2	0
Anemia	9	0	0	42	0	0	43	0	0
Lymphocyte (decrease)	44	11	0	18	0	0	47	14	4
AST/ALT elevations	11	0	0	11	0	0	43	16	0
Nausea	47	11	0	27	0	0	55	10	0
Dyspepsia/gastritis	11	0	0	16	0	0	18	0	0
Mucositis	11	0	0	11	0	0	14	6	0
Dysgeusia	9	0	0	5	0	0	24	0	0
Edema	54	0	0	10	0	0	38	2	0
Heart failure	9	9	0	0	0	0	2	0	0
Hypertension	0	0	0	16	0	0	18	0	0
Dyspnea	0	0	0	11	5	5	8	8	0
Cough	9	9	0	0	0	0	4	0	0
Wheezing	9	0	0	0	0	0	10	0	0
Grade	5			5			5		
Pulmonary fibrosis	0			1			0		
Myocardial infarction	0			0			1		
No deaths were related to selumetinib									

n, number of patients enrolled; CPK, creatinine phosphokinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

monotherapy arm had a median PFS of 4.0 months [95% CI 2.9–7.8 months] and a median OS of 10.5 months [95% CI 5.7 months to undefined]. The median number of cycles in this small cohort was 7.

Thirty patients were enrolled on combination therapy and all were assessable for response. Three patients achieved a partial response (10%) (95% CI 2.1% to 26.3%) and disease control was obtained in 13/30 (43%) (95% CI 25.5% to 52.6%). The median PFS was 2.3 months (95% CI 2.0–4.6 months) and the median OS was 21.8 months (95% CI 5.7 months to undefined). The median number of cycles was 8.

treatment efficacy in wild-type KRAS cohort

Thirty-six of the 38 patients enrolled on the KRAS wild-type cohort completed at least two cycles (Table 3 and supplementary Figure S2, available at *Annals of Oncology* online). Of the 19 patients analyzed for treatment efficacy in the erlotinib monotherapy arm, one had a partial response (5%) (95% CI 0.0% to 26.0%) and disease control was achieved in 9/19 (47%) (95% CI 24.4% to 71.1%). The median number of cycles was 3. Erlotinib demonstrated a median PFS of 2.4 months (95% CI 1.3 to 3.7 months) and a median OS of 6.3 months (95% CI 2.6 to 19.5 months).

Of the 19 patients enrolled on the combination arm, only 17 were underwent re-imaging at 2 months. Two partial responses were seen (11.7%) (95% CI 1.5% to 36.4%) of which one later was confirmed to have an activating EGFR mutation. The disease control fraction was 6/17 (35.3%) (95% CI 14.2% to 61.7%). The median PFS was 2.1 months (95% CI 1.8–5.1 months) and the median OS was 12.9 months (95% CI 3.5–25.4 months).

molecular analysis

Part of the planned analysis was to compare patients with KRAS mutations to patients with KRAS wild-type (supplementary Figure S2, available at *Annals of Oncology* online). The overall median PFS for patients enrolled in the KRAS wild-type study was 2.2 months (95% CI 1.8–3.7 months) and in the KRAS mutated study it was 3.7 months (95% CI 2.2–4.6 months) ($P = 0.48$). The median OS for patients enrolled in the KRAS wild-type study was 8.1 months (95% CI 3.5–22.2 months) and in the KRAS mutated study it was 17.0 months (95% CI 7.8 months to undefined) ($P = 0.16$).

correlative studies

Biomarker analysis was conducted on a total of 31 patients (4 patients, selumetinib monotherapy; 27 patients, combination therapy) (Figure 1). Overall, patients who received selumetinib showed a significant reduction in p-ERK both at C1D2 and C1D14. The percent Tregs also decreased after treatment at

Table 3. Summary of the results

	KRAS wild type			KRAS mutated		
	Erlotinib ($N = 19$)	Erlotinib plus selumetinib ($N = 19$[a])	P value (log-rank)	Selumetinib ($N = 11$[b])	Selumetinib plus erlotinib ($N = 30$)	P value (log-rank)
Objective response rate	1 (5%) (95% CI 0.0% to 26.0%)	2 (12%) (95% CI 1.5% to 36.4%)		0 (0%) (95% CI 0.0% to 33.6%)	3 (10%) (95% CI 2.1% to 26.3%)	
Disease control fraction	9 (47%) (95% CI 24.4% to 71.9%)	6 (35%) (95% CI 14.2% to 61.7%)		8 (89%) (95% CI 51.8% to 99.7%)	13 (43%) (95% CI 25.5% to 62.6%)	
Progression-free survival (months)	2.4 (95% CI 1.3–3.7)	2.1 (95% CI 1.8–5.1)	0.75	4.0 (95% CI 2.9–7.8)	2.3 (95% CI 2.0–4.6)	0.24
Overall survival (months)	6.3 (95% CI 2.6–19.5)	12.9 (95% CI 3.5–25.4)	0.51	10.5 (95% CI 5.7 to undef.)	21.8 (95% CI 5.7 to undef.)	0.81
Median follow-up for the 77 patients was 21.8 months						

Results displayed are median.
[a]Seventeen patients were assessable for clinical response.
[b]Nine patients were assessable for clinical response.
CI, confidence interval; undef., undefined.

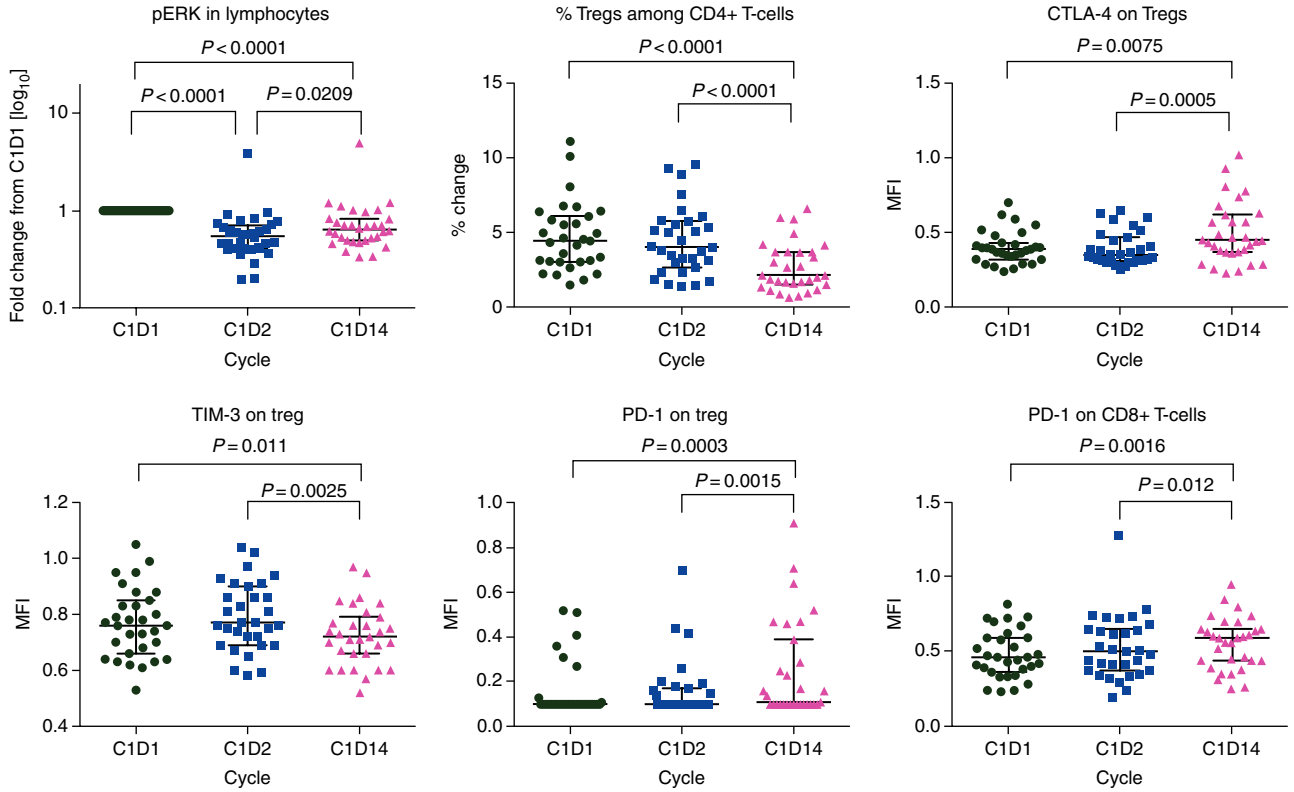

Figure 1. Changes in p-ERK, Tregs, CTLA-4, TIM-3 and PD-1 with selumetinib. (A) Phosphorylated extracellular signal-regulated kinases in circulating mononuclear lymphocytes; (B) Change in the % Tregs in CD4; (C) Change in CTLA-4 expression on Tregs; (D) Change in TIM-3 on Tregs; (E) Change in PD-1 expression on Tregs; (F) Change in PD-1 expression on CD8+ T cells.

C1D14 ($P < 0.0001$), with an increase of CTLA-4 on Tregs ($P = 0.0067$ for C1D1 to C1D14 and $P = 0.0002$ for C1D2 to C1D14). Similarly, Tim-3 on Tregs decreased at C1D14 ($P = 0.01$) and programmed cell death-1 (PD-1) on Tregs increased after treatment ($P = 0.0004$). Additionally, PD-1 on CD8+ T cells increased at C1D14 ($P = 0.0012$).

Results of additional correlative studies are presented in supplementary Materials, available at *Annals of Oncology* online. Patients with a lower PD-1 level than median on Tregs at baseline and C1D2 had improved PFS (baseline, $P = 0.0004$, C1D2 $P = 0.037$) and OS (baseline, $P = 0.024$, C1D2 $P = 0.068$) compared with those with a higher than median PD-1 (supplementary Figure S3, available at *Annals of Oncology* online). Patients with a lower Tim-3 level than median on CD8+ T cells at baseline had improved OS compared with those with a higher than median Tim-3 ($P = 0.044$).

The percent Th17 cells among CD4+ T cells did not change after treatment. A higher percent of Th17 cells among CD4+ T cells (>0.14%, median value) at baseline was associated with partial response ($P = 0.011$) (supplementary Figure S4, available at *Annals of Oncology* online). The Th17/Treg ratio increased at C1D14 compared with baseline ($P = 0.0002$). The percent of Th1 cells among CD4+ T cells increased after treatment ($P = 0.0002$). The Th1/Th2 ratio significantly increased at C1D14 ($P = 0.0012$). Patients with an increase in the percent of Th17 cells at C1D14 had improved PFS compared with those with a decrease or no change ($P = 0.028$).

discussion

Significant improvements in the outcome of advanced NSCLC have resulted from a better understanding of the molecular drivers in the adenocarcinoma subtype [21–24]. KRAS mutations are predictive of lack of response to EGFR TKIs [25], and could potentially be targeted through inhibition of its downstream kinase MEK. In our study, patients with KRAS mutations received the MEK inhibitor, selumetinib. Patients on this cohort were also the only patients to receive twice daily inhibition, the recommended dosing for phase II trials. However, no responses were seen in the selumetinib alone cohort. The lack of response in this subgroup was disappointing, but similar to another study that we recently reported [26].

The combination therapy of selumetinib and erlotinib was hypothesized to potentially improve clinical efficacy in both KRAS mutant patients and KRAS wild-type patients by a more profound blockade of the RAS/RAF/MEK/ERK signaling pathway. Owing to overlapping toxicities, MEK inhibition was only given once daily; however, significant toxicities still occurred. Both cohorts had confirmed partial responses regardless of KRAS status; however, the combination therapy failed to show improvement in PFS or ORR. The lack of efficacy of dual inhibition of the EGFR and MEK components may be secondary to the potential increased activation of the PI3K/AKT pathway [27] or induced MEK reactivation by CRAF [28]. Interestingly, recently another MEK inhibitor, trametinib, in combination with the BRAF inhibitor dabrafenib was shown to induce a response rate of 63% in BRAFV600E-mutated NSCLC patients, better than with dabrafenib alone, with manageable toxicity [29]. These results parallel what has been reported in advanced melanoma and support the continued study of MEK inhibitors in NSCLC.

Emerging evidence has revealed the importance of the interplay between anticancer chemotherapies and the host immune system [16]. Studies have revealed that lung cancer may be promoted by cytokine imbalance and Th17/Treg imbalance [30]. Additionally, the Th17/Treg ratio negatively correlates with the TNM stages of lung cancer, and targeting the Th17/Treg balance for therapeutic purposes may represent a useful tool for treatment [30]. In our study, the higher Th17 at baseline and an increase of Th17 were correlated with response and improved PFS, respectively. The Th17/Treg ratio in peripheral blood was significantly increased after therapy suggesting Th17 cells in NSCLC warrant further investigation. We also examined the pharmacodynamic impact of therapy on Tregs, CD8+ T cells and Th cell subsets and these assessments suggest that antitumor activity of selumetinib as an MEK inhibitor may be limited by an off-target effect of increasing immune checkpoint receptors on Tregs and CD8+ T cells. Thus, combination therapy with immune checkpoint blockade and selumetinib might provide a synergistic effect.

In conclusion, further study of the combination of selumetinib with erlotinib is not warranted in NSCLC, because it induced significant toxicities in 87% of patients with grade 3 and 4 occurring in over 20% of patients and did not result in improvement of ORR, PFS or OS when compared with single-agent therapy. Selumetinib therapy does demonstrate changes in the immune system regulatory balance. The exploratory analysis does provide interesting hypotheses for exploiting this effect of selumetinib or potentially more potent MEK inhibitors with immune checkpoint inhibitors.

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disclosure

The authors have declared no conflicts of interest.

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Does Gleason score at initial diagnosis predict efficacy of abiraterone acetate therapy in patients with metastatic castration-resistant prostate cancer? An analysis of abiraterone acetate phase III trials

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Background: The usefulness of Gleason score (<8 or ≥8) at initial diagnosis as a predictive marker of response to abiraterone acetate (AA) plus prednisone in patients with metastatic castration-resistant prostate cancer (mCRPC) was explored retrospectively.

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