original report

Impact of *KRAS* and *TP53* Co-Mutations on Outcomes After First-Line Systemic Therapy Among Patients With *STK11*-Mutated Advanced Non–Small-Cell Lung Cancer

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abstract

PURPOSE The *STK11* gene encodes a serine/threonine protein kinase that regulates cell polarity and functions as a tumor suppressor. Patients with non–small-cell lung cancer (NSCLC) and *STK11* mutations often have other co-mutations. We evaluated the impact of *KRAS* and *TP53* co-mutations on outcomes after first-line systemic therapy for patients with metastatic or recurrent NSCLC that harbors *STK11* mutations.

METHODS We conducted a retrospective review of patients with metastatic NSCLC and *STK11* mutations treated at the University of Pennsylvania. *STK11* mutations were identified through next-generation sequencing (NGS) in tissue or plasma. Cox proportional hazard models were used to determine the relationship between *STK11* co-mutations and survival outcomes. The Kaplan-Meier method was used to estimate overall survival (OS) and progression-free survival (PFS).

RESULTS From February 2013 to December 2016, samples from 1,385 patients with NSCLC were analyzed by NGS; of these, 77 patients (6%) harbored an *STK11* mutation (n = 56, tissue; n = 21, plasma). Of the 62 patients included, 18 had an *STK11* mutation alone, 19 had *STK11*/*KRAS*, 18 had *STK11*/*TP53*, and seven had *STK11*/*KRAS*/*TP53*. Patients with *STK11*/*KRAS* co-mutations had a worse median PFS (2.4 months) compared with *STK11* alone (5.1 months; log-rank $P = .048$), *STK11*/*TP53* (4.3 months; log-rank $P = .043$), and *STK11*/*KRAS*/*TP53* (13 months; log-rank $P = .03$). Patients with *STK11*/*KRAS* co-mutation experienced shorter median OS (7.1 months) compared with *STK11* alone (16.1 months; log-rank $P < .001$), *STK11*/*TP53* (28.3 months; log-rank $P < .001$), and *STK11*/*KRAS*/*TP53* (22 months; log-rank $P = .025$).

CONCLUSION Among patients with advanced NSCLC and *STK11* mutations treated with first-line systemic therapy, co-mutation with *KRAS* was associated with significantly worse PFS and OS. By contrast, co-mutation of *STK11* with *TP53* conferred a better prognosis.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the United States, and non–small-cell lung cancer (NSCLC) represents 80% to 85% of all lung cancer.[1] In nonsquamous NSCLC, it is routine practice to test for genetic abnormalities using comprehensive next-generation sequencing (NGS). The majority of the mutations found during routine testing are not actionable currently, but their presence likely has predictive and prognostic relevance.

STK11, also known as liver kinase B1 (LKB1), is a tumor suppressor and a negative regulator of mammalian target for rapamycin signaling. Loss-of-function mutations in germline *STK11* are associated with Peutz-Jeghers hereditary cancer syndrome. *STK11* mutations are estimated to be present in 8% to 39% of all NSCLC, with increased prevalence in smokers and patients with *KRAS* mutations.[2,3] Animal studies suggest that *STK11* mutations are critical in lung cancer differentiation, tumorigenesis, and metastasis.[4,5] Mutations in *STK11* have emerged as a potential prognostic and predictive marker in NSCLC. Somatic mutations in *STK11* have been hypothesized as primarily oncogenic through loss of function, although gain-of-function alterations through mutations in exons 1 to 2 ($STK11_{ex1\text{-}2}$) have also been described.[6] In a report by Pécuchet et al,[6] $STK11_{ex1\text{-}2}$ mutations conferred significantly worse progression-free survival (PFS) and overall survival (OS) compared with mutations in exons 3 through 9 ($STK11_{ex3\text{-}9}$) among patients undergoing curative intent surgery for

ASSOCIATED CONTENT

Appendix

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

How do *KRAS* and *TP53* co-mutations affect outcomes after first-line systemic therapy in patients with non–small-cell lung cancer and *STK11* mutations?

Knowledge Generated

Among patients with metastatic NSCLC and tumor-associated *STK11* mutations, co-mutation with *TP53* conferred better progression-free survival (PFS) and overall survival (OS) after first-line therapy compared with patients who had a *KRAS* co-mutation. *TP53* mutation in the presence of an *STK11*/*KRAS* co-mutation also conferred better PFS and OS compared with patients who had only the *STK11*/*KRAS* co-mutation.

Relevance

STK11/*KRAS* co-mutation has been associated with worse PFS after chemotherapy, but co-mutation with *TP53* may modulate outcomes after first-line chemotherapy in this group and among patients with *STK11* mutations without *KRAS* mutations.

NSCLC, which suggests that *STK11* mutations may be a more heterogeneous group than previously thought.[6,7]

Co-mutation status may be another source of heterogeneity among patients with *STK11* mutations. *KRAS* is frequently co-mutated with *STK11*, but the predictive and prognostic significance of this co-mutation is uncertain. In *KRAS*-mutant mice, co-mutation with *STK11* was associated with resistance to anticancer therapy, whereas co-mutation with *TP53* was not.[8] In humans, *STK11* mutation alone does not appear to be predictive of response to chemotherapy, whereas *KRAS*/*STK11* co-mutation has been associated with worse PFS after chemotherapy.[3,9-11] *KRAS*/*STK11* co-mutation also is associated with inferior PFS and OS after immunotherapy compared with *KRAS* alone (PFS hazard ratio [HR], 1.98; $P < .001$) or *KRAS*/*TP53* co-mutation (PFS HR, 1.77; $P = .0072$; OS duration, 6.4 *v* 16.1 *v* 16 months for *KRAS*/*STK11* v *KRAS* v *KRAS*/*TP53*, respectively).[12]

Co-mutation with *TP53* and *STK11* is less common than *KRAS*/*STK11* mutation but still may represent a distinct molecular subtype of NSCLC.[11] TP53 is a DNA binding transcription factor that regulates multiple genes involved in DNA repair, metabolism, cell cycle arrest, apoptosis, and senescence.[13,14] Gene expression studies have shown that, although *KRAS*, *STK11*, and *KRAS*/*STK11* groups share a *KRAS*-mutant gene signature, the *TP53* mutant group does not.[11] NSCLC cell lines that harbor *KRAS*/*TP53* also have a different drug sensitivity profile compared with *KRAS*/*STK11* or *KRAS*/*TP53*/*STK11* cell lines.[10] In addition, TP53 is a known regulator of *STK11* and has four potential binding sites in the *STK11* promoter.[13,15] These findings highlight the differential and context-dependent effects of a *TP53* mutation and its potential interactions with *STK11* and *KRAS*.

In this study, we evaluated patients with *STK11*-mutant NSCLC and the effect of concurrent mutations in *KRAS* and *TP53* on treatment outcomes after first-line systemic for metastatic/recurrent disease.

METHODS

Patient Population

This was a retrospective study among patients with NSCLC diagnosed and treated at the University of Pennsylvania Abramson Cancer Center between February, 2013—when NGS testing, including for *STK11*, was first performed routinely on all patients with stage IV disease—and December, 2016. Eligible patients for this study had histologically confirmed stage IV NSCLC and had NGS performed on tissue or plasma as part of routine clinical testing. Patients who received treatment outside the institution or had another concurrent malignancy were excluded.

Mutational Analysis

Plasma was analyzed by Guardant Health (Redwood City, CA) as described previously.[16] Solid tumor sequencing was performed at the Center for Personalized Diagnostics clinical laboratory at the University of Pennsylvania (Data Supplement). One *KRAS* amplification; one *KRAS* variant of unknown significance (VUS), Q61H; and one *TP53* VUS (A161S) were not considered mutations in the respective genes. *STK11* mutations were categorized as disease associated on the basis of the designation in the NGS report (ie, disease associated *v* VUS). Mutations were categorized using OncoPrinter by cBioPortal.[17,18]

Clinical Data

The following information was collected from the electronic medical record: age at diagnosis, sex, race/ethnicity, smoking status, Eastern Cooperative Oncology Group performance status at diagnosis, stage at diagnosis (TNM, according to American Joint Committee on Cancer, 7th edition, guidelines), histology, method of diagnosis, date of diagnosis, treatment (first-, second-, and third-line therapies, and chemotherapy *v* immunotherapy), and outcomes (date of progression, death, or last follow-up). All information was collected with approval from the institutional review board; informed consent was waived because of the retrospective, nontherapeutic nature of the study.

Statistical Analysis

Descriptive statistics, including mean, median, and proportions, were used to summarize patient demographics and tumor characteristics. PFS was calculated from the start of treatment of metastatic or recurrent disease to date of death or progression. The date of progression was based on radiologic progression, treatment change, or clinical deterioration that led to discontinuation of therapy, as documented in the electronic medical record. OS was calculated from the start of systemic treatment of metastatic or recurrent disease to the date of death or last follow-up. Patient data were censored at the last follow-up visit or on September 1, 2017, if still alive.

χ^2 and Kruskal-Wallis analyses were used to assess differences in baseline characteristics between the mutation groups for categoric and continuous variables, respectively. Cox proportional hazard models were used to determine the relationship of *STK11* co-mutations to survival. Kaplan-Meier method was used to estimate OS and PFS, and comparisons between groups were made using the log-rank test. The multivariable Cox regression models were selected by stepwise forward selection, and $P < .2$ was used for initial inclusion. Candidate models were refined using the likelihood ratio test for individual variables. Given the small sample size, a model with fewer covariables was selected if additional variables did not significantly change the model. The effect of co-mutation status on PFS and OS was investigated by looking at four mutation groups (*STK11* alone, *STK11/KRAS*, *STK11/TP53,* and *STK11/KRAS/TP53*) separately as well as at individual mutation effects and interactions in a Cox regression model. HRs from the Cox model were reported.

RESULTS

Baseline Characteristics

During a 42-month period, 1,385 unique patients had sequencing of a lung neoplasm in either tissue (n = 1,526 samples) or plasma (n = 245 samples). A total of 77 patients (6%) harbored an *STK11* mutation (n = 56, tissue; n = 21, plasma). Fifteen patients were excluded (Fig 1). The majority (51 of 62, or 82%) of patients received platinum doublet–based therapy as the first-line regimen (Table 1). Nine patients had a driver mutation and received targeted therapy at some point during treatment (Data Supplement). Five patients received immunotherapy as first-line systemic therapy (Data Supplement). Among the 62 included patients, 44 had tissue NGS, and 18 had plasma NGS testing (Fig 1). The baseline characteristics were well balanced among these co-mutation groups, except that patients in the *STK11* alone or *STK11/KRAS/TP53* group were slightly older ($P = .015$; Table 1). There was no statistically significant difference in the proportion of *KRAS* or *TP53* alterations detected by tissue versus plasma testing (Pearson's χ^2 test; $P = .34$ and $P = .51$, respectively).

Mutation Characteristics

A total of 46 (74%) of 62 *STK11* mutants were confirmed as disease associated (DA-*STK11*), as defined by the sequencing report. $STK11_{ex1\text{-}2}$ mutations were found in 22

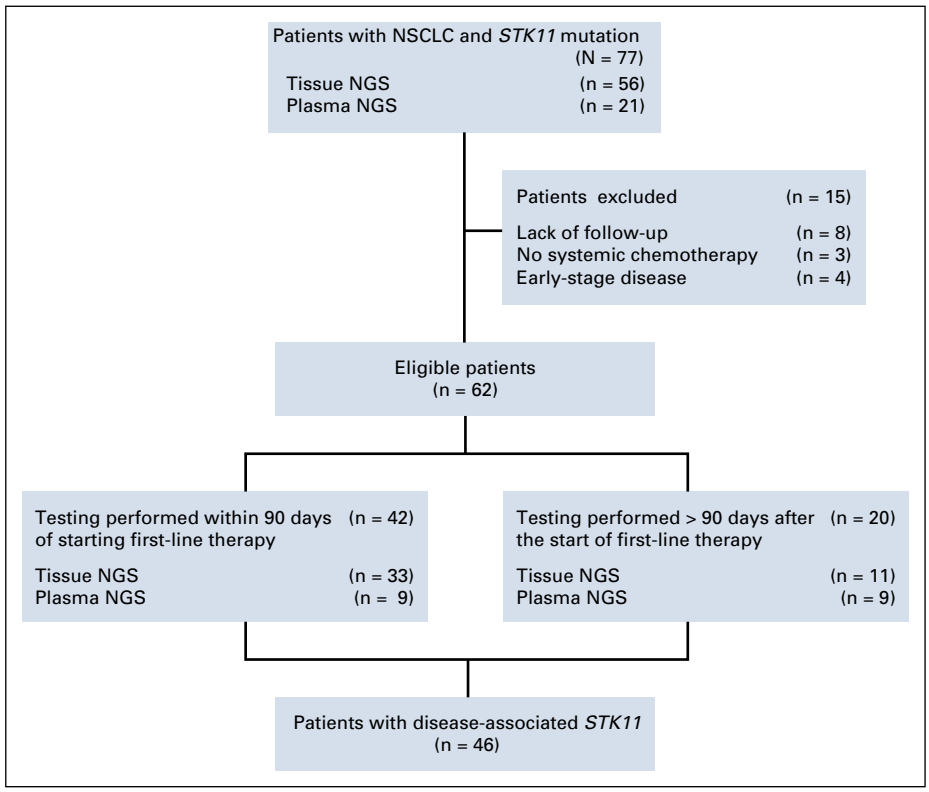

FIG 1. Flowchart of the study cohort. NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer.

TABLE 1. Baseline Characteristics of Patients With *STK11* Mutations by Co-Mutation Status

Characteristic	No. (%) of Patients				P
	***STK11* Alone** (n = 18)	***STK11/KRAS*** (n = 19)	***STK11/TP53*** (n = 18)	***STK11/KRAS/TP53*** (n = 7)	
Sex					
Female	6 (33.3)	10 (52.6)	8 (44.4)	5 (71.4%)	.345
Age at diagnosis, years					
Median (SD)	69.83 (9.1)	64.84 (6.8)	60.39 (9.5)	69.29 (7.3)	.015*
Source					
Tissue	8 (44.4)	18 (94.7)	14 (77.8)	4 (57.1%)	.006
Plasma	10 (55.6)	1 (5.3)	4 (22.2)	3 (42.9%)	
Stage at diagnosis					
I-III	6 (33.3)	7 (37)	8 (44.4)	2 (28.6%)	.863
IV	12 (66.7)	12 (63)	10 (55.6)	5 (71.4%)	
Status at enrollment					
Metastatic at diagnosis	12 (66.7)	11 (57.9)	11 (61.1)	5 (71.4)	.44
Metastatic recurrence of early stage disease	4 (22.2)	8 (42.1)	4 (22.2)	2 (28.6)	
Local recurrence requiring systemic treatment	2 (11.1)	0 (0.0)	3 (16.7)	0 (0.0)	
Histology					
Squamous	1 (5.6)	2 (10.5)	1 (5.6)	0 (0.0)	.753
Nonsquamous	17 (94.4)	17 (89.5)	17 (94.4)	7 (100.0)	
Performance status					
0	7 (38.9)	7 (38.9)	8 (44.4)	1 (20.0)	.882
1	7 (38.9)	9 (47.4)	7 (38.9)	4 (80.0)	
2-3	4(22.2)	3 (15.8)	3 (16.7)	2 (28.6)	
Smoking status†					
≤ 10 pack years	2 (11.1)	1 (5.3)	0	0	.017
10-40 pack years	10 (55.6)	11 (57.9)	6 (33.3)	1 (14.3)	
≥ 40 pack years	2 (11.1)	6 (31.6)	11 (61.1)	5 (71.4)	
First-line systemic therapy‡					
Chemotherapy	16 (88.9)	16 (84.2)	13 (72.2)	6 (85.7)	.757
Targeted therapy§	1 (5.6)	2 (10.5)	2 (11.1)	1 (14.3)	
Immunotherapy‖	1 (5.6)	1 (5.3)	3 (16.7)	0 (0.0)	

Abbreviation: SD, standard deviation.
*Kwallis *P* value used for continuous variables.
†Never smokers, n = 3; pack-years are missing for four patients.
‡Systemic therapy received as first line metastatic or recurrent disease.
§Targeted therapies included erlotinib (n = 3), crizotinib (n = 2), afatinib (n = 1).
‖Immunotherapy included pembrolizumab (n = 2), nivolumab (n = 2), and atezolizumab (n = 1).

patients, and 40 patients had $STK11_{ex3\text{-}9}$ mutations (Data Supplement). The most common *STK11* mutation was p.L282Afs*3, which resulted in a frameshift mutation in exon 6 (Fig 2).

There was no correlation between the position of the *STK11* mutation and co-mutation status. Among the 22 $STK11_{ex1\text{-}2}$ mutations identified, 17 had additional co-mutations (n = 8, *KRAS*; n = 6, *TP53*; n = 3, *KRAS/TP53*). There was also no correlation between $STK11_{ex1\text{-}2}$ or $STK11_{ex3\text{-}9}$ and the presence of a *KRAS* or *TP53* mutation (χ^2 *P* = .34 and *P* = .637, respectively). *KRAS* alterations occurred at codon positions 12, 13, 22, and 61, and each was considered disease associated by pathology report using publicly available databases. The most frequent mutation seen in *TP53* was a missense alteration of R158L or P (Fig 2).

Prognostic Relevance of Co-Mutation Status Among Patients With *STK11* Mutations

Patients with *STK11/KRAS* co-mutations had a worse median PFS (2.4 months) compared with *STK11* alone (5.1

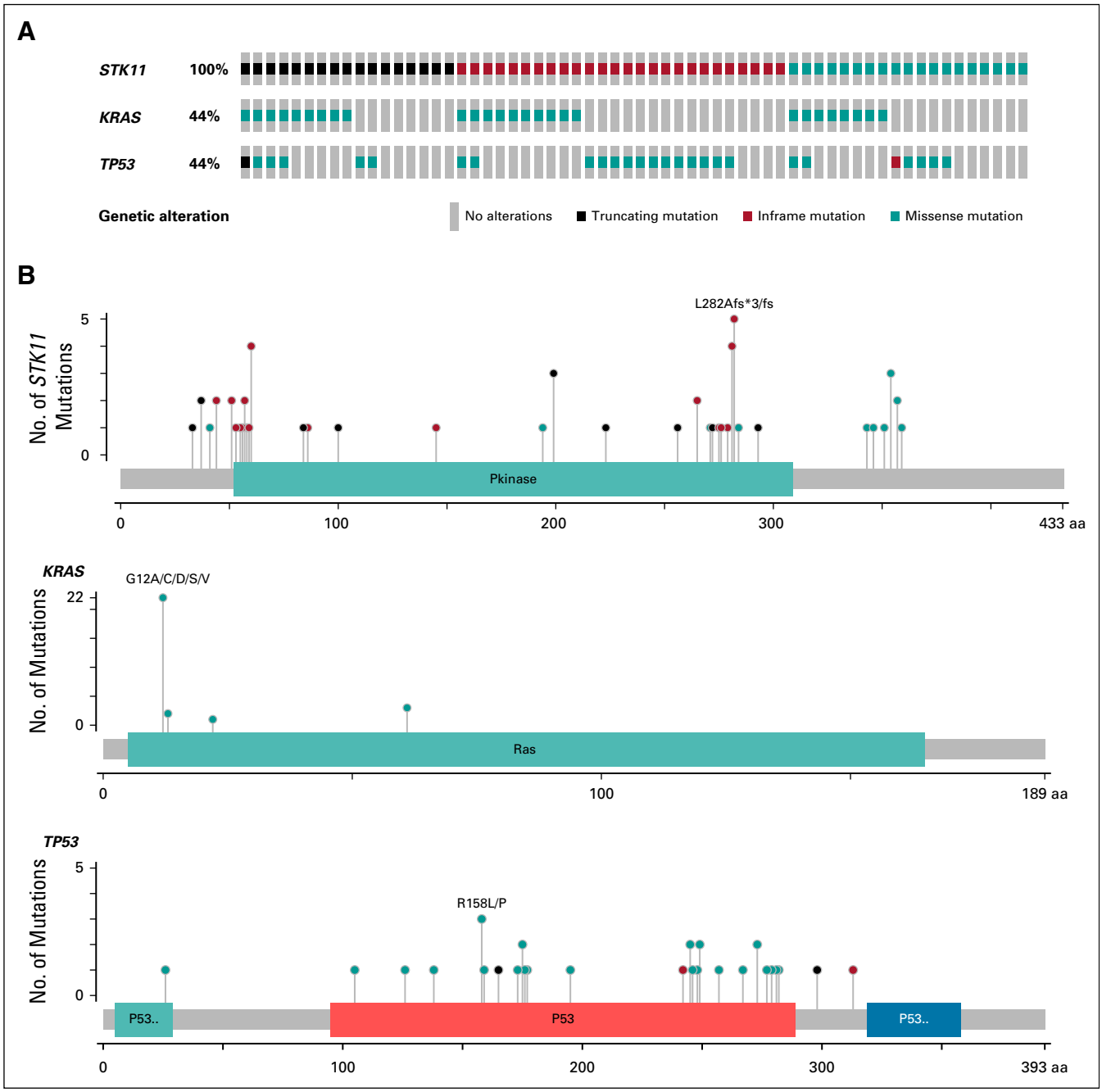

FIG 2. Distribution of *STK11*, *KRAS* and *TP53* mutations. (A) Columns represent individual patients with mutation type specified by color; missense mutations in *STK11* were found in six patients, but specific point mutations were not identified. Five missense mutations were in splice sites, and one was a deletion in exon 5. (B) Lollipop plots mapping specific mutation location (x-axis) and frequency (y-axis) for *STK11*, *KRAS* and *TP53*. aa, amino acids.

months; log-rank $P = .048$), *STK11*/*TP53* (4.3 months; log-rank $P = .043$), and *STK11*/*KRAS*/*TP53* (13 months; log-rank $P = .03$; Table 2; Fig 3A). In an unadjusted, univariable Cox proportional hazards model of PFS, male sex was the only factor independently associated with an increased risk of progression (HR, 1.82; 95% CI, 1.04 to 3.17; $P = .035$; Tables 3 and 4). This effect persisted in the multivariable model after the analysis was controlled for DA-*STK11* mutations, *KRAS* mutations, and *TP53* mutations (HR, 2.08; 95% CI, 1.15 to 3.78; $P = .016$). In the multivariable model, the interactions between DA-*STK11* and *KRAS* or DA-*STK11* and *TP53* were not statistically significant. However, when the interaction between DA-*STK11* and *KRAS* was included in the model, there was an increased risk of progression among patients with *KRAS*/DA-*STK11* mutations compared with the *KRAS*/non–DA-*STK11* group (HR, 2.03; 95% CI, 1.05 to 3.92; $P = .035$; Tables 3 and 4). There was no change in risk of progression among

TABLE 2. Median PFS and OS for *STK11* Co-Mutation Groups

	PFS (months)		OS (months)	
Mutation Group	**Median**	***P***	**Median**	***P***
STK11 alone (n = 18)	5.1	.048	16.1	.004
STK11/KRAS (n = 19)	2.4	—	7.1	—
STK11/TP53 (n = 18)	4.3	.043	28.3	< .001
STK11/KRAS/TP53 (n = 7)	13	.03	22	.025

NOTE. Median progression-free survival (PFS) and median overall survival (OS) for co-mutation groups among patients with *STK11* mutation (all mutations reported) were determined using Kaplan-Meier methodology. Using the log-rank test, the *STK11/KRAS* group experienced worse median PFS and median OS. All *P* values are compared with *STK11/KRAS*.

patients with *TP53*/DA-*STK11* compared with *TP53*/non–DA-*STK11*.

Patients with *STK11/KRAS* mutations experienced shorter median OS (7.1 months) compared with *STK11* alone (16.1 months; log-rank $P < .001$), *STK11/TP53* (28.3 months; log-rank $P < .001$), and *STK11/KRAS/TP53* (22 months; log-rank $P = .025$; Table 2; Fig 3C). Male sex conferred an increased risk of death in the univariable and multivariable models of OS (Tables 3 and 4). *KRAS* mutations were associated with an increased risk of death in the univariable model (HR, 2.46; 95% CI, 1.4 to 4.5; $P = .003$) but not in the multivariable model (Tables 3 and 4). *TP53* mutation was associated with a decreased risk of death in the univariable analysis (HR, 0.48; 95% CI, 0.25 to 0.91; P = .025) but not in the multivariable analysis. As was the case with PFS, the interaction between KRAS and DA-STK11 was not significant on its own for OS, but patients who had a KRAS mutation and a DA-STK11 mutation had an increased risk of death compared with patients who had a KRAS mutation and a non–DA-S*TK11* mutation (HR, 2.18; 95% CI, 1.08 to 4.4; $P = .031$). Interestingly, we did not find that $STK11_{ex1\text{-}2}$ mutations were associated with an increased risk of progression or death compared with $STK11_{ex3\text{-}9}$ mutations, in

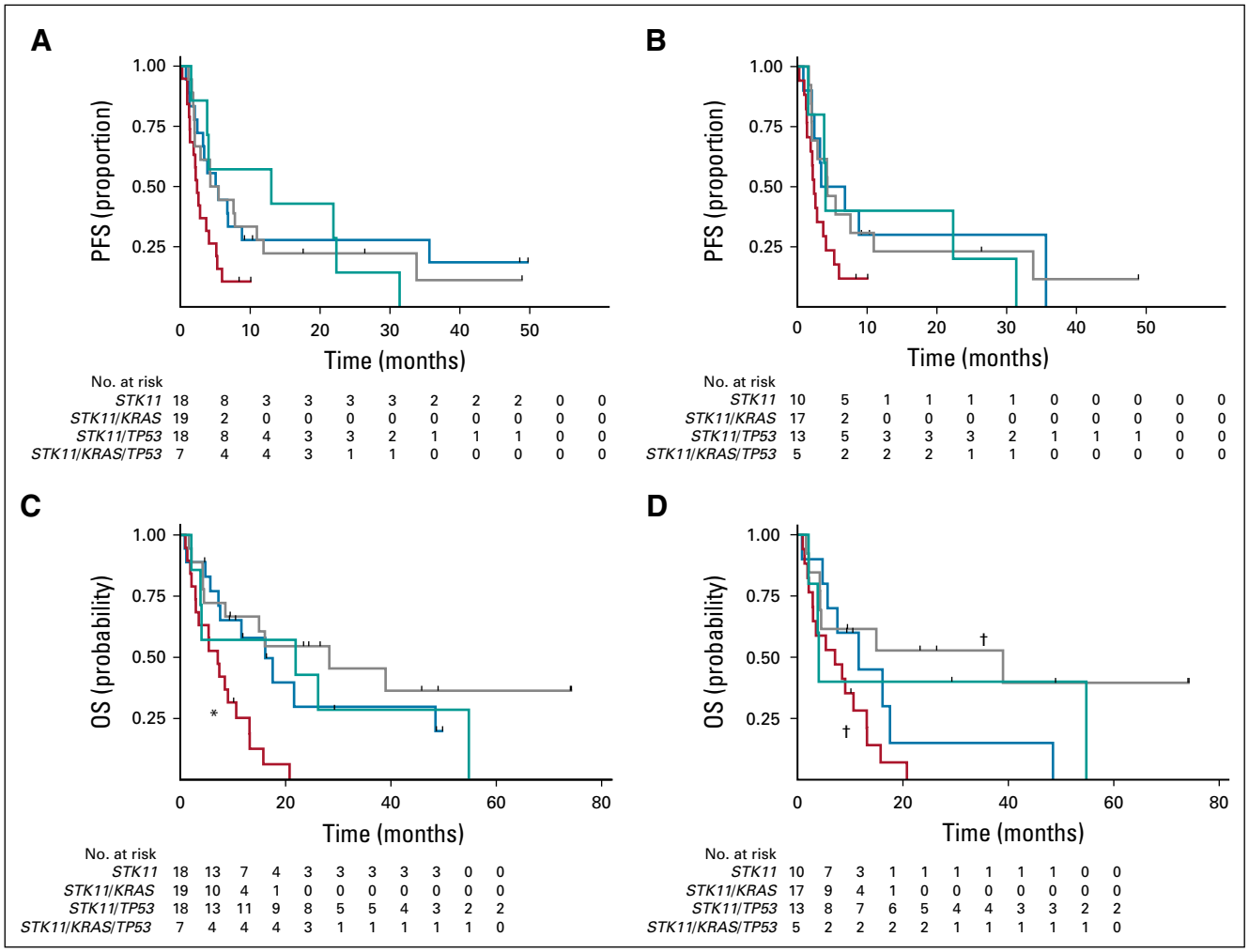

FIG 3. Progression-free survival (PFS) and overall survival (OS) by *STK11* co-mutation status. Kaplan-Meier curves of (A) PFS and (C) OS of patients with stage IV or recurrent disease and tumors with *STK11* mutation. (*) *STK11/KRAS* versus *STK11/KRAS/TP53*, log-rank $P = .03$. Kaplan-Meier curves of (B) PFS and (D) OS of patients with stage IV or recurrent disease and tumors with disease-associated *STK11* mutation. (†) *STK11/KRAS* versus *STK11/TP53*, log-rank $P = .01$.

TABLE 3. Univariable and Multivariable Models of PFS

	PFS					
	Univariable Analysis			Multivariable Model		
Variable	HR	*P*	95% CI	HR	*P*	95% CI
Source (tissue)	1.35	.310	0.76 to 2.41	1.42	.257	0.77 to 2.60
STK11 exon 1/2	1.24	.455	0.7 to 2.19			
STK11 DA	1.46	.237	0.78 to 2.75	1.18	.67	0.55 to 2.56
KRAS	1.66	.073	0.95 to 2.89	1.64	.48	0.42 to 6.44
TP53	0.74	.291	0.42 to 1.3	0.82	.51	0.46 to 1.47
Sex (reference: female)	1.82	.035*	1.04 to 3.17	2.08	.016*	1.15 to 3.78
Smoking pack-years	1.01	.163	0.997 to 1.02			
KRAS + *STK11* interaction†				1.24	.777	0.28 to 5.55
KRAS/*STK11 v KRAS*‡				2.03	.035*	1.05 to 3.92

NOTE. Univariable and multivariable Cox regression models of the effect of mutation status and covariables on the risk of progression (ie, progression-free survival). Reference for *STK11* exon 1/2 is a mutation in *STK11* exon 3-9. Reference for *STK11* DA is non-DA *STK11* mutations. Reference group for *KRAS* and *TP53* is no mutation present in these genes.

Abbreviations: DA, disease associated; HR, hazard ratio; PFS, progression-free survival.

*$P < .05$.

†Multiplicative interaction term in multivariable regression using *STK11* DA.

‡Comparison of *KRAS*/*STK11* DA versus *KRAS* in the multivariable model, including *KRAS*/*STK11* interaction.

contrast to the recent report from Pécuchet et al[6] (Appendix Fig A1). Median PFS and OS did not significantly differ according to the source of NGS (tissue *v* plasma; Tables 3 and 4; Data Supplement). Other co-factors considered in the analysis but found not to be independent predictors in the univariable model or significant contributors to the multivariable model were smoking status, performance status at diagnosis, age at diagnosis, stage at presentation, and race/ethnicity (Tables 3 and 4). Analyses that excluded patients who received immunotherapy or targeted therapy still showed superior outcomes for patients with *STK11*/*TP53* co-mutations, even in the presence of a *KRAS* mutation (Data Supplement).

Relevance of Disease-Associated Variants of *STK11*

As part of an exploratory subset analysis, we repeated the PFS and OS Kaplan-Meier analyses using only the *STK11* mutations characterized as disease associated (DA-*STK11*). When only DA-*STK11* mutations were included, there was no difference in median PFS between co-mutation groups of interest (Fig 3B). There remained a significant difference in median OS when *STK11*/*KRAS* and *STK11*/*TP53* were compared (7.1 months *v* 39 months; log-rank $P = .01$; Fig 3D).

DISCUSSION

In the era of precision medicine, it has become increasingly important to understand the full implications of an ever-increasing quantity of tumor genetic information obtained as part of routine sequencing of NSCLCs. This study includes one of the largest cohorts of patients with *STK11* mutations (n = 62), and to our knowledge it is the only study to specifically evaluate *STK11* co-mutations with *TP53* and *KRAS* and their relationship to response to first-line systemic therapy in patients with metastatic or recurrent disease. The results show that *STK11*/*KRAS* co-mutation is associated with a worse median PFS and OS after front-line chemotherapy compared with patients who had *STK11* mutation alone, whereas patients who had the *STK11*/*TP53* co-mutation had improved outcomes.

We found that, in the context of an *STK11* mutation, *TP53* mutation is associated with better outcomes even in the presence of mutant *KRAS*. The *STK11*/*TP53* and *STK11*/*KRAS*/*TP53* co-mutation groups had superior PFS and OS compared with the *STK11*/*KRAS* group. Of interest, when examined outside the context of *STK11*, *TP53* mutations reportedly have a deleterious effect on OS and response to platinum-based therapy, especially in early-stage disease.[19-22] *TP53* has not previously been found to be predictive or prognostic in the presence of a *KRAS* mutation.[9,10]

Mutations in the TP53 binding sites of the *STK11* promoter have been associated with decreased *STK11* expression in endometrial cancer.[15] In NSCLC, 82% of *TP53* mutations are in the DNA binding region; therefore, a mutation in *TP53* in NSCLC could lead to decreased expression of a deleterious STK11 protein, such as a gain-of-function *STK11* mutation in exons 1 to 2.[6,23] However, we observed a survival benefit for *TP53* co-mutation with *STK11* that was independent of the location of the *STK11* mutation. The significance of *STK11* mutation location and potential interactions with co-mutations are still poorly understood and should be explored in a larger study.

TABLE 4. Univariable and Multivariable Models of OS

	OS					
	Univariable Analysis			Multivariable Model		
Variable	HR	*P*	95% CI	HR	*P*	95% CI
Source (tissue)	1.09	.797	0.56 to 2.12	1.55	.237	0.75 to 3.22
STK11 exon 1/2	1.17	.627	0.62 to 2.19			
STK11 DA	2.02	.062	0.96 to 4.2	1.86	.204	0.72 to 4.8
KRAS	2.46	.003*	1.4 to 4.5	4.14	.060	0.94 to 18.2
TP53	0.48	.025*	0.25 to 0.91	0.6	.173	0.29 to 1.25
Sex	1.87	.048*	1 to 3.5	2.02	.047*	1 to 4
Smoking pack-years	1	.469	0.99 to 1.02			
KRAS + *STK11* interaction†				0.53	.442	0.1 to 2.7
KRAS/*STK11 v. KRAS*‡				2.18	.031*	1.08 to 4.4

NOTE. Univariable and multivariable Cox regression models of the effect of mutation status and covariables on the risk of death (ie, overall survival). Reference for *STK11* exon 1/2 is a mutation in *STK11* exon 3-9. Reference for *STK11* DA is non-DA *STK11* mutations. Reference group for *KRAS* and *TP53* is no mutation present in these genes.

Abbreviations: DA, disease associated; HR, hazard ratio; OS, overall survival.

*$P < .05$.

†Multiplicative interaction term in multivariable regression using *STK11* DA.

‡Comparison of *KRAS*/*STK11* DA versus *KRAS* in the multivariable model, including *KRAS*/*STK11* interaction.

There was no difference in outcomes when patients with $STK11_{ex1\text{-}2}$ and $STK11_{ex3\text{-}9}$ mutations were compared. The proportion of patients with *STK11* mutations in exons 1 and 2 in our cohort was similar to that of the cohort described by Pécuchet et al[6] (35% *v* 25%), who came to a different conclusion. However, the study populations differed slightly. Although some patients in our study had early-stage progression (37.1%), patients with early-stage non-progressive disease and patients who never received any systemic therapy were formally excluded (Fig 1). Therefore, if $STK11_{ex1\text{-}2}$ mutations do confer a higher risk of recurrence after early-stage disease, we would not have been able to identify this risk, given the study design.

In the context of an *STK11* mutation, we found that *KRAS* mutation in the absence of *TP53* co-mutation conferred a significantly worse PFS and OS after first-line systemic therapy for metastatic or recurrent disease. Facchinetti et al[3] also found that *STK11/KRAS* co-mutated tumors had a higher metastatic burden and a trend toward worse OS. This deleterious interaction between *STK11* and *KRAS* may be explained by previous data showing that *STK11* mutations enhance *KRAS* mutation–associated gene expression.[11] In theory, this interaction would lead to augmentation of downstream KRAS signaling driving tumorigenesis. This is also supported by the observation that an acceleration of KRAS-induced tumorigenesis and metastasis has been found in STK11-null mice as well as in humans who lack STK11 expression.[4,15]

In a separate study, Arbour et al[9] found that *KRAS/STK11* co-mutations were associated with shorter OS in univariable analysis but not in multivariable analysis. In their cohort, *STK11* co-mutation status with *KEAP1* or *NFE2L2* could have contributed to a shorter OS.[9] *KEAP1*/*NFE2L2* co-mutation occurred in 63% (60 of 95) of *STK11* mutations in their cohort and was highly correlated with the *KRAS*/*STK11* subgroup in another study.[9,10] They did not report a correlation between *KEAP1*/*NFE2L2* and *TP53* mutations. The tumor and plasma NGS panels reported in our study did not include *KEAP1* or *NFE2L2* mutations, so we were unable to assess the effect of these mutations on outcome.

Detection of *KRAS* and *TP53* mutations via plasma or tissue raises the possibility that the detected mutations may be due to clonal hematopoiesis (CH) in the blood. In another series, five of 33 *TP53* mutations detected by plasma NGS were found in peripheral-blood cells but not in the tumor.[24] The same series reported that most *JAK2*, some *TP53*, and rare *KRAS* mutations detected in cell-free DNA are from CH and not from the tumor. In our cohort, there was no significant difference in the proportion of *TP53* or *KRAS* mutations detected in tumor versus plasma (Table 1). According to the series by Hu et al,[24] it is possible that approximately one of the seven *TP53* mutations detected by plasma testing was from CH; even if true, this small proportion is unlikely to change our results. In addition, CH is associated with worse outcome after therapy, and we report better outcomes with a *TP53* mutation.[25] Therefore, this possible misclassification would bias our result toward the null and imply that the observed association may be stronger than reported.[26]

Our study has additional limitations that must be addressed. First, this analysis is based on a relatively small cohort of patients, and the results must be validated in a larger study. Second, given the retrospective nature of this

study, we used a real-world measurement of PFS defined as time from the start of treatment until radiologic progression, clinical deterioration, death, or change of therapy. This has been shown to be an appropriate surrogate for Response Evaluation Criteria in Solid Tumors (RECIST)–based PFS used in clinical trials.[27]

Many commercially available assays do not disclose how they determine whether an alteration is disease associated; thus, there may be variation among vendors in how they categorize mutations. In addition, studies that look to characterize the prognostic or predictive significance of mutations in a specific gene have used different definitions of mutation (eg, nonsynonymous, pathogenic). We initially used all nonsynonymous mutations in *STK11* but then performed a subset analysis using only disease-associated *STK11* mutations (ie, mutations classified as disease associated or pathogenic on the molecular report). With the disease-associated categorization of *STK11* mutations, there was no longer a difference in median PFS between co-mutation groups. Importantly, there was still a significant difference in median OS between *STK11*/*KRAS* and *STK11*/*TP53*. This analysis is limited by the small sample size—only five patients were in the DA-*STK11*/*KRAS*/*TP53* co-mutation group—and so should be considered exploratory. Future work must be done to standardize how classification of molecular alterations and identification of mutations that influence response to therapy and prognosis.

In summary, this study shows that the co-mutation status of *STK11*-mutated NSCLC contributes to the heterogeneity of this molecular subgroup. The study also highlights the need for a more complete understanding of the biologic interplay that multiple, seemingly unrelated mutations have on prognosis and response to therapy.

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Data analysis and interpretation: Erin Bange, Melina E. Marmarelis, Wei-Ting Hwang, Yu-Xiao Yang, Jeffrey C. Thompson, Jason Rosenbaum, Joshua M. Bauml, Christine Ciunci, Roger B. Cohen, Corey J. Langer, Erica Carpenter, Charu Aggarwal

Manuscript writing: All authors

Final approval of manuscript: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

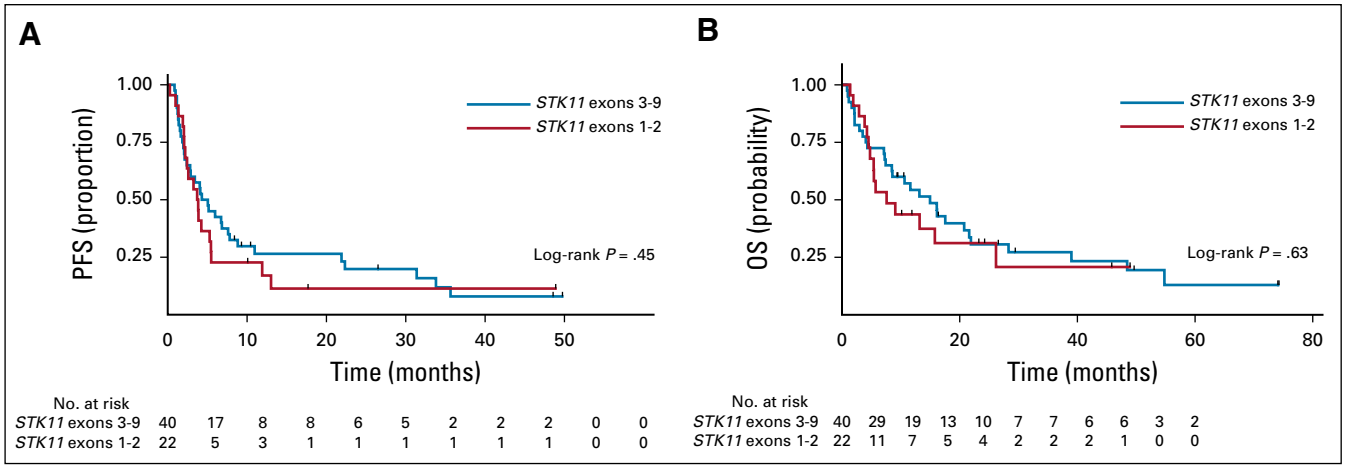

Fig A1. Kaplan-Meier curves of (A) progression-free survival (PFS) and (B) overall survival (OS) of patients with stage IV or recurrent disease and tumors with *STK11* mutations stratified by *STK11* mutation location.