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Impact of *KRAS* mutation subtype and concurrent pathogenic mutations on non-small cell lung cancer outcomes

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ABSTRACT

Objectives: Concurrent genetic mutations are prevalent in *KRAS*-mutant non-small cell lung cancer (NSCLC) and may differentially influence patient outcomes. We sought to characterize the effects of *KRAS* mutation subtypes and concurrent pathogenic mutations on overall survival (OS) and PD-L1 expression, a predictive biomarker for anti-PD-1/PD-L1 immunotherapy.

Materials and methods: We retrospectively identified patients with *KRAS*-mutant NSCLC at a single institution and abstracted clinical, molecular, and pathologic data from electronic health records. Cox regression and multinomial logistic regression were used to determine how *KRAS* mutation subtypes and concurrent pathogenic mutations are associated with OS and tumor PD-L1 expression, respectively.

Results: A total 186 patients were included. Common *KRAS* mutation subtypes included G12C (35%) and G12D (17%). Concurrent pathogenic mutations were identified in *TP53* (39%), *STK11* (12%), *KEAP1* (8%), and *PIK3CA* (4%). On multivariable analysis, *KRAS* G12D mutations were significantly associated with poor OS (hazard ratio [HR] 2.43, 95% confidence interval [CI] 1.15–5.16; $P = 0.021$), as were *STK11* co-mutations (HR 2.95, 95% CI 1.27–6.88; $P = 0.012$). Compared to no (< 1%) PD-L1 expression, *KRAS* G12C mutations were significantly associated with positive yet low (1–49%) PD-L1 expression (odds ratio [OR] 4.94, 95% CI 1.07–22.85; $P = 0.041$), and *TP53* co-mutations with high (≥50%) PD-L1 expression (OR 6.36, 95% CI 1.84–22.02; $P = 0.004$).

Conclusion: *KRAS* G12D and *STK11* mutations confer poor prognoses for patients with *KRAS*-mutant NSCLC. *KRAS* G12C and *TP53* mutations correlate with a biomarker that predicts benefit from immunotherapy. Concurrent mutations may represent distinct subsets of *KRAS*-mutant NSCLC; further investigation is warranted to elucidate their role in guiding treatment.

1. Introduction

Activating mutations in the *KRAS* oncogene are the most common genetic driver in non-small cell lung cancer (NSCLC), appearing in about 25% of adenocarcinomas and 3% of squamous cell carcinomas [1,2]. Despite their prevalence, the prognostic impact of *KRAS* mutations remains uncertain. A pooled analysis of phase III clinical trials evaluating adjuvant chemotherapy in early-stage NSCLC determined that *KRAS* mutations had a negligible impact on overall survival (OS) in patients randomized to observation [3]. Similarly, among patients with advanced NSCLC receiving placebo in the randomized phase III SATURN trial, those with *KRAS* mutations trended towards shorter OS, but the relationship was statistically insignificant [4]. However, a recent meta-analysis of 37 studies identified *KRAS* mutations as a valid but weak predictor for poor survival [5].

Further efforts have aimed to unravel the influence of individual *KRAS* mutation subtypes on patient outcomes. Preclinical studies identified distinct signaling and drug sensitivity patterns across these subtypes [6,7], suggesting that differences may arise at the level of the amino acid substitution. However, this hypothesis has garnered limited clinical support. One retrospective analysis of 677 patients with metastatic *KRAS*-mutant lung adenocarcinoma demonstrated no difference in OS based on *KRAS* mutation subtype [8]. In other retrospective reports, *KRAS* G12C mutations were linked to a shorter survival in

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patients with resected lung adenocarcinoma and in patients with advanced NSCLC receiving second- or third-line chemotherapy [9,10], and although one study found that *KRAS* G12V mutations were associated with a greater response rate to taxanes no differences were observed with OS or progression-free survival [11]. Additional studies from randomized clinical trials have examined the interaction between *KRAS* mutation subtypes and therapeutic responses, though these have yielded largely negative results [12,13].

Despite these inconsistencies, our understanding of *KRAS*-mutant NSCLC has continued to evolve over the past few years under the auspices of the precision medicine effort. A growing body of literature indicates that *KRAS*-mutant NSCLC is in fact a heterogeneous disease, comprised of molecular subgroups that may be defined by co-occurring genomic alterations. Concurrent genetic mutations, in particular, appear to differentially govern intracellular processes and influence how a tumor behaves in its microenvironment [14–16]. Accordingly, ongoing efforts aim to elucidate the role of concurrent mutations in altering responses to immunotherapy and examine their interactions with predictive biomarkers such as programmed death-ligand 1 (PD-L1). It is becoming evident that the genomic landscape in which *KRAS* mutations occur may be just as relevant for influencing response to systemic therapy and in affecting overall patient outcomes.

In this retrospective analysis, we sought to discern the effects of common *KRAS* mutation subtypes and concurrent pathogenic mutations on OS in NSCLC patients. We also examined how these genetic mutations influence tumor PD-L1 expression.

2. Materials and methods

2.1. Study design

We performed this study under an institutional review board-approved protocol. We identified patients with NSCLC and at least one *KRAS* mutation who underwent genomic testing with the Solid Tumor Actionable Mutational Panel (STAMP) assay [17,18] at the Stanford Cancer Institute between January 2015 and December 2017. Pathogenic *KRAS* mutations and variants of unknown significance were both considered for study inclusion. Patients' electronic health records were abstracted for demographic, clinical, and pathologic data, and all patients with complete baseline information were included in our analysis.

Demographic factors included age, sex, race/ethnicity, prior history of cancer, and smoking history at the time of diagnosis. Patients with a prior history of cancer included those diagnosed with at least one malignancy in any organ/system prior to evaluation for NSCLC at our institution. A prior history of lung cancer was distinguished from recurrent disease based on previously published criteria defining second primary lung cancer as "a new, distinct pulmonary malignancy that is diagnosed $\geq$5 years after the primary tumor" [19]. Smoking history was classified as never (smoked < 100 cigarettes over lifetime) versus ever ($\geq$100 cigarettes over lifetime) smoking status at the time of diagnosis.

Operative and pathology reports were reviewed to determine disease staging and histology, as classified by WHO criteria [20]. Staging conformed to the eighth edition of the American Joint Committee on Cancer/International Union Against Cancer TNM stage classification for lung cancer [21], and was summarized as local (stage I), regional (stages II and III), and distant (stage IV) disease. From pathology reports, we gathered PD-L1 tumor proportion scores (TPS) in all patients who had testing performed with the PD-L1 immunohistochemistry (IHC) 22C3 pharmDx assay (Dako, Inc.).

Patients with complete baseline information were classified as lost to follow up if no contact was made after three months from their next expected appointment with medical/surgical oncology or with another provider at the Stanford University Medical Center. Therapies that patients received for their lung cancer were categorized as localized (focal therapies to the primary lung tumor; i.e., surgical resection or radiotherapy) or systemic (i.e., chemotherapy or immunotherapy).

2.2. Molecular analysis

Molecular profiling of all tumor samples was performed using the STAMP assay as part of standard of care. STAMP uses next-generation sequencing to detect potential clinically actionable mutations in 198 genes in Version 1 and 130 genes in Version 2 [22]. For our analysis, we considered only the genes that contained overlapping coverage between both versions of the assay (47 genes), as listed in Table S1. Commonly occurring *KRAS* mutation subtypes and genes harboring known pathogenic mutations (excluding variants of unknown significance) were selected for detailed analysis. Specifically, *KRAS* G12C and *KRAS* G12D mutations were selected as commonly occurring *KRAS* transversion and transition mutations, respectively. In all analyses, patients harboring either *KRAS* mutation subtype were compared to all patients lacking that particular subtype. Concurrent mutations in *TP53*, *STK11*, *KEAP1*, and *PIK3CA* were selected for survival analysis and evaluated for their presence, as these were among the most frequently co-mutated genes in the cohort. *CDKN2A* was not selected as an insufficient number of events occurred in the subset of patients harboring *CDKN2A* co-mutations for a meaningful interpretation. Additional pathogenic mutations that were detected in genes covered only in STAMP Version 2 are listed in Table S2.

2.3. Statistical analysis

OS was measured from the date of biopsy-proven diagnosis to the date of death or last follow up, with a cut-off date of April 2018. Cox proportional hazards regression models were employed in univariable and multivariable analyses of pre-determined, clinically relevant variables and were used to estimate crude and adjusted hazard ratios (HRs) for OS. Kaplan-Meier survival curves were generated to estimate OS across genomic subsets, and the log-rank test was used for comparisons. For analysis of PD-L1 expression, a logistic regression model was developed to estimate the adjusted odds ratios (ORs) for positive PD-L1 expression, defined as $\geq$1% TPS on IHC. All molecular variables included in the survival analyses were similarly examined for their relationship with PD-L1 expression, while adjusting for smoking history. In addition, a multinomial logistic regression analysis was conducted to estimate the adjusted ORs for PD-L1 expression across three levels: none (< 1% TPS), low (1–49% TPS), and high ($\geq$50% TPS) expression. Finally, in a supplementary analysis, patient characteristics were evaluated across disease stages using Fisher's exact test for categorical variables and one-way ANOVA for continuous variables. Statistical significance was defined at a two-sided $P < 0.05$. All statistical analyses were performed using the software R Version 1.0.153 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Patient characteristics

Among 861 NSCLC patients who underwent genomic analysis with the STAMP assay at our institution, 202 patients had tumors harboring *KRAS* mutations. Among these, three patients were immediately lost to follow up and one patient died prior to complete staging. Twelve patients were evaluated at our institution for molecular profiling or for diagnostic purposes only and were excluded due to incomplete baseline data. Therefore, a total of 186 patients were included in our analysis (Figure S1).

The median age at diagnosis was 72 years (range 40–90) (Table 1). Over half the cohort was female (57%), and the majority of patients had a history of smoking (85.5%). Staging was well distributed across local (38.7%), regional (29.6%), and distant (31.7%) disease, and the tumor

Table 1
Patient Characteristics.

Characteristic	Result (N = 186)
Age (years)	72 [40-90]
Female	106 (57.0)
Race/Ethnicity	
White	146 (78.5)
Asian	24 (12.9)
Hispanic/Latino	12 (6.5)
African American/Black	4 (2.2)
Prior History of Cancer	72 (38.7)
Prior Lung Cancer	8 (4.3)
Smoking History	
Never	27 (14.5)
Ever (Current/Former)	159 (85.5)
Stage at Diagnosis	
Local	72 (38.7)
Regional	55 (29.6)
Distant	59 (31.7)
Histology	
Adenocarcinoma	176 (94.6)
Squamous Cell	4 (2.2)
Large Cell Neuroendocrine	3 (1.6)
Adenosquamous	1 (0.5)
Sarcomatoid	1 (0.5)
NOS	1 (0.5)
PD-L1 Expression	
Negative (< 1% TPS)	46 (24.7)
Low (1-49% TPS)	15 (8.1)
High (≥50% TPS)	25 (13.4)
Not Tested	100 (53.8)
Therapy Received	
Localized	133 (71.5)
Systemic	101 (54.3)

Values presented as median [range] or N (%). Percentages may not sum to 100% due to rounding. Abbreviations: *NOS* not otherwise specified, *PD-L1* programmed death-ligand 1, *TPS* tumor proportion score.

histology was predominantly adenocarcinoma (94.6%). Interestingly, almost 40% of patients in the cohort had a prior history of cancer, and 4% had been previously diagnosed with at least one separate primary lung cancer (Table S3). As expected, a greater proportion of patients with local disease (98.6%) received localized therapy throughout their treatment course, and a greater proportion of patients with regional (80.0%) or distant (76.3%) disease received systemic therapy (Table S4). Among all patient characteristics, localized and systemic therapies were the only variables that significantly differed across disease stage.

Molecular profiling identified transversion *KRAS* mutations in a majority of patients (34.9% G12C, 19.9% G12 V, 7.5% G12A) (Table 2). The G12D transition mutation was present in 17.2% of patients, and various other *KRAS* mutations characterized the remainder of the cohort. Almost all of the patients in the cohort had tumors harboring at least one pathogenic *KRAS* mutation; only one patient had a *KRAS* variant of unknown significance (Q22 L). Concurrent pathogenic mutations occurred most commonly in tumor suppressor genes, such as *TP53* (38.7%), *STK11* (11.8%), *KEAP1* (8.1%), and *CDKN2A* (5.4%) (Table 3). However, pathogenic mutations were also noted in the genes of KRAS downstream effectors, including *PIK3CA* (3.8%), *BRAF* (2.7%), and *AKT1* (1.6%). Select *KRAS* mutation subtypes (G12C and G12D) and concurrent pathogenic mutations (*TP53* and *STK11*) were approximately equally distributed across stage at diagnosis, whereas *KEAP1* co-mutations presented at a higher frequency in distant disease ($P = 0.046$; Table S4).

3.2. Survival analysis

At the time of study conclusion, 100 (53.8%) patients in the cohort were alive and 19 (10.2%) had been lost to follow up (Figure S1). The median follow-up time was 15 months. The median OS of the entire cohort was 36 months (95% confidence interval [CI] 27-not reached [NR]) (Figure S2). Stratified by stage at diagnosis, the median OS for patients with local disease was not reached, for patients with regional disease was 36 months (95% CI 27-NR), and for patients with distant disease was 10 months (95% CI 7–22), which is representative of previously published reports.

Univariable analyses identified distant disease stage (HR 5.05, 95% CI 2.68–9.53; $P < 0.001$) and *STK11* co-mutations (HR 2.18, 95% CI 1.14–4.19; $P = 0.019$) as associated with poor OS (Table 4). On multivariable analysis, distant disease stage (HR 6.21, 95% CI 2.23–17.32; $P < 0.001$) and the presence of *STK11* co-mutations (HR 2.95, 95% CI 1.27–6.88; $P = 0.012$) remained significantly associated with poor OS (Table 4). In contrast, concurrent mutations in *TP53*, *KEAP1*, and *PIK3CA* were not associated with differences in OS. After adjusting for potential confounders, *KRAS* G12D mutations were also identified as an independent factor associated with shorter OS (HR 2.43, 95% CI 1.15–5.16; $P = 0.021$), along with every one-year increase in age (HR 1.04, 95% CI 1.01–1.08; $P = 0.017$).

On Kaplan-Meier analysis, neither *KRAS* G12C nor G12D mutation subtypes were associated with significant differences in OS (Table S5,

Table 2
KRAS Mutations in the NSCLC Cohort.

Mutation	N (%)
G12	
G12C	65 (34.9)
G12V	37 (19.9)
G12D	32 (17.2)
G12A	14 (7.5)
G12F	6 (3.2)
G12R	6 (3.2)
G12S	1 (0.5)
G13	
G13D	11 (5.9)
G13C	5 (2.7)
G13E	1 (0.5)
G13I	1 (0.5)
G13R	1 (0.5)
G13V	1 (0.5)
Q61	
Q61H	3 (1.6)
Q61L	1 (0.5)
Q61R	1 (0.5)
Other	
Q22L	1 (0.5)
T50I	1 (0.5)
A146V	1 (0.5)

Percentages may not sum to 100% as three patients presented with dual *KRAS* mutations: (1) G12C and G12V, (2) G12C and G13D, and (3) A146V and T50I.

Table 3
Concurrent Pathogenic Mutations in the *KRAS*-mutant NSCLC Cohort.

Mutated Gene	N (%)
TP53	72 (38.7)
STK11	22 (11.8)
KEAP1	15 (8.1)
CDKN2A	10 (5.4)
PIK3CA	7 (3.8)
BRAF	5 (2.7)
U2AF1	5 (2.7)
AKT1	3 (1.6)
PTEN	2 (1.1)
SF3B1	2 (1.1)
CTNNB1	2 (1.1)
NFE2L2	1 (0.5)
SMAD4	1 (0.5)
TP63	1 (0.5)

Table 4
Univariable and Multivariable Analyses for Overall Survival.

	Univariable Analysis				†Multivariable Analysis			
		95% CI				95% CI		
Variable	Crude HR	Lower	Upper	*P*	Adjusted HR	Lower	Upper	*P*
Age	1.03	1.00	1.06	0.091	1.04	1.01	1.08	0.017*
Female	0.93	0.57	1.50	0.764	1.41	0.83	2.41	0.202
Ever Smoking	1.27	0.58	2.78	0.554	1.66	0.69	3.98	0.254
Stage								
Local		Reference				Reference		
Regional	1.62	0.78	3.34	0.196	1.90	0.85	4.27	0.119
Distant	5.05	2.68	9.53	< 0.001***	6.21	2.23	17.32	< 0.001***
KRAS Mutation								
G12C	0.73	0.44	1.23	0.239	1.04	0.58	1.87	0.901
G12D	1.63	0.90	2.95	0.107	2.43	1.15	5.16	0.021*
Co-mutation								
TP53	0.82	0.49	1.35	0.430	0.72	0.41	1.27	0.252
STK11	2.18	1.14	4.19	0.019*	2.95	1.27	6.88	0.012*
KEAP1	1.59	0.69	3.70	0.279	0.50	0.18	1.44	0.201
PIK3CA	1.56	0.57	4.31	0.387	0.62	0.19	2.01	0.426
Therapies								
Localized	0.26	0.16	0.42	< 0.001***	0.63	0.30	1.34	0.232
Systemic	1.58	0.95	2.62	0.077	0.73	0.37	1.47	0.380

† All variables listed were included in the multivariable regression model and selected according to the methodology described in Section 2.3. Abbreviations: *HR* hazard ratio, *CI* confidence interval. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Figures S3 and 1a). The presence of concurrent mutations in *TP53*, *KEAP1*, and *PIK3CA* were also not associated with differences in OS (Table S5, Figure S4). However, the presence of *STK11* co-mutations was associated with a significantly shorter survival (median OS: 22 months versus 36 months; log-rank $P = 0.018$; Fig. 1b).

3.3. PD-L1 expression

A total of 86 (46.2%) patients in the cohort underwent PD-L1 IHC testing at baseline (Table 1). As response to immunotherapy is highly correlated with increased PD-L1 expression [23], we were interested in discerning how *KRAS* mutation subtype and the presence of concurrent mutations are related to positive PD-L1 expression (≥1% TPS), specifically with low (1–49% TPS) and high (≥50% TPS) levels of PD-L1 expression relative to no (< 1% TPS) expression.

We found that a current or prior history of smoking, *KRAS* G12C mutations, and *TP53* co-mutations were each significantly associated with PD-L1 positivity (Table S6). In examining different levels of PD-L1 expression, we determined that *TP53* co-mutations were independently associated with high PD-L1 expression (OR 6.36, 95% CI 1.84–22.02; $P = 0.004$), whereas *KRAS* G12C mutations were independently associated low PD-L1 expression (OR 4.94, 95% CI 1.07–22.85; $P = 0.041$; Table 5). Furthermore, while adjusting for the effects of these mutations, we found that a history of smoking was significantly associated with high PD-L1 expression (OR 7.90, 95% CI 1.64–38.05; $P = 0.010$). All other mutations in this analysis had statistically insignificant relationships with either low or high levels of PD-L1 expression.

4. Discussion

In this single-institution, retrospective study, we determined that concurrent pathogenic mutations in *STK11* are associated with a significantly shorter OS in patients with *KRAS*-mutant NSCLC. Furthermore, after adjusting for the effect of commonly occurring pathogenic mutations, we identified *KRAS* G12D mutations as a poor prognostic factor for OS. Our findings add to the literature characterizing the distinct features of molecular subgroups in *KRAS*-mutant NSCLC and suggest that the impact of driver *KRAS* mutations is perhaps best understood in the context of its broader genomic landscape.

Our results provide clinical support for previous reports of *STK11* co-mutations as a marker for an aggressive tumor phenotype in *KRAS*-mutant lung cancer [24]. *STK11* is a tumor suppressor gene that encodes serine/threonine kinase 11 (also known as LKB1, or liver kinase B1), which exerts a negative regulatory role over the mTOR pathway [25]. *STK11* mutations are prevalent in NSCLC at rates ranging from 15%–35% [26], and are significantly correlated with the presence of *KRAS* mutations [27]. Clinical studies have shown a higher frequency of metastasis—including brain metastasis—in patients with advanced NSCLC harboring concurrent *KRAS* and *STK11* mutations [28]. And yet, despite demonstrating trends towards poor survival, a majority of prognostic analyses of *STK11* co-mutations in *KRAS*-mutant NSCLC were statistically insignificant and inconclusive [27–29]. In our dataset, we identified pathogenic *STK11* co-mutations as significantly detrimental on multiple measures of patient survival, including after adjustment for potential confounders in multivariable analysis.

In contrast, we did not distinguish significant relationships between the presence of other concurrent pathogenic mutations and OS in our *KRAS*-mutant NSCLC cohort. Somatic *TP53* co-mutations frequently present in *KRAS*-mutant NSCLC and in lung cancers with other activating mutations [30]. Although *TP53* mutations are indicative of poor prognosis in NSCLC [31], our results are in line with several analyses that report insignificant associations of *TP53* co-mutations with OS in NSCLC patients who harbor *KRAS* mutations [29,32]. *KEAP1* encodes an emerging tumor suppressor, which functions to inhibit Nrf2—a transcription factor encoded by *NFE2L2* [33]. A previous retrospective analysis identified *KEAP1* or *NFE2L2* co-mutations as independent prognostic factors for shorter survival in patients with advanced *KRAS*-mutant NSCLC (HR 1.96, 95% CI 1.33–2.92; $P < 0.001$) [29]. Although we did not find a similar pattern of survival in our cohort for *KEAP1* co-mutations alone, it is possible that we did not have the statistical power to detect a signal owing to a small sample size in this subgroup. As *STK11* and *KEAP1* mutations frequently co-occur in *KRAS*-mutant NSCLC [14], future studies should investigate their combined impact on patient outcomes.

We also considered the effect of concurrent *PIK3CA* mutations in *KRAS*-mutant NSCLC. *PIK3CA* encodes the p110α catalytic subunit of PI3K, an enzyme that functions downstream of KRAS in the PI3K/AKT/mTOR pathway [34]. Presumably, concurrent *KRAS* and *PIK3CA* mutations would drive tumorigenesis by upregulating the PI3K and MAPK pathways, both important for cellular survival and proliferation [35].

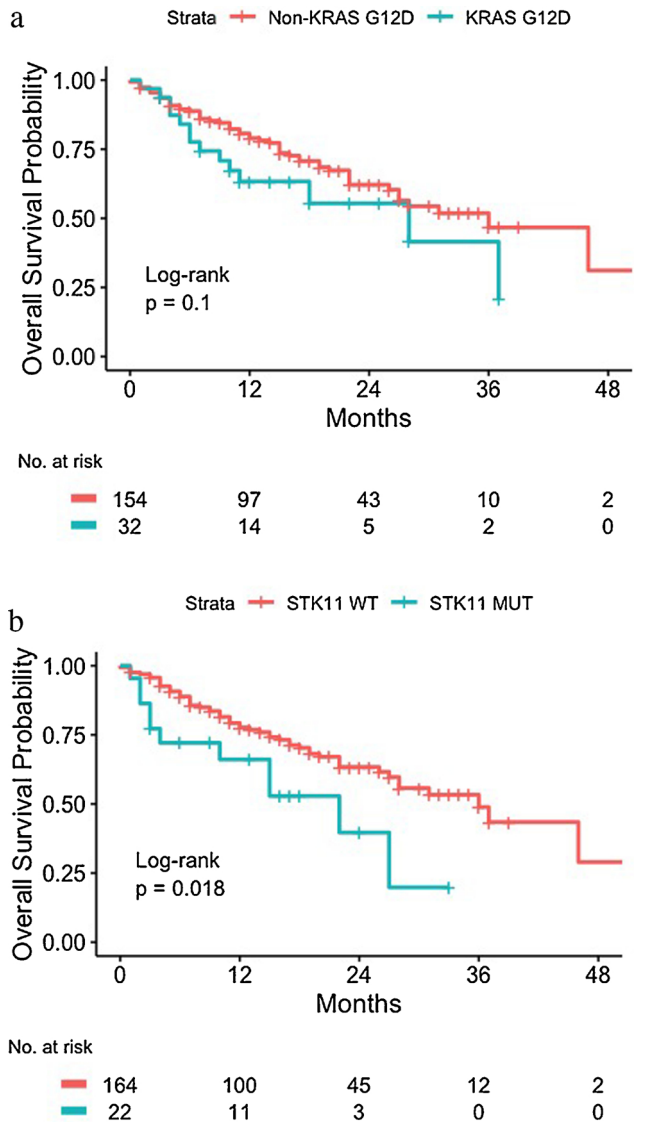

Fig. 1. Kaplan-Meier survival analysis stratified by *KRAS* G12D mutation status (**a**) and *STK11* co-mutation status (**b**). Abbreviations: *No.* number, *WT* wild-type, *MUT* mutant.

However, we were unable to find a significant relationship between *PIK3CA* co-mutations and OS in our cohort. A previous retrospective analysis suggested that *PIK3CA* mutations alone may be more detrimental compared to *PIK3CA* mutations with *EGFR/KRAS* co-mutations [36]. However, these results must be interpreted with caution as patients harboring *EGFR/KRAS* co-mutations were analyzed as a single subgroup.

In addition, we re-examined the effect of two commonly occurring *KRAS* mutation subtypes on OS while accounting for the presence of concurrent mutations. We found that *KRAS* G12D mutations were significantly associated with poor OS on multivariable analysis but not on Kaplan-Meier analysis ($P = 0.10$). It is possible that only after adjusting for potential confounders, such as disease stage and the presence of concurrent pathogenic mutations, are we able to capture the signal from *KRAS* G12D mutations. To our knowledge, this is the first study to reveal a negative prognostic effect with *KRAS* G12D mutations in NSCLC. *KRAS* G12D mutations most frequently occur in patients with NSCLC who are non-smokers [37], an association that was also observed in our cohort (data not shown). Differential activation of downstream signaling pathways has been noted across *KRAS* mutation subtypes [6], which may influence tumor biology and affect sensitivity to systemic treatment [7]. Although there has been some suggestion that *KRAS* G12C mutations confer a worse prognosis for patients with *KRAS*-mutant NSCLC [9,10], the data have been too conflicting to reach a definitive conclusion. Our results suggest that the prognostic impact of *KRAS* mutation subtypes may be best elucidated in concert with the effects of concurrent genetic mutations. Of course, these findings require validation in an independent dataset.

In the final part of our analysis, we evaluated the effects of concurrent pathogenic mutations on PD-L1 expression, a predictive biomarker for response to anti-PD-1/PD-L1 immunotherapy. In preclinical models, *KRAS* and *TP53* co-mutations correlate with increased PD-L1 mRNA and protein expression, suggesting that tumors harboring these mutations may be more responsive to immune checkpoint inhibition [14,15]. Conversely, tumors harboring concurrent *KRAS* and *STK11* mutations are associated with an immunosuppressive microenvironment [16]. Our findings confirm the association of *TP53* co-mutations with not only positive PD-L1 expression, but high levels of PD-L1 expression that are clinically meaningful, even after adjusting for smoking status. We further identified *KRAS* G12C mutations as independently associated with PD-L1 positivity, albeit with lower levels (< 50%) of PD-L1 expression. Altogether, our results correlate *TP53* and *KRAS* G12C mutations with a biomarker that predicts benefit with anti-PD-1/PD-L1 immunotherapy. This is in contrast to a previous report

Table 5
Multinomial Logistic Regression Analysis of PD-L1 Expression.

	Low (1-49% TPS) PD-L1 Expression				High (≥50% TPS) PD-L1 Expression			
		95 % CI				95% CI		
Variable	Adjusted OR	Lower	Upper	*P*	Adjusted OR	Lower	Upper	*P*
Ever Smoking	3.70	0.75	18.24	0.108	7.90	1.64	38.05	0.010**
KRAS Mutation								
G12C	4.94	1.07	22.85	0.041*	2.33	0.65	8.33	0.194
G12D	3.64	0.66	20.14	0.139	0.93	0.17	5.12	0.933
Co-mutation								
TP53	1.00	0.23	4.33	0.995	6.36	1.84	22.02	0.004**
STK11	0.17	0.01	1.95	0.155	0.61	0.12	3.18	0.556
KEAP1	0.82	0.06	10.83	0.881	0.71	0.10	5.02	0.728
PIK3CA	7.60	0.30	189.6	0.216	NE	NE	NE	NE

Adjusted ORs for low and high levels of PD-L1 expression were compared to a reference level of no (< 1% TPS) PD-L1 expression. All variables listed were included in the multinomial logistic regression model and selected according to the methodology described in Section 2.3. Abbreviations: *PD-L1* programmed death-ligand 1, *TPS* tumor proportion score, *OR* odds ratio, *CI* confidence interval, *NE* not estimable. * = $P < 0.05$; ** = $P < 0.01$.

identifying a greater mean PD-L1 expression for *KRAS*-mutant lung adenocarcinomas harboring *KRAS* G12V mutations (12.9% TPS)—a *KRAS* mutation subtype that we did not examine—compared to *KRAS* G12C mutations (8% TPS), *KRAS* G12D mutations (5.3% TPS), and *KRAS* wild-type (6% TPS; $P = 0.044$) [38]. Unfortunately, our sample size of *KRAS*-mutant NSCLC patients who received immunotherapy (n = 43) was too small to examine how these genomic subsets relate to OS following immunotherapy. However, the predictive value of *TP53* and *STK11* co-mutations was recently reported in a landmark analysis of *KRAS*-mutant NSCLC [39].

The present study describes a single institution's experience in evaluating the prognostic impact of common *KRAS* mutation subtypes and concurrent pathogenic mutations in NSCLC. Our study findings are strengthened by a relatively large cohort size, a 92% inclusion rate among all patients with *KRAS*-mutant NSCLC who underwent genomic testing at our institution, and a 90% follow-up rate for patients included in the study cohort. Our patient cohort had demographics that were largely consistent with those characteristic of *KRAS*-mutant NSCLC [4,40], but also included a fair proportion of patients who were non-white (21.5%) and non-smoking (15%). We included patients of all disease stages, which permitted examination of concurrent mutations across a range of presentations. A similar analysis was performed in patients with advanced NSCLC and revealed different prognostic patterns [29], suggesting that the impact of concurrent mutations on *KRAS*-mutant NSCLC may differ across disease stage.

One limitation of our study is that despite having a fairly large cohort, the size of each co-mutation subset was small, which may have reduced our statistical power to detect a signal. Furthermore, we selected two *KRAS* mutation subtypes that frequently occur in patients with NSCLC, but it will be important to examine an array of *KRAS* mutations and their interactions with concurrent genetic mutations. In one version of our STAMP assay, we identified pathogenic co-mutations in *NKX2.1* and *ARID1A* at rates of 8.3% and 7.3%, respectively, but we were unable to examine their impact on OS due to their inconsistent testing across the whole cohort. As these mutations are associated with aggressive tumor behaviors in various cancers [41–43], further study of their effects in *KRAS*-mutant NSCLC may be insightful. Lastly, our analysis invokes the limitations of all retrospective cohort studies, and we only infer associations from our results, not causation.

5. Conclusion

In summary, *KRAS* G12D mutations and *STK11* co-mutations are associated with poor prognosis in patients with *KRAS*-mutant NSCLC. Additionally, *KRAS* G12C mutations and *TP53* co-mutations correlate with positive PD-L1 expression and may be predictive of benefit with anti-PD-1/PD-L1 immunotherapy. Our findings further support *KRAS*-mutant NSCLC as a heterogeneous disease composed of distinct molecular subgroups and call for further assessments of their role in guiding therapeutic interventions.

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Appendix A. Supplementary data

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