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Characterization of KRAS Mutation Subtypes in Non–small Cell Lung Cancer

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ABSTRACT

KRAS is the most commonly mutated oncogene in NSCLC and development of direct KRAS inhibitors has renewed interest in this molecular variant. Different *KRAS* mutations may represent a unique biologic context with different prognostic and therapeutic impact. We sought to characterize genomic landscapes of advanced, *KRAS*-mutated non–small cell lung cancer (NSCLC) in a large national cohort to help guide future therapeutic development.

Molecular profiles of 17,095 NSCLC specimens were obtained using DNA next-generation sequencing of 592 genes (Caris Life Sciences) and classified on the basis of presence and subtype of *KRAS* mutations. Co-occurring genomic alterations, tumor mutational burden (TMB), and PD-L1 expression [22C3, tumor proportion score (TPS) score] were analyzed by *KRAS* mutation type.

Across the cohort, 4,706 (27.5%) samples harbored a *KRAS* mutation. The most common subtype was G12C (40%), followed by G12V (19%) and G12D (15%). The prevalence of *KRAS* mutations was 37.2% among adenocarcinomas and 4.4% in squamous cell carcinomas. Rates of high TMB (≥10 mutations/Mb) and PD-L1 expression varied across *KRAS* mutation subtypes. *KRAS* G12C was the most likely to be PD-L1 positive (65.5% TPS ≥ 1%) and PD-L1 high (41.3% TPS ≥ 50%). *STK11* was mutated in 8.6% of *KRAS* wild-type NSCLC but more frequent in *KRAS*-mutant NSCLC, with the highest rate in G13 (36.2%). *TP53* mutations were more frequent in *KRAS* wild-type NSCLC (73.6%).

KRAS mutation subtypes have different co-occurring mutations and a distinct genomic landscape. The clinical relevance of these differences in the context of specific therapeutic interventions warrants investigation.

Introduction

KRAS is the most common oncogenic driver in non–small cell lung cancer (NSCLC) identified in up to 25% of adenocarcinomas and 3% of squamous cell carcinomas (1, 2). *KRAS* activation results in downstream signaling to several pathways, including the *RAF-MEK-ERK* pathway. The prognostic value of *KRAS* mutations in patients with NSCLC remains unclear. Some studies have suggested worse outcomes with chemotherapy (3) while others have not (4, 5). There is similar discordance with *KRAS* co-mutation status and immunotherapy. A large retrospective study reported shorter progression-free survival (PFS) and overall survival (OS) with use of immunotherapy in patients with *KRAS*-mutant NSCLC harboring co-mutations in *STK11/LKB1* genes (6). An analysis of the KEYNOTE-189 study failed to confirm these findings, with similar benefit observed from the addition of pembrolizumab to chemotherapy, independent of *STK11* or *KEAP1* mutation status (7).

One contributing factor to these discordant results is the heterogeneity within *KRAS*. There is growing recognition of vast genetic and phenotypic heterogeneity of patients with *KRAS*-mutated NSCLC (8–10). Most frequently, *KRAS* mutations (chromosome 12p12.1) involve codons 12 and 13 and less frequently codon 61.

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Transversion mutations, including purine to pyrimidine (i.e., G>C) or pyrimidine to purine, are more common in current or former smokers, compared with transition mutations, either purine to purine (i.e., G>A) or pyrimidine to pyrimidine (i.e., T>C) which are more common in never or light smokers (11–13). Distinct *KRAS* mutations can influence the specific biology and the genomic landscape of a given cancer. This in turn can have notable therapeutic implications. With the recent development of direct KRAS inhibitors, these genomic contexts are increasingly relevant. Here, we characterize a large cohort of *KRAS*-mutant NSCLC, describing co-mutations, tumor mutational burden (TMB), and PD-L1 expression for each *KRAS* mutation subtype to help frame future treatment strategies.

Materials and Methods

Patient samples

A total of 17,095 NSCLC tumors were submitted to Caris Life Sciences for next-generation sequencing (NGS) molecular profiling between February 2015 and January 2020.

NGS

NGS was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using the NextSeq platform (Illumina). Matched normal tissue was not sequenced. A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies). The 592-gene list was custom designed to include cancer-related genes across all solid tumors that have been the best characterized for their functions and clinical relevance, including prognostic effects and targetability. All variants were detected with >99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of > 500× and an analytic sensitivity of 5%. Prior to molecular testing, tumor enrichment was achieved by harvesting targeted tissue using manual microdissection techniques. Variants detected were mapped to reference genome (hg19) and well-established bioinformatics tools such as BWA, SamTools, GATK, and snpFF were incorporated to perform variant calling functions; germline variants were filtered with various germline databases including 1000 genome and dbSNP genetic variants identified were interpreted by board-certified molecular geneticists and categorized as "pathogenic," "presumed pathogenic," "variant of unknown significance," "presumed benign," or "benign," according to the American College of Medical Genetics and Genomics standards. When assessing mutation frequencies of individual genes, "pathogenic" and "presumed pathogenic" were counted as mutations.

TMB calculation

TMB was measured by counting all non-synonymous missense, nonsense, in-frame insertions/deletions, and frameshift mutations found per tumor that had not been previously described as germline alterations in dbSNP151, Genome Aggregation Database (gnomAD) or benign variants identified by Caris geneticists. A cut-off point of ≥10 mutations (mt) per MB was used (14). Caris Life Sciences is a participant in the Friends of Cancer Research TMB Harmonization Project (15).

PD-L1 expression

IHC was performed on FFPE sections of glass slides. Slides were stained using automated staining techniques, per the manufacturer's instructions, and were optimized and validated per CLIA/CAO and ISO requirements. A board-certified pathologist evaluated all IHC results independently. The primary PD-L1 antibody clone was 22c3 (Dako). Tumor proportion score (TPS) was defined as the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The tumor was considered positive if TPS ≥ 1% and high PD-L1 expression was defined as TPS ≥ 50%.

Microsatellite instability/mismatch repair determination

A combination of multiple test platforms was used to determine the microsatellite stability (MSI) or mismatch repair (MMR) status of the tumors profiled, including fragment analysis (Promega), IHC [MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 antibody; and PMS2, EPR3947 antibody (16)], and NGS (for tumors tested with NextSeq platform, 7,000 target microsatellite loci were examined and compared with the reference genome hg19 from the University of California, Berkeley, CA).

Statistical plan

Molecular alterations among various *KRAS*-mutated groups were compared using Chi-square or Fisher exact tests [*KRAS* wild-type (WT) groups excluded from the comparative analyses] and a P value of <0.05 was considered a trending difference. Because of the large sample size of this study, P values were further corrected for multiple comparison using the Benjamini–Hochberg method and an adjusted P value (i.e., q value) of <0.05 was considered a significant difference. This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont report, and U.S. Common rule. In keeping with 45 CFR 46.101(b)(4), this study was performed utilizing retrospective, deidentified clinical data and received Institutional Review Board exemption from patient consent.

Results

Clinical characteristics

Across 17,095 NSCLC samples analyzed, 4,706 (27.5%) samples harbored a *KRAS* mutation (**Table 1**). The most common was G12C (40%), followed by G12V (19%) and G12D (15%; **Fig. 1**). *KRAS* mutations were more prevalent in female than male patients (31.35 vs. 23.7%, $P < 0.0001$) and there was no significant difference in age between *KRAS* subtypes (**Table 1**). The prevalence of *KRAS* mutations was 37.2% (3,889) among adenocarcinoma and only 4.4% (191) in squamous cell carcinoma samples; however, *KRAS* mutational distribution was similar in both histologies (**Fig. 1**). The smoking status of 1,841 patients were retrieved and categorized as never smoker, light smoker (designated as less than 15 packs per year), and current smoker (**Fig. 2**; Supplementary Table S1). The findings were not completely consistent with the known biology of *KRAS* subtypes. In our limited sample, 43% of patients with G12C mutations, 7% of patients with G12A mutations, and 8% of patients with G12D mutations were current smokers. 16% of patients with G12D mutations but only 6% of patients with G12A mutations were never or light smokers. Among *KRAS* subgroups, G12C mutations had the highest rate of current smokers, 43%.

Immunotherapy biomarkers

TMB varied significantly across the different *KRAS* mutation types ($P < 0.001$). High TMB was most common in G13 (50.0%) followed by G12 (43.7%) and least common in G12D mutations (19.1%; **Fig. 3**; Supplementary Table S2A). However, the distribution of median TMB values between different *KRAS* mutation types was narrow: 6.0 to 9.5 mt/Mb (**Fig. 4**). PD-L1 expression was also significantly different

Table 1. Patient characteristics of the NSCLC cohort studied. 4,706 (27.5%) samples of the entire cohort (17,095) had a *KRAS* mutation. Each *KRAS* mutation subtype is listed along with the frequency of patients harboring each subtype, the female versus male distribution, as well as the age range and median age.

	Total *N* (%)	Female *N* (%)	Male *N* (%)	Median age	Age range
KRAS WT	12,389 (72.5)	5,862 (47)	6,527 (53)	68.0	20–97
All KRAS mutation	4,706 (27.5)	2,677 (57)	2,029 (43)	68.0	22–97
G12C	1,882 (40)	1,102 (59)	780 (41)	68.0	27–95
G12V	915 (19)	504 (55)	411 (45)	68.0	37–92
G12D	684 (15)	386 (56)	298 (44)	69.0	22–97
G13	327 (7)	184 (56)	143 (44)	67.0	41–90
Q61	313 (7)	175 (56)	138 (44)	69.0	40–90
G12A	298 (6)	160 (54)	138 (46)	70.0	37–90
G12 Other	210 (4)	130 (62)	80 (38)	68.0	35–91
Other	77 (2)	36 (47)	41 (53)	68.0	37–89
Total	**17,095**	**8,539 (50)**	**8,556 (50)**	**68.0**	**20–97**

among *KRAS* mutations across major cutoffs, PD-L1 positive (TPS ≥ 1%), PD-L1 TPS ≥ 10%, and PD-L1 high (TPS ≥ 50%; **Fig. 3A**; Supplementary Table S2A). Of note, the *KRAS* WT population had lower PD-L1 expression than all *KRAS* mutations. *KRAS* G12C was the most likely to be PD-L1 positive, with 65.5% TPS ≥ 1%, and the most likely to be PD-L1 high, with 41.3% TPS ≥ 50% (**Fig. 3A**; Supplementary Table S2A). Immunotherapy biomarkers were compared in patients with *KRAS* G12C mutations and any other *KRAS* mutation subtype (**Fig. 3B**; Supplementary Table S2B). TMB and PD-L1 expression across major cutoffs were significantly higher in the G12C subtype compared with any other *KRAS* subtype. An additional analysis was performed to compare these biomarkers in patients with *KRAS* G12A, G12C, and G13D mutation subtypes due to different preferentially activated signaling pathways (Supplementary Table S2C; refs. 17, 18). However, additional analysis demonstrated that there were no significant differences in comparison of immune checkpoint inhibitor response markers, including PD-L1 expression, TMB, and MSI/MMR deficiency, between *KRAS* G12A and G12C mutations. In addition, after correction for multiple comparison, there were no significant differences for *KRAS* G13D compared with G12A and G12C subtypes.

Co-occurring mutations

Significant differences in *STK11 (LKB1)*, *KEAP1*, *TP53*, *BRAF*, *U2AF1*, *NF1*, and *GNAS* co-mutations were observed across *KRAS* mutational subtypes ($P < 0.0001$; **Fig. 5**; Supplementary Table S3). *STK11* was mutated in 8.6% of *KRAS* WT NSCLC but more frequently noted in every *KRAS* subtype, with the highest rate in G13 mutations (36.2%) and the lowest in G12D (14.2%). *KEAP1* was mutated most

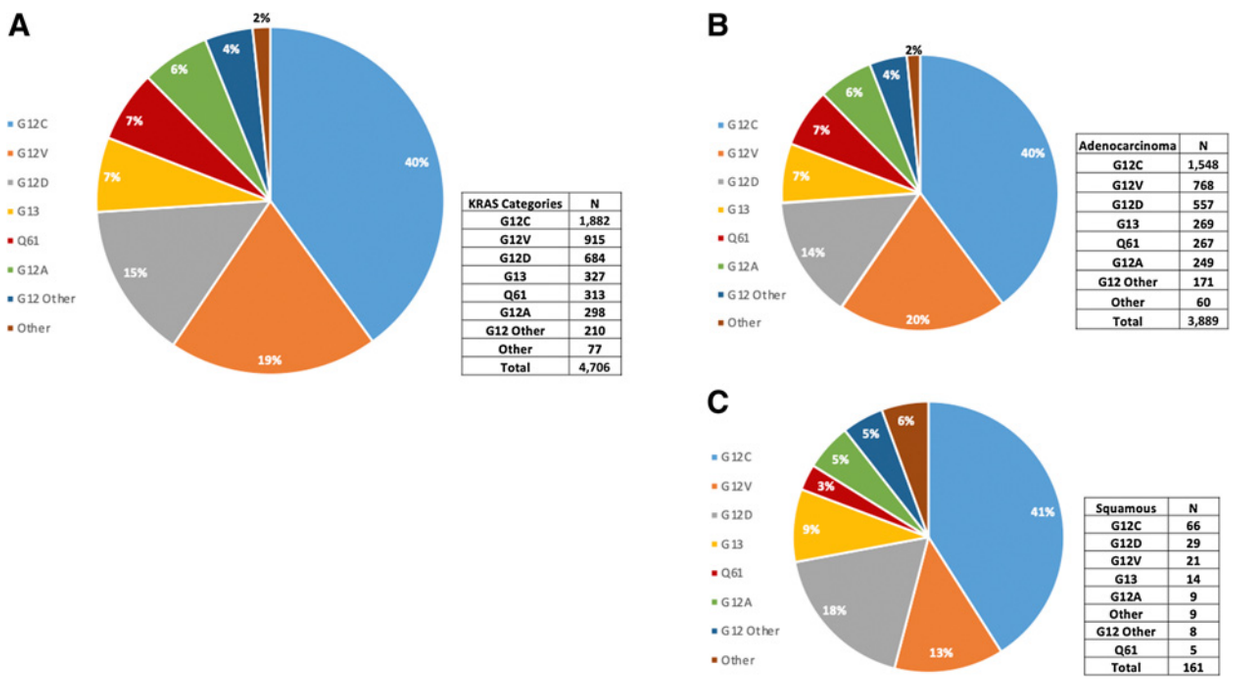

Figure 1.
KRAS mutational distribution in all NSCLC (**A**) and adenocarcinoma (**B**) and squamous cell (**C**) NSCLC histologies. The prevalence of *KRAS* mutations was 37.2% among adenocarcinoma and only 4.4% in squamous cell samples, however *KRAS* mutational distribution was similar in both histologies.

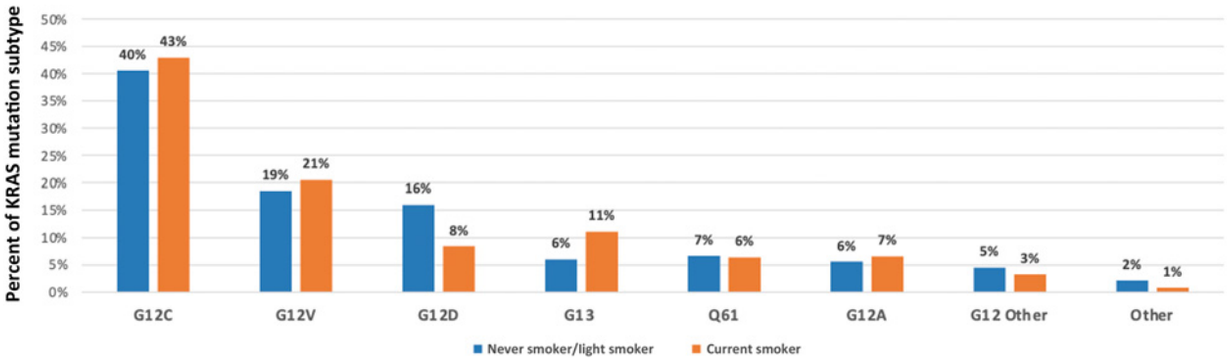

Figure 2.
Frequency of *KRAS* mutation subtypes in never smokers/light smokers (<15 packs/year) and current smokers. Smoking status data was only available in 1,841 of 4,706 patients with *KRAS* mutations in this cohort.

frequently with *KRAS* G13 (13.10%), which was more than twice the frequency in any other *KRAS* mutation subtype (3.70%–6.30%) or WT (4.20%) cases. *TP53* mutations were more frequent in *KRAS* WT NSCLC (73.6%), with the highest rate among *KRAS* mutants at 55.4% (G12other) and the lowest at 36.8% (Q61 mutations). *BRAF* and *U2AF1* mutations were much less common overall. *BRAF* mutations were most frequent in G13-mutated cases (5.20%), compared with any other *KRAS* mutation subtype (0.70%–0.260%), followed by WT cases (4.80%). *U2AF1* was mutated most frequently in *KRAS* G12other cases (7.70%), which was more than twice the frequency in any other *KRAS* mutation subtype (1.30%–3.70%) or WT (0.60%) cases. *NF1* was noted to be mutated in 21.4% of *KRAS* G13 cases, while all other *KRAS* mutations had a lower frequency of *NF1* mutations (2.8%–5.1%) than *KRAS* WT (11.5%). *GNAS* mutations were observed most frequently in G12D cases (3.4%) and less frequently in other *KRAS* mutations and WT cases (0.3%).

Significant differences in *STK11* ($P < 0.0001$), *KEAP1* ($P < 0.0001$), *TP53* ($P = 0.0002$), and *BRAF* ($P = 0.0002$) persisted across *KRAS* mutational subtypes in adenocarcinoma patients alone (Supplementary Fig. S1; Supplementary Table S4A). *STK11* was mutated most frequently in *KRAS* G13 cases (37.3%) and least frequently in G12D (15.8%). *KEAP1* mutations were more frequent

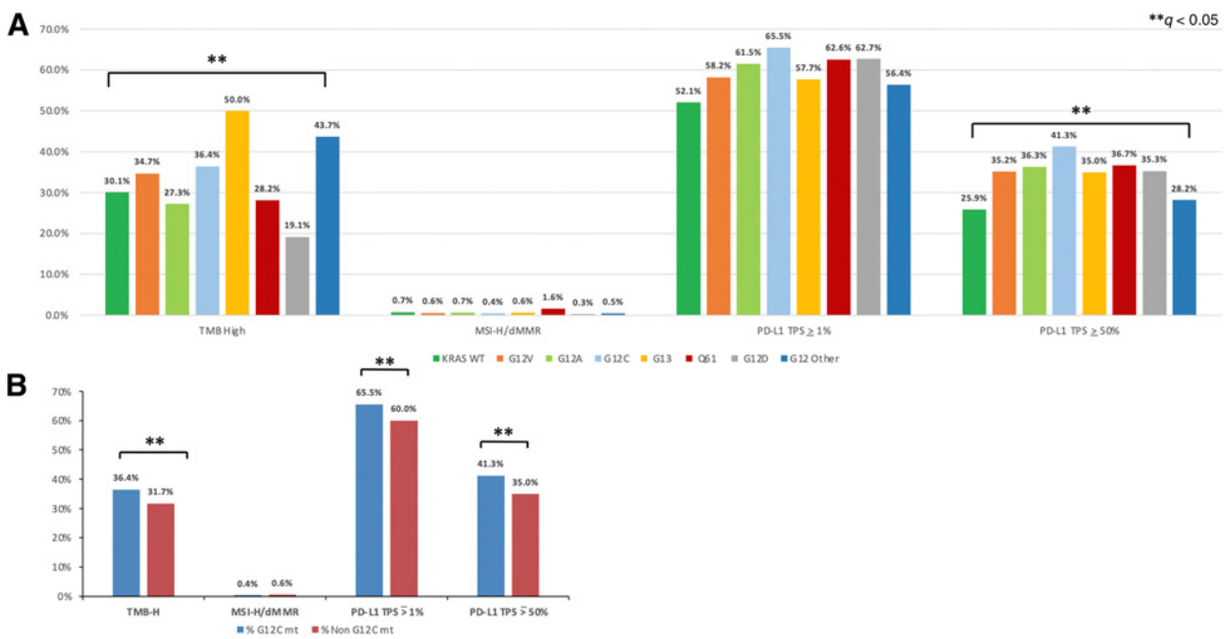

Figure 3.
Immune checkpoint therapy associated markers among *KRAS*-mutated tumors (**A**) and comparison of these markers between *KRAS* G12C mutated (G12C mt) and all other subtypes (non G12C mt; **B**). Prevalence of patients with NSCLC with high TMB (defined by ≥ 10 mt/Mb), MSI-H/MMR, and PD-L1 TPS (IHC 22c3) expression across major cut offs (TPS ≥ 1%, ≥ 10% and ≥ 50%) among each *KRAS* mutation subtype. **B,** The prevalence of patient with NSCLC with G12C mutations who had tumors with a high TMB ($P = 0.01$; $q = 0.013$), PD-L1 TPS ≥1% ($P < 0.001$; $q < 0.001$), and PD-L1 TPS ≥ 50% ($P < 0.001$; $q < 0.001$) was significantly greater than any other *KRAS* subtype. ** represents $q < 0.05$ (statistically significant).

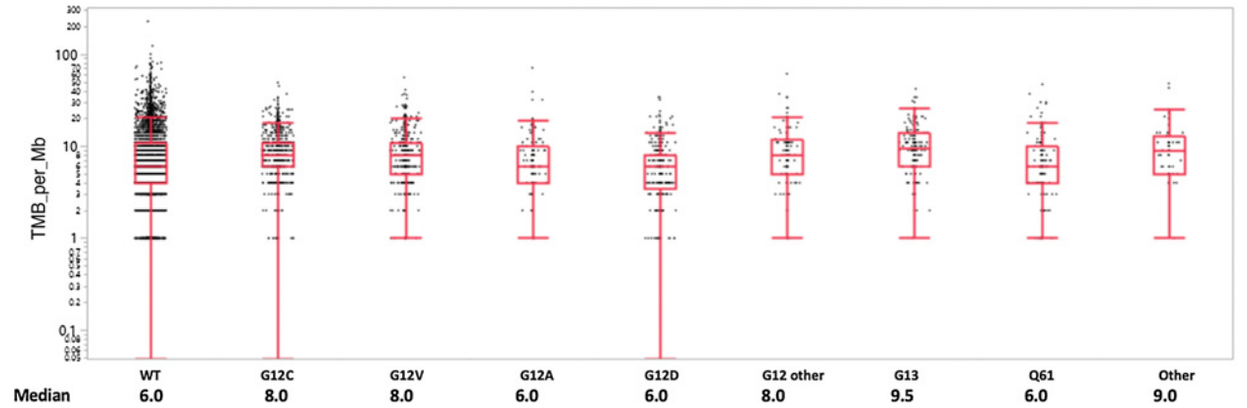

Figure 4.
TMB distribution among *KRAS* mutations. TMB distribution values with beeswarm plot displaying all patient data points. Median TMB displayed for *KRAS* WT and each *KRAS* mutation subtype.

in G13 (12.6%) which was more than twice the frequency in any other *KRAS* mutation subtype (3.4%–6.2%). *TP53* mutations were most frequent in G12other (51.8) followed closely by G13 (51.1%) and least frequent in Q61 mutations (36.0%). *BRAF* was mutated most frequently in G13 cases (4.9%) and least frequently in G12D cases (0.9%).

There were no significant differences in co-mutation frequency observed across *KRAS* subtypes in squamous NSCLC, only 4.4% of *KRAS* mutations (Supplementary Fig. S1; Supplementary Table S4B). Co-mutations that approached statistical significance included *STK11, TP53*, and *CDKN2A*. In the squamous cell carcinoma cohort, *STK11* was mutated most frequently in G12other (37.5%) while no *STK11* mutations were seen in G12D or Q61 cases. *TP53* co-mutation was seen in all G12 other mutated cases (100%) and least frequently in Q61 (40.0%). *CDKN2A* was most frequently mutated in G13 (41.7%) but was not seen at all in G12 or Q61 cases. *BRAF* was mutated most frequently in G12A (11.1%) followed by G13 (7.7%) but was not seen at all in the other *KRAS* subtypes.

Further analysis of *ATM* and *U2AF1* co-mutations was performed. Significantly different genomic alterations were found in patients with *KRAS*-mutated NSCLC with concomitant *ATM* mutations compared to *ATM* WT (Supplementary Fig. S2A). Mutations more frequently observed in *KRAS*-mutated/*ATM*-mutated patients include *CCND1* (3.8% vs. 0.8%), *FGF3* (4.2% vs. 0.8%), *FGF4* (3.5% vs. 0.7%), and *TP53* (18.4% vs. 49.7%) among others ($P < 0.0001$). There was a trend toward increased *PTCH1* mutations in co-mutated *ATM* (2.4% vs. 0.4%; $P = 0.003$, $Q = 0.063$), conversely *CDKN2A* co-mutations

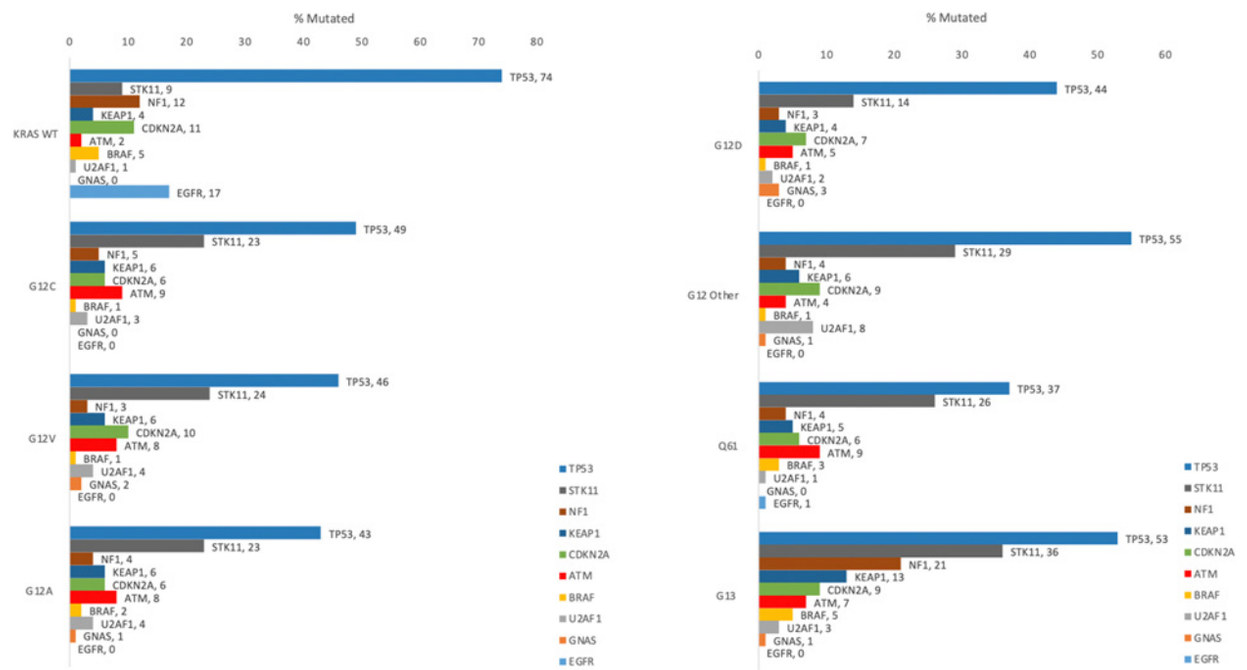

Figure 5.
Mutation rates of key biomarkers in *KRAS*-mutated NSCLC cohorts. Frequency of the 10 most common co-occurring mutations including *TP53, STK11, NF1, KEAP1, CDKN2A, ATM, BRAF, U2AF1, GNAS*, and *EGFR* are displayed for each *KRAS* mutation subtype as well as *KRAS* WT group.

trended higher in *ATM* WT compared with patients with *ATM*-mutant NSCLC (7.5% vs. 2.9%; $P = 0.002$, $Q = 0.042$). *STK11* mutations were significantly increased in patients with *KRAS* mutated but *U2AF1* WT NSCLC compared with *KRAS*- and *U2AF1*-mutant (23.6% vs. 9.9%, $P < 0.001$; Supplementary Fig. S2B). However, PD-L1–positive expression had a more frequent trend in those patients with concomitant *KRAS* and *U2AF1* mutations compared with *U2AF1* WT (72.3% vs. 60.3%; $P = 0.006$, $Q = 0.227$).

Discussion

While the role of *KRAS* mutations in tumorigenesis has been known for decades, no anti-cancer therapies targeting *KRAS* mutations have been successfully developed, until recently (19). Previous efforts to target *MEK 1/2* or *CDK 4/6* were ineffective (20). *MEK* inhibition lead to only modest efficacy with response rates of 11% and 12%, which were notably transient (21, 22). Recently, exciting phase I results have been reported with sotorasib, formerly AMG 510, a small molecule that irreversible inhibits *KRAS* G12C-mutant protein, demonstrating a 32.2% response rate and 88.1% disease control, in patients with *KRAS* G12C-mutated NSCLC (23, 24). Encouraging preclinical and clinical data has also been demonstrated using another KRAS G12C inhibitor, adagrasib, formerly MRTX849, with a recently reported response rate of 45% and disease control rate of 96.1% (25, 26). While these data are very promising for patients with *KRAS*-mutated NSCLC, there is significant variability in outcomes, duration of response, and mechanisms of resistance, all of which may be influenced by specific co-mutations present at diagnosis. Our study highlights the mutational heterogeneity that may explain prior inconsistent KRAS-targeted trial results and may influence future outcomes with subtype specific KRAS inhibitors and potentially with use of PD-1/PD-L1 inhibitors.

In *KRAS*-mutated NSCLC, there has been conflicting data on whether co-mutations influence outcomes with immunotherapy. In 2015, Skoulidis and colleagues described three major subsets of *KRAS*-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities by analysis of gene expression profiles and co-occurring genomic alterations (8). The three major *KRAS*-mutant subsets were defined by co-mutations in *STK11/LKB1* (KL subgroup), *TP53* (KP subgroup), and *CDKN2A/B* inactivation as well as low expression of *NKX2–1 (TTF1)* transcription factor (KC subgroup). The KC subgroup had biallelic deletions of *CDKN2A* (encoding for the *p16* tumor suppressor) and *CDKN2B* (encoding for the *p15* tumor suppressor), both significantly enriched in this cohort. The other two subgroups had distinct immune profiles. The KP subgroup of patients with a co-mutation in *TP53* had a higher tumor mutational load and characteristics of inflammatory response with increased expression of co-stimulatory (i.e., CD28) and co-inhibitory signals, including PD-L1. Therapeutic strategies using immune checkpoint inhibitors were appealing in this patient population given the reliance on PD-L/PD-L1 signaling and the increased immunogenicity with a large range of neoantigens. However, patients in the KL subgroup with a co-mutation in *STK11/LKB1* were found to have more alterations in *KEAP1* and *ATM* and had a "cold" immune microenvironment (relatively immune inert) with a lower rate of somatic mutations and anti-inflammatory signaling. Other studies have supported these immunogenic differences and response to immunotherapy in patients with *KRAS*-mutant NSCLC with *TP53* or *STK11* co-mutations (27, 28). In 2018, Skoulidis and colleagues showed that *STK11/LKB1* co-mutations were associated with a shorter PFS and OS when treated with immune checkpoint inhibitors (6). Recently, their group demonstrated that *STK11/LKB1* and/or *KEAP1* alterations drive primary resistance to immune checkpoint inhibitors with a lack of benefit from the addition of pembrolizumab to chemotherapy and an inferior OS in patients with non-squamous NSCLC (29). Importantly, resistance persisted in PD-L1–positive patients which emphasizes the potential significance of *STK11/LKB1* and/or *KEAP1* co-mutations and also demonstrates the challenges with the PD-L1 biomarker. Of note, there was no consistent association between common mutant *KRAS* alleles (G12C, G12V, G12D) and the three expression clusters that were originally described previously (8).

Other studies have not shown a consistent relationship between *KRAS* co-mutations in NSCLC and outcomes with immunotherapy; PD-L1 expression has not been well characterized in this particular patient subset. Arbour and colleagues found co-occurring *KEAP1* or *NFE2L2* mutations were associated with shorter OS; however, *STK11* and *TP53* were not associated with an OS difference (9). In addition, exploratory analysis of patients with NSCLC, not necessarily harboring a *KRAS* mutation, enrolled in KEYNOTE-042 and KEYNOTE-189 demonstrated better outcomes with pembrolizumab (alone or with chemotherapy, respectively) independent of *STK11* or *KEAP1* mutation status (7, 30). Thus, while *STK11* and *KEAP1* mutations alone may have prognostic value, a *KRAS* co-mutation may also predict immune checkpoint inhibitor resistance. In this article, we describe the molecular heterogeneity of each specific *KRAS* mutation subtype. Interestingly, we found patients with G13 mutations had the highest rate of *STK11* and *KEAP1* co-mutations (**Fig. 5**; Supplementary Table S3). It will be important to explore treatment outcomes with immunotherapy and chemoimmunotherapy in patients with *KRAS* G13-mutated NSCLC, as this subset may be a primary driver of the lack of benefit observed in some studies with PD-1 axis blockade (16, 31–36).

While several preclinical and clinical studies have described patients with *KRAS*-mutated NSCLC and *TP53*, *STK11*, *KEAP1* or *CDKN2A* co-mutations (6, 8, 9, 29, 37), there has been little prior work describing the potential clinical relevance of other predominant co-mutations demonstrated in our study, including *ATM* and *U2AF1 (*38). There is evidence that *ATM*-deficient lung adenocarcinoma is sensitive to PARP1 and ATR inhibitors (39). Here we have shown several mutations which are more frequent in patients with *KRAS*-mutated NSCLC with an *ATM* mutation (7.5% of *KRAS*-mutant cohort) compared with *ATM* WT (Supplementary Fig. S2A). Further exploration is needed to determine any therapeutic implications of these co-mutations. The functional role of *U2AF1* in NSCLC has not been completely elucidated (40). We have demonstrated an interesting trend in PD-L1, a biomarker of immunotherapy response, which was higher in patients with *KRAS*-mutant/*U2AF1*-mutant NSCLC (3% of *KRAS*-mutant cohort) compared with *KRAS*-mutant/*U2AF1* WT. In contrast, *STK11*, a potential marker of immunotherapy resistance, was significantly higher in patients with *KRAS*-mutant/*U2AF1* WT NSCLC (Supplementary Fig. S2B). Further studies are needed to determine the response to immune checkpoint inhibitors in these patient groups.

In terms of characterization of PD-L1 expression in *KRAS* mutation subtypes, we found that any *KRAS* mutation subtype had a greater likelihood of PD-L1 expression, across all major cutoffs, than the WT subgroup (**Fig. 3A**; Supplementary Table S2A). Specifically, G12C was the most likely to be PD-L1 positive, with 65.5% TPS > 1%, and the most likely to be PD-L1 high, with 41.3% TPS ≥ 50% (**Fig. 3B**; Supplementary Table S2B). Unfortunately, we have limited data on the patient's smoking status which is known to be associated with higher TMB and PD-L1 expression, as well as response to immunotherapy (**Fig. 2**; Supplementary Table S1; refs. 42, 43). In addition, "light smokers" categorized as less than 15 packs per year does not

accurately capture this patient population due to the loose definition. Prior studies which have included a "light smokers" category have used up to 10 pack years (13, 41). Thus, heavy smokers may also be captured in this category. While the proportions of *KRAS* mutation subtypes in each smoking category resembles some prior data, there are differences with the known biology of these subtypes (11–13). Therefore, we are unable to draw any conclusions regarding the influence of smoking status, or the *KRAS* biology itself, based on this cohort. Our data did not show a difference in immune checkpoint response markers (PD-L1, TMB, or MSI/MMR) when comparing subtypes with varying RAF signaling pathway dependence (Supplementary Table S2C). G12C and G13D subtypes have a high intrinsic GTPase hydrolysis rate and are less RAF signaling dependent compared with G12A, which has a low GTPase hydrolysis rate and is dependent on RAF signaling (17, 18). The observed difference, or lack thereof, will need to be subsequently explored in terms of clinical treatment outcomes to better understand their significance.

KRAS mutations are relatively common events in lung adenocarcinoma. Specific *KRAS* mutations exist in slightly different genomic landscapes: the rates of co-mutation of various relevant genes varied by specific *KRAS* mutation type. PD-L1 expression was also significantly different across specific *KRAS* mutations. These differences likely reflect differences in the underlying biology of each *KRAS* subset. Future therapeutic interventions must take note of these genomic differences as we further personalize cancer care.

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