Original Article

Title: Characterization of KRAS Mutation Subtypes in Non-Small Cell Lung Cancer

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Running Title: Characterization of KRAS Mutation Subtypes in NSCLC

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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 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 (NGS) of 592 genes (Caris Life Sciences) and classified based on presence and subtype of KRAS mutations. Co-occurring genomic alterations, tumor mutational burden (TMB) and PD-L1 expression (22C3, TPS score) were analyzed by KRAS mutation type.

Across the cohort, 4706 (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. Most frequently, KRAS mutations (chromosome 12p12.1) involve codons 12 and 13 and less frequently codon 61. Transversion mutations, including purine to pyrimidine (i.e. G> C) or pyrimidine to purine, are more common in current or former smokers, compared to 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 (Phoenix, AZ) for next-generation sequencing (NGS) molecular profiling between February 2015 and January 2020.

**Next generation sequencing**

NGS was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using the NextSeq platform (Illumina, Inc., San Diego, CA). Matched normal tissue was not sequenced. A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA). 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 > 500X 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 (ACMG) 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 cutoff point of ≥ 10 mutations (mt) per MB was used\(^\text{14}\). Caris Life Sciences is a participant in the Friends of Cancer Research TMB Harmonization Project \(^\text{15}\).

**PD-L1 expression**

Immunohistochemistry (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 (MSI)/Mismatch repair (MMR) determination**

A combination of multiple test platforms was used to determine the MSI or MMR status of the tumors profiled, including fragment analysis (FA, Promega, Madison, WI), IHC (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 antibody; and PMS2, EPR3947 antibody \(^\text{16}\)) and NGS (for tumors tested with NextSeq platform, 7,000 target microsatellite loci were examined and compared to the reference genome hg19 from the University of California).

**Statistical plan**

Molecular alterations amongst various KRAS-mutated groups were compared using Chi-square or Fisher Exact tests (KRAS-wild type groups excluded from the comparative analyses) and a p value of <0.05 was considered a trending difference. Due to 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 IRB exemption from patient consent.

Results

Clinical characteristics
Across 17,095 NSCLC samples analyzed, 4,706 (27.5%) samples harbored a \textit{KRAS} mutation (Table 1). The most common was G12C (40%), followed by G12V (19%) and G12D (15%) (Figure 1). \textit{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 \textit{KRAS} subtypes (Table 1). The prevalence of \textit{KRAS} mutations was 37.2% (3,889) among adenocarcinoma and only 4.4% (191) in squamous cell carcinoma samples, however \textit{KRAS} mutational distribution was similar in both histologies (Figure 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 (Figure 2, Supplemental Table 1). The findings were not completely consistent with the known biology of \textit{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 \textit{KRAS} subgroups, G12C mutations had the highest rate of current smokers, 43%.

Immunotherapy Biomarkers
TMB varied significantly across the different \textit{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%) (Figure 3, Supplementary Table 2A). However, the distribution of median TMB values between different \textit{KRAS} mutation types was narrow: 6.0 to 9.5 mt/Mb (Figure 4). PD-L1 expression was also significantly different among \textit{KRAS} mutations across major cutoffs, PD-L1 positive (TPS \geq 1%), PD-L1 TPS \geq 10% and PD-L1 high (TPS \geq 50%) (Figure 3A, Supplementary Table 2A). Of note, the \textit{KRAS} wild type (WT) population had lower PD-L1 expression than all \textit{KRAS} mutations. \textit{KRAS} G12C was the most likely to be PD-L1 positive, with 65.5% TPS \geq 1%, and the most likely to be PD-L1 high, with 41.3% TPS \geq 50% (Figure 3A, Supplementary Table 2A). Immunotherapy biomarkers were compared in patients with \textit{KRAS} G12C mutations and any other \textit{KRAS} mutation subtype (Figure 3B, Supplementary Table 2B). TMB and PD-L1 expression across major cut offs were significantly higher in the G12C subtype compared to any other \textit{KRAS} subtype. An additional analysis was performed to compare these biomarkers in patients with \textit{KRAS} G12A, G12C and G13D mutation subtypes due to different preferentially activated signaling pathways (Supplementary Table 2C)\textsuperscript{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 microsatellite instability/mismatch repair deficiency, between KRAS G12A and G12C mutations. Additionally, after correction for multiple comparison, there were no significant differences for KRAS G13D compared to 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) (Figure 5, Supplemental Table 3). 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 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 to 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 (Supplemental Figure 1, Supplemental Table 4A). STK11 was mutated most frequently in KRAS G13 cases (37.3%) and least frequently in G12D (15.8%). KEAP1 mutations were more frequent 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 (Supplemental Figure 1, Supplemental Table 4B). 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 G12other 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 (Supplemental Figure 2A). 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 trended higher in *ATM* WT compared to 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 to *KRAS* and *U2AF1* mutant (23.6% vs 9.9%, p <0.001; Supplemental Figure 2B). However, PD-L1 positive expression had a more frequent trend in those patients with concomitant *KRAS* and *U2AF1* mutations compared to *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. Previous efforts to target *MEK 1/2* or *CDK 4/6* were ineffective. *MEK* inhibition lead to only modest efficacy with response rates of 11% and 12%, which were notably transient. Recently, exciting phase I results have been reported with AMG 510, a small molecule that irreversibly inhibits *KRAS* G12C mutant protein, demonstrating a 32.2% response rate and 88.1% disease control, in patients with *KRAS* G12C mutated NSCLC. Encouraging pre-clinical and clinical data has also been demonstrated using another *KRAS* G12C inhibitor, MRTX849, with a recently reported response rate of 45% and disease control rate of 96.1%. 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 et al. described 3 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. The 3 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 bi-allelic 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 2 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-L1 signaling and the increased immunogenicity with a large range of neo-antigens. 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 \(^{28,29}\). In 2018, Skoulidis et al 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 non-squamous NSCLC patients \(^{30}\). 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 \(^{8}\).

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 et al. found co-occurring KEAP1 or NFE2L mutations were associated with shorter OS, however, STK11 and TP53 were not associated with an OS difference \(^{9}\). Additionally, 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 \(^{7,31}\). Thus, while STK11 and KEAP1 mutations alone may have prognostic value, a KRAS co-mutation may also predict immune checkpoint inhibitor resistance. In this report, 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 (Figure 5, Supplemental Table 3). 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 \(^{16,32-37}\).

While several pre-clinical and clinical studies have described patients with KRAS mutated NSCLC and TP53, STK11, KEAP1 or CDKN2A co-mutations \(^{6,8,9,30,38}\), there has been little prior work describing the potential clinical relevance of other predominant co-mutations demonstrated
in our study, including \textit{ATM} and \textit{U2AF1}\textsuperscript{39}. There is evidence that \textit{ATM} deficient lung adenocarcinoma is sensitive to PARP1 and ATR inhibitors\textsuperscript{40}. Here we have shown several mutations which are more frequent in patients with \textit{KRAS} mutated NSCLC with an \textit{ATM} mutation (7.5\% of \textit{KRAS} mutant cohort) compared to \textit{ATM} WT (Supplemental Figure 2A). Further exploration is needed to determine any therapeutic implications of these co-mutations. The functional role of \textit{U2AF1} in NSCLC has not been completely elucidated\textsuperscript{41}. We have demonstrated an interesting trend in PD-L1, a biomarker of immunotherapy response, which was higher in patients with \textit{KRAS} mutant/\textit{U2AF1} mutant NSCLC (3\% of \textit{KRAS} mutant cohort) compared to \textit{KRAS} mutant/\textit{U2AF1} WT. In contrast, \textit{STK11}, a potential marker of immunotherapy resistance, was significantly higher in patients with \textit{KRAS} mutant/\textit{U2AF1} WT NSCLC (Supplemental Figure 2B). 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 \textit{KRAS} mutation subtypes, we found that any \textit{KRAS} mutation subtype had a greater likelihood of PD-L1 expression, across all major cutoffs, than the WT subgroup (Figure 3A, Supplemental Table 2A). 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\% (Figure 3B, Supplemental Table 2B). 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 (Figure 2, Supplemental Table 1)\textsuperscript{42-44}. 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\textsuperscript{13,42}. Thus, heavy smokers may also be captured in this category. While the proportions of \textit{KRAS} mutation subtypes in each smoking category resembles some prior data, there are differences with the known biology of these subtypes\textsuperscript{11-13}. Therefore, we are unable to draw any conclusions regarding the influence of smoking status, or the \textit{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 (Supplemental Table 2C). G12C and G13D subtypes have a high intrinsic GTPase hydrolysis rate and are less RAF signaling dependent compared to G12A, which has a low GTPase hydrolysis rate and is dependent on RAF signaling\textsuperscript{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.

\textit{KRAS} mutations are relatively common events in lung adenocarcinoma. Specific \textit{KRAS} mutations exist in slightly different genomic landscapes: the rates of co-mutation of various relevant genes varied by specific \textit{KRAS} mutation type. PD-L1 expression was also significantly different across specific \textit{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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27. Janne PAea: KRYSTAL -1: Updated Safety and Efficacy Data With Adagrasib (MRTX849) in NSCLC With KRASG12C Mutation From a Phase 1/2 Study. Presented at the 32nd EORTC-NCI-AACR Symposium


Tables and Figures Legend

Table 1. Patient Characteristics of the NSCLC cohort studied.

<table>
<thead>
<tr>
<th></th>
<th>Total N (%)</th>
<th>Female N (%)</th>
<th>Male N (%)</th>
<th>Median Age</th>
<th>Age Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS WT</td>
<td>12389 (72.5)</td>
<td>5862 (47)</td>
<td>6527 (53)</td>
<td>68.0</td>
<td>20-97</td>
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<td>All KRAS mutation</td>
<td>4706 (27.5)</td>
<td>2677 (57)</td>
<td>2029 (43)</td>
<td>68.0</td>
<td>22-97</td>
</tr>
<tr>
<td>G12C</td>
<td>1882 (40)</td>
<td>1102 (59)</td>
<td>780 (41)</td>
<td>68.0</td>
<td>27-95</td>
</tr>
<tr>
<td>G12V</td>
<td>915 (19)</td>
<td>504 (55)</td>
<td>411 (45)</td>
<td>68.0</td>
<td>37-92</td>
</tr>
<tr>
<td>G12D</td>
<td>684 (15)</td>
<td>386 (56)</td>
<td>298 (44)</td>
<td>69.0</td>
<td>22-97</td>
</tr>
<tr>
<td>G13</td>
<td>327 (7)</td>
<td>184 (56)</td>
<td>143 (44)</td>
<td>67.0</td>
<td>41-90</td>
</tr>
<tr>
<td>Q61</td>
<td>313 (7)</td>
<td>175 (56)</td>
<td>138 (44)</td>
<td>69.0</td>
<td>40-90</td>
</tr>
<tr>
<td>G12A</td>
<td>298 (6)</td>
<td>160 (54)</td>
<td>138 (46)</td>
<td>70.0</td>
<td>37-90</td>
</tr>
<tr>
<td>G12 Other</td>
<td>210 (4)</td>
<td>130 (62)</td>
<td>80 (38)</td>
<td>68.0</td>
<td>35-91</td>
</tr>
<tr>
<td>Other</td>
<td>77 (2)</td>
<td>36 (47)</td>
<td>41 (53)</td>
<td>68.0</td>
<td>37-89</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17095</td>
<td>8539 (50)</td>
<td>8556 (50)</td>
<td>68.0</td>
<td>20-97</td>
</tr>
</tbody>
</table>

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.

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 out of 4,706 patients with KRAS mutations in this cohort.

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 NSCLC patients with high TMB (defined by ≥ 10 mutations/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 NSCLC patient 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 datapoints. Median TMB displayed for KRAS WT and each KRAS mutation subtype.

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.
Figure 1. KRAS mutational distribution in all NSCLC (A) and adenocarcinoma (B) and squamous cell (C) NSCLC histologies.

A. KRAS Categories N
G12C 1882
G12V 955
G12D 888
G13 327
Q61 313
G12A 298
G12 Other 210
Other 77
Total 4708

B. Adenocarcinoma N
G12C 1548
G12V 768
G12D 557
G13 269
Q61 267
G12A 249
G12 Other 175
Other 66
Total 3899

C. Squamous N
G12C 86
G12V 29
G12D 21
G13 14
Q61 9
G12A 5
G12 Other 2
Other 15
Total 161
Figure 2. Frequency of KRAS mutation subtypes in never smokers/light smokers (<15 packs/year) and current smokers
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).

A. **p<0.05

B. **
Figure 4. TMB distribution among KRAS mutations.
Figure 5. Mutation rates of key biomarkers in KRAS mutated cohorts.

% Mutated

0 10 20 30 40 50 60 70 80

KRAS WT

TP53, 74

STK11, 9

NF1, 12

K-RAS

EGFR, 17

G12D

TP53, 40

STK11, 23

NF1, 5

KEAP1, 6

GNA11, 6

ATM, 9

BRAF, 1

UBAF1, 3

GNA1, 0

EGFR, 0

G12C

TP53, 46

STK11, 24

NF1, 3

KEAP1, 6

GNA11, 10

ATM, 8

BRAF, 1

UBAF1, 4

GNA1, 0

EGFR, 0

G12V

TP53, 43

STK11, 23

NF1, 4

KEAP1, 6

GNA11, 6

ATM, 8

BRAF, 2

UBAF1, 4

GNA1, 0

EGFR, 0

G12A

TP53, 37

STK11, 26

NF1, 4

KEAP1, 5

GNA11, 6

ATM, 9

BRAF, 1

UBAF1, 3

GNA1, 0

EGFR, 0

G61

TP53, 55

STK11, 29

NF1, 4

KEAP1, 6

GNA11, 9

ATM, 4

BRAF, 1

UBAF1, 8

GNA1, 0

EGFR, 0

G13

TP53, 53

STK11, 36

NF1, 21

KEAP1, 15

GNA11, 9

ATM, 7

BRAF, 5

UBAF1, 3

GNA1, 1

EGFR, 0
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Julia Judd, Nagla Abdel Karim, Hina Khan, et al.

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