# Metabolic and tumor immune cell landscapes are significantly different amongst *KRAS* mutational variants in non-small cell lung cancer

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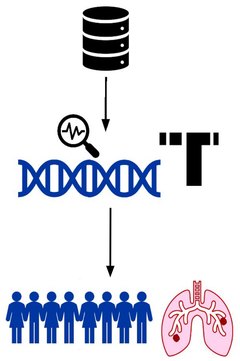
## INTRODUCTION

- Approximately 30% of patients with non-small cell lung cancer (NSCLC) have mutations in the *KRAS* oncogene.
- In NSCLC, *KRAS* mutational variants are diverse and therapeutically relevant.
- However, it is unclear how each variant is associated with the tumor biology, including lipid metabolism and the immune microenvironment.
- Since perturbed tumor immune infiltration and lipid metabolism have been previously linked to NSCLC outcomes, we evaluated and characterized *KRAS* variants and their association with lipid metabolism and immune infiltration.

## METHODS

- De-identified records of 5,925 patients diagnosed with NSCLC who also harbored *KRAS* alterations were retrospectively analyzed.
- Samples were sequenced with the Tempus xT and xR RNA assays.



- Tumor microenvironment cell proportions were estimated using QuanTIseq.
- Single-sample gene set enrichment analysis (ssGSEA) based on 775 lipid metabolic genes was used to compute enrichment scores (ES) for each pt.
- Neoantigen tumor burden (NTB) and tumor mutational burden (TMB) were analyzed as mutations per megabase (mut/Mb).
- P-values were calculated using Pearson's Chi-squared and Kruskal-Wallis rank sum test.
- Pairwise comparisons of median ES were performed using the Wilcoxon test and the FDR method was used to correct for multiple comparisons.

## SUMMARY


- Smoking history differed significantly between *KRAS* variants, with the highest proportion of never smokers observed in patients with *KRAS* G12D alterations.
- Lipid gene enrichment scores were lower in patients with *KRAS* G12C variants than in those with G12D or G12V variants.
- Immune infiltration levels differed significantly between different *KRAS* variants.
- Notably, patients with *KRAS* G12D variants had a less immunogenic immune microenvironment as indicated by a lower TMB, NTB, and proportion of CD8 T cells and M1 macrophages compared to G12C variants, which could affect immunotherapy efficacy.
- Future work should investigate whether lipid metabolism alongside a less immunogenic immune microenvironment modulates a decreased response to immunotherapy in patients with *KRAS* G12D variants.


## RESULTS

### Table 1. Demographic Information

| Characteristic | Overall N = 5,925[1] | G12A N = 464[1] | G12C N = 2,510[1] | G12D N = 937[1] | G12R N = 61[1] | G12V N = 1,251[1] | G13C N = 244[1] | G13D N = 185[1] | Q61H N = 273[1] | p-value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| **Age at Primary Diagnosis** | | | | | | | | | | 0.042 |
| Median (Q1, Q3) | 68 (62, 75) | 69 (64, 76) | 68 (62, 75) | 69 (62, 76) | 68 (61, 74) | 69 (63, 76) | 67 (62, 74) | 67 (62, 75) | 67 (61, 74) | |
| **Sex** | | | | | | | | | | 0.061 |
| Female | 3,393 (57%) | 259 (56%) | 1,463 (58%) | 516 (55%) | 31 (51%) | 728 (58%) | 134 (55%) | 91 (49%) | 171 (63%) | |
| Male | 2,532 (43%) | 205 (44%) | 1,047 (42%) | 421 (45%) | 30 (49%) | 523 (42%) | 110 (45%) | 94 (51%) | 102 (37%) | |
| **Race** | | | | | | | | | | 0.2 |
| White | 3,282 (82%) | 264 (85%) | 1,408 (83%) | 526 (81%) | 32 (84%) | 660 (81%) | 126 (78%) | 114 (91%) | 152 (83%) | |
| Black or African American | 449 (11%) | 33 (11%) | 182 (11%) | 74 (11%) | 4 (11%) | 99 (12%) | 30 (19%) | 7 (5.6%) | 20 (11%) | |
| Other Race | 194 (4.9%) | 9 (2.9%) | 84 (4.9%) | 36 (5.5%) | 2 (5.3%) | 46 (5.6%) | 6 (3.7%) | 3 (2.4%) | 8 (4.4%) | |
| Asian | 64 (1.6%) | 5 (1.6%) | 26 (1.5%) | 15 (2.3%) | 0 (0%) | 14 (1.7%) | 0 (0%) | 1 (0.8%) | 3 (1.6%) | |
| Unknown | 1,936 | 153 | 810 | 286 | 23 | 432 | 82 | 60 | 90 | |
| **Smoking Status** | | | | | | | | | | <0.001 |
| Ex-smoker | 2,220 (54%) | 165 (55%) | 984 (56%) | 335 (50%) | 22 (46%) | 466 (55%) | 80 (50%) | 60 (50%) | 108 (57%) | |
| Current-smoker | 1,564 (38%) | 110 (37%) | 738 (42%) | 182 (27%) | 21 (44%) | 313 (37%) | 76 (47%) | 51 (43%) | 73 (38%) | |
| Never-smoker | 315 (7.7%) | 24 (8.0%) | 36 (2.0%) | 154 (23%) | 5 (10%) | 74 (8.7%) | 5 (3.1%) | 8 (6.7%) | 9 (4.7%) | |
| Unknown | 1,826 | 165 | 752 | 266 | 13 | 398 | 83 | 66 | 83 | |

[2] Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's Exact Test for Count Data with simulated p-value (based on 2000 replicates)

### Figure 2. Distribution of immune cells across KRAS variants

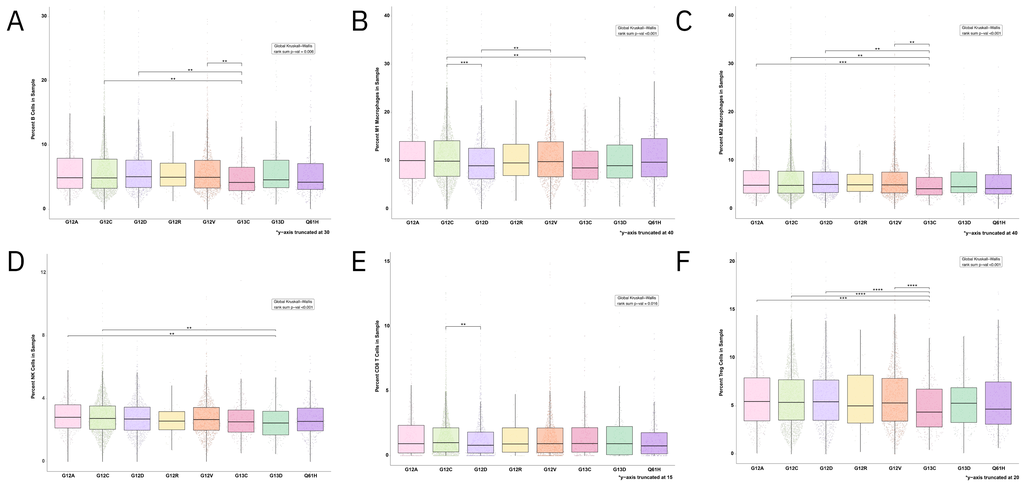



Figure 2. (A) B cells. (B) M1 macrophages. (C) M2 macrophages. (D) NK cells (E) CD8 T cells. (F) Treg cells. Statistical analysis indicates pairwise comparisons. Only q-values <0.01 are shown: **(<0.01), ***(<0.001), ****(<0.0001).
No statistically significant differences were observed for percentage of neutrophils between *KRAS* variants.

### Figure 1. Comparison of tumor immune biomarkers and lipid metabolic profiles between KRAS variants

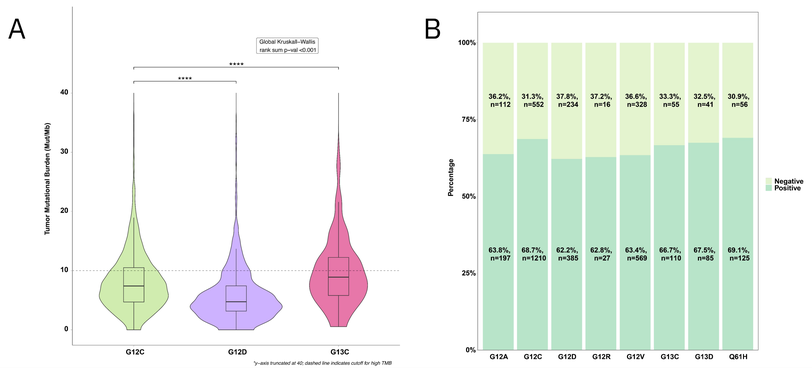



Figure 1.(A) Tumor mutational burden. (B) PD-L1 IHC positivity rates. (C) Neoantigen tumor burden. (D) Lipid metabolic gene enrichment score. KRAS G12C used as a reference category for (A), (C), and (D), and only select significant comparisons were included. FDR corrected p-values: ***(<0.001), ****(<0.0001).

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