Diminished Efficacy of Programmed Death-(Ligand)1 Inhibition in STK11- and KEAP1-Mutant Lung Adenocarcinoma Is Affected by KRAS Mutation Status

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ABSTRACT

Introduction: STK11 and KEAP1 mutations (STK11 mutant [STK11MUT] and KEAP1MUT) are among the most often mutated genes in lung adenocarcinoma (LUAD). Although STK11MUT has been associated with resistance to programmed death-(ligand)1 (PD-[L]-1) inhibition in KRASMUT LUAD, its impact on immunotherapy efficacy in KRAS wild-type (KRASWT) LUAD is currently unknown. Whether KEAP1MUT differentially affects outcomes to PD-[L]-1 inhibition in KRASMUT and KRASWT LUAD is also unknown.

Methods: Clinicopathologic and genomic data were collected from September 2013 to September 2020 from patients with advanced LUAD at the Dana-Farber Cancer Institute/Massachusetts General Hospital cohort and the Memorial Sloan Kettering Cancer Center/MD Anderson Cancer Center cohort. Clinical outcomes to PD-[L]-1 inhibition were analyzed according to KRAS, STK11, and KEAP1 mutation status in two independent cohorts. The Cancer Genome Atlas transcriptomic data were interrogated to identify differences in tumor gene expression and tumor immune cell subsets, respectively, according to KRAS/STK11 and KRAS/KEAP1 comutation status.

Results: In the combined cohort (Dana-Farber Cancer Institute/Massachusetts General Hospital + Memorial Sloan Kettering Cancer Center/MD Anderson Cancer Center) of 1261 patients (median age = 61 y [range: 22–92], 708 women [56.1%], 1065 smokers [84.4%]), KRAS mutations were detected in 536 cases (42.5%), and deleterious STK11 and KEAP1 mutations were found in 20.6% (260 of 1261) and 19.2% (231 of 1202) of assessable cases, respectively. In each independent cohort and in the combined cohort, STK11 and KEAP1 mutations were associated with significantly worse progression-free (STK11 hazard ratio [HR] = 2.04, p < 0.0001; KEAP1 HR = 2.05, p < 0.0001) and overall (STK11 HR = 2.09, p < 0.0001; KEAP1 HR = 2.24, p < 0.0001) survival to immunotherapy uniquely among KRASMUT but not KRASWT LUADs. Gene expression ontology and immune cell enrichment analyses revealed that the presence of STK11 or KEAP1 mutations results in distinct immunophenotypes in KRASMUT, but not in KRASWT, lung cancers.

Conclusions: STK11 and KEAP1 mutations confer worse outcomes to immunotherapy among patients with KRASMUT but not among KRASWT LUAD. Tumors harboring concurrent KRAS/STK11 and KRAS/KEAP1 mutations display distinct immune profiles in terms of gene expression and immune cell infiltration.

Keywords: KRAS; STK11; KEAP1; PD-[L]-1 blockade; NSCLC

Introduction

Despite marked improvements in overall survival (OS) with programmed death-(ligand)1 (PD-[L]-1) inhibition, most of the patients with metastatic NSCLC do not respond to immune checkpoint inhibition (ICI).1–4 Although tumor cell programmed death-ligand 1 (PD-L1) expression and high tumor mutational burden (TMB) are generally associated with improved benefit from ICI in NSCLC, the ability to discriminate patients who will respond to immunotherapy is limited.2,5–7 Thus, additional biomarkers of response and resistance to immunotherapy are needed to optimize treatment selection for patients with NSCLC.

KRAS mutations identify the largest subset of oncogene-driven lung adenocarcinoma (LUAD),8 and co-occurring genomic alterations in STK11 or KEAP1 genes define a unique subset of KRAS-mutant (KRASMUT) lung cancers with distinct biology and therapeutic vulnerabilities.9–12 The STK11 gene regulates diverse cellular functions including metabolism, growth, and polarity.13 STK11 loss occurs in approximately 15% of LUAD and is associated with a lack of PD-L1 expression, reduced tumor-infiltrating cytotoxic CD8+ T lymphocytes,9,14,15 and resistance to ICI in patients with KRASMUT NSCLC.16

Keap1 is a negative regulator of Nrf2, which is a master regulator of oxidative damage response.17 KEAP1 loss occurs in approximately 20% of NSCLC8 and is associated with an immunosuppressive microenvironment characterized by low infiltration of CD8+ T cells and natural killer cells in mouse models.12,18 Nevertheless, data on the correlation between KEAP1 loss and outcomes to ICI in patients with advanced LUAD are conflicting,19–21 and whether this mutation affects immunotherapy efficacy is in need of further investigation.
Because STK11 and KEAP1 mutations frequently co-occur in NSCLC, we sought to determine whether each gene mutation was independently associated with immunotherapy outcomes in NSCLC and to understand whether this impact was similar in both KRAS\textsuperscript{MUT} and KRAS wild-type (KRAS\textsuperscript{WT}) NSCLC. To unravel the potential mechanisms by which STK11 and KEAP1 alterations affect outcomes to ICI in LUAD, we also investigated the transcriptomic profiles of tumors harboring these mutations according to KRAS mutation status.

Materials and Methods

Two independent cohorts (Dana-Farber Cancer Institute and Massachusetts General Hospital [DFCI/MGH cohort] and Memorial Sloan Kettering Cancer Center and MD Anderson Cancer [MSKCC/MDACC cohort]) of patients with advanced LUAD who received PD-(L)1 inhibition and whose tumors underwent comprehensive genomic profiling were included. LUADs were characterized as STK11\textsuperscript{MUT} or KEAP1\textsuperscript{MUT} if they harbored loss-of-function alterations, including nonsense, frameshift, insertion/deletion, splice site, or pathogenic missense mutations in these genes (Supplementary Methods).

TMB was determined using the OncoPanel (DFCI) and MSK-IMPACT (MSKCC) next-generation sequencing platforms. TMB distributions were harmonized between the two platforms, as previously described.\textsuperscript{22} Determination of STK11 and KEAP1 mutation status, gene expression, and cell subset analysis from The Cancer Genome Atlas (TCGA) and detailed statistical analysis are reported in the Supplementary Methods.

Results

Patient Population

We identified a total of 1261 patients with advanced LUAD who received PD-(L)1 inhibition, with 620 (49.2\%) in a discovery cohort consisting of cases from the DFCI/MGH cohort and 641 (50.8\%) in a validation cohort from the MSKCC/MDACC cohort (Supplementary Table 1). In the combined cohort, co-occurring mutations in KRAS/STK11, KRAS/KEAP1, and STK11/KEAP1 were found in 10.9\% (138 of 1261), 8.4\% (101 of 1202), and 9.4\% (113 of 1202) of KEAP1 assessable cases, respectively (Supplementary Fig. 1).

Impact of KRAS, STK11, and KEAP1 Mutation Status on PD-L1 Expression and TMB

We first analyzed the impact of KRAS, STK11, and KEAP1 mutation status on PD-L1 expression and TMB. STK11\textsuperscript{MUT} and KEAP1\textsuperscript{MUT} LUADs had significantly lower PD-L1 expression in the DFCI/MGH, MSKCC/MDACC,
and combined cohorts, whereas \textit{KRAS}^\text{MUT} LUADs had significantly higher PD-L1 expression only in the DFCI/MGH cohort (Supplementary Fig. 2A–C). When analyzed by \textit{KRAS} status, \textit{STK11} alterations were associated with significantly lower PD-L1 expression among both \textit{KRAS}^\text{MUT} and \textit{KRAS}^\text{WT} LUADs, whereas \textit{KEAP1} mutations were associated with lower PD-L1 expression predominantly among \textit{KRAS}^\text{MUT} but not \textit{KRAS}^\text{WT} cases (Fig. 1A and B). In terms of TMB distributions, we found significantly higher median TMB in \textit{KRAS}^\text{MUT} and \textit{STK11}^\text{MUT} LUADs in the MSKCC/MDACC and in the combined cohort and among \textit{KEAP1}^\text{MUT} tumors in all the cohorts evaluated (Supplementary Fig. 2D–F). When TMB distributions were analyzed according to \textit{KRAS} status, LUADs harboring \textit{STK11} mutations had a higher TMB only among \textit{KRAS}^\text{MUT} but not \textit{KRAS}^\text{WT} cancers in the MSKCC/MDACC and in the combined cohort, whereas \textit{KEAP1}^\text{MUT} tumors had consistently higher TMB only among \textit{KRAS}^\text{MUT} but not \textit{KRAS}^\text{WT} cases, in all the cohorts evaluated (Fig. 1C and D).

\textbf{Impact of STK11 and KEAP1 Mutation Status on Clinical Outcomes to Programmed Cell Death Protein-1 Inhibition in \textit{KRAS}^\text{MUT} and \textit{KRAS}^\text{WT} LUAD}

We next analyzed the impact of \textit{STK11} mutation on ICI efficacy in all comers with LUAD and in the context of \textit{KRAS} mutation status. In both the independent cohorts, and in the combined cohort, \textit{STK11} mutation was associated with significantly shorter median progression-free survival (mPFS) and median OS (mOS) to ICI (Supplementary Fig. 3A–I). This deleterious effect of \textit{STK11} mutations on immunotherapy outcomes was largely driven by the \textit{KRAS}^\text{MUT} subgroup of LUAD. In the DFCI/MGH and MSKCC/MDACC cohorts (Supplementary Fig. 4A–D), and in the combined cohort, \textit{STK11} mutation was associated with significantly worse overall response rate (ORR), mPFS, and mOS among \textit{KRAS}^\text{MUT} LUADs but not among \textit{KRAS}^\text{WT} tumors (Figs. 2A and B and 3A–D). \textit{STK11} mutation was confirmed to be an independent predictor of shorter PFS (hazard ratio [HR] = 1.46, \( p = \text{p < 0.0001} \)).

\textbf{Figure 2. Objective response rate to PD-(L)1 inhibition according to \textit{STK11} mutation status among (A) \textit{KRAS}^\text{MUT} and (B) \textit{KRAS}^\text{WT} LUADs in the combined cohort. Objective response rate to PD-(L)1 inhibition according to \textit{KEAP1} mutation status among (C) \textit{KRAS}^\text{MUT} and (D) \textit{KRAS}^\text{WT} LUADs in the combined cohort. LUAD, lung adenocarcinoma; MUT, mutant; PD-(L)1, programmed death-(ligand)1; WT, wild-type.}
0.01) and OS (HR = 1.73, p = 0.002) to ICI in multivariable analysis in the combined cohort (Supplementary Table 2). Importantly, STK11 mutation was also associated with significantly worse clinical outcomes among KRAS\textsuperscript{MUT} LUADs across different PD-L1 expression level subgroups of less than 1%, 1% to 49%, and greater than or equal to 50%, when analyzed separately (Supplementary Fig. 5). The impact of STK11 mutation on ICI efficacy in the three most common KRAS\textsuperscript{MUT} alleles (G12C/V/D) is found in Supplementary Figure 6. Clinicopathologic characteristics of patients with KRAS\textsuperscript{MUT} and KRAS\textsuperscript{WT} LUADs according to STK11 mutation status are found in Supplementary Tables 3 and 4, whereas multivariate analyses for PFS and OS in the KRAS\textsuperscript{WT} group are found in Supplementary Table 5.

Figure 3. (A) PFS and (B) OS to PD-(L)1 inhibition according to STK11 mutation status among KRAS\textsuperscript{MUT} LUADs in the combined cohort (DFCI/MGH + MSKCC/MDACC). (C) PFS and (D) OS to PD-(L)1 inhibition according to STK11 mutation status among KRAS\textsuperscript{WT} LUADs in the combined cohort (DFCI/MGH + MSKCC/MDACC). CI, confidence interval; DFCI, Dana-Farber Cancer Institute; HR, hazard ratio; LUAD, lung adenocarcinoma; MDACC, MD Anderson Cancer Center; MGH, Massachusetts General Hospital; MSKCC, Memorial Sloan Kettering Cancer Center; MUT, mutant; OS, overall survival; PD-(L)1, programmed death-(ligand)1; PFS, progression-free survival; WT, wild-type.

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with shorter PFS (HR = 2.15, p < 0.0001) and OS (HR = 2.44, p < 0.0001) among KRAS\textsuperscript{MUT} cases in multivariable models in the combined cohort (Supplementary Table 6). When the impact of KEAP1 mutation was analyzed in the different PD-L1 subgroups, we found that KEAP1 loss was associated with worse outcomes to immunotherapy in LUADs with a PD-L1 tumor proportion score less than 1% and 1% to 49% (Supplementary Fig. 9). The impact of KEAP1 mutation on ICI efficacy according to KRAS\textsuperscript{MUT} alleles (G12C/V/D) is found in Supplementary Figure 10. Clinicopathologic characteristics of patients with KRAS\textsuperscript{MUT} and KRAS\textsuperscript{WT} LUADs according to KEAP1 mutation status are found in Supplementary Tables 7 and 8, whereas multivariate analyses for PFS and OS in the KRAS\textsuperscript{WT} group are found in Supplementary Table 9.

When the impact of STK11 and KEAP1 mutation was explored in the TCGA cohort, there was no significant effect on disease-free survival or OS (Supplementary Figs. 11A and B and 12A and B). To further explore whether STK11 and KEAP1 mutations are negative prognostic markers in the context of KRAS mutation, we also evaluated the effect of these mutations on the ORR and PFS to first-line platinum-doublet chemotherapy in a cohort of 248 patients from DFCI. We found that both

Figure 4. (A) PFS and (B) OS to PD-(L)1 inhibition according to KEAP1 mutation status among KRAS\textsuperscript{MUT} LUADs in the combined cohort (DFCI/MGH + MSKCC/MDACC). (C) PFS and (D) OS to PD-(L)1 inhibition according to KEAP1 mutation status among KRAS\textsuperscript{WT} LUADs in the combined cohort (DFCI/MGH + MSKCC/MDACC). CI, confidence interval; DFCI, Dana-Farber Cancer Institute; HR, hazard ratio; LUAD, lung adenocarcinoma; MDACC, MD Anderson Cancer Center; MGH, Massachusetts General Hospital; MSKCC, Memorial Sloan Kettering Cancer Center; MUT, mutant; OS, overall survival; PD-(L)1, programmed death-(ligand)1; PFS, progression-free survival; WT, wild-type.
STK11 and KEAP1 mutations were associated with a significantly shorter PFS among KRAS\textsuperscript{MUT} but not KRAS\textsuperscript{WT} LUADs (Supplementary Fig. 13A–D), suggesting STK11 or KEAP1 loss may also influence efficacy of chemotherapy in the setting of KRAS\textsuperscript{MUT} but not KRAS\textsuperscript{WT} advanced LUAD.

Because LUADs harbor EGFR mutations or ALK rearrangements, and LUADs from never smokers do not typically respond to ICI,\textsuperscript{2,23,24} to ensure that our findings were not due to an enrichment in EGFR/ALK-positive LUAD or in LUAD from never smokers in the KRAS\textsuperscript{WT} cohort (Supplementary Tables 4, 8, and 10), we analyzed the impact of STK11 and KEAP1 mutations on clinical outcomes in KRAS\textsuperscript{WT} NSCLC after excluding EGFR/ALK-positive cases and never smokers. We confirmed that both STK11 and KEAP1 mutations had no impact on ORR, mPFS, or mOS among KRAS\textsuperscript{WT}/EGFR\textsuperscript{WT}/ALK\textsuperscript{WT} tumors (Supplementary Figs. 14A–C and 15A–C) and among ever smokers (Supplementary Figs. 14D–F and 15D–F).

Because STK11 and KEAP1 mutations tend to co-occur (Supplementary Table 10), we lastly evaluated whether among KRAS\textsuperscript{MUT} LUADs, STK11 and KEAP1 mutations affected ICI efficacy in KEAP1\textsuperscript{WT} and STK11\textsuperscript{WT} NSCLCs, respectively. We first analyzed the impact of STK11 mutation among KRAS\textsuperscript{MUT}/KEAP1\textsuperscript{WT} LUADs and found that cases harboring and STK11 mutation had a significantly lower ORR (p = 0.004), shorter mPFS (HR = 1.93, \(p < 0.0001\)), and mOS (HR = 1.89, \(p < 0.0001\)) to ICI compared with KRAS\textsuperscript{MUT}/KEAP1\textsuperscript{WT}/STK11\textsuperscript{WT} cases (Supplementary Fig. 16A–C).

When we analyzed the effect of KEAP1 mutation among KRAS\textsuperscript{MUT}/STK11\textsuperscript{WT} cases, there was no difference in ORR to immunotherapy between KEAP1\textsuperscript{MUT} and KEAP1\textsuperscript{WT} cases (p = 0.99, Supplementary Fig. 16D). Nevertheless, the mPFS and mOS were significantly shorter among cases with KEAP1 mutation compared with KRAS\textsuperscript{MUT}/STK11\textsuperscript{WT}/KEAP1\textsuperscript{WT} cases (PFS HR = 1.80, \(p = 0.002\); mOS HR = 2.06, \(p = 0.002\), Supplementary Fig. 16E and F), suggesting the deleterious impact of KEAP1 mutation on ICI efficacy was independent from the presence of a concurrent STK11 mutation. We confirmed the independent contributions of STK11 and KEAP1 mutations to worse outcome to ICI among KRAS\textsuperscript{MUT} cases by lastly testing them in a multivariate model including the interaction between mutations in the two genes (Supplementary Fig. 17A and B). Both STK11 and KEAP1 mutations were associated with lower PFS and OS, whereas the interaction term was not associated with PFS and OS, suggesting an additive effect of STK11 and KEAP1 mutations on immunotherapy outcomes. The impact of concurrent STK11/KEAP1 mutations in these two genes on ORR, mPFS, and mOS to ICI among KRAS\textsuperscript{MUT} LUAD is found in Figure 5A–C. The overlay of KRAS status (KRAS\textsuperscript{MUT} and KRAS\textsuperscript{WT}) with KEAP1, STK11, and concurrent STK11/KEAP1 mutation is found in Supplementary Figure 18A–C.

**Gene Ontology Analysis Reveals That STK11\textsuperscript{MUT} and KEAP1\textsuperscript{MUT} LUADs Have Different Transcriptomic Profiles According to KRAS Mutation Status**

To unravel the potential mechanisms by which the deleterious impact of STK11 and KEAP1 mutations on outcomes to ICI in LUAD is primarily driven by KRAS mutation, we investigated the transcriptomic profiles of tumors harboring these mutations in KRAS\textsuperscript{MUT} and KRAS\textsuperscript{WT} LUADs. RNA sequencing data of 513 LUADs in the TCGA data set were analyzed according to KRAS/STK11 and KRAS/KEAP1 comutation status (Supplementary Methods).

**Figure 5.** (A) Objective response rate, (B) PFS, and (C) OS to PD-(L)1 inhibition according to STK11/KEAP1 comutation status, among patients with KRAS\textsuperscript{MUT} lung adenocarcinoma in the combined cohort. CI, confidence interval; MUT, mutant; OS, overall survival; PD-(L)1, programmed death-(ligand)1; PFS, progression-free survival; WT, wild-type.
We first identified genes that were differentially expressed among KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> versus KRAS<sup>MUT</sup>/STK11<sup>WT</sup> LUADs and among KRAS<sup>WT</sup>/STK11<sup>MUT</sup> versus KRAS<sup>WT</sup>/STK11<sup>MUT</sup> cancers. Next, we performed a hierarchical gene ontology analysis only on the subsets of genes which were differentially regulated in KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> tumors versus KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> but not among KRAS<sup>WT</sup>/STK11<sup>MUT</sup> versus KRAS<sup>WT</sup>/STK11<sup>MUT</sup> cancers. Among the 22 significant terminal pathways identified (Supplementary Table 11), 13 involved immune-related processes which are significantly down-regulated in KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> compared with KRAS<sup>MUT</sup>/STK11<sup>WT</sup> LUADs, but not in KRAS<sup>WT</sup>/STK11<sup>MUT</sup> compared with KRAS<sup>WT</sup>/STK11<sup>WT</sup> LUADs. We noted that genes of the class II HLA family, including CD74, HLA-DOA, HLA-DRB5, HLA-DRB1, and HLA-DMB, were significantly down-regulated in KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> versus KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> cancers. Also, genes encoding for chemokines and their receptors that are critical for T-cell, natural killer cell, and myeloid-cell recruitment and migration, such as CXCL14, CCL23, CX3CR1, and CCR6, were also significantly down-regulated in KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> tumors compared with KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> cancers. In addition, SIGLE<sup>6</sup>-14, an enhancer of inflammasome activation and macrophage interleukin-1β release, exhibited marked down-regulation among KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> tumors versus KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> tumors. The full list of genes in the 13 prioritized pathways that are significantly down-regulated in KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> tumors versus KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> tumors but not among KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> versus KRAS<sup>MUT</sup>/STK11<sup>MUT</sup> cancers is found in Supplementary Table 12.

We next identified genes that were differentially expressed among KRAS<sup>MUT</sup>/KEAP1<sup>MUT</sup> versus KRAS<sup>MUT</sup>/KEAP1<sup>WT</sup> LUADs and among KRAS<sup>WT</sup>/KEAP1<sup>MUT</sup> versus KRAS<sup>WT</sup>/KEAP1<sup>WT</sup> cancers and performed gene ontology analysis on the subsets of genes which were uniquely
up-regulated in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{WT}} \) tumors versus \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \). Among the 13 terminal pathways identified (Supplementary Table 13), 11 were involved in immune-related processes, including the following gene ontology terms: external side of plasma membrane, regulation of T-cell activation, T-cell receptor signaling, defense response to virus, regulation of leukocyte cell-to-cell adhesion, and lymphocyte migration (Fig. 6B). The log2 fold change of the individual top 20 genes included in each of the six prioritized pathways is found in Supplementary Figure 20. Interestingly, we found several genes involved in monocyte, T-cell, and dendritic cell recruitment to be significantly down-regulated in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) tumors compared with \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{WT}} \) cases, including CCL2, CXCL6, CCR1, CCR6, CCR7, and ITGAM. In addition, genes encoding proinflammatory cytokines and their receptors, such as TNF, TNFSF8, TNFRSF9, IL1B, and IL2RA, were also markedly down-regulated in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) tumors versus \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{WT}} \) cancers. Importantly, we also noted positive regulators of type I interferon and other inflammatory cytokine production, such as TMEM173 (STING), DDX58, TLR4, and TLR7, to be markedly down-regulated in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) versus \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{WT}} \) cancers. The full list of genes in the 11 pathways which are significantly down-regulated in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) tumors versus \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{WT}} \) but not among \( \text{KRAS}^{\text{WT}}/\text{KEAP1}^{\text{MUT}} \) versus \( \text{KRAS}^{\text{WT}}/\text{KEAP1}^{\text{WT}} \) is found in Supplementary Table 14.

Cell-Type Enrichment Analysis Reveals That \( \text{STK11}^{\text{MUT}} \) and \( \text{KEAP1}^{\text{MUT}} \) Tumors Have Different Immunophenotypes According to KRAS Mutation Status

We lastly evaluated whether LUADs harboring \( \text{STK11} \) or \( \text{KEAP1} \) mutations also have distinct immune cell subsets according to the presence or absence of concurrent KRAS mutation; we performed cell-type enrichment analysis by deconvoluting gene expression data into tumor-associated cell population.

First, we evaluated whether \( \text{STK11} \) mutation was associated with different cell infiltration according to KRAS mutation status and identified six immune cell types that were significantly enriched in \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{WT}} \) tumors compared with \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{MUT}} \) tumors but not in \( \text{KRAS}^{\text{WT}}/\text{STK11}^{\text{WT}} \) tumors versus \( \text{KRAS}^{\text{WT}}/\text{STK11}^{\text{MUT}} \) cancers, including M1 macrophages (\( p < 0.01 \)), M2 macrophages (\( p < 0.01 \)), granulocyte-monocyte progenitors (\( p = 0.02 \)), CD4+ effector memory cells (\( p = 0.01 \)), and B cells (\( p = 0.04 \)) (Fig. 6C). In addition, both the immune score (sum of B cells, T cells, and myeloid-derived cells) and the microenvironment score (composite score of the immune score and stroma cell signatures) were significantly enriched only in \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{WT}} \) tumors compared with \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{MUT}} \) (\( p < 0.001 \) and \( p < 0.01 \), respectively; Fig. 6C).

Conversely, \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{MUT}} \) tumors were significantly enriched in neutrophils (\( p < 0.01 \)), compared with \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{WT}} \) tumors (Fig. 6C).

We next investigated whether \( \text{KEAP1} \) mutation was also associated with a distinct pattern of infiltrating cell types in \( \text{KRAS}^{\text{MUT}} \) and \( \text{KRAS}^{\text{WT}} \) LUADs. We identified four cell types including CD8+ T cells (\( p < 0.001 \)), CD8+ central memory T cells (\( p < 0.01 \)), CD8+ naive T cells (\( p = 0.02 \)), and B cells (\( p = 0.01 \)) which were uniquely enriched in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{WT}} \) tumors versus \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) but not among \( \text{KRAS}^{\text{WT}}/\text{KEAP1}^{\text{WT}} \) tumors versus \( \text{KRAS}^{\text{WT}}/\text{KEAP1}^{\text{MUT}} \) cancers (Fig. 6D). Instead, mesenchymal stem cells were found to be significantly enriched in only \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) tumors compared with \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{WT}} \) tumors (\( p = 0.02 \)) (Fig. 6D).

Discussion

In this study, we reveal that mutations in \( \text{STK11} \) and \( \text{KEAP1} \) are frequent and define major subsets of \( \text{KRAS}^{\text{MUT}} \) LUADs, characterized by unique immune profiles and poor outcomes to ICI in two independent cohorts. Our results extend previous reports of LUAD with \( \text{STK11} \) mutations16 and identify loss-of-function mutations in \( \text{KEAP1} \) as a frequent and independent driver of resistance to ICI in patients with advanced \( \text{KRAS}^{\text{MUT}} \) LUAD. To gain insights to potential mechanisms by which \( \text{STK11} \) and \( \text{KEAP1} \) loss exerts deleterious effects on PD-(L)1 inhibition among \( \text{KRAS}^{\text{MUT}} \) but not \( \text{KRAS}^{\text{WT}} \) LUAD, we found that \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{MUT}} \) tumors had a significant down-regulation of MHC class II compared with \( \text{KRAS}^{\text{MUT}}/\text{STK11}^{\text{WT}} \), including HLA-DOA, HLA-DRB5, HLA-DRB1, and HLA-DMB. By contrast, \( \text{STK11} \) mutation was not associated with MHC class II pathway deregulation among \( \text{KRAS}^{\text{WT}} \) cases. The expression of MHC class II-restricted antigens by tumor cells is required for CD4+ T-cell activation to elicit antitumor immune responses,25 and MHC class II expression has been associated with improved PFS and OS in patients treated with ICI in multiple cancer types.26–28

\( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) LUAD was also found to have a unique gene expression profile, characterized by significant down-regulation of positive regulators of type I interferon and other inflammatory cytokines, including TMEM173 (STING), DDX58, TLR4, and TLR7. Although \( \text{STK11} \) loss has previously been reported to result in impaired tumor immunogenicity in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) LUAD, whether a similar mechanism could lead to impaired tumor immunogenicity in \( \text{KRAS}^{\text{MUT}}/\text{KEAP1}^{\text{MUT}} \) LUAD is unknown and deserves additional exploration.
These findings have implications for clinical trial interpretation and design and for treatment selection. Our study suggests that immunotherapy clinical trials should consider using stratification measures to balance randomized groups for STK11 and KEAP1 mutation status and ensure that differences in outcomes are due to therapeutic interventions rather than variations in STK11 or KEAP1 mutation frequency, especially in KRAS\textsuperscript{MUT} NSCLC. Our findings could also inform on how to sequence or combine future treatment strategies in KRAS\textsuperscript{MUT} LUAD. Preliminary data have revealed that direct KRAS inhibitors can produce responses in approximately 35% to 45% of patients with KRAS G12C\textsuperscript{MUT} NSCLC.\textsuperscript{29–31} As more effective treatment options become available for KRAS\textsuperscript{MUT} LUAD, STK11 and KEAP1 mutation status might be a useful biomarker in determining the optimal treatment sequence, and KRAS G12C inhibitors might be better used before ICI in genomic subsets of NSCLC which are predicted not to respond to PD-1 based regimens. Whether KRAS inhibition could be used in combination with immunotherapy is an area of increasing interest. Preclinical data have revealed that KRAS G12C inhibition reinvigorates the TME with CD8+ T cells, macrophages, and CD103+ cross-presenting dendritic cells, suggesting direct KRAS inhibitors may synergize with ICI.\textsuperscript{32} Particularly among genomically defined LUADs that are not predicted to respond to immunotherapy alone. Phase I/II trials of sotorasib and adagrasib in combination with pembrolizumab in patients with advanced NSCLC with KRAS G12C mutation are currently ongoing (NCT04613596). In this study, we also found that patients with KRAS\textsuperscript{MUT} LUADs and STK11 or KEAP1 mutation had worse clinical outcomes to platinum-based chemotherapy, which may argue against an only predictive nature of concurrent KRAS/STK11 and KRAS/KEAP1 alterations in LUADs. Consistently with our findings, KEAP1 and STK11 mutations have been previously reported to correlate with inferior clinical outcomes in patients treated with chemotherapy.\textsuperscript{19,33} Loss of STK11 and constitutive activation of Nrf2 in KEAP1\textsuperscript{MUT} tumors have been found to promote transcription of various cytoprotective genes that are associated with antioxidant and detoxification enzymes and protect cancer cells from ferroptosis, leading to chemoresistance in various cancers.\textsuperscript{11,34–36} Therefore, STK11 and KEAP1 mutation could potentially be predictive of worse outcomes to both chemotherapy and immunotherapy because of their pleiotropic effects on cancer cell metabolism and immune system engagement. Conversely, STK11 mutation does not seem to affect the efficacy of KRAS G12C inhibition in patients with KRAS\textsuperscript{G12C}-mutated NSCLC receiving sotorasib.\textsuperscript{37}

Limitations of this study include the retrospective design and the lack of validation from published randomized clinical trials of ICI versus chemotherapy. In addition, PD-L1 expression was not available in 35.9% of the samples. Nevertheless, to account for the potential selection bias resulting from PD-L1 tumor proportion score missingness, we used an inverse probability weighting in Cox regression analysis. Lastly, it should be acknowledged that KRAS\textsuperscript{WT} LUADs are highly heterogeneous in terms of clinicopathologic and genomic features and include subsets of tumors that typically not respond to immunotherapy, such as those harboring EGFR mutations and ALK rearrangements and those from never smokers. To address this bias, we analyzed the impact of STK11 and KEAP1 mutations on clinical outcomes in KRAS\textsuperscript{WT} NSCLC after excluding EGFR/ALK-positive cases and excluding never smokers and confirmed that both STK11 and KEAP1 mutation had no impact on immunotherapy efficacy among KRAS\textsuperscript{WT}/EGFR\textsuperscript{WT}/ALK\textsuperscript{WT} tumors and among ever smokers.

In conclusion, we reveal that STK11 and KEAP1 mutations confer worse outcomes to immunotherapy among patients with KRAS\textsuperscript{MUT} but not among KRAS\textsuperscript{WT} LUAD and that tumors harboring concurrent KRAS/STK11 and KRAS/KEAP1 mutations display distinct immune profiles. Preclinical studies are urgently needed to further dissect the molecular mechanism underlying these correlations and identify novel therapeutic vulnerabilities.

CRediT Authorship Contribution Statement

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Supplementary Data
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References


