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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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Supplementary Data

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Abstract

Introduction: *STK11* and *KEAP1* mutations (*STK11* mutant [*STK11* ^{MUT}] and *KEAP1*^{MUT}) are among the most often mutated genes in lung adenocarcinoma (LUAD). Although *STK11*^{MUT} has been associated with resistance to programmed death-(ligand)1 (PD-[L]1) inhibition in *KRAS*^{MUT} LUAD, its impact on immunotherapy efficacy in *KRAS* wild-type (*KRAS*^{WT}) LUAD is currently unknown. Whether *KEAP1*^{MUT} differentially affects outcomes to PD-(L)1 inhibition in *KRAS*^{MUT} and *KRAS*^{WT} 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 *KRAS*^{MUT} but not *KRAS*^{WT} LUADs. Gene expression ontology and immune cell enrichment analyses revealed that the presence of *STK11* or *KEAP1* mutations results in distinct immunophenotypes in *KRAS*^{MUT}, but not in *KRAS*^{WT}, lung cancers.

Conclusions: *STK11* and *KEAP1* mutations confer worse outcomes to immunotherapy among patients with *KRAS*^{MUT} but not among *KRAS*^{WT} 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 (*KRAS*^{MUT}) 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 *KRAS*^{MUT} 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 NSCLC[8] 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*^{MUT} and *KRAS* wild-type (*KRAS*^{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*^{MUT} or *KEAP1*^{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.[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*^{MUT} and *KEAP1*^{MUT} LUADs had significantly lower PD-L1 expression in the DFCI/MGH, MSKCC/MDACC, and combined cohorts, whereas *KRAS*^{MUT} LUADs had significantly higher PD-L1 expression only in the DFCI/MGH cohort (Supplementary Fig. 2A-C). When analyzed by *KRAS* status, *STK11* alterations were associated with significantly lower PD-L1 expression among both *KRAS*^{MUT} and *KRAS*^{WT} LUADs, whereas *KEAP1* mutations were associated with lower PD-L1 expression predominantly among *KRAS*^{MUT} but not *KRAS*^{WT} cases (Fig. 1A and B). In terms of TMB distributions, we found significantly higher median TMB in *KRAS*^{MUT} and *STK11*^{MUT} LUADs in the MSKCC/MDACC and in the combined cohort and among *KEAP1*^{MUT} tumors in all the cohorts evaluated (Supplementary Fig. 2D-F). When TMB distributions were analyzed according to *KRAS* status, LUADs harboring *STK11* mutations had a higher TMB only among *KRAS*^{MUT} but not *KRAS*^{WT} cancers in the MSKCC/MDACC and in the combined cohort, whereas *KEAP1* ^{MUT} tumors had consistently higher TMB only among *KRAS*^{MUT} but not *KRAS*^{WT} cases, in all the cohorts evaluated (Fig. 1C and D).

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

We next analyzed the impact of *STK11* mutation on ICI efficacy in all comers with LUAD and in the context of *KRAS* mutation status. In both the independent cohorts, and in the combined cohort, *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 *STK11* mutations on immunotherapy outcomes was largely driven by the *KRAS*^{MUT} subgroup of LUAD. In the DFCI/MGH and MSKCC/MDACC cohorts

(Supplementary Fig. 4A-D), and in the combined cohort, *STK11* mutation was associated with significantly worse overall response rate (ORR), mPFS, and mOS among *KRAS*^{MUT} LUADs but not among *KRAS*^{WT} tumors (Figs. 2A and B and 3A-D). *STK11* mutation was confirmed to be an independent predictor of shorter PFS (hazard ratio [HR] = 1.46, $p = 0.01$) 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*^{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*^{MUT} alleles (G12C/V/D) is found in Supplementary Figure 6. Clinicopathologic characteristics of patients with *KRAS*^{MUT} and *KRAS*^{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*^{WT} group are found in Supplementary Table 5.

We next analyzed the impact of *KEAP1* mutation on immunotherapy efficacy. In all comers with LUAD, *KEAP1* mutation was associated with diminished survival from ICI (Supplementary Fig. 7A-I). Here, too, *KEAP1* mutation affected immunotherapy efficacy in the *KRAS*^{MUT} subgroup but not among *KRAS*^{WT} cases in the two independent cohorts (Supplementary Fig. 8A-D) and in the combined cohort (Figs. 2C and D and 4A-D). *KEAP1* mutation also retained a significant association with shorter PFS (HR = 2.15, $p < 0.0001$) and OS (HR = 2.44, $p < 0.0001$) among *KRAS*^{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*^{MUT} alleles (G12C/V/D) is found in Supplementary Figure 10. Clinicopathologic characteristics of patients with *KRAS*^{MUT} and *KRAS*^{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*^{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 *STK11* and *KEAP1* mutations were associated with a significantly shorter PFS among *KRAS*^{MUT} but not *KRAS*^{WT} LUADs (Supplementary Fig. 13A-D), suggesting *STK11* or *KEAP1* loss may also influence efficacy of chemotherapy in the setting of *KRAS*^{MUT} but not *KRAS*^{WT} advanced LUAD.

Because LUADs harbor *EGFR* mutations or *ALK* rearrangements, and LUADs from never smokers do not typically respond to ICI,[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*^{WT} cohort (Supplementary Tables 4, 8, and 10), we analyzed the impact of

STK11 and *KEAP1* mutations on clinical outcomes in *KRAS*^{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*^{WT}/*EGFR*^{WT}/*ALK*^{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*^{MUT} LUADs, *STK11* and *KEAP1* mutations affected ICI efficacy in *KEAP1*^{WT} and *STK11*^{WT} NSCLCs, respectively. We first analyzed the impact of *STK11* mutation among *KRAS*^{MUT}/*KEAP1*^{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*^{MUT}/*KEAP1*^{WT}/*STK11*^{WT} cases (Supplementary Fig. 16A-C).

When we analyzed the effect of *KEAP1* mutation among *KRAS*^{MUT}/*STK11*^{WT} cases, there was no difference in ORR to immunotherapy between *KEAP1*^{MUT} and *KEAP1*^{WT} cases ($p = 0.99$, Supplementary Fig. 16D). Nevertheless, the mPFS and mOS were significantly shorter among cases with *KEAP1* mutation compared with *KRAS*^{MUT}/*STK11*^{WT}/*KEAP1*^{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*^{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*^{MUT} LUAD is found in Figure 5A-C. The overlay of *KRAS* status (*KRAS*^{MUT} and *KRAS*^{WT}) with *KEAP1, STK11,* and concurrent *STK11/KEAP1* mutation is found in Supplementary Figure 18A-C.

Gene Ontology Analysis Reveals That STK11^{MUT} *and* KEAP1^{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*^{MUT} and *KRAS*^{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).

We first identified genes that were differentially expressed among *KRAS*^{MUT}/*STK11*^{WT} versus *KRAS*^{MUT}/*STK11*^{MUT} LUADs and among *KRAS*^{WT}/*STK11*^{WT} versus *KRAS*^{WT}/*STK11*^{MUT} cancers. Next, we performed a hierarchical gene ontology analysis only on the subsets of genes which were differentially regulated in *KRAS*^{MUT}/*STK11*^{WT} tumors versus *KRAS*^{MUT}/*STK11*^{MUT} but not among *KRAS*^{WT}/*STK11*^{WT} versus *KRAS*^{WT}/*STK11*^{MUT}.

Among the 22 significant terminal pathways identified (Supplementary Table 11), 13 involved in immune-mediated processes were markedly down-regulated in *KRAS*^{MUT}/*STK11*^{MUT} compared with *KRAS*^{MUT}/*STK11*^{WT} LUADs, including the MHC class II protein complex, T-cell activation, immune response-activating signaling, leukocyte migration, leukocyte degranulation, and myeloid leukocyte activation (Fig. 6A). The $\log_2$ fold change in mRNA expression of the top 20 individual genes included in the six prioritized pathways which were significantly down-regulated in *KRAS*^{MUT}/*STK11*^{MUT} tumors compared with *KRAS*^{MUT}/*STK11*^{WT} LUAD is found in Supplementary Figure 19. 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*^{MUT}/*STK11*^{MUT} LUADs compared with *KRAS*^{MUT}/*STK11*^{WT} tumors. 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*^{MUT}/*STK11*^{MUT} tumors compared with *KRAS*^{MUT}/*STK11*^{WT} cancers. In addition, *SIGLEG-14,* an enhancer of inflammasome activation and macrophage interleukin-1β release, exhibited marked down-regulation among *KRAS*^{MUT}/*STK11*^{MUT} versus *KRAS*^{MUT}/*STK11*^{WT} LUADs. The full list of genes in the 13 prioritized pathways that are significantly down-regulated in *KRAS*^{MUT}/*STK11*^{MUT} tumors versus *KRAS*^{MUT}/*STK11*^{WT} but not among *KRAS*^{WT}/*STK11*^{MUT} versus *KRAS*^{WT}/*STK11*^{WT} is found in Supplementary Table 12.

We next identified genes that were differentially expressed among *KRAS*^{MUT}/*KEAP1*^{MUT} versus *KRAS*^{MUT}/*KEAP1*^{WT} LUADs and among *KRAS*^{WT}/*KEAP1*^{MUT} versus *KRAS*^{WT}/*KEAP1*^{WT} cancers and performed gene ontology analysis on the subsets of genes which were uniquely up-regulated in *KRAS*^{MUT}/*KEAP1*^{WT} tumors versus *KRAS*^{MUT}/*KEAP1*^{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 $\log_2$ 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 *KRAS*^{MUT}/*KEAP1*^{MUT} tumors compared with *KRAS*^{MUT}/*KEAP1*^{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 *KRAS*^{MUT}/*KEAP1*^{MUT} tumors versus *KRAS*^{MUT}/*KEAP1*^{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 *KRAS*^{MUT}/*KEAP1*^{MUT} versus *KRAS*^{MUT}/*KEAP1*^{WT} cancers. The full list of genes in the 11 pathways which are significantly down-regulated in *KRAS*^{MUT}/*KEAP1*^{MUT} tumors versus *KRAS*^{MUT}/*KEAP1*^{WT} but not among *KRAS*^{WT}/*KEAP1*^{MUT} versus *KRAS*^{WT}/*KEAP1*^{WT} is found in Supplementary Table 14.

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

We lastly evaluated whether LUADs harboring *STK11* or *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 *STK11* mutation was associated with different cell infiltration according to *KRAS* mutation status and identified six immune cell types that were significantly enriched in *KRAS*^{MUT}/*STK11*^{WT} tumors compared with *KRAS*^{MUT}/*STK11*^{MUT} tumors but not in *KRAS*^{WT}/*STK11*^{WT} tumors versus *KRAS*^{WT}/*STK11*^{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 *KRAS*^{MUT}/*STK11*^{WT} tumors compared with *KRAS*^{MUT}/*STK11*^{MUT} ($p < 0.001$ and $p < 0.01$, respectively; Fig. 6C). Conversely, *KRAS*^{MUT}/*STK11*^{MUT} tumors were significantly enriched in neutrophils ($p < 0.01$), compared with *KRAS*^{MUT}/*STK11*^{WT} tumors (Fig. 6C).

We next investigated whether *KEAP1* mutation was also associated with a distinct pattern of infiltrating cell types in *KRAS*^{MUT} and *KRAS*^{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 *KRAS*^{MUT}/*KEAP1*^{WT} tumors versus *KRAS*^{MUT}/*KEAP1*^{MUT} but not among *KRAS*^{WT}/*KEAP1*^{WT} tumors versus KRAS^{WT}/*KEAP1*^{MUT} cancers (Fig. 6D). Instead, mesenchymal stem cells were found to be significantly enriched in only *KRAS*^{MUT}/*KEAP1*^{MUT} tumors compared with *KRAS*^{MUT}/*KEAP1*^{WT} tumors ($p = 0.02$) (Fig. 6D).

Discussion

In this study, we reveal that mutations in *STK11* and *KEAP1* are frequent and define major subsets of *KRAS*^{MUT} LUADs, characterized by unique immune profiles and poor outcomes to ICI in two independent cohorts. Our results extend previous reports of LUAD with *STK11* mutations[16] and identify loss-of-function mutations in *KEAP1* as a frequent and independent driver of resistance to ICI in patients with advanced *KRAS*^{MUT} LUAD. To gain insights to potential mechanisms by which *STK11* and *KEAP1* loss exerts deleterious effects on PD-(L)1 inhibition among *KRAS*^{MUT} but not *KRAS*^{WT} LUAD, we found that *KRAS*^{MUT}/*STK11*^{MUT} tumors had a significant down-regulation of MHC class II compared with *KRAS*^{MUT}/*STK11*^{WT}, including *HLA-DOA, HLA-DRB5, HLA-DRB1,* and *HLA-DMB*. By contrast, *STK11* mutation was not associated with MHC class II pathway deregulation among *KRAS*^{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]

KRAS^{MUT}/*KEAP1*^{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 *STK11* loss has previously been reported to result in marked silencing of *STING* expression in *KRAS*^{MUT} LUAD, whether a similar mechanism could lead to impaired tumor immunogenicity in *KRAS*^{MUT}/*KEAP1*^{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* comutation status and ensure that differences in outcomes are due to therapeutic interventions rather than variations in *STK11* or *KEAP1* mutation frequency, especially in *KRAS*^{MUT} NSCLC. Our findings could also inform on how to sequence or combine future treatment strategies in *KRAS*^{MUT} LUAD. Preliminary data have revealed that direct KRAS inhibitors can produce responses in approximately 35% to 45% of patients with *KRAS* G12C^{MUT} NSCLC.[29-31] As more effective treatment options become available for *KRAS*^{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,[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 (NCT03600883, NCT04613596).

In this study, we also found that patients with *KRAS*^{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.[19,33] Loss of *STK11* and constitutive activation of Nrf2 in *KEAP1*^{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.[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*^{G12C}-mutated NSCLC receiving sotorasib.[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*^{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*^{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*^{WT}/*EGFR*^{WT}/*ALK*^{WT} tumors and among ever smokers.

In conclusion, we reveal that *STK11* and *KEAP1* mutations confer worse outcomes to immunotherapy among patients with *KRAS*^{MUT} but not among *KRAS*^{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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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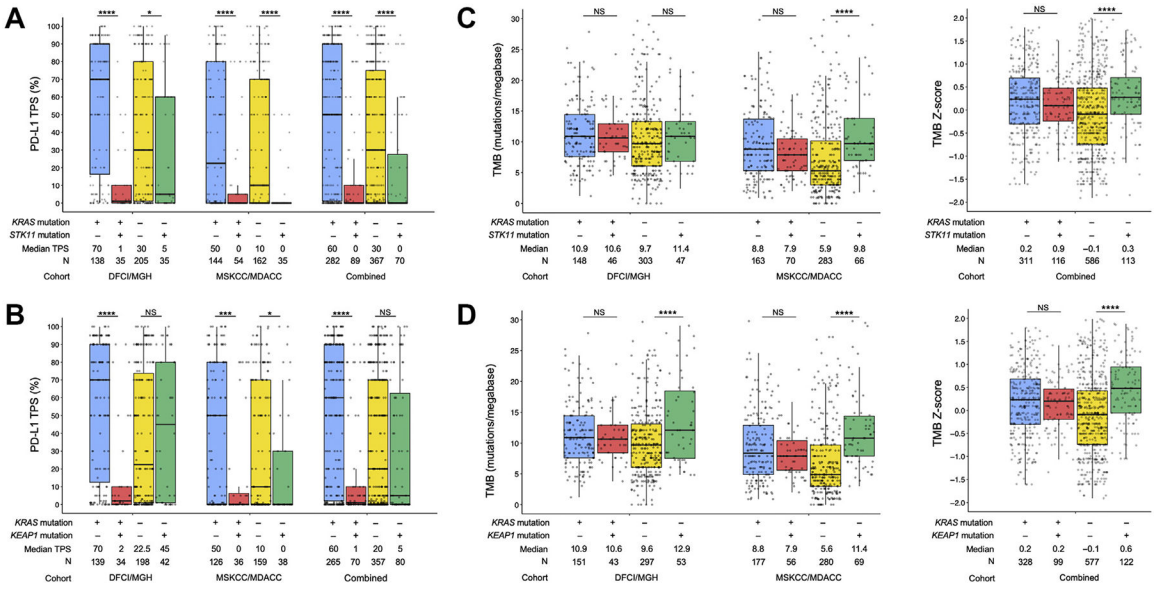

Figure 1.

PD-L1 expression according to (*A*) *KRAS/STK11* comutation status and (*B*) *KRAS/KEAP1* comutation status, in the DFCI/MGH, MSKCC/MDACC, and combined cohorts. (*C*) Tumor mutational burden according to *KRAS/STK11* comutation status, in the DFCI/MGH, MSKCC/MDACC, and combined cohorts. (*D*) Tumor mutational burden according to *KRAS/KEAP1* comutation status, in the DFCI/MGH, MSKCC/MDACC, and combined cohorts. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. DFCI, Dana-Farber Cancer Institute; MDACC, MD Anderson Cancer Center; MGH, Massachusetts General Hospital; MSKCC, Memorial Sloan Kettering Cancer Center; NS, not significant; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden; TPS, tumor proportion score

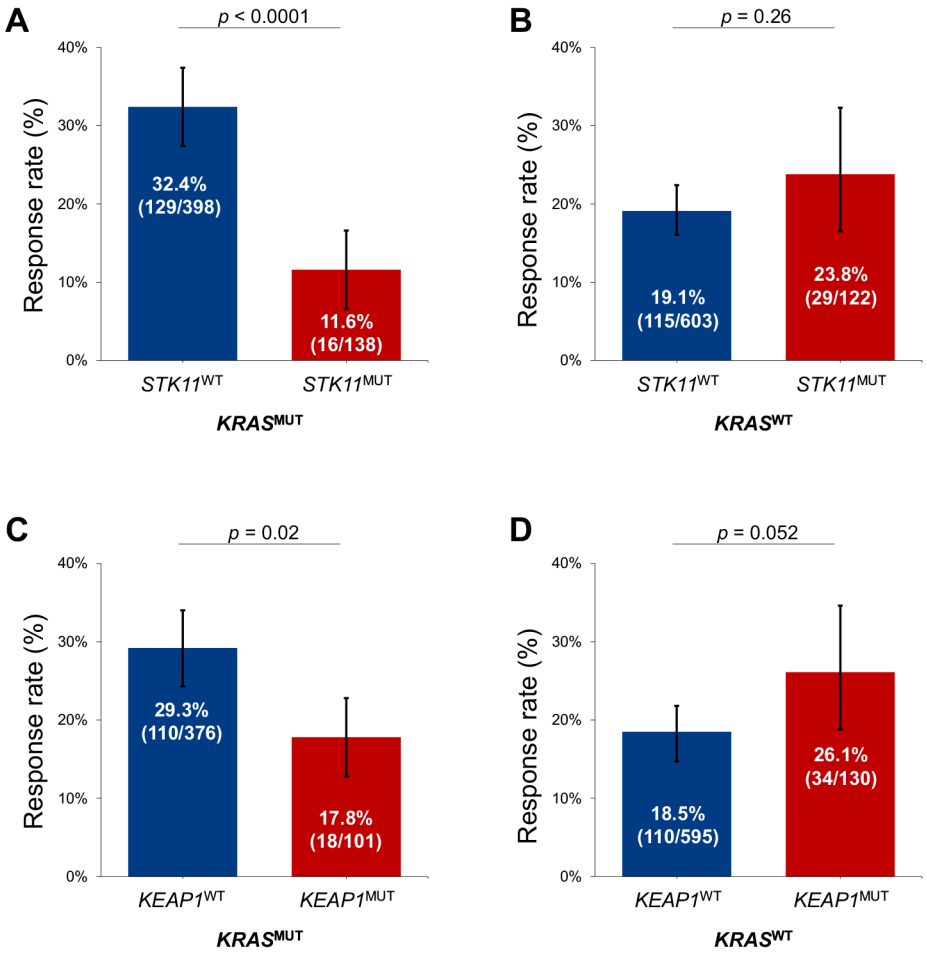

Figure 2.

Objective response rate to PD-(L)1 inhibition according to *STK11* mutation status among (*A*) *KRAS*^{MUT} and (*B*) *KRAS*^{WT} LUADs in the combined cohort. Objective response rate to PD-(L)1 inhibition according to *KEAP1* mutation status among (*C*) *KRAS*^{MUT} and (*D*) *KRAS*^{WT} LUADs in the combined cohort. LUAD, lung adenocarcinoma; MUT, mutant; PD-(L)1, programmed death-(ligand)1; WT, wild-type.

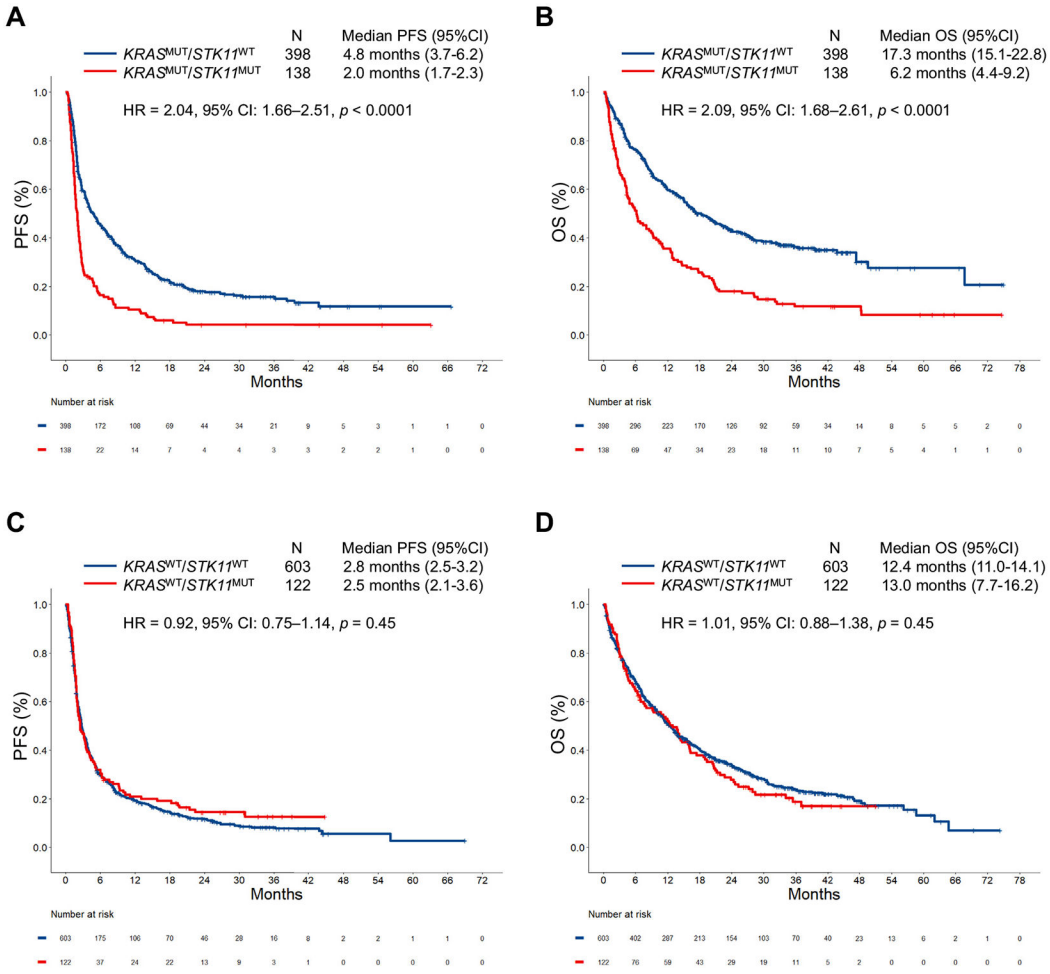

Figure 3.

(*A*) PFS and (*B*) OS to PD-(L)1 inhibition according to *STK11* mutation status among *KRAS*^{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*^{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.

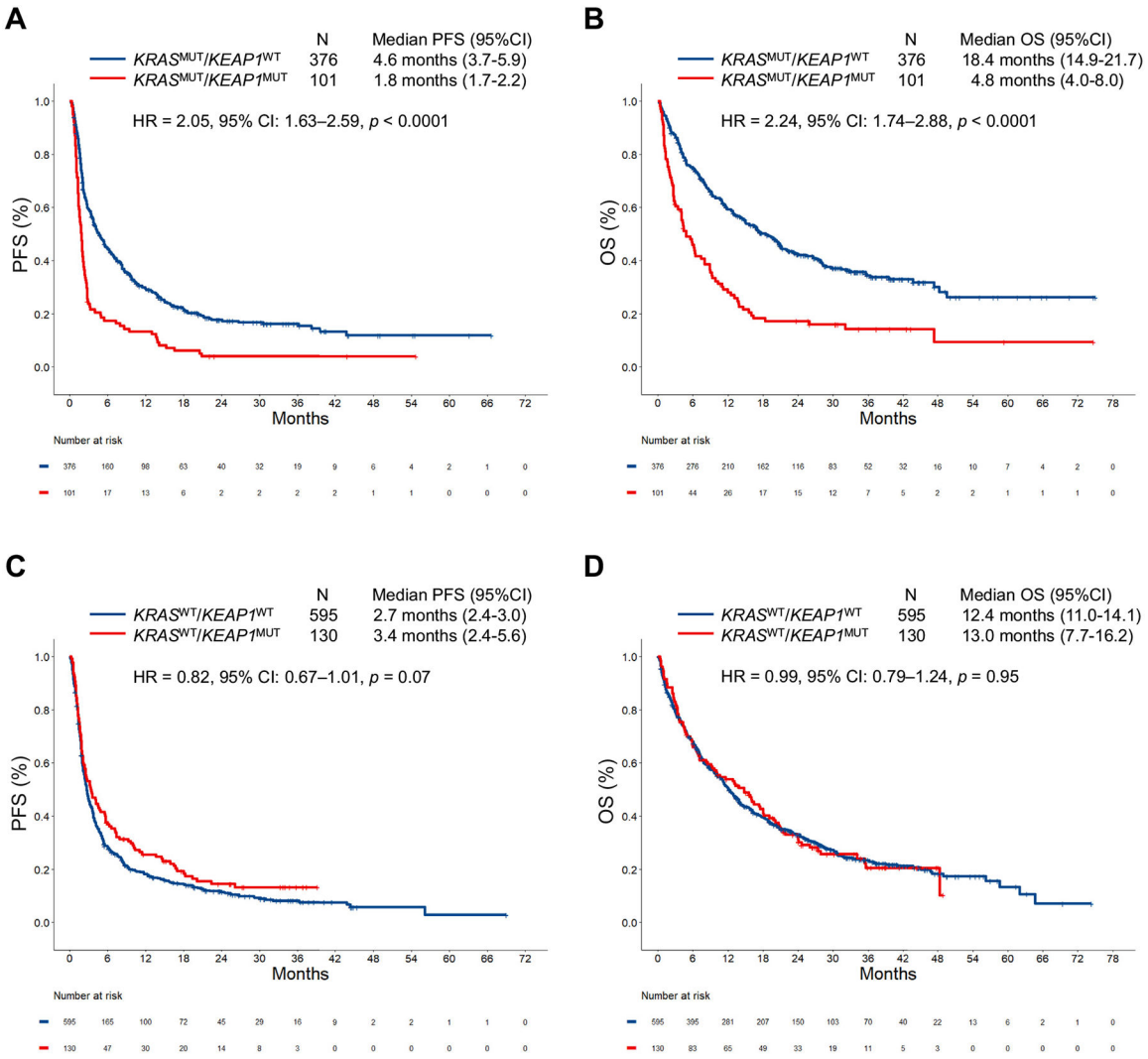

Figure 4.

(*A*) PFS and (*B*) OS to PD-(L)1 inhibition according to *KEAP1* mutation status among *KRAS*^{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*^{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.

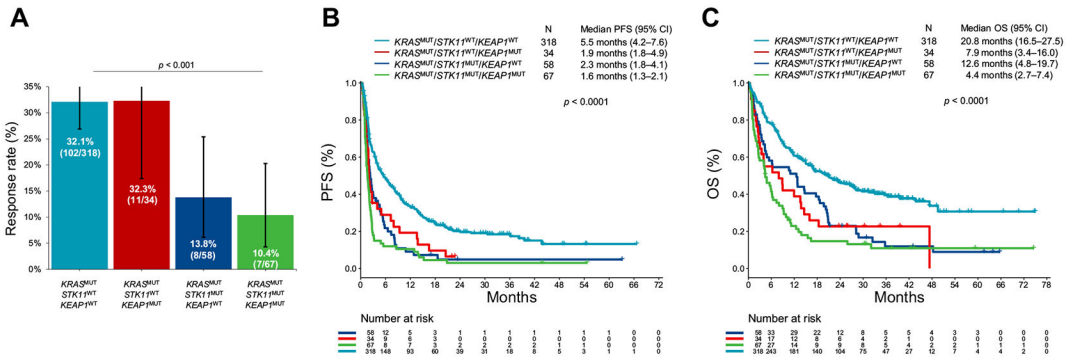

Figure 5.

(*A*) Objective response rate, (*B*) PFS, and (*C*) OS to PD-(L)1 inhibition according to *STK11/KEAP1* comutation status, among patients with *KRAS*^{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.

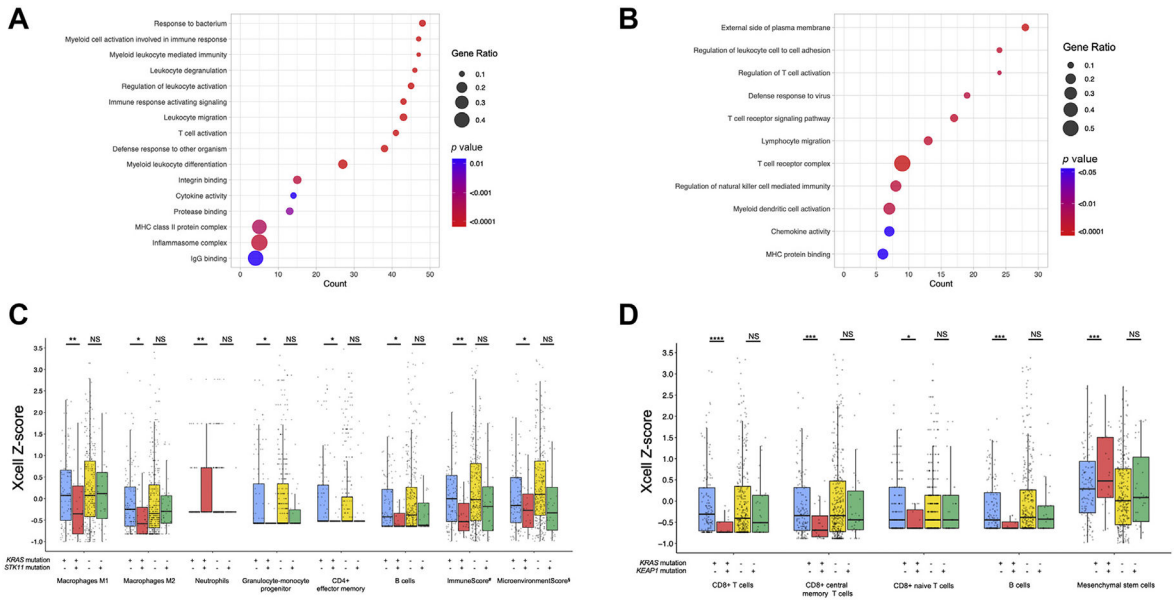

Figure 6.

(*A*) Bubble plot revealing the 13 prioritized immune-related pathways which are significantly down-regulated in *KRAS*^{MUT}/*STK11*^{MUT} compared with *KRAS*^{MUT}/*STK11*^{WT} LUADs, but not in *KRAS*^{WT}/*STK11*^{MUT} compared with *KRAS*^{WT}/*STK11*^{WT} LUADs. (*B*) Bubble plot revealing the 11 prioritized immune-related pathways which are significantly down-regulated in *KRAS*^{MUT}/*KEAP1*^{MUT} compared with *KRAS*^{MUT}/*KEAP1*^{WT} LUADs, but not in *KRAS*^{WT}/*KEAP1*^{MUT} compared with *KRAS*^{WT}/*KEAP1*^{WT} LUADs. (*C*) Cell-type enrichment analysis using xCell revealing the cell types that are uniquely enriched in *KRAS*^{MUT}/*STK11*^{WT} compared with *KRAS*^{MUT}/*STK11*^{MUT} LUADs, but not in *KRAS*^{WT}/*STK11*^{WT} compared with *KRAS*^{WT}/*STK11*^{MUT} LUADs. (*D*) Cell-type enrichment analysis using xCell revealing the cell types that are uniquely enriched in *KRAS*^{MUT}/*KEAP1*^{WT} compared with *KRAS*^{MUT}/*KEAP1*^{MUT} LUADs, but not in *KRAS*^{WT}/*KEAP1*^{WT} compared with *KRAS*^{WT}/*KEAP1*^{MUT} LUADs. LUAD, lung adenocarcinoma; MUT, mutant; NK, natural killer; NS, not significant; WT, wild-type. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Sum of B cells, CD4+ T cells, CD8+ T cells, Dendritic cells, Eosinophils, Macrophages, Monocytes, Mast cells, Neutrophils, NK cells.

§ Composite score of ImmuneScore + Stroma Score (Adipocytes, Endothelial cells, Fibroblasts)