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RESEARCH ARTICLE

STK11/LKB1 Mutations and PD-1 Inhibitor Resistance in *KRAS*-Mutant Lung Adenocarcinoma

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ABSTRACT *KRAS* is the most common oncogenic driver in lung adenocarcinoma (LUAC). We previously reported that *STK11/LKB1* (KL) or *TP53* (KP) comutations define distinct subgroups of *KRAS*-mutant LUAC. Here, we examine the efficacy of PD-1 inhibitors in these subgroups. Objective response rates to PD-1 blockade differed significantly among KL (7.4%), KP (35.7%), and K-only (28.6%) subgroups ($P < 0.001$) in the Stand Up To Cancer (SU2C) cohort (174 patients) with *KRAS*-mutant LUAC and in patients treated with nivolumab in the CheckMate-057 phase III trial (0% vs. 57.1% vs. 18.2%; $P = 0.047$). In the SU2C cohort, KL LUAC exhibited shorter progression-free ($P < 0.001$) and overall ($P = 0.0015$) survival compared with $KRAS^{MUT}$;$STK11/LKB1^{WT}$ LUAC. Among 924 LUACs, *STK11/LKB1* alterations were the only marker significantly associated with PD-L1 negativity in $TMB^{Intermediate/High}$ LUAC. The impact of *STK11/LKB1* alterations on clinical outcomes with PD-1/PD-L1 inhibitors extended to PD-L1–positive non–small cell lung cancer. In *Kras*-mutant murine LUAC models, *Stk11/Lkb1* loss promoted PD-1/PD-L1 inhibitor resistance, suggesting a causal role. Our results identify *STK11/LKB1* alterations as a major driver of primary resistance to PD-1 blockade in *KRAS*-mutant LUAC.

SIGNIFICANCE: This work identifies *STK11/LKB1* alterations as the most prevalent genomic driver of primary resistance to PD-1 axis inhibitors in *KRAS*-mutant lung adenocarcinoma. Genomic profiling may enhance the predictive utility of PD-L1 expression and tumor mutation burden and facilitate establishment of personalized combination immunotherapy approaches for genomically defined LUAC subsets. *Cancer Discov; 8(7); 822–35. ©2018 AACR.*

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INTRODUCTION

Despite improvements in overall survival (OS) and clinical responses of unprecedented duration with the use of therapeutic mAbs that target programmed cell death-1 (PD-1) or programmed cell death-1 ligand (PD-L1), the majority of patients with non–small cell lung cancer (NSCLC) fail to respond to PD-1/PD-L1 axis inhibitors (1–8). The landscape of primary resistance to PD-1 blockade in NSCLC is largely unknown, with no single factor capable of accurately segregating responders from nonresponders. Expression of PD-L1 on the membrane of tumor and immune cells is associated with enhanced objective response rates (ORR) to PD-1/PD-L1 inhibition, but is neither sensitive nor specific (1–3, 7, 9–12). A higher burden of nonsynonymous somatic mutations [tumor mutation burden (TMB)] further correlates with increased likelihood of clinical benefit and is undergoing evaluation as a predictive biomarker in many tumor types (4, 13–15).

KRAS mutations are the most prevalent oncogenic driver in NSCLC, accounting for approximately 25% of lung adenocarcinoma (LUAC; refs. 16, 17). We previously reported that co-occurring genomic alterations in *KRAS* and the

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Note: Supplementary data for this article are available at Cancer Discovery Online (http://cancerdiscovery.aacrjournals.org/).

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STK11/LKB1 (KL) or *TP53* (KP) tumor suppressor genes define subgroups of *KRAS*-mutant LUAC with distinct biology, therapeutic vulnerabilities, and immune profiles (18). *STK11/LKB1* encodes a serine threonine kinase with an established role in the regulation of cellular metabolism/energy homeostasis, growth, and polarity through phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and 12 AMPK-related kinases (19). Inactivation of *STK11* by mutational or nonmutational mechanisms is associated with an inert or "cold" tumor immune microenvironment, with reduced density of infiltrating cytotoxic $CD8^+$ T lymphocytes in both human tumors and genetically engineered murine models (18, 20, 21). On the basis of these findings, we hypothesized that *STK11/LKB1* genomic alterations may predict for lack of clinical benefit from PD-1/PD-L1 blockade in *KRAS*-mutant LUAC and conducted a study to address this hypothesis and examine the interrelationship between individual genetic alterations, TMB, and PD-L1 expression.

RESULTS

Patient Characteristics

One hundred seventy-four patients who met the prespecified eligibility criteria were included in the Stand Up To Cancer (SU2C) dataset [MD Anderson Cancer Center (MDACC; $N = 62$), Memorial Sloan Kettering Cancer Center (MSKCC; $N = 56$), Dana-Farber Cancer Institute/Massachusetts General Hospital (DFCI/MGH; $N = 56$); Table 1]. The overall cohort was representative of the general population of patients with *KRAS*-mutant LUAC with median patient age of 66 years (range, 42–87), high percentage of current/former smokers (88.5%), and typical frequencies of distinct *KRAS*-mutant alleles (Supplementary Fig. S1A and S1B; refs. 16–18, 22). Across the entire cohort, 31% of tumors were classified as KL, 32% were KP, and 37% K-only (Supplementary Fig. S1C). The majority of patients received PD-1 inhibitor monotherapy (165/174, 95%) and the remainder received combination with CTLA4 blockade (9/174, 5%; Supplementary Fig. S1A). Demographic and clinical characteristics were generally well balanced between the comutation-defined subgroups (Supplementary Fig. S1A and S1D). *STK11/LKB1* mutations were in their overwhelming majority predicted to be deleterious (Supplementary Fig. S2).

Comutations in *STK11/LKB1* Are Associated with Inferior Clinical Outcome with PD-1 Blockade in Multiple Independent Cohorts of *KRAS*-mutant LUAC

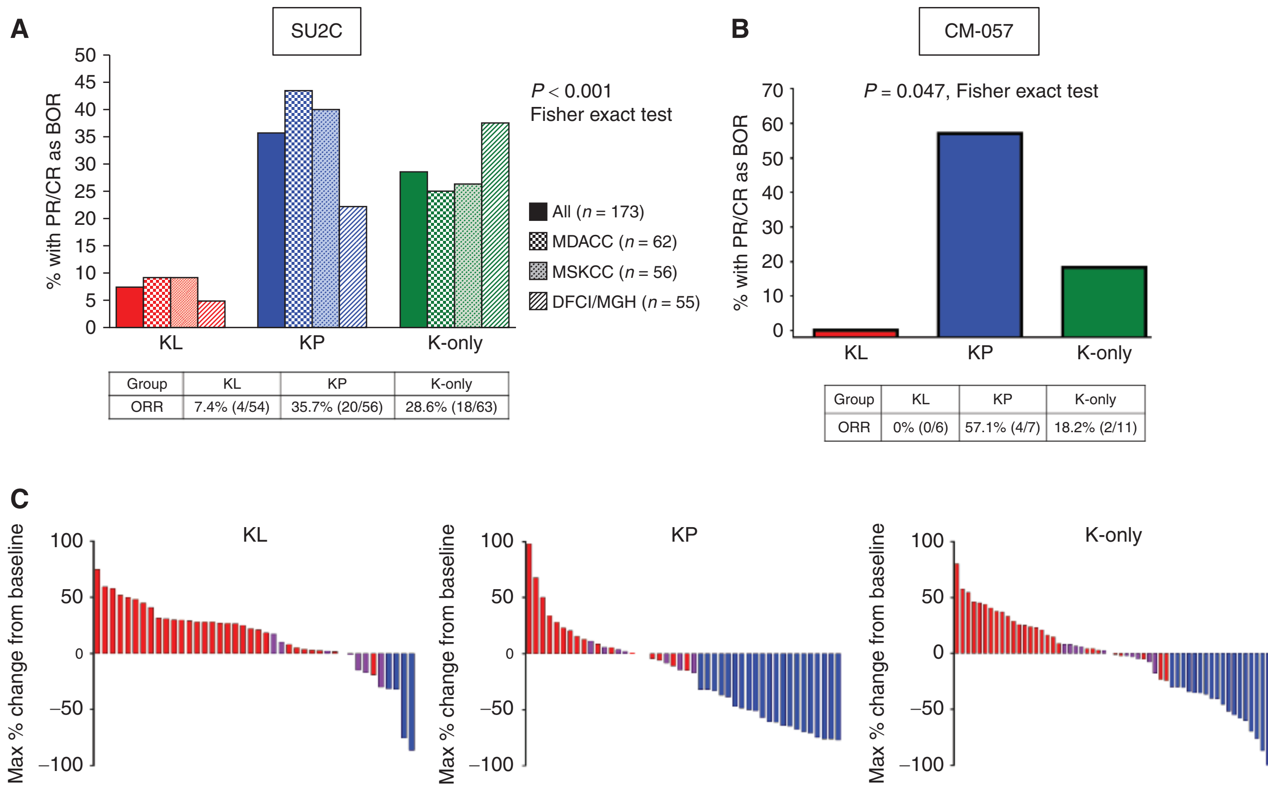
The objective response rates to PD-1 inhibition in KL, KP, and K-only groups were significantly different ($P < 0.001$, Fisher exact test; Fig. 1A and C). KL tumors were mostly resistant to PD-1 axis blockade (ORR 7.4% overall), with consistently low response rates seen in each of the three independent datasets (MDACC: 9.1%, MSKCC: 9.1%, DFCI/MGH: 4.8%). In contrast, KP LUAC were more sensitive to PD-1 inhibitors (ORR 35.7% overall). K-only tumors with no identifiable mutations in either *STK11/LKB1* or *TP53* had an intermediate response rate (28.6%). Assessment of additional co-occurring genetic alterations in the few KL tumors that responded to PD-1 blockade did not identify any obvious unifying molecular features (Supplementary Fig. S3).

To replicate these findings in the context of a randomized clinical trial, we further analyzed the impact of *STK11/LKB1* and *TP53* genetic alterations on clinical outcomes in 44 patients with *KRAS*-mutant NSCLC (96% LUAC) with available whole-exome sequencing (WES) data that were randomly assigned to treatment with nivolumab ($n = 24$) or docetaxel ($n = 20$) in the CheckMate-057 randomized phase III clinical trial (NCT01673867). In agreement with data from the SU2C cohort, ORR differed significantly between the KL, KP, and K-only subgroups in the nivolumab arm of CM-057 ($P = 0.047$), with KL tumors being refractory (ORR: 0%, 0/6) and KP more sensitive (ORR: 57.1%, 4/7) to nivolumab (Fig. 1B). Although ORR did not differ significantly among the three subgroups in the docetaxel arm ($P = 0.65$), it is relevant to note that the ORR in the KL subgroup was 0% [0/3; ORR was 0% (0/6) and 18.2% (2/11) in KP and K-only subgroups, respectively]. Given the relatively small numbers within

Table 1. Clinical cohorts included in the study

Cohort	Foundation Medicine (FM)	Stand Up To Cancer (SU2C)			Checkmate-057 (CM-057)	MDACC (PD-L1 ≥ 1%)
		MDACC	MSKCC	DFCI/MGH		
N	924 (346 *KRAS*^{MUT})	174				
		62	56	56	44	66
Nivolumab	NA	146			24	16
Pembrolizumab	NA	19			NA	40
Atezolizumab	NA	0			NA	5
anti-PD(L)-1 + anti-CTLA4	NA	9			NA	3
Docetaxel	NA	NA			20	NA
Other	NA	NA			NA	2[a]

[a]One patient with *STK11/LKB1*–mutant tumor was treated with nivolumab and NKTR-214 (CD122-based agonist) and one patient with *STK11/LKB1* wild-type tumor was treated with pembrolizumab and OX40 agonist.



Group	KL	KP	K-only
ORR	7.4% (4/54)	35.7% (20/56)	28.6% (18/63)

Group	KL	KP	K-only
ORR	0% (0/6)	57.1% (4/7)	18.2% (2/11)

Figure 1. *STK11/LKB1* comutations are associated with inferior objective response rate with PD-1 blockade in *KRAS*-mutant LUAC. **A,** Objective response rate (RECISTv1.1) to PD-1 axis blockade in the KL, KP, and K-only subgroups in the overall SU2C population ($N = 173$ response-evaluable patients) and in each of the three independent cohorts (MDACC, MSKCC, DFCI/MGH). A two-tailed Fisher exact test (computed from a 2 × 3 contingency table) was used to assess the significance of the association between group membership and best overall response (BOR; PR/CR vs. SD/PD). **B,** Objective response rate to nivolumab in the KL, KP, and K-only subgroups in the CheckMate-057 international randomized phase III clinical trial ($n = 24$). A two-tailed Fisher exact test (computed from a 2 × 3 contingency table) was used to assess the significance of the association between group membership and best overall response (PR/CR vs. SD/PD). PR, partial response; CR, complete response; SD, stable disease; PD, progressive disease. **C,** Waterfall plots illustrating individual patient-level maximal % change in tumor burden from baseline in response to PD-1/PD-L1 inhibition in the SU2C cohort. Only data from response-evaluable patients with measurable disease are graphed. Red, progressive disease; purple, stable disease; blue, partial response/complete response.

subgroups, it cannot be determined whether *STK11/LKB1* mutation is prognostic or predictive of treatment outcomes in the CM-057 dataset.

Progression-free survival (PFS) differed among the three groups in the SU2C cohort ($P = 0.0018$), with significantly shorter PFS for patients with KL compared with either KP [HR 1.77; 95% confidence interval (CI), 1.16–2.69; $P = 0.0072$] or K-only tumors (HR 1.98; 95% CI, 1.33–2.94; $P < 0.001$) in pair-wise comparisons (Fig. 2A, left). In contrast, patients with KP and K-only tumors had similar PFS. Because *STK11/LKB1* abrogation likely determines immunotherapy resistance in this context, we further compared PFS in patients with *STK11/LKB1* wild-type and mutant tumors by merging the KP and K-only cohorts. PFS was significantly shorter in KL tumors compared with *KRAS*-mutant LUAC with wild-type *STK11/LKB1* (HR 1.87; 95% CI, 1.32 to 2.66; $P < 0.001$; Fig. 2A, right). The CM-057 study had limited power to detect PFS or OS differences due to the small size of subgroup cohorts, and no significant differences were seen in PFS or OS in either arm (Supplementary Figs. S4 and S5).

OS also varied significantly among the three groups in the SU2C cohort ($P = 0.0045$; Fig. 2B, left). Median OS was 6.4 months in KL compared with 16.0 months in KP and 16.1 months in K-only LUACs. In the two-group comparison, OS was significantly shorter in *STK11/LKB1*-mutant compared with wild-type tumors (HR 1.99; 95% CI, 1.29–3.06; $P = 0.0015$; Fig. 2B, right). *KRAS* subgroup remained a significant independent predictor of OS on multivariate analysis ($P = 0.00055$). Notably, *STK11/LKB1* mutation or deficiency were not associated with worse OS in The Cancer Genome Atlas (TCGA) cohort, arguing against a purely prognostic role for *STK11/LKB1* inactivation in this setting of predominantly early-stage, surgically resected tumors (Supplementary Fig. S6), in agreement with previous studies in metastatic tumors (23–25).

Because nonmutational mechanisms can also account for STK11/LKB1 inactivation in LUAC (19), we further assessed expression of STK11/LKB1 by IHC in a subset of tumors for which archival tissue was available (26). *KRAS*^{MUT};*STK11/LKB1*^{MUT} (KL) tumors expressed low to undetectable levels of LKB1, whereas *KRAS*^{MUT};*STK11/LKB1*^{WT} tumors displayed variable levels of STK11/LKB1 expression, with 17.6% having a LKB1 H-score of zero (Fig. 3A). Patients bearing STK11/LKB1-deficient tumors (*STK11/LKB1*^{MUT} or *STK11/LKB1*^{WT} and LKB1 H-score zero) exhibited significantly shorter PFS (HR 1.80; 95% CI, 1.15–2.82; $P = 0.0094$; Fig. 3B, left) and OS (HR 2.03; 95% CI, 1.13–3.65; $P = 0.016$; Fig. 3B, right)

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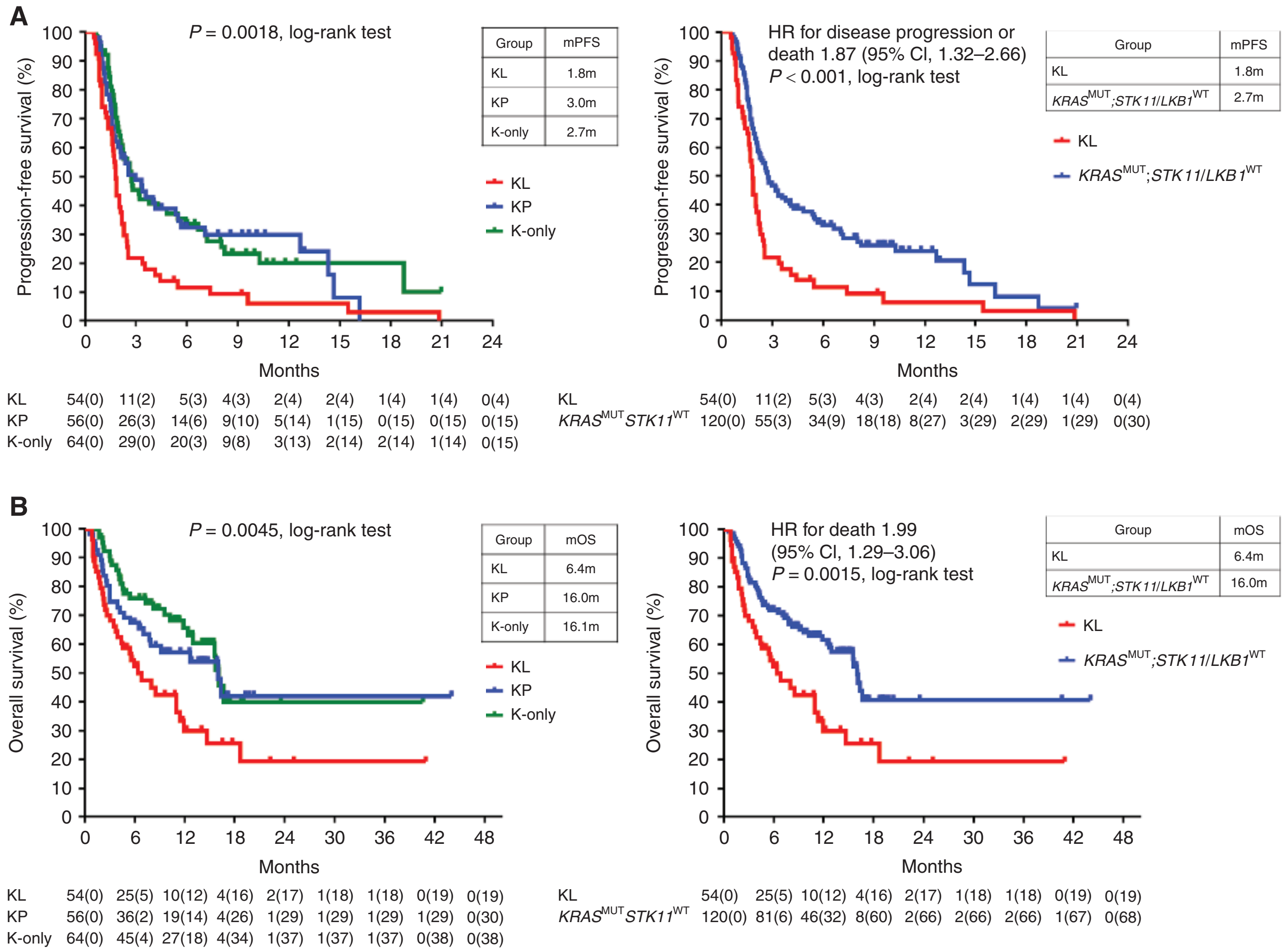

Figure 2. *STK11/LKB1* genetic alterations are associated with shorter progression-free and overall survival with PD-1 blockade among *KRAS*-mutant LUAC in the SU2C cohort. **A,** Kaplan–Meier estimates of progression-free survival with PD-1 blockade in the KL, KP, K-only subgroups (left) and in the two-group comparison between *KRAS*^{MUT};*STK11/LKB1*^{MUT} (KL) and *KRAS*^{MUT};*STK11/LKB1*^{WT} LUAC (encompassing KP and K-only tumors; right). Tick marks represent data censored at the last time the patient was known to be alive and without disease progression (date of last radiologic assessment). mPFS, median progression-free survival. **B,** Kaplan–Meier estimates of overall survival with PD-1 inhibitors in the KL, KP, K-only subgroups (left) and in the two-group comparison between *KRAS*^{MUT};*STK11*^{MUT} (KL) and *KRAS*^{MUT};*STK11/LKB1*^{WT} tumors (right). Tick marks represent data censored at the last time the patient was known to be alive. mOS, median overall survival.

compared with those harboring STK11/LKB1-proficient tumors (*STK11/LKB1*^{WT} and LKB1 H-score > 0).

STK11/LKB1 Mutations Are Significantly Enriched among TMB Intermediate/High, PD-L1–Negative Tumors

In a parallel, unbiased analysis, we sought to identify candidate genomic drivers of absent PD-L1 expression (as an indicator of a "cold" or non-T cell–inflamed immune microenvironment) in LUAC using the large Foundation Medicine (FM) dataset (Supplementary Fig. S7). We focused on TMB intermediate and high (TMB$^{I/H}$) tumors and excluded TMB low (TMB^{L}) LUAC because low TMB has been associated with impaired response to PD-1 axis inhibitors in retrospective studies, likely due to poor tumor immunogenicity (4, 13). We then compared the prevalence of individual genomic alterations in PD-L1 negative (PD-L1^{Neg}; TMB$^{I/H}$) versus high positive (PD-L1^{HP}; TMB$^{I/H}$) tumors (Fig. 4A). This analysis identified *STK11/LKB1* as the only significantly enriched gene in the PD-L1–negative group (adjusted $P < 0.001$). Further interrogation of the PD-L1/TMB landscape indicated that *STK11/LKB1* alterations are most prominently enriched in TMB^{I}; PD-L1^{Neg} samples, and LUAC-bearing *STK11/LKB1* alterations are less likely to be either PD-L1^{HP} or TMB^{L} (Fig. 4B). Furthermore, *STK11/LKB1* was significantly enriched ($P < 0.001$) for PD-L1^{Neg}; TMB$^{I/H}$-negative status even when the analysis was restricted to *KRAS*-mutant samples. Thus, we conclude that *STK11/LKB1* is associated with higher likelihood of absent PD-L1 expression among TMB$^{I/H}$ tumors irrespective of *KRAS* status.

We further analyzed PD-L1 expression and TMB in the KL/KP/K-only subgroups and their *KRAS* wild-type counterparts (Fig. 4C; Supplementary Fig. S8). PD-L1 expression varied significantly between the *KRAS* subgroups (Fig. 4C, $P < 0.001$), with KL least likely to be PD-L1^{HP} ($P < 0.001$; Supplementary Fig. S8). Among *KRAS* wild-type tumors, *STK11/LKB1* alterations were also associated with lower

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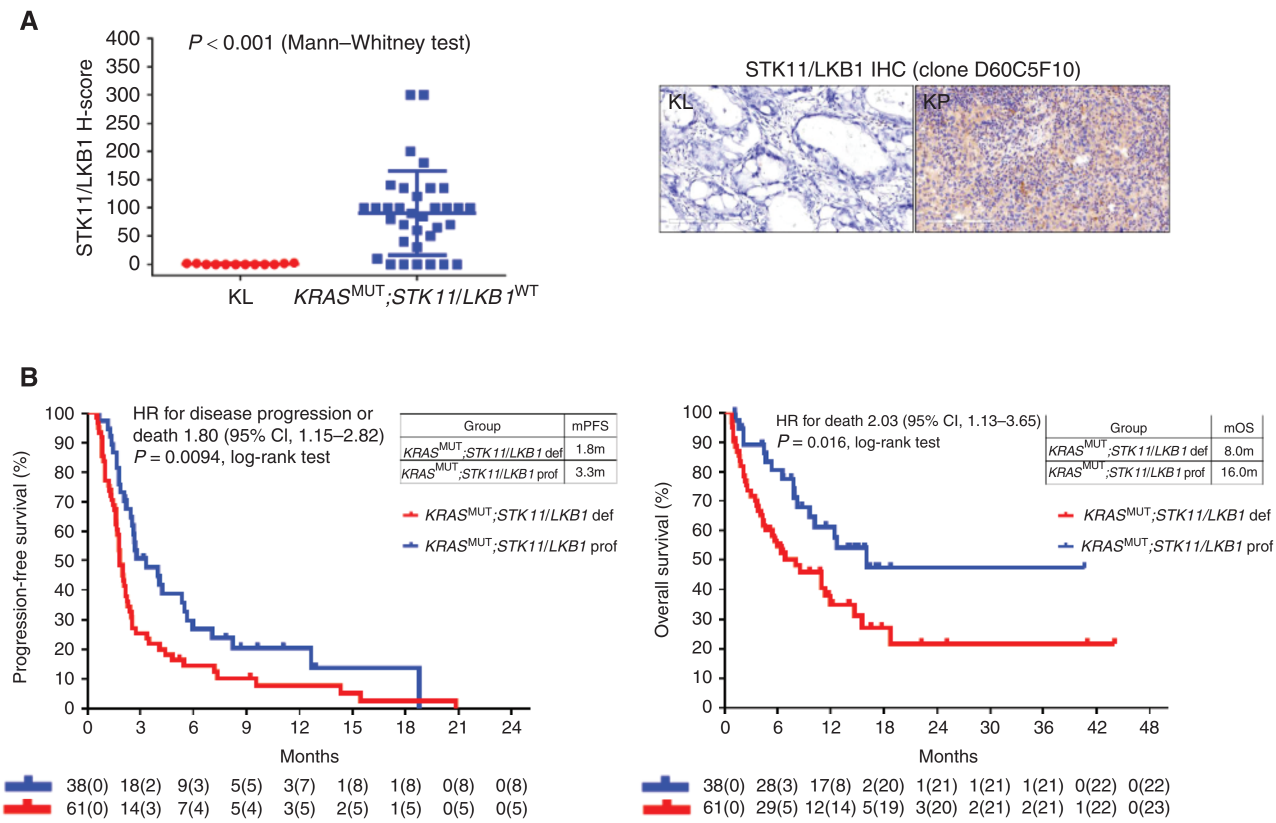

Figure 3. STK11/LKB1 expression by IHC can identify STK11/LKB1-deficient LUAC in the absence of *STK11/LKB1* alterations. **A,** LKB1 IHC expression (H-score) in *KRAS*^{MUT};*STK11/LKB1*^{MUT} (KL) and *KRAS*^{MUT};*STK11/LKB1*^{WT} LUAC. Quantitative IHC using a commercially available STK11/LKB1 rabbit mAb (clone D60C5F10, Cell Signaling Technology) is technically robust and can identify STK11/LKB1-deficient tumors with intact *STK11/LKB1* genomic locus (26). Left, KL LUACs ($n = 12$) exhibit absent or minimal cytoplasmic STK11/LKB1 staining, whereas *KRAS*^{MUT};*STK11/LKB1*^{WT} LUACs ($n = 34$) display variable LKB1 H-score. LUACs were therefore considered LKB1-proficient (prof) if they had intact *STK11/LKB1* locus and expressed STK11/LKB1 by IHC at any level (LKB1 H-score > 0) and STK11/LKB1-deficient (def) if they were *STK11/LKB1*-altered and/or exhibited LKB1 H-score = 0. Representative images of KL and KP LUAC immunostained for STK11/LKB1 are included (right). Staining was performed as described previously (26). **B,** Kaplan–Meier estimates of progression-free survival (left) and overall survival (right) with PD-1 blockade in LKB1-deficient (*STK11/LKB1*-mutant and/or STK11/LKB1 H-score = 0; $N = 61$) and STK11/LKB1-proficient (*STK11/LKB1*-wild-type and STK11/LKB1 H-score > 0; $N = 38$) *KRAS*-mutant LUAC.

likelihood of high PD-L1 expression (Supplementary Fig. S8). KP LUAC exhibited the highest rate of PD-L1 positivity (56.3% PD-L1–positive, 31.3% PD-L1^{HP}) followed by the *TP53*-altered, *KRAS* wild-type group (32.3% PD-L1-positive, 11% PD-L1^{HP}; Supplementary Fig. S8). In contrast, median TMBs across samples with *KRAS, STK11/LKB1*, or *TP53* alterations were comparable, ranging between 8.1 and 11.7 mutations/Mb (Fig. 4D; Supplementary Fig. S8).

Consistent with the observed association between *STK11/LKB1* genomic alterations and low PD-L1 expression in the FM cohort, significant difference in the rate of PD-L1 positivity (PD-L1 ≥ 1%) was also noted among KL, KP, and K-only tumors in the SU2C and CheckMate-057 cohorts (Fig. 4C; Supplementary Fig. S9), with KL exhibiting the lowest frequency of PD-L1–positive (13.6% in the SU2C and 11.1% in the CM-057 cohort) and PD-L1^{HP} tumors (0% in both cohorts; Fig. 4C; Supplementary Fig. S9).

We further directly interrogated the composition of the tumor immune microenvironment in surgically resected LUAC specimens (PROSPECT cohort) with available WES and automated quantitative IHC-based immune profiling (21, 27). In agreement with lower tumor cell PD-L1 expression, *STK11/LKB1*-mutated tumors exhibited lower densities of infiltrating CD3^{+} ($P = 0.0019$) and CD8^{+} ($P = 0.0072$) T lymphocytes but not FOXP3^{+} cells ($P = 0.7648$; Supplementary Fig. S10A). Furthermore, in the TCGA dataset *STK11/LKB1*-mutated LUAC (or *STK11/LKB1*-deficient LUAC as determined using a previously validated gene expression signature; ref. 28) exhibited lower T-cell signature scores (ref. 29; Supplementary Fig. S10B) and expressed lower *CD274* (encoding PD-L1) mRNA levels (Supplementary Fig. S10C). Thus, we provide compelling evidence from multiple independent cohorts that *STK11/LKB1* genomic alterations are associated with, and may actually promote, a non-T cell–inflamed immune microenvironment with lack of tumor cell PD-L1 expression, despite an intermediate or high TMB.

STK11/LKB1 Genomic Alterations Are Associated with Primary Resistance to PD-1 Axis Inhibitors in PD-L1–Positive NSCLC

In view of the strong association between *STK11/LKB1* genomic alterations and lack of PD-L1 expression on tumor

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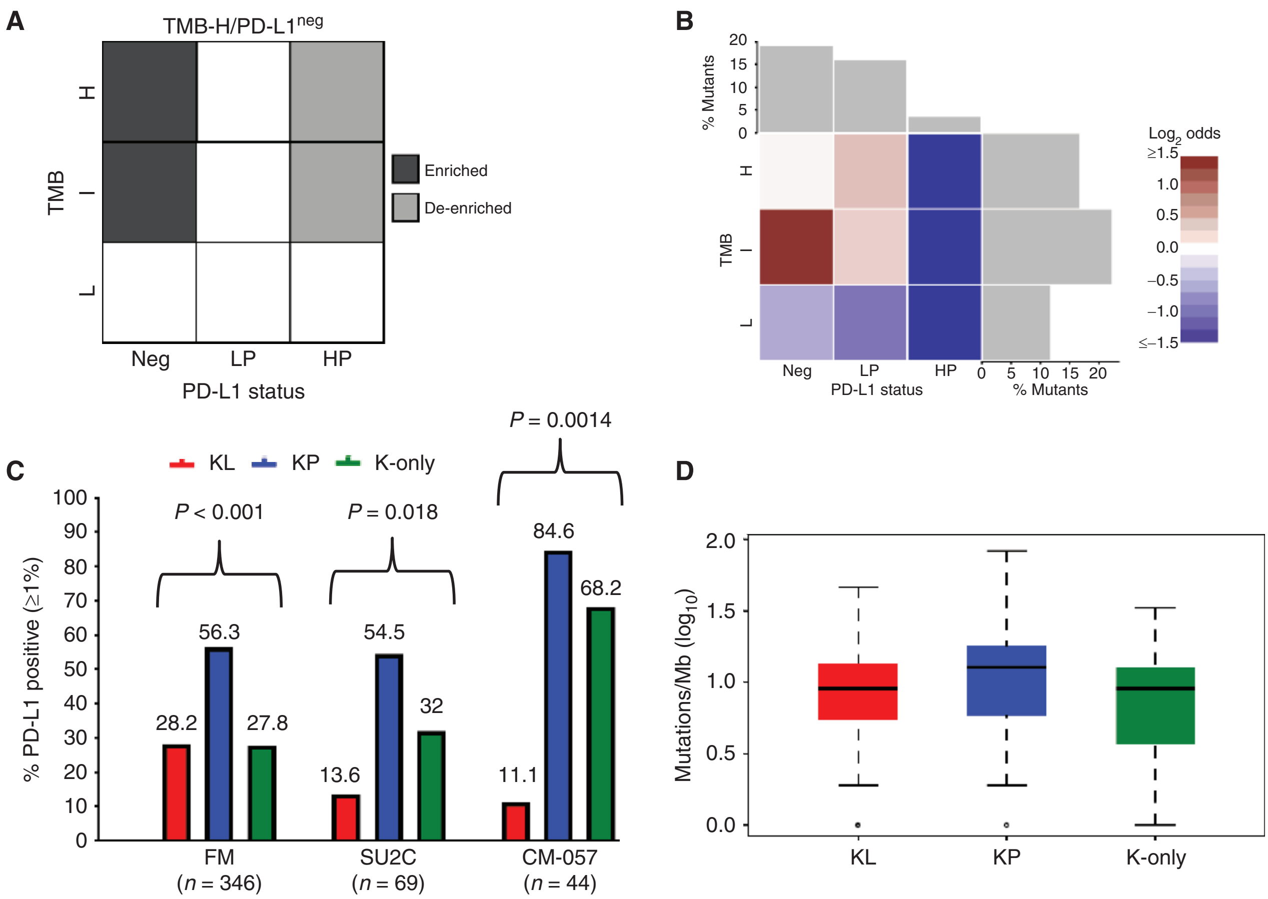

Figure 4. *STK11/LKB1* genomic alterations are enriched in LUACs with intermediate or high TMB that are negative for PD-L1 expression. **A,** PD-L1/TMB landscape matrix illustrating the enrichment analysis strategy in 924 LUAC samples with available comprehensive genomic profiling and PD-L1 expression (FM cohort). Enrichment of individual genomic alterations in PD-L1^{Neg}; TMB$^{I/H}$ versus PD-L1^{HP}; TMB$^{I/H}$ tumors was assessed using a one-sided Fisher exact test. **B,** Heat map of log-odds values reflecting the prevalence of *STK11/LKB1* alterations in different cells of the PD-L1/TMB matrix. Alterations in *STK11/LKB1* primarily cluster in TMB^{I};PD-L1^{Neg} LUAC. **C,** PD-L1 expression in the KL, KP, and K-only subgroups in the FM (*n* = 346), SU2C (*n* = 69), and CM-057 (*n* = 44) cohorts. A two-tailed Fisher exact test (computed from a 2 × 3 contingency table) was used to assess the significance of the association between group membership and PD-L1 expression status [PD-L1 positive (≥1%) or negative (0%)]. **D,** TMB (log_{10}) in the KL, KP, and K-only subgroups among 346 *KRAS*-mutant LUAC in the FM cohort.

cells, we also sought to examine the impact of *STK11/LKB1* mutations on clinical responses to PD-1 axis blockade in PD-L1 positive (≥1%) nonsquamous NSCLC. For this analysis, we identified a distinct cohort of 66 patients with nonsquamous NSCLC (irrespective of *KRAS* status) treated with PD-1/PD-L1 inhibitors at MDACC, with available *STK11/LKB1* genomic profiling and PD-L1 expression (assessed using the FDA-approved 22C3 pharmDx assay). Within this PD-L1–positive group, *STK11/LKB1*–mutated tumors exhibited significantly lower ORR to PD-1/PD-L1 blockade compared with NSCLC with intact *STK11/LKB1* (ORR 0% vs. 34.5%, $P = 0.026$, Fisher exact test; Fig. 5A), despite inclusion of PD-L1–high-expressing tumors in the *STK11/LKB1*-mutant group (Fig. 5B). Importantly, *STK11/LKB1* mutations were associated with dramatically shorter PFS (HR 4.76; 95% CI, 2.0–11.1, $P = 0.00012$, log-rank test) and OS (HR 14.3; 95% CI, 3.4–50.0, $P < 0.0001$, log-rank test) with PD-1 axis blockade (Fig. 5C and D). The effect of *STK11/LKB1* genomic alterations on PFS and OS with PD-1/PD-L1 blockade did not differ significantly across PD-L1 high (PD-L1 ≥ 50%) and low (PD-L1 < 50%) groups ($P_{interaction} = 0.48$ for PFS and $P_{interaction} = 0.59$ for OS; Supplementary Fig. S11). We therefore conclude that *STK11/LKB1* genomic alterations affect response to PD-1/PD-L1 blockade at least partially independently of PD-L1 status and that their effect likely extends to the entire population of nonsquamous NSCLC regardless of *KRAS* status. Extension of the effect of STK11/LKB1 inactivation to the broader population of nonsquamous NSCLC is further supported by data from a separate cohort of patients with TMB$^{I/H}$ nonsquamous NSCLC (without available PD-L1 expression) treated with anti–PD-1/PD-L1 therapy, whereby *STK11/LKB1* alterations (regardless of *KRAS* status) were associated with significantly shorter time on drug (HR 2.91; 95% CI, 1.22–6.92; $P = 0.0156$; Supplementary Fig. S12).

TP53 Comutations May Affect Response to PD-1 Inhibitors in PD-L1–Negative *KRAS*-Mutant LUAC

As part of an exploratory analysis we interrogated the impact of *KRAS* comutations on clinical benefit from PD-1 blockade in PD-L1–negative tumors. Among PD-L1–negative

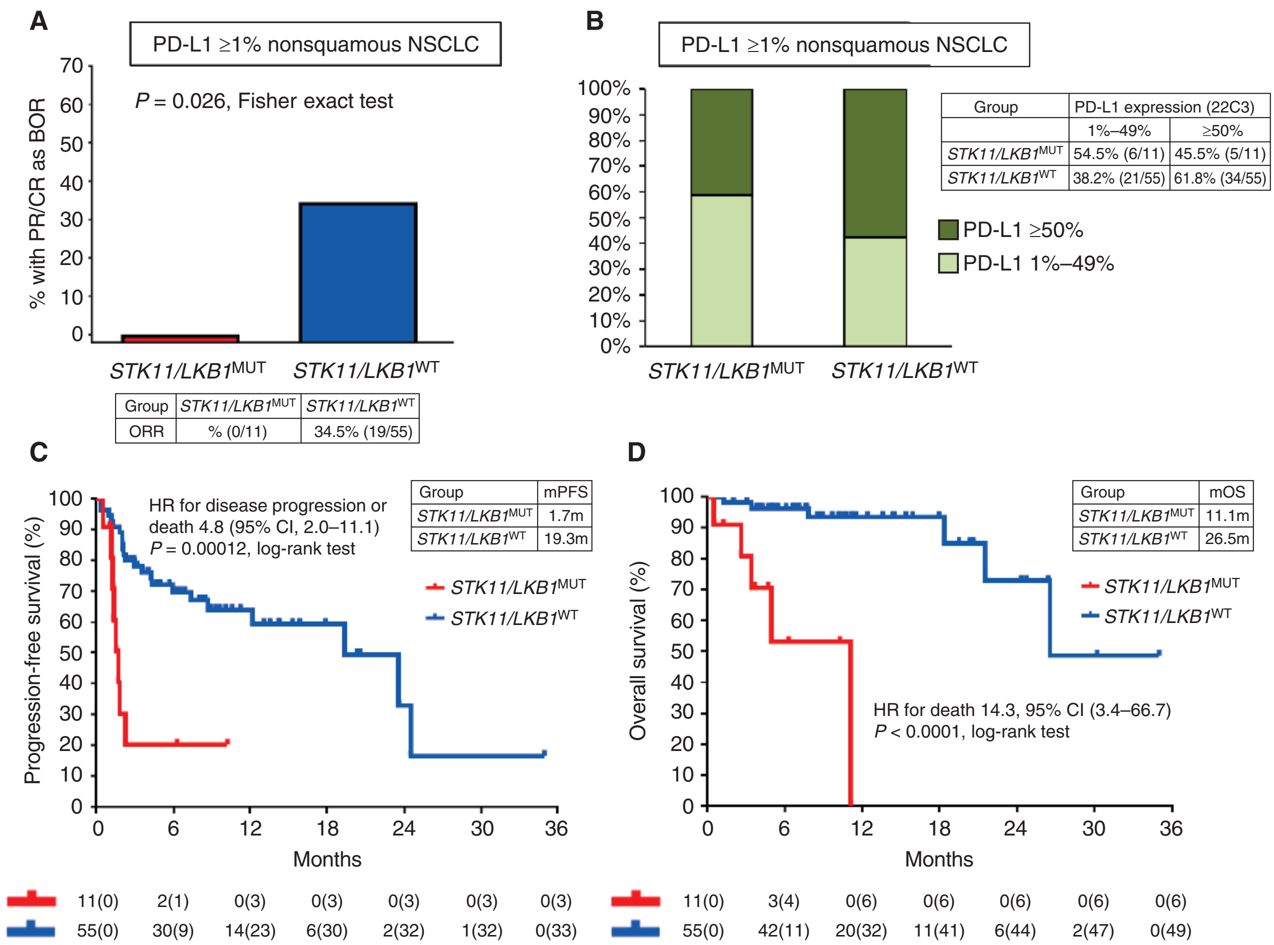

Figure 5. *STK11/LKB1* mutations are a genomic determinant of poor clinical outcome with PD-1 axis blockade in PD-L1–positive nonsquamous NSCLC, regardless of *KRAS* status. **A,** Objective response rate (RECISTv1.1) to PD-1/PD-L1 inhibitors in *STK11/LKB1*-mutant and wild-type patients with PD-L1–positive nonsquamous NSCLC (≥1%) from MDACC ($n = 66$). PD-L1 expression was assessed using the FDA-approved 22C3 pharmDx assay (Dako). A two-tailed Fisher exact test (computed from a 2 × 2 contingency table) was used to assess the significance of the association between group membership (*STK11/LKB1*-mutant versus *STK11/LKB1*–wild-type) and best overall response (PR/CR vs. SD/PD). **B,** Fractions of PD-L1 low-positive (1%–49%) and PD-L1 high-positive (≥50%) tumors in the *STK11/LKB1*-mutant and wild-type groups. **C,** Kaplan–Meier estimates of progression-free survival with PD-1/PD-L1 blockade in *STK11/LKB1*-mutant and wild-type groups. Tick marks represent data censored at the last time the patient was known to be alive and without disease progression (date of last radiologic assessment). **D,** Kaplan–Meier estimates of overall survival with PD-1 inhibitors in the *STK11/LKB1*-mutant and wild-type groups. Tick marks represent data censored at the last time the patient was known to be alive.

KRAS-mutant LUAC in the SU2C cohort ($n = 46$), disease control rate differed significantly among the subgroups ($P = 0.034$) and was highest (70%) in KP tumors (Supplementary Fig. S13). The difference in ORR also favored the KP subgroup (30%), but did not reach statistical significance ($P = 0.11$, Supplementary Fig. S13). This result further supports the notion that the predictive utility of comutations may extend beyond that of PD-L1 expression.

Stk11/Lkb1 Ablation Induces *De Novo* Resistance to PD-1 Blockade in a Syngeneic Murine Model of *KRAS*-Mutant LUAC

In order to establish whether primary resistance to immunotherapy is causally linked with STK11/LKB1 inactivation, we generated *Stk11/Lkb1*-proficient/deficient isogenic derivatives of the LKR13 *Kras*-mutant murine LUAC cell line (previously established from a spontaneously arising LUAC in the *Kras*$^{LA1/+}$ model) using CRISPR/Cas9-mediated biallelic disruption of the *Stk11/Lkb1* locus. Upon confirmation of *Stk11/Lkb1* knockout (by immunoblotting for the STK11/LKB1 protein; Supplementary Fig. S14), isogenic cell lines were implanted into the right flank of syngeneic recipient mice, and cohorts of tumor-bearing mice were randomized to treatment with anti–PD-L1 mAb or IgG control. Treatment with anti–PD-L1 mAb potently suppressed LKR13-derived tumors, but growth of *Stk11/Lkb1*-deficient LKR13 knockout (LKR13KO) continued unabated (Fig. 6A). In agreement with findings in human *STK11/LKB1*-deficient tumors, lower numbers of $CD3^+CD8^+$ and $CD3^+CD8^+/PD1^+$ T lymphocytes were present in *Stk11/Lkb1*-deficient LKR13KO tumors compared with their *Stk11/Lkb1*-proficient counterparts, whereas numbers of $CD45^+$ and $CD3^+CD4^+$ cells were not significantly different (Supplementary Fig. S15). Furthermore, we did not observe enrichment of tumor-associated neutrophils in the microenvironment of

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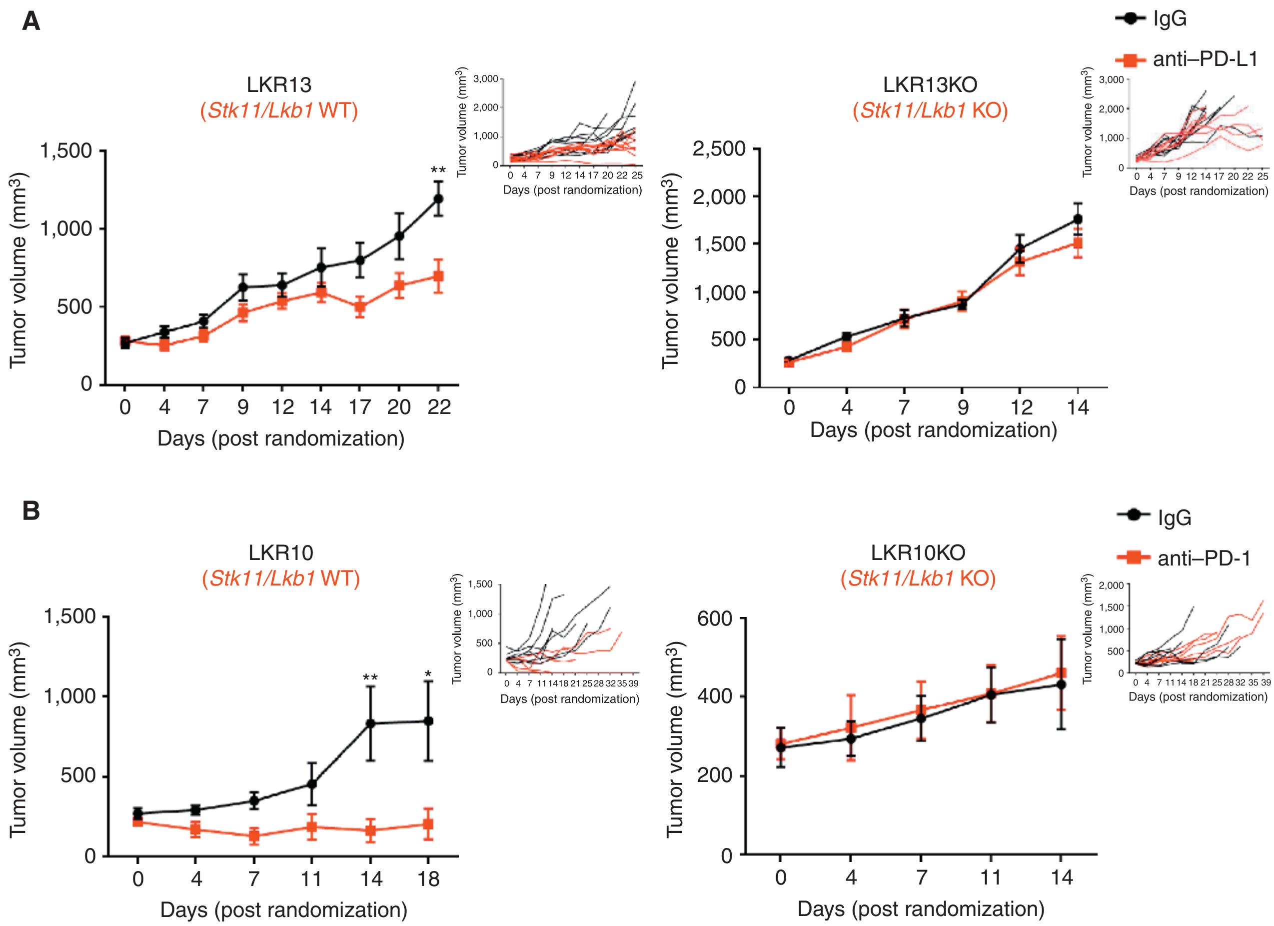

Figure 6. *Stk11/Lkb1* ablation directly promotes primary resistance to PD-L1/PD-1 blockade in immunocompetent murine models of *Kras*-mutant LUAC. *Stk11/Lkb1*-proficient/deficient isogenic derivatives of the LKR13 (**A**) and LKR10 (**B**) cell lines were used in preclinical experiments. Changes in mean (main panels) and individual (inset panels) subcutaneous tumor volume following treatment with (**A**) anti–PD-L1 (mIgG1-D265AFc clone 80) or IgG control antibody (LKR13/LKR13KO isogenic pair) and (**B**) anti–PD-1 mAb (clone RMPI-14; BioXCell) or isotype control antibody (clone 2A3; BioXCell; LKR10/LKR10KO isogenic pair) are graphed. Error bars represent SEM. Mean tumor volume plots are depicted from the time of randomization to the time that the first mouse in any of the two treatment arms was sacrificed. Spider plots indicate individual tumor volume trajectories for the entire duration of the *in vivo* experiment (25 days for the LKR13/LKR13KO and 39 days for the LKR10/LKR10KO model). Note that PD-1/PD-L1 blockade blunts the *in vivo* growth of *Stk11/Lkb1*-proficient *Kras*-mutant LUAC, whereas *Stk11/Lkb1* knockout renders tumors recalcitrant to PD-1/PD-L1 inhibition. The Mann–Whitney *U* test was used to compare mean tumor volumes between IgG control and anti–PD-L1/anti–PD-1–treated mice in each syngeneic model. Asterisks denote statistical significance at the $P \leq 0.05$ (*) and $P \leq 0.01$ (**) level.

Stk11/Lkb1-deficient tumors in this model (Supplementary Fig. S15), contrary to a previous report (20). Similar results were obtained using a second syngeneic tumor model based on the LKR10 *Kras*-mutant murine LUAC cell line in response to treatment with anti–PD-1 mAb or isotype control (Fig. 6B). Thus, *Stk11/Lkb1* loss directly promotes primary resistance to PD-1/PD-L1 blockade and fosters establishment of a non-T cell–inflamed tumor immune microenvironment in immunocompetent murine models of *KRAS*-mutant LUAC.

DISCUSSION

In this study, we identify genomic alterations in *STK11/LKB1* as a tumor cell–intrinsic determinant of primary resistance to PD-1 axis blockade in three independent retrospective cohorts of *KRAS*-mutant LUAC, a fourth cohort of PD-L1–positive NSCLC regardless of *KRAS* status, as well as in patients with *KRAS*-mutant NSCLC treated with nivolumab in the pivotal CheckMate-057 randomized phase III clinical trial. Somatic mutations in *STK11/LKB1* are prevalent in LUAC (16.7% in the large FM cohort), particularly among *KRAS*-mutant tumors (25.4% in the combined FM/SU2C cohort) and foster establishment of a non-T cell–inflamed tumor immune microenvironment with frequently undetectable tumor cell PD-L1 expression (18, 20, 21). Furthermore, we show that genetic ablation of *Stk11/Lkb1* directly promotes resistance to anti–PD-1/anti–PD-L1 therapy in two immunocompetent *Kras*-mutant LUAC murine models. Therefore, *STK11/LKB1* inactivation represents a major driver of immune escape and innate resistance to PD-1 blockade in *KRAS*-mutant LUAC.

Our work demonstrates that alterations in *STK11/LKB1* are associated with lack of PD-L1 expression in tumor cells across multiple independent cohorts, despite the presence of intermediate or high TMB. This finding is consistent with lower densities of infiltrating CD8+ CTLs in both human and murine STK11/LKB1-deficient tumors. However, the negative impact of *STK11/LKB1* genomic alterations on clinical response to PD-1 axis inhibitors also extends to PD-L1–positive NSCLC, indicating that it is at least partially independent of PD-L1 expression. In addition, in an exploratory analysis among PD-L1–negative *KRAS*-mutant LUAC, KP tumors exhibited more favorable response to PD-L1 blockade, with partial response/stable disease achieved in 7 of 10 patients. Therefore, analysis of *STK11/LKB1* and *TP53* comutations may help refine response prediction algorithms in both PD-L1 positive and negative tumors as well as in cases where tumor biopsy for assessment of PD-L1 expression is unavailable or impractical but profiling of circulating tumor DNA (liquid biopsy) has been obtained.

It is important to note that nonmutational mechanisms may also account for STK11/LKB1 inactivation in a subset of LUAC (19). Quantitative IHC for STK11/LKB1 can capture STK11/LKB1-deficient tumors in the absence of *STK11/LKB1* genomic alterations (26). Therefore, evaluation of LKB1 expression by IHC may further enhance the predictive utility of a composite biomarker panel encompassing PD-L1 expression, TMB, and *STK11/LKB1* genomic alterations.

Although our study primarily examined clinical response to PD-1 axis inhibitors in *KRAS*-mutant tumors, we anticipate that the effect of STK11/LKB1 inactivation extends to the entire LUAC population, regardless of *KRAS* status. This hypothesis is supported by (i) poor ORR and shorter PFS and OS with PD-1/PD-L1 blockade in *STK11/LKB1*-mutant tumors in a cohort of PD-L1–positive NSCLC encompassing both *KRAS*-mutant and wild-type tumors; (ii) shorter time on PD-1 inhibitor in a separate cohort of patients with *STK11/LKB1*-altered TMB$^{I/H}$ tumors; and (iii) evidence of a "cold" immune microenvironment in *STK11/LKB1*-altered LUAC irrespective of *KRAS* status, and was further proposed in a recent separate study (30). However, application to the wider population of nonsquamous NSCLC will require further validation in larger datasets.

The mechanistic basis of T-cell exclusion in STK11/LKB1-deficient tumors is under active investigation and did not constitute a focus of the current study. However, based on established and emergent STK11/LKB1 functions, a number of possibilities are proposed including altered cytokine/chemokine milieu (20), metabolic restriction of effector T cells (31), or impaired antigenicity, possibly as a result of STK11/LKB1-dependent changes in the epigenetic landscape of tumor cells (32). In a set of elegant *in vivo* experiments, inducible expression of MYC in *Kras*^{G12D}-driven murine lung adenomas triggered rapid expulsion of CD3+ T lymphocytes [as well as B cells and natural killer (NK) cells] from the tumor microenvironment via induction of IL23 and CCL9 (33). *STK11/LKB1* loss has been reported to promote transcriptional upregulation of *MYC* via the MZF1 transcription factor (34). In a colorectal cancer murine isograft model, T-cell exclusion and suppression of Th1 cell differentiation were mediated by TGFβ signaling (35), which has also been shown to be subject to modulation by STK11/LKB1 (36). Furthermore, loss of *PTEN*, which, similar to *STK11/LKB1* alterations, results in mTOR pathway activation, has been associated with impaired CD8+ T-cell recruitment in melanoma (37). In this tumor type, prior seminal work highlighted active WNT/β-catenin signaling as a key molecular driver of the non-T cell–inflamed phenotype, via ATF3-mediated suppression of CCL4 production and impaired recruitment of CD103+ dendritic cells to the tumor immune microenvironment (29); interestingly, STK11/LKB1 deficiency has previously been associated with WNT pathway activation (38). Finally, it was recently demonstrated that tumor cell–derived prostaglandin E2 (PGE2) can also impair recruitment of conventional type 1 dendritic cells (cDC1) to the tumor microenvironment both directly, through downregulation of chemokine receptor expression in cDC1, and indirectly, via attenuation of NK-cell viability and function (39). It was previously reported that expression of COX2, which catalyzes the conversion of arachidonic acid to prostaglandins, is enhanced in STK11/LKB1-deficient NSCLC cells via activated CRTC1 (40). Thus, multiple and potentially nonoverlapping mechanisms may underpin establishment and maintenance of a "cold" tumor immune microenvironment in STK11/LKB1-deficient NSCLC, and further work is required to elucidate nodal downstream effectors and signaling cascades.

Delineation of pathways and mechanisms of immune escape downstream of STK11/LKB1 inactivation is also critical in order to inform rational combination therapeutic approaches aimed at invigorating antitumor immunity. Several strategies to convert non-T cell–inflamed tumors into T cell–inflamed tumors have been proposed and are undergoing preclinical and clinical evaluation, including activation of innate immune recognition with STING agonists, TLR agonists, ionizing radiation, or expression of LIGHT in tumor cells (41–44). Such approaches, as well as efforts that tackle specific STK11/LKB1 loss–dependent immunosuppressive cascades, will require prospective evaluation in patients with STK11/LKB1–deficient NSCLC.

In contrast, evidence of preexisting CD8+ T-cell infiltrate and adaptive immune resistance in the majority of KP (and possibly K-only STK11/LKB1-proficient) tumors supports simultaneous targeting of multiple immune inhibitory pathways in this subgroup (42).

Taken together, our data reveal a novel, frequent driver of *de novo* resistance to PD-1 blockade in *KRAS*-mutant LUAC and potentially the entire LUAC population. More broadly, somatic genomic alterations in individual genes may modulate the efficacy of PD-1/PD-L1 inhibitors in given tumor types and across tumor types. Although the fully integrated set of determinants of response to these agents is not yet completely defined, our results suggest that the development of tailored immunotherapy approaches for NSCLC may be facilitated by genomic profiling to allow simultaneous characterization of specific somatic alterations including *KRAS, STK11/LKB1*, and *TP53,* in addition to TMB and PD-L1 expression.

METHODS

Patients

Patients with stage IV *KRAS*-mutant LUAC who received at least one cycle of PD-1 inhibitor therapy or combined PD-1/PD-L1 and CTLA4 blockade, were alive for ≥14 days thereafter, and had

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available molecular profiling of *KRAS, STK11/LKB1*, and *TP53* were identified by retrospective electronic medical record review. Three independent cohorts were studied from members of the Stand Up To Cancer/American Cancer Society Lung Cancer Translational Research Dream Team: MDACC, MSKCC, and a combined cohort from DFCI/MGH, cumulatively forming the SU2C cohort. A fourth cohort of 66 patients with PD-L1–positive (≥1%) nonsquamous NSCLC (regardless of *KRAS* status) from MDACC with available tumor molecular profiling was assessed to determine the impact of *STK11/LKB1* genomic alterations on clinical outcomes with anti–PD-1/PD-L1 therapy specifically in PD-L1–positive tumors. The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and its subsequent amendments. All participating patients at each institution provided written informed consent for the collection of clinical, demographic, and molecular data as well as the use of tissue for IHC and molecular studies, which proceeded in accordance with IRB-approved protocols at each of the participating institutions.

A fifth independent cohort of 44 patients with *KRAS*-mutant NSCLC (24 treated with nivolumab and 20 treated with docetaxel) with available *STK11/LKB1* and *TP53* mutational status and tumor cell PD-L1 expression from the CheckMate-057 (CM-057) international phase III randomized controlled trial (NCT01673867) was also analyzed (45).

Finally, a separate large cohort of 924 unselected patients with LUAC who submitted samples to FM for hybrid capture-based comprehensive genomic profiling (CGP) were included in an integrated analysis of TMB, PD-L1 expression, and genomic alterations of individual cancer-related genes. Duration of therapy ("time on drug") was known for a subset of patients with CGP that received PD-1/PD-L1 inhibitors. Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (protocol no. 20152817).

Study Assessments

Tumor response was assessed by dedicated thoracic radiologists (MDACC, MSKCC, DFCI) or the study investigators (MGH) using Response Evaluation Criteria in Solid Tumors, version 1.1 (RECISTv1.1). ORR was defined as the percentage of patients achieving a confirmed or unconfirmed complete or partial response. Attribution of stable disease as best overall response to therapy required a minimum interval of ≥30 days between the first day of the first cycle of treatment (C1D1) and radiologic evaluation. Patients who died before radiologic reassessment were deemed to have progressive disease. PFS was defined as the time from C1D1 to the date of disease progression or death from any cause. OS was defined as the time from C1D1 to the date of death from any cause. Efficacy endpoints for patients included in CM-057 were evaluated as described previously (45).

In the SU2C cohort, tumor cell PD-L1 expression was assessed in the most recent preimmunotherapy formalin-fixed paraffin-embedded tumor biopsy tissue at each institution using the PD-L1 E1L3N XP rabbit mAb (Cell Signaling Technology), and quantified as the percent of tumor cell membranes exhibiting specific staining of any intensity. PD-L1 expression in CM-057 samples was assessed using the Validated 28-8 pharmDx Assay (Dako; ref. 3). PD-L1 staining of tumor samples submitted to FM was performed using the VENTANA PD-L1 (SP142) assay. Tumors were characterized as PD-L1 negative (PD-L1 < 1%), low positive (1% ≤ PD-L1 < 50%), or high positive (PD-L1 ≥ 50%). Assessment of PD-L1 expression in the separate cohort from MDACC (N = 66) was based on the FDA-approved 22C3 pharmDx assay (Dako).

Molecular Profiling Platforms and Study Group Definitions

CGP of tumor and/or circulating cell-free tumor DNA utilized CLIA-certified assays available in each of the participating institutions (46–51). Samples from CM-057 underwent WES according to previously described methodology (4). Samples submitted to Foundation Medicine were processed at a CLIA-certified laboratory as described previously (52). TMB was measured by Foundation Medicine as described previously (53). Raw TMB values were measured in units of mutations per Mb and characterized as low (TMB < 6), intermediate (6 ≤ TMB < 20), or high (TMB ≥ 20).

KRAS-mutant LUAC bearing nonsynonymous somatic mutations in *STK11/LKB1* and/or mono- or biallelic loss of the *STK11/LKB1* locus were denoted as KL. *KRAS*-mutant LUAC harboring nonsynonymous somatic mutations in *TP53* and/or mono- or biallelic loss of the *TP53* locus were classified as KP. *KRAS*-mutant tumors with intact *STK11/LKB1* and *TP53* were referred to as K-only (these tumors include a multitude of additional genetic alterations in addition to mutant *KRAS*). Triple-mutant tumors (*KRAS;TP53;STK11*) were classified as KL (18). In CM-057 tumors bearing nonsynonymous somatic mutations in *STK11/LKB1* were denoted as KL. In the FM cohort, a *KRAS*-mutant LUAC sample was considered altered in *STK11/LKB1* (KL) or *TP53* (KP), if there was detection of a known nonsynonymous somatic mutation, any truncating alteration, or biallelic loss.

Preclinical Studies

LKR10/LKR10KO or LKR13/LKR13KO *Kras*-mutant murine LUAC cells (2×10^6) were injected subcutaneously into the right flank of syngeneic recipient male mice (129Sv genetic background). Mice bearing tumors ≥ 200 mm^3 were randomly assigned to intraperitoneal treatment with: (i) six doses of 200 μg anti–PD-1 (clone RMPI-14; BioXCell) or isotype control antibody (clone 2A3; BioXCell) administered twice weekly (n = 5–8 mice per group; LKR10/LKR10KO isogenic system) or (ii) six doses of 200 μg anti–PD-L1 (mIgG1-D265AFc clone 80) or IgG control antibody administered twice weekly (n = 8–9 mice per group; LKR13/LKR13KO isogenic system). Tumor caliper measurements were obtained twice weekly. Mice were sacrificed when tumor volume reached 1,500 mm^3 (LKR10/LKR10KO) or 2,000 mm^3 (LKR13/LKR13KO) or when moribund. Single-cell suspension was established from excised tumors using a commercially available Tumor Dissociation Kit (Miltenyi Biotec) and the gentleMACS Dissociator (Miltenyi Biotec), and cell suspensions were prepared corresponding to 40 mg of gross tumor per 100 μL 1 × PBS/0.05 mmol/L EDTA. One hundred microliters of cell suspension per sample was stained with an antibody cocktail including CD3-FITC (clone 17A2), CD4-PerCP55 (clone RM4-5), CD8-PECy7 (clone 53-6.7), CD45-AF700 (clone 30-F11), CD11b-FITC (clone M1/70), Ly-6G-PerCP/Cy5.5 (clone 1A8), and Zombie dye according to the manufacturer's protocol (BioLegend). Cells were analyzed using the BD FACSCanto multicolor flow cytometer and BD FACSDIVA software. The animal study was approved by the MD Anderson Institutional Animal Care and Use Committee. The LKR13 and LKR10 murine cell lines were generously provided by Dr. Tyler Jacks in 2005. All cell lines tested negative for *Mycoplasma* in March 2017 using the MycoAlert Mycoplasma Detection Kit (Lonza, LT-07-118). Cells were used in *in vivo* experiments within 10 passages from thawing.

Statistical Analysis

The significance of the association between the KL, KP, and K-only subgroup allocations and objective response to PD-1 axis blockade was assessed using the Fisher exact test. The Kaplan–Meier method was used to estimate PFS and OS. For the analysis of PFS, data for patients who were alive and had no evidence of disease progression at the time of the PFS data lockout (December 31, 2016) or who were lost to follow-up were censored at the time of the last radiologic tumor assessment. For the analysis of OS, data for patients who were alive or lost to follow-up at the time of the OS data lockout (April 25, 2017) were censored at the time of the last documented patient contact. Analysis of both PFS and OS in the separate cohort of PD-L1–positive patients from MDACC was based on a January 15, 2018 data lockout. Differences between groups in PFS and OS were assessed on the basis of the log-rank test. Bonferroni-adjusted *P* values were employed to account for multiple comparisons. HRs and the corresponding 95% CIs were computed using the Cox proportional hazards model. The Wald test was applied for testing the HR of 1. $P \leq 0.05$ was considered statistically significant for all comparisons, unless stated otherwise.

Analysis of clinical endpoints in CM-057 was conducted as previously described and corresponds to a February 18, 2016, database lock (45).

For TMB and PD-L1 analysis, statistics were calculated using R version 3.3.2 (2016-10-31). The enrichment analysis for LUAC in the PD-L1/TMB landscape was limited to genes altered in >1% of samples (100 genes).

Disclosure of Potential Conflicts of Interest

F. Skoulidis has received speakers bureau honoraria from Bristol-Myers Squibb. M.E. Goldberg has ownership interest (including patents) in Foundation Medicine, Inc. M.D. Hellmann is a consultant/advisory board member for Bristol-Myers Squibb, Merck, Genentech/Roche, AstraZeneca, Janssen, Mirati, and Shattuck Labs. M.M. Awad reports receiving a commercial research grant from Bristol-Myers Squibb and is a consultant/advisory board member for Merck, Bristol-Myers Squibb, and AstraZeneca. J.F. Gainor is a consultant/advisory board member for Bristol-Myers Squibb, Pfizer, Ariad/Takeda, Loxo, Array Biopharma, Amgen, Novartis, Genentech/Roche, and Theravance. R.J. Hartmaier has ownership interest (including patents) in Foundation Medicine. L. Gay has ownership interest (including patents) in Foundation Medicine, Inc. S.M. Ali is a Scientific Advisory Board member at Incsysus. J.A. Elvin has ownership interest (including patents) in Foundation Medicine, Inc. G. Singal has ownership interest (including patents) in Foundation Medicine. J.S. Ross has ownership interest (including patents) in Foundation Medicine. S. Kirov has ownership interest (including patents) in Bristol-Myers Squibb. J. Szustakowski has ownership interest (including patents) in Bristol-Myers Squibb. S.-H.I. Ou is a consultant/advisory board member for Foundation Medicine. N. Peled has received speakers bureau honoraria from Roche, Bristol-Myers Squibb, MSD, Pfizer, and Guardant360 and is a consultant/advisory board member for Guardant, Roche, MSD, Bristol-Myers Squibb, Takeda, Guardant360, AZ, and BI. M. Nishino reports receiving a Merck investigator studies program grant to institution, a Toshiba Medical Systems research grant to institution, and an AstraZeneca research grant to institution; is a consultant/advisory board member for WorldCare Clinical, Toshiba Medical Systems, and Daiichi Sankyo; and has received honoraria from Roche and Bayer. J.L. Sauter has ownership interest (including patents) in Merck. J. Zhang is a consultant/advisory board member for AstraZeneca. V.A. Miller is on the Board of Directors at Revolution Medicines and has ownership interest (including patents) in Foundation Medicine and Revolution Medicines. G.M. Frampton has ownership interest (including patents) in Foundation Medicine. J.D. Wolchok reports receiving a commercial research grant from Bristol-Myers Squibb and is a consultant/advisory board member for Bristol-Myers Squibb, Medimmune, and Genentech. P.A. Jänne reports receiving commercial research grants from Astellas Pharmaceuticals, AstraZeneca, Daiichi Sankyo, and PUMA; has ownership interest (including patents) in LabCorp; and is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, Roche, Pfizer, Merrimack Pharmaceuticals, Chugai, LOXO Oncology, Ignyta, and Ariad Pharmaceuticals. P.J. Stephens has ownership interest (including patents) in FMI. C.M. Rudin is a consultant/advisory board member for AbbVie, Genentech, Seattle Genetics, Bristol-Myers Squibb, AstraZeneca, Celgene, and Harpoon. L.A. Albacker has ownership interest (including patents) in Foundation Medicine Inc. J.V. Heymach is a consultant/advisory board member for Bristol-Myers Squibb, AstraZeneca, Merck, Genentech, EMD Serono, Boehringer Ingelheim, Spectrum, Lilly, Novartis, and GSK. No potential conflicts of interest were disclosed by the other authors.

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