HHS Public Access

Author manuscript

*Ann Thorac Surg*. Author manuscript; available in PMC 2021 October 01.

Published in final edited form as:

*Ann Thorac Surg*. 2020 October ; 110(4): 1131–1138. doi:10.1016/j.athoracsur.2020.03.114.

# *LKB1/STK11* Expression in Lung Adenocarcinoma and Associations with Patterns of Recurrence

**Kyle G. Mitchell, MD**[1], **Edwin R. Parra, MD PhD**[2], **Jiexin Zhang, MS**[3], **David B. Nelson, MD**[1], **Erin M. Corsini, MD**[1], **Pamela Villalobos, MD**[2], **Cesar A. Moran, MD**[4], **Ferdinandos Skoulidis, MD PhD**[5], **Ignacio I. Wistuba, MD**[2], **Junya Fujimoto, MD PhD**[2], **Jack A. Roth, MD**[1], **Mara B. Antonoff, MD**[1], **the MD Anderson Lung Cancer Immune Microenvironment Working Group**[*]

[1]Department of Thoracic and Cardiovascular Surgery, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030

[2]Department of Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, 2130 West Holcombe Blvd Suite 910, Houston, TX 77030

[3]Department of Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, 1400 Pressler St FCT 4.6000, Houston, TX 77030

[4]Department of Pathology, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030

[5]Department of Thoracic/Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, 1400 Holcombe Blvd Unit 432, Houston, TX 77030

## Abstract

**Background:** Mutations in the serine-threonine kinase *LKB1/STK11* have been implicated in mediating resistance to checkpoint blockade among patients with advanced lung adenocarcinoma. We sought to examine the associations between clinicopathologic characteristics, tumor *LKB1* expression, features of the immune microenvironment, and postoperative prognosis among patients with early-stage lung adenocarcinoma undergoing surgical therapy.

**Methods:** Formalin-fixed, paraffin-embedded specimens of patients undergoing resection of stage I-III, chemotherapy-naïve adenocarcinomas (1997–2008) were analyzed using tissue microarray sectioning. Sublobar resections were excluded. Intratumoral *LKB1/STK11* expression was quantified as H-score. In a subset, tumor associated immune cell populations were quantified using whole tumor sections in peritumoral and intratumoral compartments.

**Corresponding Author:** Mara B. Antonoff, MD, Department of Thoracic and Cardiovascular Surgery, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, mbantonoff@mdanderson.org.

[*]The MD Anderson Lung Cancer Immune Microenvironment Working Group: J. Jack Lee PhD, Ara A. Vaporciyan MD, Wayne L. Hofstetter MD, Reza J. Mehran MD, Stephen G. Swisher MD, David C. Rice MD, Boris Sepesi MD, Garrett L. Walsh MD, Carmen Behrens MD, Neda Kalhor MD, Annikka Weissferdt MD DrMed, and John V. Heymach MD PhD.



This manuscript was presented at the 55th Annual Meeting of the Society of Thoracic Surgeons; 28 January 2019; San Diego, California.

**Results:** 104 patients met inclusion criteria. *LKB1/STK11* expression (median H-score 102.9) was higher in women (median 123.3) than men (100.0, p=0.004) and in never-smokers (median 145.0) than former/current smokers (100.0, p=0.002). *LKB1/STK11* expression was positively correlated with intratumoral infiltration of $CD3^+$ (r=0.351, $P$=0.005), $CD4^+$ (r=0.436, $P$<0.001), and $CD8^+$ (r=0.263, $P$=0.049) cells. Patients with extrathoracic recurrence had lower tumor expression of *LKB1/STK11* than did other patients with recurrent disease. On multivariate analysis, low *LKB1/STK11* expression remained independently associated with poor disease-free survival and distant disease-free survival.

**Conclusions:** Low *LKB1/STK11* expression is associated with specific patient characteristics and poor postoperative prognosis in chemotherapy-naïve lung adenocarcinoma. Further investigation is warranted to delineate its clinical significance in the context of evaluating novel therapeutic agents in patients with resectable disease.

## Keywords

non-small cell lung cancer; tumor microenvironment; LKB1; STK11; adenocarcinoma

The evolving characterization of the immunogenomic landscape in non-small cell lung cancer (NSCLC) has greatly enhanced understanding of the processes driving tumorigenesis, disease progression, and tumor evasion of immunosurveillance (1). The identification of novel therapeutic targets and the development of management strategies tailored to tumor bimolecular profiles have been aided by this knowledge and, in turn, have revolutionized the management approach to patients with this disease (1–7).

Serine/threonine kinase 11 (*STK11*)/liver kinase B1 (*LKB1*) is a protein that regulates cellular metabolic processes and growth via activation of enzymes in the 5’ adenosine monophosphate-activated protein kinase family (8). *LKB1/STK11* additionally functions as a tumor suppressor, and inactivation is associated with an aggressive tumor phenotype and with prometastatic features (9–11). In the setting of advanced *KRAS*-mutant lung adenocarcinoma, coexisting *LKB1/STK11* mutations have been identified as driving local immunosuppression within the tumor microenvironment (TME) (7, 12). Importantly, these mutations have been further shown to mediate resistance to inhibitors of the PD-1/PD-L1 axis (13). However, although there have been previous efforts to characterize the role of *LKB1/STK11* in the context of advanced *KRAS*-mutant NSCLC, its associations with clinicopathologic characteristics and its prognostic significance in the context of resectable disease have not been fully elucidated. Because mutations in *LKB1/STK11* are predominantly manifested by a loss of protein expression, precise quantification and delineation of the relationships between *LKB1/STK11* expression, clinical features, and oncologic outcomes constitute a clinically salient and incompletely developed line of inquiry (11).

Antitumor immunity is marked by innate and adaptive responses that are mediated in large part by a complex interplay between T lymphocyte populations ($CD3^+$, $CD4^+$ [helper], $CD8^+$ [cytotoxic], $CD45RO^+$ [memory], $FOXP3^+$ [regulatory]), natural killer cells ($CD57^+$), tumor-associated macrophages ($CD68^+$), and tumor evasion of the immune response by activation of the PD1/PD-L1 immunoinhibitory axis (1). In this study, we hypothesized that

lung adenocarcinomas with low *LKB1/STK11* expression would be characterized by poor immune cell infiltration, an increased propensity for systemic metastases, and adverse postoperative prognosis. To that end, we analyzed relationships between tumor *LKB1/STK11* expression, tumor associated immune cell (TAIC) densities, and patterns of recurrence after curative-intent resection of lung adenocarcinoma.

## PATIENTS AND METHODS

### Patient Selection

Eligible patients for this study were those who underwent resection of primary lung adenocarcinoma at the University of Texas MD Anderson Cancer Center between 1997 and 2008 and had been previously included in a tissue microarray with *LKB1/STK11* expression data available ($n = 104$, Supplementary Figure 1). Patients who received neoadjuvant therapy (to limit possible confounding effects on the tumor microenvironment) (14) and those who underwent sublobar resection were excluded from analysis to limit possible confounding effects on oncologic outcomes. This retrospective study was approved by MD Anderson's Institutional Review Board with a waiver of individual patient consent. Tumors were retrospectively staged using the seventh edition of the American Joint Committee on Cancer's staging system (15).

### Immunohistochemical Staining and Analyses

Tissue microarrays had been previously constructed using formalin-fixed, paraffin-embedded tumor blocks using methods that have been described previously among a cohort of NSCLC patients who underwent primary resection (16, 17). Tissue microarray sections were prepared using three 1.0 mm tissue cores obtained from the center, middle, and periphery of formalin-fixed and paraffin-embedded histological sections. Cases with at least two TMA cores were included in the analysis. *LKB1/STK11* expression in tumor cells was quantified as an H-score (range, 0 to 300) by multiplying the observed staining intensity (LKB1/clone D60C5F10, dilution 1:250; Cell Signaling Technology, Beverly, MA; range 0 [no staining], $1^+$ [weak staining], $2^+$ [moderate staining], $3^+$ [strong staining]) and the percentage of cells expressing the marker (range 0–100%) (representative examples of staining levels are provided in Figure 1; Figure 1A, no staining; 1B, weak staining [$1^+$]; 1C, moderate staining [$2^+$]; 1D, strong staining [$3^+$]; 1E, biological negative control; 1F, biological positive control; stained sections of all available cases have been uploaded as Supplementary Figure 2). For a subset of cases (83/104, 80%; baseline clinical characteristics provided in Supplementary Table 1), whole tumor sections had been previously analyzed to quantify tumor-associated immune cell densities in the peritumoral and intratumoral (tumor nests and stroma of tumor) compartments using methods previously described (16). Briefly, four micrometer-thick tumor sections were stained for identification and quantification of cells expressing CD3 (polyclonal antibody, catalogue number A045201–2, dilution 1:100; Dako, Carpinteria, CA), CD4 (Novocastra, Leica Microsystems, Milton Keynes, UK; clone 4B12, dilution 1:80; Leica Biosystems, Buffalo Grove, IL), CD8 (CD8/144B, 1:20; ThermoFisher Scientific, Inc), CD45RO (UCHL1, ready to use; Leica Biosystems), CD57 (HNK-1, 1:40; BD Biosciences), CD68 (cloe PG-M1, 1:450; Dako), PD-1 (EPR4877–2, 1:250; Abcam), FOXP3 (206D, 1:50; BioLegend), and PD-L1 (E1L3N,

1:100; Cell Signaling Technology). Stained slides as well as positive and negative controls were scanned at x200 magnification using the Aperio AT2 Scanner (Leica Microsystems) and visualized and analyzed using ImageScope and Toolbox software (Leica Microsystems). As previously described, TAIC densities were examined in five 1 $mm^2$ areas and quantified as the mean density of examined areas (16).

### Outcome Definitions and Statistical Analysis

Associations between tumor expression of *LKB1/STK11* and clinicopathologic characteristics were analyzed using the Mann-Whitney U and Kruskal-Wallis tests. Pairwise correlations between *LKB1/STK11* and TAIC densities were analyzed using Spearman's correlations. For correlations between *LKB1/STK11* expression and TAIC densities, correction for multiple comparisons (9 aforementioned TAIC populations) was performed using the Benjamini-Hochberg method (18). Overall survival (OS) was defined as the time from surgery to death from any cause; patients alive at the end of the study period were censored at the date of last follow-up. Disease-free survival (DFS) was defined as the time from resection to death or recurrence; patients without a DFS event at the end of the study period were censored at the date of last follow-up. Distant DFS was defined as the time to death or distant recurrence; patients without a distant DFS event at the end of the study period were censored at the date of last follow-up. Locoregional recurrence was defined as recurrence at resection margins or within N1/N2 nodal stations at the first diagnosis of recurrence, and distant recurrence was defined as recurrence elsewhere (19). Survival times were estimated using the Kaplan-Meier method, and differences in time-to-event outcomes were analyzed using the log-rank test. Univariate Cox proportional hazards regressions were performed to examine associations between *LKB1/STK11* expression and relevant clinicopathologic features with survival. Variables with $p<0.20$ on univariate analysis were entered into a multivariate model; stepwise backwards selection was then performed with $p<0.10$ as the final selection criterion in order to optimize parsimony of the final multivariable model (parsimony assessed using Akaike's Information Criterion). For survival analysis, *LKB1/STK11* expression was as a continuous variable. All analyses were performed using R (version 3.3.0; http://www.r-project.org), SPSS (version 24.0.0; IBM, Armonk, NY), and STATA (version 14.2, StataCorp, College Station, TX). A two-tailed $P < 0.05$ was considered significant for all analyses, and a false discovery rate (FDR)-adjusted $P$ value < 0.05 was considered significant for analyses in which FDR correction was performed.

## RESULTS

### Patient, Tumor, and Treatment Characteristics

Of 104 patients who met inclusion criteria (Supplementary Figure 1), most (62, 60%) had pathologic stage I disease (Table 1). The cohort was evenly distributed by sex, and a majority of patients were former or current smokers (89, 86%). Adjuvant chemotherapy and radiotherapy were used in 23 (22%) and 13 (13%), respectively.

### Associations with Clinicopathologic Features

The median expression of *LKB1/STK11* for the entire cohort was 102.9 (*H*-score, interquartile range [IQR] 21.3–144.2). Higher *LKB1/STK11* expression was observed among women (median *H*-score 123.3 [IQR 40.0–170.0] versus 100.0 [IQR 9.2–120.0], *P*=0.004) and never-smokers (median H-score 145.0 [IQR 121.7–190.0] versus 100.0 [IQR 14.2–135.8], *P*=0.002) (Figure 2A and B). Although there were no observed differences in expression according to tumor differentiation (well/moderate 113.3 [IQR 14.2–157.5] versus poor 100.0 [IQR 25.0–123.3], *P*=0.289) or pathologic stage (stage I 110.0 [IQR 31.3–155.8] versus stage II 100.0 [IQR 0.0–124.2] versus stage III 101.3 [IQR 32.5–136.7], *P*=0.334), *LKB1/STK11* expression was inversely correlated with pathologic tumor size (r=−0.329, *P*<0.001) (Figure 2C and D). No statistically-significant difference was observed according to margin status (R0: median 105.0, IQR 22.5–147.5; R1: median 52.5, IQR 17.5–96.7; *P*=0.256).

### Associations with Densities of Tumor-Associated Immune Cells

*LKB1/STK11* expression was positively correlated with intratumoral densities of $CD3^+$ (r=0.351, *P*=0.005), $CD4^+$ (r=0.436, *P*<0.001), and $CD8^+$ (r=0.263, *P*=0.049) among the subset of cases with available TAIC IHC data in this cohort (*N* = 83/104) (Fig. 3A–C). After adjusting for multiple comparisons, no statistically-significant associations were identified with densities of $CD45RO^+$, $FOXP3^+$, $CD57^+$, and $CD68^+$ cell populations among the subgroup of study patients with IHC data available (*N* = 83/104; Supplementary Table 2).

### Associations with Postoperative Recurrence and Disease-Free Survival

After a median follow-up duration of 89.7 (IQR 34.4–135.8) months, there were 65 (63%) deaths and 66 (64%) DFS events. Median survival time (MST) and median disease-free survival time (MDFST) for the entire cohort were 87.0 (95% CI 61.3–112.8) months and 66.7 (95% CI 25.9–107.5) months, respectively. Whereas isolated locoregional recurrences were uncommon (1/104, 1%), isolated distant failure (27/104, 26%) and simultaneous locoregional and distant recurrence (11/104, 11%) were more frequently observed. Analysis of patients who had any disease recurrence (*n* = 39/104) identified *LKB1/STK11* expression to be lower among patients who suffered extrathoracic failure (*n* = 19/39, median *H*-score 40.0 [IQR 0.0–110.0]) than those that did not (*n* = 20/39, median *H*-score 120.8 [IQR 91.7–139.4], *P*=0.024). Next, we examined whether intratumoral *LKB1/STK11* expression retained prognostic significance in this cohort after controlling for other relevant clinicopathologic and treatment characteristics. On multivariate analysis, higher *LKB1/STK11* expression was independently associated with a reduced hazard of DFS events and distant disease-free survival events (Tables 2 and 3, Supplementary Tables 3 and 4).

## COMMENT

We report associations between low tumor expression of *LKB1/STK11*, baseline patient characteristics, increased distant metastases, and poor postoperative prognosis. Moreover, we identified modest associations between reduced *LKB1/STK11* expression and reduced intratumoral infiltration by populations of immune cells with key roles in the antitumor immune response ($CD3^+$ [T cells], $CD4^+$ [helper T cells], and $CD8^+$ [cytotoxic T cells]),.

Considered together, our findings suggest that diminished expression of this tumor suppressor has important effects both locally, within the tumor microenvironment, and systemically.

The results of the present analysis identified differing expression of *LKB1/STK11* according to patient clinical characteristics. Although associations with some clinicopathologic features have been consistently identified across previous studies, others have yet to be clearly defined. Previous tobacco exposure has been similarly reported to be associated with *LBK1/STK11* status (11, 20, 21). In contrast, studies have come to conflicting conclusions regarding associations between *LBK1/STK11* genomic alterations and sex (11, 20, 21). Additionally, though we did not identify lower expression among poorly differentiated tumors in the present report, previous analyses have suggested that *LKB1/STK11* mutations are more common in this subgroup (21, 22). Given these discrepancies, further study is warranted to clarify associations between *LKB1/STK11* status and patient and tumor clinicopathologic characteristics. In light of the metabolic regulatory functions of *LKB1/STK11* and emerging evidence attesting to the ability of $^{18}$F-fluorodeoxyglucose positron emission tomography to characterize features of the tumor microenvironment, additional investigation of *LKB1/STK11* status in the context of volumetric and metabolic radiographic assessment of glucose avidity remains an intriguing line of inquiry for further investigation (23, 24).

Mutations in and low expression of *LKB1/STK11* have been previously reported as associated with poor prognosis in patients with solid tumors in several contexts, including advanced NSCLC, breast cancer, and colorectal carcinoma (25, 26). In concert with the findings of the present study, relative *LKB1/STK11* deficiency has been previously identified as being associated with increased extrathoracic metastases (11). Taken in the context of work demonstrating that the antitumor immune response generates effects in the local microenvironment as well as sustained effects systemically, the associations in the present and previous reports of associations between *LKB1/STK11* status and reduced immune cell infiltration could in part explain the associations with poor prognosis (7, 11, 12, 27, 28). We speculate that these observations may reflect effects of local immunosuppression and subsequent inhibition of immune priming that are manifested as an increased propensity for systemic failures. Although further investigation is needed to elucidate these findings, the use of pretreatment biopsy as a means of identification of patients with low tumor *LKB1/STK11* expression who might be less likely to respond to novel therapeutic agents in the neoadjuvant setting is a potential avenue for future clinical application.

This study is limited by its retrospective nature, temporal and treatment heterogeneity among the study cohort, and the fact that microarray data were available in only a subset of the patients undergoing resection for primary lung adenocarcinoma during the study period. Additionally, although *LKB1/STK11* expression was independently associated with poor DFS when modeled both as a continuous and categorical variable, validation in external cohorts is needed to delineate the threshold of expression that best identifies patients at increased risk of poor postoperative prognosis. Finally, the variety of genomic alterations in *LKB1/STK11* and the complexities of intratumoral heterogeneity introduce further subtlety into the interpretation of these results. However, work by previous groups has suggested that

*LKB1/STK11* loss occurs relatively early in the progression of dysplastic lesions to carcinoma, and analysis of expression by immunohistochemistry has been validated as representative of tumor genomic status (7, 10, 11).

In summary, we identified differential expression of *LKB1/STK11* according to sex and tobacco exposure, and further observed associations between low expression and an increased risk of disease recurrence. Further examination of tumor *LKB1/STK11* expression in the context of trials of novel therapeutic agents is needed in order to identify the clinical relevance of these findings in patients with resectable disease as treatment paradigms continue to rapidly evolve.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgment:

J.V. Heymach has received research support from AstraZeneca, Bayer, GlaxoSmithKline, and Spectrum. This work was supported in part by the Cancer Prevention Research Institute of Texas Multi-Investigator Research Awards (RP160668, IIW), the National Institutes of Health/National Cancer Institute through the University of Texas Lung Specialized Programs of Research Excellence grant (P50CA70907, IIW), by generous philanthropic donations by the Mason family and anonymous donors, and by departmental funding.

## GLOSSARY OF ABBREVIATIONS

| | |
|---|---|
| **CD** | cluster of differentiation |
| **CI** | confidence interval |
| **FOXP3** | forkhead box P3 |
| **HR** | hazard ratio |
| **LKB1** | liver kinase B1, *see STK11* |
| **NSCLC** | non-small cell lung cancer |
| **PD-1** | programmed cell death protein 1 |
| **PD-L1** | programmed cell death ligand 1 |
| **STK11** | serine/threonine kinase 11, *see LKB1* |
| **TAIC** | tumor-associated immune cell |

## REFERENCES

1. Wellenstein MD, de Visser KE. Cancer-cell-intrinsic mechanisms shaping the tumor immune landscape. Immunity 2018;48(3):399–416. [PubMed: 29562192]
2. Lopes G, Wu Y-L, Kudaba I et al. Pembrolizumab (pembro) versus platinum-based chemotherapy (chemo) as first-line therapy for advanced/metastatic nsclc with a PD-L1 tumor proportion score (tps) ≥ 1%: Open-label, phase 3 Keynote-042 study. J Clin Oncol 2018;36(18_suppl):LBA4–LBA4.

3. Nilsson MB, Sun H, Diao L et al. Stress hormones promote EGFR inhibitor resistance in nsclc: Implications for combinations with beta-blockers. Sci Transl Med 2017;9(415).
4. Tang H, Xiao G, Behrens C et al. A 12-gene set predicts survival benefits from adjuvant chemotherapy in non–small cell lung cancer patients. Clin Cancer Res 2013;19(6):1577–1586. [PubMed: 23357979]
5. Reck M, Rodriguez-Abreu D, Robinson AG et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016;375(19):1823–1833. [PubMed: 27718847]
6. Forde PM, Chaft JE, Smith KN et al. Neoadjuvant PD-1 blockade in resectable lung cancer. N Engl J Med 2018;378(21):1976–1986. [PubMed: 29658848]
7. Skoulidis F, Byers LA, Diao L et al. Co-occurring genomic alterations define major subsets of kras-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. Cancer Discov 2015;5(8):860–877. [PubMed: 26069186]
8. Carretero J, Medina PP, Blanco R et al. Dysfunctional AMPK activity, signalling through mtor and survival in response to energetic stress in LKB1-deficient lung cancer. Oncogene 2006;26:1616. [PubMed: 16953221]
9. Sanchez-Cespedes M A role for lkb1 gene in human cancer beyond the Peutz–Jeghers syndrome. Oncogene 2007;26:7825. [PubMed: 17599048]
10. Ghaffar H, Sahin F, Sanchez-Cepedes M et al. Lkb1 protein expression in the evolution of glandular neoplasia of the lung. Clin Cancer Res 2003;9(8):2998–3003. [PubMed: 12912948]
11. Calles A, Sholl LM, Rodig SJ et al. Immunohistochemical loss of LKB1 is a biomarker for more aggressive biology in *KRAS*-mutant lung adenocarcinoma. Clin Cancer Res 2015;21(12):2851–2860. [PubMed: 25737507]
12. Koyama S, Akbay EA, Li YY et al. STK11/LKB1 deficiency promotes neutrophil recruitment and proinflammatory cytokine production to suppress t-cell activity in the lung tumor microenvironment. Cancer Res 2016;76(5):999–1008. [PubMed: 26833127]
13. Skoulidis F, Goldberg ME, Greenawalt DM et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in *KRAS*-mutant lung adenocarcinoma. Cancer Discov 2018;8(7):822–835. [PubMed: 29773717]
14. Parra ER, Villalobos P, Behrens C et al. Effect of neoadjuvant chemotherapy on the immune microenvironment in non-small cell lung carcinomas as determined by multiplex immunofluorescence and image analysis approaches. J Immunother Cancer 2018;6(1):48. [PubMed: 29871672]
15. Goldstraw P, Crowley J, Chansky K et al. The IASCLC lung cancer staging project: Proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol 2007;2(8):706–714. [PubMed: 17762336]
16. Parra ER, Behrens C, Rodriguez-Canales J et al. Image analysis–based assessment of PD-L1 and tumor-associated immune cells density supports distinct intratumoral microenvironment groups in non–small cell lung carcinoma patients. Clin Cancer Res 2016;22(24):6278–6289. [PubMed: 27252415]
17. Parra ER, Villalobos P, Zhang J et al. Immunohistochemical and image analysis-based study shows that several immune checkpoints are co-expressed in non-small cell lung carcinoma tumors. J Thorac Oncol 2018;13(6):779–791. [PubMed: 29526824]
18. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological) 1995;57(1):289–300.
19. Brandt WS, Yan W, Leeman JE et al. Postoperative radiotherapy for surgically resected ypN2 non-small cell lung cancer. Ann Thorac Surg 2018;106(3):848–855. [PubMed: 29807005]
20. Koivunen JP, Kim J, Lee J et al. Mutations in the *LKB1* tumour suppressor are frequently detected in tumours from caucasian but not asian lung cancer patients. Br J Cancer 2008;99:245. [PubMed: 18594528]
21. Matsumoto S, Iwakawa R, Takahashi K et al. Prevalence and specificity of *LKB1* genetic alterations in lung cancers. Oncogene 2007;26:5911. [PubMed: 17384680]

22. Jiang L, Liang X, Liu M et al. Reduced expression of liver kinase b1 and beclin1 is associated with the poor survival of patients with non-small cell lung cancer. Oncol Rep 2014;32(5):1931–1938. [PubMed: 25175672]

23. Kaira K, Serizawa M, Koh Y et al. Biological significance of 18F-FDG uptake on PET in patients with non-small-cell lung cancer. Lung Cancer 2014;83(2):197–204. [PubMed: 24365102]

24. Kim J, Hu Z, Cai L et al. Cps1 maintains pyrimidine pools and DNA synthesis in *KRAS/LKB1*-mutant lung cancer cells. Nature 2017;546:168. [PubMed: 28538732]

25. He T-Y, Tsai L-H, Huang C-C, Chou M-C, Lee H. *LKB1* loss at transcriptional level promotes tumor malignancy and poor patient outcomes in colorectal cancer. Ann Surg Oncol 2014;21(4):703–710.

26. Xiao J, Zou Y, Chen X et al. The prognostic value of decreased LKB1 in solid tumors: A meta-analysis. PLoS One 2016;11(4):e0152674. [PubMed: 27035914]

27. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: Impact on clinical outcome. Nat Rev Cancer 2012;12:298. [PubMed: 22419253]

28. Hanahan D, Coussens Lisa M. Accessories to the crime: Functions of cells recruited to the tumor microenvironment. Cancer Cell 2012;21(3):309–322. [PubMed: 22439926]

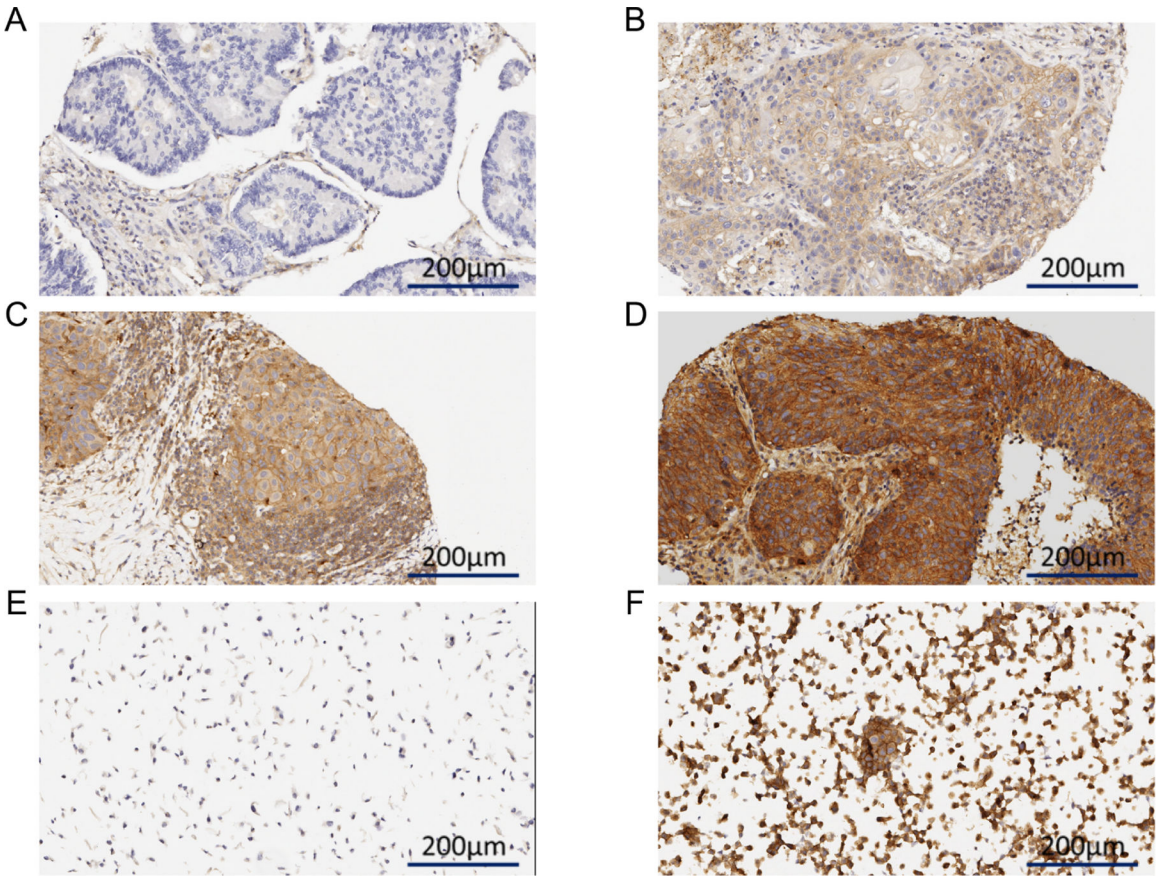


**Figure 1:**
Representative examples of LKB1/STK11 staining: **(A)** absent, **(B)** 1+, **(C)** 2+, **(D)** 3+, as well as **(E)** negative and **(F)** positive controls.

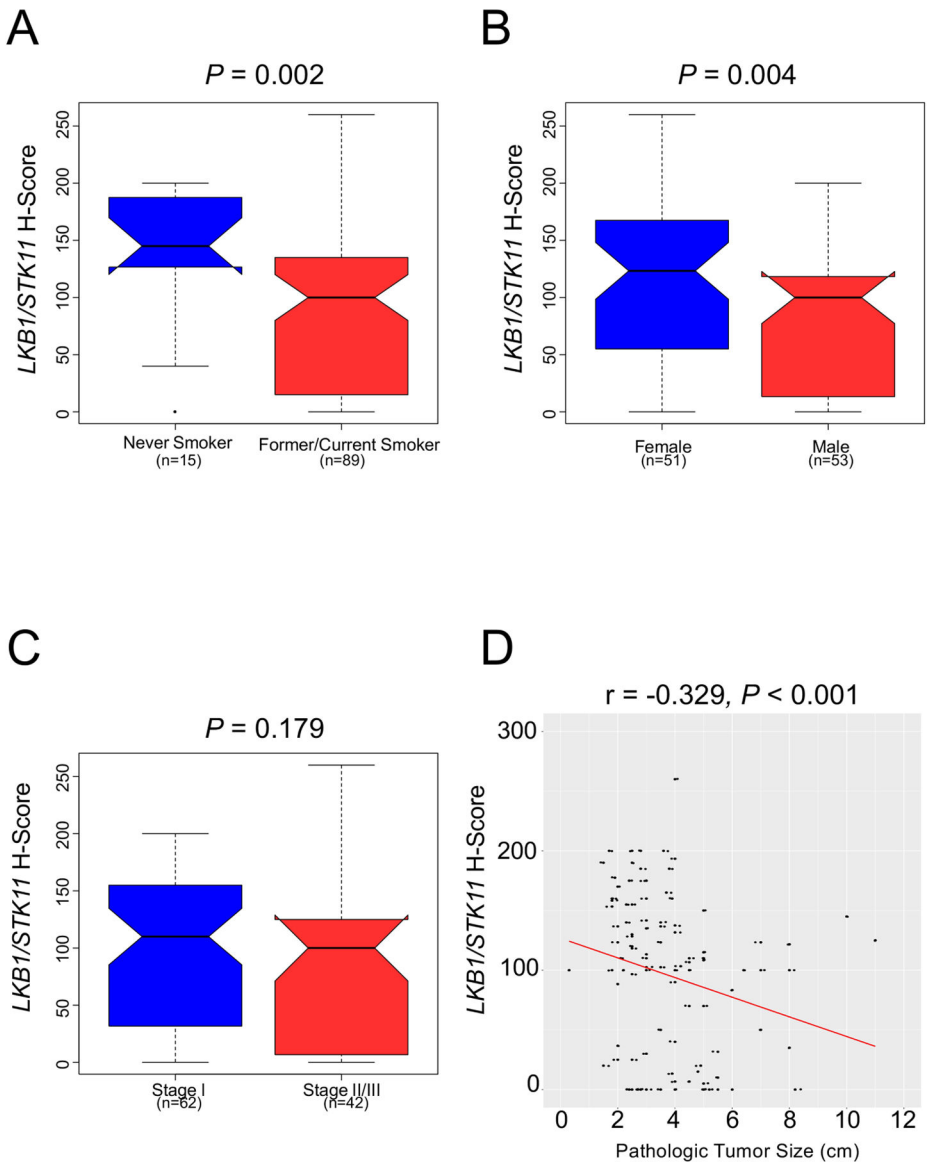


**Figure 2:**

Associations between tumor *LKB1/STK11* expression and clinicopathologic characteristics (*n* = 104). *LKB1/STK11* expression was noted to be higher **(A)** among never smokers than former or current smokers, and **(B)** among women. Although **(C)** no association was identified between *LKB1/STK11* expression and pathologic stage, its expression **(D)** was inversely correlated with pathologic tumor size. Tumor *LKB1/STK11* expression is quantified as intratumoral *H*-score.

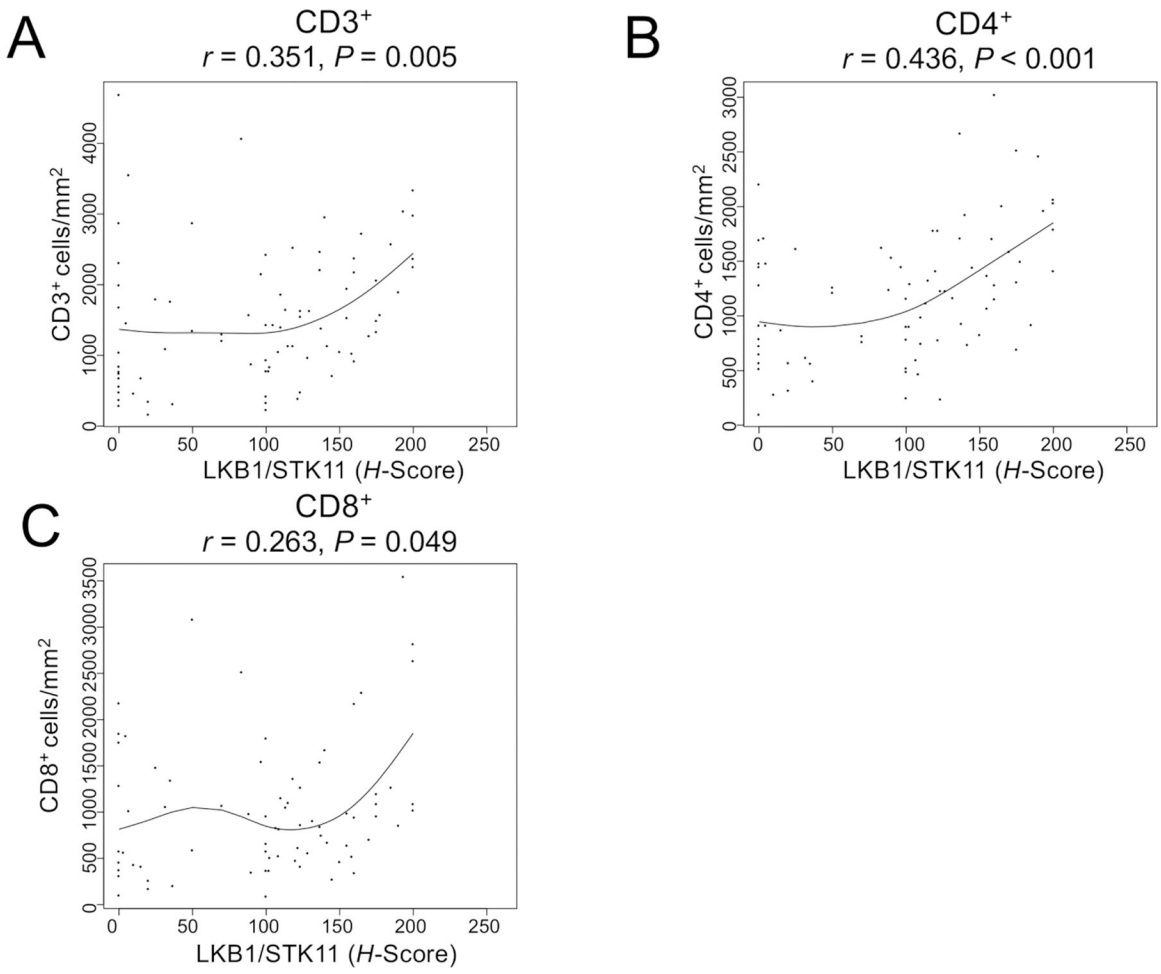


**Figure 3:**

Relationships between tumor expression of *LKB1/STK11* and intratumoral densities of cells expressing **(A)** CD3, **(B)** CD4, and **(C)** CD8. Tumor *LKB1/STK11* expression is quantified as intratumoral *H*-score. A spline fit curve is indicated in each panel.

**Table 1:**

Baseline patient, tumor, and treatment characteristics of the study cohort ($n$ = 104).

| Variable | N (%) or Median (IQR) |
|---|---|
| **Age**, median (IQR) (years) | 64.0 (56.5–73.0) |
| **Sex** | |
| Female | 51 (49.0) |
| Male | 53 (51.0) |
| **Smoking** | |
| Never | 15 (14.4) |
| Former/Current | 89 (85.6) |
| **FEV1** (% predicted) * | 87.0 (77.0–101.0) |
| **Differentiation** | |
| Poor | 43 (41.3) |
| Well/Moderate | 61 (58.7) |
| **Zubrod** | |
| 0 | 57 (54.8) |
| 1 | 47 (45.2) |
| **Extent of resection** | |
| Lobectomy/Bilobectomy | 100 (96.2) |
| Pneumonectomy | 4 (3.8) |
| **Pathologic Margin** | |
| R0 | 100 (96.2) |
| R1 | 4 (3.8) |
| **Pathologic Stage** | |
| I | 62 (59.6) |
| II | 24 (23.1) |
| III | 18 (17.3) |
| **Adjuvant therapy** | |
| Chemotherapy | 23 (22.1) |
| Radiotherapy | 13 (12.5) |

FEV1: forced expiratory volume in one second; IQR: interquartile range

* available in 103/104 (99.0)

**Table 2:**

Clinicopathologic characteristics associated with disease-free survival, with *LKB1/STK11* expression analyzed as a continuous variable ($n = 104$).

| | | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|---|
| | | **HR** | **95% CI** | **P** | **HR** | **95% CI** | **P** |
| **Age** (≥65 years) | 49 (47.1) | 1.48 | 0.91–2.41 | 0.118 | 1.56 | 0.93–2.62 | 0.09 |
| **Sex** (Male) | 53 (51.0) | 1.86 | 1.13–3.06 | 0.015 | | | |
| **Smoker** (Ever) | 89 (85.6) | 0.86 | 0.44–1.69 | 0.658 | | | |
| **Zubrod** (1) | 47 (45.2) | 1.29 | 0.79–2.10 | 0.304 | | | |
| **FEV1** (% Predicted) * | n/a | 1.00 | 0.99–1.02 | 0.879 | | | |
| **Pathologic Stage** | | | | | | | |
| I | 62 (59.6) | Reference | | | Reference | | |
| II | 24 (23.1) | 2.49 | 1.40–4.44 | 0.002 | 2.37 | 1.31–4.27 | 0.004 |
| III | 18 (17.3) | 3.43 | 1.86–6.33 | <0.001 | 2.83 | 1.40–5.73 | 0.004 |
| **Differentiation** (Poor) | 43 (41.4) | 1.11 | 0.68–1.83 | 0.668 | | | |
| **Extent of resection** (Pneumonectomy) | 4 (3.8) | 1.41 | 0.44–4.49 | 0.564 | | | |
| **Margin** (R1) | 4 (3.8) | 4.47 | 1.57–12.69 | 0.005 | 2.33 | 0.71–7.63 | 0.161 |
| ***LKB1/STK11* expression** (*H*-score, per 10 unit increase) | n/a | 0.96 | 0.93–1.00 | 0.029 | 0.96 | 0.93–1.00 | 0.044 |
| **Adjuvant Chemotherapy** | 23 (22.1) | 1.06 | 0.60–1.90 | 0.838 | 0.74 | 0.39–1.42 | 0.367 |
| **Adjuvant Radiotherapy** | 13 (12.5) | 2.78 | 1.48–5.24 | 0.002 | 2.12 | 1.02–4.39 | 0.044 |

* Data available for 103/104 (99.0%)

CI: confidence interval; FEV1: forced expiratory volume in one second; HR: hazard ratio

**Table 3:**

Clinicopathologic characteristics associated with distant disease-free survival, with *LKB1/STK11* expression analyzed as a continuous variable ($n = 104$).

| | | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|---|
| | | **HR** | **95% CI** | **P** | **HR** | **95% CI** | **P** |
| **Age** (≥65 years) | 49 (47.1) | 1.55 | 0.95–2.54 | 0.082 | 1.62 | 0.96–2.73 | 0.069 |
| **Sex** (Male) | 53 (51.0) | 1.96 | 1.18–3.25 | 0.009 | | | |
| **Smoker** (Ever) | 89 (85.6) | 0.94 | 0.48–1.85 | 0.858 | | | |
| **Zubrod** (1) | 47 (45.2) | 1.18 | 0.72–1.94 | 0.501 | | | |
| **FEV1** (% Predicted) * | n/a | 1.00 | 0.98–1.02 | 0.871 | | | |
| **Pathologic Stage** | | | | | | | |
| I | 62 (59.6) | Reference | | | Reference | | |
| II | 24 (23.1) | 2.44 | 1.37–4.35 | 0.003 | 2.38 | 1.32–4.30 | 0.004 |
| III | 18 (17.3) | 3.59 | 1.92–6.73 | <0.001 | 3.41 | 1.69–6.85 | 0.001 |
| **Differentiation** (Poor) | 43 (41.4) | 1.12 | 0.68–1.84 | 0.665 | | | |
| **Extent of resection** (Pneumonectomy) | 4 (3.8) | 1.51 | 0.47–4.82 | 0.488 | | | |
| **Margin** (R1) | 4 (3.8) | 4.33 | 1.31–14.32 | 0.016 | 2.11 | 0.56–7.90 | 0.266 |
| ***LKB1/STK11* expression** (*H*-score, per 10 unit increase) | n/a | 0.96 | 0.92–0.99 | 0.014 | 0.95 | 0.92–0.99 | 0.013 |
| **Adjuvant Chemotherapy** | 23 (22.1) | 0.86 | 0.47–1.56 | 0.617 | 0.59 | 0.31–1.14 | 0.118 |
| **Adjuvant Radiotherapy** | 13 (12.5) | 2.30 | 1.20–4.41 | 0.012 | 1.97 | 0.93–4.14 | 0.075 |

* Data available for 103/104 (99.0%)

CI: confidence interval; FEV1: forced expiratory volume in one second; HR: hazard ratio