BIOMARKERS

STK11 Inactivation Predicts Rapid Recurrence in Inoperable Early-Stage Non–Small-Cell Lung Cancer

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PURPOSE Molecular factors predicting relapse in early-stage non–small-cell lung cancer (ES-NSCLC) are poorly understood, especially in inoperable patients receiving radiotherapy (RT). In this study, we compared the genomic profiles of inoperable and operable ES-NSCLC.

MATERIALS AND METHODS This retrospective study included 53 patients with nonsquamous ES-NSCLC (stage I-II) treated at a single institution (University of Chicago) with surgery (ie, operable; n = 30) or RT (ie, inoperable; n = 23) who underwent tumor genomic profiling. A second cohort of ES-NSCLC treated with RT (Stanford, n = 39) was included to power clinical analyses. Prognostic gene alterations were identified and correlated with clinical variables. The primary clinical end point was the correlation of prognostic genes with the cumulative incidence of relapse, disease-free survival, and overall survival (OS) in a pooled RT cohort from the two institutions (N = 62).

RESULTS Although the surgery cohort exhibited lower rates of relapse, the RT cohort was highly enriched for somatic *STK11* mutations (43% *v* 6.7%). Receiving supplemental oxygen (odds ratio [OR] = 5.5), 20+ pack-years of tobacco smoking (OR = 6.1), and Black race (OR = 4.3) were associated with increased frequency of *STK11* mutations. In the pooled RT cohort (N = 62), *STK11* mutation was strongly associated with inferior oncologic outcomes: 2-year incidence of relapse was 62% versus 20% and 2-year OS was 52% versus 85%, remaining independently prognostic on multivariable analyses (relapse: subdistribution hazard ratio = 4.0, $P = .0041$; disease-free survival: hazard ratio, 6.8, $P = .0002$; OS: hazard ratio, 6.0, $P = .022$). *STK11* mutations were predominantly associated with distant failure, rather than local.

CONCLUSION In this cohort of ES-NSCLC, *STK11* inactivation was associated with poor oncologic outcomes after RT and demonstrated a novel association with clinical hypoxia, which may underlie its correlation with medical inoperability. Further validation in larger cohorts and investigation of effective adjuvant systemic therapies may be warranted.

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INTRODUCTION

Lung cancer is the most common cause of cancer-related death.[1] Although most patients with non–small-cell lung cancer (NSCLC) were diagnosed with advanced disease in past decades, the recent implementation of risk-stratified, low-dose screening computed tomography on a population-wide level has resulted in more patients being diagnosed with early-stage disease amenable to curative local therapies.[2,3] Medically fit patients are most commonly treated with surgical tumor resection, whereas those deemed medically inoperable generally undergo stereotactic body radiotherapy (SBRT).[4]

Although both surgical resection and SBRT provide an excellent likelihood for local tumor control, up to a quarter of patients experience distant metastatic failure, which generally results in death.[5-8] To date, clinical and molecular factors that predict relapse are poorly understood, and the role of additional therapies remains uncertain.[9] Adjuvant cytotoxic chemotherapies with cisplatin-based regimens do not offer clear benefit for stage I tumors (≤ 4 cm in size) and may even lead to a worse overall survival (OS) for stage IA disease.[10] The addition of immunotherapy and targeted therapies (for tumors with actionable mutations) improves disease-free survival (DFS) in early-stage resected NSCLC.[11-14] Comprehensive genomic profiling may help identify tumors susceptible to specific systemic therapies; however, existing studies have been primarily limited to surgically resected tumors.[15,16] Comparable studies in inoperable patients receiving definitive SBRT are lacking.

In this study, we characterized the genomic landscape of early-stage NSCLC (stage I and II) and present a comparative, next-generation sequencing (NGS)

ASSOCIATED CONTENT

Appendix

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

To examine the prognostic significance of genomic alterations in early-stage (stage I to II) non–small-cell lung cancer, including a novel cohort of patients receiving definitive radiotherapy.

Knowledge Generated

STK11 mutations were more prevalent in patients receiving definitive radiotherapy, compared with those receiving surgery. Increased frequencies of *STK11* mutations were observed in patients receiving supplemental oxygen, with 20+ pack-years of tobacco smoking history, or of Black race. In a unique cohort of patients receiving definitive RT, *STK11* inactivation was associated with rapid distant (rather than local) recurrence and worse overall survival.

Relevance

Since *STK11* inactivation may be associated with resistance to immune checkpoint blockade, investigation of effective adjuvant systemic therapies may be warranted in this setting. Furthermore, the novel association with chronic hypoxia suggests that hypoxia may apply a selective pressure that promotes inactivation of *STK11*, such that medically inoperable patients are more prone to develop *STK11*-mutant tumors.

analysis of patients receiving surgery and patients receiving radiotherapy (RT). We identified gene variants associated with relapse and correlated prognostic genes with various clinical factors. Importantly, we propose that prognostic genes with differential prevalence in the surgery and RT groups may be correlated with underlying clinical features associated with operability.

MATERIALS AND METHODS

Study Design and Patients

The results for this retrospective cohort study were reported following the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines.[17] Comparative analyses of molecular profiles were performed in 53 patients diagnosed with early-stage (stage I and II, node-negative) nonsquamous NSCLC and treated at our institution (University of Chicago Medical Center, Chicago, IL) with either surgery (n = 30) or hypofractionated/stereotactic body RT (n = 23) who underwent NGS on primary tumor tissue between 2015 and 2020. NGS was performed using OncoPlus, a validated 1212-gene proprietary hybrid capture genomic sequencing assay (Data Supplement).[18] Analyses were restricted to nonsquamous histologies because patients with squamous histologies did not routinely undergo molecular profiling during the study period. Demographic and clinical data were recorded with review of the electronic medical record. This study was reviewed and approved by the institutional review board. Because of the study's retrospective nature, a waiver for informed consent was provided.

To further power the association of prognostic gene variants with clinical factors and oncologic outcomes, primary analyses included a cohort of patients (n = 39) receiving RT with curative intent for early-stage nonsquamous NSCLC at Stanford Cancer Center (Stanford, CA).[19] In the Stanford cohort, mutation status was available for five genes (*KRAS*, *TP53*, *STK11*, *CDKN2A*, and *EGFR*). For correlation of gene variants with clinical factors, a total pooled cohort of all patients was analyzed (N = 92). For correlation of gene variants with clinical outcomes in the RT group, analyses were limited to the pooled cohort of patients receiving RT at University of Chicago and Stanford (N = 62). Using a pooled cohort augmented external validity of our findings through avoiding bias from subsets of the population specific to a given medical center. Bidirectional institutional review board approval was obtained to share data between institutions.

Genomic Analyses

Processing of sequencing reads and determination of tumor mutational burden and programmed death-ligand 1 expression is described in the Data Supplement.[18,20-22] To screen potentially prognostic variants, pathogenic mutations in each gene were correlated with time to relapse (Data Supplement). To correct for multiple comparisons, q-values were computed using the Benjamini-Hochberg method with the goal of keeping the false discovery rate (FDR) < 0.05. Of the genes with q-value < 0.05, only one gene (*STK11*) met our threshold of $> 10\%$ pathogenic mutation prevalence for correlation with clinical outcomes. *STK11* mutations analyzed in this study were predicted to be pathogenic, inactivating mutations.

Clinical Outcomes and Statistical Analyses

Because there was a significant imbalance in the prevalence of *STK11* mutations in the RT and surgery groups, it was hypothesized that somatic *STK11* mutations were correlated with underlying clinical features associated with operability. To investigate the association of various demographic and clinical variables with the presence of *STK11* mutations in the sequenced tumors, univariable logistic regression was performed in the total pooled cohort (N = 92) including sex, age, race and ethnicity, Eastern Cooperative Oncology Group (ECOG) performance status, body mass index, diabetes mellitus status, supplemental oxygen, pack-years of tobacco smoking, forced expiratory volume in 1 second

TABLE 1. Patient Characteristics in University of Chicago Cohort by Treatment Type

	Total	Surgery	RT	
	N = 53	n = 30	n = 23	*P*
Sex				1.00
Female	37 (70%)	21 (70%)	16 (70%)	
Male	16 (30%)	9 (30%)	7 (30%)	
Age, years, mean (SD)	70 (10)	69 (12)	73 (8)	.17
Race				.10
Non-Black	28 (53%)	19 (63%)	9 (39%)	
Black	25 (47%)	11 (37%)	14 (61%)	
ECOG				.036
0	18 (34%)	14 (47%)	4 (17%)	
1	26 (49%)	14 (47%)	12 (52%)	
2	8 (15%)	2 (7%)	6 (26%)	
3	1 (2%)	0 (0%)	1 (4%)	
FEV1, liters, mean (SD)	1.82 (0.57)	2.05 (0.54)	1.45 (0.40)	< .001
Supplemental O2				< .001
No	45 (85%)	30 (100%)	15 (65%)	
Yes	8 (15%)	0 (0%)	8 (35%)	
Smoking status				.093
Never smoking	5 (9%)	3 (10%)	2 (9%)	
Current smoking	11 (21%)	3 (10%)	8 (35%)	
Former smoking	37 (70%)	24 (80%)	13 (57%)	
Tobacco pack-years, mean (SD)	26 (23)	20 (17)	35 (27)	.013
20+ pack-years of tobacco smoking history				.57
No	19 (36%)	12 (40%)	7 (30%)	
Yes	34 (64%)	18 (60%)	16 (70%)	
Stage				.27
IA1	5 (9%)	3 (10%)	2 (9%)	
IA2	18 (34%)	7 (23%)	11 (48%)	
IA3	8 (15%)	7 (23%)	1 (4%)	
IB	8 (15%)	4 (13%)	4 (17%)	
IIA	2 (4%)	1 (3%)	1 (4%)	
IIB	12 (23%)	8 (27%)	4 (17%)	
Stage I *v* II				.55
I	39 (74%)	21 (70%)	18 (78%)	
II	14 (26%)	9 (30%)	5 (22%)	

NOTE. *P* values are calculated on the basis of Fisher's exact tests for categorical variables and two-sample *t* tests for continuous variables. ECOG, Eastern Cooperative Oncology Group; FEV1, forced expiratory volume in 1 second, RT, radiotherapy.

(FEV1; % predicted), diffusing capacity of the lung for carbon monoxide (DCLO; % predicted), and stage. Details regarding rationale for covariate inclusion are described in the Data Supplement.[23-28] Multivariable logistic regression was performed using backward selection ($P < .10$). The FEV1 % predicted (not assessed in 18 patients) and DLCO % predicted (not assessed in 31 patients) were excluded from multivariable analyses, and only univariable analyses were reported because they were not assessed in all patients.

The primary clinical analyses included the association of *STK11* mutations with time to relapse, DFS, and OS in the pooled inoperable cohort receiving RT (N = 62). Primary clinical analyses were limited to the pooled RT cohort because *STK11* mutations were only present in two patients receiving surgery. All relapses were classified as local, lobar, regional (hilar or mediastinal lymph nodes), or distant (including contralateral lung and outside of the thorax). Time to relapse was analyzed using the cumulative

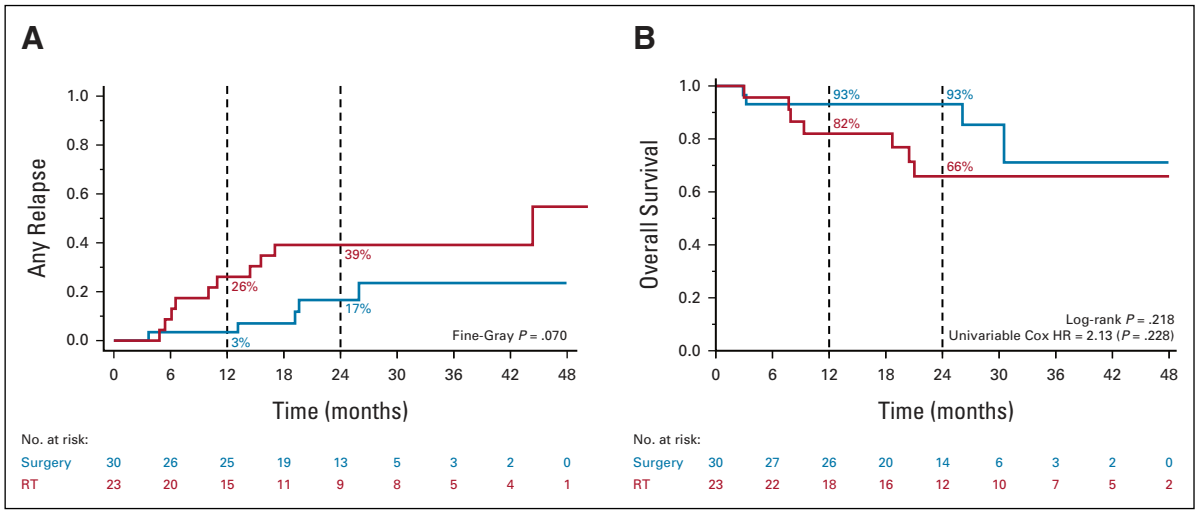

FIG 1. Clinical outcomes in the University of Chicago cohort in the medically inoperable group (patients receiving RT) and medically operable group (patients receiving surgery): (A) freedom from relapse and (B) overall survival. RT, radiotherapy.

incidence function (with death as a competing risk) using the Fine-Gray model for univariable and multivariable analyses. DFS and OS were analyzed using the Kaplan-Meier method and the log-rank *P* values were reported ($P < .05$ deemed statistically significant). To account for demographic and clinical covariates, multivariable Cox proportional hazards models were computed. Detailed definition of clinical end points and statistical analyses are described in the Data Supplement.[29,30]

RESULTS

Patient Characteristics and Outcomes by Treatment Type

From September 21, 2015, to April 13, 2020, 53 patients with nonsquamous early-stage non–small-cell lung cancer were treated definitively with either hypofractionated/stereotactic body RT (n = 23) or surgery (n = 30) and underwent NGS of tumor tissue at University of Chicago (median follow-up, 23.6 mo). Details regarding staging workup and RT regimens are described in the Data Supplement.[31] All surgical patients underwent mediastinal lymph node dissection. Overall, patients receiving RT exhibited adverse clinical features associated with worse OS, including worse performance status, lower FEV1, increased utilization of supplemental oxygen, and greater pack-years of tobacco smoking (Table 1).

Compared with patients who underwent surgery, patients receiving RT exhibited increased relapse, primarily driven by high rates of distant relapse as opposed to local failure (Fig 1). At 2 years, the cumulative incidence of relapse was 16.6% (95% CI, 5.1% to 33.9%) for surgery and 39.1% (95% CI, 19.9% to 58.0%) for RT, respectively (Fine-Gray $P = .070$). The 2-year cumulative incidence of local failure was 0% for surgery and 4.8% for RT, while the 2-year distant failure was 8.6% and 26.1%, respectively. At 2 years, the OS was 93.1% (95% CI, 75.1% to 98.2%) for surgery and 65.9% (95% CI, 41.1% to 82.2%) for RT (log-rank $P = .22$). Despite favorable local control in the RT group, the risk of distant relapse was significantly higher than expected, compared with our institutional surgical cohort and published outcomes for operable patients receiving SBRT.[29] In this context, we investigated whether there were biological differences in early-stage NSCLC between operable patients receiving surgery and inoperable patients receiving RT that could explain the differences in disease relapse.

Genomic Landscape of Early-Stage NSCLC by Treatment Type

The genomic landscape of early-stage NSCLC in the University of Chicago cohort (N = 53) is summarized in Figure 2. Pathogenic variants were detected in 60 genes and were most commonly present in *KRAS* (62%), *TP53* (42%), *STK11* (23%), *CDKN2A* (19%), and *EGFR* (15%). To screen for prognostic variants in the combined University of Chicago cohort, the freedom from relapse was analyzed via the Kaplan-Meier method for each of the 60 variants. Variants were considered potentially prognostic if their log-rank q-values were < 0.05 (ie, FDR < 0.05). These are listed in the Data Supplement. Of five potentially prognostic variants, only *STK11* variants were present with sufficient frequency (n = 12, 23%) for further clinical analyses. *STK11* also remained prognostic when screening variants by cumulative incidence of relapse with death as a competing risk (q = 0.035). Notably, *STK11* mutations were present in only 2 (6.7%) patients receiving surgery and 10 (43%) patients receiving RT. *STK11*/*KRAS* comutations were present in 7 (13%), 1 (3.3%), and 6 (26%) patients in the overall, surgery, and RT cohorts, respectively. Neither tumor mutational burden nor programmed death-ligand 1 TPS varied with *STK11* status (Data Supplement). In summary, with comprehensive molecular profiling of early-stage NSCLC, *STK11*

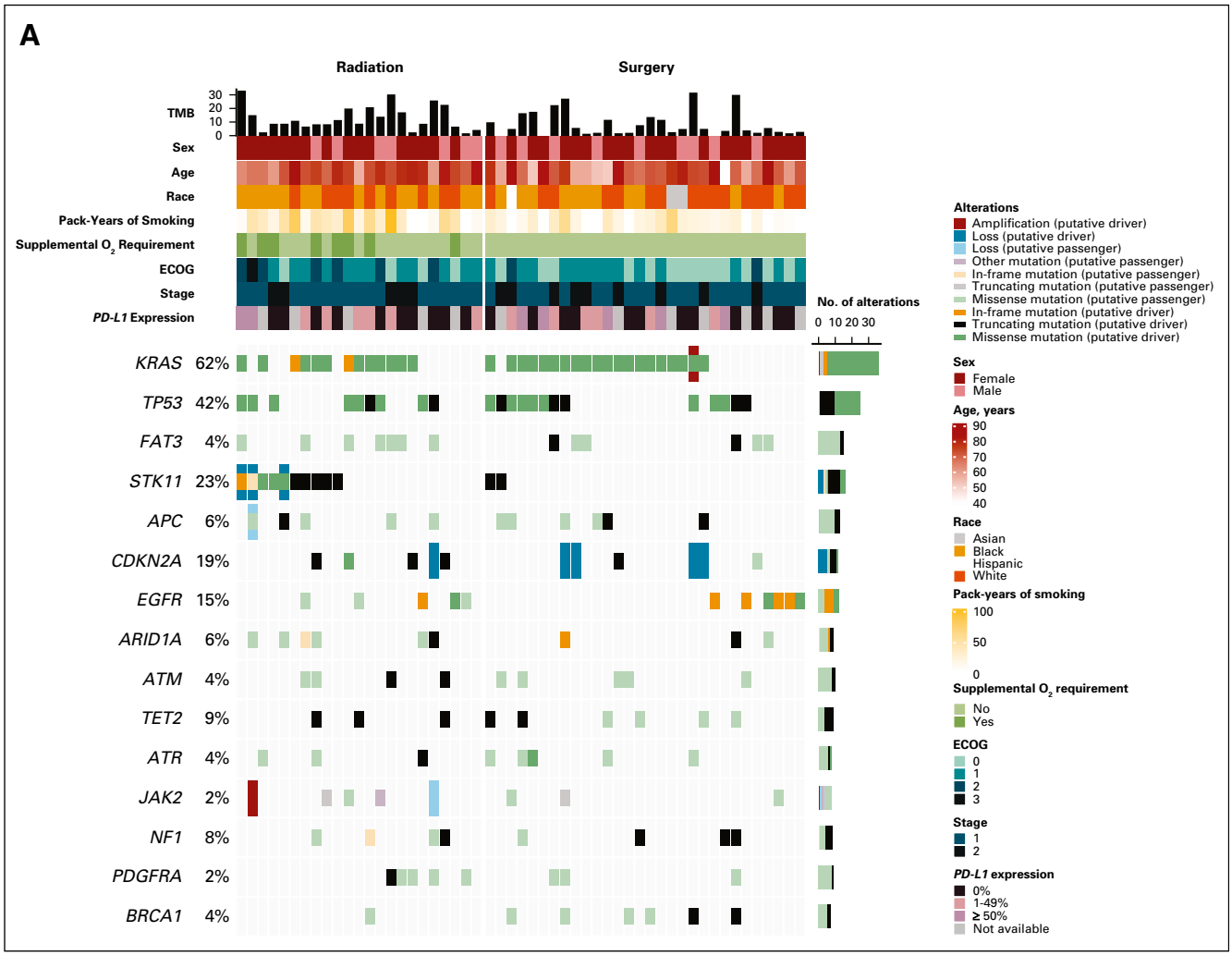

FIG 2. Genomic landscape of early-stage NSCLC in the medically inoperable group (patients receiving RT) and medically operable group (patients receiving surgery). (A) OncoPrint demonstrating top 15 recurrently altered genes in each group. All gene alterations are reported (including non-pathogenic variants); however, frequencies to the left of the plot represent pathogenic alteration prevalence. (B) Frequencies of most common gene alterations in each cohort, counting only pathogenic variants. ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed death-ligand 1; TMB, tumor mutation burden. (continued on following page)

variants were associated with relapse and were present in approximately one quarter of patients, but almost exclusively limited to medically inoperable patients.

Clinical Features Associated With *STK11* Variants

The associations of various demographic and clinical factors with *STK11* variants were investigated in the combined University of Chicago and Stanford cohorts. Univariable associations are displayed in the Data Supplement, demonstrating that treatment with RT, treatment at University of Chicago, Black race, ECOG > 1, FEV1 % predicted, supplemental oxygen, and 20+ pack-years of tobacco smoking history were associated with increased frequency of *STK11* mutations (Data Supplement). Multivariable logistic regression for presence of an *STK11* mutation was performed with backward selection ($P < .1$), including sex, age, Black race, ECOG > 1, diabetes mellitus status, body mass index, supplemental oxygen receipt, 20+ pack-years of tobacco smoking history, and stage. The final model is displayed in Table 2. Supplemental oxygen (odds ratio [OR] 5.5, $P = .027$), 20+ pack-years of tobacco smoking history (OR 6.1, $P = .03$), and Black race (OR 4.3, $P = .024$) were all associated with increased frequency of *STK11* variants in the corresponding tumors. Finally, these clinical markers of hypoxia and demographic factors did not appear to be correlated with each other (Data Supplement).

STK11 Mutation Associated With Relapse in Patients Receiving RT

We analyzed the impact of *STK11* mutations on oncologic outcomes in the pooled cohort of patients receiving RT from University of Chicago (n = 23) and Stanford (n = 39),

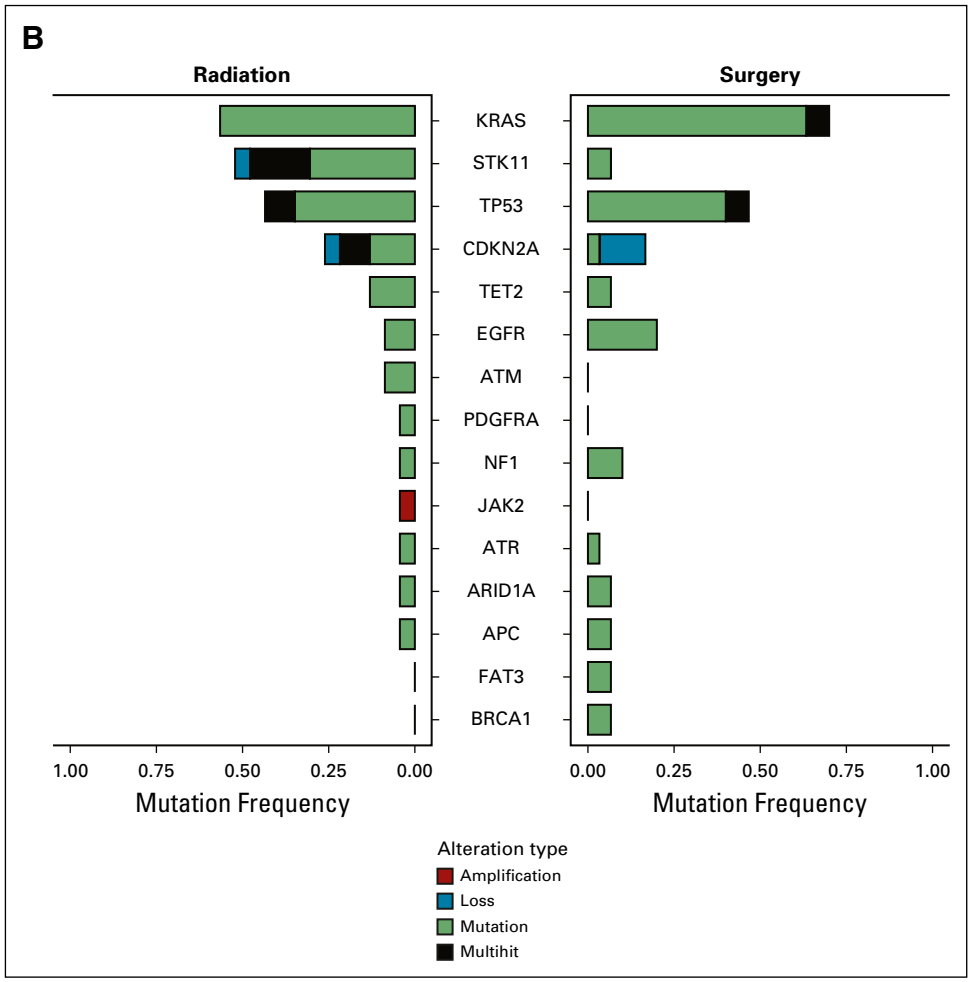

FIG 2. (Continued)

comprising 62 total patients (median follow-up, 18.9 mo). Analysis was limited to the RT cohort because only two patients in the surgery cohort exhibited *STK11* mutations. Overall, *STK11* mutations were more common in the University of Chicago cohort (43% *v* 8%, $P = .002$). Other demographic and clinical variables for each cohort were summarized in the Data Supplement.

In the pooled RT cohort, *STK11* variants were strongly associated with time to relapse, DFS, and OS (Fig 3). The 2-year cumulative incidence of relapse was 20.0% (95% CI, 9.3% to 33.7%) for *STK11* wild-type and 61.5% (95% CI, 30.8% to 81.8%) for *STK11*-mutant tumors (Fine-Gray $P = .004$). Likewise, the 2-year DFS was 71.2% (95% CI, 53.5% to 83.2%) for *STK11* wild-type and 30.8% (95% CI, 9.5% to 55.4%) for *STK11*-mutant tumors (log-rank $P = .0003$). The 2-year OS was 85.4% (95% CI, 67.7% to 93.8%) for *STK11* wild-type and 52.2% (95% CI, 18.9% to 77.6%) for *STK11*-mutant (log-rank $P = .006$). Within the individual institutional cohorts, *STK11* variants remained associated with relapse (Data Supplement). When analyzing each of the factors associated with *STK11* mutations (ie, supplemental oxygen status, 20+ pack-years of tobacco smoking history, and Black race) by mutation status, *STK11* mutations remained associated with significantly worse time to relapse, although statistical comparisons were not feasible because of small subgroup sample sizes (Data Supplement).

Multivariable regression analyses were performed to evaluate the effect of *STK11* variants while controlling for multiple covariates, using a Fine-Gray model for time to relapse and Cox models for DFS and OS. Finalized models after backward selection ($P < .1$) are displayed in Table 3, while the full models are shown in the Data Supplement. *STK11* variants exhibited a subdistribution hazard ratio (SHR) of 4.0 (95% CI, 1.5 to 10.2; $P = .0041$) for time to relapse. *STK11* variants demonstrated a hazard ratio (HR) of 6.8 (95% CI, 2.5 to 18.3; $P = .0002$) for DFS, and an HR of 6.0 (95% CI, 1.3 to 27.8; $P = .022$) for OS. Thus, *STK11* mutations remained strongly associated with increased relapse and decreased survival on multivariable analyses.

Furthermore, *STK11* mutations were predominantly associated with a distant pattern of failure, rather than local failure (Data Supplement). At 2 years, the cumulative

TABLE 2. Multivariable Logistic Regression After Backward Selection ($P < .1$) for Presence of *STK11* Variants in the Profiled Tumors

	Presence of *STK11* Variants	
Variable	**Hazard Ratio (95% CI)**	***P***
Supplemental O2		
No	Reference	
Yes	5.5 (1.2 to 24.7)	.027
Tobacco pack-years > 20		
No	Reference	
Yes	6.1 (1.2 to 31.6)	.031
Black race		
No	Reference	
Yes	4.3 (1.2 to 15.5)	.024

incidence of distant metastasis was 12.1% for *STK11* wild-type and 40.4% *STK11*-mutant tumors (Fine-Gray $P = .030$). By contrast, the 2-year cumulative incidence of local failure was 5.3% for *STK11* wild-type and 9.3% for *STK11*-mutant (Fine-Gray $P = .68$). In the *STK11*-mutant group, the single local failure presented with concomitant regional nodal failure as well. There was no association between RT dose and local failure in the pooled RT cohort (Data Supplement). Taken together, in a pooled inoperable cohort receiving definitive RT, *STK11* mutations distinguished a biologically aggressive subset of early-stage NSCLC that was associated with significantly worse OS, DFS, and cumulative incidence of relapse, with the primary mode of failure being distant recurrence.

DISCUSSION

In this study, the genomic landscapes of early-stage NSCLC (stage I to II) treated with surgery and RT were compared. Compared with the surgery cohort, patients undergoing RT demonstrated unique enrichment for *STK11* mutations (43% *v* 6.7%) that defines a poor prognostic subgroup associated with rapid distant progression. Somatic *STK11* mutations are more prevalent in patients on supplemental

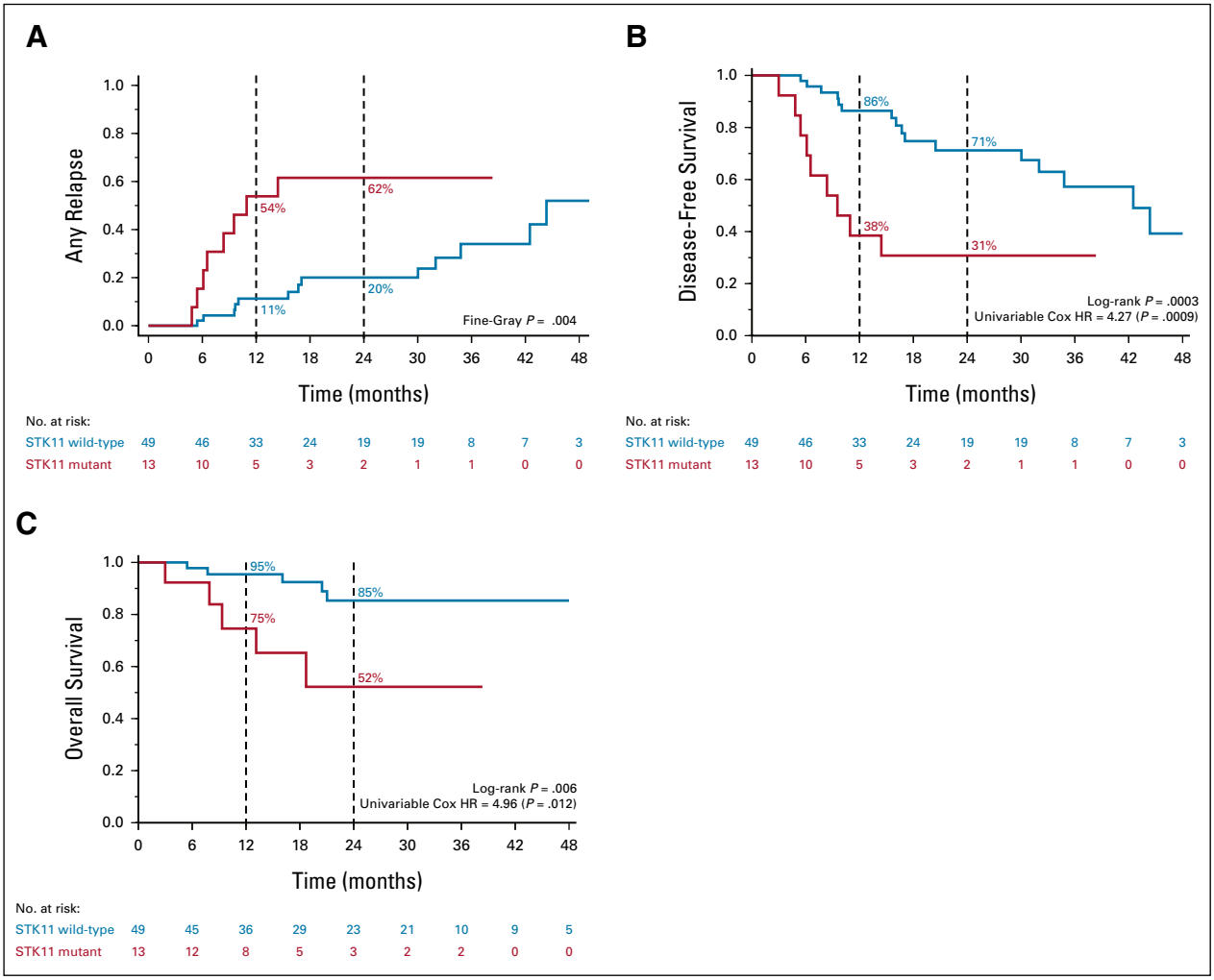

FIG 3. Clinical outcomes in the pooled University of Chicago and Stanford medically inoperable cohort by STK11 mutation status: (A) freedom from relapse, (B) disease-free survival, and (C) overall survival.

TABLE 3. Multivariable Fine-Gray Model After Backward Selection ($P < .1$) for Time to Relapse (Death Modeled as Competing Risk) and Cox Proportional Hazards Models After Backward Selection ($P < .1$) for DFS and OS

Time to Relapse		
Variable	**Subdistribution Hazard Ratio (95% CI)**	***P***
STK11 status		
Wild-type	Reference	
Mutation	4.0 (1.5 to 10.2)	.0041
DFS		
Variable	**Hazard Ratio (95% CI)**	***P***
STK11 status		
Wild-type	Reference	
Mutation	6.8 (2.5 to 18.3)	.0002
Black race		
No	Reference	
Yes	0.40 (0.14 to 1.1)	.084
OS		
Variable	**Hazard Ratio (95% CI)**	***P***
STK11 status		
Wild-type	Reference	
Mutation	6.0 (1.3 to 27.8)	.022
Institution		
University of Chicago	Reference	
Stanford	0.21 (0.05 to 0.92)	.039
Black race		
No	Reference	
Yes	0.19 (0.04 to 1.0)	.050

Abbreviations: DFS, disease-free survival; OS, overall survival.

oxygen, reporting 20+ pack-years of tobacco smoking history, and identifying as Black race. The association of *STK11* mutations with clinical hypoxia raises the novel hypothesis that the underlying clinical factors associated with medical inoperability may promote the emergence of *STK11* mutations.

STK11 (also known as *LKB1*) encodes a serine/threonine-protein kinase with potent and pleiotropic tumor suppressor functions.[25] *STK11* is one of the most frequently mutated genes in lung adenocarcinoma, and *STK11*/*KRAS* comutations in particular define a subset of advanced lung adenocarcinomas exhibiting resistance to immune checkpoint blockade.[32-34] Although these prior studies have characterized *STK11* mutations in either advanced/locally advanced or early-stage operable disease, this study uniquely characterizes its frequency and significance in early-stage disease receiving RT.

In the response to cellular stress, *STK11* plays a key role in activating the cGAS/STING pathway, an essential mediator of antitumor immunity. Therefore, mutational inactivation of *STK11* promotes an immune evasive phenotype.[35,36] Recent literature demonstrates the importance of immune evasion in early NSCLC carcinogenesis and immune-mediated pruning in the evolution of lung cancer metastasis, which suggests that immune-related mechanisms may explain the adverse outcome in *STK11*-mutant NSCLC.[37,38] Although effective therapies can be difficult to develop for loss-of-function mutations in tumor suppressor genes, multiple drugs with preclinical efficacy have been identified and may warrant further study in clinical trials.[25] These include biguanides (eg, metformin), mTOR inhibitors (eg, rapamycin), and combinatorial regimens targeting mTOR, PI3K, and MEK.[39-41] Finally, since *STK11*/*KRAS* comutations promote resistance to immunotherapy, the durable clinical benefit observed for sotorasib for KRAS p.G12C-mutant tumors (including those with *STK11* comutations) represents a promising therapeutic approach.[42]

A key finding of our analysis is that *STK11* mutations are associated with the use of supplemental oxygen (OR 5.5), 20+ pack-years of tobacco smoking history (OR 6.1), and Black race (OR 4.3). The strong association with clinical markers of hypoxia may point to distinct biological mechanisms underlying NSCLC in these cohorts. Recent studies highlight the role of hypoxia in promoting an immunosuppressive microenvironment through the induction of M2 macrophage polarization-related genes and the recruitment of myeloid-derived suppressor cells.[43] Concomitantly, there is increasing appreciation of the role of *STK11* in the cellular response to hypoxia. Preclinical data have shown that *STK11* inactivation promotes metabolic reprogramming via HIF-1a, which is the key transcriptional regulator of the cellular response to hypoxia.[44] Furthermore, the severity of underlying lung disease is correlated with the expression of HIF-1a in the non-neoplastic lung tissue of patients with lung cancer. Taken together, these data suggest that chronic hypoxia may apply a selective pressure that promotes inactivation of *STK11*, such that medically inoperable patients are more prone to develop *STK11*-mutant tumors.

The causes of racial disparities in lung cancer outcomes are certainly multifactorial, heavily influenced by inequities in access to screening, receipt of guideline-concordant treatment, and structural racism.[45-48] It is possible, however, that an elevated frequency of *STK11*-mutant tumors in Black patients contributes to these disparate lung cancer outcomes. We observed a significant association between Black race and somatic *STK11* mutations. This result is consistent with published whole-exome data demonstrating that *STK11* mutations are more frequent in tumors from African Americans (25% for adenocarcinoma and 8% for squamous cell carcinoma) relative to tumors from European Americans (13% and 1%, respectively).[27] This association appears to be functionally relevant, since relapse rates in Black versus non-Black patients are not significantly different after accounting for *STK11* status (Data Supplement).

The identification of a poor prognostic subgroup in early-stage inoperable NSCLC defined by *STK11* mutations highlights a subset of patients who may benefit from additional systemic therapies. Despite decades of investigation into predictive biomarkers of recurrence in early-stage, operable NSCLC, including promising epigenetic markers, patient selection for adjuvant chemotherapy remains largely determined on the basis of tumor size.[49,50] Immunotherapy is poised to become standard of care for resectable lung cancer, with adjuvant atezolizumab (IMpower010), neoadjuvant nivolumab (CheckMate 816), and adjuvant pembrolizumab (PEARLS/KEYNOTE-091) improving DFS/event-free survival.[11-13] Adjuvant osimertinib improved DFS for resected EGFR-mutant NSCLC in ADAURA.[14] For patients receiving SBRT, ongoing trials, such as PACIFIC-4 (ClinicalTrials.gov identifier: NCT03833154), are investigating the benefit of adjuvant immune checkpoint blockade (eg, durvalumab). The identification of *STK11* mutations as a poor prognostic factor is especially important in this context, considering the association with immunotherapy resistance in the metastatic setting.[34] Further studies are warranted to determine the level of benefit for patients with *STK11* mutations in the early-stage setting.

The unique enrichment of *STK11* mutations in the RT cohort and the association of *STK11* mutations with features that correlate with medical inoperability (eg, supplemental oxygen, pack-years of tobacco smoking history, and FEV1 % predicted) may contribute to the variation in distant failure rates and oncologic outcomes observed after SBRT. Trials of medically inoperable patients, including Radiation Therapy Oncology Group (RTOG) 0236, RTOG 0813, and RTOG 0915, exhibit distant metastasis rates of approximately 20%-40%, despite all trials requiring staging positron emission tomography.[6,51,52] By contrast, distant metastases occurred in 3%-12% in trials of medically operable patients (ie, RTOG 0618, pooled analysis of STARS and ROSEL, and revised STARS).[29,53,54] Comparisons across trials are difficult, especially with possible differences in invasive nodal staging, but differential underlying frequencies of *STK11* mutations may have partially contributed to these outcomes.

STK11 mutations were primarily associated with a distant pattern of failure, as opposed to local, and probably do not predict a decrease in the therapeutic efficacy of RT. However, it is worth acknowledging that diagnosing local failure after lung SBRT can be challenging radiographically, because of post-treatment changes and resultant fibrosis.[55,56] In a retrospective analysis of stage III patients, *STK11* mutations were associated with increased locoregional recurrence, with in vitro experiments suggesting that *STK11*-mediated radioresistance was KEAP1/NRF2-dependent.[57] *KEAP1/NFE2L2* mutations have previously been associated with high rates of local recurrence after RT but not surgery.[19] Unfortunately, *KEAP1* mutations were not profiled by our OncoPlus panel in the University of Chicago cohort. Murine models further support that *STK11/LKB1*-deficient tumors demonstrate more frequent metastases.[58] We hypothesize that *STK11* mutations portend early metastatic dissemination and distant relapse.

Limitations of this study include its retrospective design and the small fraction of early-stage patients who undergo tumor molecular profiling, even at high-volume cancer centers. To address this, a pooled RT cohort was analyzed across two institutions, University of Chicago and Stanford. Although *STK11* mutations were highly prognostic even in multivariable analyses and efforts were made to include various clinical covariates that influence the risk of relapse, unmeasured confounding variables, including those related to possible differences in patient selection between institutions, may have contributed to the poor oncologic outcomes observed in patients with *STK11* mutations. Finally, because of limited sample size, it was not possible to analyze the prognostic significance of *STK11* mutations in the surgical cohort (n = 2) and the significance of *STK11/KRAS* comutations in the pooled RT cohort (n = 7). Nonetheless, *STK11/KRAS* comutations were numerically associated with increased risk of relapse in the pooled RT cohort (57.1% *v* 25.6% at 2 years).

In conclusion, in the growing setting of early-stage, medically inoperable NSCLC receiving RT, to our knowledge, we present the first evidence for a prognostic and potentially targetable biomarker in the form of *STK11* inactivation. Although prospective, multicenter validation is warranted, if the importance of *STK11* inactivation in predicting poor outcomes in patients with early-stage NSCLC is confirmed, clinical trials of evolving adjuvant therapies targeting relevant pathways would be of significant interest.

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DATA SHARING STATEMENT

Deidentified individual participant data, including clinical and genomic data, for the University of Chicago cohort can be made available upon request.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

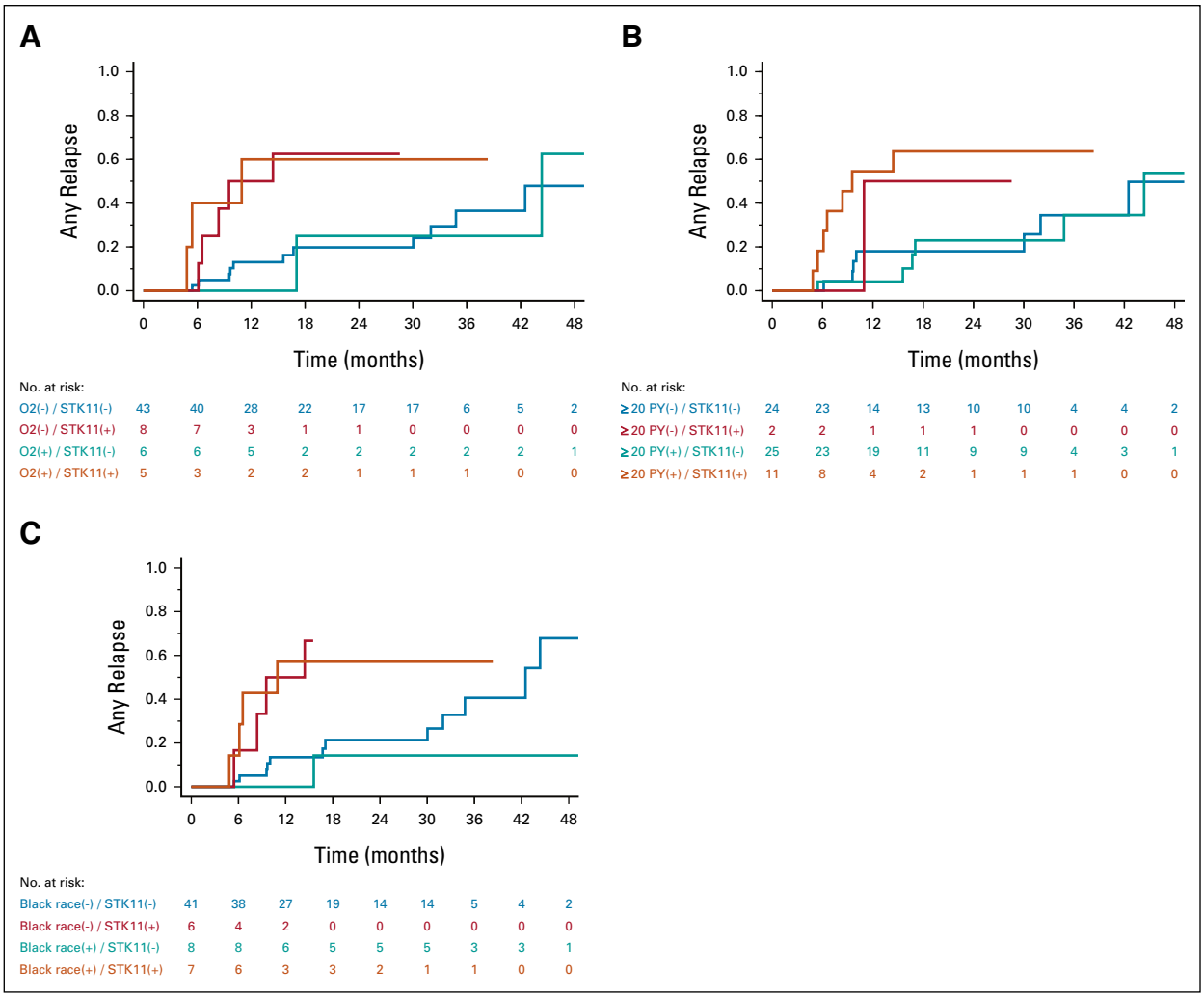

FIG A1. (A) Freedom from relapse in patients receiving supplemental oxygen or not, stratified by STK11 mutation status. (B) Freedom from relapse in patients with 20+ pack-years smoking history or not, stratified by STK11 mutation status. (C) Freedom from relapse in patients of Black race or not, stratified by STK11 mutation status. O2(–): not receiving supplemental oxygen. O2(+): receiving supplemental oxygen. ≥ 20 PY(+): 20+ pack-years smoking history. ≥ 20 PY(–): less than 20 pack-years smoking history. STK11(–): STK11 wild-type. STK11(+): STK11-mutant.

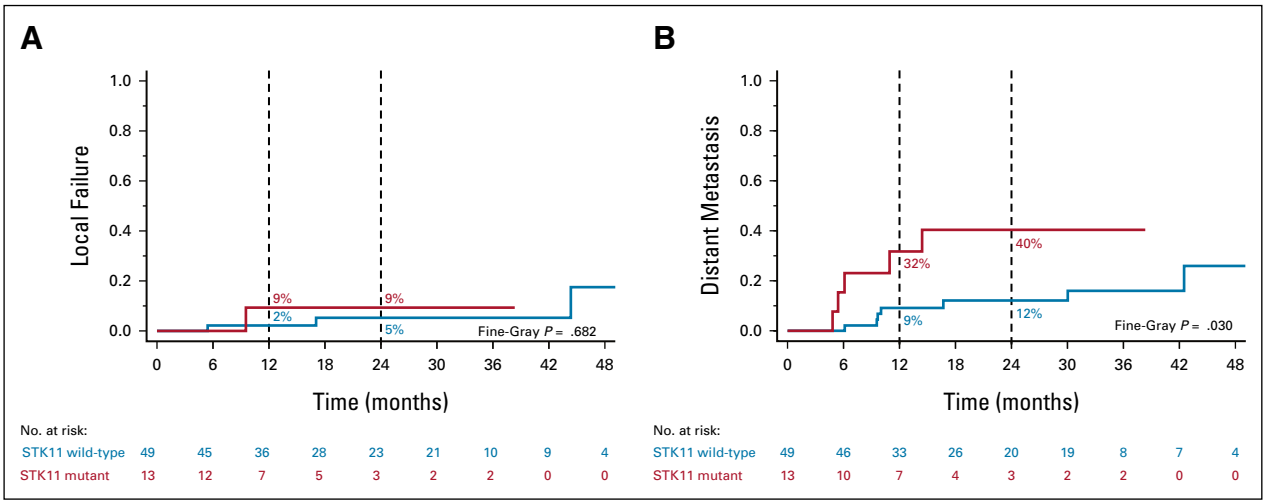

FIG A2. Patterns of progression in the pooled University of Chicago and Stanford medically inoperable cohort by STK11 mutation status: (A) freedom from local failure and (B) freedom from distant metastasis.

TABLE A1. Genes Associated With Freedom From Relapse Using Kaplan-Meier Method (FDR < 0.05)

Gene Symbol	q-Value	Variant Frequency (%)
STK11	0.0497	22.6
CTNNB1	0.006	3.8
PBRM1	0.009	3.8
GRIN2A	< 0.001	1.9
RUNX1	0.004	1.9

FDR, false discovery rate.

TABLE A2. Univariable Associations Between Various Demographic and Clinical Factors and the Presence of *STK11* Variants in the Profiled Tumors

	Total	*STK11* Wild-Type	*STK11*-Mutant	
	N = 92	n = 77	n = 15	*P*
Treatment modality				.13
Surgery	30 (33%)	28 (36%)	2 (13%)	
RT	62 (67%)	49 (64%)	13 (87%)	
Institution				.085
University of Chicago	53 (58%)	41 (53%)	12 (80%)	
Stanford	39 (42%)	36 (47%)	3 (20%)	
Sex				.57
Female	52 (57%)	42 (55%)	10 (67%)	
Male	40 (43%)	35 (45%)	5 (33%)	
Age, years, mean (SD)	73 (10)	73 (10)	73 (9)	.88
Race				.028
Non-Black	66 (72%)	59 (77%)	7 (47%)	
Black	26 (28%)	18 (23%)	8 (53%)	
ECOG > 1				.099
No	71 (77%)	62 (81%)	9 (60%)	
Yes	21 (23%)	15 (19%)	6 (40%)	
Diabetes mellitus				.19
No	68 (74%)	59 (77%)	9 (60%)	
Yes	24 (26%)	18 (23%)	6 (40%)	
BMI, mean (SD)	27 (6)	27 (6)	27 (6)	.87
FEV1, % predicted, mean (SD)[a]	84 (26)	86 (26)	72 (23)	.074
DLCO, % predicted, mean (SD)[b]	78 (27)	78 (27)	74 (28)	.64
Supplemental O2				.015
No	81 (88%)	71 (92%)	10 (67%)	
Yes	11 (12%)	6 (8%)	5 (33%)	
Smoking status				.098
Never smoking	17 (18%)	17 (22%)	0 (0%)	
Current smoking	13 (14%)	10 (13%)	3 (20%)	
Former smoking	62 (67%)	50 (65%)	12 (80%)	
20+ pack-years of tobacco smoking history				.021
No	38 (41%)	36 (47%)	2 (13%)	
Yes	54 (59%)	41 (53%)	13 (87%)	
Stage I *v* II				1.00
I	73 (79%)	61 (79%)	12 (80%)	
II	19 (21%)	16 (21%)	3 (20%)	

NOTE. *P* values represent univariable logistic regression.

BMI, body mass index; DLCO, diffusing capacity of the lung for carbon monoxide; ECOG, Eastern Cooperative Oncology Group; FEV1, forced expiratory volume in 1 second; RT, radiotherapy.

[a]FEV1 % predicted was not assessed for 18 patients.

[b]DLCO % predicted was not assessed for 31 patients.

TABLE A3. Patient Characteristics for Pooled University of Chicago and Stanford Cohort of Early-Stage NSCLC Receiving Definitive RT

	Total	UCMC	Stanford	
	N = 62	n = 23	n = 39	*P*
STK11 mutation status				.002
STK11 wild-type	49 (79%)	13 (57%)	36 (92%)	
STK11-mutant	13 (21%)	10 (43%)	3 (8%)	
Sex				.034
Female	31 (50%)	16 (70%)	15 (38%)	
Male	31 (50%)	7 (30%)	24 (62%)	
Age, years, mean (SD)	75 (9)	73 (8)	77 (9)	.067
Race				< .001
Non-Black	47 (76%)	9 (39%)	38 (97%)	
Black	15 (24%)	14 (61%)	1 (3%)	
ECOG				1.00
0	12 (19%)	4 (17%)	8 (21%)	
1	31 (50%)	12 (52%)	19 (49%)	
2	16 (26%)	6 (26%)	10 (26%)	
3	2 (3%)	1 (4%)	1 (3%)	
4	1 (2%)	0 (0%)	1 (3%)	
FEV1, liters, mean (SD)	1.50 (0.43)	1.45 (0.40)	1.58 (0.49)	.41
Supplemental O2				.013
No	51 (82%)	15 (65%)	36 (92%)	
Yes	11 (18%)	8 (35%)	3 (8%)	
Smoking status				.004
Never smoking	14 (23%)	2 (9%)	12 (31%)	
Current smoking	10 (16%)	8 (35%)	2 (5%)	
Former smoking	38 (61%)	13 (57%)	25 (64%)	
Tobacco pack-years, mean (SD)	32 (33)	35 (27)	31 (36)	.59
20+ pack-years of tobacco smoking history				.19
No	26 (42%)	7 (30%)	19 (49%)	
Yes	36 (58%)	16 (70%)	20 (51%)	
Stage				.072
IA1	4 (6%)	2 (9%)	2 (5%)	
IA2	27 (44%)	11 (48%)	16 (41%)	
IA3	12 (19%)	1 (4%)	11 (28%)	
IB	9 (15%)	4 (17%)	5 (13%)	
IIA	5 (8%)	1 (4%)	4 (10%)	
IIB	5 (8%)	4 (17%)	1 (3%)	
Stage I *v* II				.48
I	52 (84%)	18 (78%)	34 (87%)	
II	10 (16%)	5 (22%)	5 (13%)	

NOTE. *P* values are calculated on the basis of Fisher's exact tests for categorical variables and two-sample *t* tests for continuous variables.

ECOG, Eastern Cooperative Oncology Group; FEV1, forced expiratory volume in 1 second.

TABLE A4. Full Multivariable Fine-Gray Model for Time to Relapse (Death Modeled as Competing Risk) and Full Multivariable Cox Proportional Hazards Models for DFS and OS

Time To Relapse

Variable	Subdistribution Hazard Ratio (95% CI)	*P*
STK11 status		
Wild-type	Reference	
Mutation	5.0 (1.7 to 14.9)	.004
Institution		
University of Chicago	Reference	
Stanford	0.87 (0.23 to 3.3)	.84
Age (years)	1.00 (0.94 to 1.07)	.97
Stage		
I	Reference	
II	0.71 (0.13 to 3.9)	.69
Sex		
Male	Reference	
Female	0.88 (0.30 to 2.6)	.82
ECOG > 1		
No	Reference	
Yes	1.8 (0.70 to 4.5)	.21
Black race		
No	Reference	
Yes	0.43 (0.08 to 2.3)	.32
Supplemental O2		
No	Reference	
Yes	1.0 (0.29 to 3.6)	.98
Tobacco pack-years > 20		
No	Reference	
Yes	0.86 (0.33 to 2.3)	.76

DFS

Variable	Hazard Ratio (95% CI)	*P*
STK11 status		
Wild-type	Reference	
Mutation	6.1 (2.1 to 17.9)	.0010
Institution		
University of Chicago	Reference	
Stanford	0.24 (0.07 to 0.84)	.025
Age (years)	0.95 (0.89 to 1.01)	.13
Stage		
I	Reference	
II	0.45 (0.10 to 2.1)	.31
Sex		
Male	Reference	
Female	0.76 (0.30 to 1.9)	.56
ECOG > 1		
No	Reference	
Yes	2.6 (0.92 to 7.1)	.071
Black race		
No	Reference	
Yes	0.12 (0.03 to 0.54)	.0058
Supplemental O2		
No	Reference	
Yes	0.31 (0.10 to 1.0)	.054
Tobacco pack-years > 20		
No	Reference	
Yes	0.98 (0.38 to 2.5)	.96

OS

Variable	Hazard Ratio (95% CI)	*P*
STK11 status		
Wild-type	Reference	
Mutation	4.2 (0.79 to 22.7)	.093
Institution		
University of Chicago	Reference	
Stanford	0.22 (0.04 to 1.3)	.097
Age (years)	0.97 (0.87 to 1.1)	.63
Stage		
I	Reference	
II	1.7 (0.29 to 10.2)	.56
Sex		
Male	Reference	
Female	1.1 (0.25 to 4.6)	.92
ECOG > 1		
No	Reference	
Yes	2.6 (0.52 to 13.4)	.24
Black race		
No	Reference	
Yes	0.13 (0.01 to 1.4)	.095
Supplemental O2		
No	Reference	
Yes	0.77 (0.17 to 3.4)	.73
Tobacco pack-years > 20		
No	Reference	
Yes	2.2 (0.38 to 13.2)	.38

Abbreviations: DFS, disease-free survival; OS, overall survival.

(Continued in next column)