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The Genomic Landscape of *SMARCA4* Alterations and Associations with Outcomes in Patients with Lung Cancer

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Abstract

Purpose: *SMARCA4* mutations are among the most common recurrent alterations in NSCLC, but the relationship to other genomic abnormalities and clinical impact has not been established.

Experimental Design: To characterize *SMARCA4* alterations in NSCLC, we analyzed the genomic, protein expression, and clinical outcome data of patients with *SMARCA4* alterations treated at Memorial Sloan Kettering.

Results: In 4813 tumors from patients with NSCLC, we identified 8% (n= 407) patients with *SMARCA4*-mutant lung cancer. We describe two categories of *SMARCA4* mutations: Class 1 mutations (truncating mutations, fusions and homozygous deletion) and Class 2 mutations (missense mutations). Protein expression loss was associated with Class 1 mutation (81% vs 0%, ($P < 0.001$)). Both classes of mutation co-occurred more frequently with *KRAS*, *STK11*, and *KEAP1* mutations compared to *SMARCA4* wildtype tumors ($P < 0.001$). In patients with metastatic NSCLC, *SMARCA4* alterations were associated with shorter overall survival, with Class 1 alterations associated with shortest survival times ($P < 0.001$). Conversely, we found that treatment with immune checkpoint inhibitors was associated with improved outcomes in patients with *SMARCA4*-mutant tumors ($P = 0.01$), with Class 1 mutations having the best response to ICIs ($p = 0.027$).

Conclusions: *SMARCA4* alterations can be divided into two clinically relevant genomic classes associated with differential protein expression as well as distinct prognostic and treatment implications. Both classes co-occur with *KEAP1*, *STK11*, and *KRAS* mutations, but individually represent independent predictors of poor prognosis. Despite association with poor outcomes, *SMARCA4*-mutant lung cancers may be more sensitive to immunotherapy.

Introduction

Genomic abnormalities in the subunits of the *SWI/SNF* chromatin remodeling complex occur in approximately 20% of solid tumors and emerging data suggests that specific alterations within this complex might affect outcomes in certain solid tumors (1-3). For example, alterations in the *SWI/SNF* complex gene *PBRM1* have been associated with improved outcomes in patients with renal cell carcinoma treated with immune checkpoint inhibitors (ICIs); refs. (3,4). In lung cancer, inactivation of the catalytic subunit *SMARCA4* (*BRG1*), is the most common alteration within the *SWI/SNF* complex and has been associated with poor patient outcomes (1,5-10). *SMARCA4* is one of two mutually exclusive DNA-dependent ATPases, along with *SMARCA2*, involved in transcriptional regulation of gene expression (11,12). Yet, the relationship between *SMARCA4* and other alterations within the complex genomic landscape of lung cancer remains unclear.

Multiple studies have recently highlighted the importance of considering genes of interest within the context of commonly co-occurring mutations (13-18). For example, the identification of *STK11*, *KEAP1*, and *TP53* mutant subgroups has changed the paradigm of classifying *KRAS*-mutant lung cancers and non-small cell lung cancers (NSCLCs) in

general (13-15,18). These distinct subgroups correlate with differential responses to immunotherapy and long-term outcomes (13,14,17,18). Further, in *EGFR*-mutant lung cancer, mutations in *TP53* and *RBI* are associated with shorter response to TKIs and transformation to small cell carcinoma (15,16). Previous studies have shown that *SMARCA4* alterations can co-occur in *KRAS* mutant tumors, yet they also occur independently and less commonly with other driver oncogenes such as epidermal growth factor receptor (*EGFR*); refs. (5,6). However, there are only limited data on *SMARCA4*'s relationship to these other co-occurring mutations (8,10) and the significance of *SMARCA4* alterations among oncogene driven subsets of lung cancer is unknown.

Increased understanding of the relationship of *SMARCA4* in lung cancer may enable new therapeutic opportunities in the future. Recently, *SMARCA4* alterations have been shown to be oncogenic drivers in a highly aggressive subset of ovarian cancer, small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) that shows increased susceptibility to ICIs (19). Further, there have been case reports of durable responses to ICIs in thoracic *SMARCA4*-deficient undifferentiated tumors and *SMARCA4*-deficient lung carcinoma (20,21), but no studies have comprehensively evaluated treatment outcomes in a large cohort of lung cancer patients. In this study, we characterize the clinical, molecular, and histologic relationships of *SMARCA4* genomic and protein alterations in lung cancer.

Methods

We identified all patients with NSCLC of any stage with *SMARCA4* alterations detected by MSK-IMPACT NGS (22) until April of 2019 who were treated at Memorial Sloan Kettering Cancer Center (MSK) for genomic analysis (Sup. Fig. 1).

SMARCA4 alterations were classified into two groups: *SMARCA4* truncating mutations, fusions and homozygous deletions were deemed "Class 1 alteration" and 2) *SMARCA4* missense mutations or variants of unknown significance, or "Class 2 alteration" based upon categorization in OncoKB (23). Tumors with concurrent Class 1 and Class 2 alterations were classified within the Class 1 category. A retrospective pathologic analysis of expression of *SMARCA4* in all cases of with *SMARCA4* molecular alterations was performed by immunohistochemistry using the previously described methods (10).

Somatic alterations were identified using the MSK-IMPACT assay as previously described (22). Individual genes were queried for distribution and enrichment among the patients with and without *SMARCA4* alterations. Frequencies of gene alterations by *SMARCA4* alteration were considered significant with a p-value < 0.05 and, to reduce false discovery in multiple testing, FDR q-value < 0.10. Tumor mutation burden (TMB) was normalized across each version of the MSK-IMPACT panel (341, 410, or 468 genes) and defined as the total number of mutations divided by the coding region captured reported as mutations/megabase in each panel (0.897 megabases (Mb) for 341-, 1.017 Mb for 410-, and 1.139 Mb for 468-gene panel). PD-L1 expression was scored as the percentage of tumor cells with membranous staining using predominantly E1L3N antibody, as previously described (24).

Medical, pharmacy, and pathology records for all patients with metastatic NSCLC and *SMARCA4* alterations were reviewed to collect demographic, pathologic, and treatment data. A random sample of patients with metastatic NSCLC who had MSK-IMPACT without *SMARCA4* alterations and were tested during the same time period was used as a comparator group. The response to anti-PD-(L)1 therapy was determined (database lock of April 1, 2019) using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. by thoracic radiologists. This study was approved by the Institutional Review Board/Privacy Board at MSK and was in accordance with the Belmont report for retrospective review of records and waiver of consent.

Statistical Methods

Patient and tumor characteristics were compared across *SMARCA4* mutation classes (Class 1, Class 2, wild type) using Chi-square tests and Kruskal-Wallis tests. Overall survival (OS) defined from the date of metastatic diagnosis to death and accounted for the left truncation time from metastatic diagnosis to IMPACT biopsy. Patients without events were censored at their last known visit date. Survival curves and estimates of the median survival time were generated using Kaplan-Meier methods and compared across the three mutation classes using log-rank tests. A Cox proportional hazards model was adjusted for age, sex, smoking status (never smoker, former light smoker, former heavy smoker and current smoker), histology (adenocarcinoma, squamous, other), as well as co-occurring *STK11* and *KEAP1* mutations, and TMB. Hazard ratios (HR) and 95% confidence intervals (CI) are reported. Sub-analyses of OS were performed among patients with *KRAS* mutations. Patients without follow-up after their IMPACT pathology date were excluded from analyses (n = 5).

The response to immunotherapy as characterized by progression-free survival (PFS), OS, and overall response rate (ORR) was examined among the subset of patients that received immunotherapy. PFS was defined as the time from start of PD-(L)1 inhibitor to clinical or radiographic progression, death, or the end of follow-up, and OS was defined as the time from the start of PD-(L)1 inhibitor to death or the end of follow-up. PFS and OS were analyzed using Kaplan Meier methods and Cox proportional hazards model accounting for left truncation, again adjusted for age, sex, smoking status, histology, TMB, and co-occurring *STK11* and *KEAP1* mutations. Best overall response was defined as complete or partial response. Multivariable logistic regression was applied to compare the likelihood of ORR across *SMARCA4* mutation classes adjusted for age, TMB, PD-L1, *STK11*, and *KEAP1*.

To assess whether immunotherapy is associated with improved survival among patients with Class 1 or 2 *SMARCA4* mutations, we first calculated the propensity score, probability of receipt of ICIs based on available variables (mutation class, age, sex, race, smoking status, histology, TMB, co-occurring *STK11* and *KEAP1* mutations). We then adjusted for the propensity score when comparing OS for patients that received ICIs versus patients that did not via a Cox proportional hazards model accounting for left truncation. A p-value <0.05 was considered statistically significant for all analyses. Statistical analyses were performed with GraphPad Prism software version 7 (La Jolla, CA, www.graphpad.com) and R version 3.6.1 software (www.r-project.org).⁽²⁵⁾

Results

Spectrum of *SMARCA4* genomic alterations

In patients with NSCLC tested by comprehensive next-generation sequencing (NGS), 8% (n = 407 of 4813) had a *SMARCA4* alteration, with an array of *SMARCA4* alterations identified (Fig. 1). *SMARCA4* alterations were categorized into two groups based upon the type of genomic abnormality: 1) “Class 1 alterations” included truncating mutations deemed oncogenic, gene fusions, and homozygous deletions and 2) “Class 2 alterations” included all missense mutations and other variants of unknown significance based upon categorization in OncoKB (23). Tumors with concurrent Class 1 and Class 2 *SMARCA4* alterations were categorized as Class 1 tumors. In total, 212 patients (4% of total, 52% of *SMARCA4* variants) had tumors with Class 1 *SMARCA4* alterations and 195 (4% of total, 48% of *SMARCA4* variants) had tumors with Class 2 *SMARCA4* alterations (Fig. 1).

Relationship between class of *SMARCA4* genomic alteration and protein expression

We next explored the relationship between the genomic class of *SMARCA4* alteration and protein expression. Sufficient tissue for *SMARCA4* immunohistochemical analysis was available for 86 cases, including 62 tumors with Class 1 (truncating) alterations and 24 tumors with Class 2 (missense) alterations. *SMARCA4* expression loss was identified in 50 cases, all of which were tumors with Class 1 alterations (81% of Class 1 alterations). Overall, loss of *SMARCA4* expression was significantly associated with Class 1 alterations ($P < 0.001$; Fig. 1).

Molecular landscape associated with *SMARCA4* alterations

To evaluate the genomic context of *SMARCA4* alterations, we evaluated genomic profiles of tumors harboring *SMARCA4* alterations (n=407) and those without *SMARCA4* alterations (n=4406). Among commonly altered genes in lung cancer, the most frequent co-occurring mutations with *SMARCA4* alterations were *TP53* (56%), *KEAP1* (41%), *STK11* (39%) and *KRAS* (36%) (Fig. 2A and 2B).

We identified multiple genes that were associated with *SMARCA4* alterations (Fig. 2C). Mutations in *STK11* and *KEAP1* had the strongest association with *SMARCA4* mutant tumors compared to *SMARCA4* wildtype tumors ($P < 0.001$, $q < 0.001$; $P < 0.001$, $q < 0.001$; Fig. 2B and 2C). Conversely *EGFR* alterations were strongly associated within *SMARCA4* wildtype tumors compared to *SMARCA4* mutants ($P < 0.001$, $q < 0.001$). *SMARCA4* alterations occurred in the absence of *KRAS*, *STK11*, and *KEAP1* alterations in 38% of cases (Fig. 2D). *STK11* alterations occurred significantly more frequently with Class 1 than Class 2 alterations ($P < 0.001$, $q = 0.08$, Sup. Table 1). *NKX2-1* and *KEAP1* alterations also occurred more frequently with Class 1 alterations ($P = 0.002$, $q = 0.19$; $P = 0.01$, $q = 0.34$ respectively) and *EGFR* alterations were common with Class 2 alterations ($P = 0.004$, $q = 0.19$, Sup. Table 1).

Patient characteristics in advanced NSCLC by *SMARCA4* alteration class

We then investigated how the findings from our molecular and expression analyses related to clinical outcomes in patients with advanced NSCLC. Patient characteristics among stage IV

tumors with Class 1 (n=149) versus Class 2 (n=143) *SMARCA4* alterations were generally similar (Table 1). The presence of a Class 1 or 2 *SMARCA4* alteration was associated with history of smoking ($P < 0.001$) and non-adenocarcinoma histology ($P < 0.001$) compared to patients with *SMARCA4* wildtype NSCLC (n=996) (Table 1). Among patients harboring either class of *SMARCA4* mutation, 85% were smokers and 84% had adenocarcinoma; the rest had predominantly NSCLC, not otherwise classified.

Prognostic impact of Class 1 and Class 2 *SMARCA4* alterations in advanced NSCLC

Overall, we found that patients with metastatic NSCLC harboring either Class 1 or Class 2 *SMARCA4* alterations had shorter overall survival compared to patients with *SMARCA4* wildtype NSCLC ($p < 0.001$; Fig. 3A). Class 1 alterations were associated with the poorest outcomes (Fig. 3A). The differences in outcomes held in the multivariable survival analysis adjusted for age, sex, smoking status, histology, TMB, and the presence of *STK11* and/or *KEAP1* mutations (Fig. 3A).

Given the heterogeneity of co-occurring mutations, we sought to further isolate the specific impact of *SMARCA4* alterations by examining within the context of a single driver oncogene. We focused initially on 374 patients with tumors harboring *KRAS* mutations. In these patients, the presence of Class 1 or Class 2 *SMARCA4* alterations was a poor prognostic factor and remained prognostic when accounting for age, sex, smoking status, histology, TMB, and the presence of *STK11* or *KEAP1* mutations (Fig. 3B). Further, the addition of *STK11* and/or *KEAP1* was associated with decreased survival, with patients with all three *STK11*, *KEAP1*, and *SMARCA4* having the shortest survival ($P < 0.001$, Sup. Fig. 2).

Association with benefit of immunotherapy

Next, we analyzed the impact of ICIs on patient outcomes. Among patients with *SMARCA4* alterations, ICI use was associated with significantly improved survival from the start of ICIs (HR 0.67, 95% CI 0.48, 0.92, $P = 0.01$) (Fig. 4A). When evaluating known factors that predict outcomes to ICI, *SMARCA4*-mutant tumors had higher TMB ($P < 0.001$, Fig. 4B) but were more likely to be PD-L1 low or negative ($P = 0.03$, Fig. 4C). Class 1 alterations had lower expression of PD-L1 and higher median TMB compared to Class 2 alterations (Fig. 4B-C).

Finally, we sought to compare outcome among the two *SMARCA4* mutant classes and *SMARCA4* wildtype NSCLC in patients who had received ICI. Overall response was assessed in 445 out of 570 patients that received ICI. In unadjusted analyses, patients who harbored Class 1 alterations had a higher ORR in comparison to Class 2 alterations or *SMARCA4* wild-type tumors ($P = 0.027$, Fig. 4D). There was no difference in progression-free survival ($P = 0.74$) or overall survival ($P = 0.35$) on ICIs by *SMARCA4* alteration status (Fig. 4E-F).

Discussion

Here, we identify two specific classes of *SMARCA4* alterations associated with distinct protein expression and differential negative clinical outcomes in patients with metastatic

NSCLC. While both classes of *SMARCA4* alterations are associated with poor clinical outcomes, Class 1 alterations, which are associated with protein loss, are the strongest independent negative prognostic factor for patients, but respond best to ICIs. Despite the negative prognostic impact compared to patients with *SMARCA4* wildtype tumors, patients with *SMARCA4* alterations who received ICIs had better outcomes than those who did not.

This study builds upon recent data that co-occurring *STK11* and *KEAP1* mutations in lung cancer can significantly impact prognosis and responsiveness to therapy. *STK11* and *KEAP1* alterations are linked with poor prognosis and lack of response to immunotherapy in *KRAS*-mutant tumors and more recently in all patients with NSCLC. We find that *SMARCA4* alterations are associated with *STK11* and *KEAP1* mutations but are independent predictors of poor prognosis. *SMARCA4* abnormalities in combination with *STK11* and/or *KEAP1* mutations have an additive impact on shortening survival. However, unlike *STK11*, *SMARCA4* appears to be associated with increased sensitivity to immunotherapy. Future studies of *STK11* and *KEAP1* should incorporate exploration of *SMARCA4* to further delineate the role of each co-occurring mutation in influencing patient outcomes and *SMARCA4* should be identified and tested as a potential prognostic or predictive variable in prospective trials moving forward.

We observed that the spectrum of *SMARCA4* alterations differentially impact protein expression. Our findings are consistent with other recent analyses that assessed the incidence of *SMARCA4*-mutant lung cancer and frequency of protein expression loss with truncating mutations, supporting our classification schema (8,10). Interestingly, while the effect of Class 1 (truncating) alterations was most profound, we also find that, unexpectedly, patients with Class 2 (mis-sense, non-truncating) *SMARCA4* alterations had worse overall prognosis relative to patients with *SMARCA4* wild-type tumors, suggesting that function may be compromised in the setting of intact expression. Recent preclinical work provides additional mechanistic support and reveals that missense mutations of *SMARCA4* modify the open chromatin landscape and induce pro-oncogenic expression changes in *MYC* and its target genes, among others (26,27).

Our study is the first to evaluate how *SMARCA4* alterations in NSCLC influence sensitivity to ICIs. Recent analyses have shown that *SMARCA4* and *PBRM1* could be associated with improved response to immunotherapy in subtypes of ovarian cancer and renal cell cancer (4,19) and case reports have described durable responses to ICIs in a patient with a thoracic *SMARCA4*-deficient undifferentiated tumor (also referred to as a *SMARCA4*-deficient thoracic sarcoma) and a patient with NSCLC (20,21). Despite high rates of PD-L1 negativity, patients with *SMARCA4*-mutant NSCLC appear to derive significant benefit from PD-(L)1 blockade. Therefore, *SMARCA4* mutation status should be explored as a potentially novel biomarker of responsiveness to ICIs as a complement to PD-L1 expression and TMB in NSCLC.

While there are no known currently effective targeted treatments for *SMARCA4*-mutant NSCLCs, our study and others suggest *SMARCA4* is a potential target in lung cancer with distinct therapeutic vulnerabilities. For example, CDK4/6, AURKA, ATR, and EZH2 inhibition have recently shown antitumor activity in preclinical models of *SMARCA4*

deficient tumors (1,16,25,28-33). *SMARCA2* could be a synthetic lethal vulnerability in *SMARCA4*-mutant cancers. Prior reports have shown that *SMARCA2* retains expression in *SMARCA4*-mutant NSCLC and several *SMARCA2* inhibitors are currently in development to target this potential vulnerability (10,16). Future trials should explore use of these agents alone or in combination with ICIs given the efficacy of anti-PD-(L)1 antibodies in our analysis.

This study is a single-institution retrospective analysis and therefore has some inherent limitations. Unidentified factors associated with exposure and response to immunotherapy and overall survival could bias our results. Nevertheless, we accounted for all known potential variables that may influence outcomes. For example, we developed and incorporated a risk score to account for a patient's likelihood of receiving anti-PD(L)1 therapy and used a Cox proportional hazards model for multivariate analysis using the variables available. Analyses adjusting for PD-L1 expression are limited by the modest number of patients with sufficient available tissue for retrospective staining for PD-L1 and *SMARCA4*. Future studies that incorporate zygosity are also needed to understand its impact on expression and clinical outcomes.

In sum, our report highlights that *SMARCA4* alterations in lung cancer are uniquely linked to response to immunotherapy and patient outcomes. We found that the presence of *SMARCA4* abnormalities is enriched in patients with *KRAS*, *STK11*, and *KEAP1* mutations, but independently contributes to shortened overall survival with these co-occurring alterations. Despite these poor outcomes, patients with *SMARCA4*-mutant lung cancers may also be more sensitive to immunotherapy, which may enable new therapeutic options in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of translational relevance:

In this study, we characterize the clinical, molecular and histologic relationships of *SMARCA4* genomic and protein alterations in lung cancer. *SMARCA4* is the most commonly mutated member of the SWI/SNF complex, with mutations occurring in 8% of patients with NSCLC. Genomic, protein expression, and clinical outcome data identify two distinct classes of *SMARCA4* alterations. *SMARCA4* alterations often co-occur with *STK11*, *KEAP1*, and *KRAS* alterations, but they are a prognostic factor, independent of these alterations. Although patients whose tumors have Class 1 *SMARCA4* alterations (associated with protein expression loss) have a very poor prognosis, they may have higher response rates to PD-(L)1 blockade despite low PD-L1 expression.

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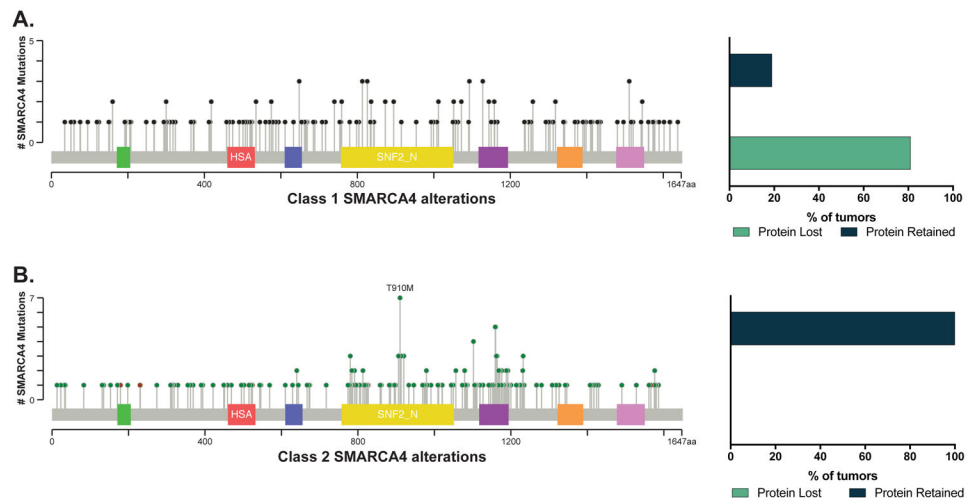


Figure 1. Spectrum of *SMARCA4* alterations by class and association with *SMARCA4* protein expression. (A) The distribution of Class 1 *SMARCA4* alterations ($n=212$) and protein expression ($n=62$). (B) The distribution of Class 2 *SMARCA4* alterations ($n=95$) and protein expression ($n=24$). Green QLQ, Gln, Leu, Gln motif; Red HSA, helicase/SANT-associated domain; Blue BRK, Brahma and Kismet domain; Yellow DEXDc, DEAD-like helicase superfamily domain; Purple SNF2_N, SNF2 family N-terminal domain; Orange HELICc, helicase superfamily C-terminal domain; Pink Bromo, bromodomain.

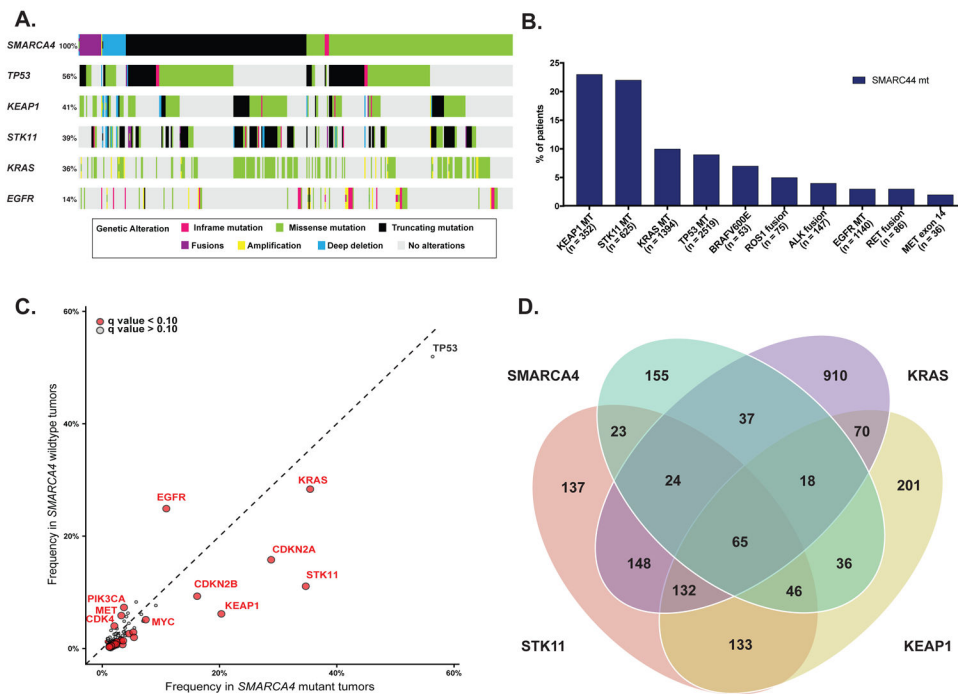


Figure 2. Genomic context of *SMARCA4* alterations. (A) Most frequent co-occurring alterations by *SMARCA4* alteration. (B) Distribution of *SMARCA4* alteration by commonly altered gene subgroups in NSCLC. (C) Frequency of altered individual genes within *SMARCA4* mutant vs *SMARCA4* wildtype subgroups. Genes labeled red were associated with significantly differential PD-L1 expression (q value < 0.10). (D) Distribution of *SMARCA4*, *STK11*, *KRAS*, and *KEAP1* alterations within NSCLC cohort.

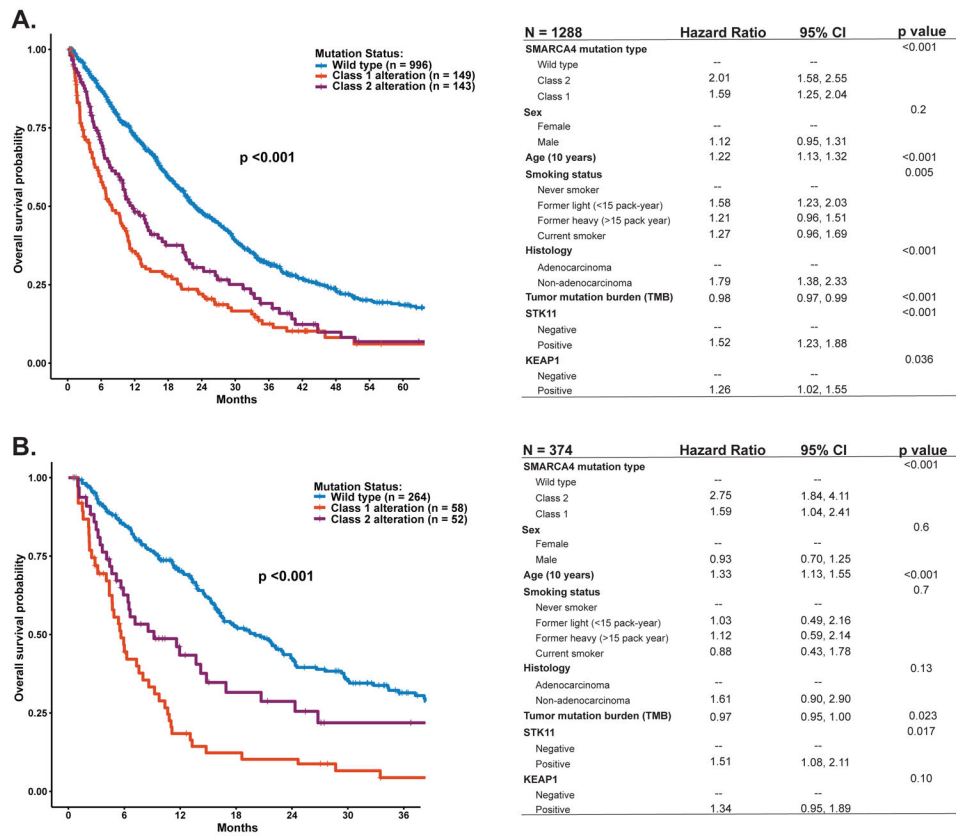


Figure 3. Survival by *SMARCA4* alteration class. (A) Overall survival among all patients, with multivariate model (right). (B) Overall survival among patients with *KRAS* mutations, with multivariate model (right).

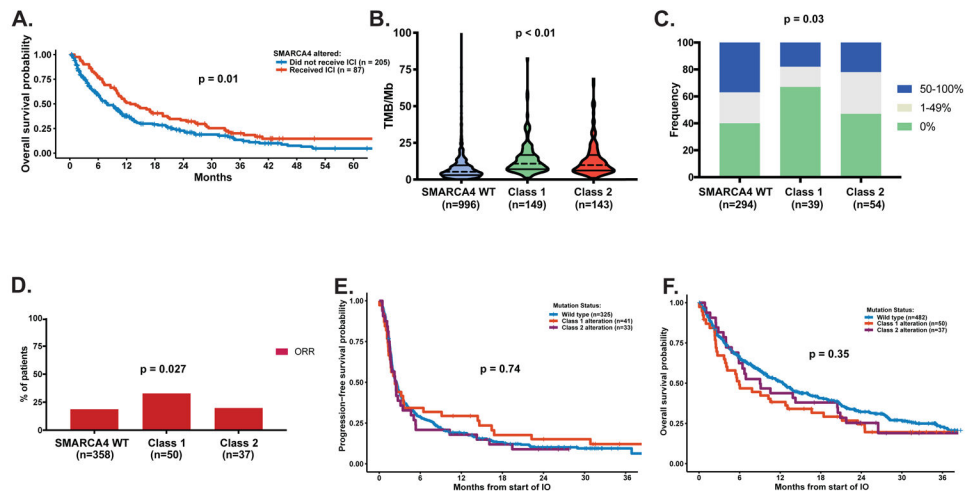


Figure 4. PD-L1 expression, tumor mutational burden, and immune checkpoint inhibitor (ICI) outcomes. (A) Overall survival among patients with *SMARCA4* alterations who did and did not receive ICIs. (B) Tumor mutational burden (TMB) by *SMARCA4* alteration class. (C) PD-L1 expression frequency by *SMARCA4* alteration class. (D) Overall response rate by *SMARCA4* alteration class. (E) Progression-free survival by *SMARCA4* alteration class. (F) Overall survival by *SMARCA4* alteration class.

Table 1:Clinical characteristics of patients with advanced NSCLC by *SMARCA4* alteration class.

Characteristic	<i>SMARCA4</i> Class 1 (N = 149)	<i>SMARCA4</i> Class 2 (N = 143)	<i>SMARCA4</i> Wild type (N = 996)	<i>P</i> -value
Median Age (Q1, Q3)	65 (58, 72)	65 (59, 72)	65 (58, 73)	0.7
Sex				0.052
Female	74 (50%)	78 (55%)	593 (60%)	
Male	75 (50%)	65 (45%)	403 (40%)	
Race				0.13
White	124 (83%)	125 (87%)	775 (78%)	
Black	7 (5%)	7 (5%)	57 (6%)	
Asian	10 (7%)	6 (4%)	101 (10%)	
Other	8 (5%)	5 (4%)	63 (6%)	
Smoking				<0.001
Never smoker	17 (11%)	26 (18%)	315 (32%)	
Former light (<15 py)	20 (13%)	19 (13%)	165 (17%)	
Former heavy (>15 py)	80 (54%)	71 (50%)	373 (37%)	
Current smoker	32 (21%)	27 (19%)	131 (13%)	
Histology				<0.001
Adenocarcinoma	121 (81%)	125 (87%)	914 (92%)	
Other	28 (19%)	18 (13%)	82 (8%)	

py: pack years