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STK11 and *KEAP1* mutations as prognostic biomarkers in an observational real-world lung adenocarcinoma cohort

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ABSTRACT

Introduction Somatic mutations in *STK11* and *KEAP1*, frequently comutated in non-squamous non-small cell lung cancer (NSQ NSCLC), have been associated with poor response to immune checkpoint blockade (ICB). However, previous reports lack non-ICB controls needed to properly ascertain the predictive nature of those biomarkers. The objective of this study was to evaluate the predictive versus prognostic effect of *STK11* or *KEAP1* mutations in NSQ NSCLC.

Methods Patients diagnosed with stage IIIB, IIIC, IVA or IVB NSQ NSCLC from a real-world data cohort from the Flatiron Health Network linked with genetic testing from Foundation Medicine were retrospectively assessed. Real-world, progression-free survival (rwPFS) and overall survival (OS) were calculated from time of initiation of first-line treatment.

Results We analysed clinical and mutational data for 2276 patients including patients treated with anti-programmed death-1 (PD-1)/anti-programmed death ligand 1 (PD-L1) inhibitors at first line (n=574). Mutations in *STK11* or *KEAP1* were associated with poor outcomes across multiple therapeutic classes and were not specifically associated with poor outcomes in ICB cohorts. There was no observable interaction between *STK11* mutations and anti-PD-1/anti-PD-L1 treatment on rwPFS (HR, 1.05; 95% CI 0.76 to 1.44; p=0.785) or OS (HR, 1.13; 95% CI 0.76 to 1.67; p=0.540). Similarly, there was no observable interaction between *KEAP1* mutations and treatment on rwPFS (HR, 0.93; 95% CI 0.67 to 1.28; p=0.653) or OS (HR, 0.98; 95% CI 0.66 to 1.45; p=0.913).

Conclusion Our results show that *STK11-KEAP1* mutations are prognostic, not predictive, biomarkers for anti-PD-1/anti-PD-L1 therapy.

Key questions

What is already known about this subject?

► Previous studies have associated mutations in *STK11* and *KEAP1* with poor outcomes in lung adenocarcinoma patients treated with immune checkpoint blockade (ICB).

What does this study add?

► We demonstrate that *STK11-KEAP1* mutations are prognostic biomarkers and not uniquely associated with inadequate response to ICB. Given mutations in *STK11* and *KEAP1* are co-occurring with each other and *KRAS*, we demonstrate the effects are independent.

How might this impact on clinical practice?

► Patients with *STK11* or *KEAP1* mutations represent a population with high unmet need. However, these mutations should not be used to exclude patients from ICB treatment.

INTRODUCTION

Advances in precision medicine have significantly changed clinical decision-making in the treatment of non-small cell lung cancer (NSCLC). Drugs for patients carrying an epidermal growth factor receptor (*EGFR*) or B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) p.V600E mutation or anaplastic lymphoma kinase or ROS proto-oncogene 1, receptor tyrosine kinase rearrangements, have significantly improved survival and established the importance of molecularly defined therapies.[1] However, not all patients with NSCLC have benefited, as a fraction of patients carry such actionable mutations. More recently, immunomodulatory cancer drugs such as anti-programmed death-1 (PD-1) have shown significant clinical benefit in NSCLC,[2] however, many patients do not show such benefit, highlighting the need for predictive biomarkers to guide patient stratification strategies.

Measuring tumour programmed death ligand 1 (PD-L1) protein expression using immunohistochemistry assays has been proposed as a rational and biologically sound approach to patient stratification.[3] Indeed, multiple PD-L1 assays are approved as companion or complementary diagnostics in NSCLC. However, PD-L1 expression alone does not always correlate with response, and additional biomarkers are needed.

Tumour mutational burden (TMB), typically assessed by tallying up all non-synonymous mutations, has also been explored as an independent predictive biomarker of response to anti-PD-1 treatment.[4] This measure serves as a proxy for the number of putative neoantigens that could be recognised by immune cells to trigger an immune response.

In addition to markers such as TMB and PD-L1, studies have assessed the role of frequently mutated genes in NSCLC as drivers of primary resistance to immune checkpoint blockade (ICB). Somatic mutations in serine/threonine kinase 11 (*STK11*) have been proposed as a potential mechanism of resistance to ICB in non-squamous (NSQ) NSCLC.[5–7] However, earlier studies in cohorts treated with non-ICB therapies identified a trend towards poor prognosis for *STK11* mutated patients or associations with a subset of *STK11* mutations.[8 9] *STK11* has been linked to multiple cellular processes, notably in lipid, glucose and cholesterol metabolism via activation of 5' AMP-activated protein kinase,[10] and has been associated with immune escape in a murine model.[11] *STK11* mutations co-occur with mutations in kelch-like ECH-associated protein 1 (*KEAP1*), which have also been associated with resistance to therapy.[7 12–14] *KEAP1* functions as a negative regulator of nuclear factor erythroid 2-related factor 2,[15] and loss-of-function mutations may contribute to an overactive cytoprotective programme.

Genomic data sets from previous studies were conducted in patient subpopulations (eg, *KRAS*-mutated)[5 12] or lacked both an ICB arm and a chemotherapy arm to ascertain the predictive nature of those biomarkers.[6–9 13] Only Arbour *et al*[12] examined the independent effects of *STK11* versus *KEAP1*. Furthermore, controlled clinical studies often lack sufficient statistical power to dissect effects of specific mutations. Thus, those studies are challenging to translate into clinical practice to inform treatment options. To assess the predictive or prognostic nature of *STK11* and *KEAP1* mutations in NSQ NSCLC, we leveraged real-world data from the Flatiron Health Clinico-Genomic Database (CGDB), which includes patients with detailed clinical information and genomic testing by Foundation Medicine.

MATERIALS AND METHODS

Cohort selection

From the advanced NSCLC CGDB[16] (April 2019 release; Flatiron Health, New York, New York, USA), we selected patients who had tumour-based genetic testing performed on the FoundationOne CDx or FoundationOne assay (Foundation Medicine, Cambridge, Massachusetts, USA) from 1 January 2011, through 31 December 2018. To mitigate the risk of prior treatments affecting results, we focused our analyses on first-line treatment. To ensure that treatment sequencing was correct, we excluded patients with an advanced diagnosis before 1 January 2011, and those who had initiated first-line treatment after 90 days following their advanced diagnosis date. Patients were further selected to have an NSQ histology and by their first-line treatment, resulting in 2276 patients across five treatment classes (online supplementary table 1).

Treatment grouping

First-line treatment data were aggregated in five broad treatment classes according to Flatiron Health rules. In summary, regimens that contained anti-PD-1 or anti-PD-L1 were considered 'PD-1/PD-L1-based therapies', those that contained *EGFR* tyrosine kinase inhibitors (TKIs) as '*EGFR* TKIs' and those that contained anti-vascular endothelial growth factor (VEGF) as 'anti-VEGF-based therapies'. Regimens including a platinum-based and any other chemotherapeutic agent, but not drugs from the above-mentioned class, were classified as 'platinum-based chemotherapy combinations'. Regimens with a single chemotherapeutic agent were considered 'single-agent chemotherapies'. A full list of regimens and treatment classes is found in online supplementary table 2.

Genomic assay

We selected patient specimens profiled on bait sets DX1, T4b, T5a or T7 of the Foundation Medicine FoundationOne CDx or FoundationOne assay, as they are performed on tumour material (as opposed to blood) and they contain tumour protein *STK11*, *KEAP1* and *KRAS* in their gene panel.

STK11 and *KEAP1* mutations

We aggregated gene-specific alterations, filtering for missense mutations, truncations and deletions. For OncoPrint visualisation, all mutations were considered. For Cox proportional hazards and Kaplan-Meier modelling, patients were labelled as mutant for a gene if they had at least one qualifying (missense, truncation and deletion) mutation in that gene. OncoPrint plots were created using ComplexHeatmap R package.[17] LollipotPlots for were created using maftools (online supplementary figure 1).[18] STK11 mutations were grouped by exon 1–2 or exon 3–9 according to their position, using *STK11* transcript id NM_000455. Other genomic alterations (deletions, fusions, splicing variants) in *STK11* were considered separately (online supplementary table 3).

KRAS mutations

For simplicity, we labelled only patients who carried a missense mutation in the hotspot locus G12-13 as *KRAS*-mutated, whereas patients carrying other alterations were considered as *KRAS*-wild type (online supplementary table 3).

Time-to-event analysis

We used real-world endpoints: real-world, progression-free survival[19] (rwPFS) and overall survival (OS),[20] as previously defined. Briefly, the date of treatment initiation was taken as the start time and the event was censored at the date of last patient activity when no progression or mortality date was present. We performed time-to-event analysis on rwPFS and OS analysis using

Cox proportional-hazards modelling in R. Kaplan-Meier curves were displayed using the R package survminer.

PD-L1 harmonisation

To obtain PD-L1 immunohistochemistry data for as many patients as possible, we harmonised the PD-L1 immunohistochemistry data from different tests by considering numerical values >50% tumour cell scoring as positive. Data were included from three PD-L1 antibody clones (28–8, 22c3 and SP142).

RESULTS

Consistent with previous estimates, *STK11* and *KEAP1* mutations were found in 20% (454 of 2276 and 451 of 2276, respectively) of patients and were frequently comutated (231 patients carried both; online supplementary figure 2). Thirty per cent (674 of 2276) of patients had tumours that carried either *STK11* and/or *KEAP1* mutations (*STK11-KEAP1*) and were enriched for male patients (53.4% vs 42.3%, p<0.001, χ^2 test), younger age at advanced diagnosis (64.9 vs 66.9 years, p<0.001, Student's t-test), smoking history (96% vs 73.3%, p<0.001, χ^2 test) and higher TMB (13.1 vs 7.94 mutations per megabase, p<0.001, Student's t-test) (table 1). Those results were consistent even when excluding *EGFR*-mutated patients (online supplementary table 4). *KRAS* mutations were found in 39% (263 of 674) of *STK11-KEAP1* patients (online supplementary figure 2). First-line treatment class was associated with *STK11-KEAP1* mutational status, explained by the finding that *EGFR* mutations are mutually exclusive with *STK11-KEAP1* and patients carrying *EGFR* mutations received *EGFR* TKIs (online supplementary figure 2 and table 5, online supplementary table 5). In a comparison of *STK11-KEAP1* versus wild-type patients, excluding *EGFR*-mutated patients, first-line treatment was not associated with *STK11-KEAP1* status (online supplementary table 4).

STK11 mutations were previously reported to be associated with low levels of T-cell inflammation and tumour PD-L1 expression.[6] Consistent with previous reports, patients with *STK11-KEAP1* mutations were enriched for negative PD-L1 staining (75.8% vs 60.8%, p<0.001, χ^2 test; table 1), as were patients with EGFR mutations (online supplementary table 4).

We performed multivariate Cox proportional hazards modelling, including age at advanced diagnosis, gender, TMB (continuous variable) and *STK11-KEAP1* mutational status for rwPFS for each treatment class independently. *STK11* and *KEAP1* mutations were both associated with shorter rwPFS across treatment classes (figure 1A). We then focused on anti-PD-1/PD-L1 and chemotherapy treatment classes and tested whether *STK11-KEAP1* mutations showed a treatment-specific effect by including an interaction term (*STK11-KEAP1* * treatment) in our previous Cox model. Consistent with the previous model, *STK11* and *KEAP1* mutations were prognostic and did not show different treatment-specific effects (figure 1B).

Table 1 Comparative table of *STK11-KEAP1* mutated patients versus wild-type patients

Characteristic	Mutant (n=674)	Wild-type (n=1602)	P value
Gender, N (%)			<0.001
Female	314 (46.6)	925 (57.7)	
Male	360 (53.4)	677 (42.3)	
Age at advanced diagnosis, median (SD), years	64.9 (9.89)	66.9 (10.5)	<0.001
Smoking, N (%)			<0.001
History of smoking	647 (96.0)	1175 (73.3)	
No history of smoking	26 (3.86)	420 (26.2)	
Unknown/not documented	1 (0.15)	7 (0.44)	
TMB score (SD)	13.1 (11.1)	7.94 (9.69)	<0.001
First-line treatment, N (%)			<0.001
Anti-VEGF-based therapies	154 (22.8)	317 (19.8)	
EGFR TKIs	15 (2.23)	296 (18.5)	
PD-1/PD-L1-based therapies	192 (28.5)	382 (23.8)	
Platinum-based chemotherapy combinations	288 (42.7)	544 (34.0)	
Single-agent chemotherapies	25 (3.71)	63 (3.93)	
PD-L1 status, N (%)			<0.001
Negative	150 (75.8)	279 (60.8)	
Positive	48 (24.2)	180 (39.2)	

EGFR, epidermal growth factor receptor; PD-1, programmed death-1; PD-L1, programmed death ligand 1; TMB, tumour mutational burden; VEGF, vascular endothelial growth factor.

We tested the independent contributions of *STK11* and *KEAP1* mutations to poor prognosis by testing them in a multivariate model including the interaction between mutations in the two genes. Both genes were associated with lower rwPFS, and *KEAP1*-only patients fared worse than patients with *STK11*-only mutations, while patients with double-mutational status had the worst outcomes (figure 1C–F). The interaction term was not associated with rwPFS, suggesting that *STK11* and *KEAP1* mutations have an additive effect. Those results were consistent across anti-PD-1/PD-L1 and chemotherapy treatment classes (figure 1C–F). Finally, we examined the differential prognostic significance of mutations located in exons 1–2 versus exons 3–9 of *STK11* (online supplementary figure 4). We observed a consistent decrease in rwPFS in both groups of mutations.

We then performed an analogous analysis using OS as the endpoint and observed the prognostic nature of *STK11-KEAP1* mutations to be highly consistent with observations for rwPFS (figure 2A–F).

We performed the analyses described above in *KRAS*-mutated patients to test the utility of *STK11-KEAP1*

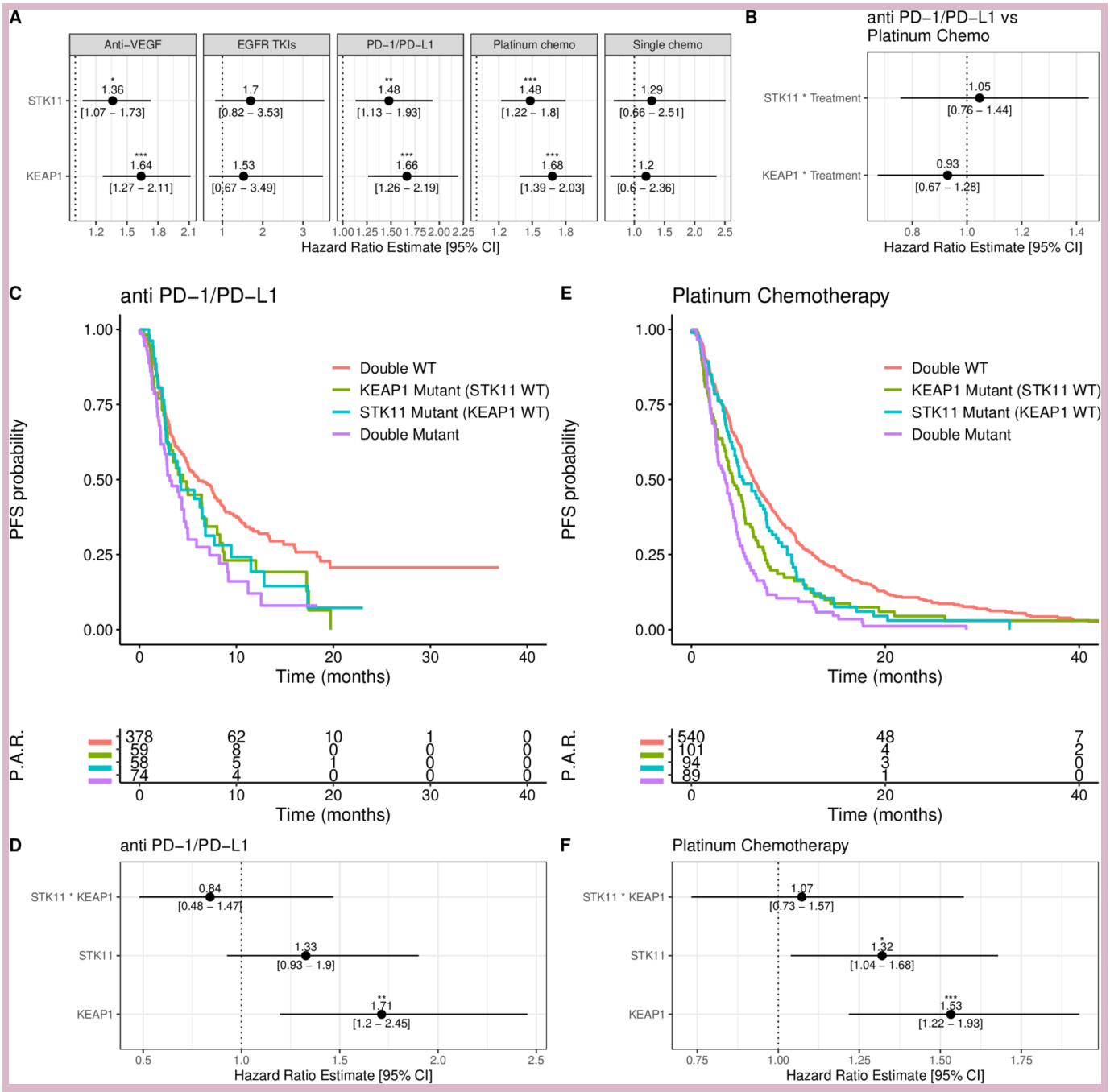

Figure 1 Effect of *STK11* and *KEAP1* somatic mutations on rwPFS in a first-line setting. (A) Forest plot of the HRs of mutations in *STK11* or *KEAP1* across different treatment classes. (B) Forest plot of the HRs of the interaction terms of *STK11* or *KEAP1* mutations and treatment (platinum chemotherapy vs PD-1/PD-L1). (C) Kaplan-Meier curves of PD-1/PD-L1-treated patients according to *STK11-KEAP1* status. (D) Forest plot of the HRs of *STK11* and *KEAP1* in PD-1/PD-L1-treated patients. (E) Kaplan-Meier curves of platinum chemotherapy-treated patients according to *STK11-KEAP1* status. (F) Forest plot of the HRs of *STK11* and *KEAP1* in platinum chemotherapy-treated patients. Stars above HRs indicate significance level (p value). ***0–0.001, **0.001–0.01, *0.01–0.05 . EGFR, epidermal growth factor receptor; PD-1, programmed death-1; PD-L1, programmed death ligand 1; rwPFS, real-world, progression-free survival; VEGF, vascular endothelial growth factor; WT, wild-type.

mutations as a predictive biomarker for this patient population (online supplementary figure 3). *STK11-KEAP1* mutations were associated with poor prognosis in this patient subset in both anti-PD-1/PD-L1-treated and chemotherapy-treated populations, consistent with our overall findings.

DISCUSSION

Our comprehensive profiling of *STK11* and *KEAP1* mutations in NSQ NSCLC demonstrated that these mutations confer a poor prognosis, regardless of treatment class. Our results extend previous reports of patients with

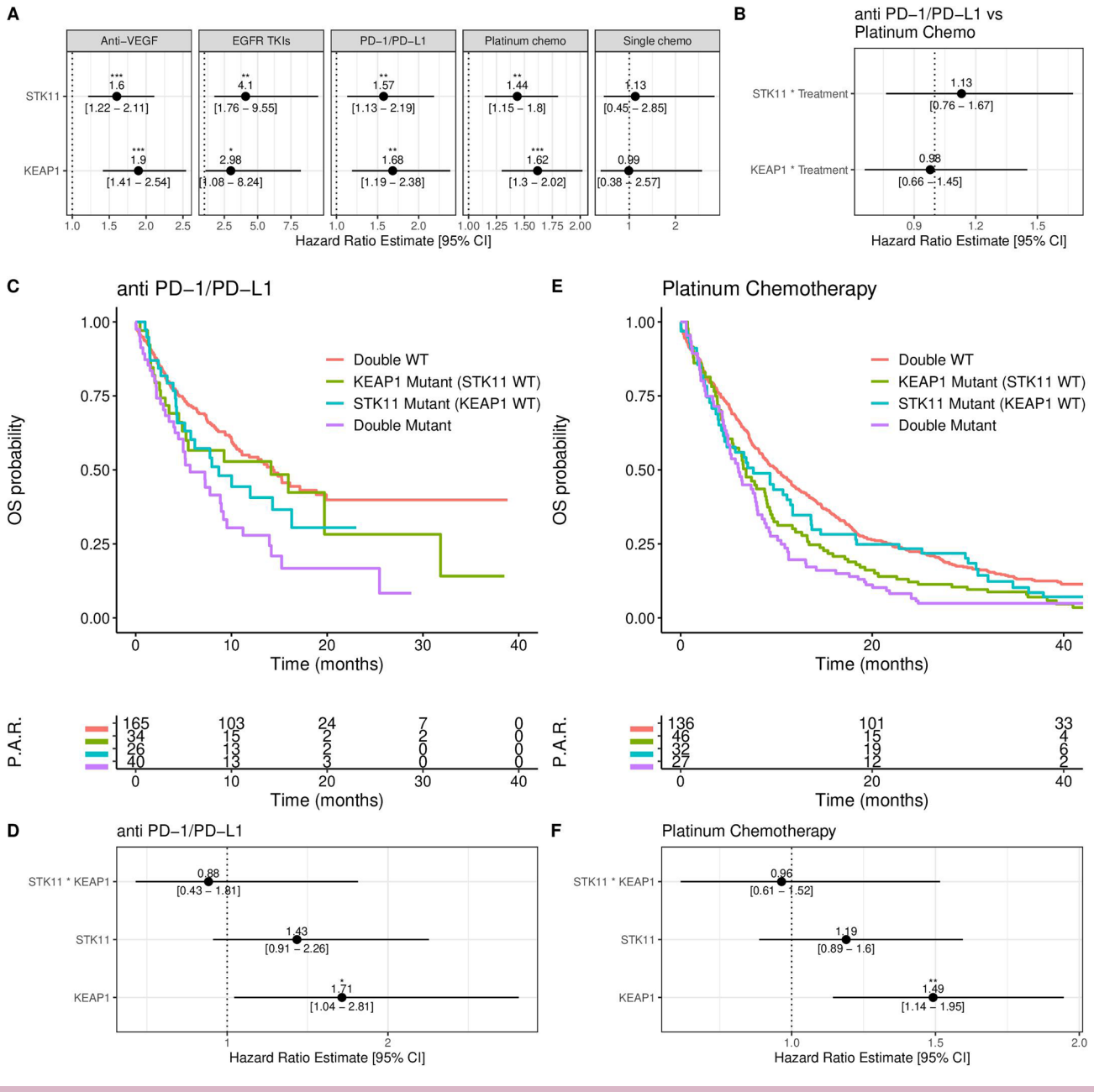

Figure 2 Effect of *STK11* and *KEAP1* somatic mutations on OS in a first-line setting. (A) Forest plot of the HRs of mutations in *STK11* or *KEAP1* across different treatment classes. (B) Forest plot of the HRs of the interaction terms of *STK11* or *KEAP1* mutations and treatment (platinum chemotherapy vs PD-1/PD-L1). (C) Kaplan-Meier curves of PD-1/PD-L1-treated patients according to *STK11-KEAP1* status. (D) Forest plot of the HRs of *STK11* and *KEAP1* in PD-1/PD-L1-treated patients. (E) Kaplan-Meier curves of platinum chemotherapy-treated patients according to *STK11-KEAP1* status. (F) Forest plot of the HRs of *STK11* and *KEAP1* in platinum chemotherapy-treated patients. Stars above HRs indicate significance level (p value). ***0–0.001, **0.001–0.01, *0.01–0.05. EGFR, epidermal growth factor receptor; PD-1, programmed death-1; PD-L1, programmed death ligand 1; rwPFS, real-world, progression-free survival; VEGF, vascular endothelial growth factor; WT, wild-type.

STK11 or *KEAP1* mutations[5–9 12 13] by examining a larger cohort across multiple treatment types, broader patient populations and examining the additive effect of *STK11* and *KEAP1*.

There are some limitations to our study. Real-world data are retrospective and observational and thus may not offer the same robustness as prospective randomised clinical trials. Factors that influence clinical decision-making but are not explicitly captured by real-world data sets may exist and thus confound analyses. Other factors such as tumour evolutionary dynamics between specimen collection, diagnosis and treatment start or during treatment may influence the associations. Moreover, although the cohort was large, it might not be sufficiently powered

to capture a low-effect-size interaction between *STK11* and *KEAP1* mutations.

In conclusion, our results provide evidence against previous reports suggesting that *STK11-KEAP1* mutations are predictive biomarkers for anti-PD-1/PD-L1 therapy.[5–7 12] Using a cohort of 2276 patients with NSCLC, we show that *STK11* and *KEAP1* mutations are associated with poor prognosis across all therapy classes and should not be used as a patient selection marker for ICB.

Correction notice Figure 2 has been updated with correct panels D & F. The new corrected figure does not impact the interpretation of results nor any conclusion of the work.

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