ORIGINAL ARTICLE

Associations of Tissue Tumor Mutational Burden and Mutational Status With Clinical Outcomes With Pembrolizumab Plus Chemotherapy Versus Chemotherapy For Metastatic NSCLC

Check for updates

Marina C. Garassino, MD,[a,*] Shirish Gadgeel, MD,[b] Silvia Novello, MD, PhD,[c]
Balazs Halmos, MD,[d] Enriqueta Felip, MD,[e] Giovanna Speranza, MD,[f] Rina Hui, PhD,[g]
Edward B. Garon, MD,[h] Hidehito Horinouchi, MD, PhD,[i]
Shunichi Sugawara, MD, PhD,[j] Delvys Rodriguez-Abreu, MD, PhD,[k] Martin Reck, MD,[l]
Razvan Cristescu, PhD,[m] Deepti Aurora-Garg, PhD,[m] Andrey Loboda, PhD,[m]
Jared Lunceford, PhD,[m] Julie Kobie, PhD,[m] Mark Ayers, MS,[m] Bilal Piperdi, MD,[m]
M. Catherine Pietanza, MD,[m] Luis Paz-Ares, MD, PhD[n]

[a]Section of Hematology/Oncology, Thoracic Oncology program, University of Chicago, Chicago, Illinois, and IRCCS Istituto Nazionale dei Tumori, Milano
[b]Division of Hematology/Oncology, Department of Internal Medicine, Henry Ford Cancer Institute/Henry Ford Health System, Detroit, Michigan

[c]*Department of Oncology, University of Turin, Orbassano, Italy*
[d]*Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, New York*
[e]*Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron University, Barcelona, Spain*
[f]*Centre intégré de cancérologie de la Montérégie, Université de Sherbrooke, Greenfield Park, Quebec, Canada*
[g]*Westmead Hospital and University of Sydney, Sydney, New South Wales, Australia*
[h]*David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, California*
[i]*Department of Thoracic Oncology, National Cancer Center Hospital, Tokyo, Japan*
[j]*Department of Pulmonary Medicine, Sendai Kousei Hospital, Miyagi, Japan*
[k]*Complejo Hospitalario Universitario Insular Materno-Infantil de Gran Canaria, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain*
[l]*LungenClinic, Airway Research Center North, German Center for Lung Research, Grosshansdorf, Germany*
[m]*Merck & Co., Inc., Rahway, New Jersey*
[n]*Hospital Universitario 12 de Octubre, Spanish National Cancer Research Center, Universidad Complutense and Ciberonc, Madrid, Spain*

ABSTRACT

Introduction: We evaluated tissue tumor mutational burden (tTMB) and mutations in *STK11, KEAP1,* and *KRAS* as biomarkers for outcomes with pembrolizumab plus platinum-based chemotherapy (pembrolizumab-combination) for NSCLC among patients in the phase 3 KEYNOTE-189 (ClinicalTrials.gov, NCT02578680; nonsquamous) and KEYNOTE-407 (ClinicalTrials.gov, NCT02775435; squamous) trials.

Methods: This retrospective exploratory analysis evaluated prevalence of high tTMB and *STK11, KEAP1*, and *KRAS* mutations in patients enrolled in KEYNOTE-189 and KEYNOTE-407 and the relationship between these potential biomarkers and clinical outcomes. tTMB and *STK11, KEAP1*, and *KRAS* mutation status was assessed using whole-exome sequencing in patients with available tumor and matched normal DNA. The clinical utility of tTMB was assessed using a prespecified cutpoint of 175 mutations/exome.

Results: Among patients with evaluable data from whole-exome sequencing for evaluation of tTMB (KEYNOTE-189, $n = 293$; KEYNOTE-407, $n = 312$) and matched normal DNA, no association was found between continuous tTMB score and overall survival (OS) or progression-free survival for pembrolizumab-combination (Wald test, one-sided $p > 0.05$) or placebo-combination (Wald test, two-sided $p > 0.05$) in patients with squamous or nonsquamous histology. Pembrolizumab-combination improved outcomes for patients with tTMB greater than or equal to 175 compared with tTMB less than 175 mutations/exome in KEYNOTE-189 (OS, hazard ratio = 0.64 [95% confidence interval (CI): 0.38–1.07] and 0.64 [95% CI: 0.42–0.97], respectively) and KEYNOTE-407 (OS, hazard ratio = 0.74 [95% CI: 0.50–1.08 and 0.86 [95% CI: 0.57–1.28], respectively) versus placebo-combination. Treatment outcomes were similar regardless of *KEAP1, STK11,* or *KRAS* mutation status.

Conclusions: These findings support pembrolizumab-combination as first-line treatment in patients with metastatic NSCLC and do not suggest the utility of tTMB, *STK11, KEAP1,* or *KRAS* mutation status as a biomarker for this regimen.

Introduction

Pembrolizumab plus platinum-based chemotherapy (pembrolizumab-combination) is a standard-of-care first-line treatment for patients with metastatic NSCLC irrespective of tumor programmed death-ligand 1 (PD-L1) expression.[1,2] The role of pembrolizumab-combination in treatment is supported by two placebo-controlled randomized phase 3 studies that reported improved overall survival (OS) and progression-free survival (PFS) among patients with previously untreated metastatic nonsquamous NSCLC without sensitizing *EGFR* mutation or *ALK* alteration (KEYNOTE-189; NCT02578680) or metastatic squamous NSCLC (KEYNOTE-407; NCT02775435) irrespective of PD-L1 expression levels.[3,4]

Tumor mutational burden (TMB), defined as the number of somatic mutations in the tumor genome, is of interest as a biomarker for immune checkpoint inhibitors.[5–7] There is discordant evidence for TMB as a biomarker for treatment outcomes with first-line immunotherapy versus immunotherapy plus chemotherapy in advanced NSCLC.[8] Using whole-exome sequencing (WES) of patients with NSCLC who received pembrolizumab monotherapy, tissue TMB (tTMB) was found to be

associated with OS and PFS benefit.[9] Findings from other studies of immunotherapy with anti−PD-(L)1 agents alone or in combination with anti−CTLA-4 therapy suggest that tTMB may have clinical utility as a biomarker for treatment outcomes.[5,10–14]

Mutations in driver genes, including in *STK11* (also known as *LKB1*), *KEAP1*, and *KRAS*, occur in a meaningful proportion of patients with advanced or metastatic NSCLC and are of interest as potential biomarkers for outcomes with anti−PD-(L)1 therapy.[15–17] Mutations in *STK11* and *KRAS* are more common in patients with nonsquamous histology (including adenocarcinoma) than those with squamous histology.[18] *KRAS* G12C is the most frequently occurring *KRAS* mutation in NSCLC, comprising approximately 35% of identified *KRAS* mutations.[18,19] Mutations in *KRAS* are a common oncogenic driver in nonsquamous NSCLC,[18] and some studies have suggested that *KRAS* mutations may be associated with improved outcomes with anti−PD-(L)1 therapy plus chemotherapy.[17] *STK11* and *KEAP1* mutations have been associated with poor outcomes in nonsquamous NSCLC, may occur concurrently with *KRAS* mutations, and have been associated with a potential lack of benefit with anti−PD-(L)1 therapy plus chemotherapy.[15–17,20,21]

To investigate the prevalence and potential clinical utility of tTMB and *STK11, KEAP1*, and *KRAS* mutations as biomarkers of outcomes, we conducted separate exploratory analyses of the KEYNOTE-189 and KEYNOTE-407 trials in patients with metastatic NSCLC who received pembrolizumab or placebo plus platinum-based chemotherapy.

Materials and Methods
Study Design and Patients

The KEYNOTE-189 (ClinicalTrials.gov, NCT02578680)[3] and KEYNOTE-407 (ClinicalTrials.gov, NCT02775435)[4] trials were randomized, double-blind, placebo-controlled phase 3 trials enrolling patients with previously untreated metastatic NSCLC regardless of tumor PD-L1 expression. Patients had nonsquamous NSCLC without sensitizing *EGFR* or *ALK* alterations in KEYNOTE-189 and squamous NSCLC in KEYNOTE-407. The study protocols and all amendments were approved by the appropriate ethics committee at each study site. Patients provided written informed consent before participation.

Treatment

In KEYNOTE-189, patients were randomized 2:1 to receive four 3-week cycles of intravenous pembrolizumab 200 mg or placebo, plus pemetrexed 500 mg/m^2 and either cisplatin (75 mg/m^2) or carboplatin (area under the concentration−time curve = 5 mg/mL/min) followed by pembrolizumab or placebo once every 3 weeks for an additional 31 cycles (35 cycles in total) and indefinite pemetrexed maintenance therapy.

In KEYNOTE-407, patients were randomized 1:1 to four 3-week cycles of intravenous pembrolizumab 200 mg or placebo plus carboplatin (area under the concentration−time curve = 6 mg/mL/min) and either paclitaxel (200 mg/m^2) or nab-paclitaxel (100 mg/m^2) followed by pembrolizumab or placebo once every 3 weeks for an additional 31 cycles (35 cycles in total).

Assessments

tTMB and select single-gene mutations (*STK11, KEAP1,* and *KRAS*) were assessed centrally by WES of tumor tissue and matched normal DNA as previously described.[22] tTMB was assessed using a prespecified cutpoint of 175 mutations/exome (mut/exome) to define subgroups with high tTMB (≥175 mut/exome; tTMB-high) versus low tTMB (<175 mut/exome; tTMB-low). This cutpoint was derived using GEP and WES TMB data from a training set of patients with multiple tumor types across the pembrolizumab clinical program, in which 175 mut/exome yielded the most statistically significant difference in the distribution of a gene expression profile comprising 18 genes.[6,23–25] This cutpoint most closely approximates the 10 mutations per megabase used by the updated pipeline FoundationOne F1Dx_v3.2 assay (FoundationMedicine, Cambridge, MA).[26–28] Full methodology for WES analysis is included in Supplementary Methods.

End Points

The clinical objectives of KEYNOTE-189 and KEYNOTE-407 have been reported previously.[3,4] The objectives of these analyses were to evaluate the prevalence of high tTMB and *STK11, KEAP1*, and *KRAS* mutations in patients enrolled in KEYNOTE-189 and KEYNOTE-407 and to evaluate the relationship between these potential biomarkers and clinical outcomes (OS, PFS, and objective response rate [ORR]) in patients treated with pembrolizumab-combination and placebo-combination. Additional objectives were to investigate the relationship between tTMB and tumor PD-L1 expression, the association between tTMB and treatment efficacy, and the clinical utility of tTMB as a predictor of efficacy. Exploratory biomarker analyses were prespecified in the study protocol for each study. The statistical analysis plan was prespecified before merging clinical and biomarker data.

Statistical Analysis

Efficacy was assessed in the biomarker-evaluable populations, which comprised randomized patients

Table 1. Demographics and Baseline Clinical Characteristics in the tTMB-Evaluable Populations in Each Study

Characteristics	KEYNOTE-189			KEYNOTE-407		
	tTMB-Evaluable Population (n = 293)	Single-Gene Mutation-Evaluable Population (n = 289)	Total Population (n = 616)	tTMB-Evaluable Population (n = 312)	Single-Gene Mutation-Evaluable Population[a] (n = 285)	Total Population (n = 559)
Median age, y (IQR)	64 (56-69)	63 (56-69)	64 (57-69)	66 (60-71)	66 (60-71)	65 (60-71)
Male	166 (56.7)	162 (56.1)	363 (58.9)	252 (80.8)	230 (80.7)	455 (81.4)
ECOG performance status 1	164 (55.9)	162 (56.1)	346 (56.2)	215 (68.9)	194 (68.1)	396 (70.8)
Former or current smoker	260 (88.7)	256 (88.6)	543 (88.1)	291 (93.3)	265 (93.0)	518 (92.7)
PD-L1 TPS						
<1%	99 (33.8)	98 (33.9)	190 (30.8)	111 (35.6)	100 (35.1)	194 (34.7)
1%-49%	910 (31.1)	90 (31.1)	186 (30.2)	117 (37.5)	111 (38.9)	207 (37.0)
≥50%	98 (33.4)	96 (33.2)	202 (32.8)	83 (26.6)	74 (26.0)	146 (26.1)
Could not be evaluated[b]	5 (1.7)	5 (1.7)	38 (6.2)	0 (0.0)	0 (0.0)	12 (2.1)

Note: Data are presented as *n* (%) unless otherwise noted.
[a]*KRAS* and *STK11* mutation data were excluded for KEYNOTE-407 because these mutations are rare in squamous NSCLC, and the number of patients with these mutations who also had evaluable WES data from both tumor and normal DNA was small.
[b]Specimens had an inadequate number of tumor cells or no tumor cells.
ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; PD-L1 TPS, programmed death-ligand 1 tumor proportion score; tTMB, tissue tumor mutational burden; WES, whole-exome sequencing.

who had evaluable samples for WES and received one or more doses of study treatment. The association between tTMB, assessed as a continuous $\log_{10}$-transformed variable, and treatment efficacy were evaluated separately for each trial, with the significance level set at 0.05 and no multiplicity adjustment. Wald tests on the tTMB regression coefficients were used to calculate one-sided *p* values for pembrolizumab, under the hypothesis that higher tTMB positively associates with improved outcomes. Two-sided *p* values were calculated for chemotherapy because there was no a priori hypothesis regarding the direction of the association. Descriptive analyses were performed to assess the association between *STK11*, *KEAP1*, and *KRAS* status and clinical outcomes (OS, PFS, and ORR). The prespecified statistical analysis plan is described in Supplementary Methods.

Results
Patients
In KEYNOTE-189, 293 of 616 (47.6%) randomized patients had evaluable WES data and were included in the tTMB-evaluable population (pembrolizumab, *n* = 207; control, *n* = 86), and 289 (46.9%) had matched normal DNA and were included in the single-gene mutation-evaluable population (*STK11, KEAP1,* and *KRAS*). The data cutoff for all analyses from KEYNOTE-189 was September 21, 2018 (Supplementary Fig. 1A). In KEYNOTE-407, 312 of 559 (55.8%) randomized patients

with evaluable WES data were included in the tTMB-evaluable population (pembrolizumab, *n* = 143; control, *n* = 169), and 285 (46.9%) were included in the single-gene mutation-evaluable population (*KEAP1*; *STK11* and *KRAS* were not evaluated in patients with squamous NSCLC owing to the low prevalence of these mutations in squamous NSCLC). The data cutoff date for all analyses from KEYNOTE-407 was May 9, 2019 (Supplementary Fig. 1B). Demographics and baseline clinical characteristics are described in Table 1.

Clinical Outcomes in the tTMB-Evaluable Population and Association of tTMB With Efficacy
In each study, clinical outcomes (i.e., OS, PFS, and ORR) in the tTMB-evaluable groups for pembrolizumab-combination versus placebo-combination were similar to those in the intent-to-treat population (Supplementary Table 1). tTMB and PD-L1 tumor proportion score (TPS) were not strongly associated with one another in either treatment arm in either study (Supplementary Fig. 2A and B). For assessment of the association of tTMB with efficacy, on the basis of the area under the receiver operating characteristic curve for ORR, higher tTMB assessed as a continuous variable was not associated with ORR in either treatment arm (Fig. 1A and C). No association was found between tTMB (assessed as a continuous variable) and ORR (in logistic regression analyses) or OS and PFS (in Cox proportional hazard regression analyses) in either
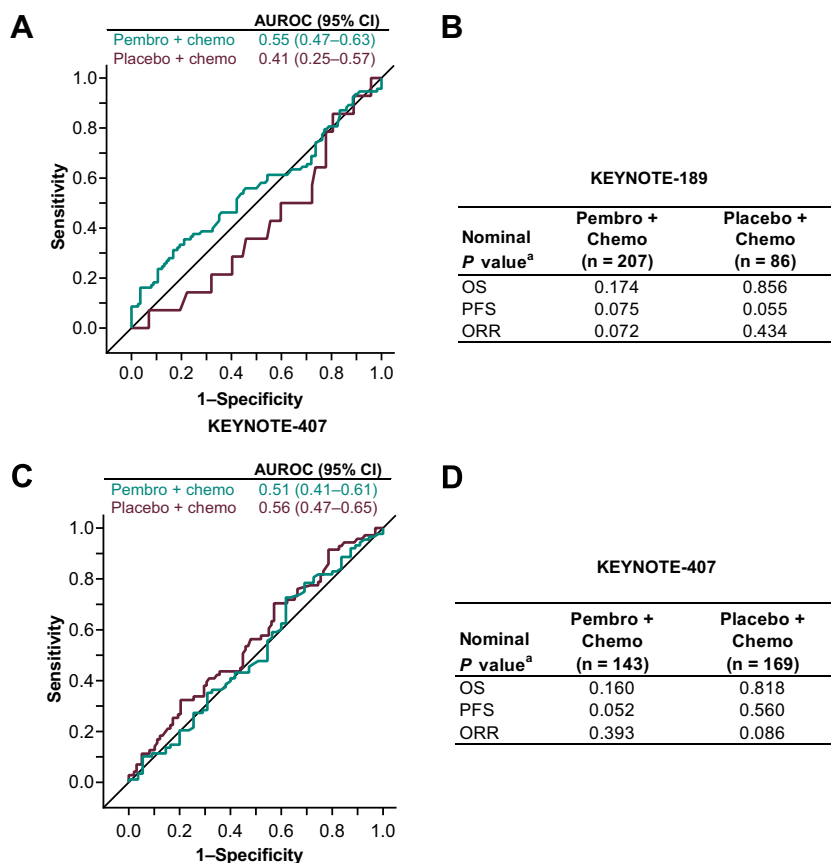
Figure 1. Association of tTMB with efficacy outcomes in (*A*) and (*B*) KEYNOTE-189 and (*C*) and (*D*) KEYNOTE-407. In panels *A* and *C*, the graph illustrates the area under the ROC curve for ORR. Panels *B* and *D* provide *p* values for OS, PFS, and ORR in each respective study from logistic regression analysis. [a]*p* values were calculated using the Wald test and are one-sided for pembrolizumab-combination (a priori hypothesis that tTMB was positively associated with improved outcomes for pembrolizumab-combination) and two-sided for placebo-combination (no a priori hypothesis regarding the direction of the association between tTMB and outcomes) with significance level set at 0.05 and no multiplicity adjustment. tTMB was graphed on a $\log_{10}$ scale for the ROC curve. AUC, area under the curve; CI, confidence interval; ORR, objective response rate; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; r, correlation coefficient; ROC, receiver operating characteristics; tTMB, tissue tumor mutational burden; TPS, tumor proportion score.

treatment arm in either study (Wald test one-sided, $p > 0.05$ for the pembrolizumab-combination arm and two-sided $p > 0.05$ for the placebo-combination arm in each study; Fig. 1*B* and *D*).

Clinical Outcomes in Patients With tTMB Greater Than or Equal to 175 Mutations/Exome and tTMB Less Than 175 Mutations/Exome

In KEYNOTE-189, 134 patients had tTMB greater than or equal to 175 mut/exome (pembrolizumab-combination, $n = 100$; placebo-combination, $n = 34$) and 159 had tTMB less than 175 mut/exome (pembrolizumab-combination, $n = 107$; placebo-combination, $n = 52$). Hazard ratios (HRs) (95% confidence interval [CI]) for OS favored the pembrolizumab-combination group in patients with tTMB greater than or equal to 175 mut/exome (0.64, 0.38–1.07)

and in patients with tTMB less than 175 mut/exome (0.64, 0.42–0.97) (Fig. 2*A*). HRs (95% CI) for PFS favored the pembrolizumab-combination group in patients with tTMB greater than or equal to 175 mut/exome (0.32, 0.21–0.51) and in patients with tTMB less than 175 mut/exome (0.51, 0.35–0.74) (Fig. 2*B*). In the tTMB greater than or equal to 175 mut/exome group, the ORR (95% CI) was 50.0% (39.8%–60.2%) with pembrolizumab-combination versus 11.8% (3.3%–27.5%) with placebo-combination. For patients with tTMB less than 175 mut/exome, the ORR (95% CI) was 40.2% (30.8%–50.1%) versus 19.2% (9.6%–32.5%), respectively (Supplementary Fig. 3*A*).

In KEYNOTE-407, 162 patients had tTMB greater than or equal to 175 mut/exome (pembrolizumab-combination, $n = 73$; placebo-combination, $n = 89$) and 150 patients had tTMB less than 175 mut/exome (pembrolizumab-combination, $n = 70$; placebo-
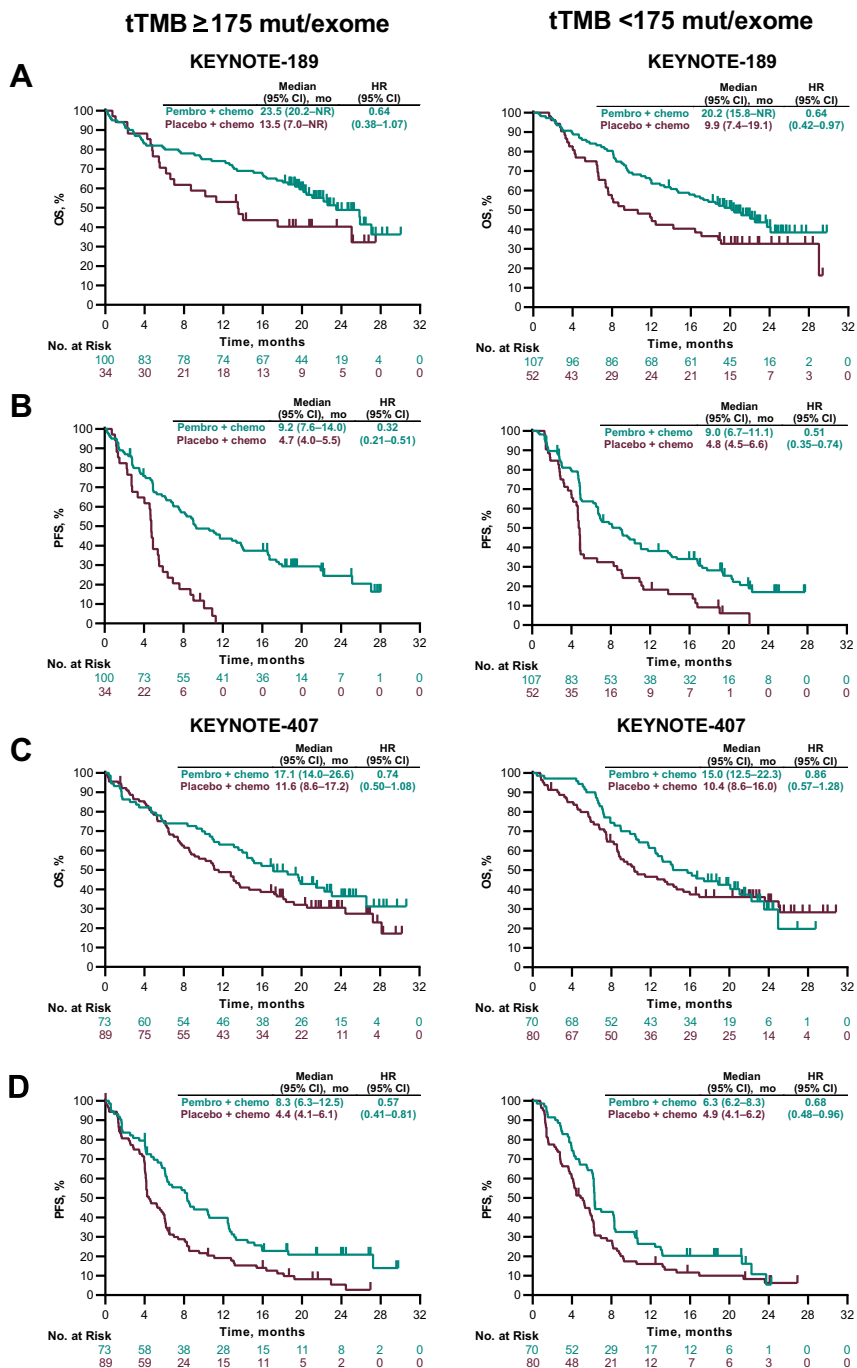
Figure 2. Clinical utility of tTMB for OS and PFS in each study at cutpoints of greater than or equal to 175 mut/exome and less than 175 mut/exome. Kaplan-Meier estimates of OS (*A*) and PFS (*B*) in KEYNOTE-189. Kaplan-Meier estimates of OS (*C*) and PFS (*D*) in KEYNOTE-407. Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; NR, not reached; OS, overall survival; PFS, progression-free survival; tTMB, tissue tumor mutational burden.

combination, *n* = 80). The HRs for OS favored pembrolizumab-combination in the tTMB greater than or equal to 175 mut/exome group (0.74; 95% CI 0.50–1.08) and less than 175 mut/exome group (0.86; 95% CI: 0.57–1.28) (Fig. 2*C*). PFS was improved with pembrolizumab-combination among patients with tTMB greater than or equal to 175 mut/exome (HR = 0.57; 95% CI: 0.41–0.81) and less than 175 mut/ exome (HR = 0.68; 95% CI: 0.48–0.96) (Fig. 2*D*). In the tTMB greater than or equal to 175 mut/exome group, ORR (95% CI) was 58.9% (46.8%–70.3%) with pembrolizumab-combination versus 44.9% (34.4%–

A

B

Figure 3. Kaplan-Meier estimates of OS and PFS by *STK11* status in the single-gene mutation-evaluable population in KEYNOTE-189. (*A*) OS and (*B*) PFS. Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; NR, not reached; OS, overall survival; mut, mutation; Pembro, pembrolizumab; PFS, progression-free survival; wt, wild-type.

55.9%) with placebo-combination. For patients with tTMB less than 175 mut/exome, ORR (95% CI) was 64.3% (51.9%–75.4%) versus 38.8% (28.1%–50.3%), respectively (Supplementary Fig. 3*B*).

Clinical Outcomes in Patients With Versus Without Single-Gene Mutations

STK11. Of the 289 evaluable patients in KEYNOTE-189, 54 (18.7%) had *STK11* mutations. In KEYNOTE-407, 8 of 285 (2.8%) evaluable patients had *STK11* mutations. Because *STK11* mutations occurred infrequently in KEYNOTE-407, associations between *STK11* status and PD-L1, tTMB, or outcomes were not evaluated.

In KEYNOTE-189, the median (interquartile range [IQR]) PD-L1 TPS tended to be numerically lower (0% [0–16] versus 15% [0–75]) and the median (IQR) TMB scores (209 [132–265] versus 146 [89–264] mut/exome)

tended to be numerically higher among patients with versus without an *STK11* mutation (Supplementary Fig. 4*A*). The prevalence of *STK11* mutations by PD-L1 (TPS) and tTMB score (mut/exome) in the *STK11*-evaluable population is illustrated in Supplementary Figure 4*B*.

In KEYNOTE-189, the HRs (95% CI) for OS among patients with an *STK11* mutation were 0.75 (0.37–1.50) and 0.59 (0.41–0.85) with wild-type *STK11* (Fig. 3*A*). The HRs (95% CI) for PFS were 0.81 (0.44–1.47) in patients with an *STK11* mutation and 0.38 (0.27–0.52) with wild-type *STK11* (Fig. 3*B*). The ORRs (95% CI) for pembrolizumab-combination versus placebo-combination were 30.6% (16.4%–48.1%) versus 16.7% (3.6%–41.4%), respectively, in the *STK11* mutation group and 48.8% (41.0%–56.6%) versus 16.4% (8.5%–27.5%), respectively, in the *STK11* wild-type group (Supplementary Fig. 5).

Figure 4. Kaplan-Meier estimates of OS and PFS by *KEAP1* status in the single-gene mutation-evaluable populations. OS in (*A*) KEYNOTE-189 and (*B*) KEYNOTE-407. PFS in (*C*) KEYNOTE-189 and (*D*) KEYNOTE-407. Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; NR, not reached; OS, overall survival; Pembro, pembrolizumab; PFS, progression-free survival; wt, wild-type.
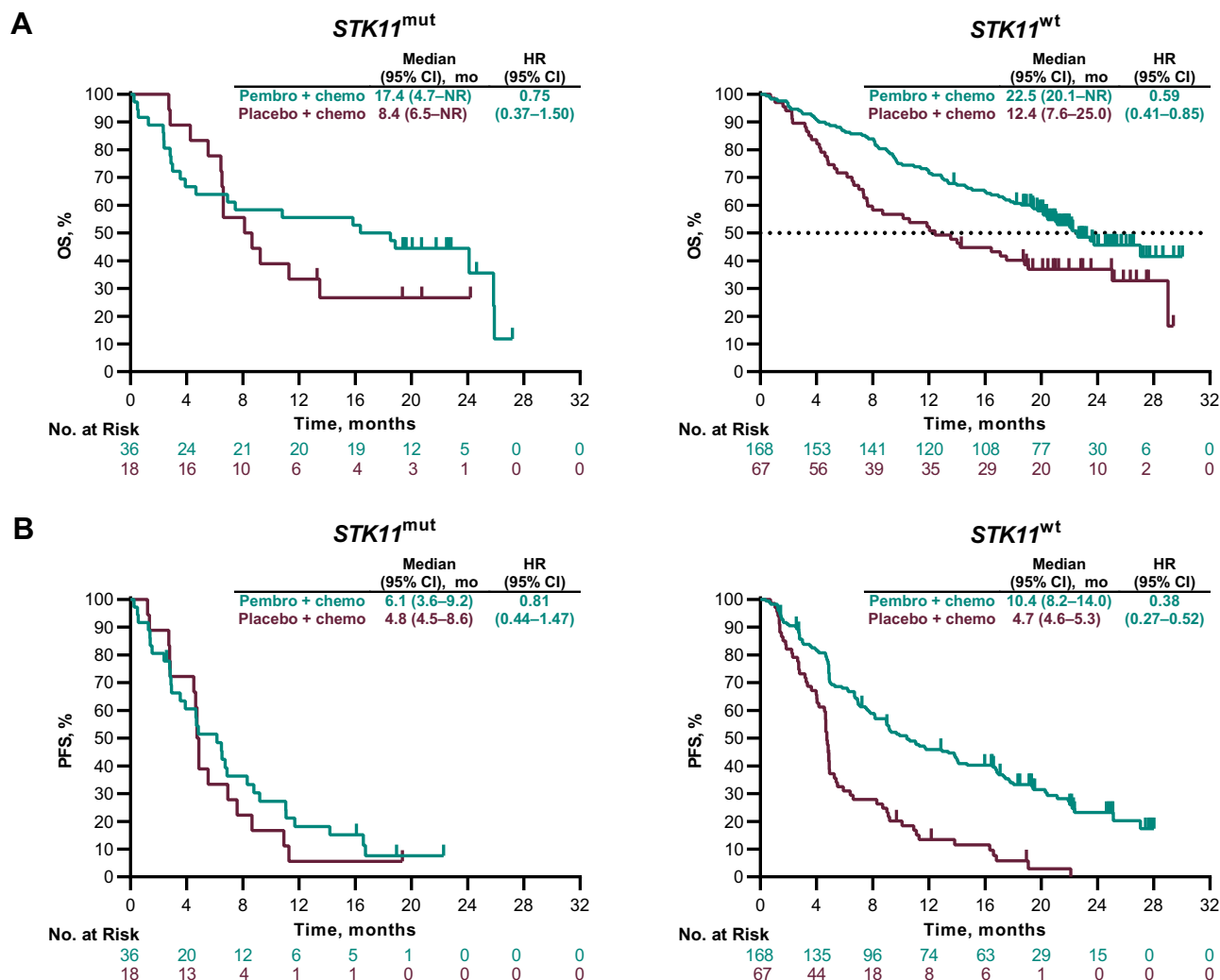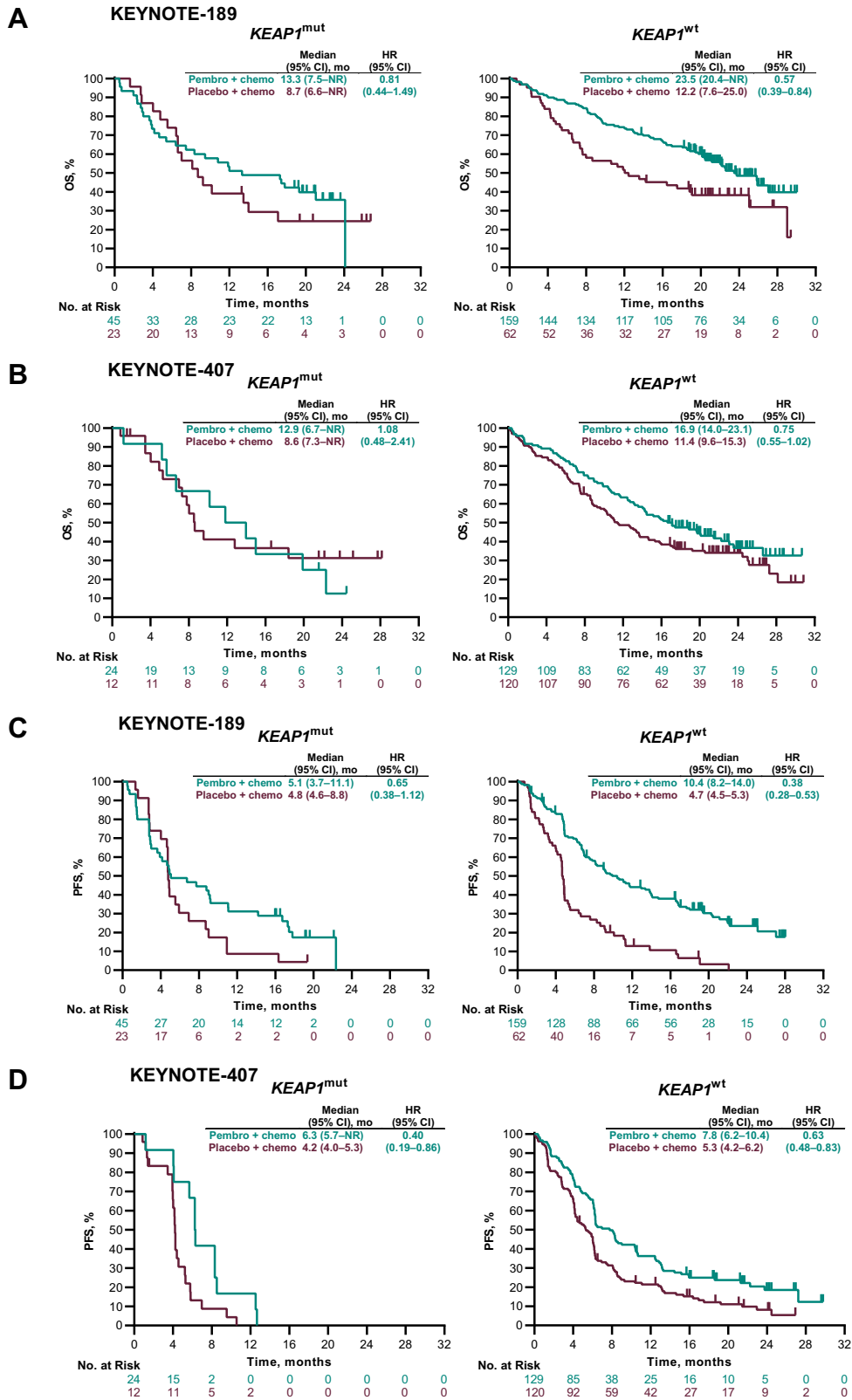
Figure 5. Kaplan-Meier estimates of OS and PFS by *KRAS* status in the single-gene mutation-evaluable population in KEYNOTE-189. (*A*) OS and (*B*) PFS. Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; NR, not reached; OS, overall survival; Pembro, pembrolizumab; PFS, progression-free survival; wt, wild-type.
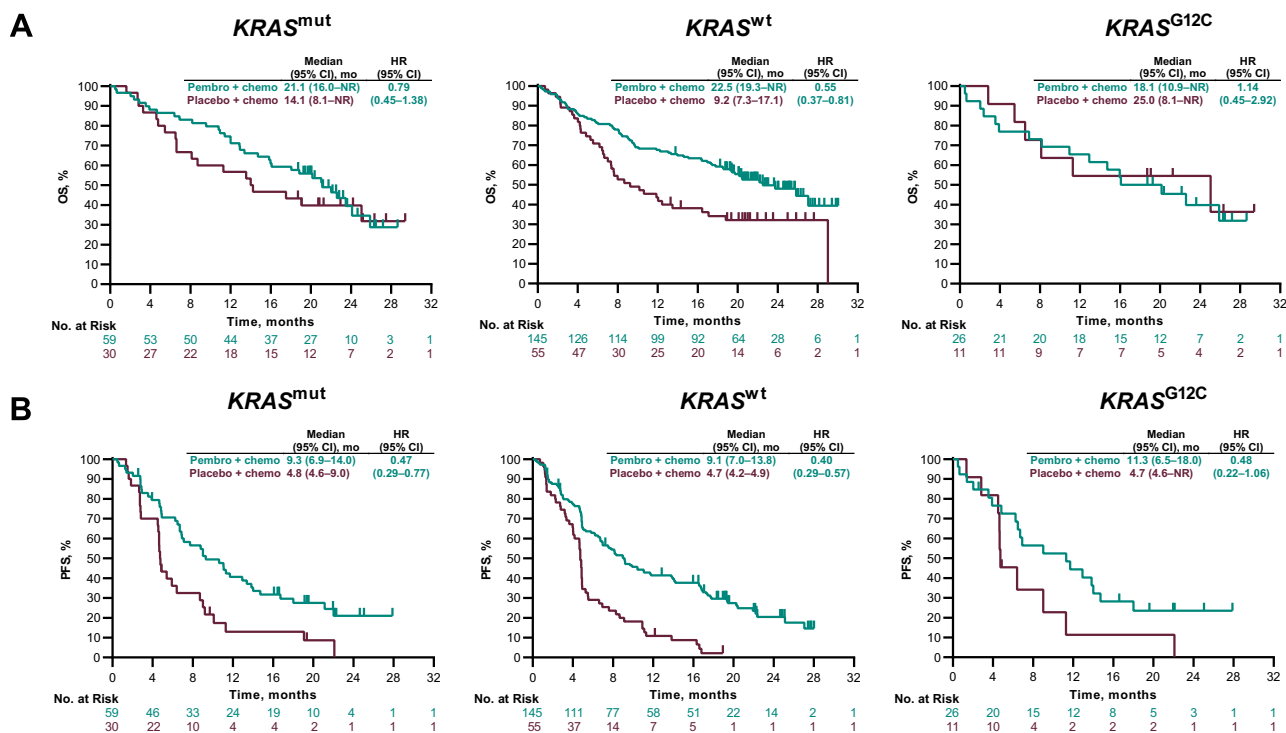
KEAP1. Of the 289 patients in KEYNOTE-189 with evaluable WES data from matched tumor and normal DNA, 68 (23.5%) had *KEAP1* mutations. In KEYNOTE-407, 285 patients had evaluable WES data from matched tumor and normal DNA, and 36 (12.6%) had *KEAP1* mutations.

Among patients in KEYNOTE-189 with *KEAP1* mutations, the median ([IQR]) PD-L1 TPS was numerically lower (1% [0–13] versus 20% [0–75]), and the median (IQR) tTMB score was numerically higher versus wild-type *KEAP1* (173 [124–267] versus 147 [89–263] mut/exome) (Supplementary Fig. 6*A*). The prevalence of *KEAP1* mutations by PD-L1 (TPS) and tTMB score (mut/exome) in the *KEAP1*-evaluable population is illustrated in Supplementary Figure 6*B*. Among patients in KEYNOTE-407 with *KEAP1* mutations, the median (IQR) PD-L1 TPS (11% [1–57]) and median (IQR) tTMB scores (205 [140–296]) were numerically higher versus patients with wild-type *KEAP1* (Supplementary Fig. 6*C*). No association between PD-L1 (TPS) and tTMB score (mut/exome) in the *KEAP1*-evaluable population was observed (Supplementary Fig. 6*D*).

In KEYNOTE-189, pembrolizumab-combination was associated with improved OS and PFS compared with placebo-combination, regardless of *KEAP1* mutation status (*KEAP1* mutation HR [95% CI] for OS, 0.81 [0.44–

1.49]; *KEAP1* wild-type HR [95% CI] for OS, 0.57 [0.39–0.84]) (Fig. 4*A*). The HRs (95% CI) for PFS were 0.65 (0.38–1.12) in patients with *KEAP1* mutations and 0.38 (0.28–0.53) with *KEAP1* wild-type (Fig. 4*C*). The ORR (95% CI) for pembrolizumab-combination versus placebo-combination was 35.6% (21.9%–51.2%) versus 17.4% (5.0%–38.8%), respectively, in patients with *KEAP1* mutations and 48.4% (40.4%–56.5%) versus 16.1% (8.0%–27.7%), respectively, in patients with wild-type *KEAP1* (Supplementary Fig. 7*A*).

In KEYNOTE-407, the HRs (95% CI) for OS were 1.08 (0.48–2.41) in patients with *KEAP1* mutations and 0.75 (0.55–1.02) for wild-type *KEAP1* (Fig. 4*B*). The HRs (95% CI) for PFS were 0.40 (0.19–0.86) in patients with *KEAP1* mutations and 0.63 (0.48–0.83) in patients with wild-type *KEAP1* (Fig. 4*D*). The ORRs (95% CI) for pembrolizumab-combination versus placebo-combination were 66.7% (34.9%–90.1%) versus 54.2% (32.8%–74.5%), respectively, in patients with *KEAP1* mutations and 61.7% (52.4%–70.4%) versus 41.9% (33.2%–50.9%), respectively, in patients with wild-type *KEAP1* (Supplementary Fig. 7*B*).

KRAS. Of the 289 evaluable patients in KEYNOTE-189, 89 (32.2%) had *KRAS* mutations, of which 37 (12.8%) were *KRAS* G12C mutations. In KEYNOTE-407, 14 out of

285 (4.9%) patients had *KRAS* mutations; none were *KRAS* G12C. Because *KRAS* occurred infrequently in KEYNOTE-407 (squamous NSCLC), associations between *KRAS* status and PD-L1, tTMB, or outcomes were not evaluated.

In KEYNOTE-189, the median (IQR) PD-L1 TPS (30% [1%−71%] versus 5% [0%−60%]) and median (IQR) TMB scores (204 [137−276] versus 141 [85−252] mut/exome) tended to be higher in patients with versus without *KRAS* mutations (Supplementary Fig. 8A). Joint association between PD-L1 (TPS) and tTMB score (mut/exome) for *KRAS*-mutant and *KRAS* wild-type patients is illustrated in Supplementary Figure 8B.

The HRs (95% CI) for OS were 0.79 (0.45−1.38) for any *KRAS* mutation and 0.55 (0.37−0.81) for *KRAS* wild-type (Fig. 5A). For PFS, the HRs (95% CI) were 0.47 (0.29−0.77) for any *KRAS* mutation and 0.40 (0.29−0.57) for *KRAS* wild-type (Fig. 5B). The ORR (95% CI) for pembrolizumab-combination versus placebo-combination was 40.7% (28.1%−54.3%) versus 26.7% (12.3%−45.9%) for any *KRAS* mutation and 47.6% (39.2%−56.0%) versus 10.9% (4.1%−22.3%) for wild-type *KRAS* (Supplementary Fig. 9).

For the subgroup of patients with *KRAS* G12C mutation (pembrolizumab-combination, $n = 26$; placebo-combination, $n = 11$), the HRs for patients who received pembrolizumab-combination or placebo-combination were 1.14 (0.45−2.92) and 0.48 (0.22−1.06) for the OS and PFS, respectively (Fig. 5A and B). The corresponding ORRs were 50.0% (29.9%−70.1%) and 18.2% (2.3%−51.8%), respectively (Supplementary Fig. 9).

Discussion

Among patients with advanced NSCLC in the KEYNOTE-189 (nonsquamous) and KEYNOTE-407 (squamous) studies, first-line treatment with platinum-based chemotherapy with or without pembrolizumab revealed no association between tTMB, *KEAP1* mutation (nonsquamous or squamous) or *STK11,* or *KRAS* mutation (nonsquamous) and treatment outcomes. There was no significant association between tTMB for either treatment arm and NSCLC histology. Furthermore, there was no strong correlation between tTMB and PD-L1 TPS in either treatment arm in either study. Pembrolizumab-combination revealed improved clinical benefit versus placebo-combination irrespective of mutations in *STK11, KEAP1*, and *KRAS*. These findings do not support the clinical utility of tTMB as a biomarker for pembrolizumab plus platinum-based chemotherapy for metastatic squamous or nonsquamous NSCLC.

The prevalence of tTMB, *STK11, KEAP1,* and *KRAS* mutations were generally consistent with that previously reported.[7,17,29] The predictive value of tTMB as a biomarker for outcomes with anti−PD-(L)1 therapy may vary when administered as monotherapy or in combination with chemotherapy. In an exploratory analysis of biomarker-evaluable data from the phase 3 KEYNOTE-042 trial of pembrolizumab monotherapy in patients with PD-L1 TPS greater than or equal to 1% advanced NSCLC that used a similar analytical approach, higher tTMB levels were associated with improved outcomes with pembrolizumab but not with chemotherapy. Moreover, patients with tTMB greater than or equal to 175 mut/exome had improved OS and PFS compared with chemotherapy, whereas those with tTMB less than 175 mut/exome did not.[30] In other studies of anti−PD-(L)1 therapies in NSCLC, a relationship between tissue or plasma TMB and clinical outcomes has been reported for studies of both monotherapies, including pembrolizumab,[9] nivolumab,[14] and atezolizumab,[12] and immunotherapy combination therapies, such as nivolumab plus ipilimumab[11,13,31] and durvalumab plus tremelimumab.[32] A review of multiple studies of anti−PD-(L)1 therapy given as single agents across various solid tumor types, including nonsquamous and squamous NSCLC, revealed a significant correlation between increasing TMB and increasing ORR ($p < 0.001$).[33] This finding of an association between tTMB and outcomes with pembrolizumab monotherapy but not with pembrolizumab-combination represents a parallel to the utility of PD-L1 as a biomarker in first-line NSCLC: PD-L1 provides a biomarker for response with pembrolizumab monotherapy,[34−36] but its predictive value is diminished among patients receiving pembrolizumab-combination.[3,4] For patients with PD-L1−negative disease (who are not eligible for pembrolizumab monotherapy), pembrolizumab plus chemotherapy remains an appropriate treatment option irrespective of tTMB.

We also investigated relationships between mutations in *STK11, KEAP1,* and *KRAS* and clinical outcomes in KEYNOTE-189 and KEYNOTE-407, each of which has been suggested to be potentially associated with outcomes among patients receiving anti−PD-(L)1 therapy.[17] Our results indicate that OS benefit persisted among patients who received pembrolizumab-combination regardless of *STK11* or *KEAP1* mutation status. There was no difference in PFS; however, given the relatively small number of patients, there is low precision for estimating the HRs for OS and PFS, as reflected in the very wide confidence intervals. Pembrolizumab-combination was generally associated with improved clinical outcomes compared with placebo-combination regardless of *STK11, KEAP1*, or *KRAS* mutation status; nevertheless, the magnitude of benefit in some groups remains uncertain. In KEYNOTE-407, the HR for OS was 0.96 versus 0.76 among patients with *KEAP1* mutations

versus wild-type *KEAP1*. However, given the small number of patients with a mutation, there is insufficient evidence to support the hypothesis of no benefit for pembrolizumab-combination in patients with squamous NSCLC with *KEAP1* mutations or vice versa. KEYNOTE-189 did not provide evidence of an association between *KRAS* mutation status and outcomes with pembrolizumab-combination. Among patients with *KRAS* G12C mutation, the HR (95% CI) for OS was 1.14 (0.45–2.92), although the sample size was too small to make definitive conclusions. The improvement in OS, PFS, and ORR with pembrolizumab-combination versus placebo-combination was observed irrespective of *KRAS* mutation status. These findings are consistent with other studies of the associations between these mutations and response and resistance to anti−PD-(L)1 therapies, with *KRAS* mutations generally associated with improved outcomes and *STK11* and *KEAP1* mutations being associated with poorer outcomes compared with the corresponding wild-types.[9,17,37–40] In contrast with studies that have suggested *STK11* and *KEAP1* mutations confer resistance to anti−PD-(L)1 therapies, patients with these mutations were found to have improved outcomes with pembrolizumab monotherapy versus chemotherapy in patients with advanced NSCLC in the KEYNOTE-042 study.[41]

These analyses were exploratory with few patients in some groups. Biomarker analyses were prespecified in the study protocol for both KEYNOTE-407 (squamous) and KEYNOTE-189 (nonsquamous) and the analysis plan was prespecified before the clinical and biomarker data were merged. Furthermore, our analysis only included patients with WES-evaluable samples, resulting in small sizes for certain groups. Notably, improvements in clinical outcomes observed with pembrolizumab-combination versus placebo-combination in the biomarker-evaluable populations were similar to the total populations of each study. As discussed, there is discordant evidence for TMB as a biomarker for treatment outcomes with first-line immunotherapy versus chemotherapy compared with immunotherapy plus chemotherapy versus chemotherapy in advanced NSCLC.[8] Although WES is considered the gold-standard measurement of TMB, this technique is time-consuming, costly, and laborious.[5] In addition, although there can be variations across cancer types,[42] TMB 175 mut/exome assessed by WES has been shown to be well aligned with the FoundationOne CDx (Foundation Medicine, Cambridge, MA) TMB cutpoint of 10 mutations per megabase that is known to enrich for response across multiple solid tumor types, including NSCLC.[28]

In conclusion, the results of this exploratory analysis suggest that tTMB and *STK11, KEAP1*, and *KRAS* mutation status have limited clinical utility as biomarkers for patients treated with first-line pembrolizumab plus platinum-based chemotherapy in metastatic non-squamous and squamous NSCLC. Our findings support the use of pembrolizumab plus platinum-based chemotherapy as a standard first-line combination therapy for patients with metastatic nonsquamous NSCLC, regardless of tTMB or *STK11, KEAP1*, or *KRAS* mutation status.

CRediT Authorship Contribution Statement

Marina C. Garassino, Shirish Gadgeel, Silvia Novello, Balazs Halmos, Enriqueta Felip, Giovanna Speranza, Rina Hui, Edward B. Garon, Hidehito Horinouchi, Shunichi Sugawara, Delvys Rodriguez-Abreu, Martin Reck, Razvan Cristescu, Deepti Aurora-Garg, Andrey Loboda, Jared Lunceford, Julie Kobie, Mark Ayers, Bilal Piperdi, M. Catherine Pietanza, Luis Paz-Ares: Writing - review & editing.

Julie Kobie, Razvan Cristescu: Data curation, Formal analysis.

Marina C. Garassino, Shirish Gadgeel, Silvia Novello, Balazs Halmos, Enriqueta Felip, Giovanna Speranza, Rina Hui, Edward B. Garon, Hidehito Horinouchi, Shunichi Sugawara, Delvys Rodriguez-Abreu, Martin Reck, Luis Paz-Ares: Supervision, Investigation.

Razvan Cristescu, Deepti Aurora-Garg, Andrey Loboda, Jared Lunceford, Julie Kobie, Mark Ayers, Bilal Piperdi, M. Catherine Pietanza: Methodology.

Data-Sharing Statement

Data will be made available for request after product approval in the United States and European Union or after product development is discontinued. There are circumstances that may prevent Merck Sharp & Dohme from sharing requested data, including country or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan that is collaboratively developed by the requestor and Merck Sharp & Dohme subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, Merck Sharp & Dohme will either perform the proposed analyses and share the results with the requestor or will construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

Acknowledgments

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2022.100431.

References

1. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology [NCCN Guidelines]: non-small-cell lung cancer, version 3.2019. https://www.nccn.org/patients/guidelines/cancers.aspx#nsclc. Accessed May 22, 2020.
2. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2019;30:863–870.
3. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378:2078-2092.
4. Paz-Ares L, Luft A, Vicente D, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med*. 2018;379:2040-2051.
5. Fancello L, Gandini S, Pelicci PG, Mazzarella L. Tumor mutational burden quantification from targeted gene panels: major advancements and challenges. *J Immunother Cancer*. 2019;7:183.
6. Stenzinger A, Allen JD, Maas J, et al. Tumor mutational burden standardization initiatives: recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions. *Genes Chromosomes Cancer*. 2019;58:578-588.
7. Yarchoan M, Albacker LA, Hopkins AC, et al. PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. *JCI Insight*. 2019;4:e126908.
8. Galvano A, Gristina V, Malapelle U, et al. The prognostic impact of tumor mutational burden (TMB) in the first-line management of advanced non-oncogene addicted non-small-cell lung cancer (NSCLC): a systematic review and meta-analysis of randomized controlled trials. *ESMO Open*. 2021;6:100124.
9. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015;348:124-128.
10. Berland L, Heeke S, Humbert O, et al. Current views on tumor mutational burden in patients with non-small cell lung cancer treated by immune checkpoint inhibitors. *J Thorac Dis*. 2019;11(suppl 1):S71–S80.
11. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med*. 2018;378:2093-2104.
12. Kowanetz M, Zou W, Shames D, et al. Tumor mutation burden (TMB) is associated with improved efficacy of atezolizumab in 1l and 2l+ NSCLC patients. *J Thorac Oncol*. 2017;12(suppl):S321-S322.
13. Ready N, Hellmann MD, Awad MM, et al. First-line nivolumab plus ipilimumab in advanced non-small-cell lung cancer (CheckMate 568): outcomes by programmed death ligand 1 and tumor mutational burden as biomarkers. *J Clin Oncol*. 2019;37:992–1000.
14. Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med*. 2017;376:2415-2426.
15. Campbell JD, Alexandrov A, Kim J, et al. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nat Genet*. 2016;48:607-616.
16. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature*. 2014;511:543-550.
17. Skoulidis F, Heymach JV. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. *Nat Rev Cancer*. 2019;19:495-509.
18. La Fleur L, Falk-Sorqvist E, Smeds P, et al. Mutation patterns in a population-based non-small cell lung cancer cohort and prognostic impact of concomitant mutations in KRAS and TP53 or STK11. *Lung Cancer*. 2019;130:50-58.

19. Aredo JV, Padda SK, Kunder CA, et al. Impact of KRAS mutation subtype and concurrent pathogenic mutations on non-small cell lung cancer outcomes. *Lung Cancer*. 2019;133:144-150.

20. Best SA, De Souza DP, Kersbergen A, et al. Synergy between the KEAP1/NRF2 and PI3K pathways drives non-small-cell lung cancer with an altered immune microenvironment. *Cell Metab*. 2018;27:935-943.e4.

21. Kadara H, Choi M, Zhang J, et al. Whole-exome sequencing and immune profiling of early-stage lung adenocarcinoma with fully annotated clinical follow-up. *Ann Oncol*. 2017;28:75-82.

22. Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science*. 2018;362:eaar3593.

23. Merino DM, McShane LM, Fabrizio D, et al. Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. *J Immunother Cancer*. 2020;8:e000147.

24. Ayers M, Lunceford J, Nebozhyn M, et al. IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest*. 2017;127:2930-2940.

25. Panda A, Betigeri A, Subramanian K, et al. Identifying a clinically applicable mutational burden threshold as a potential biomarker of response to immune checkpoint therapy in solid tumors. *JCO Precis Oncol*. 2017;2017:PO.17.00146.

26. Marabelle A, Fakih M, Lopez J, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol*. 2020;21:1353-1365.

27. Aurora-Garg D, Albright A, Qiu P, et al. Large-scale evaluation of concordance of genomic scores in whole exome sequencing and Foundation Medicine comprehensive genomic platform across cancer types. Paper presented at: Society for Immunotherapy of Cancer (SITC); November 6-10, 2019. National Harbor, MD.

28. Cristescu R, Aurora-Garg D, Albright A, et al. Tumor mutational burden predicts the efficacy of pembrolizumab monotherapy: a pan-tumor retrospective analysis of participants with advanced solid tumors. *J Immunother Cancer*. 2022;10:e003091.

29. Papillon-Cavanagh S, Doshi P, Dobrin R, Szustakowski J, Walsh AM. STK11 and KEAP1 mutations as prognostic biomarkers in an observational real-world lung adenocarcinoma cohort. *ESMO Open*. 2020;5:e000706.

30. Herbst RS, Lopes G, Kowalski DM, et al. Association between tissue TMB (tTMB) and clinical outcomes with pembrolizumab monotherapy (pembro) in PD-L1-positive advanced NSCLC in the KEYNOTE-010 and −042 trials. *Ann Oncol*. 2019;30:v916-v917.

31. Hellmann MD, Nathanson T, Rizvi H, et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. *Cancer Cell*. 2018;33:843-852.

32. Rizvi NA, Cho BC, Reinmuth N, et al. Durvalumab with or without tremelimumab vs standard chemotherapy in first-line treatment of metastatic non-small cell lung cancer: the MYSTIC Phase 3 randomized clinical trial. *JAMA Oncol*. 2020;6:661-674.

33. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med*. 2017;377:2500-2501.

34. 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;375:1823-1833.

35. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Updated analysis of KEYNOTE-024: pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with PD-L1 tumor proportion score of 50% or greater. *J Clin Oncol*. 2019;37:537-546.

36. Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet*. 2019;393:1819-1830.

37. Aggarwal C, Thompson JC, Chien AL, et al. Baseline plasma tumor mutation burden predicts response to pembrolizumab-based therapy in patients with metastatic non-small cell lung cancer. *Clin Cancer Res*. 2020;26:2354-2361.

38. Skoulidis F. Inactivating STK11/LKB1 genomic alterations are a major driver of primary resistance to PD-1 axis blockade in non-squamous non-small cell lung cancer. *Cancer Res*. 2019;79:Abstract nr SY42-02.

39. Rizvi H, Sanchez-Vega F, La K, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *J Clin Oncol*. 2018;36:633-641.

40. Miao D, Margolis CA, Vokes NI, et al. Genomic correlates of response to immune checkpoint blockade in microsatellite-stable solid tumors. *Nat Genet*. 2018;50:1271-1281.

41. Cho BC, Lopes G, Kowalski DM, et al. Relationship between STK11 and KEAP1 mutational status and efficacy in KEYNOTE-042: pembrolizumab monotherapy versus platinum-based chemotherapy as first-line therapy for PD-L1-positive advanced NSCLC. *Cancer Res*. 2020;80:CT084.

42. Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet*. 2019;51:202-206.