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ORIGINAL ARTICLE

Associations of tissue tumor mutational burden and mutational status with clinical outcomes in KEYNOTE-042: pembrolizumab versus chemotherapy for advanced PD-L1-positive NSCLC☆

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Background: We evaluated whether tissue tumor mutational burden (tTMB) and *STK11*, *KEAP1*, and *KRAS* mutations have clinical utility as biomarkers for pembrolizumab monotherapy versus platinum-based chemotherapy in patients with programmed death ligand 1 (PD-L1)-positive (tumor proportion score $\geq$1%) advanced/metastatic non-small-cell lung cancer (NSCLC) without *EGFR*/*ALK* alterations in the phase III KEYNOTE-042 trial.

Patients and methods: This retrospective exploratory analysis assessed prevalence of tTMB and *STK11*, *KEAP1*, and *KRAS* mutations determined by whole-exome sequencing of tumor tissue and matched normal DNA and their associations with outcomes in KEYNOTE-042. Clinical utility of tTMB was assessed using a prespecified cut point of 175 mutations/exome.

Results: Of 793 patients, 345 (43.5%) had tTMB $\geq$175 mutations/exome and 448 (56.5%) had tTMB <175 mutations/exome. No association was observed between PD-L1 expression and tTMB. Continuous tTMB score was associated with improved overall survival (OS) and progression-free survival among patients receiving pembrolizumab (Wald test, one-sided $P < 0.001$) but not those receiving chemotherapy (Wald test, two-sided $P > 0.05$). tTMB $\geq$175 mutations/exome was associated with improved outcomes for pembrolizumab versus chemotherapy, whereas tTMB <175 mutations/exome was not {OS: hazard ratio, 0.62 [95% confidence interval (CI) 0.48-0.80] and 1.09 (95% CI 0.88-1.36); progression-free survival: 0.75 (0.59-0.95) and 1.27 (1.04-1.55), respectively}. Improved OS [hazard ratio (95% CI)] for pembrolizumab versus chemotherapy was observed regardless of *STK11* [*STK11* mutant ($n = 33$): 0.37 (0.16-0.86), *STK11* wild-type ($n = 396$): 0.83 (0.65-1.05)]; *KEAP1* [*KEAP1* mutant ($n = 64$): 0.75 (0.42-1.35), *KEAP1* wild-type ($n = 365$): 0.78 (0.61-0.99)], or *KRAS* [*KRAS* mutant ($n = 69$): 0.42 (0.22-0.81); *KRAS* wild-type ($n = 232$): 0.86 (0.63-1.18)] mutation status.

Conclusion: tTMB with a cut point of $\geq$175 mutations/exome is a potential predictive biomarker for pembrolizumab monotherapy for advanced/metastatic PD-L1 tumor proportion score $\geq$1% NSCLC. Pembrolizumab is a standard first-line treatment in this setting regardless of *STK11*, *KEAP1*, or *KRAS* mutation status.

Key words: tissue tumor mutational burden, single-gene genetic alterations, pembrolizumab, locally advanced or metastatic non-small-cell lung cancer, biomarker

INTRODUCTION

Pembrolizumab monotherapy has demonstrated clinical benefit in patients with advanced/metastatic programmed death ligand-1 (PD-L1)-positive [PD-L1 tumor proportion score (TPS) $\geq$1%] non-small-cell lung cancer (NSCLC) without sensitizing *EGFR* mutations or *ALK* rearrangements, with approval in the United States based on PD-L1 TPS $\geq$1% and in the European Union based on PD-L1 TPS $\geq$50%.[1-4] In the international, randomized, open-label, phase III KEYNOTE-042 study, pembrolizumab monotherapy improved overall survival (OS) compared with platinum-based

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chemotherapy in patients with previously untreated PD-L1 TPS ≥1% advanced/metastatic NSCLC.[1] Among patients with PD-L1 TPS ≥1%, the hazard ratio (HR) was 0.81 [95% confidence interval (CI) 0.71-0.93; $P = 0.0018$) with pembrolizumab versus chemotherapy and 0.69 (95% CI 0.56-0.85; $P = 0.0003$) in patients with PD-L1 TPS ≥50%, respectively.[1]

There has been significant interest in identifying additional biomarkers for response to pembrolizumab in patients with advanced NSCLC. Tumor mutational burden (TMB), which has been defined as the number of somatic mutations in the tumor exome, is a promising biomarker for immune checkpoint inhibitors (ICIs).[5,6] Higher TMB has been associated with higher levels of neoantigens in patients with a broad range of tumor types, although not yet specifically in patients with NSCLC,[7,8] which are targets for an immune system activated by ICIs, including pembrolizumab.[5,9] Associations between tissue TMB (tTMB) and clinical outcomes with anti-PD-(L)1 therapies have been reported.[10-13] Because lung cancer is associated with high tTMB,[14] tTMB may have clinical utility as a predictive biomarker for ICIs. Retrospective analyses from several studies evaluating ICIs in the first-line or subsequent setting as monotherapy or in combination with anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) therapies have provided supporting evidence.[11,12,15-19]

Other potential predictive biomarkers include mutations in genes such as *STK11*, *KEAP1*, and *KRAS*. *STK11* (also known as *LKB1*) and *KEAP1* have been associated with chemoresistance and poor outcomes in patients with NSCLC.[20-23] *KRAS* mutations are more commonly observed in NSCLC with nonsquamous than squamous histology[24,25] and have been reported in ∼15%-30% of lung adenocarcinomas in Western populations, with *KRAS* G12C being the most frequently occurring.[26-30] To date, preclinical and small retrospective clinical studies have provided equivocal evidence regarding clinical outcomes and/or sensitivity and resistance to chemotherapy or ICIs in patients with NSCLC with *KRAS* mutations,[31] and a pooled analysis reported a higher response rate to anti-PD-(L)1 therapy and a higher 6-month progression-free survival (PFS) rate in *KRAS* mutant patients than *KRAS* wild-type patients with NSCLC.[32]

We undertook a retrospective exploratory biomarker analysis of the KEYNOTE-042 study to assess the prevalence of high tTMB [defined as ≥175 mutations per exome (mut/exome)] and *STK11*, *KEAP1*, and *KRAS* (nonsquamous only) mutations in patients with PD-L1 TPS ≥1% advanced/metastatic NSCLC who received pembrolizumab monotherapy or chemotherapy and to evaluate associations of these potential biomarkers with clinical outcomes.

METHODS

Study design and patients

KEYNOTE-042 (ClinicalTrials.gov, NCT02220894) is a randomized, open-label, phase III study that enrolled adult patients with previously untreated locally advanced/metastatic NSCLC and PD-L1 TPS ≥1% without sensitizing *EGFR* mutation or *ALK* translocation.[1] The protocol and all amendments were approved by the appropriate ethics committee at each center, the study was conducted in accordance with the standards of Good Clinical Practice, and patients provided written informed consent.

Treatment

Patients were randomized 1 : 1 to pembrolizumab 200 mg every 3 weeks for 35 cycles or investigator's choice of carboplatin AUC 5-6 mg/ml/min plus paclitaxel 200 mg/m^2 or pemetrexed 500 mg/m^2 (nonsquamous histology only) every 3 weeks for 4-6 cycles, followed by optional maintenance therapy of pemetrexed 500 mg/m^2 for patients with nonsquamous histology, until disease progression, unacceptable adverse events, or patient withdrawal.

Assessments

PD-L1 expression was evaluated using PD-L1 IHC 22C3 pharmDx (Agilent Technologies, Carpinteria, CA). TMB was evaluated in tumor tissue from patients with PD-L1 TPS ≥1% advanced/metastatic NSCLC with a matched germline in a subset of patients. tTMB and single gene (*STK11*, *KEAP1*, and *KRAS*) mutation status were evaluated centrally by whole-exome sequencing (WES) of tumor tissue using formalin-fixed, paraffin-embedded pretreatment tumor samples using either ImmunoSelect™-R (Personal Genome Diagnostics, Baltimore, MD) or ACE Cancer Exome™ (Personalis, Menlo Park, CA). The clinical utility of tTMB was assessed using a prespecified cut point of 175 mut/exome to define patient subgroups with high tTMB (≥175 mut/exome; tTMB-high) versus low tTMB (<175 mut/exome; tTMB-low). This cut point was derived using GEP and WES TMB data from a training set of patients with multiple tumor types who received treatment with pembrolizumab across the pembrolizumab clinical program[5,33-35] and most closely approximates the 10 mut/Mb cut point by targeted sequencing as per the FoundationOne F1Dx_v3.2 assay (Foundation Medicine, Cambridge, MA).[10,36,37]

Objectives

The clinical objectives of KEYNOTE-042 have been previously described.[1] The objectives of the current analyses were to assess the prevalence of high tTMB and *STK11*, *KEAP1*, and *KRAS* mutations in patients with PD-L1 TPS ≥1% advanced/metastatic NSCLC in KEYNOTE-042 and to investigate the relationship between these biomarkers and clinical outcomes [OS, PFS, and objective response rate (ORR) per RECIST version 1.1 by blinded independent central review (BICR)] in patients treated with pembrolizumab or chemotherapy. Exploratory biomarker analyses were prespecified in the study protocol and, for each analysis, the statistical analysis plan and tTMB cut points were prespecified before clinical and biomarker data were merged.

Statistical analyses

The biomarker-evaluable populations comprised all patients with PD-L1 TPS ≥1% who were randomized to pembrolizumab or chemotherapy (i.e. all patients analyzed as treated) and had evaluable samples for tTMB using WES,

and also had matched normal DNA available for evaluation of *STK11*, *KEAP1*, and *KRAS* (in nonsquamous disease) somatic mutations. For the association of tTMB with outcomes, tTMB was assessed as a continuous $\log_{10}$-transformed variable. 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. The statistical analysis plan for tTMB analysis is described in Supplementary Material, Methods, available at https://doi.org/10.1016/j.annonc.2023.01.011.

RESULTS

Patients

Of 1274 patients with advanced squamous and nonsquamous NSCLC and PD-L1 TPS ≥1% who were randomized to pembrolizumab or chemotherapy [the intent-to-treat (ITT) population], 793 (62.2%) had samples evaluable for tTMB analysis using WES (pembrolizumab, $n = 414$; chemotherapy, $n = 379$). A total of 429/793 patients (54.0%) had matched normal DNA and were assessable for *STK11* and *KEAP1*, and 301/793 (38.0%) had nonsquamous disease with *KRAS*-evaluable data (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2023.01.011). The data cut-off date for all analyses was 4 September 2018. Demographics and baseline clinical characteristics are described in Table 1.

Clinical outcomes in biomarker-evaluable populations

The clinical outcomes in the biomarker-evaluable population for pembrolizumab versus chemotherapy (i.e. HRs for OS, PFS, and ORR in each treatment group) were similar to those in the ITT population (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2023.01.011).

Table 1. Patient demographics and baseline clinical characteristics

Characteristic, *n* (%)	tTMB-assessable population (*n* = 793)	*STK11/KEAP1*-assessable population (*n* = 429)	*KRAS*-assessable population[a] (*n* = 301)	Total population (*N* = 1274)
Age, median (IQR), years	63 (57-69)	63 (56-69)	62 (56-68)	63 (57-69)
Male	560 (70.6)	304 (70.9)	196 (65.1)	902 (70.8)
ECOG PS 1	535 (67.5)	283 (66.0)	199 (66.1)	884 (69.4)
Former/current smoker	607 (76.5)	334 (77.9)	224 (74.4)	992 (77.9)
Squamous histology	287 (36.2)	128 (29.8)	—	491 (38.5)
PD-L1 TPS				
1%-49%	428 (54.0)	233 (54.3)	160 (53.2)	675 (53.0)
≥50%	365 (46.0)	196 (45.7)	141 (46.8)	599 (47.0)

ECOG PS, Eastern Cooperative Oncology Group performance score; IQR, interquartile range; PD-L1, programmed death ligand-1; TPS, tumor proportion score; tTMB, tissue tumor mutational burden.
[a]Nonsquamous histology only; data for squamous histology are not presented. *KRAS* mutations were more prevalent for nonsquamous histology.

Relationships between tTMB, tumor PD-L1 expression, and efficacy in patients with advanced NSCLC and PD-L1 TPS ≥1%

There was no correlation between tTMB and PD-L1 TPS for pembrolizumab ($r = 0.05$) or chemotherapy ($r = 0.04$; Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2023.01.011).

The area under the receiver operating characteristic (AUROC) curve for ORR and tTMB was 0.67 (95% CI 0.61-0.73) for pembrolizumab and 0.57 (95% CI 0.50-0.63) for chemotherapy (Figure 1A). When assessed as a continuous variable, tTMB was associated with better OS, PFS, and ORR (Wald test, one-sided $P < 0.001$ in each case; Figure 1B) for pembrolizumab. tTMB was not associated with improved OS or PFS for chemotherapy ($P = 0.060$ and $P = 0.174$, respectively; two-sided *P* value; Figure 1B).

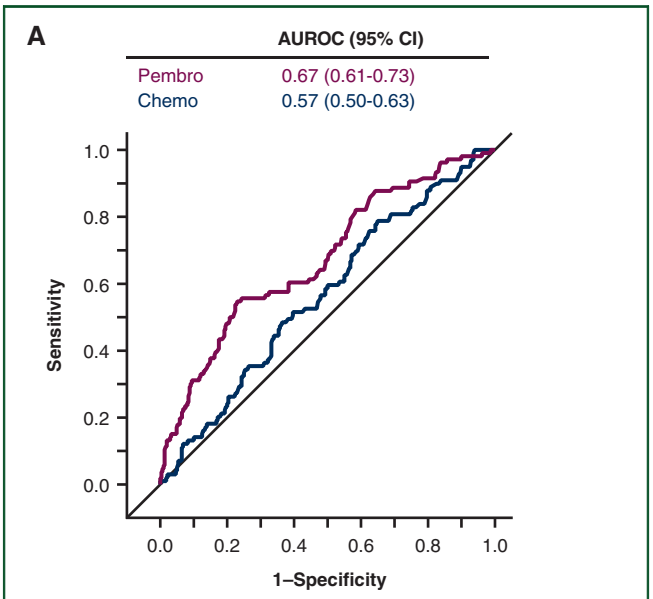
Clinical outcomes in patients with tTMB ≥175 mut/exome and tTMB <175 mut/exome

The clinical utility of tTMB to predict clinical outcomes was investigated using a predefined cut point of 175 mut/exome from 793 evaluable tumor samples from patients with advanced NSCLC and PD-L1 TPS ≥1%. Overall, 345 patients (43.5%: pembrolizumab, $n = 180$; chemotherapy, $n = 165$) had tTMB ≥175 mut/exome. A total of 448 (56.5%) patients (pembrolizumab, $n = 234$; chemotherapy, $n = 214$) had tTMB <175 mut/exome. The proportion of patients with PD-L1 TPS ≥50% in the tTMB ≥175 mut/exome and tTMB <175 mut/exome groups was similar (49.3% versus 43.5%).

The HR (95% CI) for OS was 0.62 (0.48-0.80) for pembrolizumab versus chemotherapy in the tTMB ≥175 mut/exome group and 1.09 (0.88-1.36) in the tTMB <175 mut/exome group (Figure 2A). HRs (95% CI) for PFS were 0.75 (0.59-0.95) for the tTMB ≥175 mut/exome group and 1.27 (1.04-1.55) for the tTMB <175 mut/exome group (Figure 2B). ORRs for pembrolizumab and chemotherapy were higher in the tTMB ≥175 mut/exome group (34.4% versus 30.9%) than in the tTMB <175 mut/exome group (18.8% versus 22.4%; Figure 2C).

Clinical outcomes in patients with versus without single-gene mutations

STK11. Of 429 patients with evaluable WES data from tumor and matched normal DNA, 33 patients (7.7%) had *STK11* mutations (pembrolizumab, $n = 16$; chemotherapy, $n = 17$). Twelve patients (2.8%) had both *STK11* and *KEAP1* mutations. In patients with nonsquamous tumors, 27/301 (9.0%) had an *STK11* mutation; among squamous tumors, 6/128 (4.7%) had an *STK11* mutation. In patients with *STK11* mutations, PD-L1 TPS was lower than in patients with *STK11* wild-type [median (interquartile range; IQR) PD-L1 TPS 15% (3%-50%) versus 40% (10%-80%), respectively]. tTMB score (mut/exome) was higher in patients with *STK11* mutations versus *STK11* wild-type [median (IQR) 191 (104-272) versus 146 (72−253), respectively; Supplementary Figure S3A, available at https://doi.org/10.1016/j.annonc.2023.01.011]. Prevalences of *STK11* mutations by PD-L1 (TPS) and tTMB



B

Nominal *P* value[a]	Pembro (*n* = 414)	Chemo (*n* = 379)
OS	<0.001 (one-sided)	0.060 (two-sided)[b]
PFS	<0.001 (one-sided)	0.174 (two-sided)[b]
ORR	<0.001 (one-sided)	0.035 (two-sided)

Figure 1. (A) Association of tTMB with efficacy outcomes based on AUROC curve for ORR. Graph shows area under the ROC curve for ORR. (B) Association of tTMB with clinical outcomes with pembrolizumab versus chemotherapy. Table provides *P* values for OS, PFS, and ORR from logistic regression analysis.

AUROC, area under the receiver operating characteristic; Chemo, chemotherapy; CI, confidence interval; mut, mutation; ORR, overall response rate; OS, overall survival; PD-L1, programmed death ligand-1; Pembro, pembrolizumab; PFS, progression-free survival; TMB, tumor mutational burden; TPS, tumor proportion score; tTMB, tissue TMB.

[a]Wald test. *P* values are one-sided for pembrolizumab because the *a priori* hypothesis was that higher tTMB was positively associated with improved outcomes of pembrolizumab. *P* values are two-sided for chemotherapy because there was no *a priori* hypothesis regarding the direction of the association between tTMB and outcomes of chemotherapy. TMB was assessed as a continuous, $\log_{10}$-transformed variable.

[b]tTMB showed negative directions of association with PFS and OS in the chemotherapy arm.

score (mut/exome) in the *STK11*-evaluable population are shown in Supplementary Figure S3B, available at https://doi.org/10.1016/j.annonc.2023.01.011.

The HR (95% CI) for OS between the pembrolizumab and chemotherapy groups was 0.37 (0.16-0.86) in the *STK11* mutant group and 0.83 (0.65-1.05) in the *STK11* wild-type group (Figure 3A). HRs (95% CI) for PFS were 0.75 (0.36-1.57) and 0.91 (0.74-1.13), respectively (Figure 3B). ORR for pembrolizumab versus chemotherapy was 31.3% versus 5.9% in patients with *STK11* mutation and 29.4% versus 23.6% for *STK11* wild-type (Figure 3C).

KEAP1. Of 429 patients with evaluable WES data from matched tumor and normal DNA, 64 (14.9%) had *KEAP1* mutation (pembrolizumab, $n = 31$; chemotherapy, $n = 33$). In nonsquamous tumors, 47/301 (15.6%) had a *KEAP1* mutation; among squamous tumors, 17/128 (13.3%) had a *KEAP1* mutation. Outcomes were similar when outcomes in squamous and nonsquamous patients were analyzed separately. Median (IQR) PD-L1 TPS was similar in patients with and without *KEAP1* mutation [40% (10%-81%) versus 40% (10%-80%), respectively; Supplementary Figure S4A, available at https://doi.org/10.1016/j.annonc.2023.01.011], whereas median (IQR) tTMB score was 183 (114-283) mut/exome among patients with *KEAP1* mutations versus 142 (68-252) mut/exome without *KEAP1* mutations (Supplementary Figure S4A, available at https://doi.org/10.1016/j.annonc.2023.01.011). Prevalence of *KEAP1* mutations by PD-L1 (TPS) and tTMB score (mut/exome) in the *KEAP1*-evaluable population is shown in Supplementary Figure S4B, available at https://doi.org/10.1016/j.annonc.2023.01.011.

For OS, HR (95% CI) between the pembrolizumab and chemotherapy arms was 0.75 (0.42-1.35) in the *KEAP1* mutant group and 0.78 (0.61-0.99) in the *KEAP1* wild-type group (Figure 4A). For PFS, HRs (95% CI) between the pembrolizumab and chemotherapy arms were 0.67 (0.38-1.17) and 0.96 (0.77-1.20), respectively (Figure 4B). ORR was 35.5% for pembrolizumab versus 18.2% for chemotherapy in patients with *KEAP1* mutation and 28.6% versus 22.9% for *KEAP1* wild-type patients, respectively (Figure 4C).

KRAS. Of 301 patients with nonsquamous histology who were evaluable for *KRAS* mutation status, 69 (22.9%) had *KRAS* mutations [pembrolizumab, $n = 30$ (10.0%); chemotherapy, $n = 39$ (13.0%)]; 29/69 (42.0%) with a *KRAS* G12C mutation (pembrolizumab, $n = 12$; chemotherapy, $n = 17$). The distribution of PD-L1 expression levels (TPS) and tTMB scores (mut/exome) was higher in *KRAS* mutant patients compared with *KRAS* wild-type [PD-L1 TPS: median (IQR) 60% (10%-95%) versus 35% (10%-80%), respectively; TMB: median (IQR) 191 (129-288) versus 105 (56-226) mut/exome, respectively; Supplementary Figure S5A, available at https://doi.org/10.1016/j.annonc.2023.01.011]. Joint association between PD-L1 (TPS) and tTMB score (mut/exome) for *KRAS* mutant and *KRAS* wild-type patients is shown in Supplementary Figure S5B, available at https://doi.org/10.1016/j.annonc.2023.01.011.

HRs (95% CI) for OS for pembrolizumab versus chemotherapy were 0.42 (0.22-0.81) in the group with any *KRAS* mutation, 0.28 (0.09-0.86) in the *KRAS* G12C mutation group, and 0.86 (0.63-1.18) in the *KRAS* wild-type group (Figure 5A). HRs (95% CI) for PFS between the pembrolizumab and chemotherapy groups were 0.51 (0.29-0.87), 0.27 (0.10-0.71), and 1.00 (0.75-1.34), respectively (Figure 5B). ORRs in the pembrolizumab and chemotherapy groups were 56.7% and 18.0%, 66.7% and 23.5%, and 29.1% and 21.0%, respectively (Figure 5C).

DISCUSSION

This exploratory biomarker analysis of the KEYNOTE-042 study demonstrated that higher tTMB (defined by a cut point of $\geq$175 mut/exome) was associated with improved clinical outcomes among patients with advanced NSCLC and PD-L1 TPS $\geq$1% receiving pembrolizumab monotherapy. There was no apparent association between tTMB and PD-L1 TPS, similar to previous reports.[38-40] Higher tTMB further

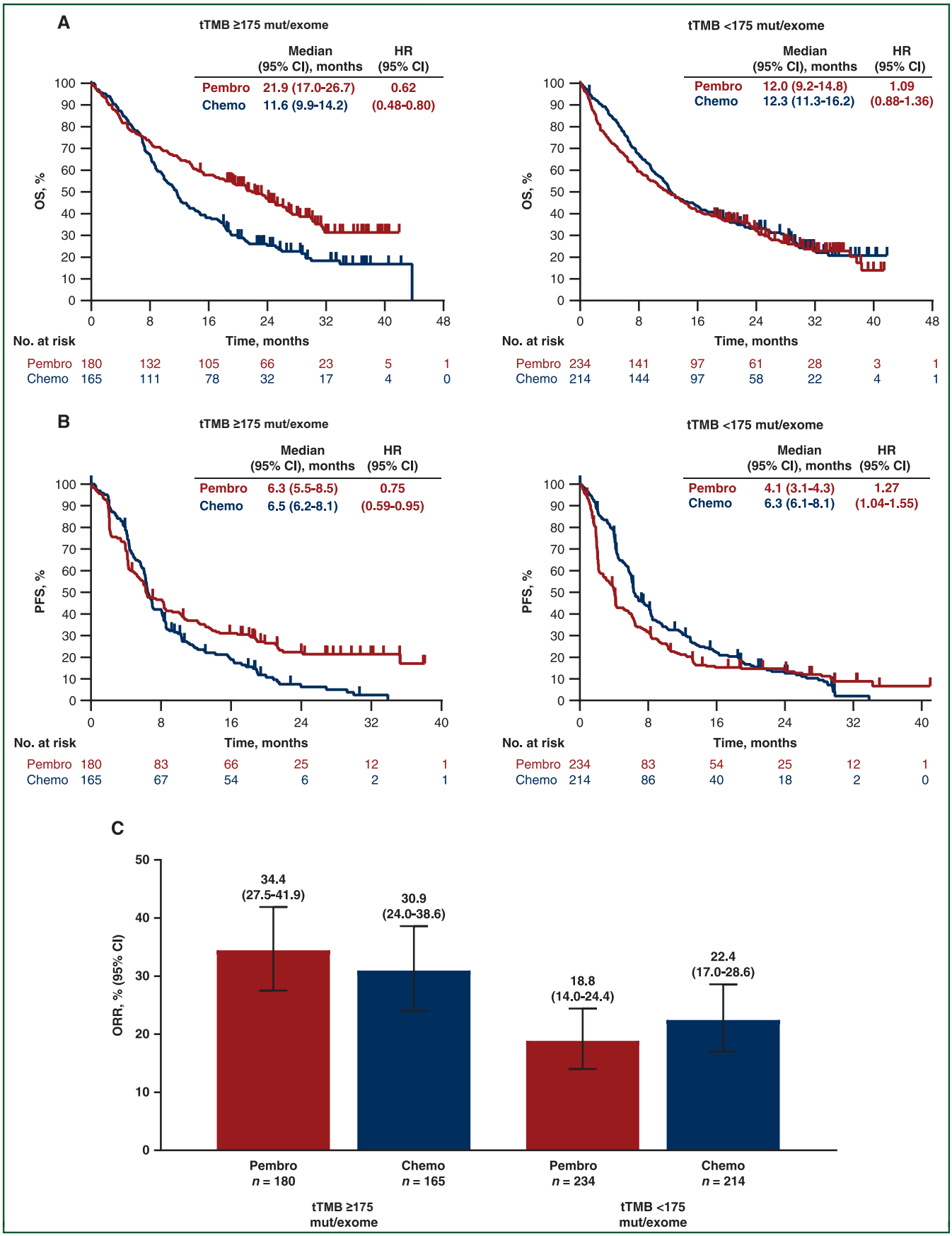


Figure 2. (A) OS, (B) PFS per RECIST version 1.1 by blinded independent central review, and (C) ORR per RECIST version 1.1 by blinded independent central review in patients with tTMB ≥175 mut/exome and tTMB <175 mut/exome.

Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; ORR, overall response rate; OS, overall survival; Pembro, pembrolizumab; PFS, progression-free survival; tTMB, tissue tumor mutational burden.

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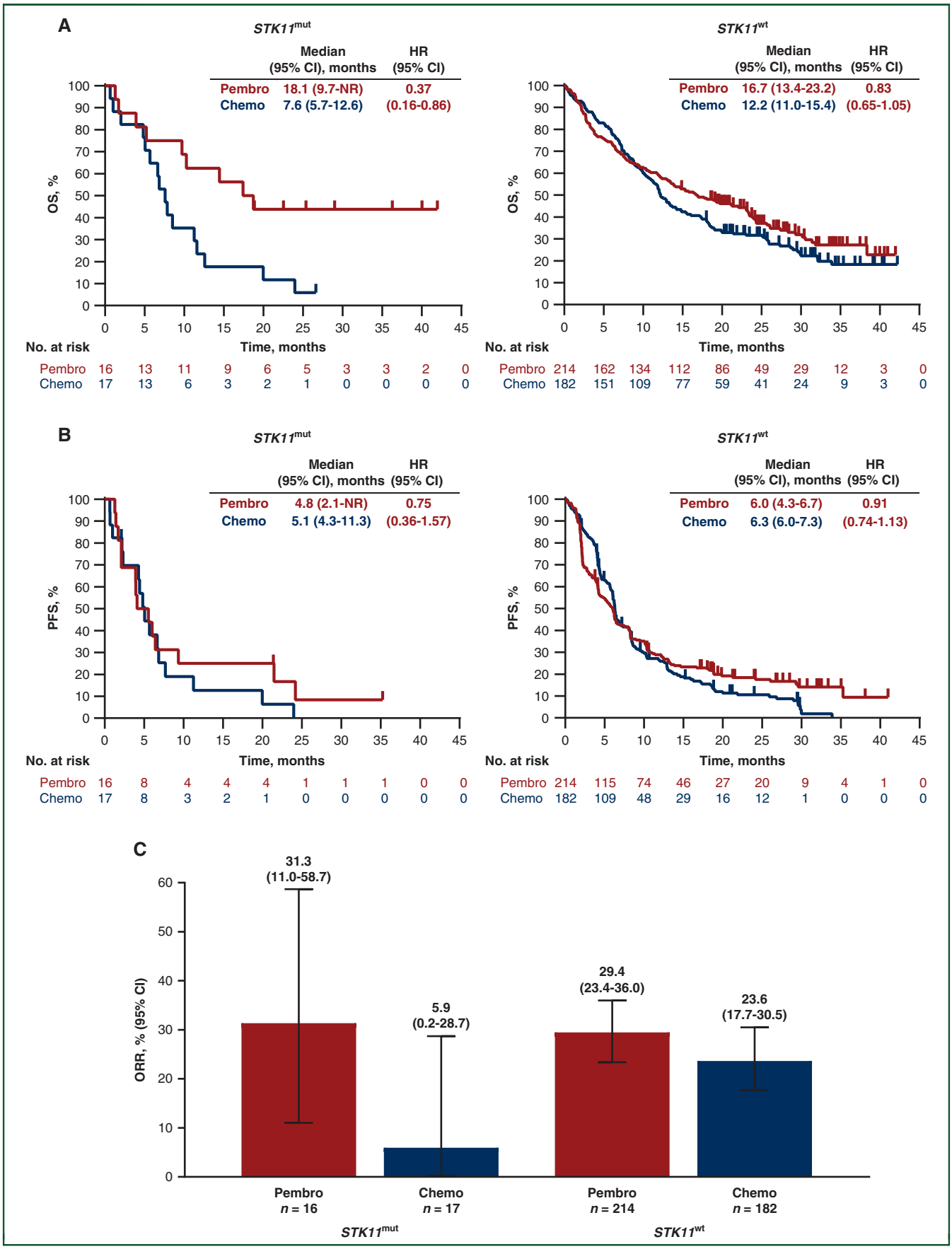

Figure 3. (A) OS, (B) PFS per RECIST version 1.1 by blinded independent central review, and (C) ORR per RECIST version 1.1 by blinded independent central review in patients with and without *STK11* mutation.

Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; NR, not reached; ORR, objective response rate; OS, overall survival; Pembro, pembrolizumab; PFS, progression-free survival; wt, wild-type.

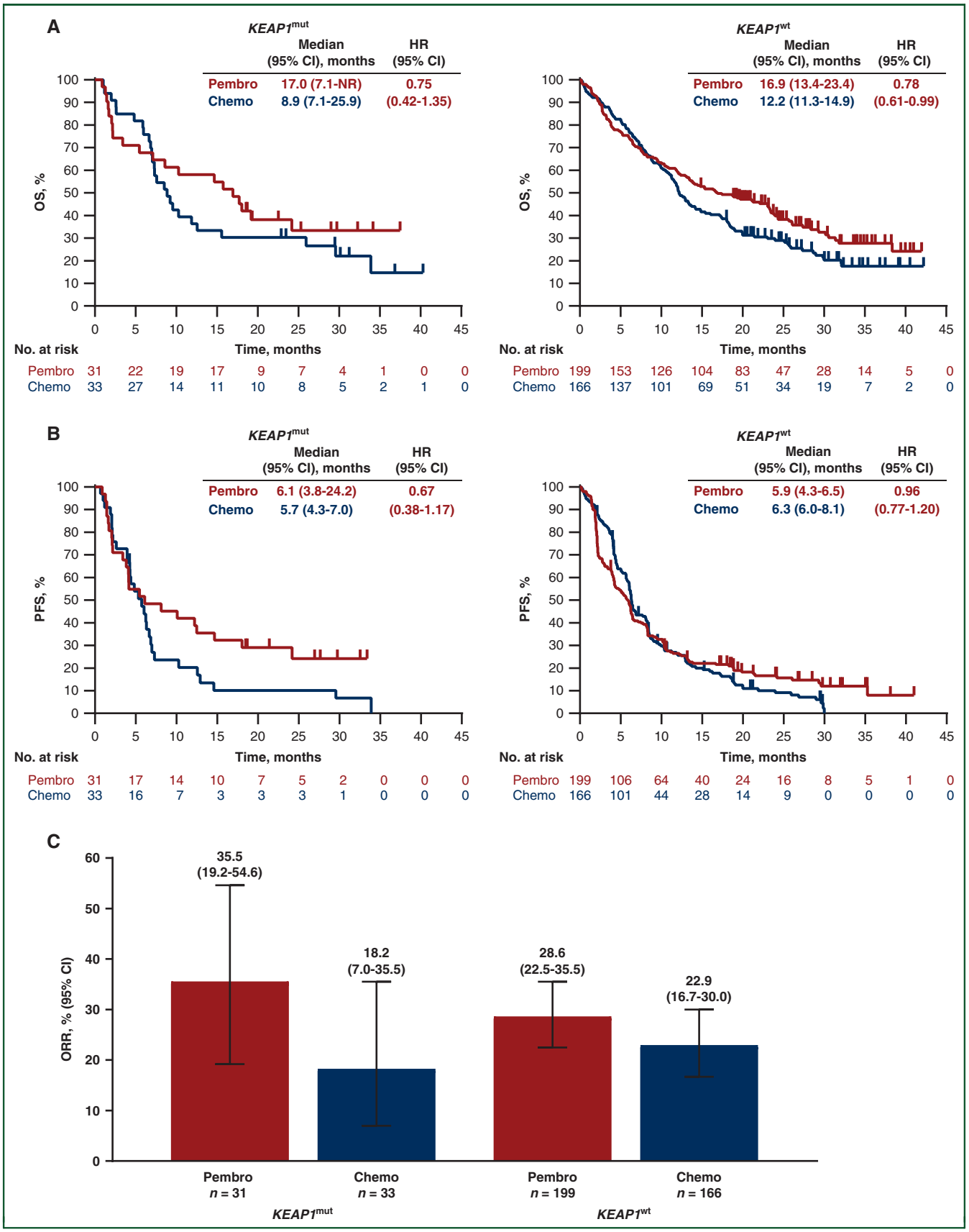

Figure 4. (A) OS, (B) PFS per RECIST version 1.1 by blinded independent central review, and (C) ORR per RECIST version 1.1 by blinded independent central review in patients with and without *KEAP1* mutation.

Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; NR, not reached; ORR, overall response rate; OS, overall survival; Pembro, pembrolizumab; PFS, progression-free survival; wt, wild-type.

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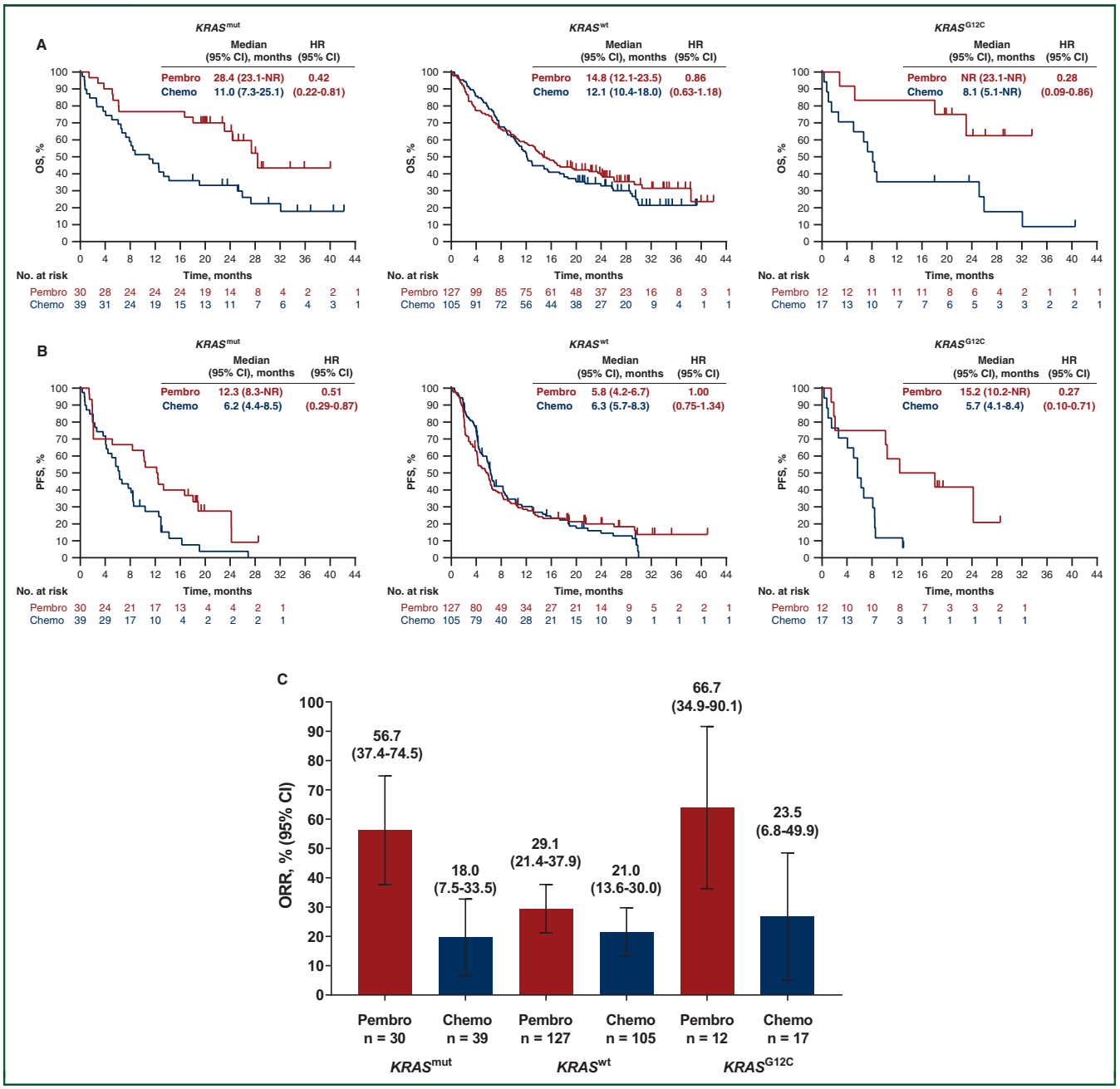

Figure 5. (A) OS, (B) PFS per RECIST version 1.1 by blinded independent central review, and (C) ORR per RECIST version 1.1. by blinded independent central review in patients with and without *KRAS* mutation.

Chemo, chemotherapy; CI, confidence interval; HR, hazard ratio; mut, mutation; NR, not reached; ORR, objective response rate; OS, overall survival; Pembro, pembrolizumab; PFS, progression-free survival; wt, wild-type.

enriched a patient population who had improved clinical benefit from pembrolizumab in advanced/metastatic NSCLC with PD-L1 TPS $\geq$1%. Furthermore, the clinical benefit from pembrolizumab over standard platinum-based chemotherapy observed in the overall population was maintained when evaluating outcomes by mutational status in *STK11*, *KEAP1*, and, in patients with nonsquamous NSCLC, *KRAS*.

In patients with NSCLC, the relationship between tTMB and clinical outcomes has been examined in studies evaluating pembrolizumab monotherapy,[41] atezolizumab monotherapy,[12,42] nivolumab monotherapy,[16] nivolumab plus ipilimumab,[11,17,18] and durvalumab plus tremelimumab[19] with varying results; of note, some studies used targeted sequencing (as opposed to WES). Additionally, in an evaluation of multiple studies of anti-PD-(L)1 therapies on multiple solid tumor types, a significant correlation was found between increasing tTMB and increasing ORR ($P < 0.001$) with the correlation coefficient of 0.74 suggesting that approximately half of the differences in ORR between tumor types may be related to tTMB.[13] Although these studies used a range of analytic techniques to assess tTMB and evaluated various cut points for tTMB, taken together these results support tTMB as a potential predictive biomarker for anti-PD-(L)1 therapy in patients with

advanced/metastatic NSCLC. WES is considered the gold standard method for sequencing cancer genetics, however, it can be limited in routine clinical practice due to high costs, long turnaround time, and the requirement for a large tumor sample.[5,35,43] Although tTMB by targeted sequencing has become more prevalent in assessing tTMB, prior studies have demonstrated panel size can greatly affect TMB assessment, and recommend a minimum panel size of 1 Mb for TMB estimation.[44] tTMB remains to be validated in a prospective randomized controlled trial in this setting, however, and PD-L1 remains the key biomarker used to select patients in the clinical setting for pembrolizumab monotherapy.

Recent findings from the prospective phase III BFAST trial concluded that blood-based TMB (bTMB) at a cut-off of $\geq$16 mut/exome was not a predictive biomarker for clinical outcomes with atezolizumab in patients with previously untreated metastatic NSCLC [HR for PFS, 0.77 (95% CI 0.59-1.00); HR for OS, 0.87 (95% CI 0.64-1.17)].[45] A central difference between the current analysis and the BFAST trial is that in our study we evaluated tumor TMB whereas in the BFAST study blood TMB was evaluated. Some evidence has supported a correlation between tTMB and bTMB.[46,47] Further investigation will be required to evaluate whether tTMB and bTMB provide different predictive information for outcomes with immunotherapy.

Overall, pembrolizumab was generally associated with improved OS compared with chemotherapy regardless of *STK11*, *KEAP1*, or *KRAS* mutation status in patients with PD-L1 TPS $\geq$1% advanced NSCLC. Whereas the treatment effect for pembrolizumab versus chemotherapy was greater among patients with *STK11*, *KEAP1*, and *KRAS* mutations, the treatment effects in patients with wild-type *STK11*, *KEAP1*, and *KRAS* were very similar to those in the overall population (HR for OS, 0.81). For groups with or without *STK11* or *KEAP1* mutation, pembrolizumab was associated with better clinical outcomes than chemotherapy regardless of *STK11* or *KEAP1* mutation status, and OS, PFS, and ORR with pembrolizumab were similar in patients with or without *STK11* or *KEAP1* mutation. The efficacy of chemotherapy, however, was lower in patients with *STK11* mutation than in those with wild-type *STK11*, consistent with prior reports.[48,49]

Among patients with nonsquamous histology and PD-L1 TPS $\geq$1%, pembrolizumab was generally associated with improved efficacy in patients with *KRAS* mutations compared with those with wild-type *KRAS*. As noted above, the HR for OS in patients with *KRAS* wild-type status was very similar to the overall population (*KRAS* wild-type, 0.86; overall population, 0.81). Moreover, *KRAS* mutations were associated with higher PD-L1 TPS, suggesting the improved outcomes in patients with *KRAS* mutations may be associated with higher PD-L1 TPS. In the pembrolizumab arm, patients with any *KRAS* mutation and patients with *KRAS* G12C mutation had improved clinical outcomes (improved ORR and longer PFS and OS) compared with those with *KRAS* wild-type NSCLC. These findings suggest that pembrolizumab monotherapy is an effective standard treatment option as a first-line therapy for PD-L1 TPS $\geq$1% advanced nonsquamous NSCLC with *KRAS* mutation. Pembrolizumab monotherapy or pembrolizumab-containing combinations should be considered a standard comparator for first-line comparative studies on *KRAS*-targeted therapy for patients with advanced/metastatic NSCLC.

Prior clinical evidence has suggested that tumors with *STK11* and *KEAP1* mutations may be less responsive to immunotherapy.[50] Retrospective observational studies have suggested potential associations between *STK11*[51,52] and *KEAP1* mutation[53] and reduced clinical benefit from immunotherapy.[20,21] In this analysis of a randomized, controlled trial, pembrolizumab was associated with better outcomes than chemotherapy regardless of *STK11* or *KEAP1* mutation status in patients with PD-L1 TPS $\geq$1% NSCLC. Similarly, the randomized phase III MYSTIC study of durvalumab versus durvalumab-tremelimumab versus chemotherapy also found no association between *STK11* or *KEAP1* mutation status and response to immunotherapy.[54] Notably, MYSTIC did not meet the prespecified endpoint for OS; the lack of treatment effect may have limited the potential to identify an association of OS with tTMB.[19] Evidence supporting an association between *KRAS* status and response to immunotherapy from other studies has been equivocal.[31] Although our results demonstrated a trend towards better clinical outcomes with pembrolizumab in patients with *KRAS* mutation, the results were confounded by the associations of *KRAS* mutation status with higher PD-L1 expression and higher tTMB score.

The finding from the current analysis that higher tTMB was associated with improved outcomes with pembrolizumab monotherapy differs from the findings from exploratory analyses of two phase III studies of first-line pembrolizumab plus platinum-based chemotherapy in patients with nonsquamous (KEYNOTE-189) or squamous (KEYNOTE-407) metastatic NSCLC.[55] Analyses of associations between tTMB and outcomes in those trials used similar statistical approaches to this study but did not find an association between tTMB and outcomes in patients receiving pembrolizumab plus chemotherapy or placebo plus chemotherapy. The reasons for the difference in predictive value for tTMB between the pembrolizumab monotherapy and pembrolizumab plus chemotherapy studies are uncertain, but the results suggest that combining pembrolizumab with chemotherapy abrogates the putative predictive value of tTMB. In contrast, results of our analyses of KEYNOTE-042 are consistent with analyses of KEYNOTE-189 and KEYNOTE-407 with regard to the influence of *STK11*, *KEAP1*, or *KRAS* mutational status on outcomes.

A limitation of the current report was the exploratory nature of these biomarker analyses. Biomarker analyses were prespecified in the study protocol and, for each of the specific analyses presented here, a statistical analysis plan and the tTMB cut points were prespecified before merging the clinical and biomarker datasets. Furthermore, WES data were only available in a subset of patients within the ITT population in KEYNOTE-042. The baseline characteristics were well

balanced in biomarker-evaluable subgroups, however, and our findings for OS, PFS, and ORR were consistent with those reported for the overall ITT population.[1] Due to low prevalence of specific single-gene mutations of interest, the sample size is relatively small in these subgroups with resultant wide confidence intervals and should be interpreted with caution. Finally, this analysis evaluated tTMB only, and therefore we are unable to ascertain whether tTMB levels align with bTMB levels in this setting.

Overall, the results of these exploratory biomarker analyses of the KEYNOTE-042 study suggest that tTMB may have potential clinical utility as a biomarker for pembrolizumab monotherapy as a first-line treatment in patients with PD-L1 TPS $\geq$1% advanced/metastatic NSCLC. Pembrolizumab monotherapy is an effective first-line treatment option regardless of *STK11* or *KEAP1* status and for patients with nonsquamous histology regardless of *KRAS* status.

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DATA SHARING

Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA (MSD) is committed to providing qualified scientific researchers access to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers. The MSD data sharing website (available at: http://engagezone.msd.com/ds_documentation.php) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for request after product approval in the US and EU or after product development is discontinued. There are circumstances that may prevent MSD 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 MSD subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, MSD 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.

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