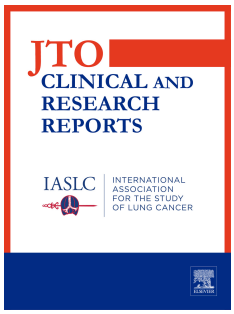

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Associations of Tissue Tumor Mutational Burden and Mutational Status With Clinical Outcomes With Pembrolizumab Plus Chemotherapy Versus Chemotherapy For Metastatic NSCLC

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Abbreviations: AUC, area under the concentration–time curve; AUROC, area under the receiver operating characteristic; CTLA-4, cytotoxic T lymphocyte antigen 4; GEP, gene expression profile; HR, hazard ratio; ICI, immune checkpoint inhibitors; NSCLC, non–small-cell lung cancer; OS, overall survival; ORR, objective response rate; PD-(L)1, programmed death (ligand) 1; PFS, progression-free survival; TPS, tumor proportion score; tTMB, tissue tumor mutational burden; WES, whole-exome sequencing.

ABSTRACT (250/250 limit)

Introduction: We evaluated tissue tumor mutational burden (tTMB) and mutations in *STK11, KEAP1,* and *KRAS* as biomarkers for outcomes with 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 (WES) in patients with available tumor and matched normal DNA. Clinical utility of tTMB was assessed using a prespecified cutpoint of 175 mutations/exome.

Results: Among patients with evaluable data from WES 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 (PFS) 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 ≥175 compared with tTMB <175 mutations/exome in KEYNOTE-189 (OS, HR: 0.64 [95% CI, 0.38–1.07] and 0.64 [95% CI, 0.42–0.97], respectively) and KEYNOTE-407 (OS, HR: 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 utility for tTMB, *STK11*, *KEAP1*, or *KRAS* mutation status as a biomarker for this regimen.

Keywords: tissue tumor mutational burden, single-gene genetic alterations, pembrolizumab, metastatic non–small-cell lung cancer, biomarker

INTRODUCTION

Pembrolizumab plus platinum-based chemotherapy (pembrolizumab-combination) is a standard-of-care first-line treatment for patients with metastatic NSCLC irrespective of tumor PD-L1 expression.[1,2] The role of pembrolizumab-combination in treatment is supported by two placebo-controlled randomized phase 3 studies that demonstrated improved 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 (ICI).[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, 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–cytotoxic T lymphocyte antigen 4 (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/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 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/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 (AUC, 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 (AUC, 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 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 mut/Mb used by the updated pipeline FoundationOne F1Dx_v3.2 assay (Foundation Medicine, Cambridge, MA).[26-28] Full methodology for WES analysis is included in **Supplemental Methods**.

Endpoints

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 who had evaluable samples for WES and received ≥1 dose 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 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 **Supplemental Methods**.

RESULTS

Patients

In KEYNOTE-189, 293/616 (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 (**Supplemental Figure-1A**). In KEYNOTE-407, 312/559 (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 squamous patients due to low prevalence of these mutations in squamous NSCLC). The data cutoff date for all analyses from KEYNOTE-407 was May 9, 2019 (**Supplemental Figure-1B**). Baseline demographics and 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 (ie, OS, PFS, and ORR) in the tTMB-evaluable groups for pembrolizumab-combination versus placebo-combination were similar to that in the intent-to-treat [ITT] population (**Supplemental Table-1**). tTMB and PD-L1 TPS were not strongly associated with one another in either treatment arm in either study (**Supplemental Figure 2A, B**). For assessment of association of tTMB with efficacy, based on the area under the receiver operating characteristic (AUROC) curve for ORR, higher tTMB assessed as a continuous variable was not associated with ORR, in either treatment arm **(Figure 1A, 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 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; **Figure 1B, D**).

Clinical Outcomes in Patients With tTMB ≥175 Mutations/Exome and tTMB <175 Mutations/Exome

In KEYNOTE-189, 134 patients had tTMB ≥175 mut/exome (pembrolizumab-combination, *n*=100; placebo-combination, *n*=34]) and 159 with tTMB <175 mut/exome (pembrolizumab-combination, *n*=107; placebo-combination, *n*=52). HRs (95% CI) for OS favored the pembrolizumab-combination group in patients with tTMB ≥175 mut/exome (0.64, 0.38–1.07) and in patients with tTMB <175 mut/exome (0.64, 0.42–0.97; **Figure 2A**). HRs (95% CI) for PFS favored the pembrolizumab-combination group in patients with tTMB ≥175 mut/exome (0.32, 0.21–0.51) and in patients with tTMB <175 mut/exome (0.51, 0.35–0.74; **Figure 2B**). In the tTMB ≥175 mut/exome group, 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 <175 mut/exome, ORR (95% CI) was 40.2% (30.8%–50.1%) versus 19.2% (9.6%–32.5%), respectively (**Supplemental Figure 3A**).

In KEYNOTE-407, 162 patients had tTMB ≥175 mut/exome (pembrolizumab-combination, *n*=73; placebo-combination, *n*=89) and 150 patients had tTMB <175 mut/exome (pembrolizumab-combination, *n*=70; placebo-combination, *n*=80). HRs (95% CI) for OS favored pembrolizumab-combination in the tTMB ≥175 mut/exome group (0.74, 0.50–1.08) and <175 mut/exome group (0.86, 0.57–1.28; **Figure 2C**). PFS was improved with pembrolizumab-combination among patients with tTMB ≥175 mut/exome (HR, 0.57; 95% CI, 0.41–0.81) and <175 mut/exome (HR, 0.68; 95% CI, 0.48–0.96; **Figure 2D**). In the tTMB ≥175 mut/exome

group, ORR (95% CI) was 58.9% (46.8%–70.3%) with pembrolizumab-combination versus 44.9% (34.4%–55.9%) with placebo-combination. For patients with tTMB <175 mut/exome, ORR (95% CI) was 64.3% (51.9%–75.4%) versus 38.8% (28.1%–50.3%), respectively (**Supplemental Figure 3B**).

Clinical Outcomes in Patients With Versus Without Single-Gene Mutations

STK11

Of 289 evaluable patients in KEYNOTE-189, 54 (18.7%) had *STK11* mutations. In KEYNOTE-407, 8/285 (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, median (interquartile range [IQR]) PD-L1 TPS tended to be numerically lower (0% [0–16] vs 15% [0–75]) and median (IQR) TMB scores (209 [132–265] vs 146 [89–264] mut/exome) tended to be numerically higher among patients with versus without an *STK11* mutation; **Supplemental Figure 4A**). Prevalence of *STK11* mutations by PD-L1 (TPS) and tTMB score (mut/exome) in the *STK11*-evaluable population is shown in **Supplemental Figure 4B**.

In KEYNOTE-189, HRs (95% CI) for OS among patients with an *STK11* mutation was 0.75 (0.37–1.50), and 0.59 (0.41–0.85) with wild-type *STK11* (**Figure 3A**). HRs (95% CI) for PFS was 0.81 (0.44–1.47) in patients with an *STK11* mutation and 0.38 (0.27–0.52) with wild-type *STK11* (**Figure 3B**). 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 **(Supplemental Figure 5**).

KEAP1

Of 289 patients in KEYNOTE-189 with evaluable WES data from matched tumor and normal DNA, 68 (23.5%) had *KEAP1* mutation. In KEYNOTE-407, 285 patients had evaluable WES data from matched tumor and normal DNA, 36 (12.6%) had *KEAP1* mutations.

Among patients in KEYNOTE-189 with *KEAP1* mutations, median ([IQR]) PD-L1 TPS was numerically lower (1% [0–13] vs 20% [0–75]) and median (IQR) tTMB score was numerically higher versus wild-type *KEAP1* (173 [124–267] vs 147 [89–263] mut/exome; **Supplemental Figure 6A**). Prevalence of *KEAP1* mutations by PD-L1 (TPS) and tTMB score (mut/exome) in the *KEAP1*-evaluable population is shown in **Supplemental Figure 6B**. Among patients in KEYNOTE-407 with *KEAP1* mutations, 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* (**Supplemental Figure 6C**). No association between PD-L1 (TPS) and tTMB score (mut/exome) in the *KEAP1*-evaluable population was observed (**Supplemental Figure 6D)**.

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]; **Figure 4A**). HRs (95% CI) for PFS was 0.65 (0.38–1.12) in patients with *KEAP1* mutation and 0.38 (0.28–0.53) with *KEAP1* wild-type (**Figure 4C**). 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* (**Supplemental Figure 7A**).

In KEYNOTE-407, HRs (95% CI) for OS were 1.08 (0.48–2.41) in patients with *KEAP1* mutation, and 0.75 (0.55–1.02) for wild-type *KEAP1* (**Figure 4B**). HRs (95% CI) for PFS was 0.40 (0.19–0.86) in patients with *KEAP1* mutations and 0.63 (0.48–0.83) for wild-type *KEAP1* **(Figure 4D)**. ORRs (95% CI) for pembrolizumab-combination versus placebo-combination was 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, for wild-type *KEAP1* (**Supplemental Figure 7B)**.

KRAS

Of 289 evaluable patients in KEYNOTE-189, 89 (32.2%) had *KRAS* mutations, of which 37 (12.8%) were *KRAS* G12C mutations. In KEYNOTE-407, 14/285 (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, 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* mutation (**Supplemental Figure 8A)**. Joint association between PD-L1 (TPS) and tTMB score (mut/exome) for *KRAS* mutant and *KRAS* wild-type patients is shown in **Supplemental Figure 8B**.

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 (**Figure 5A**). For PFS, 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 (**Figure 5B**). 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* **(Supplemental Figure 9)**.

For the subgroup of patients with *KRAS* G12C mutation (pembrolizumab-combination, *n*=26; placebo-combination, *n*=11), 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 OS and PFS, respectively (**Figure 5A, B**). Corresponding ORRs were 50.0% (29.9%–70.1%) and 18.2% (2.3%–51.8%), respectively **(Supplemental Figure 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 showed 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 demonstrated improved clinical benefit versus placebo-combination irrespective of mutations in *STK11*, *KEAP1*, and *KRAS*. These findings do not support clinical utility of tTMB as a biomarker for pembrolizumab plus platinum-based chemotherapy for metastatic squamous or nonsquamous NSCLC.

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 therapies 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 ≥1% advanced NSCLC that employed a similar analytical approach, higher tTMB levels were associated with improved outcomes with pembrolizumab but not with chemotherapy. Moreover, patients with tTMB ≥175 mut/exome had improved OS and PFS compared with chemotherapy, whereas those with tTMB <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 therapies given as single agents across various solid tumor types, including nonsquamous and squamous NSCLC, demonstrated 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 use 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 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 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; nonetheless, the magnitude of benefit in some groups remains uncertain. In KEYNOTE-407, 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, HR (95% CI) for OS was 1.14 (0.45–2.92), although 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 to studies that have suggested *STK11* and *KEAP1* mutations confer resistance to anti–PD-(L)1 therapies, patients with these mutations were shown 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 to immunotherapy plus chemotherapy versus chemotherapy in advanced NSCLC.[8] While WES is considered the gold-standard measurement of TMB, this technique is time-consuming, costly, and laborious.[5] Additionally, while 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 TMB cutpoint of 10 mut/Mb 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 as well as *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 nonsquamous 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*, and *KRAS* mutation status.

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DATA SHARING STATEMENT

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

	KEYNOTE-189			KEYNOTE-407		
Characteristic	**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/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)	12 (2.1)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; PD-L1 TPS, programmed death ligand 1 tumor proportion score; tTMB, tissue tumor mutational burden.

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.

FIGURE LEGENDS

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, graph shows 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 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; 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.

Figure 2. Clinical utility of tTMB for OS and PFS in each study at cutpoints of ≥175 mut/exome and <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.

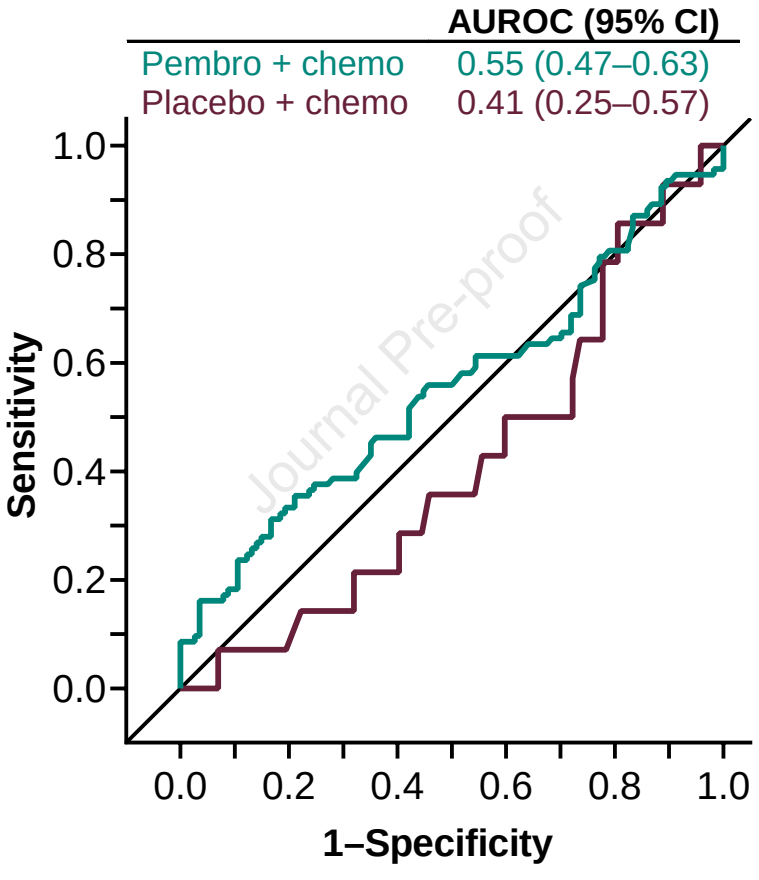
Figure 3. Kaplan-Meier estimates of OS and PFS by *STK11* status in the single-gene mutation-evaluable population in KEYNOTE-189. (A**)** OS and (B**)** PFS. Chemo,

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

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.

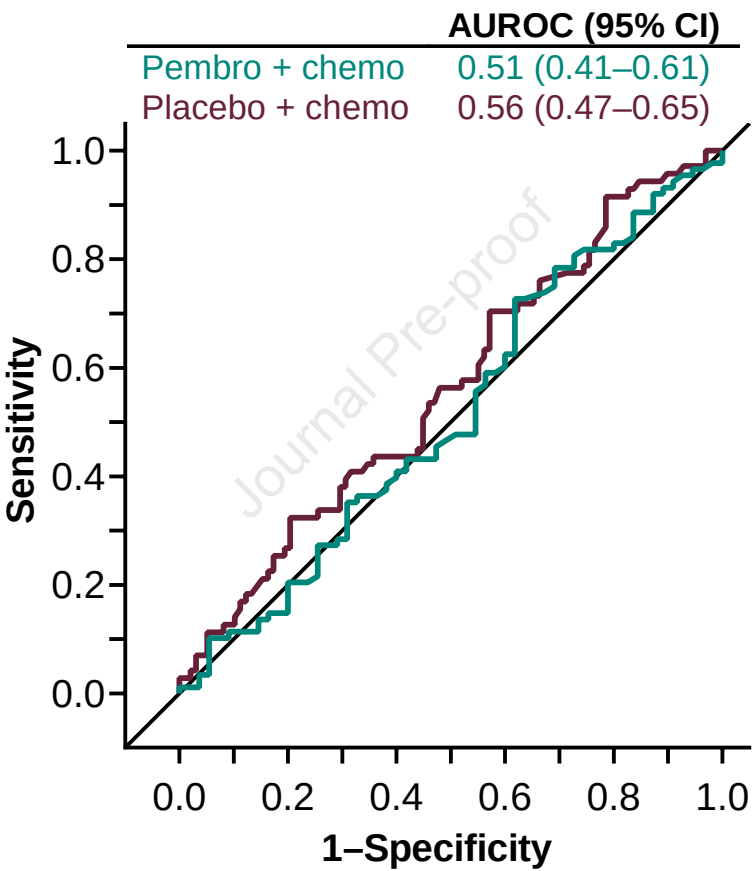
A.



B. **KEYNOTE-189**

Nominal *P* value[a]	Pembro + Chemo (n = 207)	Placebo + Chemo (n = 86)
OS	0.174	0.856
PFS	0.075	0.055
ORR	0.072	0.434

C.



D.

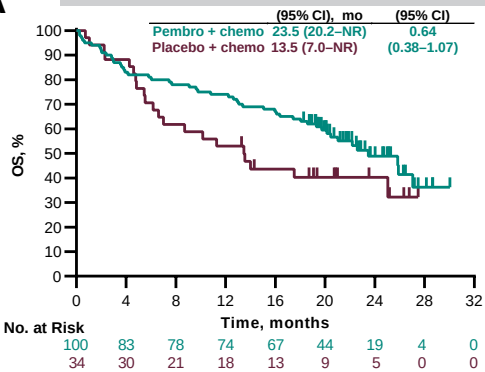
KEYNOTE-407

Nominal *P* value[a]	Pembro + Chemo (n = 143)	Placebo + Chemo (n = 169)
OS	0.160	0.818
PFS	0.052	0.560
ORR	0.393	0.086

tTMB ≥175 mut/exome

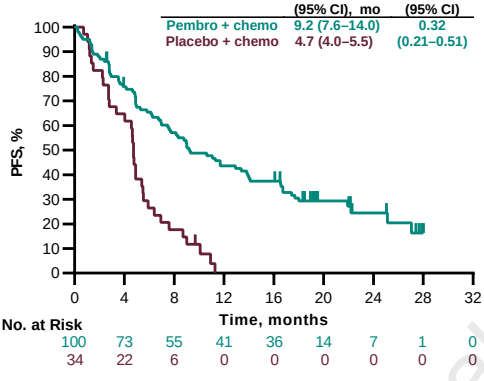
KEYNOTE-189

A



No. at Risk								
100	83	78	74	67	44	19	4	0
34	30	21	18	13	9	5	0	0

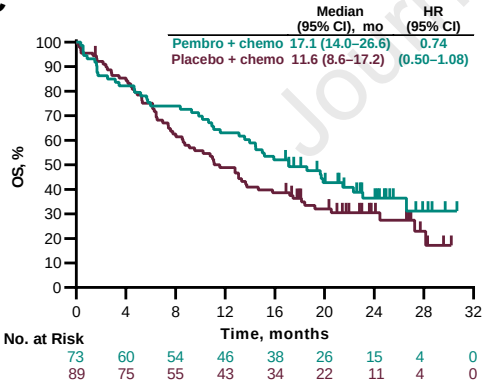
B



No. at Risk								
100	73	55	41	36	14	7	1	0
34	22	6	0	0	0	0	0	0

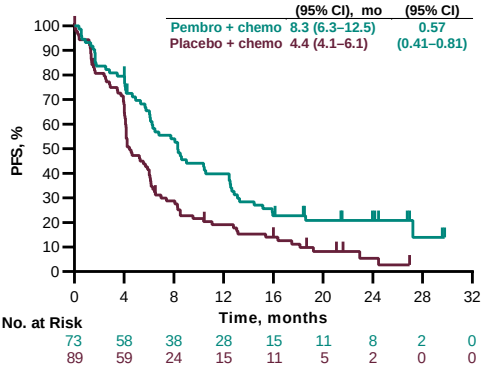
KEYNOTE-407

C



No. at Risk								
73	60	54	46	38	26	15	4	0
89	75	55	43	34	22	11	4	0

D

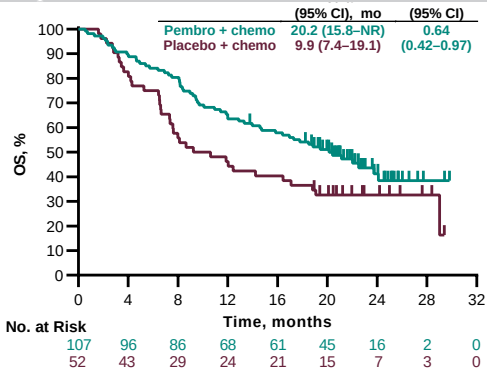


No. at Risk								
73	58	38	28	15	11	8	2	0
89	59	24	15	11	5	2	0	0

tTMB <175 mut/exome

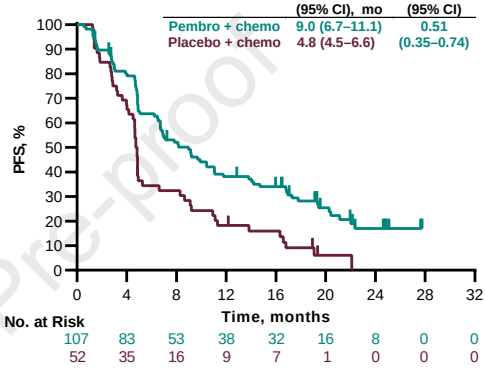
KEYNOTE-189

A



No. at Risk								
107	96	86	68	61	45	16	2	0
52	43	29	24	21	15	7	3	0

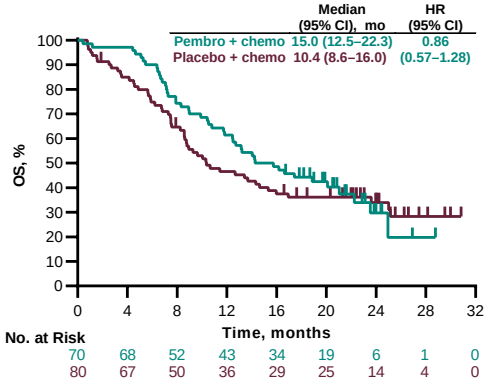
B



No. at Risk								
107	83	53	38	32	16	8	0	0
52	35	16	9	7	1	0	0	0

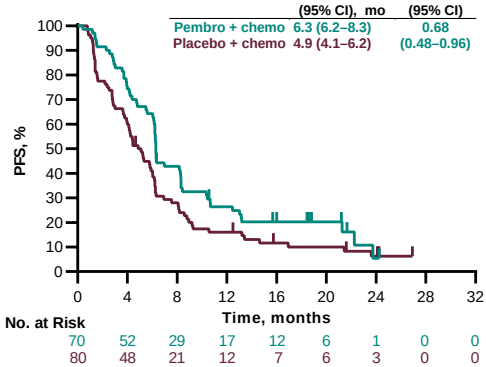
KEYNOTE-407

C



No. at Risk								
70	68	52	43	34	19	6	1	0
80	67	50	36	29	25	14	4	0

D



No. at Risk								
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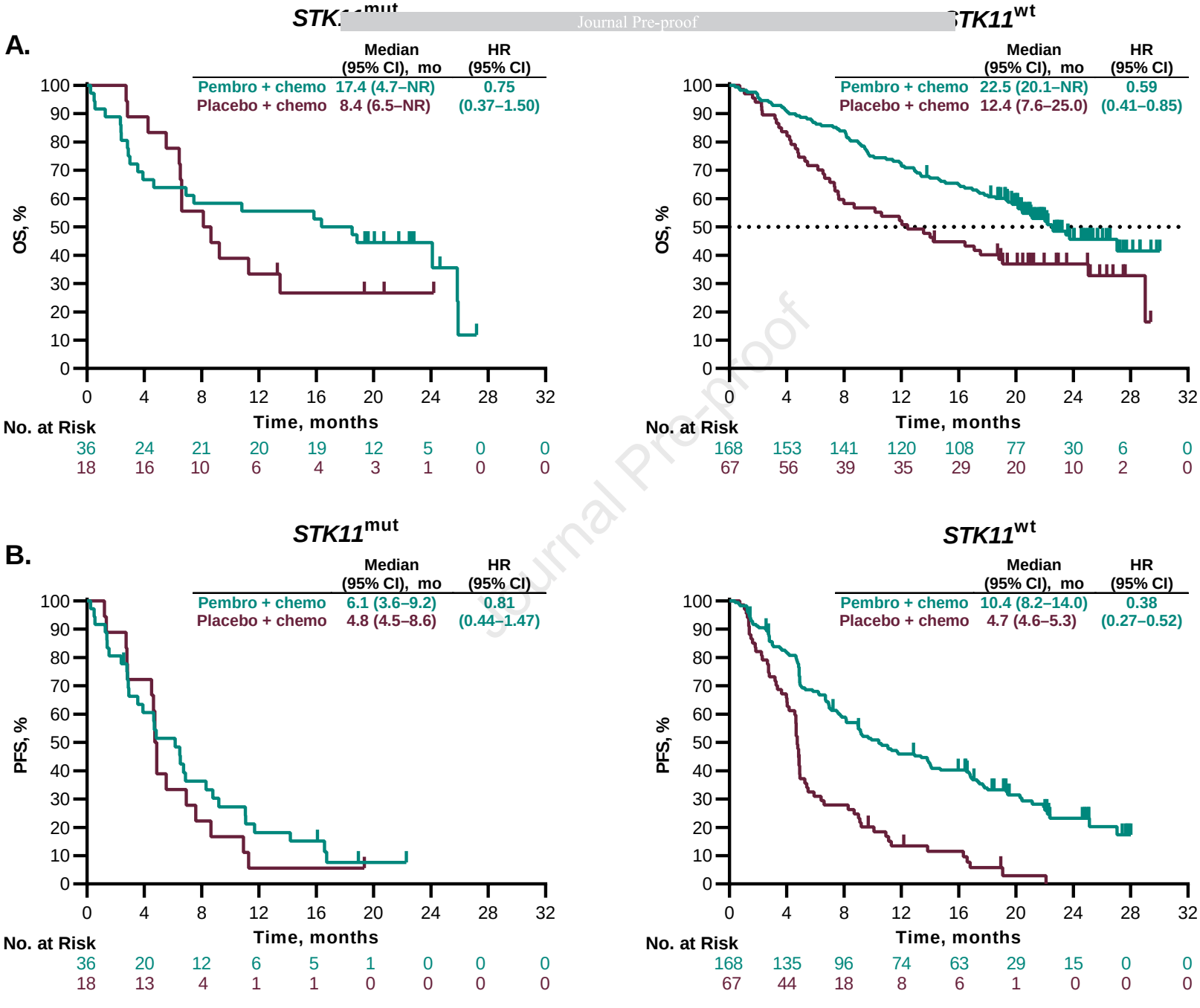
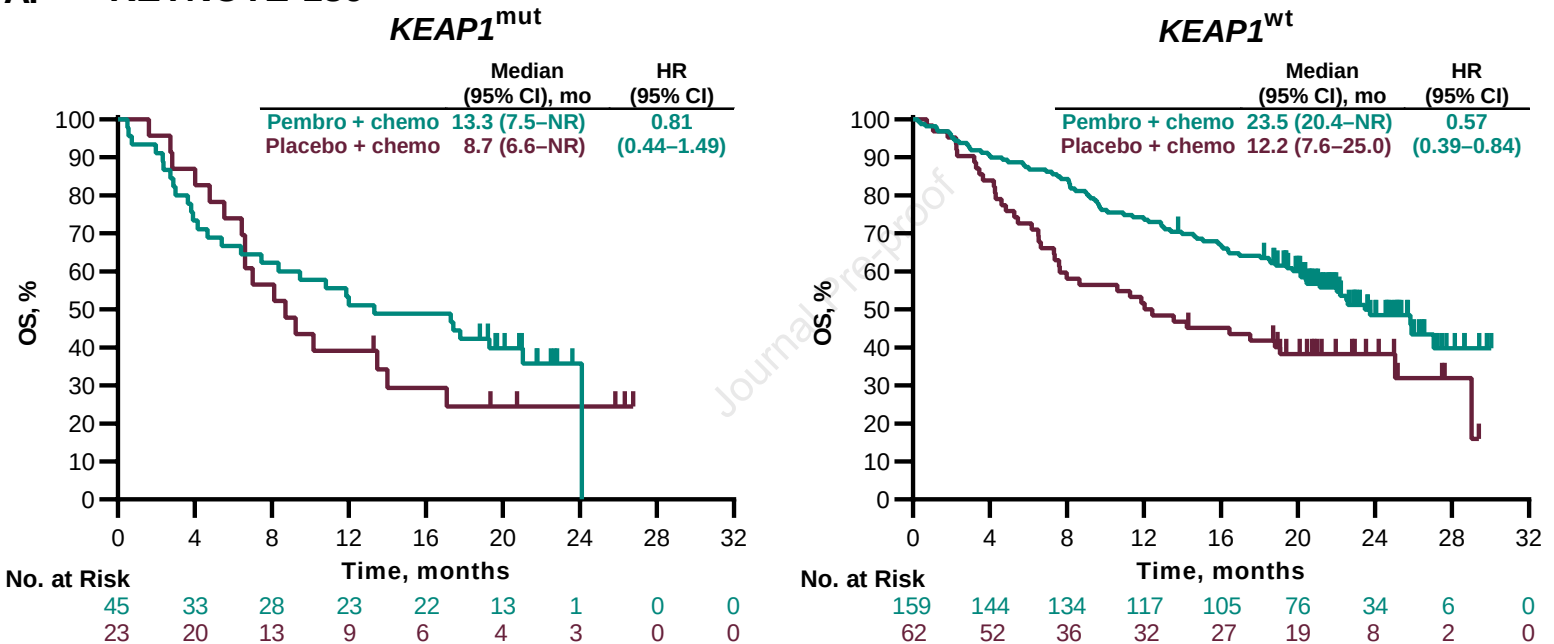
80	48	21	12	7	6	3	0	0



A. KEYNOTE-189



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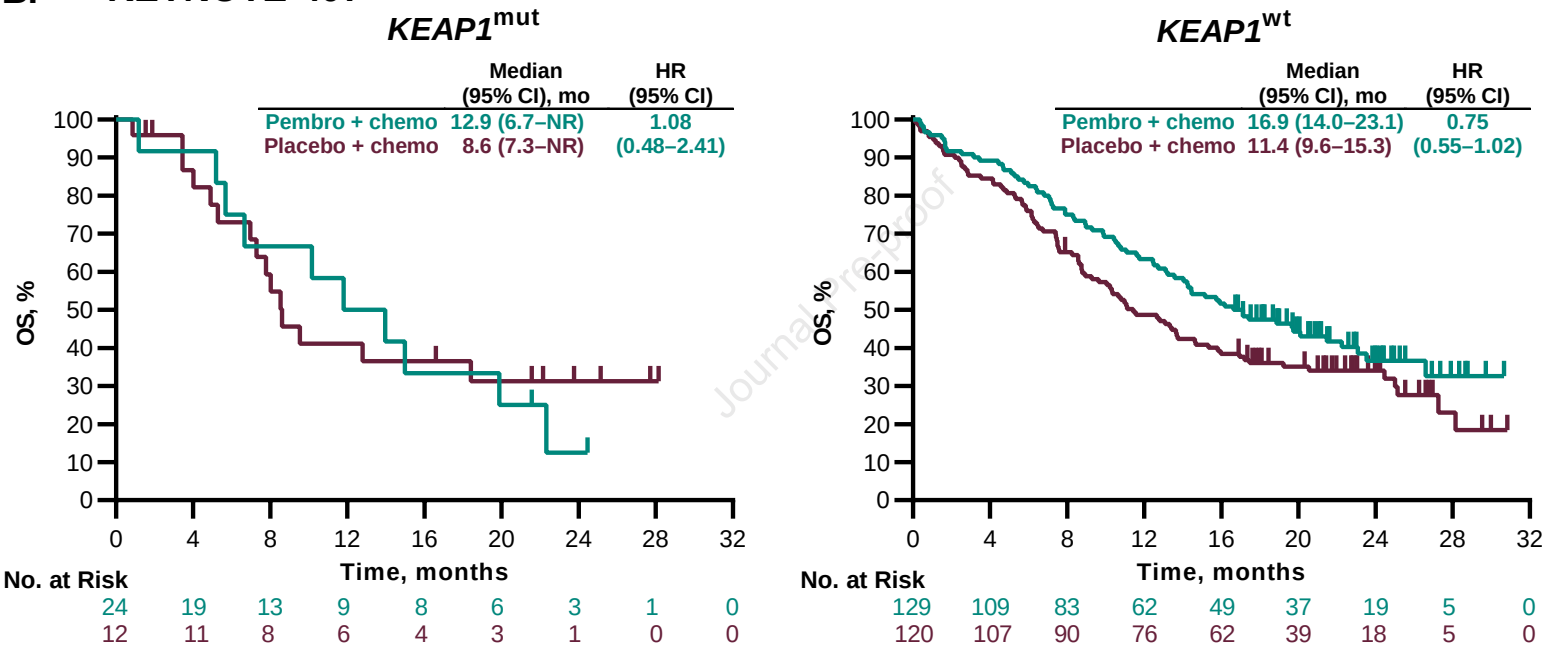
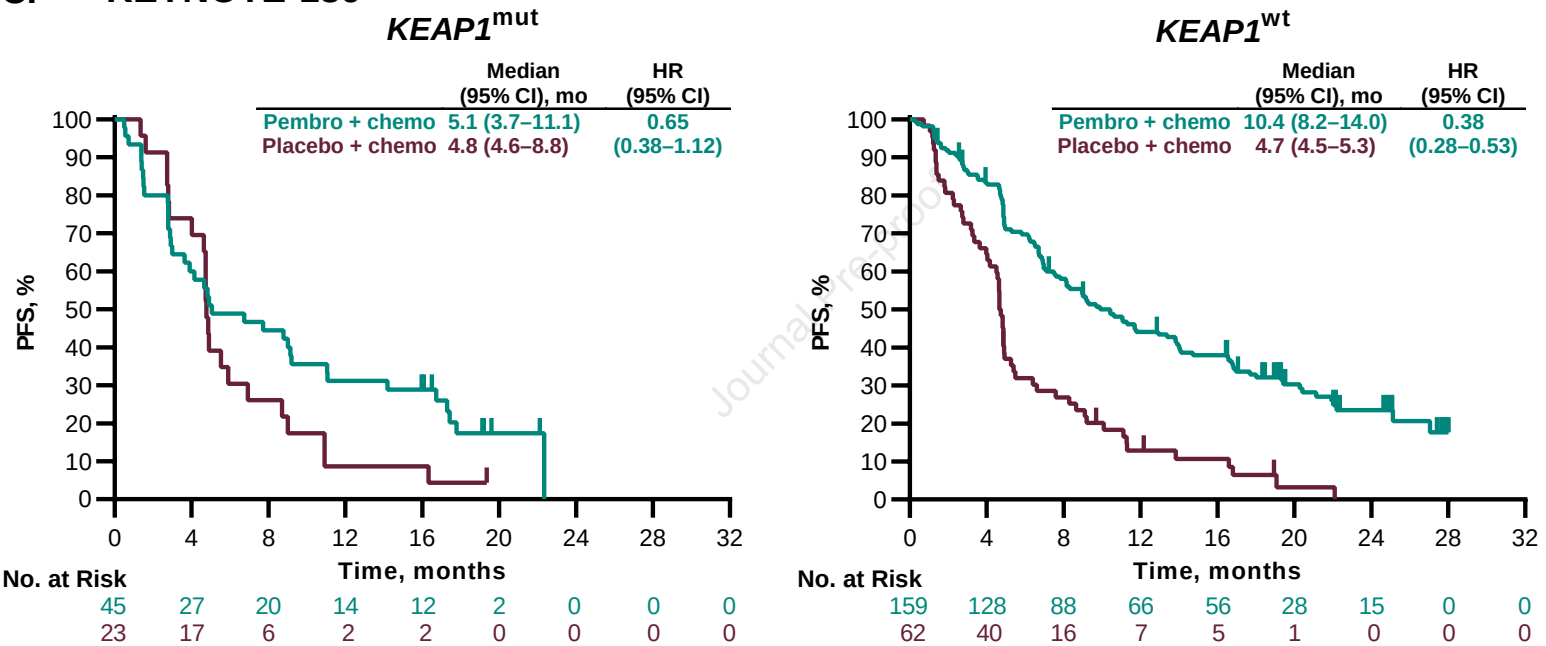


C. KEYNOTE-189



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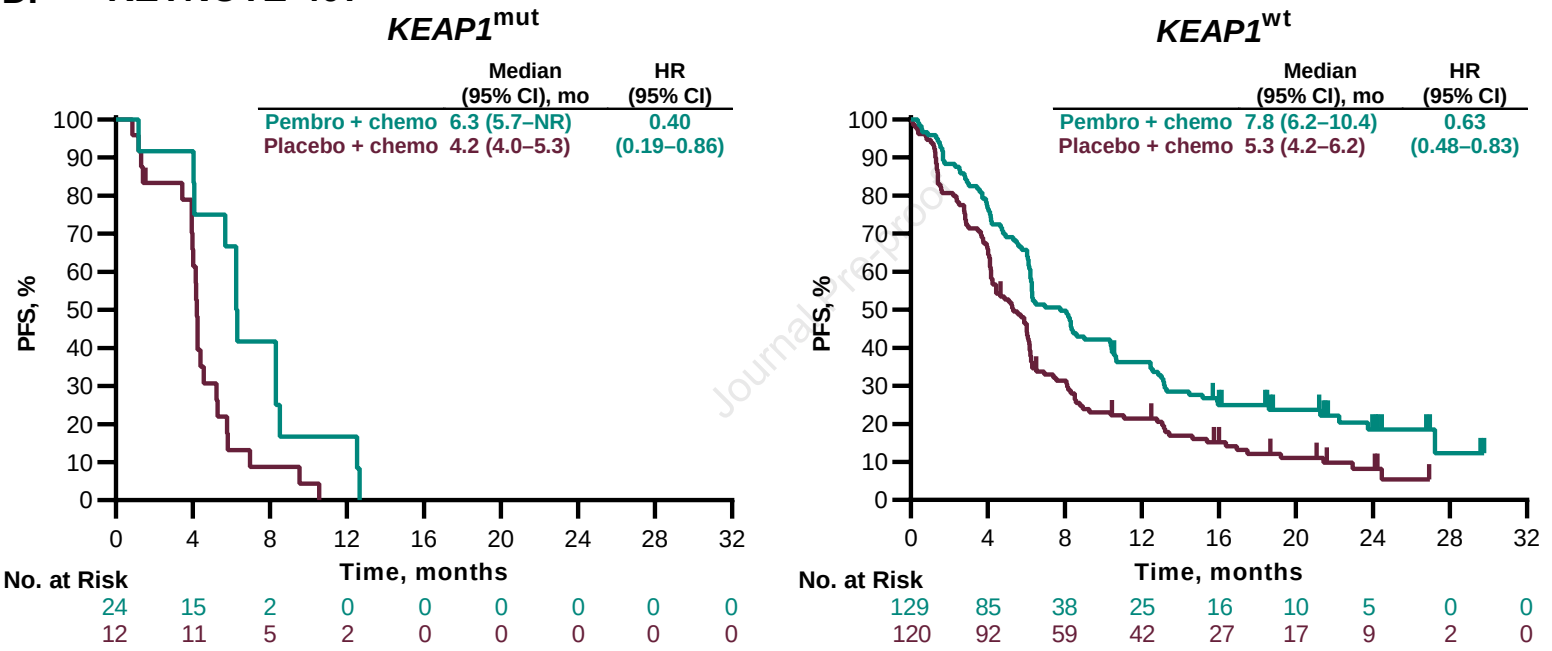
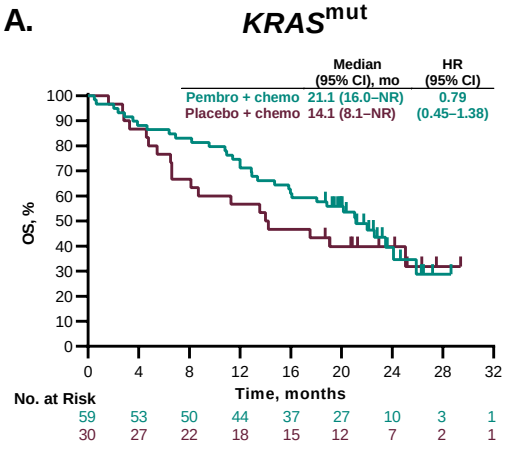
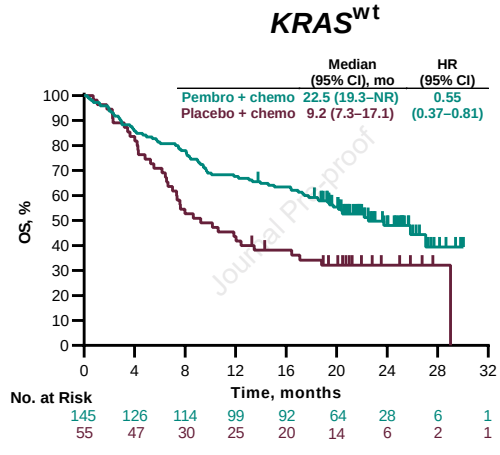
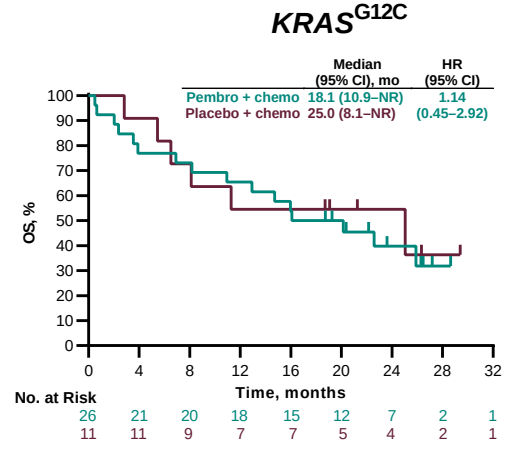


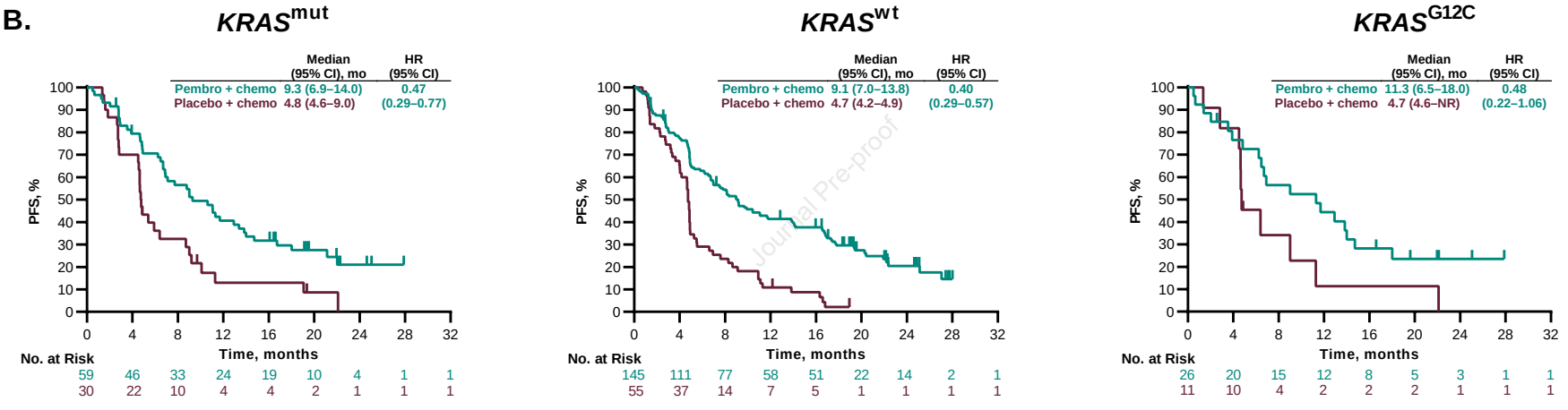
A.







B.



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