

ORIGINAL ARTICLE

Use of archival versus newly collected tumor samples for assessing PD-L1 expression and overall survival: an updated analysis of KEYNOTE-010 trial

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Background: In KEYNOTE-010, pembrolizumab versus docetaxel improved overall survival (OS) in patients with programmed death-1 protein (PD)-L1-positive advanced non-small-cell lung cancer (NSCLC). A prespecified exploratory analysis compared outcomes in patients based on PD-L1 expression in archival versus newly collected tumor samples using recently updated survival data.

Patients and methods: PD-L1 was assessed centrally by immunohistochemistry (22C3 antibody) in archival or newly collected tumor samples. Patients received pembrolizumab 2 or 10 mg/kg Q3W or docetaxel 75 mg/m² Q3W for 24 months or until progression/intolerable toxicity/other reason. Response was assessed by RECIST v1.1 every 9 weeks, survival every 2 months. Primary end points were OS and progression-free survival (PFS) in tumor proportion score (TPS) $\geq 50\%$ and $\geq 1\%$; pembrolizumab doses were pooled in this analysis.

Results: At date cut-off of 24 March 2017, median follow-up was 31 months (range 23–41) representing 18 additional months of follow-up from the primary analysis. Pembrolizumab versus docetaxel continued to improve OS in patients with previously treated, PD-L1-expressing advanced NSCLC; hazard ratio (HR) was 0.66 [95% confidence interval (CI): 0.57, 0.77]. Of 1033 patients analyzed, 455 (44%) were enrolled based on archival samples and 578 (56%) on newly collected tumor samples. Approximately 40% of archival samples and 45% of newly collected tumor samples were PD-L1 TPS $\geq 50\%$. For TPS $\geq 50\%$, the OS HRs were 0.64 (95% CI: 0.45, 0.91) and 0.40 (95% CI: 0.28, 0.56) for archival and newly collected samples, respectively. In patients with TPS $\geq 1\%$, OS HRs were 0.74 (95% CI: 0.59, 0.93) and 0.59 (95% CI: 0.48, 0.73) for archival and newly collected samples, respectively. In TPS $\geq 50\%$, PFS HRs were similar across archival [0.63 (95% CI: 0.45, 0.89)] and newly collected samples [0.53 (95% CI: 0.38, 0.72)]. In patients with TPS $\geq 1\%$, PFS HRs were similar across archival [0.82 (95% CI: 0.66, 1.02)] and newly collected samples [0.83 (95% CI: 0.68, 1.02)].

Conclusion: Pembrolizumab continued to improve OS over docetaxel in intention to treat population and in subsets of patients with newly collected and archival samples.

Trial registration: ClinicalTrials.gov: NCT01905657.

Key words: pembrolizumab, tumor samples, PD-L1 expression

Introduction

Pembrolizumab is a humanized monoclonal antibody against programmed death-1 protein (PD-1) that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab monotherapy is currently approved in several countries, including the United States, Europe and Japan, for the first-line treatment of patients with metastatic non-small-cell lung cancer (NSCLC) whose tumors express high levels of PD-L1 [i.e. tumor proportion score (TPS) $\geq 50\%$]; and for the treatment of patients with metastatic NSCLC with TPS $\geq 1\%$ and disease progression on/after platinum-containing chemotherapy. A companion diagnostic test [PD-L1 IHC 22C3 pharmDx assay; Agilent Technologies (formerly Dako), Carpinteria, CA] is approved to aid in selection of patients with NSCLC for pembrolizumab treatment based on PD-L1 expression in formalin-fixed, paraffin-embedded tumor samples [1]. This assay utilizes qualitative immunohistochemistry (IHC) and the murine 22C3 anti-human PD-L1 monoclonal antibody to detect and score levels of PD-L1 expression on tumor cells [2]. PD-L1 expression is measured as the TPS, defined as the percentage of tumor cells with membranous PD-L1 staining of any intensity.

In the randomized, phase II/III KEYNOTE-010 study, pembrolizumab 2 and 10 mg/kg given every 3 weeks (Q3W) demonstrated superior overall survival (OS) over standard-of-care docetaxel treatment in patients with previously treated, PD-L1-positive NSCLC [3]. This study enrolled 1034 patients with TPS $\geq 1\%$, with the final 593 enrolled patients stratified by TPS 1%–49% and $\geq 50\%$. The primary results showed median OS in the total population (i.e. TPS $\geq 1\%$) was higher for both pembrolizumab doses compared with docetaxel [10 mg/kg: 12.7 versus 8.5 months; hazard ratio (HR) 0.61, $P < 0.0001$; 2 mg/kg: 10.4 versus 8.5 months; HR 0.71, $P = 0.0008$]. Likewise, for patients with at least 50% tumor cells expressing PD-L1, OS was significantly longer with pembrolizumab 2 mg/kg (14.9 versus 8.2 months; HR 0.54, $P = 0.0002$) and 10 mg/kg (17.3 versus 8.2 months; HR 0.50, $P < 0.0001$) than with docetaxel. With respect to progression-free survival (PFS) in the total population (i.e. TPS $\geq 1\%$), there was no significant difference for either dose of pembrolizumab compared with docetaxel (10 mg/kg: 4.0 versus 4.0 months; HR 0.79, $P = 0.004$; 2 mg/kg: 3.9 versus 4.0 months; HR 0.88, $P = 0.07$). Furthermore, PFS was improved in the TPS $\geq 50\%$ subset of studied patients receiving pembrolizumab 2 mg/kg (5.0 versus 4.1 months; HR 0.59, $P < 0.0001$) and 10 mg/kg (5.2 versus 4.1 months; HR 0.59, $P < 0.0001$) compared with docetaxel. Initially, any tumor sample (i.e. archival or newly collected) was permitted for PD-L1 testing in KEYNOTE-010. Based on epidemiology data suggesting some discordance between PD-L1 expression levels in pretreatment surgical tumor specimens and specimens collected at time of relapse (surgical tumor specimens or core needle biopsy samples), the study

protocol was later amended to require PD-L1 assessment in newly collected tumor samples except when it risked patient safety [4]. Unlike other well-known oncological biomarkers, PD-L1 tumor expression is dynamic and may be affected by previous treatment or disease stage [5–9]. Given that previously collected archival tissue is often the most convenient and easily accessible tissue source for biomarker testing in the second-line setting, the determination of whether archival samples can be substituted for those collected contemporaneously is an important question as it relates to identifying patients with a greater likelihood of response to pembrolizumab. Thus, the current analysis was undertaken to compare the treatment response to pembrolizumab by PD-L1 expression in archival and newly collected tumor samples in the KEYNOTE-010 study. Specifically, the primary objectives of this pre-specified, exploratory analysis were to (i) compare the prevalence of PD-L1 TPS $\geq 50\%$ and $\geq 1\%$ in archival versus newly collected tumor samples and (ii) evaluate the relative clinical benefit of pembrolizumab over docetaxel for OS, PFS and overall response rate (ORR) based on archival and newly collected tumor samples using updated survival data from KEYNOTE-010. The ability to use archival samples instead of newly collected samples for the determination of PD-L1 expression would expedite treatment decisions and improve patient care.

Methods

Study design

KEYNOTE-010 (ClinicalTrials.gov identifier, NCT01905657) is an international, open-label, phase II/III study of pembrolizumab versus docetaxel that randomized 1034 patients with previously treated advanced NSCLC with a PD-L1 TPS $\geq 1\%$ between 28 August 2013 and 27 February 2015 [3]. Patients were randomized (1 : 1 : 1) to one of the two doses of pembrolizumab (2 mg/kg or 10 mg/kg Q3W administered intravenously over 30 min for a maximum of 24 months) or docetaxel (75 mg/m² Q3W administered intravenously over 1 h for the maximum number of cycles permitted by the local regulatory authorities). Patients were treated until confirmed disease progression, intolerable toxicity, patient withdrawal or physician decision. Patients were stratified by Eastern Oncology Cooperative Group (ECOG) performance status (0 versus 1) and region (East Asia versus non-East Asia). A third stratification variable, extent of PD-L1 expression (TPS $\geq 50\%$ versus 1%–49%), was added after 441 patients were allocated and the PD-L1 IHC assay cut point was established in KEYNOTE-001 study.

Patients

Eligible patients (≥ 18 years) had locally advanced or metastatic NSCLC with progression as per Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1) after ≥ 2 cycles of platinum-doublet chemotherapy, as well as an appropriate tyrosine kinase inhibitor (TKI) for those with *EGFR*-sensitizing mutation or *ALK* gene rearrangement;

measurable disease as per RECIST; ECOG performance status of 0–1; provision of a signed informed consent and a tumor sample with PD-L1 TPS $\geq 1\%$. Initially, any tumor sample was permitted for PD-L1 testing; however, the study protocol was later amended to require a newly collected tumor sample except when it risked patient safety. To be considered a newly collected sample, it was required to be from a recent biopsy with no intervening treatment between the time of sample collection and the initiation of study therapy. The only exception was patients on a TKI before biopsy collection. They could resume that agent after the biopsy. Key exclusion criteria included previous treatment with PD-1 checkpoint inhibitors or docetaxel, known active brain metastases, history of pneumonitis or active autoimmune disease.

PD-L1 status for eligibility was assessed using the anti-PD-L1 antibody clone 22C3 (Merck & Co. Inc., Kenilworth, NJ) and a clinical trial version of the approved IHC assay (pharmDx assay; Dako, Carpinteria, CA). PD-L1 positivity was defined as membranous staining on at least 1% of tumor cells [10].

End points

In the previous report, primary end points included OS (i.e. time from randomization to death due to any cause) and PFS (time from randomization to radiologically confirmed progressive disease or death due to any cause) both in the total population (i.e. TPS $\geq 1\%$) and in the TPS $\geq 50\%$ stratum for both pembrolizumab doses [3]. Secondary end points included ORR and duration of response in the TPS $\geq 1\%$ population and $\geq 50\%$ stratum. Exploratory end points included evaluation of OS and PFS in patients enrolled based on archival and newly collected tumor samples. Because OS and PFS results were similar between pembrolizumab doses for the primary end points [3], the 2 and 10 mg/kg doses were pooled for the purpose of the current analysis to minimize variability.

Assessments

Tumor response was assessed every 9 weeks per RECIST, v1.1 by independent central review [11]. Survival was assessed every 2 months after treatment discontinuation.

Statistics

The Kaplan–Meier method was used to estimate OS and PFS in the archival and newly collected sample populations. For OS, data for patients who were alive or lost to follow-up were censored at the time of last confirmed contact. For PFS, data for patients who had not progressed or were lost to follow-up were censored at the time of last tumor assessment. The proportions of patients with archival and newly collected tumor samples were tallied and summated using summary statistics. A stratified Cox proportional hazard model with Efron's method of tie handling was used to calculate HRs and associated 95% confidence intervals (CIs). Statistical analyses were carried out using SAS (version 9.3).

Results

Subject characteristics

Baseline demographics and disease characteristics were similar for patients enrolled in the combined pembrolizumab groups and the docetaxel group (Table 1). One patient was excluded from the efficacy analysis because it was not possible to assess tumor response due to GCP noncompliance at the study site. Of the 1033 patients analyzed, 455 (44%) were enrolled based on archival samples and 578 (56%) on newly collected tumor samples. The proportions of patients enrolled based on archival samples occurred with a similar frequency in patients with squamous

Table 1. Baseline demographics and disease characteristics in the overall PD-L1 TPS $\geq 1\%$ population

Characteristic, n (%)	Pembrolizumab ^a N = 690	Docetaxel ^b N = 343
Age, median (range), years	63 (20–88)	62 (33–82)
Men	425 (62)	209 (61)
ECOG performance status		
0	232 (34)	116 (34)
1	454 (66)	224 (65)
≥ 2	4 (<1)	2 (<1)
Smoking history		
Current or former	564 (82)	269 (78)
Never	123 (18)	67 (20)
Unknown	3 (<1)	7 (2)
Histology		
Squamous	156 (23)	66 (19)
Nonsquamous	484 (70)	240 (70)
Other/not specified	50 (7)	37 (11)
EGFR-sensitizing mutation	60 (9)	26 (8)
ALK translocation	6 (<1)	2 (<1)
No. of lines of previous treatment		
Neoadjuvant	2 (<1)	0
Adjuvant	13 (2)	3 (<1)
1	478 (69)	235 (69)
≥ 2	196 (28)	104 (30)
Unknown	1 (<1)	1 (<1)

^aCombined data across the 2 and 10 mg/kg dose.

^bOne patient in the docetaxel arm had an unknown ECOG performance status; for five of the six patients who had an ECOG performance status ≥ 2 during screening, the score improved to 1 by the time the patients were randomly allocated to treatment.

and nonsquamous histology (Figure 1A). The median time between sample collection and PD-L1 assessment was 250 days (range 3–2510) for archival samples and 11 days (range 1–371) for new samples. Approximately 40% of archival samples and 45% of newly collected tumor samples were PD-L1 TPS $\geq 50\%$ (Figure 1B). The data in this report were analyzed using a cut-off date of 24 March 2017 [median follow-up 31 months (range 23–41)]. Patients with newly collected tumor samples [28 months (range 24–41)] had a shorter mean follow-up time versus those with archival samples [34 months (range 24–41)] due to the protocol amendment (see above).

Outcome analysis

Kaplan–Meier estimates of OS for the overall population of patients with TPS $\geq 50\%$, TPS $\geq 1\%$ and TPS 1%–49% irrespective of sample type are shown in Figure 2A, C and E, respectively. For patients with TPS $\geq 50\%$, the OS HR was 0.50 (95% CI: 0.39, 0.64) favoring pembrolizumab over docetaxel. The median values were 17.1 months (95% CI: 12.4, 21.8) and 8.2 months (95% CI: 6.6, 10.4), respectively. For patients with TPS $\geq 1\%$, the OS HR is 0.66 (95% CI: 0.57, 0.77) favoring pembrolizumab over docetaxel with median values of 11.9 months (95% CI: 10.4, 13.3) and

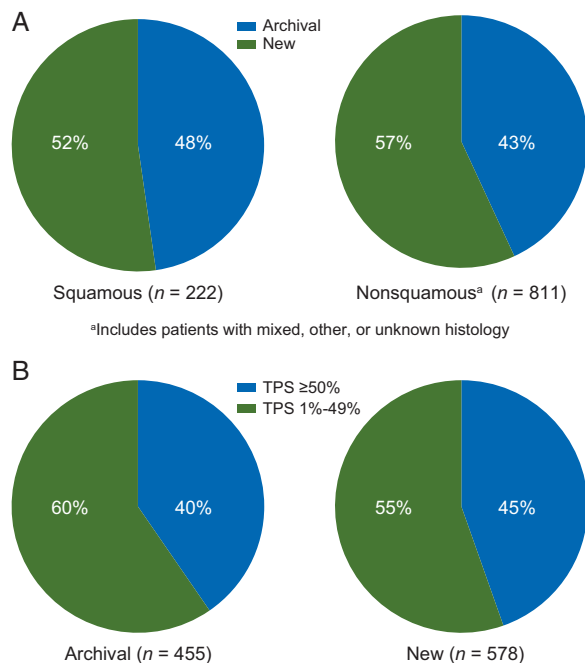


Figure 1. (A) Prevalence of archival and newly collected tumor samples used for assessment of PD-L1 expression by histology. (B) Prevalence of PD-L1 TPS $\geq 50\%$ and TPS 1%–49% in archival and newly collected tumor samples.

8.6 months (95% CI: 7.9, 9.8), respectively. For patients with TPS 1%–49%, the OS HR is 0.76 (95% CI: 0.62, 0.92) with median values of 10.2 months (95% CI: 9.3, 11.8) and 8.7 months (95% CI: 8.0, 10.7), respectively.

Kaplan–Meier estimates of OS for the populations of patients with TPS $\geq 50\%$ and $\geq 1\%$ and further analyzed by archival versus newly collected tumor samples are shown in Figure 3. For TPS $\geq 50\%$ patients enrolled based on PD-L1 expression in archival samples, the OS HR was 0.64 (95% CI: 0.45, 0.91) favoring pembrolizumab over docetaxel. The median values were 11.8 months (95% CI: 9.0, 15.9) and 7.5 months (95% CI: 4.6, 12.2), respectively (Figure 3A). For TPS $\geq 50\%$ patients enrolled based on PD-L1 expression in new samples, the OS HR was 0.40 (95% CI: 0.28, 0.56), with median values of 28.1 months (95% CI: 17.1, NR) and 8.3 months (95% CI: 6.3, 11.7), respectively (Figure 3B). For TPS $\geq 1\%$ patients enrolled based on PD-L1 expression in archival samples, the OS HR was 0.74 (95% CI: 0.59, 0.93) favoring pembrolizumab with median values of 10.5 months (95% CI: 9.3, 12.0) and 8.5 months (95% CI: 7.3, 9.9), respectively (Figure 3C). For TPS $\geq 1\%$ patients enrolled based on PD-L1 expression in new samples, the OS HR was 0.59 (95% CI: 0.48, 0.73), with median values of 13.3 months (95% CI: 11.2, 17.1) and 8.7 months (95% CI: 7.6, 10.7), respectively (Figure 3D).

Kaplan–Meier estimates of PFS for the overall population of patients with TPS $\geq 50\%$, $\geq 1\%$ and 1%–49% irrespective of sample type are shown in Figure 3B, D and F, respectively. For patients with TPS $\geq 50\%$, the PFS HR was 0.58 (95% CI: 0.46, 0.73) favoring pembrolizumab over docetaxel. The median values were 5.3 months (95% CI: 4.2, 6.7) and 4.1 months (95% CI: 3.6, 4.6), respectively. For patients with TPS $\geq 1\%$, the PFS HR is 0.83

(95% CI: 0.72, 0.96) with median values of 3.9 months (95% CI: 3.1, 4.1) and 4.1 months (95% CI: 3.6, 4.3), respectively. For patients with TPS 1%–49%, the PFS HR is 1.02 (95% CI: 0.85, 1.24) with median values of 2.6 months (95% CI: 2.1, 3.4) and 4.1 months (95% CI: 2.7, 5.4), respectively.

Kaplan–Meier estimates of PFS for the populations of patients with TPS $\geq 50\%$ and $\geq 1\%$ and further analyzed by archival versus newly collected tumor samples are shown in Figure 4. When evaluated in patients with TPS $\geq 50\%$, PFS was observed to be longer with pembrolizumab than docetaxel, irrespective of whether patients were enrolled based on archival or newly collected tumor samples. In archival sample patients, the PFS HR was 0.63 (95% CI: 0.45, 0.89) with median values of 4.0 month for each treatment. In new sample patients, the PFS HR was 0.53 (95% CI: 0.38, 0.72) with median values of 6.9 months for pembrolizumab and 4.3 months for docetaxel (Figure 4A and B). When evaluated in patients with TPS $\geq 1\%$, there was no difference in PFS between pembrolizumab and docetaxel irrespective of whether patients were enrolled based on archival or newly collected tumor samples. In archival sample patients, the PFS HRs were 0.82 (95% CI: 0.66, 1.02) and 0.83 (95% CI: 0.68, 1.02) in patients with archival and new samples, respectively, with median values that ranged from 3.1 to 4.2 months for both treatments (Figure 4C and D).

In both the TPS $\geq 50\%$ and TPS $\geq 1\%$ populations irrespective of sample type, pembrolizumab led to a greater improvement in ORR compared with docetaxel (Figure 5). For the TPS $\geq 50\%$, the ORR was 32.4% (94/290; 95% CI: 27.1, 38.1) for pembrolizumab versus 8.6% (13/152; 95% CI: 4.6, 14.2) for docetaxel. For the TPS $\geq 1\%$, the ORR was 20.9% (144/690; 95% CI: 17.9, 24.1) for pembrolizumab versus 9.3% (32/343; 95% CI: 6.5, 12.9) for docetaxel. When analyzed across the individual populations defined by TPS $\geq 1\%$ / $\geq 50\%$ and archival/newly collected tumor samples, improvements in ORR were observed with pembrolizumab versus docetaxel for all populations examined except for patients with TPS $\geq 1\%$ and archival samples. In the docetaxel group, similar proportions of patients had responses ranging from $\sim 8\%$ to $\sim 10\%$ across the individual populations. For pembrolizumab, the ORR was 15.7% (47/300; 95% CI: 11.7, 20.3) for patients with TPS $\geq 1\%$ and archival samples, 22.7% (27/119; 95% CI: 15.5, 31.3) for patients with TPS $\geq 50\%$ and archival samples, 24.9% (97/390; 95% CI: 20.7, 29.5) for patients with TPS $\geq 1\%$ and newly collected tumor samples, and 39.2% (67/171; 95% CI: 31.8, 46.9) for patients with TPS $\geq 50\%$ and newly collected tumor samples.

Discussion

The amount of PD-L1 expression may theoretically change over time. The question is whether this happens with sufficient magnitude and with sufficient frequency that newly acquired tissue is mandatory. The motivation for this analysis was in large part an attempt to answer this question. Cho et al. [4] attempted to address this question by looking at the change in PD-L1 status (using 22C3) in paired lung samples but could not directly assess the predictive value of PD-L1 status for response to pembrolizumab. Temporal effects, along with spatial tumor heterogeneity and analytical factors, represent unavoidable practical limitations

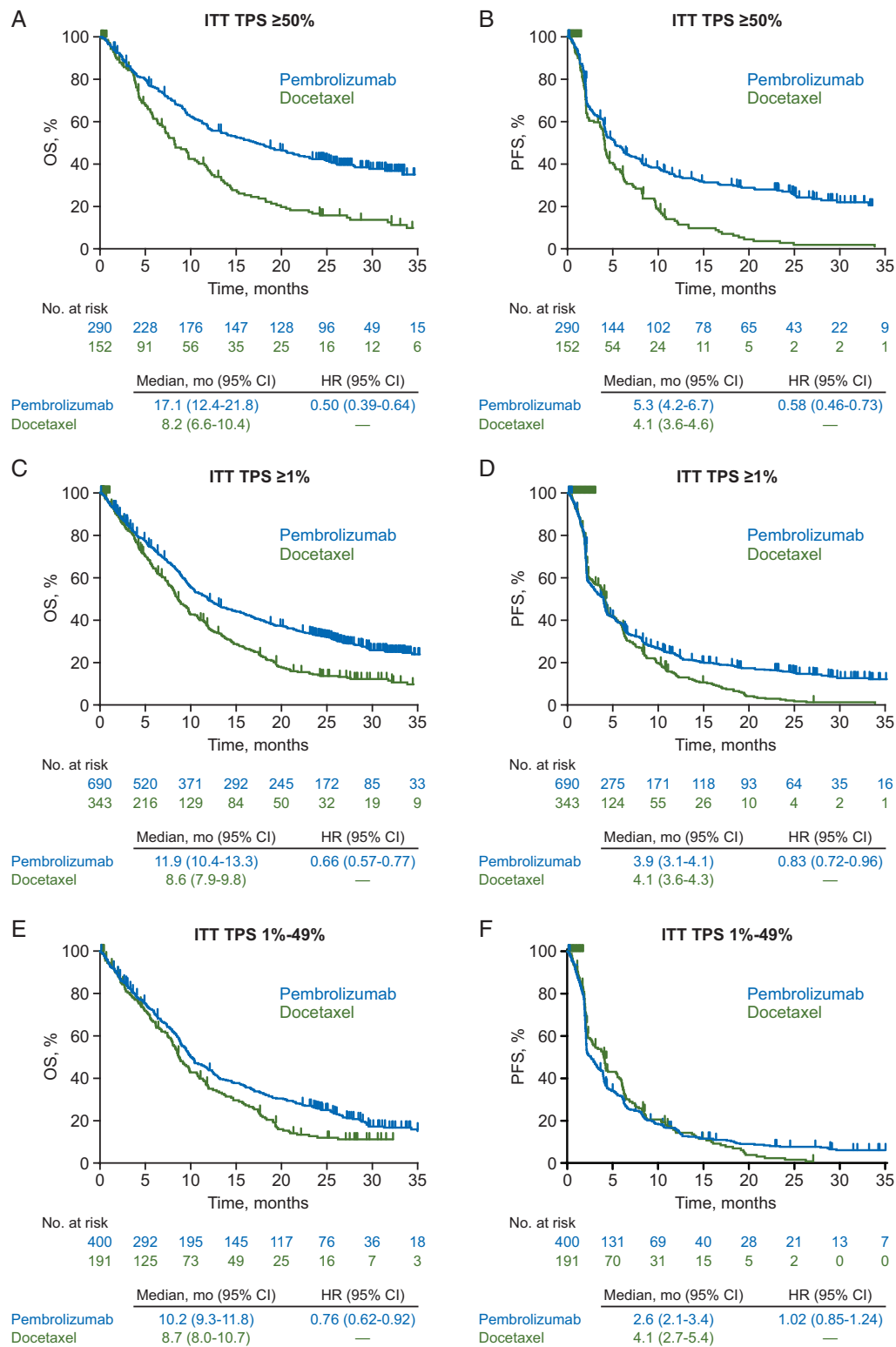


Figure 2. Kaplan–Meier estimates of (A) overall survival (OS) and (B) progression-free survival (PFS) for the ITT population (TPS ≥50%), and (C) OS and (D) PFS for the ITT population (TPS ≥1%) and (E) OS and (F) PFS for the ITT population (TPS 1%–49%). ITT, intention to treat.

for assessing PD-L1 status. In addition, other, more subtle factors may also come into play. For the most part, newly acquired biopsies were obtained primarily to assess PD-L1 status, and thus testing was assessed on a more optimal section from the block, more

representative of PD-L1 expression by the total body burden of tumor. Conversely, PD-L1 testing from archival biopsies may often be assessed from tissue blocks which are nearly exhausted, and thus contain a minimal amount of tissue.

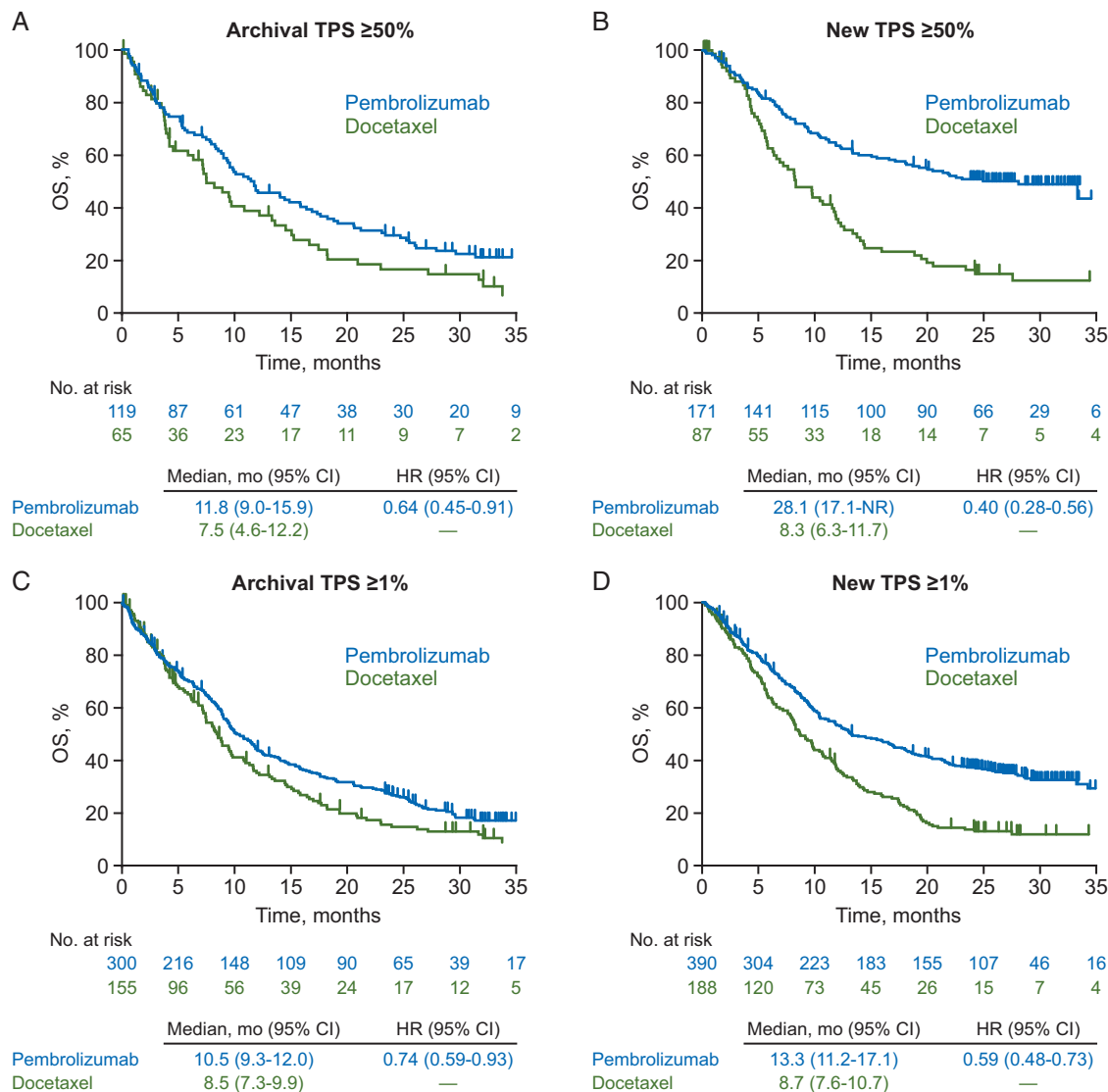


Figure 3. Kaplan–Meier estimates of overall survival for archival and newly collected tumor samples presented by PD-L1 TPS. (A) Archival tumor samples, TPS $\geq 50\%$. (B) Newly collected tumor samples, TPS $\geq 50\%$. (C) Archival tumor samples, TPS $\geq 1\%$. (D) Newly collected tumor samples, TPS $\geq 1\%$.

Prior studies have demonstrated a clear association between PD-L1 tumor expression and antitumor efficacy of pembrolizumab in patients with metastatic NSCLC [3, 10]. The KEYNOTE 010 study demonstrated improved OS, PFS and ORR with pembrolizumab over docetaxel in patients with PD-L1 positive, refractory NSCLC in the primary analysis (using data cut point of 30 September 2015 representing ~ 13.1 months of follow-up) [3], for patients in the TPS $\geq 50\%$ stratum, and improved OS for patients in the TPS $\geq 1\%$ population. The final 442 patients were enrolled into strata based on a PD-L1 expression level of $\geq 1\%$ – 49% or $\geq 50\%$ as assayed in either an archival or recently collected tumor sample. One remaining question that has not yet been evaluated to date is the comparability of the treatment response to pembrolizumab in patients with archival and newly collected tumor samples by PD-L1 expression. The ability to utilize archival tumor samples for assaying PD-L1 expression instead of requiring newly collected tumor samples would greatly benefit

patient health and streamline treatment decisions. Thus, this pre-specified exploratory analysis examined OS, PFS and ORR in patient populations defined based on PD-L1 expression (TPS $\geq 1\%$ and $\geq 50\%$) and tumor sample type (archival and newly collected tumor samples) using the updated data cut point of 24 March 2017 (additional 18 months of follow-up from primary analysis).

The results of this analysis show that the distributions of PD-L1 expression levels (i.e. TPS $\geq 1\%$ and $\geq 50\%$) were similar among both archival (60% and 45%, respectively) and newly collected (55% and 45%, respectively) tumor samples. This finding demonstrates that PD-L1 expression was adequately preserved following months of storage (median 8 months between sample collection and assay) and was possibly unaffected by initiation of additional treatments following tumor sample collection, and potentially worsening disease stage. Additionally, similar proportions of patients with squamous and nonsquamous histology were enrolled based on archival and newly collected tumor

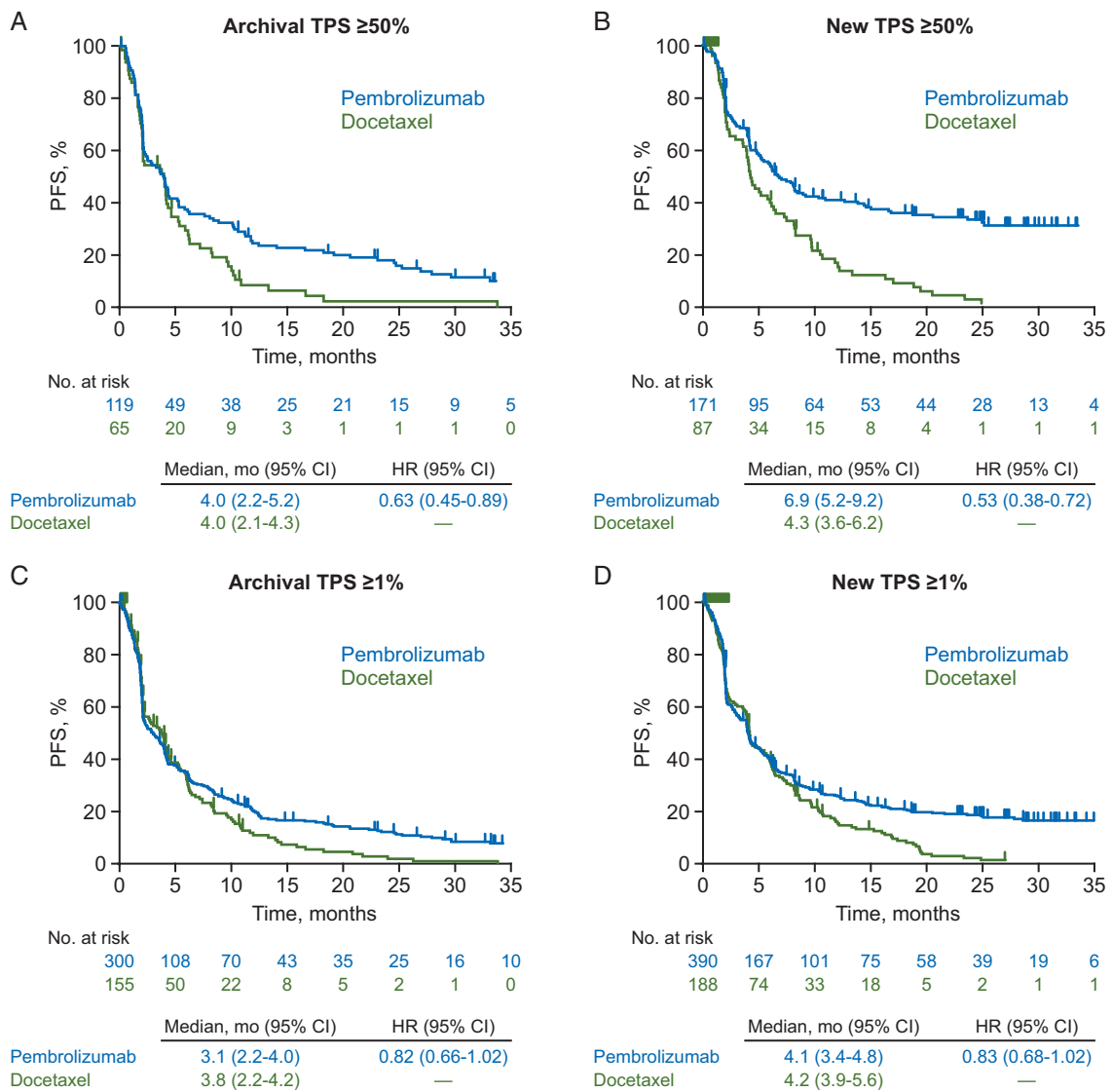


Figure 4. Kaplan–Meier estimates of progression-free survival for archival and newly collected tumor samples presented by PD-L1 TPS. (A) Archival tumor samples, TPS $\geq 50\%$. (B) Newly collected tumor samples, TPS $\geq 50\%$. (C) Archival tumor samples, TPS $\geq 1\%$. (D) Newly collected tumor samples, TPS $\geq 1\%$.

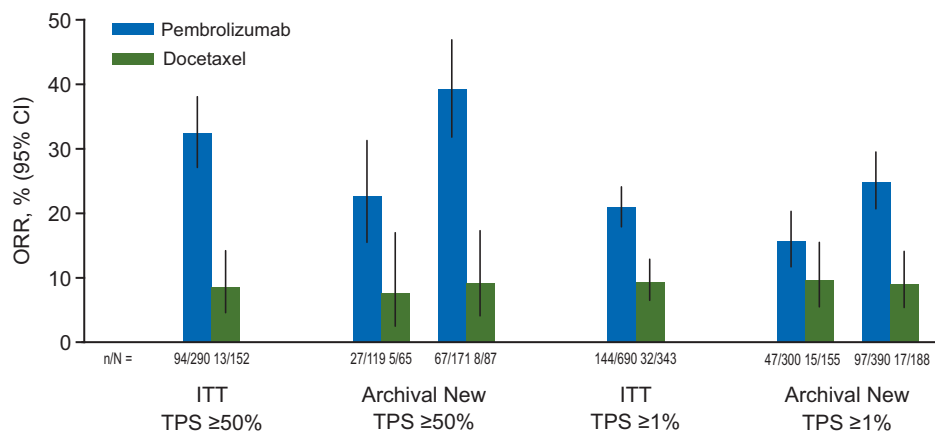


Figure 5. Overall response rate (95% CI) for archival and newly collected tumor samples presented by PD-L1 TPS $\geq 50\%$ and TPS $\geq 1\%$. ITT, intention to treat.

samples showing there was no obvious baseline imbalance related to tumor histology that may have skewed the analysis results.

Treatment with pembrolizumab provided an OS benefit relative to docetaxel in the overall study population as well as patients with TPS $\geq 1\%$ and $\geq 50\%$ regardless of the age of the tumor sample. Within both the archival and newly collected tumor sample populations, a trend toward longer OS was observed in patients with TPS $\geq 50\%$ versus $\geq 1\%$. The fact that the newly acquired biopsy was somewhat more predictive may reflect the fact that the newly acquired biopsy better reflects the PD-L1 status of the tumor at the time of treatment. However, this does not negate the validity of using archival biopsies to assess PD-L1 status. This finding is consistent with the results seen in the overall study population [10]. Similarly, OS appeared more pronounced in the newly collected tumor sample versus archival populations when examined within each of the TPS $\geq 1\%$ and TPS $\geq 50\%$ categories. Although the OS survival benefit appeared numerically greater in patients with newly collected tumor samples, the CIs for the HRs excluded 1.0 and overlapped across all the patient populations examined in this analysis. Median OS with pembrolizumab ranged from 10.5 to 28.1 months. In contrast, median OS with docetaxel was similar across the patient populations, with a median survival time ranging from 7.5 to 8.7 months, which is consistent with that reported in a previous study (9 months) [12].

With regard to PFS and ORR, improved outcomes were observed with pembrolizumab over docetaxel in patients with TPS $\geq 50\%$ regardless of whether PD-L1 expression was assessed in archival or newly collected tumor samples. By comparison, the PFS and ORR benefit of pembrolizumab appeared less pronounced in patients with TPS $\geq 1\%$ across the archival and newly collected tumor sample populations. This is consistent with the findings in the overall study population [10]. Given that OS with pembrolizumab was improved relative to docetaxel across all PD-L1 expression and tumor sample populations, these data suggest that PFS may not appropriately reflect the true benefit of anti-PD-1 treatment. A previous study of nivolumab and docetaxel in patients with nonsquamous NSCLC also reported the lack of a PFS benefit despite a significant improvement in OS [13].

Although the results of this analysis are compelling, several limitations should be considered before extrapolating these findings more broadly. The Blueprint PD-L1 ICH Assay Comparison Project recently showed variability in the clinical diagnostic performance of the four PD-L1 IHC assays used in clinical trials of patients with NSCLC [14]. Despite similar analytical performances of various PD-L1 expression assays, interchanging the assays and cutoff values resulted in misclassification of PD-L1 status in some patients. This finding suggests that the TPS cutoff values used in the current analysis may not be broadly applicable to other PD-L1 IHC assays. While it is tempting to attribute between-assay discordance to short-term sources of variation (analytical run-to-run, pathologist read-to-read, block section-to-section), differences in the stability of assay target antigens may also come into play. The Blueprint Project does not address this issue, and the rationale for using archival samples critically depends on antigen stability. Therefore, the ability of archival samples to accurately predict response to treatment can only be extrapolated to the IHC 22C3 assay at this point in time. Future analyses are needed to determine whether similar findings are

observed for different PD-L1 assays. In addition, a recent study showed heterogeneity in PD-L1 expression within fields of view of the same tumor sample, suggesting that a minimal representative area (not yet determined) must be sampled to accurately predict response to therapy [15]. Therefore, it is possible that differences in heterogeneity within archival versus newly collected tumor samples may limit the applicability of these findings.

The situation has become much simpler now that pembrolizumab is approved for first-line treatment. Even if there are multiple biopsies, the temporal effects are minimal. The interval between biopsies will be relatively short, there is no intervening therapy, and other factors such as total tumor body burden and immune exhaustion do not come into play. Furthermore, pembrolizumab itself has become standard of care, so assessment of PD-L1 expression is prioritized along with other testing (e.g. EGFR mutations and ALK gene rearrangements), and PD-L1 is carried out on more optimal tissue sections. The most important factor for making first-line treatment decisions is to perform PD-L1 testing on an optimal tissue section, i.e. one containing as many viable tumor cells as possible.

In conclusion, the results of this analysis show the OS benefit of pembrolizumab over docetaxel for both TPS $\geq 50\%$ and TPS $\geq 1\%$, regardless of whether PD-L1 was assessed in archival or newly collected tumor samples. Compared with newly collected tumor samples, archival samples were not associated with loss of PD-L1 expression and thereby enabled an accurate estimation of response to treatment. Although there appeared to be a more pronounced clinical benefit in the TPS $\geq 50\%$ stratum based on newly collected tumor samples, there was still clinical benefit when TPS was determined from archival specimens. Newly diagnosed patients with advanced NSCLC should be evaluated for PD-L1 using the 22C3 antibody. Whether to obtain a new biopsy in such a setting can be discussed with patients with the knowledge that clinical benefit was observed with archival biopsies.

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