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Plasma tumor mutation burden is associated with clinical benefit in patients with non-small cell lung cancer treated with anti-programmed death-1 monotherapy

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Abstract

Background: The clinical utility of plasma tumor mutational burden (pTMB) requires further validation. Herein, the pTMB and genetic alterations were investigated as predictive biomarkers for anti-PD-1 monotherapy outcome in metastatic non-small cell lung cancer (NSCLC).

Methods: The GuardantOMNI panel (Guardant Health) was used to identify pTMB and genetic alterations. Data from 99 patients with metastatic NSCLC treated with pembrolizumab or nivolumab in first-, second-, or third-line settings between June 2016 and December 2020 were collected. Associations between pTMB and clinical benefit rate (CBR, stable disease ⩾6 months or partial response), progression-free survival (PFS), and overall survival (OS) were assessed.

Results: Median pTMB in 84 patients was 10.8 mutations/megabase (mut/Mb). Histological analyses revealed that 61 and 36% of the patients had adenocarcinomas and squamous NSCLC, respectively. Most patients were treated with nivolumab (74%) and most anti-PD-1 agents were administered as second-line treatment (70%). The median follow-up duration was of 10.9 months (range, 0.2–40.7). Patients with high pTMB (⩾19 mut/Mb) had a higher CBR (69%) compared with low pTMB patients (33%; $p = 0.01$). *ARID1A* ($p = 0.007$) and either *ERBB2* or *KIT* mutations ($p = 0.012$) were positive and negative determinants, respectively, for clinical benefit. Multivariate analysis further showed that high pTMB was an independent predictive biomarker for both PFS [hazard ratio (HR) = 0.44, 95% confidence interval (CI): 0.22–0.88, $p = 0.02$] and OS (HR = 0.37, 95% CI: 0.18–0.76, $p = 0.007$).

Conclusion: High pTMB (⩾19 mut/Mb) is significantly associated with CBR in patients with NSCLC treated with anti-PD-1 agents.

Keywords: anti-PD-1, clinical benefit, genetic alterations, non-small cell lung cancer, plasma tumor mutational burden

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Introduction

Immunotherapy, particularly programmed death 1 (PD-1) receptor and program death ligand 1 (PD-L1)-targeting agents, has revolutionized treatment options for metastatic non-small cell lung cancer (NSCLC).[1–4] For patients without driver mutations, such as epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), and *ROS1*, the first-line treatment is PD-1/PD-L1 inhibitors, with or without chemotherapy, depending on the level of PD-L1 expression. That is, for patients with a PD-L1 tumor

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proportion score (TPS) ⩾50%, pembrolizumab, an anti-PD-1 agent, is the standard of care. Meanwhile, patients with 1–49% TPS are treated with PD-1/PD-L1 inhibitors in combination with chemotherapy. Although these treatment options have succeeded in prolonging overall survival (OS) and have durable response in a subset of patients, PD-L1 expression remains an imperfect biomarker.[5] Thus, an unmet need persists for the identification of more effective biomarkers to facilitate improved patient selection.

In addition to PD-L1, two notable prognostic markers have received Food and Drug Administration (FDA) approval. For patients harboring advanced tumors that are microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR), pembrolizumab is a promising option, regardless of tumor origin.[6] Recently, the FDA also approved the use of pembrolizumab in patients with tumors carrying high tumor mutational burden (TMB-H), defined as ⩾10 mutations/megabase (mut/Mb).[7] The pan-cancer approval was based on the FoundationOne CDx assay in the exploratory KEYNOTE-158 study, which included 102 patients with advanced solid tumors previously treated with other regimens. However, the study included 10 cancer types, the majority of which were small cell lung cancer, gynecological cancers, and anal cancer. Furthermore, the clinical benefit of the treatment was not consistent across all tumor types. Characterization of the prognostic value of TMB alone, without considering factors such as the possibility that the TMB cut-off points may differ according to histology, tumor heterogeneity, as well as the complex interplay within the tumor microenvironment, is an ongoing challenge that requires further investigation.[8,9]

Currently, several trials have explored the clinical utility of plasma TMB (pTMB) as a possible biomarker for NSCLC.[10] The MYSTIC trial retrospectively explored pTMB using the GuardantOMNI panel (Guardant Health, Redwood, CA) and discovered that patients with a cut-off value ⩾20 mut/Mb exhibited a higher OS when treated with durvalumab and tremelimumab, compared to patients with <20 mut/Mb treated with chemotherapy.[11] A retrospective analysis with patients treated with second-line atezolizumab enrolled in POPLAR and OAK clinical trials using the FoundationOne CDx assay (Foundation Medicine, Inc, Cambridge, MA) showed that the pTMB cut-off of 16 mut/sample had the strongest progression-free survival (PFS).[12] Subsequently, B-F1RST, a prospective study evaluating pTMB, used a cut-off of 16 mut/sample (roughly equivalent to 14.5 mut/Mb) in patients treated with first-line atezolizumab and showed prolonged PFS and OS.[13] However, the role of pTMB in patients with NSCLC treated with anti-PD-1 monotherapy in real-world settings requires further validation.

In this study, we used the Guardant OMNI™ panel (Guardant Health) to investigate pTMB as a predictive biomarker for clinical response, PFS, and OS by anti-PD-1 agents, namely, pembrolizumab or nivolumab in NSCLC. In addition, we analyzed the genetic alterations and correlated these findings with the clinical outcomes to immunotherapy.

Materials and methods

Patients and study design

Data for patients with histologically confirmed stage IV squamous and non-squamous NSCLC with no prior exposure to immunotherapy, treated with anti-PD-1 agents, pembrolizumab or nivolumab, between June 2016 and December 2020 at Yonsei Cancer Center and St. Vincent's Hospital were collected retrospectively. Clinicopathological variables, such as age, sex, smoking, Eastern Cooperative Oncology Group (ECOG) performance score, histology, molecular alterations of *EGFR*, *ALK*, PD-L1 expression, and line of chemotherapy were collected. Prior to treatment with anti-PD-1 agents, plasma samples were collected for analysis of pTMB.

Patients were administered either 200 mg fixed dose or 2 mg/kg of pembrolizumab or 3 mg/kg of nivolumab intravenously in 3-week or 2-week cycles, respectively. The treatment continued until radiologic disease progression, unacceptable toxicity, or at such time that the physician or patient elected to discontinue. All patients underwent baseline computed tomography scans and had subsequent imaging every three or four cycles for pembrolizumab and nivolumab, respectively. Response evaluation was assessed with Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and the best responses were measured and categorized as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).[14]

The PD-L1 immunohistochemistry (IHC) 22C3 PharmDx assay (Agilent Technologies, Santa Clara, CA), as well as the SP263 and SP142 assay (Ventana Medical Systems, Tucson, AZ, USA) were performed as previously described.[15] PD-L1 was defined as positive if the TPS was ⩾1%. *EGFR* mutations in tissues were analyzed using a real-time polymerase chain reaction via a peptide nucleic acid Clamp™ *EGFR* Mutation Detection Kit (Panagene Inc., Daejeon, Korea).[16] To identify *ALK* rearrangement, IHC was performed with ALK (rabbit monoclonal, clone D5F3, Cell Signaling Technology, Danvers, MA, USA) antibodies.[17] Fluorescence in situ hybridization was performed using a break-apart ALK probe (Vysis LSI Dual Color, Break Arrangement Probe, Abbott Molecular, Abbott Park, IL, USA).

Plasma TMB calculation

GuardantOMNI (Guardant Health), a 500-gene panel with a 2.145 Mb sequence output, was used to report the small-nucleotide variants (SNVs), insert/deletions (indels), copy number variants, fusions, MSI-H status, and TMB.[18,19] QIAmp Circulating Nucleic Acid Kit (Qiagen, Inc., Hilden, Germany), labeled with non-random oligonucleotide barcodes (IDT, Inc., Coralville, IA), was used to prepare sequencing libraries. The libraries were then enriched by hybrid capture (Agilent Technologies, Inc., Carpinteria, CA), pooled, and sequenced by paired-end synthesis (NovaSeq 6000, Illumina, Inc., San Diego, CA) with a typical depth of 20,000× reads. All variant detection analyses were performed using the locked clinical GuardantOMNI™ bioinformatics pipeline and reported unaltered by post hoc analyses. Base call files generated by Illumina's RTA software (v3.3.5) were demultiplexed using *bcl2fastq* (v2.20) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads). Processed reads were then aligned to hg19 using BWA-MEM (0.7.15; arXiv:1303.3997v2) and were used to build double-stranded consensus representations of original, unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions. SNVs and indels were classified as somatic or germline using a statistical beta-binomial model.[20] Plasma TMB was reported as mutations per Mb by the GuardantOMNI algorithm that includes all somatic synonymous and non-synonymous SNVs and indels, excluding germline, CHIP, driver, and resistance mutations, with statistical adjustment for sample-specific tumor shedding and molecular coverage. Samples with low tumor shedding, including all somatic mutations <0.3% of the maximum somatic allele fraction or low unique molecule coverage, were identified as pTMB-unevaluable. Validation of pTMB and MSI has been previously described.[19,20] To identify the enrichment of functional genomic alterations in patients with NSCLC treated with anti-PD1 agents, genomic correlation analysis was performed with the results from somatic mutations, including non-synonymous SNVs, indels, amplifications, and translocations.

Statistical analysis

Baseline characteristics and pTMB were analyzed using chi-square test, Fisher's exact test, Mann-Whitney *U* test, and Kruskal-Wallis test for categorical variables and continuous variables as indicated. Overall response rate (ORR) and disease control rate (DCR) were defined as proportion of CR and PR and CR, PR, and SD, respectively. Clinical benefit rate (CBR) was defined as proportion of CR, PR, and SD of ⩾6 months, as previously described in other studies.[21–23] The cut-off point of pTMB was selected based on the best cut-off point that reflected improved CBR, and the lowest hazard ratio (HR) for PFS and OS. The primary objective of this study was to find the clinical association between pTMB and CBR, PFS, and OS in anti-PD-1-monotherapy-treated NSCLC patients. The secondary objective was to identify the genomic alterations that correlates with response to anti-PD-1-monotherapy. The Kaplan-Meier method with log-rank test was used to estimate PFS and OS. Cox regression was used for multivariate analysis of PFS and OS. PFS was defined as the time interval between first treatment with anti-PD-1 to disease progression or death. OS was defined as first treatment with anti-PD-1 until last follow-up or death. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 27 (IBM, Chicago, IL, USA) and GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, CA) and were considered significant if two-sided *p*-value was <0.05.

Results

Patient characteristics according to pTMB

Table 1 shows the baseline characteristics of the 99 patients involved in this study. Most patients

Table 1. Baseline characteristics of patients.

characteristics no.	All patients		Low pTMB		High pTMB		*p*-Value
	n = 84	%	*n* = 67	%	*n* = 17	%	
Age (years)							
<65	41	49	33	49	8	47	0.872
⩾65	43	51	34	51	9	53	
Sex							
Male	67	80	50	75	17	100	0.037
Female	17	20	17	25	0	0	
Smoking status							
Current, ex-smoker	66	79	50	74	16	94	0.104
Never	18	21	17	26	1	6	
Histology							
Adenocarcinoma	49	58	39	59	10	59	0.768
Squamous	33	39	26	39	7	41	
Sarcomatoid	2	3	2	2	0	0	
ECOG performance							
0–1	77	92	63	94	14	82	0.143
2	7	8	4	6	3	18	
EGFR mutation							
Wild	63	75	50	91	13	100	0.575
Mutation	5	6	5	9	0	0	
ALK fusion							
Wild	64	76	54	98	10	100	1
Fusion	1	1	1	2	0	0	
PD-L1 expression							
<1%	16	19	13	19	3	18	0.938
1–49%	37	44	30	45	7	41	
⩾50%	31	37	24	36	7	41	
Immunotherapy							
Pembrolizumab	22	26	18	27	4	24	1
Nivolumab	62	74	49	73	13	77	
Line of treatment							
1	8	10	7	10	1	6	0.758
2	58	69	45	67	13	77	
3	18	21	15	22	3	18	

Percentages may not sum to 100 because of rounding.
ALK, anaplastic lymphoma kinase; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; PD-L1, programmed death ligand 1; pTMB, plasma tumor mutational burden.

were male ($n=78$, 79%) and were either current or ex-smokers ($n=77$, 78%). Histology results revealed adenocarcinoma ($n=60$, 61%), squamous ($n=36$, 36%), and sarcomatoid ($n=3$, 3%) NSCLC. Among the patients with genetic alterations, seven had *EGFR* mutations (L858R, $n=5$, exon 20 insertion, $n=2$) and one had *ALK* fusion, who were all classified as low pTMB. High PD-L1 expression was observed in 36 patients (36%) with TPS $\geq 50\%$. PD-L1 was expressed in 21% patients ($n=21$) with TPS $<1\%$ and in 42% patients ($n=42$) with TPS 1–49%, respectively.

Median pTMB was 10.8 mut/Mb (Supplemental Table S1). A cut-off of 19 mut/Mb was selected for pTMB analysis based on the forest plot showing high pTMB that best reflected improved CBR, in addition to the tendency, but not statistically significant PFS and OS (Supplemental Figure 1A–C). In high pTMB (>19 mut/Mb), there was a tendency for improved PFS [HR 0.56, 95% confidence interval (CI): 0.31–1.01, $p=0.053$] (Supplemental Figure S1A), and OS (HR 0.58, 95% CI: 0.31–1.09, $p=0.09$) (Supplemental Figure S1B), and statistically significant improved CBR (odds ratio, OR 4.4, 95% CI: 1.41–15.33, $p=0.014$) in patients with higher TMB (Supplemental Figure S1C). Among the 99 patients, 84 patients had pTMB results with 67 and 17 patients defined as low pTMB (<19 mut/Mb) and high pTMB ($\geq$19 mut/Mb), respectively. Overall, there was no statistical difference between the baseline characteristics, such as age, smoking status, histology, ECOG performance, *EGFR* mutation, *ALK* fusion, PD-L1 expression by TPS, type of immunotherapy, and line of treatment by high pTMB and low pTMB, with the exception of sex ($p=0.037$; Table 1). There was a trend for higher pTMB in current and ex-smokers, although the small sample size in the subsets limited the statistical strength between two comparisons. Patients grouped according to PD-L1 by TPS of 50% showed statistical differences in smoking status ($p=0.044$), type of immunotherapy regimen ($p\leq 0.001$), and line of treatment ($p=0.025$; Supplemental Table S2).

Plasma TMB and PD-L1 on tumor cells and response to immunotherapy

The distribution of pTMB and PD-L1 is depicted in Supplemental Figure S2A. Patient distribution by TPS 1% (Supplemental Figure S2B) and 50% (Supplemental Figure S2C) are similar for both high TMB and low TMB groups.

Ninety-three patients had measurable lesions for assessment of response evaluation following either pembrolizumab or nivolumab. The median treatment duration was 3.5 months (range, 0.4–35), and the follow-up duration was 10.9 months (range 0.2–40.7). Overall, 20 patients (22%) achieved a PR, and 42 (45%) and 31 (33%) patients had SD and PD, respectively (Table 2). None of the patients in our study achieved CR. Figure 1 shows the waterfall plot, depicting the best percentage of tumor shrinkage, and tumor shrinkage was seen in both the low and high pTMB. Subgroup analysis showed that the CBR was significantly higher in pTMB-high patients (69%) than in pTMB-low patients (33%; $p=0.01$). However, the objective response rate (ORR) [pTMB-high (25%) *versus* pTMB-low (21%), $p=0.738$] and the DCR [pTMB-high (75%) *versus* pTMB-low (64%), $p=0.386$] were not significantly different between pTMB-high and -low patients. Patients were also categorized by PD-L1 cut-off by TPS 1 and 50% (Supplemental Table S3). We observed no difference in CBR, ORR, and DCR between PD-L1 of TPS $<1\%$ *versus* $\geq 1\%$ and TPS $<50\%$ *versus* $\geq 50\%$.

Plasma TMB, PD-L1, and survival outcomes

Patients with high pTMB based on 19 mut/Mb cut-off had favorable survival outcomes, both median PFS ($p=0.052$) and median OS ($p=0.089$; Figure 2(a) and (b)). The median PFS was 8.0 (95% CI: 4.3–11.7) and 3.5 months (95% CI: 2.5–4.5) for high and low pTMB, respectively, whereas the median OS was 24.1 months (95% CI: 0–49.6) for the high pTMB and 9.9 months (95% CI: 7.4–12.4) for low pTMB.

Supplemental Figure S3 shows the subgroup analysis of PD-L1 expression in patients with TPS 1 and 50%. The median PFS was longer in patients with TPS $\geq 50\%$ than those with TPS $<50\%$ (4.9 *versus* 3.6 months, $p=0.011$; Supplemental Figure S3A). However, no difference was observed in median OS between the two groups (15.3 *versus* 10.4 months, $p=0.218$; Supplemental Figure S3B). Furthermore, no difference in median PFS (4.6 *versus* 3.6 months, $p=0.07$) or OS (10.9 *versus* 13.2 months, $p=0.776$) was detected in patients with TPS ≥ 1 and $<1\%$ (Supplemental Figure S3C, D).

Patients were also subdivided according to PD-L1 expression with PD-L1^{low} defined as those with

Table 2. Summary of best response by high and low pTMB.

	Total ($n=93$)		Low TMB ($n=63$)		High TMB ($n=16$)		p-Value
	No.	%	No.	%	No.	%	
Best overall response							0.764
PR	20	22	13	21	4	25	
SD	42	45	27	43	8	50	
PD	31	33	23	37	4	25	
CBR[a]	42	45	21	33	11	69	0.01
ORR	20	22	13	21	4	25	0.738
DCR	62	67	40	64	12	75	0.386
PFS, months, median (95% CI)	4.6	2.9–6.3	3.5	2.5–4.5	8.0	4.3–11.7	0.052
PFS rate at 6 months, % (95% CI)	40.0	30.4–49.6	28.4	17.6–39.2	63.3	39.8–86.8	
PFS rate at 12 months, % (95% CI)	24.6	16.0–33.2	20.9	11.1–30.7	31.7	8.8–54.6	
OS, months, median (95% CI)	13.0	8.4–17.6	9.9	7.4–12.4	24.1	0–49.6	0.089
OS rate at 12 months, % (95% CI)	50.1	40.1–60.1	44.8	32.8–56.8	56.7	32.4–81.0	
OS rate at 24 months, % (95% CI)	24.5	16.1–32.9	17.9	8.7–27.1	50.4	25.9–74.9	

[a]CBR was defined as SD ⩾6 months, PR, or CR.
CBR, clinical benefit rate; CI, confidence interval; DCR, disease control rate; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; pTMB, plasma mutational burden; SD, stable disease.

TPS <50% and PD-L1^{high} as those with TPS ⩾50%. Further analysis of the four subgroups (PD-L1^{low}/TMB^{low}, PD-L1^{low}/TMB^{high}, PD-L1^{high}/TMB^{low}, and PD-L1^{high}/TMB^{high}) showed that PD-L1^{low}/TMB^{low} had the shortest median PFS of 3.1 months (95% CI: 1.8–4.4), and PD-L1^{high}/TMB^{high} had the longest median PFS of 36.1 months (95% CI: 10.5–61.7; $p=0.026$; Figure 2(c)). There was no difference in median OS between the subgroups ($p=0.175$), but PD-L1^{high}/TMB^{high} had the longest median OS of 36.1 months (95% CI: 10.5–61.7; Figure 2(d)).

Genomic correlates with response to anti-PD-1 agent

Figure 1 shows the distribution and frequency of genetic alterations. *TP53* (73.7%) was the most common genetic alteration, followed by *LRP1B* (23.2%), *KMT2D* (21.2%), and *EGFR* (19.2%). Molecular alterations according to clinical response, including PFS, OS, CBR, ORR, and DCR were evaluated, and genes that showed statistical significance with the parameters of clinical responses were included (Figure 3). Patients with *ARID1A* alteration ($p=0.007$) had higher CBR compared with the wild type. *KMT2D* and *LRP1B* alterations were identified as possible predictors for higher CBR, although the results were not statistically significant. In contrast, *ERBB2* or *KIT* alterations ($p=0.012$) were predictors of lower CBR (Figure 3(a)). The differences in TMB were also evaluated according to genetic alterations (Figure 3(b)). Patients with *KMT2D* ($p<0.001$) and *LRP1B* ($p<0.001$) alterations had higher TMB than those with no alterations (wild type). PFS was prolonged in patients with immune checkpoint inhibitors (ICIs)-sensitive markers such as *ARID1A*, *KMT2D*, and *LRP1B* alterations (Supplemental Figure S4). Patients with *ERBB2* alteration ($p=0.045$) and either *ERBB2* or *KIT* alterations ($p=0.007$) had significantly lower PFS.

Univariate and multivariate analyses of factors affecting survival

The univariate analysis of PFS showed a statistically significant difference in sex (female *compared with* male, HR = 2.1, 95% CI: 1.27–3.48,

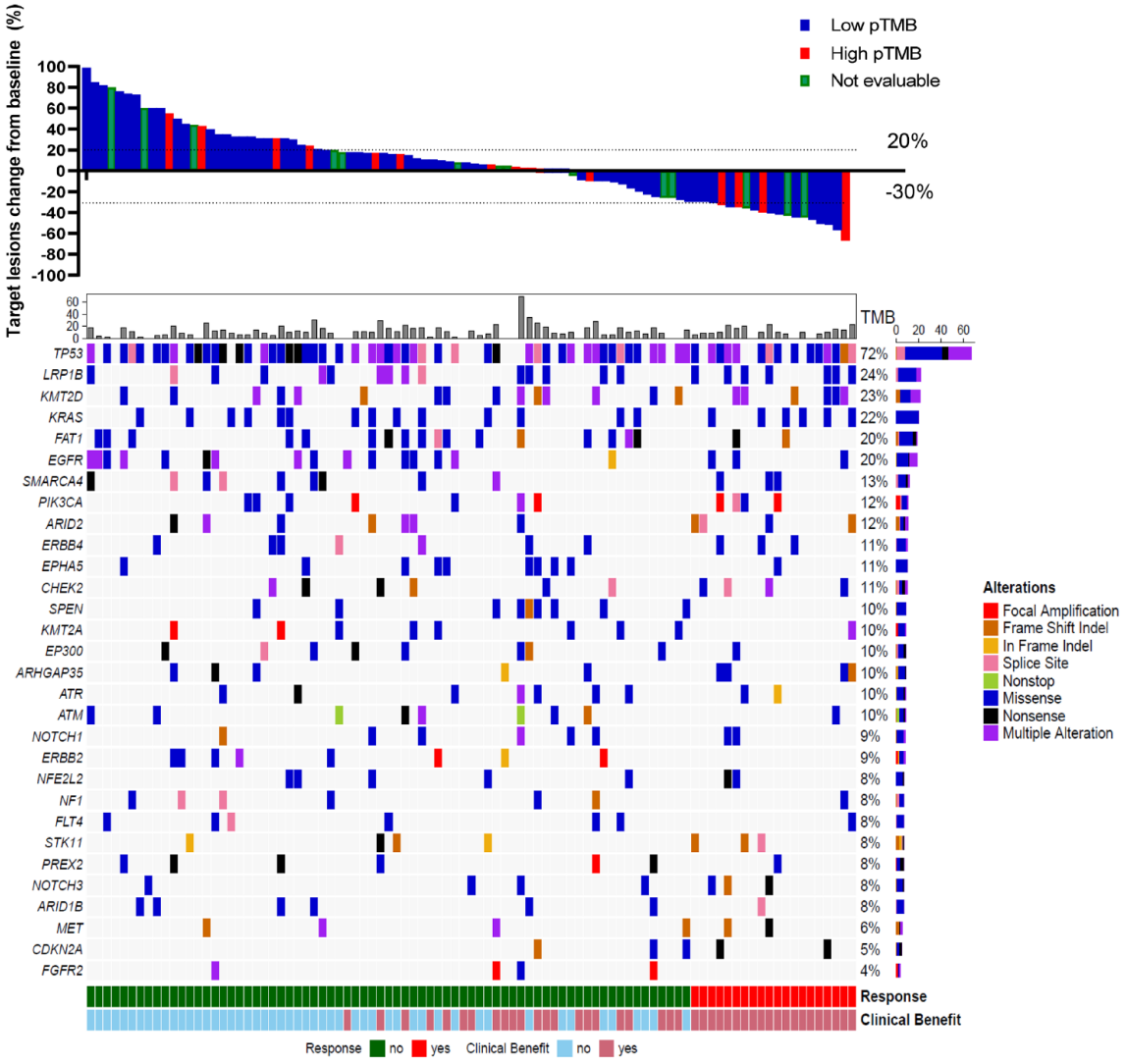

Figure 1. Waterfall plot shows the best percent changes in target tumor burden. Landscape of distribution and frequency of genetic alterations.
mut/Mb, mutation/megabase; pTMB, plasma tumor mutational burden.

$p=0.004$), smoking status (never smoker *compared with* current/ex-smoker, HR = 1.96, 95% CI: 1.19–3.23, $p=0.008$), ECOG (PS of 2 *compared with* 0 or 1, HR = 2.78, 95% CI: 1.27–6.09, $p=0.011$), and PD-L1 expression levels (⩾50% *compared with* <50%, HR = 0.56, 95% CI: 0.36–0.88, $p=0.012$; Table 3). Plasma TMB was a marginally significant factor in the univariate analysis ($p=0.057$). In the multivariate analysis, pTMB (high pTMB *compared with* low pTMB, HR = 0.44, 95% CI: 0.22–0.88, $p=0.02$) was a significant factor in addition to the ECOG (PS of 2, HR = 5.24, 95% CI: 2.06–13.32, $p<0.001$) and sex (female, HR = 2.08, 95% CI: 1.17–3.71, $p=0.013$). PD-L1 did not remain a significant independent factor for PFS in the multivariate analysis.

In the univariate analysis of OS, ECOG PS of 2 (HR = 5.63, 95% CI: 2.48–12.79, $p=0.001$) and third-line treatment with anti-PD1 (HR = 2.46, 95% CI: 1.46–4.14, $p=0.001$) were associated with worse outcomes (Table 3). Plasma TMB was also a marginally significant predictive factor in univariate analysis for OS; however, higher pTMB (HR = 0.37, 95% CI: 0.18–0.76, $p=0.007$) was associated with improved OS in the multivariate analysis.

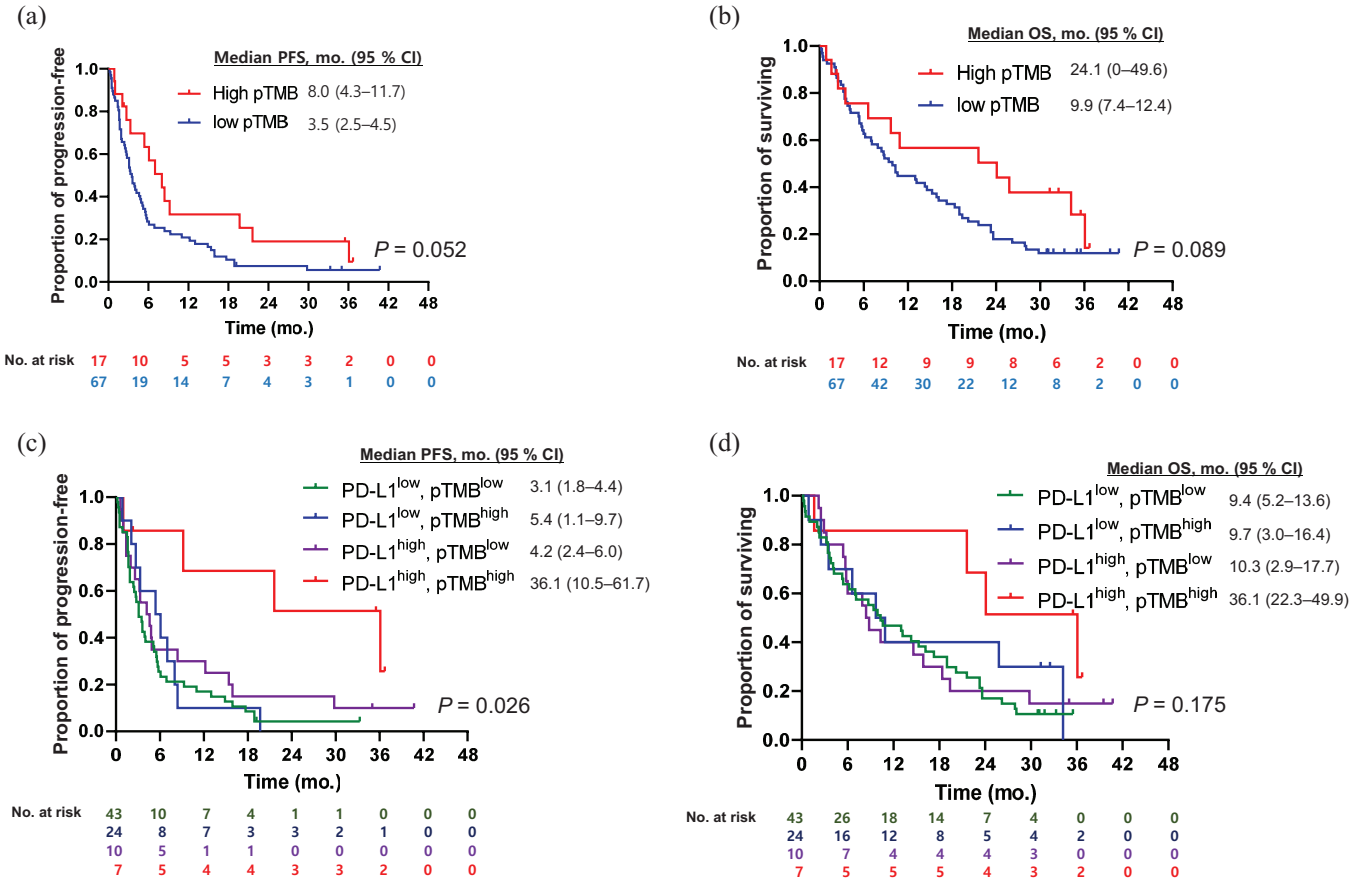

Figure 2. Kaplan-Meier survival analysis according to pTMB and combination of pTMB and PD-L1 expression. Plasma TMB and PD-L1 expression was categorized by cut-off point of 19 mut/Mb and 50%, respectively. (a) PFS and (b) OS between patients with high and low pTMB. (c) PFS and (d) OS by combination of pTMB and PD-L1 expression. Patients were categorized into four subtypes; PD-L1^{low}/TMB^{low}, PD-L1^{low}/TMB^{high}, PD-L1^{high}/TMB^{low}, and PD-L1^{high}/TMB^{high}.

CI, confidence interval; mo., months, mut/Mb, mutation/megabase; OS, overall-survival; PD-L1, programmed death ligand 1; PFS, progression-free survival; pTMB, plasma tumor mutational burden

Discussion

To our knowledge, this is the first real-world study to assess the role of pTMB as a predictive biomarker for anti-PD-1 monotherapy. A cut-off of 19 mut/Mb was selected for pTMB analysis based on the forest plot showing high pTMB that best reflected improved CBR. Our study revealed a correlation between high pTMB (⩾19 mut/Mb) and clinical benefit (SD ⩾6 months or PR) in patients with NSCLC treated with pembrolizumab or nivolumab. In addition, we showed that pTMB represents a significant predictive biomarker for not only PFS but also OS in multivariate analysis. In contrast, patients with PD-L1 TPS ⩾50% had prolonged PFS; however, this was not observed for PFS in the multivariate analysis. Further analysis on genetic alterations showed that *ARID1A* alteration is associated with improvement in CBR, while either *ERBB2* or *KIT* alterations were associated with lower CBR and shorter PFS.

Prior to the validation of TMB and dMMR/MSI-H as predictive biomarkers for ICIs, PD-L1 IHC was the sole predictive biomarker available for NSCLC.[24] Despite the preselected population with PD-L1 expression, only a subset of patients responded to immunotherapy and showed durable responses.[25] Thus, PD-L1 remains an imperfect biomarker in terms of selecting patients who will respond best to ICIs. TMB-H tumors enriched with immunogenic neoantigens attract host T-cells and activate immune response.[26]

TMB-H tumors enriched with immunogenic neoantigens attract host T-cells and activate immune response.[26] Several studies have demonstrated the clinical significance of tissue

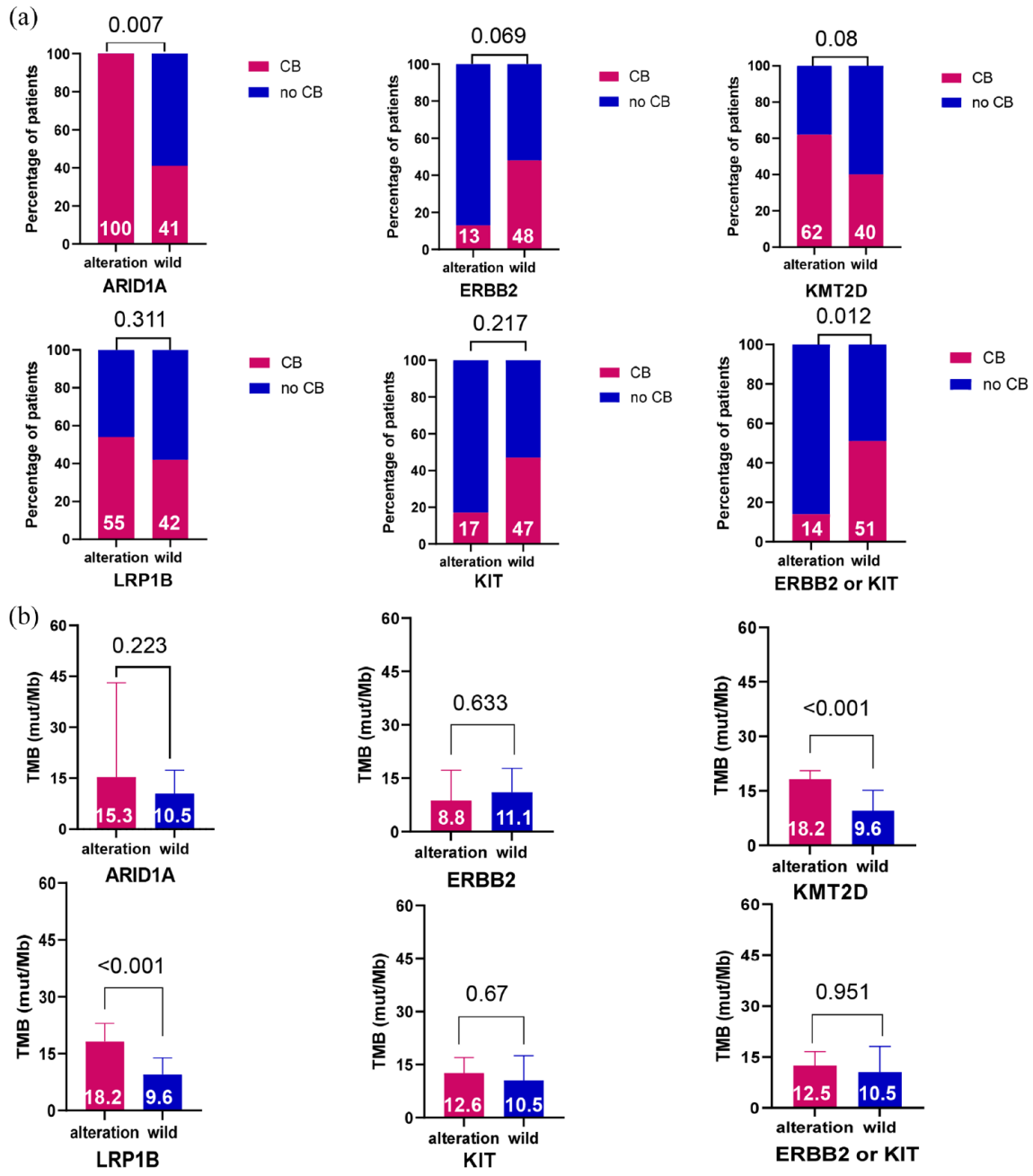

Figure 3. (a) Clinical benefit and (b) pTMB by genetic alterations, including *ARID1A, ERBB2, KMT2D, LRP1B, KIT*, and either *ERBB2* or *KIT*. *ARID1A* alteration ($p = 0.007$) and either *ERBB2* or *KIT* alterations ($p = 0.012$) were associated with CBR. Chi-square test or Fischer's exact test was used to compare two groups. *KMT2D* ($p < 0.001$) and *LRP1B* ($p < 0.001$) alterations were associated with higher pTMB. Mann–Whitney *U* test was used to compare two groups

Data points in (b) are presented as median with interquartile range.

CBR, clinical benefit rate; mut/Mb, mutations per megabase; pTMB, plasma tumor mutational burden.

TMB (tTMB) and pTMB, but with different cut-off points, for defining TMB.[10] The first promising data on tTMB were obtained from the retrospective analysis of patients treated with pembrolizumab in front-line settings in NSCLC.[27] The study showed that improved durable clinical benefit (DCB) and PFS were seen in patients with high non-synonymous mutation burden detected by whole exome sequencing (WES).

Table 3. Univariate and multivariate analyses of PFS and OS.

Variable	Category	PFS						OS					
		Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
		HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age	<65 *versus* ⩾65 years	1.06	0.71–1.60	0.768				1.39	0.90–2.15	0.136			
Sex	Male *versus* female	2.10	1.27–3.48	0.004	2.08	1.17–3.71	0.013	1.41	0.83–2.38	0.203			
Smoking status	Ex-smoker/current smoker *versus* never smoker	1.96	1.19–3.23	0.008				1.44	0.86–2.42	0.164			
Histology	Adenocarcinoma *versus* others	0.90	0.59–1.38	0.632				0.96	0.62–1.49	0.857			
ECOG	0, 1 *versus* 2	2.78	1.27–6.09	0.011	5.24	2.06–13.32	<0.001	5.63	2.48–12.79	0.001	10.81	4.09–28.62	<0.001
PD-L1 expression	TPS <50% *versus* TPS ⩾50%	0.56	0.36–0.88	0.012				0.75	0.47–1.19	0.221			
Immunotherapy	Nivolumab *versus* pembrolizumab	0.78	0.45–1.17	0.185				0.94	0.58–1.54	0.815			
Line of treatment	1, 2 *versus* 3	1.59	0.95–2.65	0.079				2.46	1.46–4.14	0.001			
pTMB	Low (<19 mut/Mb) *versus* high (⩾19 mut/Mb)	0.57	0.31–1.02	0.057	0.44	0.22–0.88	0.02	0.58	0.31–1.10	0.094	0.37	0.18–0.76	0.007

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; PD-L1, programmed death ligand 1; pTMB, plasma mutational burden, TPS, tumor proportion score.

Subsequently, the retrospective analysis of CheckMate 026 study on first-line nivolumab *versus* platinum doublet chemotherapy in NSCLC resulted in prolonged PFS, but not OS in patients with high TMB, defined as greater than 243 missense mutations by WES.[28] The phase II study of low-dose ipilimumab and nivolumab as first-line treatment in Checkmate 568 demonstrated that the cut-off for TMB ⩾10 mut/Mb by FoundationOne CDx assay (Foundation Medicine, Inc.) resulted in improvement of ORR for high TMB patients.[29] Using the prespecified TMB cut-off value from Checkmate 568, the Check Mate 227 study assessed the prognostic impact of TMB as its co-primary endpoints with PD-L1 in the patients treated with low-dose ipilimumab and nivolumab, compared with standard chemotherapy as first-line treatment in NSCLC.[30] Although 42% of the patients failed to obtain the TMB score with targeted NGS panel, higher ORR and improvement in PFS were seen in the TMB-high group. The final analysis of OS showed that patients treated with low-dose ipilimumab and nivolumab had benefits in ORR, PFS, and OS, regardless of TMB or PD-L1.[19]

Currently, the pan-cancer analysis with tTMB utilizing FoundationOne CDx in the phase II KEYNOTE 158 study showed that patients harboring TMB ⩾10 mut/Mb benefit from pembrolizumab irrespective of tumor types.[7] In contrast to tTMB, pTMB is non-invasive, and it does not require tissue sampling for assessment of mutation burden. Furthermore, pTMB may represent the entire somatic mutations and provide complete mutational landscape, as opposed to tTMB which represents the somatic mutations of the obtained primary or metastatic tissue samples.[31]

The utilization of pTMB as a biomarker in NSCLC showed promising results in the MYSTIC trial, which explored both tTMB and pTMB in patients treated with durvalumab and a combination of durvalumab and tremelimumab compared with chemotherapy in front-line settings.[11,32] Using the GuardantOMNI™ panel (Guardant Health), the cut-off value for high pTMB defined as ⩾16 mut/Mb, and patients with high pTMB exhibited significantly improved OS.[32] Further analysis revealed an increase in OS with higher pTMB cut-offs, and pTMB as a

biomarker independent of PD-L1. Similarly, pTMB was also assessed retrospectively in the POPLAR and OAK trial in a subset of patients; the pTMB cut-off of ⩾16 mut/sample by the FoundationOne CDx assay (Foundation Medicine, Inc.) showed correlation with improved PFS in patients with high pTMB treated with second-line atezolizumab.[12]

Recently, the prospective analysis of prespecified pTMB with a cut-off ⩾16 mut/Mb, using the GuardantOMNI™ panel (Guardant Health) in patients with NSCLC treated with first-line pembrolizumab-based treatment demonstrated that patients with ⩾16 mut/Mb had DCB, defined as response CR, PR, and SD for more than 6 months, and prolonged median PFS (14.1 *versus* 4.7 months; HR, 0.30 [0.16–0.60; $p < 0.001$]).[21] Although the same panel was used for analysis, the cut-off value for pTMB differed in predicting responses to anti-PD-1 agents. In our study, higher CBR was seen in the pTMB-high group, defined as ⩾19 mut/Mb. Since there is no standardized cut-off value for pTMB, we conducted the retrospective analysis to determine the subset of patients with high pTMB who clinically benefit from anti-PD-1 agents in terms of higher CBR. Our results showed that pTMB at 19 mut/Mb yields the lowest HR in both PFS and OS, and high pTMB (>19 mut/Mb) was associated with improved clinical benefit. The discrepancy between our results and those of a previous study may be attributed to the different lines of treatment, ICIs monotherapy, and different patient population characteristics, including ethnicity and socioeconomic status. In many studies that evaluated pTMB in patients treated with both chemotherapy and ICIs, chemotherapy has been identified as a possible confounding factor for the evaluation of pTMB. In contrast, our study only included patients treated with anti-PD-1 monotherapy. Thus, our results have an advantage of finding the response predictors specifically for the anti-PD-1 agent. Considering the wide array of platforms used to define high TMB and the different agents of ICIs used in various clinical settings, the pTMB remains inconclusive as a predictive biomarker and requires further validation.[10]

Previous studies have shown that *ARID1A, KMT2D*, and *LRP1B* alterations are associated with favorable outcomes to ICIs.[21,33,34] These patient subsets were more likely to be MSI-H or high TMB compared with patients without alterations. Similarly, our study showed that clinical benefit was more common in patients with *ARID1A* alteration, while *ARID1A, KMT2D*, and *LRP1B* alterations were marginally associated with improved PFS. In addition, *KMT2D* and *LRP1B* alterations were associated with increased TMB. These correlations provide a possible mechanism of action for the alterations as a sensitive biomarker of anti-PD-1 agent. Oncogenic driver alterations have been reported as negative predictors for ICIs, causing resistance to ICIs.[35] This study showed that patients with *KIT* alterations and either *ERBB2* or *KIT* alterations were also resistant to anti-PD1 agents, with less frequent clinical benefit and shorter PFS than patients without alteration. Other alterations such as STK11 or KIF5B, which are also considered poor prognostic markers, were not detected or small in numbers for proper analysis in our study. Further studies are warranted to validate our results.

This study is limited by its retrospective analysis from two centers, treatment with two different anti-PD-1 agents, and the line in which these agents were administered. Our study also did not include tissue sample analysis for investigation of tumor microenvironment or TMB. Furthermore, the correlation between pretreatment plasma and matched tissue may provide insights into pTMB as a predictive biomarker for immunotherapy. Further studies, including prospective studies with a larger sample size are required for validating pTMB in patients treated with anti-PD-1 agents in metastatic NSCLC.

In conclusion, the findings of this study reveal that pTMB predicts clinical outcomes in terms of clinical benefit in patients with metastatic NSCLC treated with anti-PD-1 monotherapy, irrespective of PD-L1 expression.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Yonsei Cancer Center (4-2016-0678) and St. Vincent's Hospital (VC16TISI0208). All patients provided written informed consent.

Consent for publication

Not applicable.

Author contribution(s)

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplemental material

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