Original Study

Association of Tumor Mutational Burden With DNA Repair Mutations and Response to Anti–PD-1/PD-L1 Therapy in Non–Small-Cell Lung Cancer

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Abstract

Tumor mutational burden (TMB) has emerged as a biomarker for response to immune checkpoint blockade in clinical trials. We broadened the potential impact of TMB by evaluating the utility of commercial comprehensive genomic profiling in non–small-cell lung cancer in clinical practice. We analyzed 72 patients and 34 treated with anti–programmed cell death 1/programmed death ligand 1 therapy, with higher TMB predicting longer overall survival.

Purpose: To examine clinical predictors of tumor mutational burden (TMB), to explore the association between TMB and DNA repair mutations, and to analyze TMB as a biomarker for response to immune checkpoint blockade in non–small-cell lung cancer. **Patients and Methods:** TMB scores were determined retrospectively for 72 consecutive patients at our institution with next-generation sequencing comprehensive genomic profiling testing by Foundation Medicine. TMB scores were correlated with a number of clinical variables and presence of DNA repair mutations. Thirty-four patients were treated with anti–programmed cell death 1 (PD-1)/programmed death ligand 1 (PD-L1) therapies, and survival analyses based on TMB score were performed. In addition, tissue immunohistochemical analysis was performed for a subset of patients. **Results:** History of smoking, but not other clinical variables, including prior treatment lines, stage of disease, and number of metastatic sites, predicted higher TMB score. Higher TMB score was significantly associated with greater number of DNA repair mutations. In the subset of patients treated with immune checkpoint blockade, higher TMB score significantly predicted overall survival, but not progression-free survival (hazard ratio = 0.10, $P = .003$; hazard ratio 1.1, $P = .84$, respectively). In a small subset of patients, PD-1/PD-L1 staining did not independently predict progression-free survival or overall survival. **Conclusion:** Tissue TMB was significantly associated with smoking history and number of DNA repair mutations. TMB is a promising biomarker for response to anti–PD-1/PD-L1 therapy, with higher TMB score predicting longer overall survival.

Clinical Lung Cancer, Vol. 20, No. 2, 88-96
Keywords: Biomarker, Immunotherapy, NGS, NSCLC, TMB

Introduction

Immune checkpoint blockade has resulted in a paradigm shift toward utilizing therapies that manipulate immune cells to control tumor growth. Therapies targeting the programmed death ligand 1 (PD-1)/programmed death ligand 1 (PD-L1) checkpoints have produced impressive responsive rates in patients with metastatic disease refractory to multiples lines of therapy.[1] Responses have been observed in patients with non–small-cell lung cancer

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Submitted: Feb 24, 2018; Revised: Aug 20, 2018; Accepted: Sep 15, 2018; Epub: Sep 25, 2018

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1525-7304/$ - see frontmatter
https://doi.org/10.1016/j.cllc.2018.09.008

(NSCLC), melanoma, renal-cell carcinoma, bladder carcinoma, and microsatellite instability (MSI)-high colorectal cancer, among others. In order to optimally select patients for treatment, biomarkers that predict immune response to NSCLC tumors are critical.

Identifying patients who preferentially respond to checkpoint blockade necessitates a better understanding of how peripheral effector T cells interact with the tumor microenvironment. A variety of clinical and genetic predictors have been considered as playing a role in this interaction. In NSCLC, smoking history has been associated with a > 10 times increase in mutations compared to nonsmokers.[2] Furthermore, genomic-wide sequencing studies across large populations have demonstrated that tumor histologies more likely to respond to anti–PD-1/PD-L1 therapy, including NSCLC, have a particularly high number of somatic mutations.[3] Studies have also examined PD-L1 immunohistochemistry (IHC) as a biomarker, with mixed response. While PD-L1, particularly at high levels of expression, may indicate a greater likelihood of response to anti–PD-1/PD-L1 therapy, the correlation between PD-L1 expression by IHC varies on the basis of tumor histologic features as well as spatial and temporal factors (eg, location and time frame of biopsy).[4-6] In addition, some patients with minimal or no PD-L1 expression experience therapeutic benefit. Clearly, challenges remain in order to identify reliable, cost-effective biomarkers to identify which patients are most likely to receive therapeutic benefit from checkpoint blockade and avoid unnecessary immune-related adverse events.[7]

Tumor mutational burden (TMB) has emerged as a potential measure of genomic instability and neoantigens to predict response to immunotherapy. Neoantigens arise as a result of tumor-specific mutations that are absent from the normal human genome.[8,9] While there are many challenges associated with direct neoantigen prediction, TMB may serve as an indirect, probabilistic measure of immunogenicity. Therefore, TMB assesses a measure of individual tumor genomic instability, which may suggest the potential for cytotoxic T lymphocytes to target the tumor with checkpoint blockade. Previous research has shown that higher nonsynonymous mutation burden as assessed by whole-exome sequencing was associated with objective response and progression-free survival (PFS) in patients with NSCLC treated with pembrolizumab, melanoma patients treated with PD-1, and cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) blockade, atezolizumab in urothelial carcinoma, and other diverse cancers.[10-15] Predictive accuracy of mutational burden has also been reported using comprehensive genomic profiling in NSCLC in addition to whole-exome sequencing.[16]

In addition, DNA repair mutations and MSI have been implicated as drivers for response to immunotherapy.[17] Particular DNA repair mutations, including *POLD1*, *BRCA2*, *POLE*, *PRKDC*, *MSH2*, *RAD51C*, *LIG3*, *RAD17*, and *POLE 4* have been associated with genomic instability in NSCLC.[10] In addition, clinical studies have associated mismatch repair deficiency and response to PD-1 blockade with pembrolizumab in both MSI-high colorectal and noncolorectal tumors.[18,19] MSI-high tumors produced higher objective response rates, thereby validating the use of immune checkpoint blockade for tumors with DNA repair defects in this population. The mechanisms underlying this response included a dense T-cell immune infiltration, cytokine-rich tumor microenvironment, and approximately 20 times higher number of mutation-associated neoantigens. However, further validation of the interaction among TMB, DNA repair mutations, and tissue IHC PD-1/PD-L1 staining are necessary in commercial applications to expand clinical utility.

We used comprehensive genomic profiling to assess TMB for patients with NSCLC using a Clinical Laboratory Improvement Amendments–certified next-generation sequencing (NGS) platform. TMB was correlated with clinical variables, DNA repair mutations, lung cancer driver mutations, IHC markers of the tumor microenvironment, and clinical response to anti–PD-1/PD-L1 therapy. The goals of this study were to examine the association of TMB with response to checkpoint blockade in NSCLC and to correlate TMB with mutational status across a comprehensive list of genes involved in DNA repair.

Patients and Methods

Patient Selection and Study Design

The institutional review board of Northwestern University Feinberg School of Medicine approved the study. Written informed consent from patients was waived per the institutional review board for this retrospective review of molecular analyses. Studies were performed in concordance with the Health Insurance Portability and Accountability Act and the Declaration of Helsinki. All patients were treated at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. The cohort consisted of 82 consecutive patients with NSCLC identified retrospectively with commercial NGS testing by FoundationOne (Foundation Medicine, Cambridge, MA) between 2013 and 2016. Of the 82 patients, 72 passed the FoundationOne quality control to accurately determine TMB. At the time of our analyses, TMB was calculated for research purposes only, and the assay was not clinically indicated for TMB. However, since that time, TMB is now reported for all patients undergoing FoundationOne testing. Clinical characteristics were collected via electronic medical record review.

In addition, PFS and overall survival (OS) were determined for 35 patients who were treated with anti–PD-1/PD-L1 therapies. One patient was excluded because the sample did not pass the quality control by FoundationOne. Therefore, the final samples size for survival analyses was 34 patients. PFS was calculated on the basis of time of disease progression on immune checkpoint blockade or until last follow-up or patient death. OS was determined on the basis of the date of last follow-up or patient death.

Next-Generation Sequencing

All patients underwent NGS testing performed by Foundation Medicine. The gene panels varied depending on the date the sequencing was performed. Number of genes ranged from 236 to 315. The panel expanded to 315 genes in approximately August 2014. The tests sequenced approximately 1.1 million base pairs of DNA.[20] The clinical reports consisted of nonsynonymous potentially functional mutations and nonsynonymous variants of unknown significance (VUS).

Definition of TMB

TMB was determined by Foundation Medicine. TMB was calculated by including all substitutions and indels over the entire

somatic, coding, sequencing length.[20] Synonymous mutations were included given their potential to promote genomic instability. Noncoding alterations were excluded. In addition, predicted germline mutations and somatic alterations in COSMIC and truncations in tumor suppressor genes were not counted.[20,21] TMB was calculated in mutations per megabase pair. TMB categories were determined by Foundation Medicine on the basis of the following ranges, rounded to the nearest whole number: very low (0-1), low (2-5), intermediate (6-14), high intermediate (15-19), high (20-34), and very high (35 and above).

DNA Repair Mutations

Genomic alterations were further classified into direct and indirect DNA repair mutations. Direct DNA repair mutations were mutations involved in mismatch repair, homologous recombination, Fanconi anemia, nucleotide excision repair, base excision repair, nonhomologous end joining, or direct reversal DNA pathways. In addition, indirect or caretaker mutations implicated in maintaining genomic stability were also included. These classifications were determined using a list of 193 DNA repair genes known to have increased associations with mutational burden across multiple tumor types.[22] Of these, 122 mutations were categorized as direct and 71 as indirect DNA repair mutations.

Tissue IHC

Tissue staining was retrospectively performed for a subset of 14 patients with accessible tissue for IHC. All of these patients were treated with immune checkpoint blockade. Of the 34 patients treated with immune checkpoint blockade, 20 were excluded as a result of inaccessible pathology or insufficient sample for IHC (such as samples acquired by fine-needle aspiration biopsy). Tissue staining was performed for PD-1, PD-L1 (tumor), PD-L1 (immune), CD3, CD4, CD8, CD20, CD56, and FOXP3. Samples were read and interpreted by an attending pathologist. Data were reported as the percentage of cells staining for each particular marker.

Statistical Analysis

Two-sample *t* tests and chi-square tests were used to test the association of TMB with clinical or DNA repair mutation variables. Univariate and multivariate logistic regression were performed to assess the significance of predictors on survival status. Survival analyses compared TMB and survival. PFS and OS were assessed by Kaplan-Meier estimation, with patients censored at last follow-up. Log-rank tests and Cox proportional hazards regression were performed to compare survival distributions based on TMB-low and TMB-high. All analyses were performed using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org/) and the "base" (v3.3.1), "stats" (v3.3.1), "survival" (v2.38), and "ggplot2" (v2.2.1) packages, as well as required dependencies.

Table 1 Clinical Characteristics of 82 NSCLC Patients Who Underwent NGS Testing

Characteristic	Value
Age (Y)	
Median	64.5
Range	37-88
Sex	
Female	49 (59.8)
Male	33 (40.2)
Lung Cancer Histology	
Adenocarcinoma	64 (78.0)
Squamous	11 (13.4)
NOS	7 (8.5)
Smoking Status	
Minimal/never	26 (31.7)
Current/former	56 (68.3)
Patients Receiving Immunotherapy	
Total	35 (42.4)
Nivolumab	21 (60.0)
Atezolizumab	7 (20.0)
Pembrolizumab	5 (14.3)
Durvalumab	2 (5.7)
No. Prior Lines of Treatment	
0-1	74 (90.2)
2+	8 (9.8)
Prior Radiotherapy	
Yes	19 (23.2)
No	63 (76.8)
Disease Stage	
III	20 (24.4)
IV	62 (75.6)
No. Metastatic Sites	
0	20 (24.4)
1	42 (51.2)
2+	20 (24.4)
Presence of *EGFR/KRAS* Mutations	
EGFR mutant/wild type	14/68
KRAS mutant/wild type	12/70
TMB Score	
Median	8
Mean	11.6
Range	< 1 to 55
FoundationOne Quality Control Status	
Passed quality control	72 (87.8)
Did not pass quality control	10 (12.2)

Data are presented as n (%) unless otherwise indicated.
Abbreviations: NGS = next-generation sequencing; NOS = not otherwise specified; NSCLC = non–small-cell lung cancer; TMB = tumor mutational burden.

Results

Patient Characteristics

Table 1 shows the patient and tumor characteristics of the 82 patients included in the study, including 72 (87.8%) of 82 patients who passed the internal quality control by Foundation Medicine to accurately determine TMB. Median age was 64.5 years; there was a slight predominance of women (59.8%). In terms of histology, the majority of patients had adenocarcinoma (78.0%), followed by squamous-cell carcinoma (13.4%) and other histologies (8.5%). Of

the 35 patients treated with single-agent immune checkpoint blockade, 21 received nivolumab, 7 received atezolizumab, 5 received pembrolizumab, and 2 received durvalumab. The majority of patients had metastatic disease at the time of TMB determination (75.6%).

Tumor Mutational Burden

TMB scores were determined by Foundation Medicine in mutations per megabase pair, including all substitutions and indels over the entire sequencing length of DNA with germ-line and driver variants filtered out. For the entire sample, TMB ranged from < 1 to 55 mutations per megabase pair with a median of 8 and a mean of 11.6. Foundation Medicine had predefined TMB ranges: very low: 0-1, low: 2-5, intermediate: 6-14, high intermediate: 15-19, high: 20-34, and very high: 35 and above. The majority of patients (74.4%, 61 of 82) had TMB-low or -intermediate (Figure 1A).

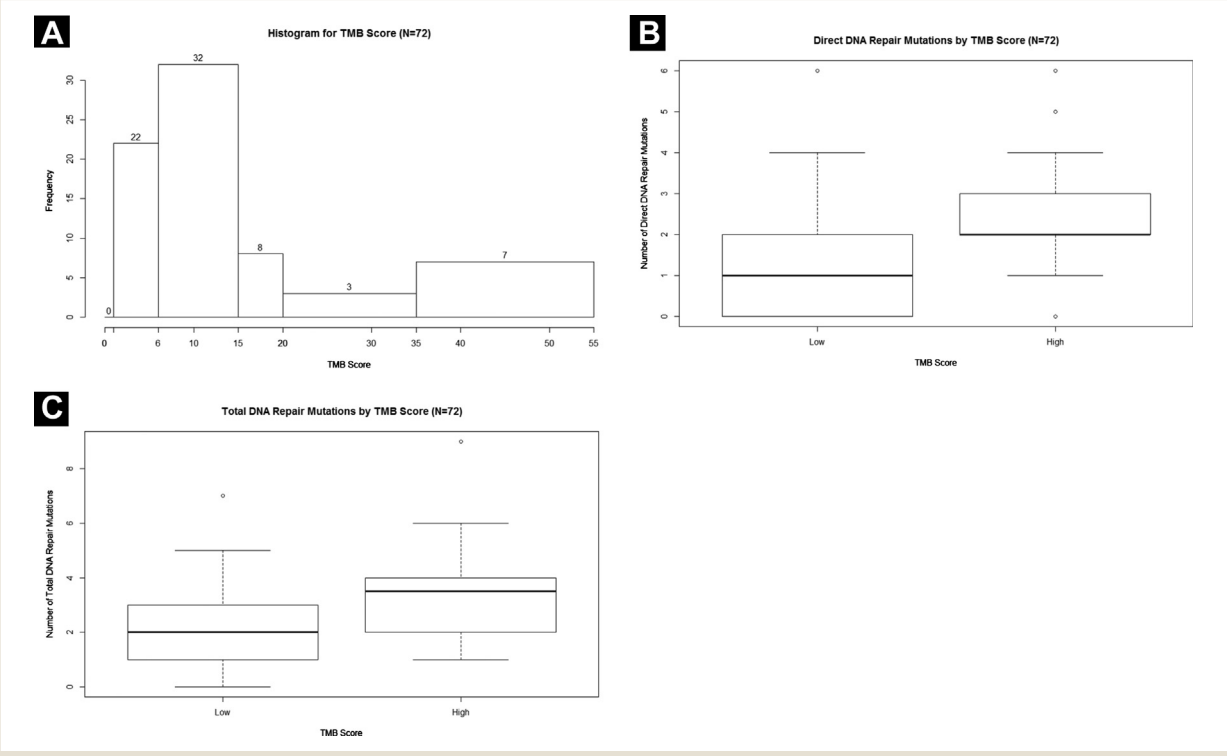
Association of TMB With Patient Characteristics

TMB scores were analyzed retrospectively and associated with clinical characteristics stratified on the basis of TMB ranges of 0 to 14 (very low to intermediate) versus 15 and above (high intermediate to very high) according to our initial hypothesis that only relatively high TMB samples defined in this way would be more likely to respond to immune checkpoint blockade (Table 2). Scores were also compared on the basis of the median of the sample (Supplemental Table 1). TMB was significantly associated with current or former history of smoking ($P = .011$, chi-square test, Supplemental Figure 1). TMB was not correlated with number of treatment lines before tissue collection, stage of disease, or number of metastatic sites ($P > .5$). These findings were consistent when testing TMB as a continuous variable, with only smoking being significantly associated with TMB score ($P < .001$).

Association of TMB With Tumor Mutations

TMB was correlated with reported mutations that were available to clinical providers on FoundationOne reports, given that TMB determination includes many alterations not included on clinical reports. TMB was significantly associated with number of potentially functional mutations, number of VUS mutations, and total reported mutations ($P < .003$, 2-sample t test). The presence of known lung cancer driver mutations in *EGFR* or *KRAS* trended toward lower TMB but was not statistically significant ($P = .156$). Additionally, one patient had an *ALK* fusion with low TMB score, 3 patients had *ROS1* fusions (2 with low TMB and 1 intermediate), and 6 patients had *BRAF* mutations with TMB range 1 to 18, with

Figure 1 **TMB Landscape and Association of TMB Score and DNA Repair Mutations in Patients With NSCLC (A) Landscape of TMB scores. TMB was Calculated in Mutations per Megabase Pair. TMB Categories Were Determined as Follows: Very Low (0-1), low (2-5), Intermediate (6-14), High Intermediate (15-19), High (20-34), and Very High (≥35). (A) High TMB Scores (Above 15 Mutations per Megabase Pair) in Our Sample Were Significantly Associated With (B) Greater Number of Direct DNA Repair Mutations (*t* test, $P = .001$) and (C) Total DNA Repair Mutations (*t* test, $P < .001$). DNA Repair Mutations Were Categorized Into Direct and Indirect**



Abbreviations: NSCLC = non–small-cell lung cancer; TMB = tumor mutational burden.

Table 2 TMB by Patient Characteristics and Mutation Status

Characteristic	Variable	TMB < 15	TMB ≥ 15	All	*P*
Smoker	Minimal/never	22	1	23	.013[a]
	Current/former	32	17	49	
	All	54	18	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.311
No. of prior lines of treatment	0-1	49	15	64	.665
	2+	5	3	8	
	All (N)	54	18	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.456
Disease stage	I-III	11	4	15	1.000
	IV	43	14	57	
	All	54	18	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.240
Metastatic sites	0	11	5	16	.741
	1	27	9	36	
	2+	16	4	20	
	All	54	18	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.377
Presence of *EGFR/KRAS* mutations	Yes	20	3	23	.189
	No	34	15	49	
	All	54	18	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.297
No. of potential functional direct + indirect DNA repair mutations	Mean (N)	0.81 (54)	1.28 (18)	0.93 (72)	.051[b]
No. of VUS direct + indirect DNA repair mutations	Mean (N)	1.41 (54)	2.33 (18)	1.64 (72)	.011[b]
No. of direct DNA repair mutations	Mean (N)	1.17 (54)	2.50 (18)	1.50 (72)	.002[b]
No. of All DNA repair mutations	Mean (N)	2.22 (54)	3.61 (18)	2.57 (72)	.010[b]
No. of potentially functional mutations	Mean (N)	5.28 (54)	8.17 (18)	6.00 (72)	.008[b]
No. of VUS mutations	Mean (N)	9.76 (54)	25.11 (18)	13.60 (72)	< .001[b]
Total reported mutations	Mean (N)	15.04 (54)	33.28 (18)	19.60 (72)	< .001[b]

Abbreviations: TMB = tumor mutational burden; VUS = variant of unknown significance.
[a]Pearson chi-square test.
[b]Welch 2-sample *t* test.

1 patient categorized as very low, 1 patient as low, 3 patients as intermediate, and 1 patient as high intermediate.

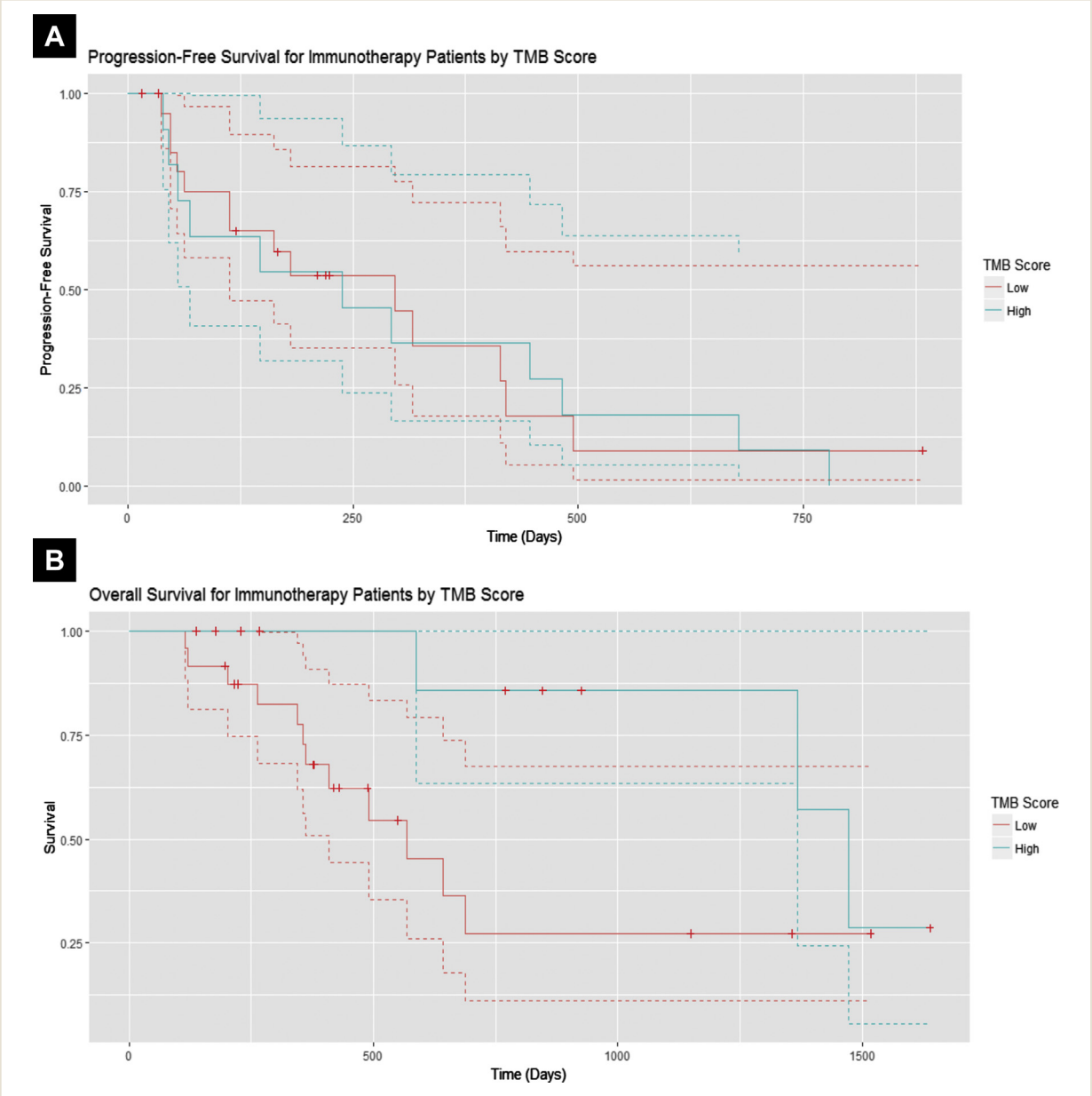
Tumor mutations were further subdivided on the basis of whether they were implicated in DNA repair. Mutations were classified as either direct DNA repair or indirect DNA repair mutations (Supplemental Table 2). Direct DNA repair mutations included mutations involved in mismatch repair, homologous recombination, Fanconi anemia, nucleotide excision repair, base excision repair, nonhomologous end joining, or direct reversal DNA pathways. In addition, indirect DNA mutations were those implicated in maintaining genomic stability. TMB was significantly associated with all combinations of direct and indirect DNA repair mutations, regardless of potential functionality (Table 2, $P < .05$, 2-sample *t* test). Box plots demonstrating a significant association between presence of DNA repair mutations and higher TMB are shown in Figure 1B and 1C.

Table 3 Survival Analyses for 34 Patients Treated With Immune Checkpoint Blockade

Survival	Variable	Value
Overall survival	Log-rank test	*P* = .003
		HR = 0.10
	Proportional hazards regression	95% CI = 0.01-0.76
		P = .026
Progression-free survival	Log-rank test	*P* = .839
		HR = 1.08
	Proportional hazards regression	95% CI = 0.48-2.46
		P = .849

Abbreviations: CI = confidence interval; HR = hazard ratio.

Figure 2 Survival Curves Including PFS and OS for Patients Treated With Immune Checkpoint Blockade. TMB was Significantly Correlated With Longer OS but not PFS. PFS and OS Were Assessed by Kaplan-Meier Estimation. Log-rank Tests and Cox Proportional Hazards Regression Were Performed to Compare Survival Distributions Based on TMB-Low and TMB-High



Abbreviations: OS = overall survival; PFS = progression-free survival; TMB = tumor mutational burden.

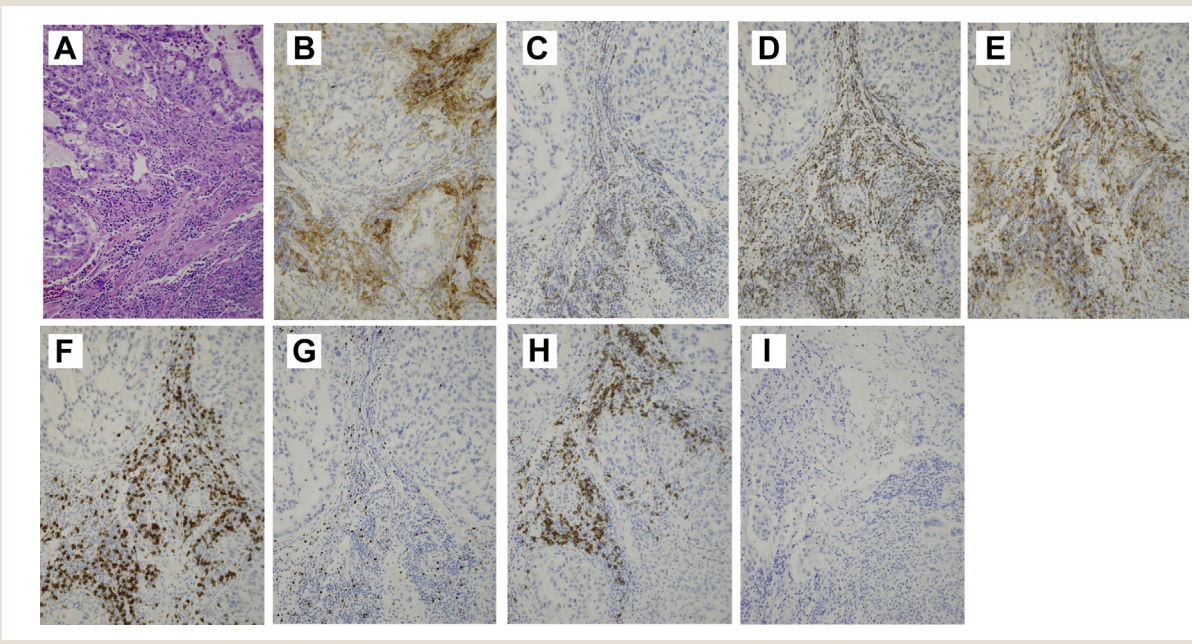
Survival Analysis for Patients Treated With Immune Checkpoint Blockade

For all patients treated with anti−PD-1/PD-L1 therapy (n = 35), PFS and OS were obtained retrospectively. Of these samples, 34 (97.1%) of 35 passed FoundationOne quality control testing and were included in the final analyses. Higher TMB was associated with significantly longer OS (hazard ratio = 0.10; 95% confidence interval, 0.01-0.76; $P = .026$) (Table 3). PFS was not significantly associated with TMB (hazard ratio = 1.08; 95% confidence interval, 0.48-2.46; $P = .839$). The univariate analysis between survival and the clinical predictors smoking, number of prior lines of treatment, stage of disease, and metastatic sites were not significant. Survival curves are shown in Figure 2A and 2B. Using the median of our sample (TMB score of 8), TMB above this threshold was not significantly associated with longer OS (hazard ratio = 0.50; 95% confidence interval, 0.17-1.49; $P = .214$).

Tissue IHC

In an exploratory analysis, tissue IHC was performed on samples from 14 available patients (Figure 3). Staining was performed on the

Figure 3 Tissue Immunohistochemistry of Tumor Microenvironment. Representative Images Shown for Single Patient With Following Stains: H&E (A), PD-L1 (B), PD-1 (C), CD3 (D), CD4 (E), CD8 (F), Foxp3 (G), CD20 (H), and CD56 (I)



Abbreviations: H&E = hematoxylin and eosin; PD-1 = programmed cell death 1; PD-L1 = programmed death ligand 1.

following: hematoxylin and eosin, PD-L1, PD-1, CD3, CD4, CD8, FOXP3, CD20, and CD56 (Supplemental Table 3). In univariate analyses, PD-1 and PD-L1 cutoffs were selected on the basis of what evenly split the sample. There was no significant association between PD-1/PD-L1 staining using 5% and 10% cutoffs with PFS or OS (Supplemental Table 4). Multivariate analyses including the covariates TMB and PD-1/PD-L1 expression are shown in Supplemental Table 5. TMB was significantly associated with PD-L1 tumor cell staining > 20% and PD-L1 immune cell staining > 5%. Other markers including CD3, CD4, CD8, FOXP3, CD20, and CD56 were not significantly associated with survival.

Discussion

Clinical validation of TMB using comprehensive genomic profiling is critical to explore TMB for widespread use as a viable biomarker for response to immune checkpoint blockade in NSCLC. Existing biomarkers including PD-L1 staining and tumor lymphocyte infiltration into the microenvironment have shown early promise as predictive biomarkers.[23-25] However, limitations remain with respect to defining adequate thresholds, standardized clinical use, and missing patients who may still benefit from therapy.[26] The primary aims of our study were to examine predictors of TMB score, correlate TMB with DNA repair mutations, and associate TMB with patient survival for patients treated with immune checkpoint blockade.

In terms of clinical predictors, we found that a history of smoking was significantly associated with higher TMB scores. This finding has been validated by other work previously.[2] The mechanism of this relates to mutagens inducing DNA damage, and therefore a higher number of DNA mutations. Interestingly, TMB was not correlated with number of treatment lines before tissue collection, stage of disease, or number of metastatic sites. This finding suggests that TMB may be associated with DNA damage as opposed to advanced disease.

TMB was further correlated with mutations available on reports to clinical providers. TMB was significantly associated with potentially functional mutations and number of VUS. However, driver mutations including *EGFR* and *KRAS* were not associated with TMB score. The few *ALK* and *ROS1* fusions observed in our sample also tended to have low TMB scores. Prior work has also demonstrated that driver mutations are associated with low TMB.[27] We further examined the correlation between a list of direct and indirect DNA repair mutations. As we hypothesized, in both cases, mutations in DNA repair mutations predicted higher TMB scores. The mechanism of this is likely related to genomic instability associated with impaired DNA repair mechanisms, in part due to oncogene-induced DNA replication stress.[28] Specifically, higher TMB is associated with an increased number of immunogenic neoantigens.[29] This is particularly pronounced in patients with DNA repair mutations who generate a greater number of neoantigens for T cell reactivity.[8] On this basis, there is likely an increase of $CD8^+$ T cells at baseline and during therapy in patients with disease more likely to respond to immune checkpoint blockade, but we were unable to confirm this hypothesis with our limited sample size of patients with available tissue.[30]

In the subset of patients treated with immune checkpoint blockade, higher TMB was associated with significantly longer OS using our predefined TMB threshold, but not PFS. Larger sample

sizes will need to explore why TMB did not appear to predict PFS. One potential mechanistic explanation for this can be delayed effect of immunotherapy, or pseudoprogression. In addition, prior research has shown that nivolumab did not increase PFS, but only OS when compared to standard chemotherapy with docetaxel for advanced nonsquamous NSCLC (CheckMate 057).[31] Interestingly, in multivariate analysis, stage of disease, number of metastatic sites, and other clinical variables did not predict response to immune checkpoint blockade. The finding that only TMB predicted longer OS for patients treated with anti−PD-1/PD-L1 correlates with recent trial data indicating that patients with MSI-high colorectal and other tumor types respond well to immune checkpoint blockade.[18,19] In addition, recent phase 3 data demonstrated that high TMB score was a predictor of PFS for nivolumab and ipilimumab in patients with NSCLC.[32] TMB may serve as an indirect marker to predict neoantigens and an influx of a greater number of cytotoxic T lymphocytes to the tumor.[33]

There were several limitations of our study. First, the study was retrospective and may be prone to unknown bias. Second, the sample size for our tissue IHC studies were limited because they were based on samples with available tissue. Therefore, our conclusions from these analyses should be viewed as exploratory. Third, we combined several anti−PD-1/PD-L1 therapies in our analyses, and further similar studies may need to explore each anti−PD-1/PD-L1 antibody separately. Fourth, it is possible that number of mutations, as opposed to DNA repair mutations in particular, biased this association with TMB, but this is relatively unlikely given the sequencing length and number of genes in the comprehensive genomic profiling panel. Last, our findings reflect comprehensive genomic profiling from a single tissue NGS company and therefore may not apply to other commercially available sequencing platforms.

Conclusion

We validated the feasibility of using commercial tissue TMB determination as a biomarker to predict response to immune checkpoint blockade in NSCLC. Higher TMB score was correlated with smoking history and number of DNA repair mutations. Furthermore, higher TMB was associated with prolonged OS for patients treated with immune checkpoint blockade, but not PFS. Further studies are needed to validate these findings across other commercial NGS platforms.

Clinical Practice Points

- Treatment with immune checkpoint blockade has produced significant responses in a proportion of patients across various tumor types in the metastatic setting.
- Evaluating biomarkers for response to immune checkpoint blockade in NSCLC is critical to determine which patients have disease that will optimally respond to therapy.
- TMB has emerged a potential biomarker in clinical trials for response to immune checkpoint blockade.
- We performed a retrospective analysis using a commercially available NGS platform to determine clinical predictors of TMB score and the association of TMB with DNA repair mutations and tissue IHC.
- Higher TMB was significantly associated with a history of smoking, but not other clinical variables, and greater number of DNA repair mutations.
- In addition, higher TMB predicted longer OS, but not PFS, for patients treated with immune checkpoint blockade.
- For a small subset of patients, tissue IHC for PD-1, PD-L1, and other markers was not associated with OS.
- These and other data indicate the potential to utilize commercial NGS platforms to measure TMB in clinical practice as a viable biomarker for response to immune checkpoint blockade in NSCLC.

Acknowledgment

Supported in part by Developmental Therapeutics Funds, Northwestern University Feinberg School of Medicine.

Disclosure

Y.K.C. has received consulting fees from Foundation Medicine, Guardant Health, Biodesix, AstraZeneca, and Genentech. The other authors have stated that they have no conflict of interest.

Supplemental Data

A supplemental figure and tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2018.09.008.

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Supplemental Table 1 TMB by Patient Characteristics and Mutation Status Based on TMB Above and Below Median

Characteristic	Variable	TMB (Mutations per Megabase)			*P*
		TMB (≤8)	TMB (> 8)	All	
Smoker	Minimal/never	22	1	23	< .013[a]
	Current/former	11	38	49	
	All	33	39	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.311
No. of prior lines of treatment	0-1	30	34	64	.900
	2+	3	5	8	
	All	33	39	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.456
Stage of disease	I-III	7	8	15	1.000
	IV	26	31	57	
	All	33	39	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.240
Metastatic sites	0	7	9	16	.970
	1	17	19	36	
	2+	9	11	20	
	All	33	39	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.377
Presence of *EGFR/KRAS* mutations	Yes	14	9	33	.133
	No	19	30	49	
	All	33	39	72	
	Kaplan-Meier log-rank test (immunotherapy only)				.297
No. of potential functional direct + indirect DNA repair mutations	Mean	0.66 (n = 33)	1.15 (n = 39)	0.93 (n = 72)	.002[b]
No. of VUS direct + indirect DNA repair mutations	Mean	1.15 (n = 33)	2.05 (n = 39)	1.64 (n = 72)	.005[b]
No. of direct DNA repair mutations	Mean	1.00 (n = 33)	1.92 (n = 39)	1.50 (n = 72)	.005[b]
No. of all DNA repair mutations	Mean	1.82 (n = 33)	3.21 (n = 39)	2.57 (n = 72)	< .001[b]
No. of potentially functional mutations	Mean	4.85 (n = 33)	6.97 (n = 39)	6.00 (n = 72)	.017[b]
No. of VUS mutations	Mean	7.70 (n = 33)	18.59 (n = 39)	13.60 (n = 72)	< .001[b]
Total reported mutations	Mean	12.55 (n = 33)	25.56 (n = 39)	19.60 (n = 72)	< .001[b]

Abbreviations: TMB = tumor mutational burden; VUS = variant of unknown significance.
[a]Pearson chi-square test.
[b]Welch 2-sample *t* test.

Supplemental Table 2 DNA Repair Genes and Pathways in Cancer

DNA Repair Genes	DNA Repair Pathways
Direct	
ATM	4
ATR	5
RPA1	3,4
RPA2	3,4
RPA3	3,4
RPA4	3,4
BRCA1	4
BRCA2	4,5
MRE11A	4
RFC1	1,2,3
RFC2	1,2,3
RFC3	1,2,3
RFC4	1,2,3
RFC5	1,2,3
XRCC1	1
XRCC2	4
XRCC3	4
XRCC4	6
XRCC5	6
XRCC6	6
PCNA	1,2,3
PARP1	1
PARP2	1
PARP3	1
PMS1	2
PMS2	2
MLH1	2
MLH3	2
MSH2	2
MSH3	2
MSH4	2
MSH5	2
MSH6	2
EX01	2
NBN	4
RAD23A	3
RAD23B	3
RAD50	4
RAD51	4
RAD51B	4
RAD51C	4,5
RAD51D	4
RAD52	4
RAD54B	4
RAD54L	4
CHEK1	5
CHEK2	4

Supplemental Table 2 Continued

DNA Repair Genes	DNA Repair Pathways
FANCI	5
FANCD2	5
FANCA	5
FANCB	5
FANCC	5
FANCE	5
FANCF	5
FANCL	5
FANCG	5
FANCM	5
ERCC1	3,5
ERCC2	3
ERCC3	3
ERCC4	3
ERCC5	3
ERCC6	3
ERCC8	3
APEX1	1
APEX2	1
FEN1	1
XPA	3
XAB2	3
XPC	3
GTF2H1	3
GTF2H2	3
GTF2H3	3
GTF2H4	3
GTF2H5	3
PALB2	4,5
PRKDC	6
LIG1	1,3
LIG3	1,3
Lig4	6
FAAP24	5
BRIP1	5
SLX4	5
FAN1	5
MUS81	4,5
EME1	4,5
POLE	1,3
POLD1	1,2,3
MGMT	7
OGG1	1
UNG	1
SMUG1	1
MBD4	1
TDG	1
MUTYH	1
NTHL1	1

Supplemental Table 2 Continued

DNA Repair Genes	DNA Repair Pathways
MPG	1
NEIL1	1
NEIL2	1
NEIL3	1
PNKP	1
APLF	1
ALKBH2	7
ALKBH3	7
CETN2	3
DDB1	3
DDB2	3
PMS2P3	2
CDK7	3
CCNH	3
MNAT1	3
UVSSA	3
MMS19	3
DMC1	4
SHFM1	4
RBBP8	4
SLX1A	4
SLX1B	4
GEN1	4
FAAP20	5
DCLRE1C	6
NHEJI	6
Indirect[a]	
PAXIP1	
BLM	
MLL3	
CRIP1	
CDK12	
BAP1	
BARD1	
WRN	
BUB1	
CENPE	
ZW10	
TTK	
KNTC1	
AURKB	
POLB	
POLH	
POLQ	
TDP1	
TDP2	
NUDT1	
DUT	
RRM2B	
POLG	
REV3L	
MAD2L2	
REV1	
POLI	
POLK	
POLL	
POLM	
POLN	
TREX1	
TREX2	
APTX	
SPO11	
ENDOV	
UBE2A	
UBE2B	
UBE2V2	
UBE2N	
RAD18	
SHPRH	
HLTF	
RNF168	
SPRTN	
RNF8	
RNF4	
H2AFX	
CHAF1A	
SETMAR	
RECQL4	
MPLKIP	
DCLRE1A	
DCLRE1B	
PRPF19	
RECQL	

Supplemental Table 2 Continued

DNA Repair Genes	DNA Repair Pathways
RECQL5	
HELQ	
RDM1	
NABP2	
ATRIP	
MDC1	
RAD1	
RAD9A	
HUS1	
RAD17	
TP53	
TP53BP1	
TOPBP1	
CLK2	
PER1	

Listed are 193 DNA repair genes separated as direct DNA repair genes or indirect DNA repair genes (regulators of genomic stability). DNA repair pathways for each direct DNA repair gene are listed: 1 = base excision repair, 2 = mismatch repair, 3 = nucleotide excision repair, 4 = homologous recombination, 5 = Fanconi anemia, 6 = nonhomologous end joining, 7 = direct reversal.

[a]Genes indirectly associated with maintenance of genomic stability.

Supplemental Table 3 Immunohistochemistry Staining

Antibody/Tumor Marker	Titer, Dilution, or RTU	Source/Company
PD-1	1:250	Abcam
PD-L1	1:200	Cell Signaling
FOXP3	1:50	Abcam
CD4	RTU	Leica
CD8	RTU	Leica
CD56	1:50	Dako
CD3	1:300	Dako
CD20	1:2000	Dako

Abbreviation: RTU, ready-to-use.

Supplemental Table 4 Survival Analyses by PD-1/PD-L1 Immunohistochemical Staining (N = 14)

Survival	Analysis	Results
OS (PD-1 immune—5%)	Proportional hazards regression	HR = 1.60 95% CI = 0.35-7.27 *P* = .542
PFS (PD-1 immune—5%)	Proportional hazards regression	HR = 1.11 95% CI = 0.29-4.20 *P* = .882
OS (PD-1 immune—10%)	Proportional hazards regression	HR = 1.04 95% CI = 0.23-4.68 *P* = .961
PFS (PD-1 immune—10%)	Proportional hazards regression	HR = 0.89 95% CI = 0.22-3.61 *P* = .872
OS (PD-L1 tumor—20%)	Proportional hazards regression	HR = 1.19 95% CI = 0.26-5.43 *P* = .823
PFS (PD-L1 tumor—20%)	Proportional hazards regression	HR = 1.27 95% CI = 0.33-4.80 *P* = .728
OS (PD-L1 immune—5%)	Proportional hazards regression	HR = 0.57 95% CI = 0.13-2.57 *P* = .465
PFS (PD-L1 immune—5%)	Proportional hazards regression	HR = 0.54 95% CI = 0.14-2.03 *P* = .360
OS (PD-L1 immune—10%)	Proportional hazards regression	HR = 1.46 95% CI = 0.32-6.59 *P* = .623
PFS (PD-L1 immune—10%)	Proportional hazards regression	HR = 0.48 95% CI = 0.10-2.41 *P* = .374

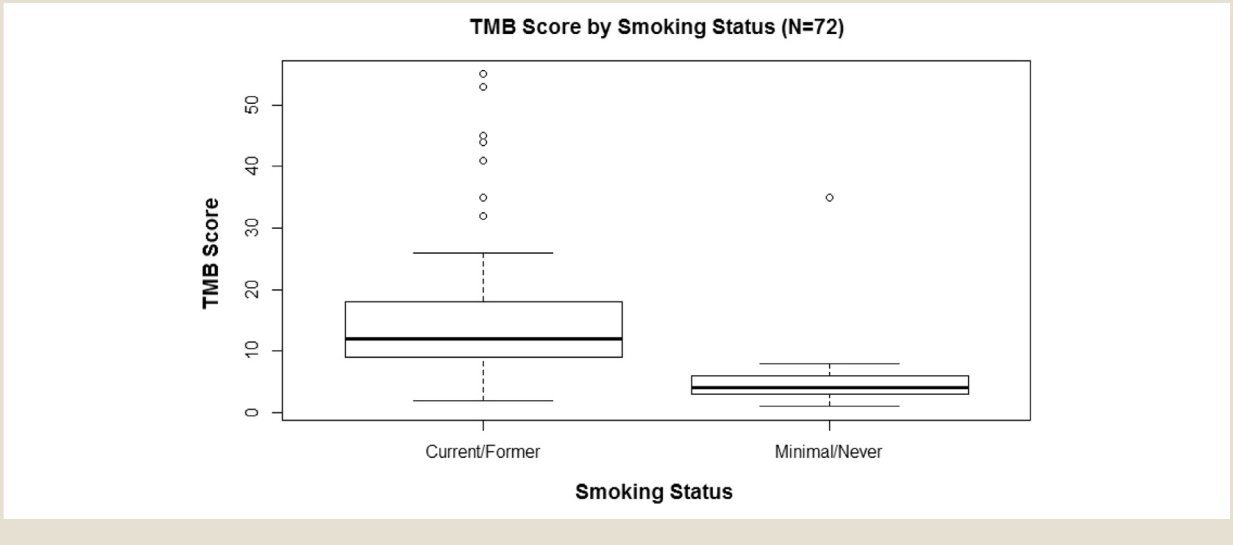
Abbreviations: CI = confidence interval; HR = hazard ratio; OS = overall survival; PD-1 = programmed cell death 1; PD-L1 = programmed death ligand 1; PFS = progression-free survival.

Supplemental Table 5 Multivariate Analyses Based on TMB Score and PD-1/PD-L1 Staining (N = 14)

Characteristic	Predictor	HR (95% CI)	*P*
Overall Survival (N = 14)			
TMB + PD-1 immune cell (5%)	TMB	0.13 (0.01, 1.24)	.077
	PD-1	1.17 (10.25, 5.55)	.842
TMB + PD-1 immune cell (10%)	TMB	0.11 (0.01, 1.06)	.057
	PD-1	0.66 (0.14, 3.13)	.597
TMB + PD-L1 tumor cell (20%)	TMB	0.05 (0.00, 0.94)	.045*
	PD-L1	0.27 (0.02, 3.03)	.287
TMB + PD-L1 immune cell (5%)	TMB	0.09 (0.01, 0.87)	.038*
	PD-L1	0.33 (0.07, 1.68)	.183
TMB + PD-L1 immune cell (10%)	TMB	0.09 (0.01, 1.02)	.052
	PD-L1	0.54 (0.09, 3.20)	.499
Progression-Free Survival (N = 12)			
TMB + PD-1 immune cell (5%)	TMB	2.28 (0.37, 14.07)	.376
	PD-1	1.60 (0.34, 7.51)	.553
TMB + PD-1 immune cell (10%)	TMB	2.13 (0.31, 14.91)	.445
	PD-1	1.35 (0.24, 7.46)	.730
TMB + PD-L1 tumor cell (20%)	TMB	2.53 (0.40, 15.83)	.321
	PD-L1	1.92 (0.41, 8.99)	.406
TMB + PD-L1 immune cell (5%)	TMB	1.28 (0.19, 8.82)	.799
	PD-L1	0.61 (0.12, 3.03)	.543
TMB + PD-L1 immune cell (10%)	TMB	0.63 (0.02, 24.98)	.804
	PD-L1	0.32 (0.01, 12.59)	.543

Abbreviations: CI = confidence interval; HR = hazard ratio; PD-1 = programmed cell death 1; PD-L1 = programmed death ligand 1; TMB = tumor mutational burden.
*Statistically significant.

Supplemental Figure 1 TMB Score by Smoking Status (N = 72)



Abbreviation: TMB = tumor mutational burden.