Tumor Mutational Burden and *PTEN* Alterations as Molecular Correlates of Response to PD-1/L1 Blockade in Metastatic Triple-Negative Breast Cancer

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ABSTRACT

Purpose: Few patients with metastatic triple-negative breast cancer (mTNBC) benefit from immune checkpoint inhibitors (ICI). On the basis of immunotherapy response correlates in other cancers, we evaluated whether high tumor mutational burden (TMB) ≥10 nonsynonymous mutations/megabase and *PTEN* alterations, defined as nonsynonymous mutations or 1 or 2 copy deletions, were associated with clinical benefit to anti-PD-1/L1 therapy in mTNBC.

Experimental Design: We identified patients with mTNBC, who consented to targeted DNA sequencing and were treated with ICIs on clinical trials between April 2014 and January 2019 at Dana-Farber Cancer Institute (Boston, MA). Objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) were correlated with tumor genomic features.

Results: Sixty-two women received anti-PD-1/L1 inhibitors alone (23%) or combined with targeted therapy (19%) or chemotherapy (58%). High TMB (18%) was associated with significantly longer PFS (12.5 vs. 3.7 months; $P = 0.04$), while *PTEN* alterations (29%) were associated with significantly lower ORR (6% vs. 48%; $P = 0.01$), shorter PFS (2.3 vs. 6.1 months; $P = 0.01$), and shorter OS (9.7 vs. 20.5 months; $P = 0.02$). Multivariate analyses confirmed that these associations were independent of performance status, prior lines of therapy, therapy regimen, and visceral metastases. The survival associations were additionally independent of PD-L1 in patients with known PD-L1 and were not found in mTNBC cohorts treated with chemotherapy ($n = 90$) and non-ICI regimens ($n = 169$).

Conclusions: Among patients with mTNBC treated with anti-PD-1/L1 therapies, high TMB and *PTEN* alterations were associated with longer and shorter survival, respectively. These observations warrant validation in larger datasets.

Introduction

Patients with metastatic triple-negative breast cancer (mTNBC) have limited treatment options and a poor prognosis with a median overall survival of 13 to 18 months (1). Despite the success of PD-1/L1 inhibitors in other cancers, their single-agent efficacy in mTNBC is low: monotherapy responses range from 5% in unselected cohorts to 25% in patients with PD-L1-positive and/or treatment-naïve disease (2–5).

Recently the IMpassion130 study showed that adding atezolizumab to nab-paclitaxel improved progression-free survival (PFS) and overall survival (OS) in patients with treatment-naïve PD-L1-positive mTNBC (6). On the basis of these data, this combination was granted accelerated approval for the treatment of mTNBC with ≥ 1% PD-L1 expression on immune cells (6). However, there are still open questions surrounding the broad utility of PD-L1 testing for selecting patients for immune checkpoint inhibitors (ICI), and additional biomarkers to predict benefit are being investigated.

Given its close association with neoantigen burden and T-cell infiltration (7–10), tumor mutational burden (TMB) is one marker of tumor antigenicity (11, 12). A growing body of evidence has shown that high TMB correlates with response to PD-1/L1 inhibitors (13–18), but not non-ICI therapies (18), across different cancer types. Prior work has also shown that loss of the tumor suppressor *PTEN* may be linked to poor responses to PD-1 blockade in patients with melanoma and uterine leiomyosarcoma (19, 20) and *PTEN* is frequently altered in TNBC (21). However, in mTNBC, data about the relationship of high TMB and *PTEN* alterations with immunotherapy response are lacking. Therefore, the aim of this work was to evaluate the association of high TMB and *PTEN* alterations with ICI efficacy in patients with mTNBC.

Materials and Methods

Study cohort

We included patients with mTNBC, defined as the absence of *HER2* amplification and estrogen and progesterone receptor expression

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

This study investigates whether high tumor mutational burden (TMB) and *PTEN* alterations affect response to anti-PD-1/L1 therapies among patients with metastatic triple-negative breast cancer (mTNBC). High TMB and PTEN alterations correlate with clinical responses to immune checkpoint inhibitors in other tumors, but these associations have not been well studied in breast cancer. In this cohort of 62 women with mTNBC treated with anti-PD-1/L1 therapies, high TMB was associated with improved progression-free survival, while *PTEN* alterations were associated with reduced responses and progression-free and overall survival. These associations were independent of clinical confounders, as well as PD-L1 in patients with known PD-L1, and were not found in patients treated with nonimmunotherapy regimens. Overall, high TMB and *PTEN* alterations were associated with better and worse outcomes, respectively, among patients with mTNBC treated with anti-PD-1/L1 therapies. These results warrant validation in larger prospective studies, including ongoing trials investigating whether AKT inhibitors reverse resistance to PD-1 blockade.

(<1%), treated with anti-PD-1/L1 therapy as monotherapy or in combination with chemotherapy or targeted therapy at the Dana-Farber Cancer Institute (Boston, MA). Eligible patients prospectively provided written consent for research tumor genomic sequencing under protocol #11-104 and underwent targeted DNA sequencing (OncoPanel) on either an archival metastatic (47%), primary (45%), local recurrence (6%), or unknown (2%) tumor sample. This current project was performed after receiving approval by the Dana-Farber/Harvard Cancer Center Institutional Review Board (DF/HCC Protocol #18-082) and conducted in accordance with the ethical guidelines outlined by the Belmont Report.

Genomic and PD-L1 assessment

Performed in a Clinical Laboratory Improvement Amendments-certified laboratory environment, OncoPanel uses targeted exome sequencing to detect copy number alterations, single nucleotide variants, and translocations across the full coding regions and selected intronic regions of a predefined subset of cancer-related genes with tumor-derived DNA (22). In this study, the majority of patients (n = 44) had testing done using OncoPanel version 2 (23), which targets the full coding regions or selected intronic regions of 335 genes (exonic coverage region = 0.82 megabase [Mb]). Four patients were assessed with OncoPanel version 1 (24, 25), which targets 305 genes (exonic coverage region = 0.75 Mb), and 14 patients were evaluated with OncoPanel version 3 (22), targeting 507 genes (exonic coverage region = 1.3 Mb). TMB was calculated as the number of nonsynonymous somatic mutations per megabase of exonic sequence data covered by each panel. All nonsynonymous mutations, including nonsense, missense, frame-shift, splice site, and nonstop changes, were considered. High TMB was defined as ≥10 mutations/Mb, and *PTEN* alterations were defined as nonsynonymous mutations or 1 or 2 copy deletions, based on prior work showing that partial *PTEN* deletions are associated with poor prognosis in breast cancer (26). All patients but one had OncoPanel performed on samples collected before exposure to immunotherapy.

PD-L1 expression was centrally evaluated (Q2 Solutions) during screening on patients treated with pembrolizumab (n = 37) using the PD-L1 IHC22C3 pharmDx kit (Agilent, Carpinteria, CA). Expression was measured by the combined positive score (CPS), defined as the ratio of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) to the total number of tumor cells. PD-L1 positivity was defined as CPS > 1.

Statistical analysis

Responses were prospectively assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 during each clinical trial. PFS was defined as the date of starting immunotherapy to the date of progression, death, or last follow-up. OS was defined as the date of starting immunotherapy until the date of death or last follow-up. Patients alive and without progression at last follow-up were censored for PFS, and those still alive were censored for OS. The associations of high TMB and *PTEN* alterations with objective response rate (ORR), PFS, and OS were assessed with logistic regression for ORR and the Kaplan-Meier method, log-rank tests, and Cox proportional hazards regression for PFS and OS. Multivariate regression models adjusted for the following clinical factors: Eastern Cooperative Oncology Group performance status (ECOG PS) at trial enrollment (≥1 vs. 0), therapy regimen (monotherapy versus combination therapy, which showed the same significant associations as adjustment by individual therapy regimen, data not shown), number of prior systemic metastatic therapies (≥1 vs. 0), and presence of visceral metastasis (yes vs. no). Analyses were performed in RStudio Version 1.2.5001.

Results

Patient characteristics

Between April 2014 and January 2019, 62 women with mTNBC met the inclusion criteria for this analysis. These women were enrolled on 6 different clinical trials with anti-PD-1/L1 therapy: 14 (23%) patients received ICIs as monotherapy [pembrolizumab (n = 7, NCT02447003); atezolizumab (n = 7; NCT01375842)], and 48 (77%) received ICIs in combination with chemotherapy [pembrolizumab plus eribulin (n = 30; NCT02513472); atezolizumab plus nab-paclitaxel (n = 6; NCT01633970)] or targeted therapy [nivolumab plus cabozantinib (n = 9; NCT03316586); pembrolizumab plus niraparib (n = 3; NCT02657889)]. At baseline, the median age was 55 years (range 32-76); 68% had an ECOG PS of 0; 74% had visceral metastasis; and 60% had received one or more prior systemic therapies for metastatic disease (**Table 1**). The median follow-up was 13.5 months, and there were 54 progression events and 44 deaths. Eight patients remained free of disease progression: 3 patients continued on immune checkpoint inhibitor therapy, 3 stopped treatment per protocol after 2 years of therapy, and 2 stopped due to toxicity. Overall, the median PFS and OS for the entire cohort were 4.2 and 16.0 months, respectively.

TMB, PTEN alterations, and PD-L1 status

The median TMB was 6 mutations/Mb, and 12 (18%) patients were classified as having high TMB. The most commonly mutated genes were *TP53* (51; 82%), *BRCA1* (10; 16%), and *ATM* (8; 13%; Supplementary Fig. S1). A total of 18 (29%) patients had *PTEN* alterations, including 10 patients with 1 copy deletions, 6 patients with nonsynonymous alterations, 1 patient with a 2 copy deletion, and 1 patient with a 1 copy deletion and a nonsynonymous alteration. Of the 18 patients with *PTEN* alterations, 3 also had high TMB. Patients with PTEN alterations had the same mean TMB (7.5 vs. 7.3 mutations/Mb) and a higher median TMB (8.2 vs. 5.3 mutations/Mb) than patients without PTEN alterations.

Table 1. Baseline characteristics.

Characteristic	Total ($n = 62$)	Not High TMB ($n = 50$)	High TMB ($n = 12$)	PTEN WT ($n = 44$)	PTEN Altered ($n = 18$)
Age, y, median (range)	55 (32-76)	55 (32-71)	58 (42–76)	56 (32-76)	52 (37-76)
Female, *N* (%)	62 (100)	50 (100)	12 (100)	44 (100)	18 (100)
ECOG-PS, *N* (%)					
0	42 (68)	32 (64)	10 (83)	31 (70)	11 (61)
1	20 (32)	18 (36)	2 (17)	13 (30)	7 (39)
Visceral metastases	46 (74)	35 (70)	11 (92)	31 (70)	15 (83)
Prior therapies for metastatic disease					
Median (range)	1 (0-6)	1 (0-6)	1 (0-2)	1 (0-6)	1 (0-6)
0, *N* (%)	25 (40)	20 (40)	5 (42)	17 (39)	8 (44)
1, *N* (%)	19 (31)	15 (30)	4 (33)	14 (32)	5 (28)
2, *N* (%)	13 (21)	10 (20)	3 (25)	10 (23)	3 (17)
≥3, *N* (%)	5 (8)	5 (10)	0 (0)	3 (7)	2 (11)
Previous therapy, *N* (%)					
Neo(adjuvant) therapy	57 (92)	64 (92)	11 (92)	41 (93)	16 (89)
Taxanes	56 (90)	44 (88)	12 (100)	39 (89)	17 (94)
Anthracycline	51 (82)	40 (80)	11 (92)	36 (82)	15 (83)
Regimen, *N* (%)					
Monotherapy	14 (23)	10 (20)	4 (33)	12 (27)	2 (11)
Combination	49 (77)	40 (80)	8 (67)	32 (73)	16 (89)
PD-L1 status[a], *N* (%)					
Positive	14 (38)	11 (41)	3 (30)	11 (42)	3 (27)
Negative	23 (62)	16 (59)	7 (70)	15 (58)	8 (73)
ORR (%)	35	30	58	48	6

Abbreviations: ECOG-PS, Eastern Cooperative Oncology Group performance status; ORR, objective response rate; TMB, tumor mutational burden; WT, wild type; yrs, years

[a]The PD-L1 analysis included 37 patients.

PD-L1 expression was assessed on 37 tumors and was positive in 14 (38%) cases (**Table 1**). The cohort of patients with known PD-L1 status was generally representative of the overall cohort (Supplementary Table S1), except for a slightly higher portion of patients receiving immunotherapy as first-line treatment for metastatic disease (54% vs. 40% in the overall cohort). The ORR was numerically higher in PD-L1–positive tumors (57%) versus PD-L1–negative tumors (35%; $P = 0.3$ by Fisher exact test). Among tumors with high TMB and known PD-L1 status ($n = 10$), 3 (30%) were PD-L1 positive, while among those without high TMB ($n = 27$), 11 (41%) tumors were PD-L1 positive ($P = 0.7$ by Fisher exact test). The median TMB was also not statistically different between patients with PD-L1–positive and negative tumors (Wilcoxon test $P = 0.7$; Supplementary Fig. S2). Likewise, among tumors with *PTEN* alterations and known PD-L1 status, 3 (27%) were PD-L1 positive, while among those without *PTEN* alterations, 11 (42%) tumors were PD-L1 positive ($P = 0.5$ by Fisher exact).

High TMB and PTEN alterations associate with ORR, PFS, and/or OS in ICI cohort

The ORR was numerically higher for patients with high TMB (58%) versus those with low TMB (30%; $P = 0.09$ by Fisher's exact) and significantly lower for patients with *PTEN* alterations (6%) versus those without *PTEN* alterations (48%; $P = 0.001$ by Fisher exact test). Univariate analyses showed that patients with high TMB had a 3.3 times higher odds of response than patients without high TMB [OR = 3.27; 95% confidence interval (CI), 0.90-12.67; $P = 0.07$], and patients with *PTEN* alterations had a 94% lower odds of response than patients without *PTEN* alterations (OR = 0.06; 95% CI, 0.003–0.36; $P = 0.01$). In multivariate analyses, patients with high TMB had a 4 times higher odds of response than patients without high TMB (OR = 4.32; 95% CI, 1.05–19.89; $P = 0.05$), and patients with *PTEN* alterations had a 94% lower odds of response than patients without *PTEN* alterations (OR = 0.06; 95% CI, 0.003–0.34; $P = 0.01$), independent of clinical factors. In the 37 patients with known PD-L1 status, high TMB was associated with a numerically higher odds of response (OR = 3.17; 95% CI, 0.61-19.57; $P = 0.18$), and PTEN alterations were still associated with a significantly lower odds of response (OR = 0.07; 95% CI, 0.003–0.51; $P = 0.02$) after adjustment for clinical factors and PD-L1.

Patients with high TMB experienced longer median PFS (12.5 months, 95% CI 6.3–not reached) versus patients without high TMB (3.7 months, 95% CI 2.3–5.8, log-rank $P = 0.03$; **Fig. 1A**), while patients with *PTEN* alterations experienced shorter median PFS (2.3 months, 95% CI 2.0–4.2) versus patients without *PTEN* alterations (6.1 months; 95% CI 3.9–9.1, log-rank $P = 0.01$; **Fig. 1B**). Similarly, patients with high TMB also experienced longer survival (median OS 29.2 months, 95% CI 20.5–not reached) versus patients without high TMB (median OS, 14.2 months; 95% CI, 11.6–24.5, log-rank $P = 0.06$; **Fig. 1C**), while patients with *PTEN* alterations experienced shorter survival (median OS, 9.7 months; 95% CI, 5.0–34.6) versus patients without *PTEN* alterations (median OS, 20.5 months; 95% CI, 13.8–33.2, log-rank $P = 0.01$; **Fig. 1D**).

In univariate analyses, patients with high TMB had significantly longer PFS (HR, 0.46; 95% CI, 0.22–0.95; $P = 0.04$) and numerically higher OS (HR = 0.48; 95% CI, 0.22–1.05; $P = 0.07$) versus those without high TMB, while patients with *PTEN* alterations had significantly shorter PFS (HR = 2.04; 95% CI, 1.15–3.63; $P = 0.01$) and significantly worse OS (HR = 2.19; 95% CI, 1.16–4.13; $P = 0.02$) versus patients without *PTEN* alterations (**Table 2**). Multivariate analyses confirmed that patients with high TMB experienced significantly longer PFS (HR = 0.42; 95% CI, 0.19–0.93; $P = 0.03$) and numerically

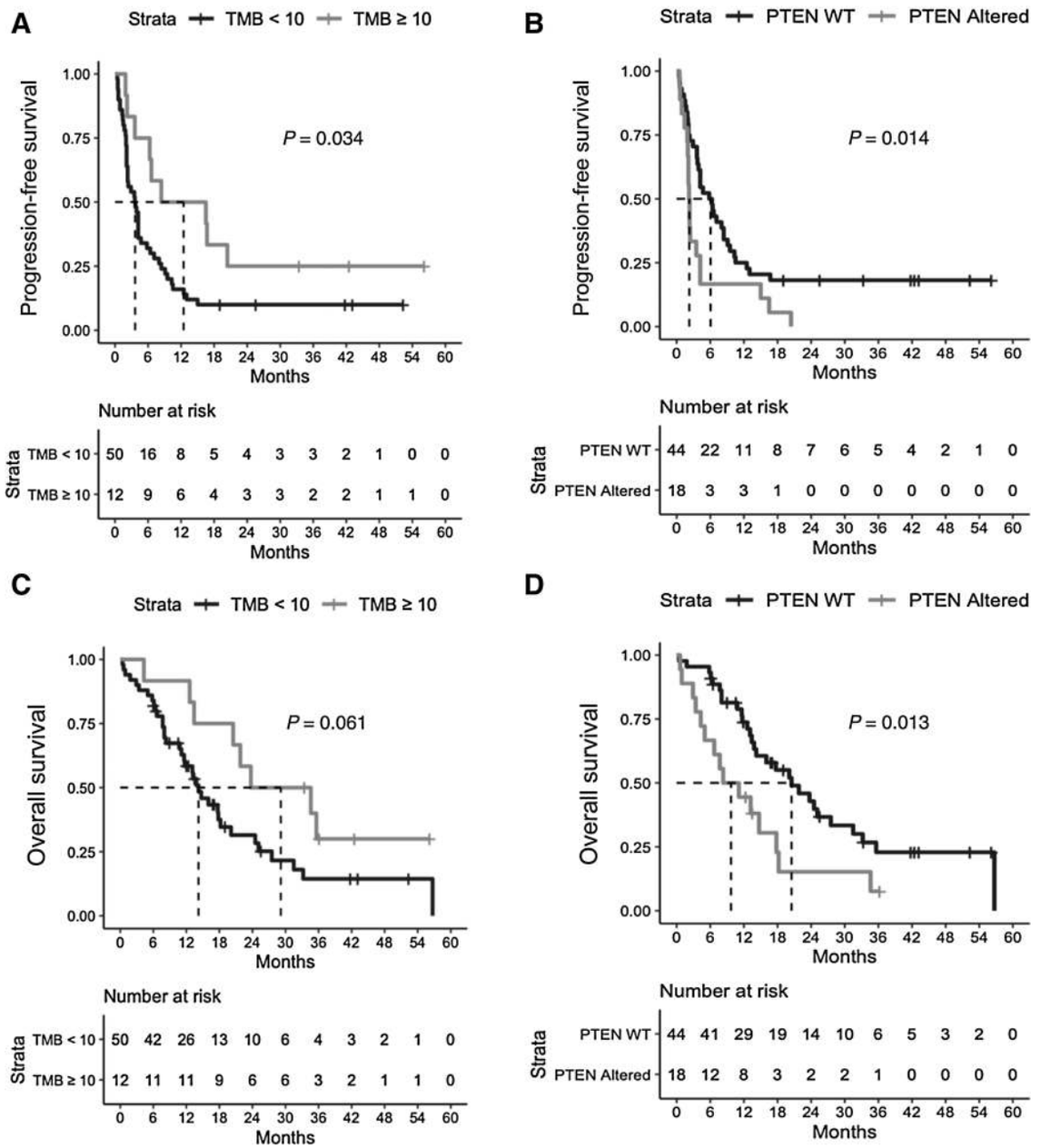

Figure 1.

Kaplan–Meier curves for progression-free and overall survival by biomarker status in anti-PD-1/L1-treated cohort. Progression-free survival (**A**) and overall survival (**C**) by tumor mutational burden status (<10 vs. ≥10 mutations/megabase), progression-free survival (**B**), and overall survival (**D**) by *PTEN* alteration (absent vs. present). Abbreviations: TMB, tumor mutational burden; WT, wild type.

higher OS (HR = 0.54; 95% CI, 0.23–1.26; $P = 0.15$), while patients with *PTEN* alterations had significantly shorter PFS [HR = 2.71; 95% CI, 1.44–5.10; $P = 0.002$) and OS (HR = 3.26; 95% CI, 1.59–6.68; $P = 0.001$), independent of clinical factors (**Fig. 2**). In two final multivariate models, the first including both high TMB and *PTEN* alterations in addition to clinical factors and the second adding PD-L1 status to the first, both high TMB and *PTEN* alterations remained significantly associated with longer and shorter PFS, respectively, and *PTEN* alterations remained significantly associated with shorter OS (**Table 2**). Alterations in other immunotherapy-related pathways did not show statistically significant associations with response (See Results and Supplementary Table S2 in the Supplementary Data).

To explore whether high TMB and *PTEN* alterations are predictive or prognostic, we examined the association of high TMB and *PTEN* alterations with PFS and OS in previously published mTNBC cohorts treated with chemotherapy ($n = 90$) and non-ICI regimens ($n = 169$, see Methods in the Supplementary Data; ref. 27). We analyzed PFS and OS in 90 patients with mTNBC, who underwent pretreatment targeted

Table 2. Univariate and multivariate analysis of factors associated with progression-free and overall survival following immune checkpoint inhibitor–based therapies.

	Progression-free survival		Overall survival	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Univariate models ($n = 62$)				
High TMB	0.46 (0.22–0.95)	0.04	0.48 (0.22–1.05)	0.07
PTEN alteration	2.04 (1.15–3.63)	0.01	2.19 (1.16–4.13)	0.02
ECOG PS	1.72 (0.58–3.05)	0.06	3.24 (1.74–6.04)	0.0002
Previous lines of therapy	1.57 (0.91–2.71)	0.11	1.39 (0.75–2.56)	0.30
Regimen	1.31 (0.69–2.49)	0.41	1.14 (0.57–2.26)	0.72
Visceral metastases	1.10 (0.59–2.06)	0.77	1.11 (0.56–2.20)	0.77
High TMB multivariate model ($n = 62$)				
High TMB	0.42 (0.19–0.93)	0.03	0.54 (0.23–1.26)	0.15
ECOG PS	1.64 (0.89–3.00)	0.11	2.93 (1.53–5.63)	0.001
Previous lines of therapy	1.57 (0.89–2.76)	0.12	1.29 (0.68–2.45)	0.43
Regimen	1.83 (0.90–3.73)	0.10	1.54 (0.71–3.35)	0.27
Visceral metastases	1.50 (0.79–2.86)	0.22	1.35 (0.65–2.82)	0.42
PTEN Alteration multivariate model ($n = 62$)				
PTEN alteration	2.71 (1.44–5.10)	0.002	3.26 (1.59–6.68)	0.001
ECOG PS	1.98 (1.08–3.62)	0.03	3.84 (1.99–7.39)	0.00006
Previous lines of therapy	1.83 (1.02–3.29)	0.04	1.57 (0.81–3.06)	0.18
Regimen	1.84 (0.90–3.74)	0.09	1.69 (0.78–3.62)	0.18
Visceral metastases	1.21 (0.64–2.30)	0.56	1.05 (0.52–2.14)	0.88
High TMB and PTEN alteration multivariate model ($n = 62$)				
High TMB	0.37 (0.17–0.80)	0.01	0.48 (0.20–1.14)	0.10
PTEN alteration	3.07 (1.62–5.81)	0.0006	3.44 (1.67–7.07)	0.0008
ECOG PS	1.67 (0.91–3.05)	0.10	3.34 (1.72–6.49)	0.0004
Previous lines of therapy	1.79 (1.00–3.21)	0.05	1.48 (0.76–2.87)	0.25
Regimen	2.38 (1.15–4.96)	0.02	2.05 (0.92–4.55)	0.08
Visceral metastases	1.45 (0.75–2.78)	0.27	1.28 (0.61–2.67)	0.52
High TMB, PTEN alteration, and PD-L1 multivariate model ($n = 37$)				
High TMB	0.37 (0.15–0.91)	0.03	0.42 (0.16–1.11)	0.08
PTEN alteration	2.82 (1.16–6.85)	0.02	2.93 (1.14–7.53)	0.03
PD-L1	0.78 (0.31–1.93)	0.59	1.28 (0.48–3.43)	0.63
ECOG PS	1.16 (0.47–2.87)	0.74	2.27 (0.90–5.73)	0.08
Previous lines of therapy	1.41 (0.62–3.18)	0.41	1.39 (0.59–3.30)	0.45
Regimen	1.18 (0.41–3.42)	0.76	1.70 (0.58–4.97)	0.33
Visceral metastases	0.71 (0.27–1.88)	0.50	1.00 (0.39–2.59)	0.99

Note: High TMB: ≥ 10 mutations/megabase vs. < 10 mutations/megabase; PTEN alteration (nonsynonymous mutation or 1 or 2 copy deletion): present vs. absent; PD-L1: positive vs. negative; ECOG PS: ≥ 1 vs. 0; Previous lines of therapy: ≥ 1 vs. 0; Regimen: monotherapy vs. combination therapy; Visceral metastases: present vs. absent.

Abbreviations: CI, confidence interval; ECOG-PS: Eastern Cooperative Oncology Group performance status; TMB, tumor mutational burden.

DNA sequencing (MSK-IMPACT; 57% with 410-gene version 2 covering 1.016478 Mb of exon) on either a metastatic (62%) or primary (34%) tumor sample and were treated with single-agent chemotherapy (71%) or combination chemotherapy (29%) that was not labeled as neoadjuvant or adjuvant treatment (27). We also analyzed OS in 169 patients with mTNBC treated with regimens that did not include immune checkpoint inhibitors (ICIs) (27). In these cohorts, neither high TMB nor *PTEN* alterations were associated with PFS and/or OS in univariate or multivariate analyses adjusted for prior lines of metastatic therapy (≥1 vs. 0) and chemotherapy regimen (monotherapy vs. combination; Supplementary Table S3; Supplementary Figs. S3-S5).

Discussion

Prior work has shown that high TMB is associated with higher response rates and prolonged PFS following anti-PD-1/L1 therapy across different tumor types (7, 10, 13–15, 28–32), and that *PTEN* loss is linked to inferior responses to PD-1 blockade and resistance to T-cell–mediated immunotherapy (19, 20). However, the relationship of high TMB and *PTEN* alterations with immunotherapy response in mTNBC has not previously been well characterized. In this mTNBC cohort with comprehensive clinical and genomic annotations, we observed a significant positive association of high TMB with longer PFS and a significant negative association of *PTEN* alterations with lower ORR and shorter PFS and OS among patients with mTNBC treated with anti-PD-1/L1 therapies. Importantly, these associations remained significant after adjustment for PD-L1 status and clinical confounders, including monotherapy versus combination regimen and first versus higher treatment line, indicating that these factors are unlikely explanations for the observed associations.

The identification of biomarkers that predict clinical benefit to ICI-based therapies is needed to better select patients who are more likely to benefit from therapy and spare patients less likely to benefit from immunotherapy toxicity. To date, there are only 2 validated and clinically available biomarkers that predict benefit to ICI: mismatch repair deficiency (dMMR) (33) and PD-L1 expression (34). Yet dMMR

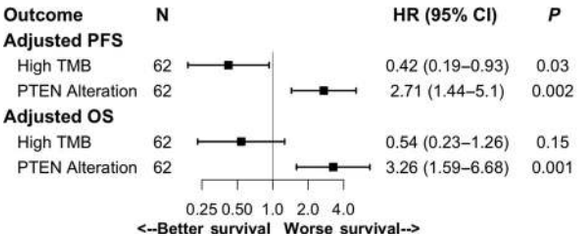

Figure 2.

Adjusted HR for progression-free survival (PFS) and overall survival (OS) by biomarker status in anti-PD-1/L1–treated cohort. PFS and OS HRs by TMB ≥10 versus <10 mutations/megabase and *PTEN* alterations (present vs. absent), adjusted for ECOG PS (≥ 1 vs. 0), previous lines of therapy (≥ 1 vs. 0), regimen (monotherapy vs. combination therapy), and visceral metastases (present vs. absent). Abbreviations: ECOG-PS, Eastern Cooperative Oncology Group performance status; TMB, tumor mutational burden.

is rare in breast cancer, occurring in < 2% of tumors, more commonly in early-stage disease (33). As for PD-L1, the phase III IMpassion130 study showed that the combination of atezolizumab plus nab-paclitaxel was superior to nab-paclitaxel alone only for patients with treatment-naïve mTNBC tumors with ≥ 1% PD-L1-positive immune cells by the SP142 antibody (6). This led to the recent FDA approval for this regimen in PD-L1-positive patients. However, a minority of patients with TNBC have PD-L1–positive tumors by the approved companion diagnostic PD-L1 SP142 assay, ranging from 40% in IMpassion130 (6), which included primary and metastatic tumors, to 25% of mTNBC tumors at our institution. Concerns remain regarding the utility of PD-L1 expression as a reliable biomarker, including its dynamic nature with varying expression over time, the discordance among different PD-L1 assays, and the fact that some PD-L1-negative patients respond to ICIs (35).

Thus, there is an unmet need to define better biomarkers to predict benefit and resistance to immunotherapy in mTNBC, and high TMB and *PTEN* alterations are possible candidates. Using publicly available genomic data from 3969 patients with breast cancer from 6 different cohorts, our group previously showed that 5% of breast cancers have high TMB and that metastatic tumors have a greater prevalence of high TMB than primary tumors (8.4% vs. 2.9%; ref. 36). In addition, that study showed that high TMB tumors had greater immune cytolytic activity (7), as measured by the RNA expression of the CD8-positive T-cell effectors *GZMA* and *PRF1*, suggesting that these patients are more likely to respond to ICI therapies. Likewise, *PTEN* loss correlated with decreased T-cell infiltration, reduced T-cell expansion, and worse response to PD-1 inhibitors in other tumors (19, 20). Thus, there is reason to hypothesize that TMB and *PTEN* alterations may be correlates of response to ICIs.

This study showed that high TMB was significantly associated with longer PFS independent of clinical factors and PD-L1 status. These results are supported by the TAPUR study, a prospective clinical trial of single-agent pembrolizumab in patients with heavily pretreated metastatic breast cancer with TMB ≥ 9 mutations/Mb, which reported an overall response rate of 21% and a durable clinical benefit rate of 37% (37). In addition, other studies have shown that TMB and PD-L1 expression are independent predictive markers of response to ICI therapies and have low correlation across multiple cancers (16, 17). In fact, higher TMB has been associated with worse response to non-ICI treatments in metastatic breast cancer (38). Samstein and colleagues similarly showed that there was no association between TMB and OS in a cohort of 860 patients with breast cancer treated with non-ICI therapies (18), which we similarly concluded examining only patients with TNBC from the same institution. The Samstein and colleagues study also had a small cohort of patients with metastatic breast tumors treated with ICIs ($n = 45$), including 25 patients with ER-negative tumors, which did not demonstrate an association between high TMB and OS. However, only 4 of the 45 patients included in this cohort had tumors with TMB ≥10 mutations/Mb, 20 patients (45%) received single-agent anti-CTLA-4 therapy, and the clinical outcome data were not directly accessed from structured clinical trials. In contrast, all patients in the present study received anti-PD-1/L1-based regimens on a clinical trial.

This study also demonstrated that *PTEN* alterations were significantly associated with lower ORR and shorter PFS and OS independent of clinical factors and PD-L1 status. Prior analyses have demonstrated that partial *PTEN* deletions associate with worse OS in breast cancer (26). These findings highlight the importance of determining whether *PTEN* alterations are predictive or prognostic and prompted our analyses of *PTEN* alterations in patients with mTNBC treated with chemotherapy and non-ICI therapies, which, although underpowered, suggested that *PTEN* alterations are not prognostic. Regardless, the present findings about *PTEN* alterations underlying ICI resistance are directly applicable to the current clinical development of immunotherapy combinations in mTNBC. Consistent with the finding that PI3K/AKT pathway inhibition reversed resistance to T-cell–mediated immunotherapy in murine models (19), a phase Ib study of the AKT-inhibitor ipatasertib and (nab)-paclitaxel combined with atezolizumab in 26 patients with mTNBC demonstrated an impressive ORR of 73%, which represents an improvement over doublet regimens of taxane chemotherapy combined with atezolizumab or ipatasertib across biomarker subgroups (39). Several larger trials are currently being developed to further investigate the combination of AKT inhibitors, PD-1/L1 inhibitors, and chemotherapy in patients with mTNBC. Whether future trials confirm that *PTEN*-altered mTNBC harbors ICI resistance that may be reversed by PI3K/AKT/mTOR inhibitors remains to be determined.

Limitations

This analysis of prospectively treated patients has several limitations, including the small sample size, the heterogeneity of prior and current therapy regimens including monotherapy and combination immunotherapy regimens, and the lack of functional validation that the observed *PTEN* alterations, which included single copy deletions, led to decreased PTEN expression in tumors. In addition, the prevalence of high TMB in this study was higher than previously reported. Possible explanations include that OncoPanel only evaluates tumor without concurrent germline DNA and that high TMB tumors were assessed with OncoPanel versions covering < 1 Mb of exome, which can overestimate TMB versus whole exome sequencing (32, 40). Moreover, the ideal cutoff for defining high TMB in mTNBC is unknown. We used the same cutoff as reported in the large pan-cancer analysis by Campbell and colleagues (41). Overall, our study alone does not prove that TMB and *PTEN* alterations are predictive biomarkers. Instead, additional validation studies, including analyses of TMB and *PTEN* alterations in larger cohorts of immunotherapy and nonimmunotherapy treated patients, are required to establish these correlates as predictive biomarkers of response to ICIs in mTNBC.

Conclusion

As the first genomic analysis of anti-PD-1/L1 response in a mTNBC cohort with in-depth clinical annotations, this study found that

patients with versus without high TMB were more likely to experience longer PFS and that patients with versus without *PTEN* alterations were more likely to progress and experience shorter PFS and OS, even after adjustment for clinical heterogeneity and PD-L1 status. Prospective studies are required to validate the associations of high TMB and *PTEN* alterations with ICI response in mTNBC and to determine whether these findings are generalizable to early stage TNBC. To elucidate the role of high TMB, we designed a currently enrolling multicenter phase II trial of nivolumab plus ipilimumab in metastatic HER2-negative breast cancers with high TMB (NIMBUS, NCT03789110). Similarly, the role of *PTEN* alterations may be clarified in ongoing clinical trials investigating whether AKT inhibitors reverse resistance to PD-1 blockade.

Disclosure of Potential Conflicts of Interest

R. Barroso-Sousa is an employee/paid consultant for Roche and Eli Lilly, and reports receiving speakers bureau honoraria from Roche, Eli Lilly, Bristol-Myers Squibb, Pfizer, and Novartis. S. Pernas reports receiving other remuneration from Polyphor. S. Hodi is an employee/paid consultant for Bristol-Myers Squibb, Merck, EMD Serono, Novartis, Sanofi, Takeda, Genentech, Surface, Compass Therapeutics, Apricity, Aduro, Pionyr, 7 Hills Pharma, Verastem, Torque, and Amgen; reports receiving commercial research grants from Bristol-Myers Squibb (to institution) and Novartis (to institution); and holds ownership interest (including patents) in Novartis and Bristol-Myers Squibb (to institution per institutional policies). I.E. Krop is an employee/paid consultant for Bristol-Myers Squibb, Daiichi/Sankyo, Macrogenics, Genentech/Roche, Novartis, Merck, Context Therapeutics, and Taiho Oncology, and reports receiving commercial research grants from Genentech/Roche and Pfizer. D. Dillon is an employee/paid consultant for Oncology Analytics, Inc. and Novartis. E.P. Winer is an employee/paid consultant for Carrick Therapeutics, Genentech/Roche, Genomic Health, GlaxoSmith Kline, Jounce, Leap, Lilly, Merck, and Seattle Genetics, and reports receiving commercial research grants from Genentech/Roche and Merci. N. Wagle is an employee/paid consultant for Novartis, Eli Lilly, Relay Therapeutics; reports receiving commercial research grants from Novartis and Puma Biotechnology; and holds ownership interest (including patents) in Foundation Medicine and Relay Therapeutics. N.U. Lin is an employee/paid consultant for Seattle Genetics, Puma and Daichii Sankyo, and reports receiving commercial research grants from Genentech, Merck, Pfizer, and Seattle Genetics. E.A. Mittendorf is an employee/paid consultant for AstraZeneca, Merck, Genentech, Genomic Health, and Sellas Lifesciences, and reports receiving commercial research grants from GlaxoSmithKline. E.M. Van Allen is an employee/paid consultant for Tango Therapeutics, Genome Medical, Invitae, Janssen, and Ervaxx; reports receiving commercial research grants from Bristol-Myers Squibb and Novartis; reports receiving speakers bureau honoraria from Illumina; and holds ownership interest (including patents) in Genome Medical, Ervaxx, Tango Therapeutics, and Syapse. S.M. Tolaney is an employee/paid consultant for AstraZeneca, Eli Lilly, Merck, Nektar Therapeutics, Novartis, Pfizer, Genentech, Immunomedics, Bristol-Myers Squibb, Eisai, Nanostring, Puma, Sanofi, Paxman, Odonate, Seattle Genetics, Silverback Therapeutics, G1 Therapeutics, AbbVie, Outcomes4Me, and Anthenex, and reports receiving commercial research grants (all to institutions) from AstraZeneca, Eli Lilly, Merck, Nektar Therapeutics, Novartis, Pfizer, Genentech, Immunomedics, Exelixis, Bristol-Myers Squibb, Eisai, Nanostring, Cyclacel, and Odonate. No potential conflicts of interest were disclosed by the other authors.

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