Baseline Plasma Tumor Mutation Burden Predicts Response to Pembrolizumab-based Therapy in Patients with Metastatic Non–Small Cell Lung Cancer

Charu Aggarwal[1], Jeffrey C. Thompson[2], Austin L. Chien[1], Katie J. Quinn[3], Wei-Ting Hwang[4], Taylor A. Black[1], Stephanie S. Yee[1], Theresa E. Christensen[1], Michael J. LaRiviere[5], Benjamin A. Silva[1], Kimberly C. Banks[3], Rebecca J. Nagy[3], Elena Helman[3], Abigail T. Berman[5], Christine A. Ciunci[1], Aditi P. Singh[1], Jeffrey S. Wasser[6], Joshua M. Bauml[1], Corey J. Langer[1], Roger B. Cohen[1], and Erica L. Carpenter[1]

ABSTRACT

Purpose: The role of plasma-based tumor mutation burden (pTMB) in predicting response to pembrolizumab-based first-line standard-of-care therapy for metastatic non–small cell lung cancer (mNSCLC) has not been explored.

Experimental Design: A 500-gene next-generation sequencing panel was used to assess pTMB. Sixty-six patients with newly diagnosed mNSCLC starting first-line pembrolizumab-based therapy, either alone or in combination with chemotherapy, were enrolled (Clinicaltrial.gov identifier: NCT03047616). Response was assessed using RECIST 1.1. Associations were made for patient characteristics, 6-month durable clinical benefit (DCB), progression-free survival (PFS), and overall survival (OS).

Results: Of 66 patients, 52 (78.8%) were pTMB-evaluable. Median pTMB was 16.8 mutations per megabase (mut/Mb; range, 1.9–52.5) and was significantly higher for patients achieving DCB compared with no durable benefit (21.3 mut/Mb vs. 12.4 mut/Mb, $P = 0.003$). For patients with pTMB ≥ 16 mut/Mb, median PFS was 14.1 versus 4.7 months for patients with pTMB < 16 mut/Mb [HR, 0.30 (0.16–0.60); $P < 0.001$]. Median OS for patients with pTMB ≥ 16 was not reached versus 8.8 months for patients with pTMB < 16 mut/Mb [HR, 0.48 (0.22–1.03); $P = 0.061$]. Mutations in *ERBB2* exon 20, *STK11, KEAP1*, or *PTEN* were more common in patients with no DCB. A combination of pTMB ≥ 16 and absence of negative predictor mutations was associated with PFS [HR, 0.24 (0.11–0.49); $P < 0.001$] and OS [HR, 0.31 (0.13–0.74); $P = 0.009$].

Conclusions: pTMB ≥ 16 mut/Mb is associated with improved PFS after first-line standard-of-care pembrolizumab-based therapy in mNSCLC. *STK11/KEAP1/PTEN* and *ERBB2* mutations may help identify pTMB-high patients unlikely to respond. These results should be validated in larger prospective studies.

Introduction

Immunotherapy is the current standard first-line treatment for patients with metastatic non–small cell lung cancer (mNSCLC) whose tumors lack therapeutically targetable mutations. In the United States, pembrolizumab is currently approved for treatment of mNSCLC with PD-L1 tumor proportion score (TPS) ≥ 1%, and in combination with chemotherapy regardless of PD-L1 TPS. In practice, pembrolizumab monotherapy is reserved for patients with a PD-L1 TPS ≥ 50% (1); patients with PD-L1 < 50% are usually treated with histology-specific platinum-doublet therapy in combination with pembrolizumab (2, 3). Nevertheless, PD-L1 TPS is an imperfect biomarker, as evidenced by a significant benefit of chemoimmunotherapy across all PD-L1 levels (4). Therefore, there is a need to develop novel biomarkers to better identify patients likely to respond to immunotherapy. Tumor mutation burden (TMB), the number of somatic mutations per megabase (mut/Mb), is one such emerging biomarker. In retrospective studies, tissue-based TMB (tTMB) was directly related to clinical outcomes following checkpoint blockade in mNSCLC (5). Specific negative predictor mutations in *STK11, KEAP1*, and other genes have also been evaluated in tissue as biomarkers for immunotherapy (6–10).

Tissue samples often provide inadequate DNA for sequencing and may underrepresent tumor molecular heterogeneity (11, 12). Circulating cell-free tumor DNA (ctDNA), shed into blood by tumor cells, is increasingly utilized to identify actionable mutations and predict response to therapy (4, 13). Recently, ctDNA-based next-generation sequencing (NGS) was used to determine TMB in plasma; patients with pTMB ≥ 16 mut/Mb receiving atezolizumab on the OAK and POPLAR trials had improved overall survival (OS) compared with pTMB-low patients (14, 15). On the basis of this study, using a prespecified plasma TMB cutoff of ≥ 16 mut/Mb, clinical trials are underway. Preliminary analyses from this proof-of-concept trial reveal a numerical benefit for response rate and survival outcomes in a prospectively selected population of patients with high pTMB receiving atezolizumab for mNSCLC (16). A similar benefit was seen with combination durvalumab and tremelimumab compared with

[1]Division of Hematology/Oncology, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania. [2]Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania. [3]Guardant Health Inc., Redwood City, California. [4]Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, Pennsylvania. [5]Division of Radiation Oncology, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania. [6]Neag Comprehensive Cancer Center, UConn Health, University of Connecticut, Farmington, Connecticut.

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

Corresponding Author: Charu Aggarwal, Raymond and Ruth Perelman School of Medicine at the University of Pennsylvania, 3400 Civic Center Blvd, 10-137, South Pavilion, Philadelphia, PA 19104. Phone: 215-662-6318; Fax: 215-349-5326; E-mail: charu.aggarwal@pennmedicine.upenn.edu

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Translational Relevance

Pembrolizumab-based therapy is currently standard-of-care first-line therapy for patients with metastatic non–small cell lung cancer (mNSCLC) whose tumors lack therapeutically targetable mutations. Tissue-based testing of PD-L1 tumor proportion score can be used to stratify patients onto single-agent pembrolizumab versus combination pembrolizumab-chemotherapy. However, it is an imperfect biomarker, and there is a need for additional predictive clinical biomarkers to aid clinical decision-making. High tumor mutation burden is associated with response to therapy, but testing requires sufficient tissue, which can be difficult to obtain. Here we report on the plasma TMB (pTMB) of 66 prospectively enrolled patients with mNSCLC who received first-line pembrolizumab monotherapy or in combination with chemotherapy. High-baseline pTMB was associated with improved response rate (by RECIST) and progression-free survival. Although a larger validation study is needed, our results show the potential clinical utility of a plasma-based TMB test to help inform therapy selection.

chemotherapy on the MYSTIC trial using the 2.145 Mb GuardantOMNI assay at pTMB cutoffs of ≥ 16 and ≥ 20 mut/Mb (12). To our knowledge, the role of plasma-based TMB and negative predictor mutations for predicting response to pembrolizumab-based therapy including in combination with chemotherapy in a real-world setting has not been explored.

Here we evaluated a plasma-based 2.145-Mb 500-gene NGS panel to measure baseline pTMB and specific negative predictor mutations for 66 patients with mNSCLC receiving first-line pembrolizumab-based treatment as standard-of-care.

Materials and Methods

Patients and study design

Patients were enrolled from March 1, 2017 to October 11, 2018 and included if they had pathologically confirmed mNSCLC, and received pembrolizumab-based therapy as standard-of-care first-line treatment (Clinicaltrial.gov identifier: NCT03047616). RECIST, version 1.1 was used to perform independent radiographic response assessments. Efficacy was also defined as durable clinical benefit [DCB; complete response (CR), partial response (PR), or stable disease (SD) lasting > 6 months] or no durable benefit (NDB; PD or SD lasting ≤ 6 months; ref. 17). OS was calculated from the date of first pembrolizumab infusion to the time of death or censored at most recent follow-up; PFS was calculated from the date of first pembrolizumab infusion to the time of death or first documented disease progression, whichever came first, or censored at most recent follow-up. Patients were followed for a minimum of 6 months. We followed the REporting recommendations for tumor MARKer prognostic studies guidelines (18). This study was approved by the Institutional Review Board of the University of Pennsylvania (Philadelphia, Pennsylvania) and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Plasma-based mutation detection and statistical analysis

Plasma was obtained at baseline, prior to initiation of pembrolizumab-based therapy. Sequencing was performed using the 2.145 Mb GuardantOMNI panel (19, 20). The mutation count included all coding somatic single-nucleotide variants (SNV; including silent SNVs) and indels. Germline alterations were filtered out (21). Driver and resistance mutations were excluded, as well as putative clonal hematopoiesis mutations, which were identified using a curated database and in-sample context (22). Raw mutation count was corrected for sample-specific tumor shedding and molecule coverage, with the corrected count reported as pTMB (units mut/Mb; ref. 22). Samples with low tumor shedding (all somatic mutations < 0.3% maximum somatic allele fraction) or low unique molecule coverage were identified as pTMB-unevaluable (Supplementary Fig. S1A).

Validation of plasma-based panel

Reproducibility was assessed using 11 deidentified retrospective plasma samples from multiple tumor types, including NSCLC (Supplementary Fig. S1B; ref. 23). *In silico* analysis was conducted to assess concordance of pTMB with whole exome sequencing (WES)-determined TMB for 513 NSCLC tissue samples from The Cancer Genome Atlas (12, 15, 24, 25; Supplementary Fig. S1C). We conducted additional *in silico* analysis to simulate a comparison of TMB scores using a publicly available, retrospective cohort (17) of patients with advanced NSCLC. (Supplementary Fig. S1D). This builds on previous reports of a positive correlation between TMB scores for matched plasma (as measured by GuardantOMNI) and tissue, as measured by either WES (24) or FoundationOne tissue panel (12, 15) on the MYSTIC trial.

Statistics

Descriptive statistics were computed for patient, tumor, and treatment characteristics. Associations between these characteristics and pTMB were examined using Spearman ρ correlation for continuous variables, or Wilcoxon rank sum or Kruskal–Wallis tests for categorical variables due to a departure from a normal distribution for pTMB scores (**Table 1**). Comparisons of pTMB between 9-week response status (CR/PR vs. SD/PD) and 6-month DCB were determined using a nonparametric bootstrap test of the medians. The OR of binary response status with pTMB was estimated using a logistic regression. We also examined the association of a continuous pTMB with outcomes after confirming the linearity assumption using a restricted cubic spline (26). Using the endpoint of PFS, an optimal cutoff in the range of 15 to 16 mut/Mb was identified such that the log-rank test statistic was maximized in the current data. The cutoff of 16 mut/Mb was selected for the additional analyses on this basis and also based on the existing literature (14, 15). Kaplan–Meier curves for PFS and OS were generated and compared between patients with high pTMB (≥ 16 mut/Mb) and low pTMB (< 16 mut/Mb) using log-rank test. HRs and the associated 95% confidence intervals (CI) were estimated using Cox proportional hazard (PH) model. PH assumption was checked using Schoenfeld residuals–based score test and no violation was identified. Potential confounders including age (≥ 65, < 65), sex, Eastern Cooperative Oncology Group (ECOG) status (≥ 2, < 2), treatment (pembrolizumab monotherapy, pembrolizumab–chemotherapy), number of metastatic sites (≥ 3, < 3), PD-L1 TPS (< 50%, > 50%), and tumor–node–metastasis (TNM) stages (M1a, M1b/c) were examined individually with the binary pTMB group using multivariate Cox or logistic regression. To determine the association of negative predictors with response to immunotherapy, mutations in *ERBB2, STK11, KEAP1, PTEN, KRAS*, and *PIK3CA* (Supplementary Table S1) were tested for association with PFS and OS using a Cox PH model. Increased chromosomal aneuploidy [fraction genome aneuploidy (FGA)] has been associated with inferior

Table 1. Patient baseline characteristics.

Characteristics	All patients $N = 66$	Pembrolizumab monotherapy $N = 31$	Pembrolizumab + chemotherapy $N = 35$	pTMB evaluable $N = 52$	Median pTMB 16.76	Association with pTMB, P^a
Age						
Median	67	68	66	66.5	NA	0.830
Range	47–89	54–89	47–85	47–83	NA	
Sex						
Male	33	15	18	28	17.24	0.762
Female	33	16	17	24	15.67	
Race						
White	48	20	28	36	15.67	0.679
Black or African American	15	9	6	13	21.07	
Pacific Islander	1	0	1	1	11.34	
Other	2	2	0	2	14.85	
Smoking status						
Active	14	6	8	10	19.76	0.122
Former	47	23	24	40	15.67	
Never	5	2	3	2	6.63	
Histology						
Adenocarcinoma	54	22	32	42	17.36	0.069
Squamous	7	7	0	6	10.06	
Poorly Differentiated	4	1	3	3	25.80	
Spindle Cell Neoplasm	1	1	0	1	4.79	
ECOG performance status at therapy start						
0	19	7	12	15	17.24	0.739
1	34	15	19	26	17.85	
2	9	8	1	7	17.24	
≥3	1	1	0	1	9.58	
Unknown[b]	3	0	3	3	13.24	
Tissue PD-L1%						
<1%	19	0	19	16	21.07	0.325
1–49%	12	0	12	7	11.34	
≥50%	34	31	3	28	15.67	
Unknown[b]	1	0	1	1	4.49	
Number of metastatic sites at blood draw						
1	6	3	3	3	16.13	0.283
2	29	11	18	19	20.11	
3	19	11	8	18	13.67	
4	7	3	4	7	13.24	
≥5	5	3	2	5	17.24	
TNM classification						
M1a	12	6	6	8	15.38	0.238
M1b/c	54	25	29	44	17.24	

Abbreviation: pTMB, plasma tumor mutation burden.
[a]Spearman ρ rank correlation for continuous variables, Wilcoxon rank sum test or Kruskal–Wallis test for categorical variables.
[b]Patients for whom this characteristic is unknown were excluded from analysis of the association with pTMB in rightmost column.

outcomes (5), and was also analyzed. Two-sided $P < 0.05$ were considered significant. Statistical analyses were performed using Stata, version 15 (Stata Corp) and GraphPad Prism, version 7.

Results

Baseline pTMB associated with response to pembrolizumab-based therapy

Sixty-six consecutive patients with mNSCLC were enrolled in this single-center prospective biomarker trial (**Table 1**). Thirty-one patients (47.0%) received pembrolizumab monotherapy [median, 4.1 (0–29.4) months on therapy] and 35 (53.0%) received pembrolizumab with platinum pemetrexed–based chemotherapy [median, 7.1 (2.0–21.7) months on therapy] with median OS of 22.1 months and 21.9 months, respectively (Supplementary Fig. S2). Fifty-two of 66 patients (78.8%) were pTMB evaluable (see Materials and Methods). pTMB could not be evaluated in 14 patients due to low tumor shedding (all somatic mutations < 0.3% maximum somatic allele fraction) or low unique molecule coverage. The median pTMB was 16.8 mut/Mb (range, 1.9–52.5; Supplementary Fig. S3A). No samples were found to be microsatellite instability–high (ref. 27). Consistent with previous reports (14), there was no correlation between pTMB and tissue PD-L1 status ($P = 0.348$; Supplementary Fig. S3B).

To assess outcomes following pembrolizumab-based therapy, we first analyzed whether baseline pTMB was associated with RECIST-determined response to therapy. Of all enrolled patients, 45 patients had RECIST evaluable disease at week 9. Median pTMB for patients achieving a week 9 CR/PR (responders) was 21.5 mut/Mb (range, 7.7–52.5), compared with 13.9 mut/Mb (range, 1.9–31.6) for patients with SD/PD (nonresponders; $P = 0.037$). Using a logistic regression model,

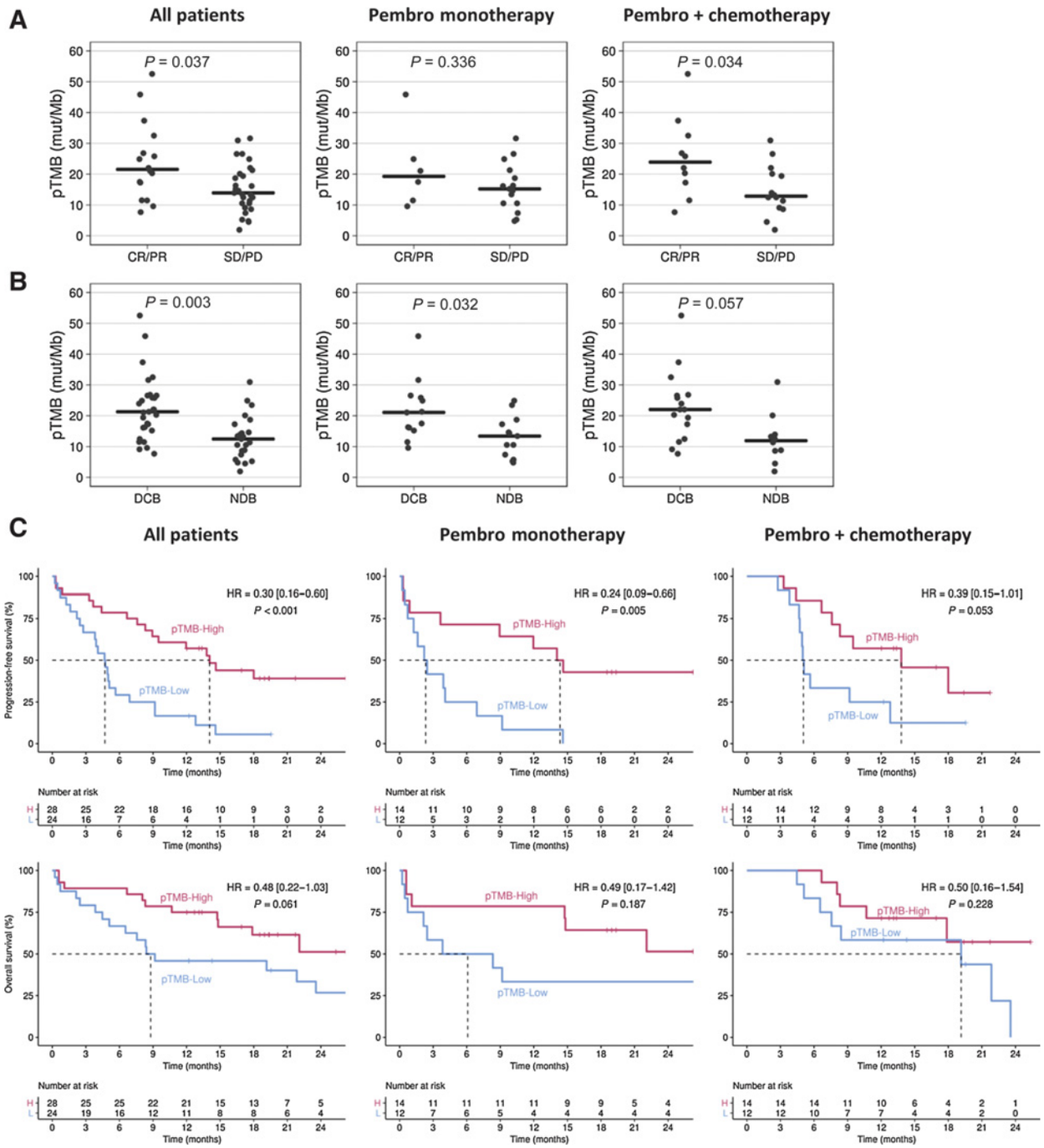

Figure 1.

pTMB and response to pembrolizumab (Pembro). **A,** 45 RECIST-evaluable patients (21 pembrolizumab monotherapy and 24 platinum pemetrexed–based chemotherapy) at week 9 on therapy were categorized as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD), and pTMB levels assessed. **B,** 52 RECIST-evaluable patients (26 pembrolizumab monotherapy and 26 platinum pemetrexed–based chemotherapy) at month 6 on therapy were categorized as durable clinical benefit (DCB; CR, PR, or SD as of 6 months) or no durable benefit (NDB; PD). Horizontal lines indicate median values. **C,** Kaplan–Meier survival curves using a cutoff of 16 mut/Mb for PFS (top) and OS (bottom) for 52 pTMB-evaluable patients (26 pembrolizumab monotherapy and 26 platinum pemetrexed–based chemotherapy). Left, all patients; middle, patients who received pembrolizumab monotherapy; right, patients who received platinum pemetrexed–based chemotherapy.

pTMB score was significantly associated with week 9 response with an OR of 1.09 (95% CI, 1.02–1.08; $P = 0.018$) per one-unit increase in pTMB. Of 21 RECIST-evaluable patients at 9 weeks who received pembrolizumab monotherapy, median pTMB for responders was 19.3 (range, 9.6–45.9), and for nonresponders, it was 15.2 (range, 4.8–31.6); this difference did not reach statistical significance ($P = 0.336$). Median pTMB for the 24 RECIST evaluable patients receiving platinum pemetrexed–based chemotherapy who were responders was 23.9 mut/Mb (range, 7.7–52.5) compared with 12.8 mut/Mb (range, 1.9–31.0; $P = 0.034$; **Fig. 1A**) for nonresponders. The OR per one-unit increase in pTMB was 1.07 ($P = 0.22$) for patients treated with pembrolizumab monotherapy and 1.11 ($P = 0.05$) for platinum pemetrexed–based chemotherapy.

Among the 52 RECIST-evaluable patients at month 6, median pTMB for patients achieving a durable clinical benefit (DCB; CR/PR/SD lasting more than 6 months; ref. 17) was higher than for those with NDB at 21.3 mut/Mb (range, 7.7–52.5) versus 12.4 mut/Mb (range, 1.9–30.9), respectively ($P = 0.003$). The difference in median pTMB was also significant for the 26 patients who received pembrolizumab monotherapy, with median pTMB of 21.1 mut/Mb (range, 9.6–45.9) for those achieving DCB and a median pTMB of 13.4 mut/Mb (range, 4.8–24.9) for patients with NDB ($P = 0.032$). Among the 26 patients who received platinum pemetrexed–based chemotherapy, those with DCB had a higher median pTMB of 22.0 mut/Mb (range, 7.7–52.5) versus 11.9 mut/Mb (range, 1.9–30.9) for those with NDB ($P = 0.057$; **Fig. 1B**). In the multivariate logistic regression analyses, none of the covariates examined (see Materials and Methods) changed the OR of DCB for pTMB more than 15%; thus, adjusted OR was not computed.

We next assessed whether pTMB was associated with PFS and OS. When analyzed as a continuous variable, baseline pTMB was significantly associated with PFS (HR, 0.93 per one-unit change; 95% CI, 0.90–0.97; $P = 0.001$). To assess pTMB as a binary variable, we first identified the appropriate cut-off value. Because the optimal cutoff for measurement of TMB, whether in tissue or plasma, is a function of panel size, sequencing approach, and other factors, we utilized the endpoint of PFS in our own dataset to identify the optimal cutoff (see Materials and Methods). Using this cutoff of 16 mut/Mb to assess pTMB as a binary variable, we determined a median PFS of 14.1 versus 4.7 months for pTMB ≥ 16 versus < 16 mut/Mb (HR, 0.30; 95% CI, 0.16–0.60; $P < 0.001$). In our dataset, 28 of 52 pTMB-evaluable patients (53.8%) had a pTMB ≥ 16 mut/Mb. In the multivariate Cox model analyses, the inclusion of covariates did not significantly change the estimated HRs when comparing pTMB ≥ 16 versus < 16 mut/Mb. A similar association for PFS comparing pTMB ≥ 16 versus < 16 mut/Mb was observed among the 26 patients who received pembrolizumab monotherapy (median PFS of 14.1 vs. 2.2 months; HR, 0.24; 95% CI, 0.09–0.66; $P = 0.005$) and the 26 patients who received platinum pemetrexed–based chemotherapy (median PFS of 13.8 vs. 5.0 months; HR, 0.39; 95% CI, 0.15–1.01; $P = 0.053$). There was no significant difference in median OS as a function of pTMB level overall [median not reached for pTMB-high vs. 8.8 months for pTMB-low; HR, 0.48 (0.22–1.03); $P = 0.061$], or among patients receiving pembrolizumab monotherapy [median NR vs. 6.1 months; HR, 0.49 (0.17–1.42); $P = 0.187$] or platinum pemetrexed–based chemotherapy [median NR vs. 19.2 months; HR, 0.50 (0.16–1.54); $P = 0.228$; **Fig. 1C**].

Exploratory analysis of association of ctDNA-detected mutations with response to pembrolizumab-based therapy

In our cohort, 6 pTMB-high patients did not achieve DCB (**Fig. 1B**). Similar lack of response for some pTMB-high patients was also recently reported (28). Primary and acquired resistance to PD1 blockade can be associated with specific mutations in *JAK1* and *JAK2* (29, 30), although these mutations were not detected in our cohort. This led us to hypothesize that mutational profiling might improve pTMB association with response. We focused on loss-of-function mutations in *STK11, KEAP1,* and *PTEN*, and an activating *ERBB2* exon 20 insertion, all previously shown to be associated with lack of response to checkpoint inhibitors; we also focused on *KRAS* and *PIK3CA* mutations, previously correlated with improved response to checkpoint inhibitors (Supplementary Table S1; refs. 5–10, 31). Increased chromosomal aneuploidy (FGA) has been associated with inferior outcomes (5), and was therefore assessed (**Fig. 2A**). FGA was not significantly associated with response ($P = 0.198$). While the nine putative negative predictor mutations detected for our patient cohort (Supplementary Table S1) were not significantly associated with PFS ($P = 0.110$), we next explored the effects on PFS of a combination of these negative predictor mutations and pTMB. The median PFS for patients with pTMB ≥ 16 and no negative predictor mutations was 18.0 versus 4.7 months for patients with pTMB < 16 or with any negative predictor mutations. This resulted in a HR of 0.24 (0.11–0.49; $P < 0.001$) for the combined predictors versus 0.30 (0.16–0.60) for pTMB alone. Median OS for patients with pTMB ≥ 16 and absence of negative predictor mutations was not reached, versus median OS of 8.4 months for pTMB < 16 mut/Mb or any negative predictor mutations. This resulted in a HR of 0.31 (0.13–0.74; $P = 0.009$) for the combined predictors versus a HR of 0.48 (0.22–1.03) for pTMB alone (**Fig. 2B**).

Discussion

To our knowledge, this prospective study is the largest to correlate pTMB to outcomes after first-line standard-of-care pembrolizumab-based combination therapy in mNSCLC. Overall response rate, median PFS, and median OS were similar to those observed in large phase III trials (1, 2). Using a 2.145-megabase NGS panel and analyzing pTMB as a continuous variable, we determined that median pTMB was significantly higher for patients who experienced a response at 9 weeks ($P = 0.037$) and at 6 months on therapy ($P = 0.003$). Using a pTMB cutoff of 16 mut/Mb (14, 15), we demonstrate that patients with pTMB ≥ 16 mut/Mb had improved PFS (HR = 0.30), and were more likely to sustain DCB (OR = 8.9). We also demonstrate that combining loss-of-function mutations in *STK11/KEAP1/PTEN* and activating *ERBB2* exon 20 insertion mutations with pTMB improved the ability to predict response. Similar to previous reports (5), there was no correlation between pTMB and tumor PD-L1 expression. Taken together, our results suggest pTMB is associated with response to first-line pembrolizumab-based therapy in mNSCLC.

Pembrolizumab-based immunotherapy and chemoimmunotherapy have become standard first-line therapy for mNSCLC patients without a targetable driver mutation (2). Lack of biomarkers beyond the current standard of tissue PD-L1 has limited our ability to select patients who benefit most from immunotherapy. TMB is a promising biomarker; higher tTMB was associated with efficacy of single-agent atezolizumab compared with chemotherapy (17, 32, 33). Similarly, using a cutoff of 10 mut/Mb for tTMB, first-line treatment with nivolumab plus ipilimumab was associated with longer PFS and improved response rate compared with standard platinum-based chemotherapy (5). However, in these trials, tissue samples were only evaluable for TMB in a subset of patients; Hellman and colleagues and Rizvi and colleagues reported 57.7% and 41.1% of tissue samples, respectively, as TMB-evaluable (5, 12, 15). In contrast, Gandara and

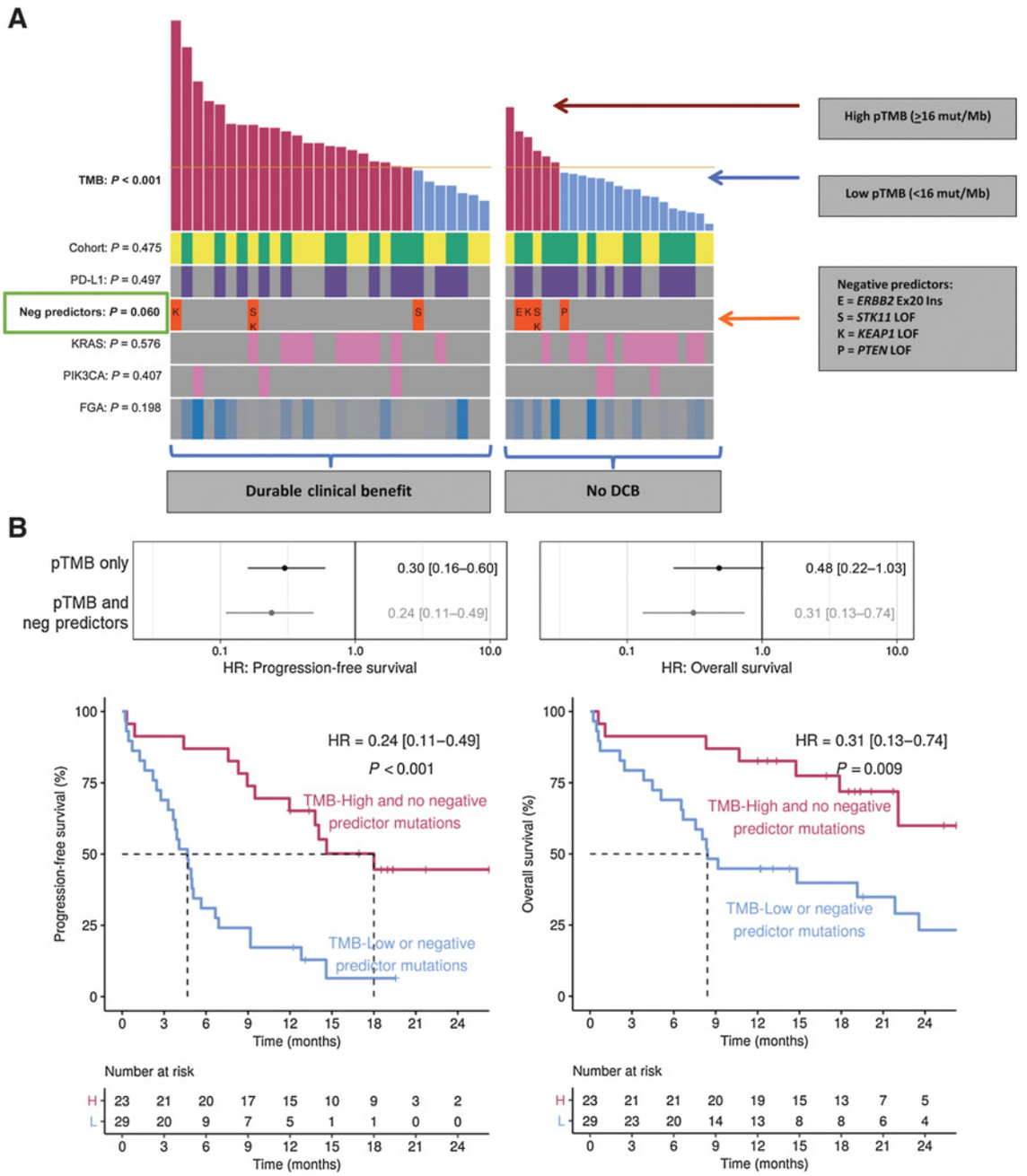

Figure 2.

Mutational analysis and response to pembrolizumab. **A,** pTMB scores are represented by the height of the bars (red = pTMB ≥ 16 mut/Mb, blue = pTMB < 16 mut/Mb) and arranged in decreasing order with 29 patients who achieved a durable clinical benefit (left) and 23 patients with no durable benefit (right). Yellow horizontal line indicates pTMB = 16 mut/Mb. Rows indicate: pembrolizumab cohort with pembrolizumab monotherapy in green and platinum pemetrexed–based chemotherapy in yellow; PD-L1 ≥ 50% patients in purple; patients with a negative predictor mutation in *ERBB2, STK11, KEAP1*, or *PTEN* in orange; *KRAS* or *PIK3CA* mutations in pink; and fraction genome aneuploidy (FGA; analyzed as a continuous variable) in blue (lighter blue = lower FGA, darker blue = higher FGA). For negative predictor mutations, capital letter indicates specific mutation detected. **B,** Forest plots (top) and Kaplan–Meier survival curves (bottom) for PFS and OS. For the forest plots, black indicates the HR and 95% CI for pTMB alone, and gray indicates results for pTMB and negative predictors.

colleagues reported 77.3% and 73.1% of patients on the POPLAR and OAK studies, respectively, as TMB-evaluable from plasma (14). In our study, 52 of 66 patients (79.8%) were pTMB-evaluable (15), suggesting that pTMB may provide a noninvasive option for predicting response in patients for whom tissue-based TMB is impossible. Although a recent report casts doubt on the association of tTMB with response to pembrolizumab plus chemotherapy (34), pTMB in our study is correlated with 9-week response ($P = 0.034$) and 6-month durable

clinical benefit ($P = 0.057$). Just as we and others have demonstrated that plasma-based mutation analysis may provide broader sampling of the tumor mutational profile than tissue (4, 35), pTMB may associate more strongly with response than tTMB, although additional studies with matched plasma and tissue TMB measurements will be necessary.

Aside from serving as a noninvasive biomarker when tissue is lacking, pTMB may have other advantages. High spatial and intratumoral heterogeneity of the immune microenvironment may challenge reliance on a single tissue biopsy to predict immune signatures (36, 37). pTMB may overcome this by more comprehensively capturing overall tumor antigenicity, including primary and metastatic sites. WES is still considered the most robust assessment of TMB, but is currently infeasible for clinical decision-making. Panel-based TMB measurements have emerged, leading to debate on panel size, variant-type inclusion, interchangeability of scores from different panels, and determination of appropriate cut-off points. Until consensus is reached, utility of a panel's TMB score must be assessed against clinical outcomes.

Wang and colleagues reported on a NSCLC population that spanned multiple lines of therapy (first-line, second-line, and beyond), in which a 150-gene panel with a pTMB cutoff of 6 mut/Mb could accurately predict response (28). However, the assay required the SNV allele fraction to be >1.0%, and lacked adjustment of TMB score for low shedding. Moreover, their dataset had 15 nonresponders with high pTMB, suggesting additional genomic factors that may not have been accounted for. Mutations in *STK11/KEAP1* have been associated with inferior outcomes in patients treated with pembrolizumab-based chemotherapy, including among tTMB-high and PD-L1–positive patients (10). Data from the MYSTIC trial confirmed the negative prognostic role of *KEAP1* using plasma NGS in patients with mNSCLC receiving combination immunotherapy; however, it did not clearly confirm the predictive role for *STK11*, but rather showed that this may be a prognostic biomarker, with overall worse outcomes seen in patients with *STK11* mutation (38). The divergent data on mutations and their interplay with outcomes following chemotherapy, chemoimmunotherapy, and immunotherapy combinations can be potentially explained by the complex molecular interactions that exist within the tumor microenvironment. While others have demonstrated an association between a subset of mutations, to our knowledge, the combination of pTMB and specific negative predictor mutations in *ERBB2* exon 20, *STK11, KEAP1,* and *PTEN* from plasma has not been reported. These are small numbers and the analysis should be considered purely exploratory. Adding mutation analysis might enhance the ability of pTMB to predict outcomes from immunotherapy. These observations, if validated, suggest that including these genomic biomarkers in the predictive algorithms, may improve identification of pTMB-high patients unlikely to respond.

Our study does have certain limitations. It is a single-center, nonrandomized study. Matched tissue TMB was not able to be performed as it is not yet a part of the routine clinical tissue NGS testing performed at our institution. Further study is required to validate our findings, including pTMB cutoff, in a larger dataset. Although combining tissue TMB and PD-L1 has shown improved prediction of immunotherapy response, this analysis could not be performed here, as the treatment regimens (pembrolizumab monotherapy vs. platinum pemetrexed–based chemotherapy) were largely dictated by tumor PD-L1 TPS. Our study also does not consider characteristics of the tumor microenvironment, immune competence, including MHC status or the microbiome. Nevertheless, our results do argue for larger-scale validation of plasma-based TMB in the context of prospective pembrolizumab-based therapy in mNSCLC; if substantiated, this assay should be integrated into routine clinical management of patients with mNSCLC.

Disclosure of Potential Conflicts of Interest

C. Aggarwal is an advisory board member/unpaid consultant for Bristol-Myers Squibb, Celgene, Eli Lilly, Merck, Roche, and AstraZeneca. J.C. Thompson is a paid advisory board member at Guardant Health. K.C. Banks is an employee/paid consultant for Guardant Health. K.J. Quinn and E. Helman are employees/paid consultants for and hold ownership interest (including patents) in Guardant Health. R.J. Nagy holds ownership interest (including patents) in Guardant Health. A.T. Berman is an employee/paid consultant for AstraZeneca, IMX, and Imedex, and reports receiving speakers bureau honoraria from Varian. J.S. Wasser holds ownership interest (including patents) in Merck. J.M. Bauml is an employee/paid consultant for Bristol-Myers Squibb, AstraZeneca, Celgene, Merck, Janssen, Genentech, Guardant Health, Boehringer Ingelheim, Takeda, Regeneron, and Ayala, and reports receiving commercial research grants from Merck, Incyte, Novartis, Bayer, Janssen, AstraZeneca, Takeda, and Amgen. C.J. Langer is an employee/paid consultant for AstraZeneca, Merck, Takeda, Gilead, and Genentech, reports receiving commercial research grants from Guardant, and other commercial research support from Merck, Lilly, Takeda, Cellgene, Inovio, and Trizell. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C. Aggarwal, J.C. Thompson, K.J. Quinn, R.J. Nagy, J.S. Wasser, E.L. Carpenter

Development of methodology: C. Aggarwal, J.C. Thompson, K.J. Quinn, W-T. Hwang, K.C. Banks, E. Helman

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Aggarwal, J.C. Thompson, A.L. Chien, K.J. Quinn, T. Christensen, M.J. LaRiviere, R.J. Nagy, C.A. Ciunci, J.M. Bauml, C.J. Langer, R.B. Cohen, E.L. Carpenter

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Aggarwal, J.C. Thompson, A.L. Chien, K.J. Quinn, W-T. Hwang, M.J. LaRiviere, K.C. Banks, R.J. Nagy, E. Helman, J.M. Bauml, C.J. Langer, E.L. Carpenter

Writing, review, and/or revision of the manuscript: C. Aggarwal, J.C. Thompson, A.L. Chien, K.J. Quinn, W-T. Hwang, T.A. Black, S.S. Yee, T. Christensen, M.J. LaRiviere, B.A. Silva, K.C. Banks, R.J. Nagy, E. Helman, A.T. Berman, C.A. Ciunci, A.P. Singh, J.S. Wasser, J.M. Bauml, C.J. Langer, R.B. Cohen, E.L. Carpenter

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Aggarwal, A.L. Chien, T.A. Black, S.S. Yee, T. Christensen, B.A. Silva, A.T. Berman

Study supervision: C. Aggarwal, J.C. Thompson, E.L. Carpenter

Other (head of thoracic team under whose aegis this research occurred): C.J. Langer

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