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Baseline plasma tumor mutation burden predicts response to pembrolizumab-based therapy in patients with metastatic non-small cell lung cancer

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

[1.]Division of Hematology/Oncology, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA

[2.]Division of Pulmonary, Allergy and Critical Care Medicine, Thoracic Oncology Group, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA

[3.]Division of Radiation Oncology, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA

[4.]Guardant Health Inc., Redwood City, USA

[5.]Neag Comprehensive Cancer Center, UConn Health, University of Connecticut, Farmington, USA

[6.]Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania, Philadelphia, USA

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 (NGS) 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).

Corresponding Author: Charu Aggarwal, MD, MPH, Division of Hematology-Oncology, Department of Medicine, University of Pennsylvania Perelman School of Medicine, 10-137 South Pavilion, 10th Floor, 3400 Civic Center Blvd, Philadelphia, PA, 19104, United States, (215) 662-6318, charu.aggarwal@pennmedicine.upenn.edu.

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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 to 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 vs. 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 vs. 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.

Keywords

Non-small cell lung cancer (NSCLC); liquid biopsy; pembrolizumab; next-generation sequencing (NGS); tumor mutation burden (TMB); plasma

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 US, 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 Tumor Proportion Score (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 chemo-immunotherapy 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 under-represent 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 to pTMB-low patients[14, 15]. Based on this study, using a pre-specified plasma TMB cut-off 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 to 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 3/1/17 – 10/11/18 and included if they had pathologically confirmed mNSCLC, received pembrolizumab-based therapy as standard of care first-line treatment (Clinicaltrial.gov identifier: NCT03047616). Response Evaluation Criteria in Solid Tumors (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)[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 six months. We followed the REporting recommendations for tumor MARKer prognostic studies (REMARK) guidelines[18]. This study was approved by the Institutional Review Board (IRB) of the University of 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 (SNVs; including silent SNVs) and indels. Germline alterations were filtered out[21]. Driver and resistance mutations were excluded, as well as putative clonal hematopoiesis (CH) 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)[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 (Supplemental Figure 1A).

Validation of plasma-based panel

Reproducibility was assessed using 11 de-identified retrospective plasma samples from multiple tumor types, including NSCLC (Supplemental Figure 1B)[23]. In silico analysis

was conducted to assess concordance of pTMB with WES-determined TMB for 513 NSCLC tissue samples from The Cancer Genome Atlas (TCGA)[12, 15, 24, 25] (Supplemental Figure 1C). We conducted additional in-silico analysis to simulate a comparison of TMB scores using a publicly available, retrospective cohort[17] of advanced NSCLC patients. (Supplemental Figure 1D). 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's rho correlation for continuous variables, 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 versus SD/PD) and 6-month DCB were determined using a non-parametric bootstrap test of the medians. The odds ratio 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. Hazard ratios 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, ECOG status (≥2, <2), treatment (pembrolizumab monotherapy, pembrolizumab-chemotherapy), number of metastatic sites (≥3, <3), PD-L1 TPS (<50%, >50%) and 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* (Supplemental Table 1) 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 outcomes[5], and was also analyzed. Two-sided p-values <0.05 were considered significant. Statistical analyses were performed using Stata, version 15 (Stata Corp, College Station, TX) 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 (P; median 4.1 (0 – 29.4) months on therapy) and 35 (53.0%) received pembrolizumab with platinum-pemetrexed-based chemotherapy (PC; median 7.1 (2.0 – 21.7) months on therapy)

with median OS of 22.1 months and 21.9 months respectively (Supplemental Figure 2). Fifty-two of 66 patients (78.8%) were pTMB evaluable (see 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 to 52.5; Supplemental Figure 3A). No samples were found to be Microsatellite Instability-High (MSI-high)[27]. Consistent with previous reports[14], there was no correlation between pTMB and tissue PD-L1 status (P=0.348; Supplemental Figure 3B).

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 to 13.9 mut/MB (range 1.9 – 31.6) for patients with SD/PD (non-responders; P=0.037). Using a logistic regression model, pTMB score was significantly associated with week 9 response with an odds ratio (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 P, median pTMB for responders was 19.3 (range 9.6 – 45.9), and for non-responders, it was 15.2 (4.8 – 31.6); this difference did not reach statistical significance (P=0.336). Median pTMB for the 24 RECIST evaluable patients receiving PC who were responders was 23.9 mut/MB (7.7 – 52.5) compared to 12.8 mut/MB (1.9 – 31.0; P=0.034; Figure 1A) for non-responders. The OR per one-unit increase in pTMB was 1.07 (P=0.22) for patients treated with P and 1.11 (P=0.05) for PC.

Among the 52 RECIST evaluable patients at month 6, median pTMB for patients achieving a durable clinical benefit (DCB; CR/PR/SD lasting >6 months[17]) was higher than for those with no durable benefit (NDB) at 21.3 mut/Mb (range 7.7 – 52.5) vs. 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 P, 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 PC, those with DCB had a higher median pTMB of 22.0 mut/MB (range 7.7 – 52.5) vs. 11.9 mut/MB (range 1.9 – 30.9) for those with NDB (P=0.057); (Figure 1B). In the multivariate logistic regression analyses, none of the covariates examined (see 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 cutoff value. Since 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 data set to identify the optimal cutoff (see Methods). Using this cutoff of 16 mut/Mb to assess pTMB as a binary variable, we determined a median PFS of 14.1 vs 4.7 months for pTMB ≥16 vs <16 mut/Mb; HR=0.30, 95% CI: 0.16 – 0.60, P<0.001). In our data set, 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 vs <16 mut/Mb. A similar association for PFS comparing pTMB ≥16 vs <16 mut/Mb was observed among the 26 patients who received P (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 PC (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 P (median NR vs 6.1 months, HR 0.49 [0.17–1.42]; *P*=0.187) or PC (median NR vs 19.2 months, HR 0.50 [0.16–1.54]); *P*=0.228; (Figure 1C).

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

In our cohort, six pTMB-high patients did not achieve DCB (Figure 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 (Supplemental Table 1)[5–10, 31]. Increased chromosomal aneuploidy (fraction genome aneuploidy; FGA) has been associated with inferior outcomes[5], and was therefore assessed (Figure 2A). FGA was not significantly associated with response (*P*=0.198). While the nine putative negative predictor mutations detected for our patient cohort (Supplemental Table 1) 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 (Figure 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 chemo-immunotherapy 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 to 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 to standard platinum-based chemotherapy[5]. However, in these trials, tissue samples were only evaluable for TMB in a subset of patients; Hellman et al. and Rizvi et al. reported 57.7% and 41.1% of tissue samples, respectively, as TMB-evaluable[5, 12, 15]. By contrast, Gandara et al. 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 non-invasive 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 non-invasive biomarker when tissue is lacking, pTMB may have other advantages. High spatial and intra-tumoral 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-points. Until consensus is reached, utility of a panel's TMB score must be assessed against clinical outcomes.

Wang et al. reported on a NSCLC population that spanned multiple lines of therapy (first, 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 non-responders 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 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, chemo-immunotherapy 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, non-randomized 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 (P vs. PC) 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Translational Relevance

Pembrolizumab based therapy is currently standard of care frontline therapy for patients with metastatic NSCLC (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 TMB 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 frontline pembrolizumab monotherapy or in combination with chemotherapy. High baseline pTMB was associated with improved response rate (by RECIST) and PFS. 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.

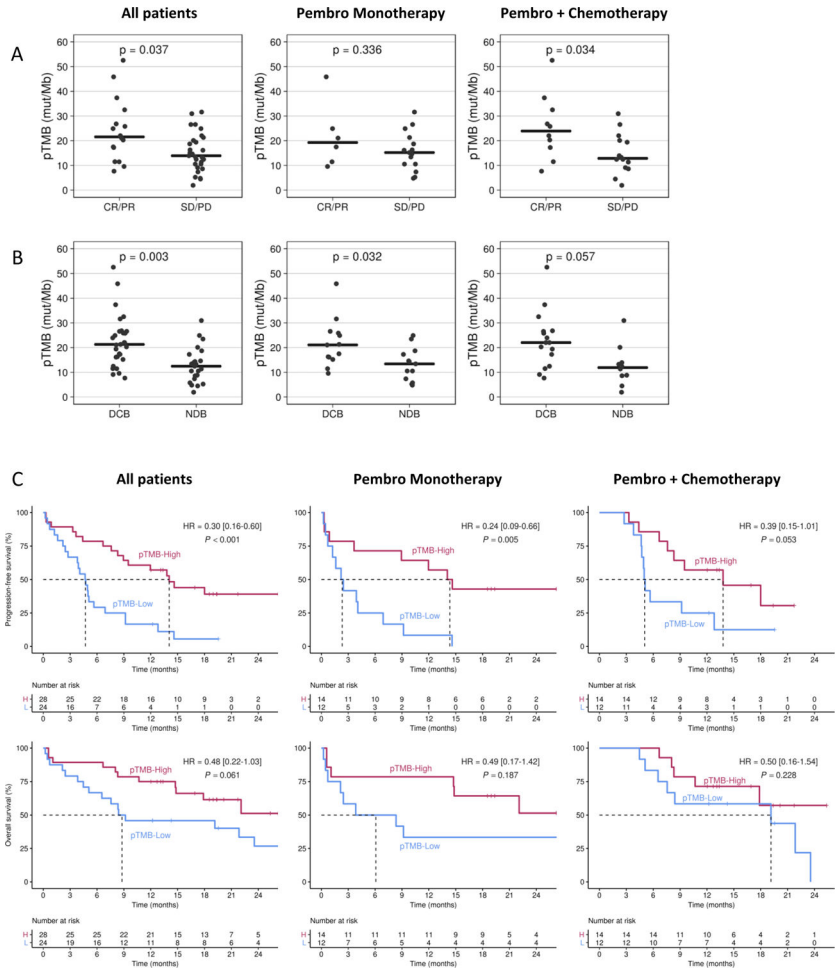

Figure 1. pTMB and response to pembrolizumab.

A) 45 RECIST-evaluable patients (21 P and 24 PC) 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 P and 26 PC) at month-6 on therapy were categorized as durable clinical benefit (DCB; CR, PR, or SD as of 6 months) or no durable benefit (progressive disease; NDB). Horizontal lines indicate median values. **C**) Kaplan-Meier survival curves using a cutoff of 16 mut/Mb for PFS (above) and OS (below) for 52 pTMB-evaluable patients (26 P and 26 PC). **Left** panel is all patients, **middle** includes patients who received P, and **right** panel includes patients who received PC.

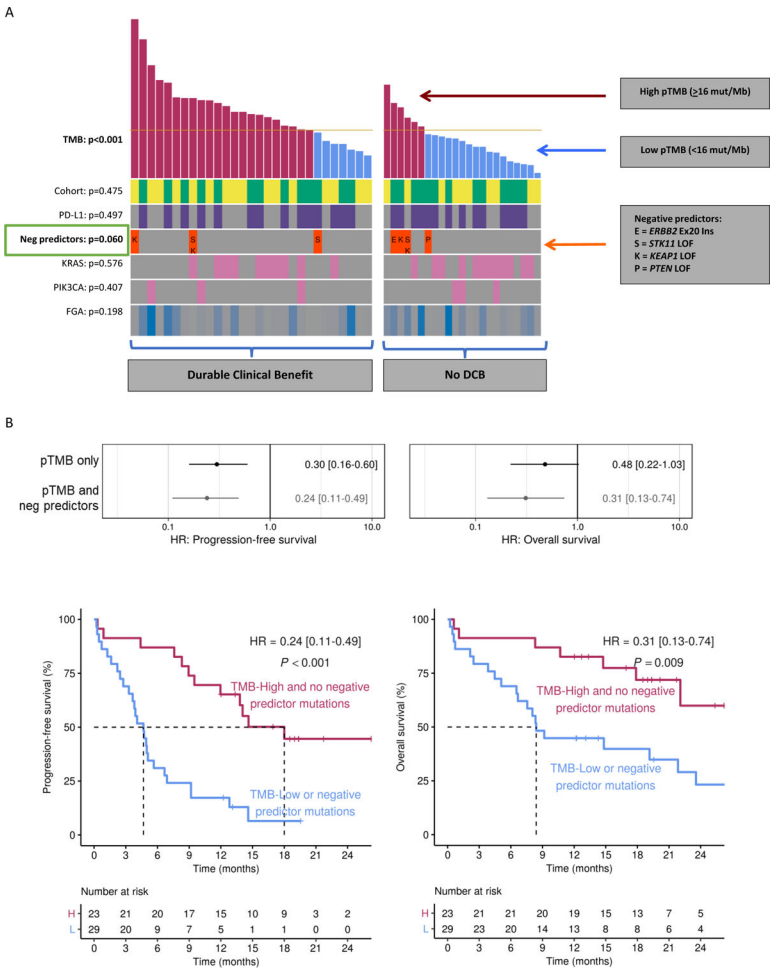

Figure 2. Mutational analysis and response to pembrolizumab

A) pTMB scores are represented by the height of the bars (red = pTMB≥16 mut/Mb, blue = pTMB<16) 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 P in green and PC 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 (with lighter blue = lower FGA, darker blue = higher FGA). For negative predictor mutations, capital letter indicates specific mutation detected. **B**) Forest plots (above) and Kaplan-Meier survival curves (below) for PFS and OS. For the forest plots, black indicates the hazard ratio and 95% confidence interval for pTMB alone, and grey indicates results for pTMB and negative predictors.

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*-value[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
American	Black or African	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	

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*-value[a]
Number of Metastatic Sites at blood draw						
1	6	3	3	3	16.13	
2	29	11	18	19	20.11	
3	19	11	8	18	13.67	0.283
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	

Abbreviations: pTMB, plasma tumor mutation burden.

[a]Footnote: Spearman's rho 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 right-most column.