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Predictive biomarkers for PD-1/PD-L1 checkpoint inhibitor response in NSCLC: an analysis of clinical trial and real-world data

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ABSTRACT

Background Many biomarkers have been proposed to be predictive of response to anti-programmed cell death protein-1 (PD-1)/anti-programmed death ligand-1 (PD-L1) checkpoint inhibitors (CPI). However, conflicting observations and lack of consensus call for an assessment of their clinical utility in a large data set. Using a combined data set of clinical trials and real-world data, we assessed the predictive and prognostic utility of biomarkers for clinical outcome of CPI in non-small cell lung cancer (NSCLC).

Methods Retrospective cohort study using 24,152 patients selected from 71,850 patients with advanced NSCLC from electronic health records and 9 Roche atezolizumab trials. Patients were stratified into high and low biomarker groups. Correlation with treatment outcome in the different biomarker groups was investigated and compared between patients treated with CPI versus chemotherapy. Durable response was defined as having complete response/partial response without progression during the study period of 270 days.

Results Standard blood analytes (eg, albumin and lymphocyte) were just prognostic, having correlation with clinical outcome irrespective of treatment type. High expression of PD-L1 on tumors (≥50% tumor cell staining) were specifically associated with response to CPI (OR 0.20; 95% CI 0.13 to 0.30; p<0.001). The association was stronger in patients with non-squamous than squamous histology, with smoking history than non-smokers, and with prior chemotherapy than first-line CPI. Higher tumor mutational burden (TMB) (≥10.44 mut/Mb) was also specifically associated with durable response to CPI (OR=0.40; 95% CI 0.29 to 0.54; p<0.001). The combination of high TMB and PD-L1 expression was the strongest predictor of durable response to CPI (OR=0.04; 95% CI 0.00 to 0.18; p<0.001). There was no significant association between PD-L1 or TMB levels with response to chemotherapy, suggesting a CPI-specific predictive effect.

Conclusions Standard blood analytes had just prognostic utility, whereas tumor PD-L1 and TMB specifically predicted response to CPI in NSCLC. The combined high TMB and PD-L1 expression was the strongest predictor of durable response. PD-L1 was also a stronger predictor in patients with non-squamous histology, smoking history or prior chemotherapy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Many biomarkers have been proposed to be predictive of response to checkpoint inhibitors (CPI). However, conflicting observations and lack of consensus call for an assessment of their clinical utility in a large data set.

WHAT THIS STUDY ADDS

⇒ Among the subpopulations, programmed death ligand-1 (PD-L1) was a stronger predictor in patients with non-squamous histology, smoking history or prior chemotherapy. PD-L1 and tumor mutational burden (TMB) combined is a very strong predictor.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Standard blood analytes are just prognostic biomarkers, whereas tumor PD-L1 and TMB predicted durable response to CPI in real-world and clinical trial patients with non-small cell lung cancer.

INTRODUCTION

The advent of cancer treatments directed at programmed cell death protein 1 (PD-1) and its ligand (PD-L1) has dramatically improved clinical outcomes for many types of cancers including non-small cell lung cancer (NSCLC).[1 2] Checkpoint inhibitors (CPI) targeting PD-1 and PD-L1 have been demonstrated to be effective in first and subsequent lines of therapy for NSCLC.[2–5] However, the clinical responses to CPI vary considerably among patients. Selection criteria for patients who can potentially benefit the most from these therapies remain unclear.

Various standard blood analytes have also been reported or proposed to be predictive biomarkers of response to CPI in several cancer types, including NSCLC. They included monocytes,[6] neutrophils,[6] neutrophil to lymphocyte ratio,[7 8] lung immune prognostic index,[9] albumin,[10 11]lactate dehydrogenase and C-reactive protein.[12]

The expression of PD-L1 on tumors has been used as a biomarker in diverse cancer types including lung cancer.[5] Previous reports on the association between tumor PD-L1 expression and clinical outcomes have been variable, with some trials demonstrating predictive value of PD-L1 expression which was not observed in others.[13] The association between biomarker expression and patient outcomes has been largely studied using data from clinical trials with a stringent selection of patient populations. Even then, the estimated predictive value of PD-L1 expression has been inconsistent, with some trials reporting it to be strongly predictive of clinical outcomes whereas other trials indicating weak or non-existent association.[14]

Tumor mutational burden (TMB) which is the number of mutations per coding region of a cancer cell, has been demonstrated to correlate with clinical response and extended survival, suggesting that TMB could be a predictive biomarker for CPI.[15] However, the value of TMB as a predictive biomarker has been controversial.[16 17]

Therefore, the aim of this study was to assess the clinical utility of these biomarkers for predicting response to CPI among adults with advanced-stage NSCLC. To assess the clinical utility with statistical confidence and their general use in both real-world and randomized control trial settings, we used data from electronic health records (EHR) databases and clinical trials of atezolizumab. To distinguish the predictive (specific to the treatment) versus prognostic (independent of the treatment)[18] nature of the biomarkers,[19] we also compared the effect in patients treated with chemotherapy. In addition, we assessed the influence of smoking status and histology (non-squamous vs squamous) in this population.

This analysis is the largest study of this nature, to our knowledge, with data of more than 70,000 patients with NSCLC used as the starting material for the analyses.

METHODS

Data sources and study design

Three separate sources of data were used for this study. Results from the individual data sources were consistent with each other and were combined to gain more statistical power. The first data source consists of nine in-house historical atezolizumab advanced NSCLC clinical trials. They are the five phase II or III atezolizumab monotherapy studies,[3 5 20–22] and four phase III atezolizumab combo-therapy studies.[23–26] Patients were consented for treatment and data usage, and were treated in accordance with the Declaration of Helsinki. In-house clinical trial data were used in accordance to internal processes and guidelines. The trial data were analyzed with all patients treated, regardless of intention-to-treat status.

The second source of data was the US nationwide, de-identified, EHR-derived Flatiron Health (FH) database.[27] It is a longitudinal database comprizing patient-level structured (eg, laboratory values and prescribed medications) and unstructured data (eg, biomarker reports), curated via technology-enabled abstraction. During the study period, the de-identified data originated from approximately 280 US cancer clinics (~800 sites of care).

The third source of data was the integrated FH/Foundation Medicine (FMI) Clinico-Genomic Database (CGDB).[27 28] It consists of patients from the FH database who had genomic data derived from FMI comprehensive genomic profiling. CGDB provides de-identified patient-level data (from FH database) linked to FMI genomic data from the same patient.[28] Since CGDB patients were part of the FH database, to avoid including duplicated patients from the two databases, we excluded the patients in FH database with FMI tests. Patient treatment data between January 2011 and February 2020 (data collection cut-off date) from the two databases were used for the analyses. Both databases consist of retrospective observational de-identified anonymized patient-level data; as such, this study was exempt from informed consent and Institutional Review Board requirements. Contracts governing the access and proper use of data were signed and the rules were respected. Patients from these combined sources were then selected according to various criteria, such as data availability, as illustrated in the top part of figure 1A.

For the study design, for each biomarker, patients were stratified into high and low biomarker groups. For PD-L1, they were PD-L1 high versus PD-L1 negative. For biomarkers with continuous values, they were third tertile versus first tertile. For TMB, the first and third tertiles were equivalent to TMB of ≤4.35 and ≥10.44 mut/Mb, respectively. The correlation of biomarker levels with treatment outcome was explored ('Analysis' part of figure 1A). The high and low biomarker groups were balanced on patients' baseline characteristics that are known prognostic factors that can confound the biomarker effect (age, sex, race, disease stage at initial diagnosis, smoking history, histology and Eastern Cooperative Oncology Group (ECOG) performance status (PS)) using multivariate propensity score-based algorithm[29 30] (see 'Statistical analysis' section for detail). All patient counts in the result plots are weighted counts. Two sets of analyses were performed: (1) response analysis based on durable response definition which delineates 'responders' and 'non-responders'; (2) progression analysis based on durable clinical benefit definition which delineates 'non-progressors' and 'progressors' (figure 1AC). To distinguish CPI-specific predictive effect versus general prognostic effect of biomarkers, analysis of standard-of-care chemotherapy cohorts was also performed.

To focus on the association of the biomarker with specific drug response rather than with overall survival (OS), which depends on diverse prognostic factors, we removed patients who died early regardless of the treatment received. It has been previously reported[31] that the CPI and chemotherapy survival curves did not differentiate well until after 18 weeks (also confirmed in our data set: figure 1B). We therefore restricted the analysis to patients who were alive beyond 18 weeks. We confirmed

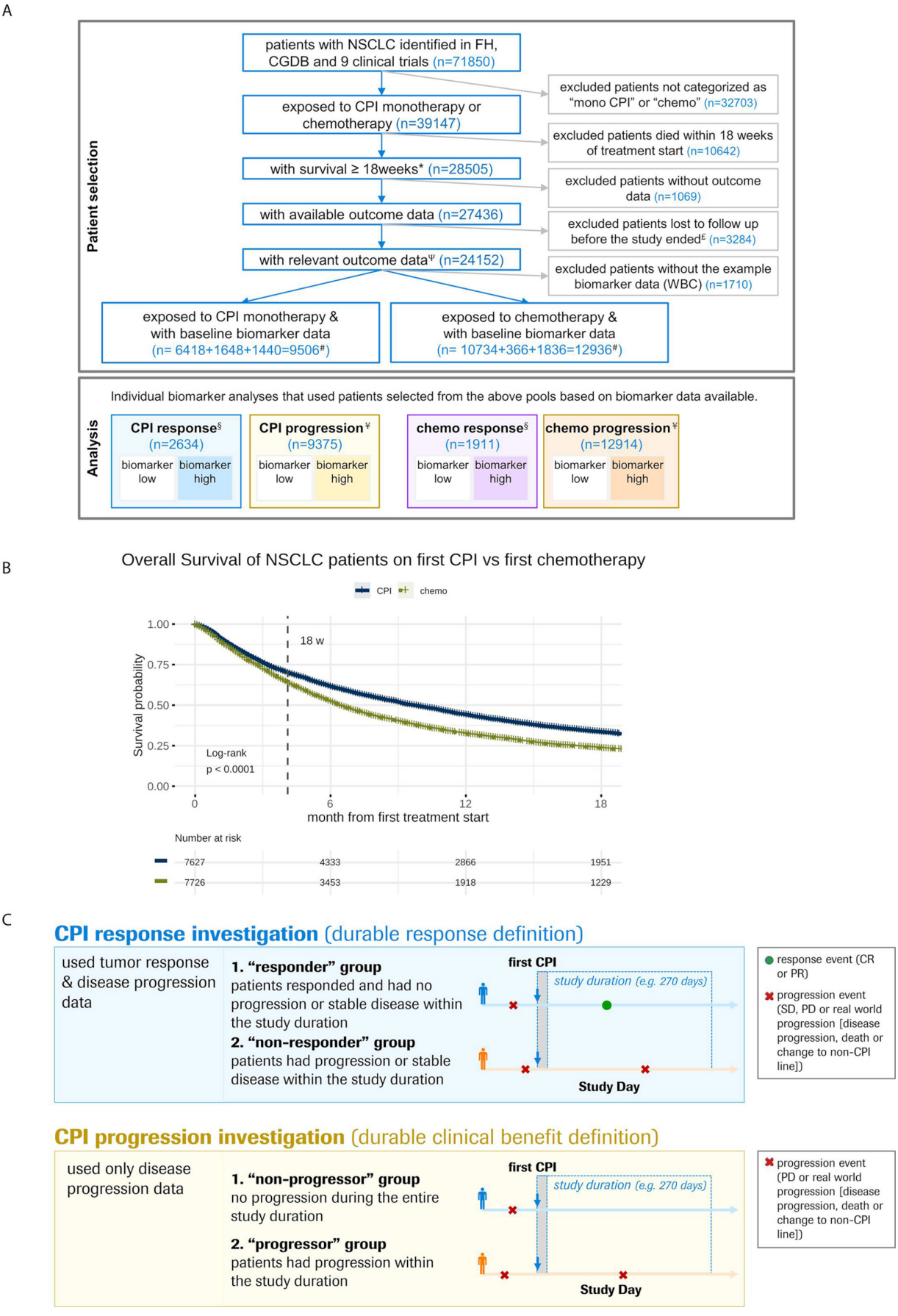

Figure 1 (Continued)

Figure 1 Patient selection and treatment outcome group definitions. (A) Patient selection for the study cohorts. Blue numbers in parentheses denote numbers of patients combined from the three data sources (CGDB, the nine in-house atezolizumab studies and FH database) at each step of patient selection and analysis. The numbers at the end of patient selection step with 'baseline biomarker data' show the numbers of patients with the most commonly available biomarker analyzed (white blood cell (WBC)). For the other biomarkers, the patient numbers are illustrated in each analysis figure. In each biomarker analysis, patients were stratified into high and low biomarker groups, and the correlation between biomarker levels and treatment outcome groups was studied. Patients with intermediate biomarker levels (eg, second tertile) were not analyzed. The biomarker high and low groups were balanced on baseline characteristics using propensity score-based SMRW method. Patients in the bottom 'response' analysis were also included in the 'progression' analysis since they had both response and progression outcome data. The 'progression' analysis included additional patients from the FH database because disease progression data is available. (B) Kaplan-Meier plot of overall survival of FH patients treated with CPI or chemotherapy with their first CPI or chemotherapy started during 2015 to February 2020 when the therapies were both used. Dotted vertical line indicates the cut-off used to exclude early deaths, when the two survival curves can be well differentiated. The two groups were balanced on their baseline characteristics using propensity score-based SMRW weights. (C) Treatment outcome group definitions. Each group is represented by a schematic patient journey. Same definition is used for CPI or chemotherapy, but CPI is used as an example for illustration. Patients were aligned on their first CPI treatment day (↓). Disease progression during the first 14 days following CPI initiation was excluded as recommended by Flatiron Health (grayed period). Example of disease progression (x) and/or tumor response events (●) are indicated on the journey. The analysis of response to CPI (blue box) used both tumor response and disease progression data. A durable response was defined as having a CR or PR within the study duration. The analysis of progression on CPI (yellow box) used only progression data. Progressors were defined as patients with a progression event during the study duration. Footnotes for figure 1A: *Patients who died within 18 weeks from treatment start were removed (see Methods and Discussion for rationale). £Under the durable outcome definition (figure 1C), durable response or clinical benefit requires the entire study duration (270 days in this study) to confirm the durability. ΨPatient characteristics are provided in table 1. #Number of patients with the most commonly available biomarker (WBC) from FH, CGDB and trials, respectively. §Analysis performed on patients using durable response definition in figure 1C. ¥Analysis performed on patients using durable clinical benefit definition in figure 1C. CGDB, Clinico-Genomic Database; CPI, checkpoint inhibitors; CR, complete response; FH, Flatiron Health; NSCLC, non-small cell lung cancer; PD, progressive disease; PR, partial response; SMRW, Standardized Mortality Ratio Weighting.

that the inclusion of early deaths is a confounding effect in the sensitivity analysis (online supplemental figure 1).

Patient treatment and demographics

For the real-world data (RWD), patients with no record of CPI or other immunotherapy were assigned to the chemotherapy cohort if they were treated with cytotoxic monotherapy or combinations with other cytotoxics or targeted therapies. Patients in the CPI cohorts were treated with nivolumab, (~35%) pembrolizumab (~40%), atezolizumab (~20%) or durvalumab (~5%). Assignment to CPI cohort was regardless of other therapies before or after the CPI line. The analysis used the initial line of exposure to CPI or chemotherapy for the treatment of NSCLC. In CPI-treated patients, slightly greater than 50% of the patients had their first CPI in the first line and ~35% in the second line, and among the chemotherapy patients, ~95% had their first chemotherapy in the first line. All RWD patients included in our analysis were at an advanced stage when they started the treatment. There were 1114 stage I–III patients from the atezolizumab studies, which constitute <5% of the 24,152 patients selected for the study, figure 1A.

In the atezolizumab trials, there were a mix of chemo-refractory and chemo-naïve patients. All CGDB data and seven of nine atezolizumab studies,[22 24] included patients with epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) mutations. To determine whether we can account for the impact of EGFR or ALK mutation on the relationship between PD-L1 and/or TMB on one side and the response or progression on the other side, we analyzed the FH data set. EGFR or ALK mutation (prevalence of 6.7% and <1%) test results were available for 70% or 66% of the patients, respectively. The impact of the mutation status on the biomarker effect of CPI response was minimal, potentially due to the low mutation prevalence, (≤0.01 max difference in the ORs) and was therefore not pursued further.

Outcome measures

Tumor response data included Response Evaluation Criteria in Solid Tumours (RECIST) response assessments from atezolizumab trials and real-world response (rwR)[32] data from CGDB.[28] Disease progression was defined using the following data extracted from the databases: real-world progression (rwP)[33] abstracted by FH from patient records, death or change to a treatment line of a different drug category. The durable response definition dichotomized patients into responders (defined as having rwR or RECIST complete or partial response (CR/PR) without progression during the entire study duration of 270 days) and non-responders (having progressive disease (PD) or stable disease (SD) in the trials or disease progression in RWD during the study). The duration of 270 days was to ensure prolonged response that would include multiple tumor scans and a duration for which balanced groups can be obtained. Patients who had a response and had no PD/SD/disease progression but lost to follow-up before the study ended were not included in the analysis.

The durable clinical benefit definition dichotomized the patients into non-progressors and progressors, similar for clinical benefit rate. Specifically, non-progressors

had no PD (in trials) or disease progression (in RWD) throughout the study duration, and progressors had PD or disease progression. The main difference between the two definitions is that the durable clinical benefit definition considers durable SD as non-progressors, whereas the durable response definition requires durable CR or PR to be 'responders'. The two treatment outcome definitions are illustrated in figure 1C.

Biomarker measurements

PD-L1 on tumor tissue was detected by immunohistochemistry using anti-PD-L1 antibody clones 22C3, SP142, SP263 or 28–8. Sensitivity analysis using the different antibody clones showed similar patterns of association with response. They were combined to increase power. Samples were considered 'negative' if they had a percentage tumor cell with PD-L1 staining <1%, 'low/mod' if it was ≥1% and <50%, and 'high' it was ≥50%. The Roche trials used the SP142 PD-L1 assay (Ventana Medical Systems, Tucson, Arizona, USA). FH and CGDB patients used mainly 22C3, SP142 or SP263 assays (results extracted from the medical records in the databases). Tumor PD-L1 expression measured closest to and no more than 1-year prior to the index start date of treatment was used for the analyses. In case of multiple records from the same date, the records from the primary site and the most commonly used antibody were selected.

TMB was quantified by FMI using FoundationOne panel next-generation sequencing,[34] and defined as the number of non-driver somatic coding mutations detected per megabase of a tumor genome extrapolated from targeted sequenced genome. TMB data from the most recent specimens collected before start of treatment was used. Research Use Only calculations based on FMI's research algorithm at the time of collection were analyzed.[28 35]

Measurements from standard blood analytes were extracted from the three data sources. Data from the most recent measurement no more than 1 year before the start of treatment was used. In case of multiple values from the same date, the median was used.

Statistical analysis

The statistical analysis approach we used comprised of two steps. In the first step, patient groups with high and low biomarkers were balanced on their baseline characteristics using the multivariate propensity score-based cohort balancing SMRW (Standardized Mortality Ratio Weighting) algorithm to minimize potential confounders between these comparison groups.[30 36] The second step used univariate analysis but made use of the weights coming from the cohort balancing for each patient. Baseline characteristics included age, sex, race, disease stage at the initial diagnosis, smoking history, histology and ECOG PS. These are available demographics and covariates that can potentially impact the outcome (having durable response or not). The descriptive statistics of the baseline characteristics are in table 1 (for the 24,152 patients with relevant outcome data) and online supplemental table 1 (for the initial 71,850 patients) and online supplemental table 2 (for the 8121 patients analyzed for tumor PD-L1).

There were differences in patient characteristics between different data sources, especially between atezolizumab trials and RWD (FH and CGDB). Since patients from the different data sources were present in both the high and low biomarker groups, the differences between these two analysis groups were readily balanced with methods like SMRW. All the results from the association and survival analyses presented in the main body of the paper used balanced cohorts (by applying SMRW weights). It was noted that there were typically no major differences in results between balanced and unbalanced cohorts. An example for unbalanced cohort has been provided in online supplemental figure 2.

Association of biomarkers with the treatment outcomes was explored using Fisher's exact test. Null hypothesis was complete independence between the groups. Sensitivity analysis using multivariate logistic regression adjusted by the same set of baseline covariates used in propensity score-based balancing mentioned above produced very similar results (ORs were mostly ≤ ±0.03 compared with Fisher's exact test using propensity score balanced data). In the mosaic plot for visualizing the association (vcd R package[37]), colors reflect Pearson's χ^2 residuals and indicate extent of deviation from expected frequency under complete independence. Forest plot for summarizing the ORs from the Fisher's exact tests was performed using forestplot R package.[30 38] Statistical significance of difference between ORs was estimated using z-test when independence assumption was true (using the asymptotic normal distribution for log odds). Otherwise, bootstrapping was used to estimate the mean and variance of the log OR differences, which were used to calculate the p value. Overlapping and unique patients in each of the data set were sampled separately and then recombined accordingly to capture the dependence between the two data sets. Survival analysis used univariate Cox proportional hazards model with individual biomarkers investigated in separate models. The models were corrected for any potential imbalance in baseline characteristics between the low and high biomarker groups using the weights from the propensity score-based SMRW outputs. The survival curves were performed using Kaplan-Meier method.[39 40] All analyses were conducted using R software V.3.6 or 4.0.

RESULTS

Patient selection

A total of 71,850 patients with advanced NSCLC were pooled from the FH, CGDB and nine atezolizumab trials. Characteristics of these patients are provided in online supplemental table 1. The selection of patients from the data sources is depicted in figure 1A. Characteristics of the 24,152 patients selected for biomarker analysis are

Table 1 Characteristics of the patients selected for biomarker analysis from the three data sources (the 24,152 patients 'with relevant outcome data' in figure 1A)

	Atezo* (N=3294)	CGDB† (N=2111)	FH‡ (N=18,747)
Age§			
Mean (SD)	63.2 (9.27)	67.4 (9.93)	68.1 (9.23)
Median (min, max)	64.0 (25.0, 90.0)	68.0 (27.0, 85.0)	69.0 (25.0, 85.0)
Sex			
Female	1204 (36.6%)	1095 (51.9%)	8866 (47.3%)
Male	2090 (63.4%)	1016 (48.1%)	9880 (52.7%)
Unknown	0 (0%)	0 (0%)	1 (0.0%)
Race			
African American	65 (2.0%)	146 (6.9%)	1713 (9.1%)
Asian	583 (17.7%)	66 (3.1%)	383 (2.0%)
Caucasian	2525 (76.7%)	1477 (70.0%)	13,762 (73.4%)
Other	20 (0.6%)	299 (14.2%)	1507 (8.0%)
Unknown	101 (3.1%)	123 (5.8%)	1382 (7.4%)
ECOG PS¶			
0	1300 (39.5%)	577 (27.3%)	4219 (22.5%)
1	1983 (60.2%)	1037 (49.1%)	6865 (36.6%)
2	8 (0.2%)	267 (12.6%)	2329 (12.4%)
3	1 (0.0%)	60 (2.8%)	483 (2.6%)
4	0 (0%)	2 (0.1%)	28 (0.1%)
Missing	2 (0.1%)	168 (8.0%)	4823 (25.7%)
Disease stage**			
Stage 0	0 (0%)	0 (0%)	2 (0.0%)
Stage I	247 (7.5%)	190 (9.0%)	1598 (8.5%)
Stage II	251 (7.6%)	148 (7.0%)	1027 (5.5%)
Stage III	616 (18.7%)	525 (24.9%)	4808 (25.6%)
Stage IV	2125 (64.5%)	1201 (56.9%)	10,732 (57.2%)
Unknown	55 (1.7%)	47 (2.2%)	580 (3.1%)
Smoking history			
Previous/current	2763 (83.9%)	1775 (84.1%)	16672 (88.9%)
Never	531 (16.1%)	333 (15.8%)	1902 (10.1%)
Unknown	0 (0%)	3 (0.1%)	173 (0.9%)
Histology			
Non-squamous	2404 (73.0%)	1560 (73.9%)	12,633 (67.4%)
Squamous	848 (25.7%)	484 (22.9%)	5258 (28.0%)
Unknown	42 (1.3%)	67 (3.2%)	856 (4.6%)

*Atezo—atezolizumab NSCLC trials.
†CGDB—Clinico-Genomic Database by Flatiron Health-Foundation Medicine.
‡FH—Flatiron Health electronic health record database.
§Age—age at treatment start.
¶ECOG PS—baseline Eastern Cooperative Oncology Group performance status before treatment start.
**Disease stage—group stage at initial diagnosis for the RWD sources (FH and CGDB), and at study start for the atezolizumab trials.
NSCLC, non-small cell lung cancer ; RWD, real-world data.

provided in table 1. The exclusive nature of the therapies was chosen to facilitate better comparison and delineation of biomarker associations with outcomes to CPI versus chemotherapy. Characteristics of patients for whom tumor PD-L1 data were available and who were treated with mono-CPI or chemotherapy are provided in online supplemental table 2.

Standard blood analytes are only prognostic

Using the large patient cohorts from the FH database, we first examined standard blood analytes, many of which have been suggested to be predictive biomarkers of response to CPI in several cancer types. We tested the association of categorical levels (first tertile vs third tertile) of the blood analytes with disease progression

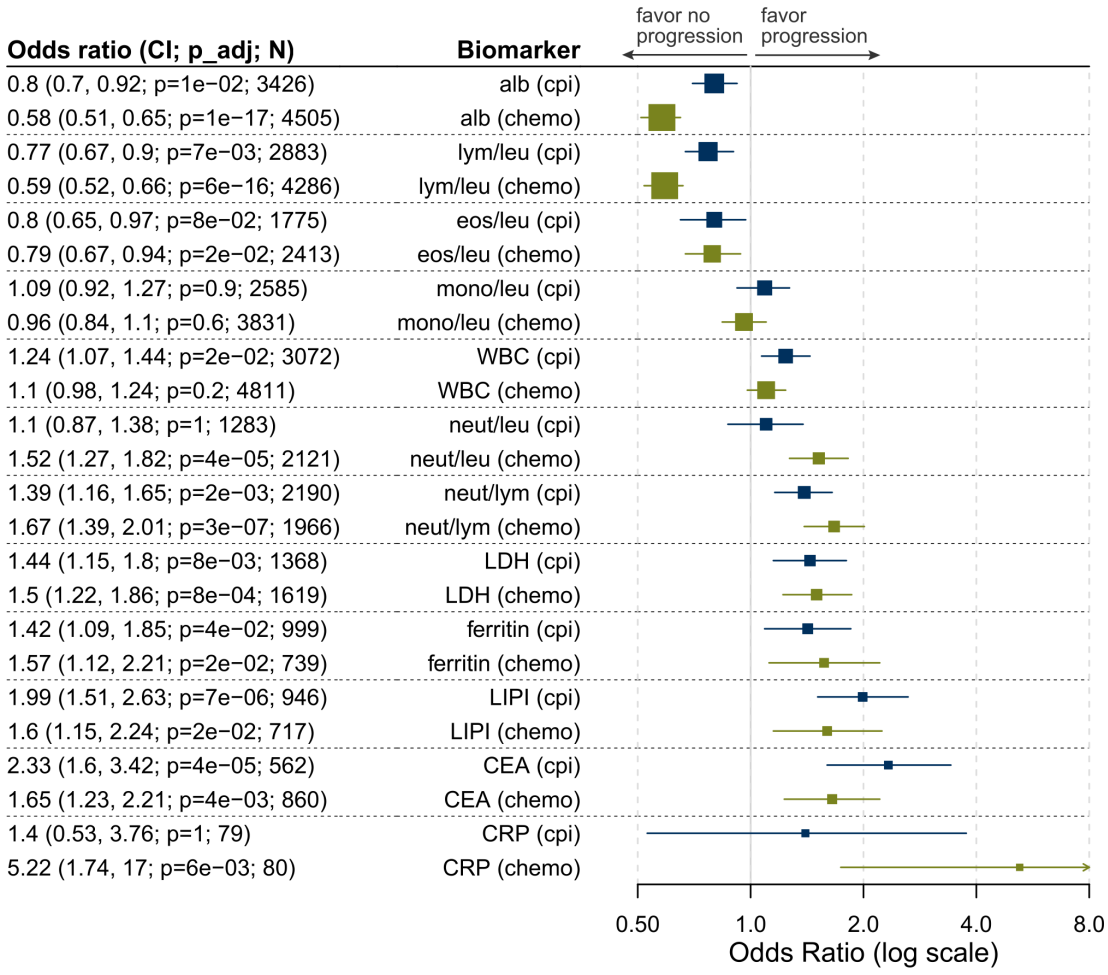

Figure 2 Prognostic effect of standard blood analytes. Forest plot of association of standard blood analytes with CPI progression in Flatiron Health patients. OR <1 indicates association of high biomarker levels (third tertile) with greater odds for no disease progression, compared with low biomarker expression (first tertile). OR >1 indicates the opposite and OR of 1 indicates no impact of the biomarker on clinical outcome. The groups of patients with high and low biomarker levels were balanced on their baseline characteristics using Standardized Mortality Ratio Weighting algorithm. P values were multiple testing corrected by the Holm method.[51] CEA, carcinoembryonic antigen; CPI, checkpoint inhibitors; CRP, C-reactive protein; LDH, lactate dehydrogenase; LIPI, Lung Immune Prognostic Index; PWBC, white blood cell.

(using the durable clinical benefit definition described in figure 1C). Categorical data were used to reduce noise or differences between data sets due to subtle differences between outcome measurements in clinical studies versus RWD or between different studies. Serum albumin levels were correlated with disease progression to both CPI (OR 0.80, 95% CI 0.70 to 0.92; adjusted p=0.01) and chemotherapy outcomes (OR 0.58, 95% CI 0.51 to 0.65; adjusted p<0.001; figure 2). This confirmed its prognostic value since higher albumin levels correlated with better outcome irrespective of the nature of treatment and was not specific for the CPI response. Other evaluated blood analytes did not differentiate association with CPI outcomes from those with chemotherapy (figure 2).

Predictive effect of tumor PD-L1 and TMB

We then examined tumor PD-L1 and TMB levels using CGDB and atezolizumab trial data. The OR was 0.20 (95% CI 0.13 to 0.30; p<0.001) which denotes that patients with high tumor expression of PD-L1 (≥50% tumor cells with positive staining) had approximately one-fifth (20%) the odds of being non-responders to CPI therapies compared with patients with no PD-L1 tumor expression. The area under the receiver operating characteristic (ROC) curve was 0.65 (95% CI 0.61 to 0.69). Conversely, tumor PD-L1 had only a weak correlation with response to chemotherapy (OR 0.69, 95% CI 0.49 to 0.97; p=0.03; figure 3A; ROC AUC=0.56, 95% CI 0.52 to 0.60). Similarly, patients who had a high TMB (third tertile, ≥10.44 mut/Mb) had less than half the odds (OR 0.40, 95% CI 0.29 to 0.54; p<0.001; ROC AUC=0.62, 95% CI 0.59 to 0.65) of not responding to CPI therapy compared with patients who had low TMB (first tertile). However, TMB had no correlation with response to chemotherapy (OR 0.95, p=0.9; figure 3B; ROC AUC=0.52, 95% CI 0.46 to 0.58).

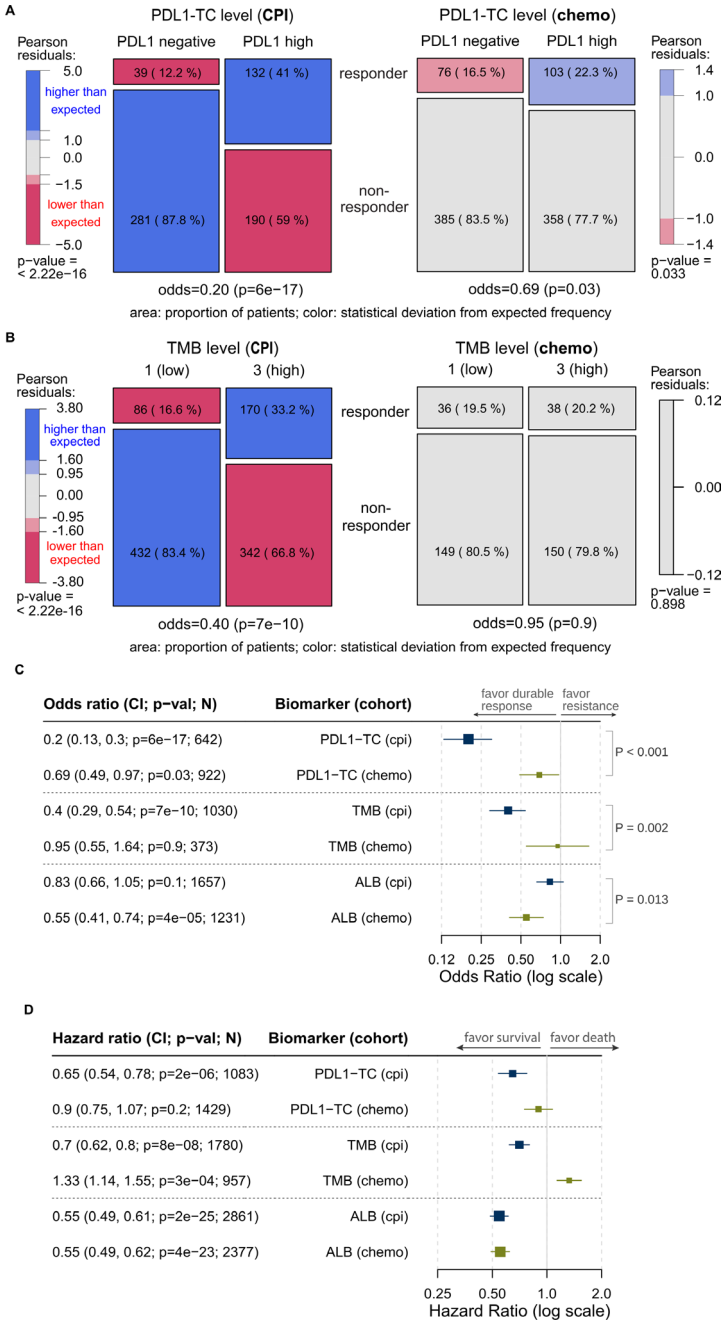

Figure 3 Predictive effect of PD-L1 and TMB for responses to CPI or chemotherapy in CGDB and atezolizumab trial patients. (A) Distribution of CPI (left plot) and chemotherapy (right plot) durable responders and non-responders among the PD-L1-high and PD-L1-negative patients. (B) Distribution of CPI (left plot) and chemotherapy (right plot) durable responders and non-responders among the TMB high and low patients. (C) Forest plot of association of baseline tumor PD-L1 and TMB, and blood albumin with response to CPI versus chemotherapy. The differences in each pair of CPI and chemo ORs were statistically significant (z-test). (D) Forest plot of association of baseline tumor PD-L1 and TMB, and blood albumin with overall survival. For A–D, the groups of patients with high and low biomarker levels were balanced on their baseline characteristics using Standardized Mortality Ratio Weighting algorithm, which outputs weights for each patient. For both A and B, the numbers in each of the cells of the mosaic plot indicate the weighted patient counts and percentages in the corresponding biomarker group. The areas indicate the proportion of the weighted patient counts in each of the four groups. X^2 p value (under the color legend scale bar) indicates overall statistical significance of any association between PD-L1 or TMB levels and CPI response. Color indicates individual statistical significance associated with each cell. Blue indicates significantly higher patient numbers and red indicates significantly lower numbers than if the distribution was random. Color intensity indicates the extent of significance from expected (light and dark color correspond to confidence levels of 90% and 99%, respectively). The OR is from Fisher's exact test of the weighted patient counts. For C and D, OR (C) or HR (D) <1 indicates association of high biomarker levels with greater odds for clinical response (C) or overall survival (D), compared with low biomarker level. OR/HR >1 indicates the opposite and ratio of 1 indicates no impact of the biomarker on clinical outcome. ALB, albumin; CGDB, Clinico-Genomic Database; CPI, checkpoint inhibitors; PD-L1, programmed death ligand-1; TC, tumor cells; TMB, tumor mutational burden.

All the reported results used high and low biomarker level cohorts which were balanced using patients' baseline characteristics. The analysis results from using unbalanced cohorts showed the same pattern (online supplemental figure 2).

The predictive nature of tumor PD-L1 and TMB is summarized and compared side-by-side with the prognostic nature of blood albumin in figure 3C. Comparing PD-L1 and TMB, tumor PD-L1 expression was significantly better as an independent predictive biomarker for response to CPI than TMB (OR 0.20 vs OR 0.40). This was still the case even when the PD-L1 high group was compared with the combined PD-L1 negative and low/mod groups (OR 0.35, 95% CI 0.26 to 0.46; p<0.001; online supplemental figure 3).

We have also examined the OS of these patient groups and their association with biomarkers. Patients with early deaths were included for the OS analysis. Normal or higher blood albumin level strongly correlated with increased OS with both CPI and chemotherapy compared with low albumin level (HR 0.55 in both cases; p<0.001). It is established that normal or higher albumin levels correlate with good prognosis. High tumor PD-L1 expression also correlated with increased OS with CPI therapy compared with low expression (HR 0.65, 95% CI 0.54 to 0.78; p<0.001), but no statistically significant association was observed with chemotherapy (figure 3D). Similar correlation between high TMB and increased OS was also observed with CPI therapy (HR 0.70, 95% CI 0.62 to 0.80; p<0.001). Interestingly, there was an inverse correlation between OS and TMB in patients receiving chemotherapy (HR 1.33, 95% CI 1.14 to 1.55; p<0.001). Our finding is consistent with some of the previous reports[41 42] although others have shown no significant differences.[43 44]

Combined, PD-L1 and TMB had stronger predictive effect

The combination of high tumor PD-L1 expression and high TMB had a higher predictive power than the two biomarkers assessed independently. Taken independently, the OR for tumor PD-L1 expression and TMB in patients who had both data were 0.19 and 0.37 whereas the combined high PD-L1 and TMB had an OR of 0.04 (95% CI 0 to 0.18; p<0.001; figure 4A). This means that the odds of having no durable response to CPI therapy was 4% of those with combined negative PD-L1 and low TMB. The increased predictive potential of the combination of PD-L1 and TMB was statistically significant compared with PD-L1 alone (p=0.01; figure 4A). Figure 4B presents the percentage of durable responders and non-responders in the different combinations of low versus high levels of PD-L1 and TMB. The dual-high ('hihi') compared with dual-low ('lolo') groups had a strong difference in per cent CPI response (OR=0.04). Patients with high expression of one biomarker and low expression of the other had an intermediate response to CPI. There was a significantly large difference in CPI response between the dual-high and the TMB-only high patients ('hihi' vs 'lohi'; OR 0.19, 95% CI 0.07 to 0.46; p<0.001). In contrast, the difference between dual-high and PD-L1-only high patients was more moderate and not statistically significant ('hihi' vs 'hilo'; OR 0.50, 95% CI 0.19 to 1.26; p=0.13). This is consistent with the observation above that PD-L1 was a better independent predictive biomarker for response to CPI than TMB.

PD-L1 predictive effect influenced by prior therapy, histology and smoking history

We aimed to further investigate key factors that influenced the predictive effect of the biomarkers. We used the large FH cohorts that enabled greater statistical confidence in the detection of predictive effects in subpopulations. Prior to the analysis, we first confirmed the predictive effect of tumor PD-L1 and TMB on disease progression to CPI (figure 5A). We used patients who had both response and progression data and could be studied for either response or progression (CGDB and trial patients) using the two outcome definitions. Interestingly, PD-L1 had a stronger correlation to response to CPI (CR or PR; OR 0.20) than to progression (OR 0.37). This difference is statistically significant (p=0.009). TMB, however, was a comparable predictor of tumor response as it was for progression. This was because more SD non-progressors were PD-L1 negative than PD-L1 high (61% vs 39%). These PD-L1 negative SD patients were not considered responders, leading to fewer PD-L1-negative responders, resulting in a stronger correlation with response to CPI. However, fewer SD non-progressors were TMB low than TMB high (45% vs 55%).

We then combined patients from all three sources for the subpopulation progression analysis. We found that tumor PD-L1 expression had a stronger inverse correlation with disease progression in patients with prior chemotherapy than those with first-line CPI (figure 5B; OR 0.34 vs 0.57; p=0.003). Moreover, the predictive effect was stronger in patients with non-squamous than squamous disease (OR 0.37 vs 0.58; p=0.012). This difference in predictive effect associated with histology was not strongly confounded by smoking history as the analysis of smokers alone reflected a similar stronger correlation of PD-L1 expression with non-squamous disease. In addition, the predictive effect was significantly stronger in patients with a previous or current history of smoking than non-smokers (OR 0.39 vs 0.70; p=0.019).

We further analyzed the TMB predictive effect in these subpopulations in the CGDB and atezolizumab trials. We used the clinical benefit outcome definition (progression analysis) because the predictive strength was comparable to that using durable response definition (figure 5A) and there were more patients. Overall, the outcome was similar to our findings with PD-L1 for prior therapy and histology subpopulations, but potentially due to smaller sample size, it had weaker statistical significance (figure 5C). Accordingly, TMB levels had a stronger inverse correlation with disease progression in patients with prior chemotherapy than those with first-line CPI (OR 0.36 vs 0.62; p=0.03), but did not differentially correlate with progression in

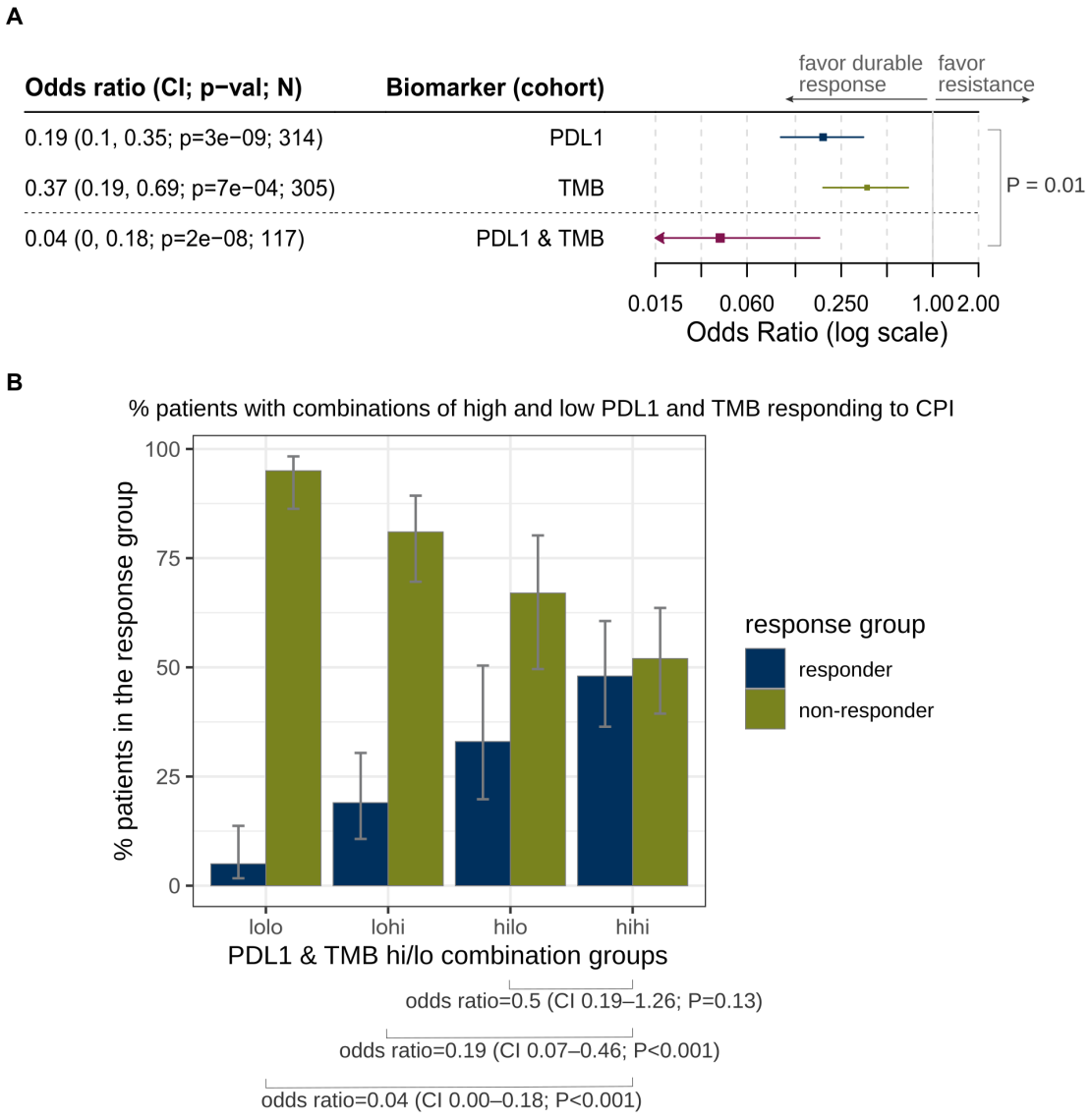

Figure 4 Combined effect of tumor PD-L1 and TMB for responses to CPI. (A) Forest plot showing OR and 95% CI for individual and combined biomarker effects in patients with both PD-L1 and TMB data. The top two rows are patients with high versus low level of individual biomarkers. ORs <1 indicate association of high biomarker levels with greater odds for clinical response, compared with low biomarker levels. The combined PD-L1 and TMB patients (dual-high compared with dual-low) in the bottom row showed a much stronger predictive effect than each individual biomarker in the top two rows (OR 0.04 vs OR 0.2 and 0.4, respectively). The difference in the ORs is statistically significant (bootstrap p=0.01). All high and low biomarker patient groups in each analysis were balanced on their baseline characteristics using Standardized Mortality Ratio Weighting algorithm. (B) Bar chart shows the per cent of CPI durable responder/non-responder groups in each of the four combined high and low expression groups: first indicates PD-L1-TC ('lo'=PD-L1 negative; 'hi'=PD-L1 high) and second TMB ('lo'=first tertile; 'hi'=third tertile). Error bars indicate 95% confidence that the true proportion are within the intervals. ORs indicate the odds of being non-responding in patients who had both biomarkers high versus those with only one of the biomarkers high or both low. CPI, checkpoint inhibitors; PD-L1, programmed death ligand-1; TC, tumor cells; TMB, tumor mutational burden.

patients with non-squamous than squamous disease (OR 0.38 vs 0.48; p>0.05). Interestingly, high TMB levels had a much stronger predictive effect for no CPI progression (or CPI response) in non-smokers than smokers (OR 0.19 vs 0.50; p=0.002).

DISCUSSION

Notwithstanding the limitations of the present study, such as the retrospective nature and the heterogeneity of the patient populations inherent to RWD analysis, there are some important associations revealed by our analyses. The utility of tumor PD-L1 expression and TMB is confirmed as predictive biomarkers for response specific to CPI, combining real-world settings with clinical trials. Our data however show that standard blood analytes that have been described previously as predictive biomarkers of response to CPI were in fact prognostic (independent of treatment type or not specific to CPI). We found that including early

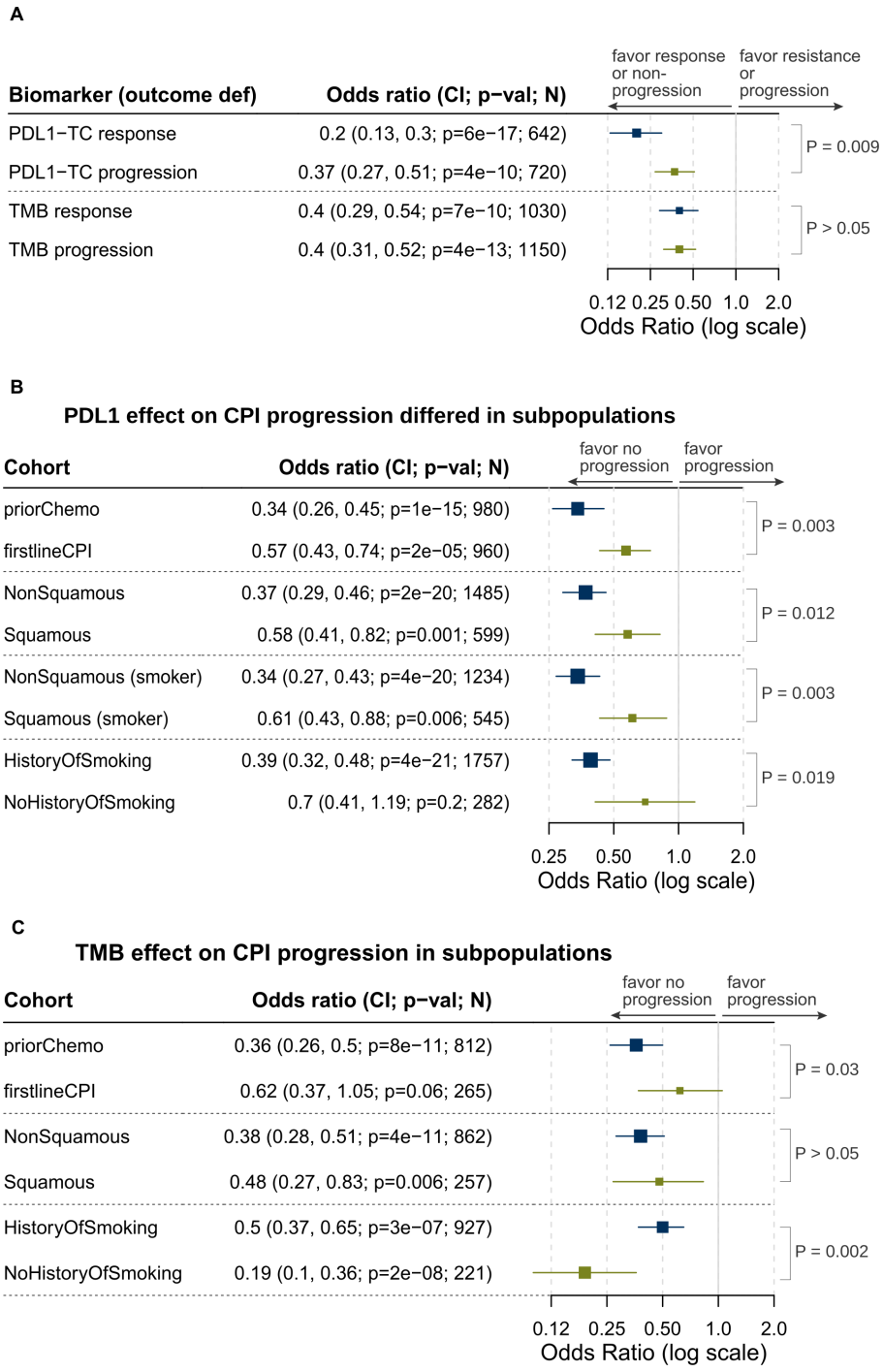

Figure 5 Factors influencing predictive effect of PD-L1 and TMB on progression following CPI therapy. (A) Confirmation of predictive effect of PD-L1 and TMB on disease progression outcome. Treatment outcome was defined using two different definitions: having response (CR and PR) versus having non-progression (equivalent to CR, PR and SD), as illustrated in figure 1C. Patients who had both response and progression data (in CGDB and clinical trials) were used for these comparisons. The forest plot shows the ORs of patient groups with high versus low biomarker levels, their odds of having either response or progression. Only PD-L1 levels showed a significant difference in the two ORs for the two outcome definitions (p=0.009). (B) Forest plot showing ORs comparing CPI progression in high PD-L1 versus negative PD-L1 in different subpopulations. Patients from all three data sources were used. The differences in each pairs of ORs were statistically significant (z-test ≤0.05). (C) Forest plot showing ORs comparing CPI progression in third tertile versus first tertile of TMB in different subpopulations. Patients from CGDB and atezolizumab trials who had TMB data were used. The prior therapy and analysis of smoking history showed significant difference in the two ORs (z-test p<0.05). For all the plots, higher ORs on the >1 side indicate stronger biomarker predictive effect on CPI progression. In each pair of analyses, the high and low biomarker level groups were balanced by their baseline characteristics using the Standardized Mortality Ratio Weighting algorithm. CGDB, Clinico-Genomic Database; CPI, checkpoint inhibitors; CR, complete response; PD-L1, programmed death ligand-1; PR, partial response; SD, stable disease; TMB, tumor mutational burden.

deaths (online supplemental figure 1) only strengthened the association of serum albumin with both CPI and chemotherapy outcomes, without affecting the association of PD-L1 or TMB with treatment outcomes. This is reflected by the lower ORs for albumin and no obvious change of ORs for PD-L1 and TMB (online supplemental figure 1 vs figure 3C). This increased association was also observed in other blood analytes (data not shown) and reflects the association of these blood analytes with OS (eg, low baseline albumin predicted the early deaths). This also suggests that PD-L1 and TMB are specific for response to CPI and not as much for OS. Removal of the early deaths from the analysis allowed us to focus more on the biomarkers' effect on response to CPI, and better differentiate between CPI-specific predictive biomarkers versus general prognostic biomarkers.

Our results add to the body of evidence and demonstrate the clinical utility of TMB[15 45 46] as a biomarker. While TMB was equally effective as a biomarker for predicting both disease progression and response to CPI, high tumor PD-L1 expression emerged as a stronger correlate of clinical response (CR or PR) than disease progression. Additionally, tumor PD-L1, in our analysis, had a better correlation to response in comparison to TMB. This might be because high PD-L1 is not just a direct escape mechanism of the tumor through PD-1 engagement, but also indirectly reflect the more inflamed tumor microenvironment as PD-L1 is upregulated by inflammatory cytokines such as interferon-γ and tumor necrosis factor-α and is more amenable to successful cancer immunotherapy. On the other hand, although high TMB typically reflects higher immunogenicity of the tumor by potentially producing de novo epitopes, it also reflects a greater likelihood of escape by aggressive and antigen-loss variants. While tumor PD-L1 and TMB are independent biomarkers,[2 47] our results show that pairing both improves the predictability of response to CPI therapy. Our results are in keeping with a previous study demonstrating the complementarity of the two biomarkers.[15] Indeed, the percentage of patients responding to CPI who had high tumor PD-L1 or TMB alone was approximately half of the percentage of patients whose tumors were high for both biomarkers (figure 4B). Our results also revealed that the predictive power of PD-L1 was the greatest in patients who had non-squamous histology, history of smoking and prior chemotherapy. It could be speculated that this might be due to the histology-specific mutational landscape associated with these different groups. Our own data revealed that KRAS mutations are much more prevalent in patients with NSCLC of non-squamous histology and those with smoking history (OR=4.19, 95% CI 3.58 to 4.92, p<0.001; OR=3.47, 95% CI 2.95 to 4.10, p<0.001, respectively), which is in keeping with previous reports.[48] Intriguingly, high TMB is associated with better response to CPI in non-smokers as compared with smokers. One might speculate that this reflects that tumors in non-smokers harbor neoepitopes with higher immunogenicity or that they have better antigen presenting machinery, potentially due to a less suppressed tumor microenvironment.

Finally, there is the need for approaches to make CPI more effective in patients with low tumor PD-L1 and/or TMB. Recent reports have demonstrated that many chemotherapy agents used to treat lung cancer can upregulate PD-L1 on tumor cells.[49 50] The use of chemo-immunotherapy and CPI-immunotherapy sequentially or in combination may provide the best outcome for patients with NSCLC. Analyzing these questions in RWD and clinical study data in this patient group on treatment with CPI-chemo combination therapy can test this hypothesis.

In conclusion, despite heterogeneity of patient population and PD-L1 assays, high PD-L1 and TMB were found to correlate with durable response, with greater correlation in non-squamous patients and those with prior chemotherapy or smoking history. PD-L1 and TMB in combination had much stronger correlation with tumor response than either biomarker considered independently. PD-L1 was also better at predicting tumor responses compared with disease progression, whereas TMB showed no distinction. Cumulatively, this report reinforced the clinical utility of tumor PD-L1 and TMB, for predicting response to CPI in patients with NSCLC.

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Contributors WVS processed all the data and performed all the analyses in the study, takes responsibility for the integrity and accuracy of data analyses and is the studyguarantor. DD contributed to the statistical analysis of the data and reviewed the manuscript. ER contributed to the discussion and revision of clinical aspects and definition of clinical outcomes and JC is responsible for the conception and design of the work.

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Ethics approval All clinical studies reported have already been published and the relevant references[5 20–27] are provided in the manuscript. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon request. The RWD used in the study have been originated by Flatiron Health Inc and Foundation Medicine Inc. These de-identified data may be made available upon request, and are subject to a license agreement with Flatiron Health and Foundation Medicine; interested researchers should contact dataaccess@flatiron.com and cgdb-fmi@flatiron.com to determine the licensing terms. For the Roche data, qualified researchers may request access to individual patient-level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/ourmember/roche/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/innovation/process/clinical-trials/data-sharing/).

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