

Prediction performance comparison of biomarkers for response to immune checkpoint inhibitors in advanced non-small cell lung cancer

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Abstract

Background: The aim of the present study was to compare the predictive accuracy of PD-L1 immunohistochemistry (IHC), tissue or blood tumor mutation burden (tTMB, bTMB), gene expression profile (GEP), driver gene mutation, and combined biomarkers for immunotherapy response of advanced non-small cell lung cancer (NSCLC).

Methods: In part 1, clinical trials involved with predictive biomarker exploration for immunotherapy in advanced NSCLC were included. The area under the curve (AUC) of the summary receiver operating characteristic (SROC), sensitivity, specificity, likelihood ratio and predictive value of the biomarkers were evaluated. In part 2, public datasets of immune checkpoint inhibitor (ICI)-treated NSCLC involved with biomarkers were curated ($N = 871$). Odds ratio (OR) of the positive versus negative biomarker group for objective response rate (ORR) was measured.

Results: In part 1, the AUC of combined biomarkers (0.75) was higher than PD-L1 (0.64), tTMB (0.64), bTMB (0.68), GEP (0.67), and driver gene mutation (0.51). Combined biomarkers also had higher specificity, positive likelihood ratio and positive predictive value than single biomarkers. In part 2, the OR of combined biomarkers of PD-L1 plus TMB (PD-L1 cutoff 1%, 0.14; cutoff 50% 0.13) was lower than that of PD-L1 (cutoff 1%, 0.33; cutoff 50% 0.24), tTMB (0.28), bTMB (0.48), *EGFR* mutation (0.17) and *KRAS* mutation (0.47), for distinguishing ORR of patients after immunotherapy. Furthermore, positive PD-L1, tTMB-high, wild-type *EGFR*, and positive PD-L1 plus TMB were associated with prolonged progression-free survival (PFS).

Conclusion: Combined biomarkers have superior predictive accuracy than single biomarkers for immunotherapy response of NSCLC. Further investigation is warranted to select optimal biomarkers for various clinical settings.

KEYWORDS

prediction biomarkers, immune checkpoint inhibitors, non-small cell lung cancer

INTRODUCTION

The striking development of immunotherapy, especially immune checkpoint inhibitors (ICIs), has brought obvious curative effect and prolonged overall survival (OS) for advanced non-small cell lung cancer

(NSCLC) while some patients do not obtain benefit from immunotherapy.¹ In response to the urgent need for precision medicine, determination of an optimal biomarker to enrich beneficial population of immunotherapy is essential to enhance healthcare cost-effectiveness.²

Programmed death ligand-1 (PD-L1) expression measured by immunohistochemistry (IHC), the first FDA-approved companion diagnostic for immunotherapy, has been widely utilized in clinical trials and real-world clinical practice.^{3,4} However, the divergent antibodies (22C3, 28-8, SP263 and SP142) and cutoff values of PD-L1 IHC test have confused some clinicians.⁵ Apart from that, a cohort study of 1552 patients with NSCLC found that high tumor mutation burden (TMB) (>19.0 mutations per megabase) was associated with improved efficacy of immunotherapy irrespective of PD-L1 expression levels.⁶ Moreover, the phase 2 B-FIRST trial suggested that blood TMB (bTMB) ≥ 16 indicated longer OS after the treatment of atezolizumab, which pushed forward the predictive biomarker of immunotherapy to the field of liquid biopsy.⁷ In addition, in previous studies, the T cell-inflamed gene expression profile (GEP), a focused gene set related to antigen presentation, IFN γ signal, and cytolytic effector molecules, was found to hold enormous potential to predict response from immunotherapy across multiple tumor types.^{8,9} Since most clinical trials have excluded patients with *EGFR* or *KRAS* mutations,^{10–12} the application of ICIs on patients with driver gene mutations is controversial. Noticeably, the ORIENT-31 trial reported improved progression-free survival (PFS) in patients with *EGFR* mutations after receiving immunotherapy.¹³ The impact of *KRAS* mutation on immunotherapy response has also been extensively investigated.¹⁴

Above all, multiple biomarkers have demonstrated contribution to the prediction of immunotherapy efficacy. A study of over 10 solid tumor types compared the predictive accuracy of PD-L1, TMB, GEP, multiplex IHC (mIHC)/immunofluorescence (IF) and multimodality biomarkers for anti-PD-1/PD-L1 treatment, which revealed superior predictive value of mIHC/IF and multimodality biomarkers.¹⁵ Here, we systematically compared the predictive performance of these biomarkers in advanced NSCLC, for the first time, based on the ICI-treated clinical trials and integrated public datasets of NSCLC. Furthermore, we provide the added value of bTMB and driver gene mutations in predicting the efficacy of ICI-based therapy.

METHODS

Clinical trials and dataset curation

In part 1, we searched PubMed, Embase, and Cochrane Central from inception to December 6, 2023 for eligible studies following the PRISMA-DTA guideline.¹⁶ The search strategy is provided in Table S1. Study inclusion criteria was as follows: (1) registered on ClinicalTrials.gov. (2) Advanced or metastatic NSCLC patients treated with mono-ICI, or ICI-based combination therapy. (3) Not less than two categories of biomarkers were explored. (4) The sample size was ≥ 40 . Studies including PD-L1 positive patients only were excluded.

In part 2, public datasets of ICI-treated advanced/metastatic NSCLC patients involved with biomarker exploration were included^{12,17–19} to further evaluate the predictive value of the biomarkers.

Data collection

In part 1, basic information about the included studies was extracted: first author, published year, size of analyzed patients, ICI treatment strategy, investigated biomarker, corresponding assay and threshold. Objective response rate (ORR), if not, surrogated OS rate, or PFS rate of patients according to the biomarker (+ vs. –) were extracted to present a 2 by 2 contingency table of TP (true-positive), FP (false-positive), FN (false-negative) and TN (true-negative). As to the driver gene mutation status, *EGFR* wild-type and *KRAS* mutated type were defined as positive, respectively. For PD-L1 IHC, tTMB or bTMB with multiple cutoff values investigated, the threshold with the biggest Youden statistic was chosen.

In part 2, biomarker relevant data and ORR were curated from the original published literatures. tTMB/bTMB were stratified as high versus low according to the threshold defined in the source study. *EGFR* mutations investigated here referred to exon 19 deletion (19del), exon 20 insertion (20ins), L858R, L861Q/S768I/E709X/G719X/exon 18 deletion (18del), T790M, and C797S variants. *KRAS* mutations referred to all types of genomic variants of *KRAS*.

Statistical analysis

In part 1, the sensitivity against specificity of individual biomarker was calculated and fitted into the summary receiver operating characteristic (SROC) curve of each category of biomarker. The area under the curve (AUC) with 95% confidence interval (CI) was measured. In addition, pooled analyses of sensitivity and specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR), positive predictive value (PPV) and negative prediction value (NPV) of each category of biomarker were conducted. Bivariate model developed by Reitsm et al.²⁰ was applied to produce synthesized measurements mentioned above. Nonoverlapping of confidence interval (CI) indicated statistical significance ($p < 0.05$). A Deeks' funnel plot was performed to measure the publication bias of the included studies. Statistical analysis was conducted with Stata software version 14.0 using Midas module. The quality of primary studies was measured by the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool, using Review Manager 5.3 (Cochrane Community).

In part 2, the significance of the categorical biomarkers was determined by chi-squared test, and the predictive value was measured by odds ratio (OR) (95% CI), where $OR < 1$ indicated favorable predictive effect. Progression-free survival was measured by the Kaplan–Meier method. The hazard ratio (HR) (95% CI) according to the biomarker (+

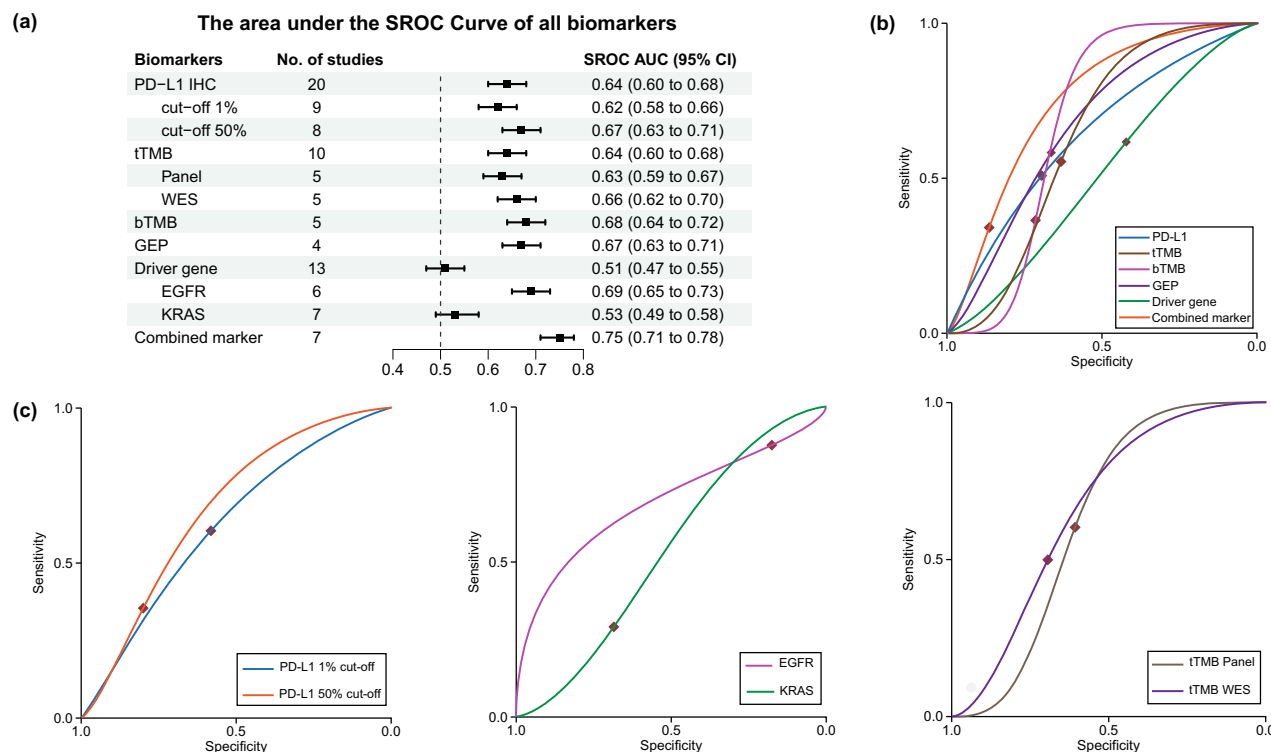


FIGURE 1 Comparison of SROC AUC across all biomarkers and subgroup analyses. (a) Diamonds represent pooled AUC of each kind of biomarker, and horizontal lines indicate 95% CIs. Nonoverlapping 95% CIs indicated a significant difference, $p < 0.05$. (b) SROC of six kinds of biomarkers were summarized in one coordinate system based on individual SROC shown in Figure S2. (c) Subgroup analyses of PD-L1, driver gene mutation, and tTMB assays. AUC, area under the curve; CIs, confidence intervals; SROC, summary receiver operating characteristic; tTMB, tissue tumor mutation burden.

vs. —) was retrieved by the Cox proportional hazards model, and the p -value was determined by the log-rank test with the R package survival (version 3.5–5).

RESULTS

SROC curves and AUC values

In part 1, following rigorous selection steps, 36 articles^{10–12,19,21–50} were filtered from a total of 7698 manuscripts for data extraction (Figure S1). Finally, 59 individual biomarker results were processed, including PD-L1 IHC ($n = 20$), tTMB ($n = 10$), bTMB ($n = 5$), GEP ($n = 4$), driver gene mutation (*EGFR*, $n = 6$ and *KRAS*, $n = 7$) and combined marker ($n = 7$, PD-L1 + tTMB/bTMB/GEP). The basic characteristics of all the studies, predictive outcomes (TP, FP, FN, TN), assay and cutoff value of the biomarkers are summarized in Table S2. The sensitivity and specificity of the individual studies were calculated and plotted to fit SROC curves of six kinds of biomarkers, respectively (Figure S2). The AUC of each biomarker modality was measured to determine the performance of predicting response. Combined biomarkers showed higher AUC (95% CI) (0.75 [0.71–0.78]) than PD-L1 (0.64 [0.60–0.68], $p < 0.05$), tTMB (0.64 [0.60–0.68], $p < 0.05$), bTMB (0.68 [0.64–0.72]),

GEP (0.67 [0.63–0.71]), and driver gene mutation (0.51 [0.47–0.55], $p < 0.05$). The AUC of driver gene mutation was significantly lower than other biomarkers (Figure 1a,b). Furthermore, we conducted subgroup analyses and found that PD-L1 IHC predicted ICIs response more effectively when the cutoff was 50% than 1% (AUC [95% CI], 0.67 [0.63–0.71] vs. 0.62 [0.58–0.66]) (Figure 1c). To investigate the predictive effect differences between *EGFR* and *KRAS* mutation, we performed subgroup analysis of driver genes to determine the predictive value. Our results showed that *EGFR* had significantly higher AUC (95% CI) than *KRAS* (0.69 [0.65–0.73] vs. 0.53 [0.49–0.58], $p < 0.05$). Furthermore, we found that tTMB measured by whole exome sequencing (WES) had slightly higher AUC (95% CI) than that measured by targeted gene panel (0.66 [0.62–0.70] vs. 0.63 [0.59–0.67]) (Figure 1c).

The predictive accuracy of the biomarkers was further compared for patients receiving ICI-based combination therapy. The AUC (95% CI) of combined biomarkers (0.70 [0.66–0.74]) remained higher than single biomarkers, and the AUC (95% CI) of bTMB (0.66 [0.62–0.70]) still ranked second. PD-L1 with cutoff 50% (0.68 [0.63–0.72]) still had higher AUC than that of cutoff 1% (0.59 [0.55–0.64]), and the AUC of TMB measured by WES (0.65 [0.61–0.70]) remained slightly higher than that measured by targeted gene panel (0.63 [0.58–0.67]) (Figure S3).

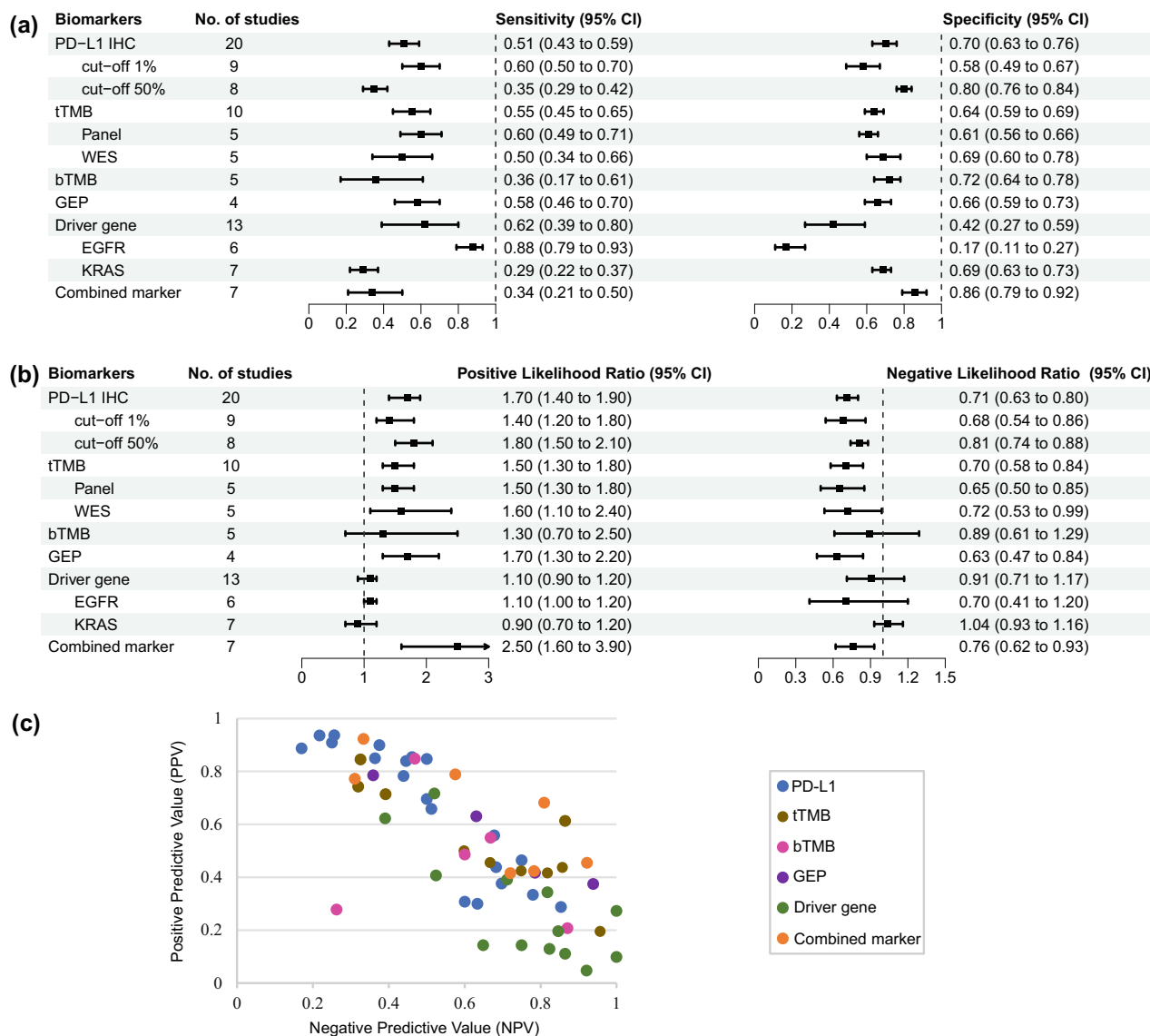


FIGURE 2 Comparison of the pooled sensitivity and specificity, PLR and NLR, PPV and NPV. (a, b) Diamonds represent pooled sensitivity, specificity, PLR, NLR of each kind of biomarker, and horizontal lines indicate 95% CIs. Nonoverlapping 95% CIs indicated a significant difference, $p < 0.05$. (c) Dots represent positive predictive value against negative predictive value of the biomarkers. CIs, confidence intervals; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.

Pooled sensitivity and specificity, LRs and PVs

The pooled sensitivity and specificity of each kind of biomarker were compared (Figure 2a). EGFR mutation had the highest sensitivity (0.88; 95% CI: 0.79–0.93) and the lowest specificity (0.17; 95% CI: 0.11–0.27). The specificity of combined biomarkers (0.86; 95% CI: 0.79–0.92) was significantly higher than PD-L1 IHC (0.70; 95% CI: 0.63–0.76; $p < 0.05$), tTMB (0.64; 95% CI: 0.59–0.69; $p < 0.05$), bTMB (0.72; 95% CI: 0.64–0.78; $p < 0.05$), GEP (0.66; 95% CI: 0.59–0.73; $p < 0.05$) and driver gene mutation (0.42; 95% CI: 0.27–0.59; $p < 0.05$). For PD-L1 IHC, the sensitivity of cutoff 1% (0.60; 95% CI: 0.50–0.70) was significantly higher than that of cut-off 50% (0.35; 95% CI: 0.29–0.42; $p < 0.05$) while the specificity was significantly lower (0.58; 95% CI: 0.49–0.67

vs. 0.80; 95% CI: 0.76–0.84; $p < 0.05$). The sensitivity of EGFR (0.88; 95% CI: 0.79–0.93) was significantly higher than that of KRAS (0.29; 95% CI: 0.22–0.37; $p < 0.05$) while the specificity of EGFR (0.17; 95% CI: 0.11–0.27) was significantly lower than that of KRAS (0.69; 95% CI: 0.63–0.73; $p < 0.05$).

The pooled PLR and NLR of all biomarkers were also compared (Figure 2b). The pooled PLR of combined biomarkers (2.50; 95% CI: 1.60–3.90), PD-L1 IHC (1.70; 95% CI: 1.40–1.90), tTMB (1.50; 95% CI: 1.30–1.80), and GEP (1.70; 95% CI: 1.30–2.20) were all higher than that of driver gene (1.10; 95% CI: 0.90–1.20, $p < 0.05$). KRAS mutation showed higher NLR (1.04; 95% CI: 0.93–1.16) than PD-L1 IHC (0.71; 95% CI: 0.63–0.80, $p < 0.05$), tTMB (0.70; 95% CI: 0.58–0.84, $p < 0.05$), GEP (0.63; 95% CI: 0.47–0.84,

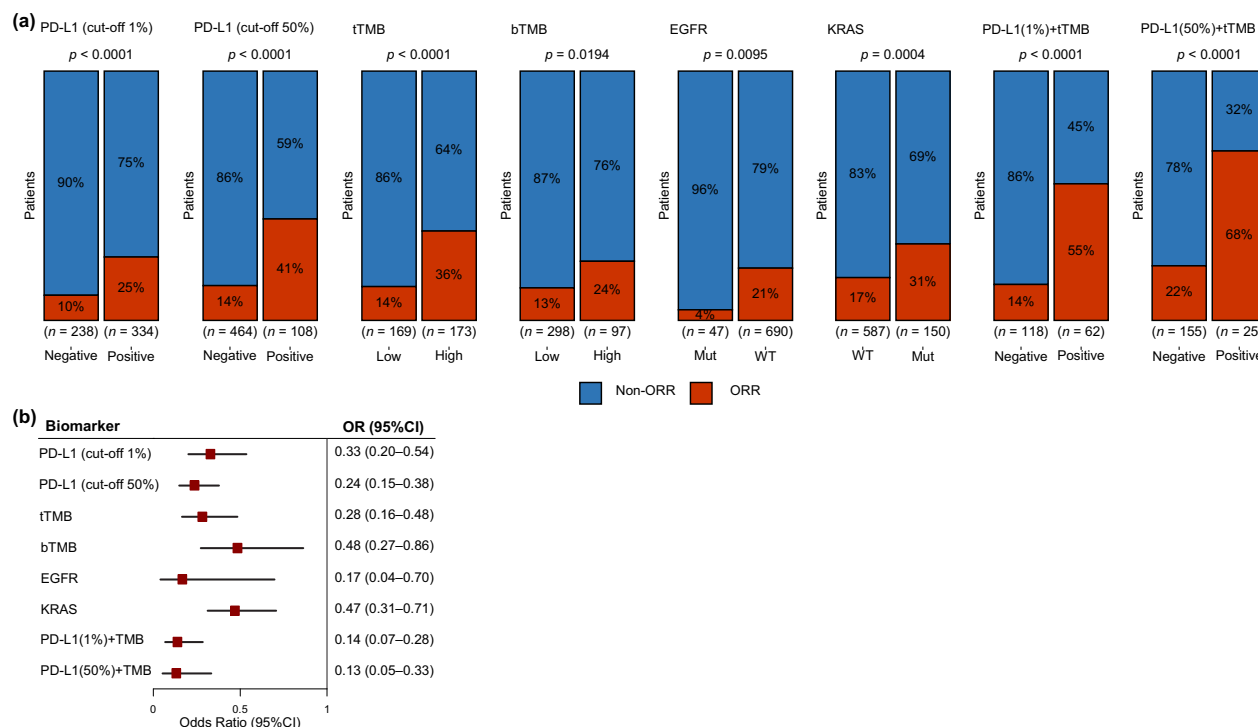


FIGURE 3 The predictive value of the biomarkers for ORR in public datasets. (a) Stacked bar plots show the ORR of patients from different biomarker groups. The corresponding p -value was calculated from chi-squared test. (b) The forest plot shows the OR (95% CI) of the biomarkers for ORR of immunotherapy. CI, confidence interval; OR, odds ratio; ORR, objective response rate.

$p < 0.05$), and combined biomarker (0.76; 95% CI: 0.62–0.93).

The PPV and NPV of all biomarkers from individual studies are displayed in Figure 2c. From the scatter plot distribution we found that the PPV of combined biomarkers was predisposed to be higher than the other biomarkers while the PPV of driver gene mutation was predisposed to be lower.

Quality assessment of the included studies is summarized (Figure S4). The Deeks' funnel plot did not present substantial asymmetry and the test showed no evidence of publication bias ($p = 0.27$) (Figure S5).

Predictive performance validation

We curated four public datasets of NSCLC receiving ICI-based treatment^{12,17–19} and selected a total of 831 patients with available ORR. The baseline characteristics of patients are summarized (Table S3). We found that patients with positive PD-L1 defined by $>1\%$ or $>50\%$ had higher ORR than those with negative PD-L1 (25% vs. 10%, 41% vs. 14%, respectively). Both tTMB (χ^2 test, $p < 0.0001$) and bTMB (χ^2 test, $p = 0.0194$) were predictive of ORR from ICI-based therapy. Patients with EGFR mutation had lower ORR (4% vs. 21%) while KRAS mutated patients had higher ORR (31% vs. 17%) than wild-type patients. Positive

combined biomarker of PD-L1 plus TMB was enriched with more responsive patients (PD-L1 cutoff 1%, 55%; cutoff 50%, 68%) (Figure 3a). Furthermore, PD-L1 with cutoff 50% performed superior predictive value than that of cutoff 1% (OR, 0.24 vs. 0.33). The predictive efficacy of bTMB was lower than that of tTMB (OR, 0.48 vs. 0.28). The status of EGFR mutation could better distinguish ICI beneficiaries than that of KRAS (OR, 0.17 vs. 0.47). Combined biomarkers of PD-L1 plus TMB exhibited the most prominent predictive performance, evidenced by the lowest OR values (0.14, 0.13 for PD-L1 cutoff 1%, 50%, respectively) (Figure 3b).

We further investigated the predictive value of these biomarkers for PFS of patients after receiving ICI-based therapy (Figure 4). Patients with positive PD-L1 had prolonged PFS than those with negative PD-L1 (cutoff 1%, HR, 0.67; 95% CI: 0.50–0.90; cutoff 50%, HR, 0.67; 95% CI: 0.45–0.99; $p < 0.05$). tTMB distinguished ICI beneficiaries (HR, 0.58; 95% CI: 0.46–0.74; $p < 0.05$) while bTMB was not predictive for PFS after ICI treatment ($p > 0.05$). With regard to the driver gene mutations, EGFR mutant patients exhibited shorter PFS (WT vs. Mut, HR, 0.56; 95% CI: 0.36–0.89; $p < 0.05$), while KRAS mutation did not distinguish ICI beneficiaries. Moreover, patients with positive combined biomarkers of PD-L1 (defined by $>1\%$ or $>50\%$) plus TMB displayed superior PFS benefit to those with negative combined biomarkers (HR, 0.34; 95% CI: 0.22–0.51; HR, 0.36; 95% CI: 0.19–0.69, respectively).

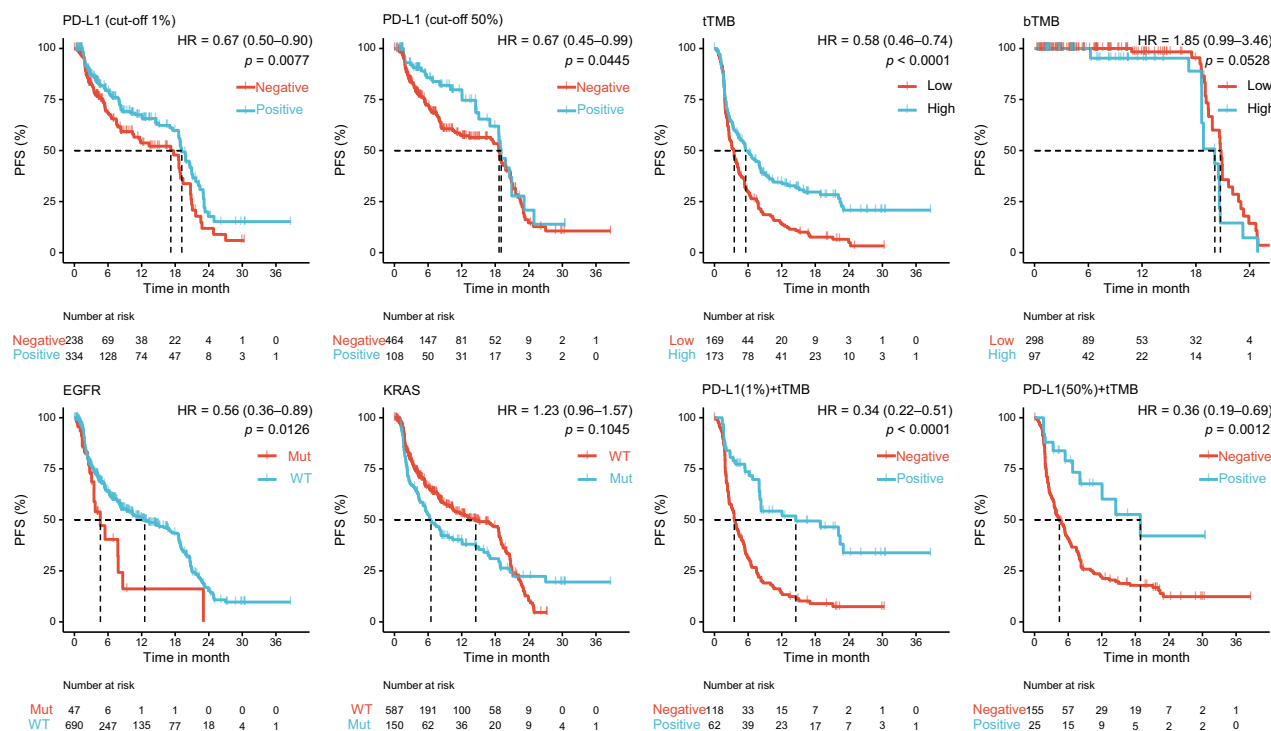


FIGURE 4 The association between the biomarkers and PFS after immunotherapy. p -value was determined via the log-rank test. PFS, progression-free survival.

DISCUSSION

This study conducted a comprehensive comparison across various biomarkers for advanced NSCLC receiving immunotherapy, which may provide convincing evidence to define the most valuable predictive biomarker. This study had a couple of advantages in terms of methodology. In part 1, previous studies on this topic included both clinical trials and retrospective studies on diverse cancer types,¹⁵ while our research focused on the predictive biomarkers for advanced NSCLC, and all the included data came from clinical trials, which abrogated the cancer-specific bias and confounding factors from retrospective studies. In addition, our rigorous inclusion criteria, which required clinical trials involved with more than two categories of biomarkers exploration, counteracted the potential bias introduced by the covariates such as age, sex, smoking history, tumor stage and study design between compared studies. Moreover, the prediction performance of biomarker was measured predominantly by the AUC of SROC calculated by bivariate model,²⁰ which preserved the two-dimensional nature of original data and considered the correlation between sensitivity and specificity, more rational than the conventional SROC model. In part 2, we provided robust evidence for the predictive value of the biomarkers based on a sizable integrated dataset, which further validated the results in part 1.

In part 1, we found that combined biomarkers comprised of “PD-L1 + TMB” or “PD-L1 + GEP” or “PD-L1 + CD8” had a higher AUC than single PD-L1 and TMB, demonstrating that a composite of candidate biomarkers generated more effective discrimination of responders from nonresponders to ICIs.^{17,49,51} In addition, combined biomarkers with higher specificity than single biomarkers indicated a lower false positive rate; in this case, patients selected to receive ICIs but would not respond. Also, patients with positive combined biomarkers enriched further benefit from ICIs based on the tendency of higher PLR and PPV. In part 2, positive versus negative combined biomarkers of PD-L1 plus TMB also exhibited the lowest odds ratio for ORR of immunotherapy, and significantly distinguished patients with prolonged PFS. Rizvi et al.¹⁸ also validated that PD-L1 and TMB were independent biomarkers, the union of which produced a superior predictive utility than separately used. In addition to this union modality, Liu et al.⁵² verified remarkable feasibility of the alliance of TMB and copy-number alteration (CNA), since superior survival was observed in patients with TMB^{high}CNA^{low} than those with TMB^{low}CNA^{low}, TMB^{high}CNA^{high} and TMB^{low}CNA^{high}. Due to the complex interaction between tumor and host immune environment,^{53,54} combination of biomarkers opened up new ways to dissolve the predicament. More importantly, with the development of artificial intelligence and its application in medicine, machine-learning and deep learning models that integrated tumor- and T cell-intrinsic

features from multiomics sequencing and multimodality data would develop a comprehensive predictive system and play a dominant role in selecting beneficial patients.⁵⁵

The intrinsic heterogeneity of PD-L1 expression, as well as coexistence of multiple immune checkpoints such as LAG3, IDO1⁵⁶ may distract the predictive performance of PD-L1. In part 1, a higher cutoff (50%) with higher specificity and PPV excluded those who would not benefit from ICIs, negating the exposure of adverse effect of ICIs and economic burden, while a lower cutoff (1%) with higher sensitivity ensured more patients with opportunity to receive ICIs.⁵⁷ However, PD-L1 with cutoff 50% failed to distinguish long-term PFS after immunotherapy in public datasets and biomarkers for long-term survival were desired to select those who truly benefit from immunotherapy.⁵⁸ Among the single biomarkers explored, the utilization of TMB in real-world practice was challenging due to the various targeted gene panel and corresponding algorithm used, although it proved to have a significant correlation with that of WES.⁵⁹ Here, our analysis found that TMB measured by gene panel displayed slightly lower predictive value of AUC to that of WES, indicating an approximate estimate of TMB measured by WES, but more economical and accessible in clinical practice. Furthermore, bTMB showed promise as a candidate biomarker with AUC ranking only second to the combined biomarker in part 1 and was validated in the public datasets with a considerably low OR for predicting ORR in part 2. More importantly, bTMB determined via sequencing of circulating cell-free DNA has emerged as a noninvasive means to measure TMB, particularly for patients with inaccessible tissue biopsies.⁶⁰ Moreover, dynamic changes of bTMB during treatment also indicate the prognosis of lung squamous cell carcinoma patients receiving immunotherapy.⁴²

In addition, GEP is an indicator of the inflamed TME profile, and reflects the common features of reinvigorated antitumor immunity.⁶¹ “Preliminary IFN- γ ” signature (10-gene set) and “preliminary expanded immune” signature (28-gene set) have also validated that immune-related gene signatures can distinguish responders from nonresponders.⁶² Since the involved GEP consisted of three to eight genes and was calculated using various algorithms with different thresholds, the ground use of GEP deserves further optimization of calculation and validation in larger cohort.⁶² Noticeably, the application of immunotherapy on patients with *EGFR* mutations have attracted broad interest. In addition, the effect of *KRAS* and comutation of *KRAS* with *STK11* or *KEAP1* on immunotherapy have been widely studied.⁶³ Here, the significantly discrepant predictive effect between *EGFR* and *KRAS* warned us to discriminate the implication of various driver gene mutation. In part 2, patients with *EGFR* mutations had significantly lower ORR while patients with *KRAS* mutations had significantly higher ORR than wild-type patients, indicating the implication of *EGFR* mutation to exclude patients while *KRAS* mutation to include patients for immunotherapy, respectively. Furthermore, *EGFR* held obviously higher sensitivity

and lower specificity than *KRAS*, and *KRAS* failed to predict PFS after immunotherapy.

Limitations

First, the cutoff values of PD-L1 IHC partly depended on the assays using diverse antibodies. Thus, the effect of different thresholds of PD-L1 was hard to disentangle from the effect of used antibodies. Then, some of the included studies enrolled only nonsquamous NSCLC or patients without known driver gene mutation, which may have introduced patient selection bias. Finally, bTMB failed to predict PFS after immunotherapy in part 2, indicating that refinement of bTMB was desired to select ICI beneficiaries. A modified bTMB algorithm, LAF-bTMB (mutation counts with an AF $\leq 5\%$) defined by our team, could help ensure better selection of patients most likely to benefit from ICI,⁶⁴ which warrants further exploration.

In conclusion, a wide variety of candidate predictive biomarkers, however, have led to confusion for clinical application. This study draws a conclusion that combined biomarkers provide further enriched benefit from ICIs. The treatment of ICIs in *EGFR/KRAS*-mutated patients and the application of bTMB as a predictive biomarker warrant prospective investigation and validation. As we look ahead, taking into consideration various molecular underpinnings, further design of composite modality and artificial intelligence-based predictive model would unequivocally foster more efficient predictors. Moreover, evaluation of the biomarkers and models in prospective clinical trials are desired to provide more robust evidence for directing precision immunotherapy in clinical practice.

AUTHOR CONTRIBUTIONS

Jie Zhao: Conceptualization, methodology, investigation, software, formal analysis, writing-original draft, writing-review and editing, visualization. Wei Zhuang: Methodology, software, formal analysis, writing-original draft, writing-review and editing, visualization. Boyang Sun: Methodology, formal analysis, writing-original draft, writing-review and editing, visualization and validation. Hua Bai, Zhijie Wang, Jia Zhong, Rui Wan and Lihui Liu: Project administration, resources, writing-review and editing. Jianchun Duan: Conceptualization, supervision, resources, data curation, writing-review and editing, funding acquisition. Jie Wang: Conceptualization, supervision, resources, data curation, writing-review and editing, funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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