An exploration of LAF-bTMB as a predictor for the efficacy of immunotherapy combined with chemotherapy in non—small cell lung cancer

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Abstract

Background: Immune checkpoint inhibitor (ICI) combined with chemotherapy is one of the standards of care for advanced non—small cell lung cancer (NSCLC) without driver mutations. However, the biomarker of combination therapy is still unknown. Although previous studies have confirmed that low allele frequency adjusted blood-based tumor mutational burden (LAF-bTMB) is associated with the efficacy of ICI monotherapy, there has been no report on the correlation between the efficacy of LAF-bTMB and ICI combined chemotherapy. This study aimed to explore whether LAF-bTMB can be used as a predictive biomarker for the efficacy of immunotherapy combined with chemotherapy in advanced NSCLC.

Methods: This study enrolled patients diagnosed with advanced NSCLC and who received ICI combined with chemotherapy for first-line therapy from May 2020 to December 2021 at Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. Clinical information, treatment information, survival data, and peripheral blood samples of every patient before treatment were collected. Next-generation sequencing was performed on plasma samples to estimate bTMB and LAF-BTMB.

Results: A total of 42 patients with NSCLC were enrolled. In this cohort, 19 patients achieved partial response (PR), and the objective response rate (ORR) was 45.2%. The median progression-free survival (PFS) of all patients was 13.4 months (95% CI, 7.49–19.72). Both PFS and the overall survival (OS) were significantly longer in the responder (R) group than in the non-responder (NR) group (median PFS, 16.4 months vs. 7.2 months, \( p = 0.028 \); median OS, NE vs. 9.3 months, \( p = 0.016 \)). There was no significant difference in bTMB and LAF-BTMB between the R and NR group. The ORR of patients with LAF-bTMB ≤8muts/Mb was significantly higher than that of patients with LAF-bTMB >8muts/Mb (ORR, 61% vs. 26%, respectively, \( p = 0.033 \)). When LAF-bTMB ≤8muts/Mb or >20muts/Mb, ORR was significantly higher than that of patients with LAF-bTMB between 8 and 20muts/Mb (ORR were 57% and 21%, \( p = 0.047 \)). No correlation has been found between LAF-bTMB and PFS or OS.

Conclusions: This study confirmed that neither bTMB nor LAF-bTMB is feasible as a potential predictor of first-line immunochemotherapy for advanced NSCLC. More suitable biomarkers need to be explored to screen patients with better efficacy of immunotherapy combined with chemotherapy in the future.

Keywords: bTMB, immune checkpoint inhibitor, LAF-bTMB, non—small cell lung cancer
INTRODUCTION

According to the latest national cancer epidemiological survey report[^4] in 2022, lung cancer is the malignant tumor with the highest mortality rate in China. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer and accounts for about 80% of all lung cancer types.[^5] The National Comprehensive Cancer Network guidelines recommend genetic testing for patients with newly diagnosed advanced adenocarcinoma of the lung and targeted therapy for those with positive driver genes. Immune checkpoint inhibitor (ICI) alone or ICI combination therapy is preferred in first-line therapy for squamous cell carcinoma and adenocarcinoma without targetable mutations. At present, only 15% to 20% of unselected patients may respond to ICI monotherapy,[^3] so it is necessary to find biomarkers to predict the efficacy of ICI. Serval clinical trials[^7][^8] have proved that ICI monotherapy is preferred for patients with programmed death ligand 1 (PD-L1) ≥50%. However, in the KEYNOTE-189[^9] and KEYNOTE-407[^10] clinical trials, significantly improved progression-free survival (PFS) or overall survival (OS) in patients with advanced NSCLC treated with ICI combination therapy was observed, and the efficacy was independent of PD-L1 expression. The major challenge for PD-L1 is the observation that PD-L1 may not always be associated with ICI responsiveness.

Tumor mutation burden (TMB) was defined as the total number of non-synonymous mutations present in tumor tissue.[^11] The higher the TMB, the more neoantigens, and the more easily the tumor cells are recognized by the immune system. Rizvi et al.[^12] first published the results of their study that explored the efficacy of TMB and NSCLC immunotherapy. In their study, TMB higher than the median of mutations detected by whole exome sequencing was defined as TMB-high, and TMB-high patients who received ICI had longer PFS than that in TMB-low. The same conclusion has been drawn in the CheckMate 026 study.[^13] Even in the CheckMate 227 study,[^14] which investigated the correlation between TMB and the efficacy of programmed cell death protein 1 (PD-1) antibody plus CTLA-4 antibody, TMB can still be used as a predictor of PFS in dual immunotherapy. Nonetheless, it is necessary to highlight that TMB has limitations as a predictive biomarker, especially when used in predicting the efficacy of combining the ICI with platinum-based chemotherapy.[^9][^10] In addition, driver genes detection for patients with advanced NSCLC is based on tissue samples, therefore, there may not be any more tissue left for TMB testing. Blood TMB (bTMB) calculated based on liquid biopsy using circulating tumor DNA (ctDNA) has also been used in clinical as a substitute for TMB.[^15] Previous studies[^[16] demonstrated that patients who can benefit from ICI monotherapy tend to harbor a high level of bTMB. Contradictory results were observed from OAK and POPULAR studies.[^17] These two studies indicated that bTMB failed to differentiate patients with OS benefits.

Wang et al.[^18] found that bTMB measured by ctDNA was affected by the interference of maximum somatic allele frequency (MSAF), which affected the predictive effect of bTMB. Once the interference of MSAF was removed by modification of the TMB algorithm, low allele frequency adjusted blood-based tumor mutational burden (LAF-bTMB) can serve as a reliable predictor of OS in ICI monotherapy. However, whether LAF-bTMB is associated with the outcome of ICI plus chemotherapy is unclear. In the current study, next-generation sequencing (NGS) was performed on plasma collected in a cohort of 42 patients to evaluate LAF-bTMB. We investigated the potential of LAF-bTMB in predicting the responses and outcomes to ICI plus chemotherapy in advanced NSCLC patients.

MATERIALS AND METHODS

Patient cohorts

Patients diagnosed with advanced NSCLC at Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College between May 2020 and December 2021 who met the following inclusion criteria were enrolled in this study, (i) patients were between the age of 18 to 80; (ii) patients had received no previous systemic therapy; (iii) patients had at least one measurable lesion; and (iv) patients received anti-PD-1/PD-L1 plus chemotherapy for the first-line therapy. Blood samples were collected from each patient before the initiation of the combination therapy. This study was approved by the Ethics Committee of the Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. All patients had signed informed consent.

The responses to therapy were evaluated at baseline and every 2 cycles of treatments, using computed tomography (CT) or magnetic resonance imaging (MRI). The clinical outcomes of patients were evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1).[^19] The effect evaluation includes complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Disappearance of all target lesions was defined as CR. A reduction of at least 30% in the total diameter of the target lesion was considered as PR. The total diameter of the target lesions increased by at least 20% or new lesion was considered as PD. Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD was defined as SD. SDA was defined as patients who have stable disease and tumor size reduction. SDb was defined as those who have stable disease with tumor size increase. Objective response rate (ORR) was defined as the percentage of patients with CR and PR. Disease control rate (DCR) was defined as the percentage of patients with CR, PR, and SD. PFS was defined as the interval between the initiation of the therapy and the time of PD or the last follow-up. OS was defined as the interval between the initiation of the therapy and the time of death.

The group of response was defined according to the following criteria. The patients who confirmed PR as their best...
response and those who did not confirm PR were divided into response group (R) and non-response group (NR), respectively. Group A included patients achieved PR and SDa and the patients with the best efficacy SDb and PD were classified into group B. Patients who achieved SD for more than 6 months and patients with PR were regrouped into the same group renamed as durable clinical benefit (DCB). The no-durable benefit group (NDB) included patients who got SD, but developed PD within 6 months and patients who archived PD as the best response.

Blood sample processing and cell-free DNA isolation

Blood samples were centrifuged in Streck tubes within 2 hours of collection at 1600 × g at 4°C for 10 minutes. Approximately 5 mL plasma supernatant was transferred to a new 5 mL microfuge tube and centrifuged at 16,000 × g at 4°C for 10 minutes to remove residual cells and debris. Supernatant was transferred into a new tube, followed by cell-free DNA (cfDNA) extraction using the QiAmp Circulating Nucleic Acid Kit (Qiagen) following the manufacturer’s instructions. DNA concentration was quantified with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). Genomic DNA (gDNA) from white blood cells was extracted using the QIAamp DNA Mini Kit (Qiagen).

Library preparation and targeted capture

cfDNA libraries were prepared using the KAPA Hyper Prep Kit (KAPA Biosystems) following the manufacturer’s protocol and were individually barcoded with unique molecular identifiers (UMI). In brief, 30 to 60 ng of cfDNA were subjected to end-repairing, A-tailing, and ligation with indexed adapters. The libraries were then polymerase chain reaction (PCR)-amplified and purified for target enrichment. The concentration and size distribution of each library were determined using a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and a LabChip GX Touch HT Analyzer (PerkinElmer), respectively.

For targeted capture, indexed libraries were subjected to probe-based hybridization with a customized NGS panel covering whole exons of 733 cancer-related genes. The probe baits were individually synthesized 5’ biotinylated 120 bp DNA oligonucleotides (IDT). Repetitive elements were filtered out from intronic baits according to the annotation by UCSC Genome RepeatMasker. The xGen Hybridization and Wash Kit (IDT) were used for hybridization enrichment. Briefly, 500 ng indexed DNA libraries were pooled to obtain 2 μg of DNA. The pooled DNA sample was then mixed with Human Cot-1 DNA and xGen Universal Blockers-TS Mix and dried down in a SpeedVac system. The Hybridization Master Mix was added to the samples and incubated in a thermal cycler at 95°C for 10 minutes, before being mixed and incubated with 4 μL of probes at 65°C overnight. Target regions were captured following the manufacturer’s instructions. The concentration and fragment size distribution of the final library was determined using a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and a LabChip GX Touch HT Analyzer (PerkinElmer), respectively.

DNA sequencing, data processing, and variant calling

The captured libraries were loaded onto a NovaSeq 6000 platform (Illumina) for 100 bp paired-end sequencing. Raw data of paired samples were mapped to the reference human genome hg19 using the Burrows-Wheeler Aligner. An in-house developed software was used to generate duplex consensus sequences based on dual UMI integrated at the end of the DNA fragments. To improve specificity, especially for variants with low allele frequency in the ctDNA, an in-house loci specific variant detection model based on binomial test was applied. The variants were subsequently filtered by their supporting count, strand bias status, base quality, and mapping quality. In addition, variant calling was also optimized to detect variants at short tandem repeat regions. Single-nucleotide polymorphism (SNPs) and indels were annotated by ANNOVAR against the following databases: dbSNP (v138), 1000Genome and ESP6500 (population frequency >0.015). Only missense, stopgain, frameshift, and non-frameshift indel mutations were kept. Copy number variations (CNVs) and gene rearrangements and LAF-bTMB were detected as described previously.

Statistical analysis

Survival data was analyzed using the Kaplan–Meier method. The log-rank test was used to compare the PFS and OS between patients in different response group and between group PIK3CA-AKT1 WT or MUT. The 95% confidence intervals (CIs) of the ORR and DCR were calculated using the Clopper and Pearson method. The comparison of LAF-bTMB levels between group R and group NR was examined by the Mann–Whitney test. The χ² test or Fisher exact test was used to test the difference of categorical variables. All reported p-values were based on two-tailed testing, and a p-value below 0.05 was deemed statistically significant. All statistical analyses were performed using the GraphPad Prism software 8.3.0 (GraphPad Software) and R software, version 4.1 (R Foundation for Statistical Computing).

RESULTS

Patient characteristics

Between May 2020 and December 2021, a total of 42 patients diagnosed with advanced NSCLC and treated with ICI plus
Chemotherapy as first-line treatment were prospectively enrolled in this study. The median age of all patients was 63 years (ranging from 46 to 79 years). A total of 79% of patients were male (33/42) and 21% were female (9/42). A total of 0.28 patients were diagnosed with adenocarcinoma and 14 with squamous cell carcinoma. Eastern Cooperative Oncology Group Performance Status scores ranged from 0 to 2, with 26 patients (62%) having a score of 0 and 4 patients (9.5%) with brain metastases at initial diagnosis. PD-L1 expression was assessed in 30 patients (71.5%). PD-L1 expression was positive (≥1%) in 18 patients (43%), of which seven patients (17%) were PD-L1 ≥50%. All patients received first-line PD-1 inhibitor combined with chemotherapy including 23 with pembrolizumab, 10 with sintilimab, five with tislelizumab, three with camrelizumab, and one with toripalimab. Seven patients received immunotherapy combined with chemotherapy and antiangiogenic therapy. Clinical information was summarized in Table 1.

Mutational profiling and correlation between mutations and response

Collectively, mutations detected in 42 patients including single nucleotide variants (SNV) and CNV, gene fusion, and SNV is the most common mutation type. The most commonly mutated genes were TP53 (69.0%, n = 29), KRAS (31.0%, n = 13), CDKN2A (16.7%, n = 7), and EGFR (14.3%, n = 6). In patients harboring KRAS alteration, five patients evaluated PR as their best response to the first-line treatment and seven patients had SD, and only one archived PD. There were seven patients with TP53 and KRAS co-mutation, four patients achieved PR, six patients achieved SD, one patient achieved PD, and the ORR was 36%. There were three cases with STK11 mutation, including two cases with KRAS co-mutation, and the best outcome evaluation of those two patients was SD. EGFR amplification was performed on three patients, and the best outcome evaluation was PR. Among the six patients with EGFR alteration (SNV and CNV), three patients with EGFR amplification and three patients harboring rare nonsense mutations in EGFR, five of them were evaluated as PR. Three patients had copy number amplification of PIK3CA, and the best treatment evaluation was PR. Variants detected in at least two patients are shown in Figure 1, a total of 34 patients.

The correlation between bTMB, LAF-bTMB, and efficacy

Among all the patients, 19 patients achieved PR, 22 patients achieved SD, and one patient was rated as PD. ORR among the evaluable patients was 45.2% and DCR was 97.6%. The PFS of group R and group NR were 16.4 months and 7.2 months, respectively (p = 0.028). The OS of group R was longer than that of group NR (9.3 months and not reached, p = 0.016), respectively (see Figures S1 and S2 for details).

The median bTMB of group R and group NR were 6.711 muts/Mb and 10.07 muts/Mb, respectively. There was no significant difference in bTMB between the two groups (p = 0.683). The median bTMB values of the group A and B were 10.07 muts/Mb and 5.034 muts/Mb, respectively. No difference was observed in group A and B (p = 0.421). Regrettably, there was still no difference observed in the bTMB distribution between DCB and NDB (p = 0.623). The results above were all displayed in Figure 2.

The predictive performance between bTMB and ORR was evaluated with a series of cutoff points for bTMB. No correlation was found between bTMB and ORR according

| Characteristics   | N (%) |
|-------------------|-------|
| Age (years)       |       |
| Median (range)    | 63 (46–79) |
| Gender            |       |
| Male              | 33 (79%) |
| Female            | 9 (21%) |
| Histological subtype |     |
| Squamous cell carcinoma | 14 (33%) |
| Adenocarcinoma   | 28 (67%) |
| Smoking history   |       |
| Yes               | 29 (69%) |
| No                | 13 (31%) |
| Brain metastasis  |       |
| Yes               | 4 (9.5%) |
| No                | 38 (90.5%) |
| ECOG PS           |       |
| <1                | 26 (62%) |
| ≥1                | 16 (38%) |
| PD-L1 expression  |       |
| <1%               | 12 (28.5%) |
| 1%–49%            | 11 (26%) |
| ≥50%              | 7 (17%) |
| Unknown           | 12 (28.5%) |
| PD-1 inhibitor    |       |
| Pembrolizumab     | 23 (55%) |
| Sintilimab        | 10 (24%) |
| Tislelizumab      | 5 (12%) |
| Camrelizumab      | 3 (7%) |
| Toripalimab       | 1 (2%) |
| Anti-angiogenic therapy |    |
| Bevacizumab       | 7 (16.7%) |
| No                | 35 (83.3%) |

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death ligand 1; PD-1, programmed cell death protein 1.
to dichotomous stratification. By tertile stratification, there was a trend toward higher ORR in patients with bTMB-low or bTMB-high compared to those with TMB-median, but there was no difference by statistical analysis (Figure 3).

Based on the different bTMB cutoffs, we analyzed the correlation between bTMB and PFS and found no significant correlation between bTMB and immune combination chemotherapy PFS (Figure 4). As can be seen from the

**FIGURE 1**  Mutational profile and clinical response.

**FIGURE 2**  Correlations between bTMB and clinical benefit in different groups. (a) bTMB in group R and group NR. (b) bTMB in group A and group B. (c) bTMB in group DCB and group NDB. bTMB, blood-based tumor mutational burden; R, response; NR, non-response; DCB, durable clinical benefit; NDB, no-durable benefit group.
previous results, the group bTMB-low and bTMB-high have a tendency to benefit more compared to group bTMB-median. After further adjustment for trichotomies, the top one-fourth bTMB was defined as bTMB-high, the bottom one-fourth bTMB as bTMB-low, and the rest as bTMB-median. PFS was longer for bTMB-high and bTMB-low than for bTMB-medium, but this was still not statistically significant (Figure S3).

Analysis of the LAF-bTMB by the three grouping scenarios mentioned previously, no significant association with efficacy was found regardless of whether LAF-bTMB was high or low (Figure S4). We further assessed the correlation between LAF-bTMB and ORR. There is a tendency for the ORR to increase when the LAF-bTMB is low or very high, whereas it is relatively low when the LAF-bTMB is intermediate. At a cutoff value of 8muts/Mb, the ORR was found to be significantly higher for LAF-bTMB ≤8 muts/Mb than for LAF-bTMB >8 muts/Mb (ORR, 61% vs. 26%, p = 0.033).

Further analysis revealed a significantly higher ORR for LAF-bTMB ≤8 muts/Mb or >20 muts/Mb than for LAF-bTMB between 8 and 20 muts/Mb (ORR, 57% vs. 21%, p = 0.047) (Figure 6). Various LAF-bTMB cutoff values were set to analyze the correlation between LAF-bTMB and PFS, whereas there was no significant correlation between LAF-bTMB and PFS of immunotherapy combined with chemotherapy (Figure 7). Furthermore, PFS for LAF-bTMB ≤8 muts/Mb or LAF-bTMB >20 muts/Mb had no statistical difference from PFS for LAF-bTMB between 8 and 20 muts/Mb (Figure S4).

Exploratory analysis: potential positive factors for predicting immune response

Previous results showed that patients with PIK3CA or AKT1 variants all achieved PR. Therefore, we assumed that PIK3CA and AKT1 variants were potential positive predictors of immunotherapy, and then we analyzed the correlation between these two mutations and ORR and PFS. Patients carrying either PIK3CA or AKT1 mutation were classified as group MUT, and the rest patients were classified as group WT. The ORR of group MUT was significantly higher than that of group WT (ORR, 100% vs. 38%, respectively, p = 0.014). The median PFS of group MUT and group WT were 21.1 months and 13.3 months, respectively (hazard ratio [HR], 3.619; 95% CI, 1.074–12.19; p = 0.113).

There was no manifest difference observed in bTMB or LAF-bTMB between the group MUT and WT (Figure 8). We further explored whether gene mutations related to the PI3K-AKT–mTOR pathway can be used as predictive markers of immunotherapy efficacy. Several genes were involved in PI3K-AKT–mTOR pathway, including PIK3CA,
PIK3R1, PIK3R2, PTEN, PDPK1, AKT1/2, MTOR, RICTOR, TSC1/2, RHEB, RPTOR, and MLST8. In this study, eight of 42 patients were detected with variants of this pathway, containing PIK3CA, AKT1, AKT2, PTEN, mTOR, and RICTOR genes. These eight patients were defined as group PI3K-AKT-mTOR MUT, and the rest of the patients

**FIGURE 5** Correlations between LAF-bTMB and clinical benefit in different groups. (a) LAF-bTMB in group R and group NR. (b) LAF-bTMB in group R and group NR. (c) LAF-bTMB in group DCB and group NDB. LAF-bTMB, low allele frequency adjusted blood-based tumor mutational burden; R, response; NR, non-response; DCB, durable clinical benefit; NDB, no-durable benefit group.

**FIGURE 6** Correlation between the ORR and LAF-bTMB with a series of cutoff points for the LAF-bTMB. ORR, objective response rate; LAF-bTMB, low allele frequency adjusted blood-based tumor mutational burden.

**FIGURE 7** Correlation between the progression-free survival and LAF-bTMB with a series of cutoff points for the LAF-bTMB. LAF-bTMB, low allele frequency adjusted blood-based tumor mutational burden.

**FIGURE 8** Association of bTMB and LAF-bTMB with PIK3CA/AKT1 mutation. (a) bTMB in group PIK3CA/AKT1 WT and group PIK3CA/AKT1 MUT. (b) LAF-bTMB in group PIK3CA/AKT1 WT and group PIK3CA/AKT1 MUT. LAF-bTMB, low allele frequency adjusted blood-based tumor mutational burden; WT, wild-type; MUT, mutation.
DISCUSSION

The previous study\textsuperscript{18} has indicated that LAF-bTMB could be a meaningful predictor of clinical outcomes after treatment with ICI monotherapy. However, whether LAF-bTMB could be a predictive biomarker for ICIs combined chemotherapy remain undetermined. To our knowledge, this is the first study to explore the potential predictive value of LAF-bTMB as a biomarker to identify advanced NSCLC patients who are more likely to benefit from ICIs combined with chemotherapy in the first-line treatment. Regrettably, neither bTMB nor LAF-bTMB was associated with the efficacy of ICI in combination with chemotherapy.

In this study, we indicated that bTMB was unable to predict ORR and OS benefits from immune-chemotherapy. Previous studies on the predictive role of bTMB as a biomarker in immune response were still controversial. The study published in JAMA oncology \textsuperscript{2019}\textsuperscript{16} suggested that when bTMB ≥ 6 muts/Mb was defined as the bTMB-high, the ORR and PFS of the bTMB-high patients received ICI alone were better than those of the bTMB-low. However, the B-FIRST study,\textsuperscript{23} in which researchers evaluated bTMB as a predictive biomarker for first-line atezolizumab monotherapy, revealed that the ORR was significantly higher in patients with bTMB ≥ 16 muts/Mb than those with bTMB < 16 muts/Mb, but there was no significant difference in PFS between the two groups. Unlike the ICI monotherapy, bTMB was not correlated with ORR and PFS in the clinical trial,\textsuperscript{24} which investigated the efficacy of camrelizumab combined with chemotherapy for the first-line treatment in advanced NSCLC. The above studies proved that bTMB could not be a predictor of the efficacy of immuno-chemotherapy, which was consistent with observations in the present study.

Detection of tumor mutations in the blood depends on the amount of ctDNA released by tumor cells. It has been reported that the high level of ctDNA amount was correlated with poor survival.\textsuperscript{25,26} In the blood, the ctDNA amount was reflected by the MSAF, which has been proven to be the interference of bTMB prediction.\textsuperscript{18} Once MSAF interference is removed, bTMB was able to predict OS benefit of ICI monotherapy. In this paper, we analyzed the relationship between LAF-bTMB and the efficacy of the ICI combination treatment and we found that when the cutoff value of LAF-bTMB was 8 muts/Mb, the ORR of patients with high LAF-bTMB was worse than that with low LAF-bTMB. Similar trends were observed in recurrent glioblastoma that a very low level of TMB predicts benefit from ICI.\textsuperscript{27} However, it is unknown whether this phenomenon is unique to this specific tumor type. In addition, no semblable data were found in NSCLC. One potential reason may lie in that a high level of TMB is a poor prognostic factor of NSCLC.\textsuperscript{28} When the cutoff value is 25 muts/Mb, the ORR of patients with high LAF-bTMB was much higher than that in patients with low LAF-bTMB, although without statistical meaning. Patients with a very high level of LAF-bTMB seem more likely to respond to ICI therapy, which may be because of the very high level of bTMB provoking a more adequate immune response. However, a high level of LAF-bTMB failed to identify patients who would get PFS benefit from immunotherapy. Therefore, LAF-bTMB is not a perfect predictor of efficacy in the treatment mode of immunotherapy combined with chemotherapy.

One retrospective study\textsuperscript{29} explored the relationship between TMB and the efficacy of chemotherapy. Among 294 patients with different tumor types who received chemotherapy, TMB beyond 10 muts/Mb were defined as a high TMB level. Regardless of the level of TMB, no difference was observed in clinical response and outcomes. Jiang et al.\textsuperscript{24} found that the bTMB measured after two cycles of treatment (on-treatment bTMB) was associated with the efficacy of ICI combined with chemotherapy. Patients treated with camrelizumab plus chemotherapy had a better ORR, PFS, and OS if they had low level of on-treatment bTMB. In addition, the lower the ΔbTMB (the difference between on-treatment bTMB and pre-treatment bTMB), the greater the therapeutic benefit patients would obtain. Although in the combined mode of chemotherapy and ICI, the cytotoxicities of chemotherapy will contribute to tumor cells releasing more DNA into the blood, and then more neoantigens were produced,\textsuperscript{30} which will further increase the probability of recognition by the immune system. The bTMB or LAF-bTMB assessed in the baseline blood samples could not reflect the efficacy of chemotherapy alone or the synergistic efficacy increased by combination therapy. The post-treatment and dynamically monitored bTMB may be the appropriate biomarker for predicting the efficacy of combined immunotherapy.

Furthermore, patients carrying PI3K\textsuperscript{18} and AKT\textsuperscript{18} gene variants all achieved PR in this study. The latest research data of CHOICE-01\textsuperscript{31} published at the American Society of Clinical Oncology Congress in 2022 demonstrated that patients carrying PI3K-AKT–mTOR pathway gene mutations could obtain better clinical benefits when treated with toripalimab combined with chemotherapy. We conducted an exploratory analysis and found that the MUT group tended to have longer PFS than the WT group, but there was no statistically significant difference. A previous study\textsuperscript{32} indicated that patients with specific mutations related to immunotherapy (including PI3K-AKT–mTOR pathway gene mutations) and lower TMB level (<10 muts/Mb) have longer OS. However, in this study, no difference in bTMB level was observed between group MUT and WT.

There are still some limitations in our study. Although this is a prospective study, only 42 patients were enrolled in this study within one and a half years. The sample size is
relatively small, which may lead to potential bias. The follow-up time needs to be extended. At the time of data analysis, some patients are still receiving immune maintenance therapy, so their PFS data are not available. Patients treated with five different anti-PD-1 therapies were combined in this study. It is not yet possible to analyze whether the efficacy is related to receiving different PD-1 inhibitors.

In conclusion, bTMB or LAF-bTMB measured before treatment could not serve as a potential biomarker for predicting the efficacy of ICI combined with chemotherapy in the first line for advanced NSCLC. Other more feasible biomarkers should be explored to predict the efficacy of immunotherapy combined with chemotherapy in the future.

CONFLICTS OF INTEREST
The authors have no conflicts of interest to declare.

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REFERENCES
1. Zheng R, Zhang S, Zeng H, Wang S, Sun K, Chen R, et al. Cancer incidence and mortality in China. 2016. J Natl Cancer Inst. 2022;2:1–9.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
3. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373:1627–39.
4. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373:123–35.
5. Garon EB, Rizvi NA, Hui R, Leigh N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372:2018–28.
6. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet. 2017;389:255–65.
7. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019;393:1819–30.
8. Herbst RS, Giacccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, et al. Atezolizumab for first-line treatment of PD-L1-selected patients with NSCLC. N Engl J Med. 2020;383:1328–39.
9. Garassino MC, Gadgel S, Esteban E, Felip E, Speranza G, Domine M, et al. Patient-reported outcomes following pembrolizumab or placebo plus pemetrexed and platinum in patients with previously untreated, metastatic, non-squamous non-small-cell lung cancer (KEYNOTE-189): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2020;21:387–97.
10. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gümüş M, Mazières J, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. N Engl J Med. 2018;379:2040–51.
11. Jardim DL, Goodman A, de Melo Gagliato D, Kurzrock R. The challenges of tumor mutational burden as an immunotherapy biomarker. Cancer Cell. 2021;29:154–73.
12. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124–8.
13. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-line Nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med. 2017;376:2415–26.
14. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab plus Ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med. 2018;378:2693–104.
15. Chae YK, Davis AA, Agte S, Pan A, Simon NI, Iams WT, et al. Clinical implications of circulating tumor DNA tumor mutational burden (ctDNA TMB) in non-small cell lung cancer. Oncologist. 2019;24:820–8.
16. Wang Z, Duan J, Cai S, Han M, Dong H, Zhao J, et al. Assessment of blood tumor mutational burden as a potential biomarker for immunotherapy in patients with non-small cell lung cancer with use of a next-generation sequencing cancer gene panel. JAMA Oncol. 2019;5:696–702.
17. Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med. 2018;24:1441–8.
18. Wang Z et al. Allele frequency-adjusted blood-based tumor mutational burden as a predictor of overall survival for patients with NSCLC treated with PD-(L)1 inhibitors. J Thorac Oncol. 2020;15:556–67.
19. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228–47.
20. Karolchik D, Hinrichs AS, Purye TS, Roskin KM, Sugnet CW, Haussler D, et al. The UCSC table browser data retrieval tool. Nucleic Acids Res. 2004;32:D493–6.
21. Li H, Durbin R. Fast and accurate short read alignment with burrows-wheelers transform. Bioinformatics (Oxford, England). 2009;25:1754–60.
22. Su D, Zhang D, Chen K, Lu J, Wu J, Cao X, et al. High performance of targeted next generation sequencing on variance detection in clinical tumor specimens in comparison with current conventional methods. J Exp Clin Cancer Res. 2017;36:121.
23. Kim ES, Velcheti V, Mekhail T, Yun C, Shagan SM, Hu S, et al. Blood-based tumor mutational burden as a biomarker for atezolizumab in non-small cell lung cancer: the phase 2 B-FIRST trial. Nat Med. 2022;28:939–45.
24. Jiang T, Chen J, Xu X, Cheng Y, Chen G, Pan Y, et al. On-treatment blood TMB as predictors for camrelizumab plus chemotherapy in advanced lung squamous cell carcinoma: biomarker analysis of a phase III trial. Mol Cancer. 2022;21:4.
25. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA: in early- and late-stage human malignancies. Sci Transl Med. 2014;6:224ra24.
26. Dawson SJ, Tsui DWY, Murtaza M, Biggs H, Rueda OM, Chin SF, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med. 2013;368:1199–209.
27. Strickler JH, Hanks BA, Khasraw M. Tumor mutational burden as a predictor of immunotherapy response: is more always better? Clin Cancer Res. 2021;27:1236–41.
28. Owada-Ozaki Y, Muto S, Takagi H, Inoue T, Watanabe Y, Audigier-Valette C, et al. Nivolumab plus Ipilimumab in lung cancer – a multicentre randomised study. Lancet Oncol. 2022;23:1198–204.
29. Zhu S, Zhang T, Zheng L, Liu H, Song W, Liu D, et al. Combination strategies to maximize the benefits of cancer immunotherapy. J Hematol Oncol. 2021;14:4156.
30. Wang J, Wang Z, Wu L, Li B, Cheng Y, Li X, et al. Final progression-free survival, interim overall survival, and biomarker analyses of...
CHOICE-01: a phase 3 study of toripalimab versus placebo in combination with first-line chemotherapy for advanced NSCLC without EGFR/ALK mutations. J Clin Oncol. 2022;40:9028–8.

32. Pan D, Hu AY, Antonia SJ, Li CY. A gene mutation signature predicting immunotherapy benefits in patients with NSCLC. J Thorac Oncol. 2021;16:419–27.

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

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