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RESEARCH ARTICLE

Tumor mutational burden as a predictive biomarker for non-small cell lung cancer treated with immune checkpoint inhibitors of PD-1/PD-L1

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Abstract

Background The significant clinical benefits of PD-1/PD-L1 immune checkpoint inhibitors (ICIP) in non-small cell lung cancer (NSCLC) have been widely recognized, emphasizing the urgent need for a reliable biomarker. In this study, we find the remarkable capacity of tumor mutational burden (TMB) to serve as an accessible and streamlined indicator.

Patients and methods We designed a retrospective cohort study, consisting of 600 NSCLC patients treated with ICIP. Association between TMB and overall survival (OS), progression-free survival (PFS), objective response rate (ORR), and disease control rate (DCR) has been explored.

Results A strong positive correlation between TMB levels and OS, PFS rates, clinical benefit has been found when TMB > = 16(TMB > = 16 mutations/megabase (mut/Mb)). However, when TMB < 16, increasing TMB values did not exhibit a gradual stepwise increase in OS and PFS rates. The median months of OS in the TMB > = 16 and < 16 are 35.58, and 10.71 months respectively with average 12.39 months ($p < 0.0001$). The median months of PFS in the TMB > = 16 and < 16 are not-obtained, and 2.79 months respectively with an average of 3.32 months ($p < 0.0001$). The DCR in the TMB > = 16 and < 16 are 71.4% and 44.2% respectively with an average of 47.7% ($p < 0.0001$). The ORR in the TMB > = 16 and < 16 are 49.4% and 20.8% respectively with an average of 24.5% ($p < 0.0001$).

Conclusion The TMB > = 16 shows significantly associated with optimal ICIP treatment outcomes, including higher patient survival rates, delayed disease progression, and significant clinical benefits. These results present the potential of TMB as a promising biomarker candidate for NSCLC patients undergoing ICIP treatment.

Keywords TMB · NSCLC · Lung cancer · Biomarker · Immunotherapy

Introduction

Non-small cell lung cancer (NSCLC) represents a complex and challenging disease that requires a multifaceted approach to treat [1]. The current treatment modes for NSCLC encompass a range of interventions, including surgery, chemotherapy, targeted therapy, radiotherapy, immunotherapy, anti-angiogenesis therapy, etc. [1–6]. In recent years, it has been remarkable progress in the field of immunotherapy, with advanced NSCLC patients achieving long-term survival and ushering in a new era of chronic disease management [7]. Nowadays, the emergence of programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) immune checkpoint inhibitors has markedly altered the therapeutic outlook for NSCLC, offering tremendous hope and promise for patients worldwide [8]. However, the clinical benefit rates of immune checkpoint inhibitors remain relatively low with the objective response rate (ORR) of 20% [9]. The identification and validation of biomarkers will be critical in guiding personalized treatment decisions and ultimately improving outcomes for NSCLC patients.

Tumor mutational burden (TMB) is defined as the relative number of gene mutations in a specific tumor tissue,

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calculated as follows: TMB (Mut/Mb) = total number of mutations (including synonymous, non-synonymous point mutations, substitutions, insertions, and deletions)/target region coding region size [10, 11]. Recently, TMB has been identified as a biomarker for ICIP in melanoma. The FDA has also approved pembrolizumab in all cancers with a TMB > 10Mut/Mb, emphasizing the potential predictive power of this biomarker [12, 13]. TMB has been shown to be particularly valuable in predicting prognosis in advanced NSCLC patients receiving adjuvant immunotherapy [14]. Further investigation to validate and expand upon these findings will be critical in fully unlocking the potential of TMB as a powerful tool for prognostication and therapeutic guidance in cancer patients.

In this paper, the aim of this retrospective cohort study is to explore the correlation between TMB levels and survival and response in NSCLC patients treated with ICIP. Our results showed A strong positive correlation between

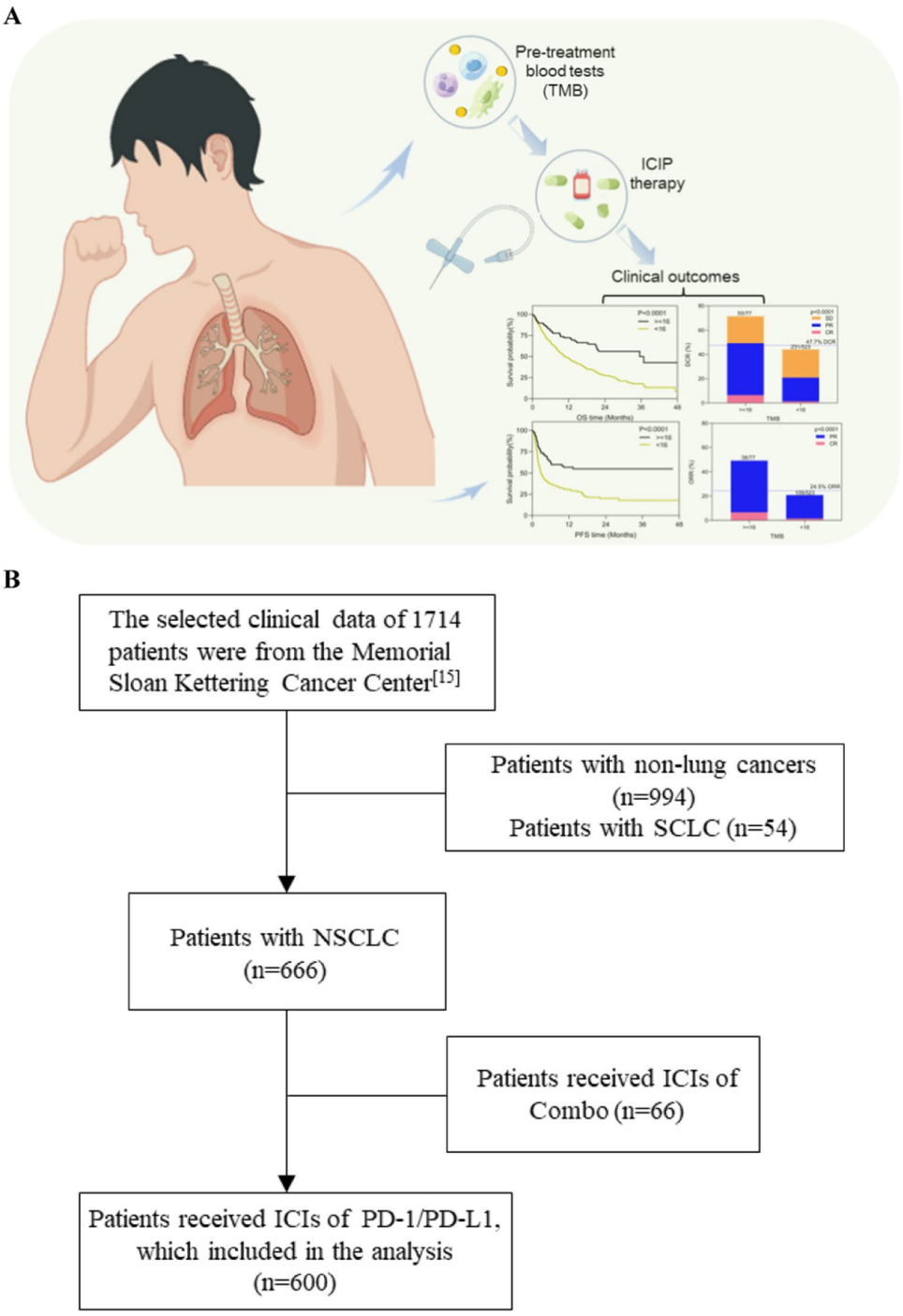


Fig. 1 Clinical model for predicting clinical outcomes of ICIP treatment using pre-treatment blood tests (**A**) and Flow diagram for clinical data filter (**B**). TMB values were gathered from the nearest blood test preceding the initial ICIP infusion. We present a precision medicine-based approach utilizing the TMB as a critical prognostic marker in ICIP treatment. Through a systematic and data-driven analysis of NSCLC patients ($n = 600$), we have demonstrated the powerful predictive value of TMB in guiding treatment decisions and improving clinical outcomes, including OS, PFS, DCR, and ORR. ICIs, Immune checkpoint inhibitors; NSCLC, Non-small cell lung cancer; SCLC, Small cell lung cancer; Combo, Combination of anti-PD-1/PD-L1 and anti-CTLA-4; CTLA-4, Cytotoxic T lymphocyte-associated antigen-4

Table 1 Characteristics of patients in the study ($n = 600$)

Characteristic	No. of patients (%)
Sex	
Female	300 (50)
Male	300 (50)
Age, years	
≤ 65	287 (47.8)
> 65	313 (52.2)
ICI line of treatment	
First line	169 (28.2)
Subsequent line	431 (71.8)
Stage	
II + III	128 (21.3)
IV	472 (78.7)
Performance status	
ECOG = 0	136 (22.7)
ECOG = 1	330 (55)
ECOG ≥ 2	40 (6.7)
Unknown	94 (15.6)
TMB	
TMB ≥ 16	77 (12.8)
TMB < 16	523 (87.2)

TMB levels and OS, PFS rates, clinical benefit has been found when TMB > = 16. However, when TMB < 16, there is no significant correlation between the TMB and OS and PFS rates. Our results underscore the potential utility of TMB as a valuable prognostic marker for patients being treated with ICIP, providing a promising avenue for further research in identifying high-risk patient subgroups for targeted intervention.

Methods

Data source and patients selection

The clinical data of 600 NSCLC patients who received ICIP treatment were sourced from the Memorial Sloan Kettering Cancer Center [15]. TMB, derived from DNA in tumor tissue, was defined as the total number of somatic non-synonymous tumor mutations normalized to the exonic coverage of the respective MSK-IMPACT panel in megabases (mutations/megabase) [15]. TMB was obtained from the nearest blood test preceding the initial ICIP infusion. Patients selected for the study were all those who had been selected by Luc G.T. Morris and his colleagues [15]. We excluded patients with a history of SCLC, and those who had received combination immune checkpoint inhibitors of PD-1/PD-L1 and CTLA-4 (Fig. 1A, B).

Survival analysis

The Kaplan–Meier method was utilized for survival analyses of OS and PFS of NSCLC patients treated with ICIP. The Cox proportional hazards regression model was subsequently applied to adjust for potential confounding factors, including sex, age, tumor stage, drug class of ICIP, and ICIP as the first or subsequent line of treatment. The response was classified using the Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1 criteria [16].

Validation cohort

To verify the above results, the analysis of 400 randomly resampled cohorts was conducted by the bootstrap resampling method for validation of the TMB thresholds analyzed in the original cohort by SPSS. We reevaluated the correlation between TMB and OS, PFS, DCR, and ORR, then investigated the differences between the validation cohort and original cohort with median months of OS and PFS, as well as ORR and DCR.

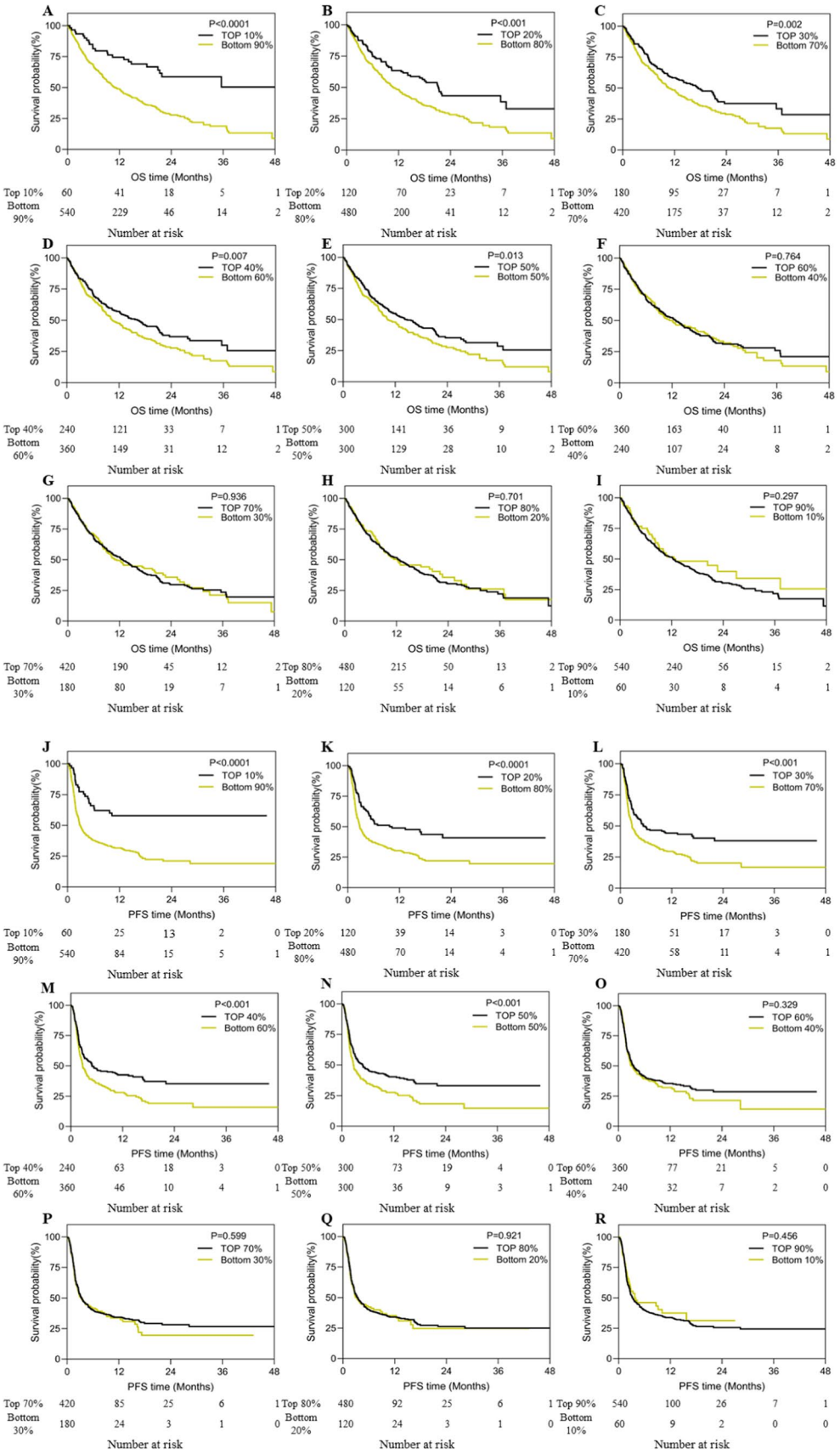
Statistical analysis

Pearson's χ^2-test was utilized to compare response and clinical outcomes between groups. Kaplan–Meier analysis was employed to calculate the corresponding survival rates. The log-rank test compares survival rates between groups. Cox proportional hazard regression model was thereafter utilized to calculate hazard ratios (HRs). All statistical analyses were conducted by SPSS 24.0.

Results

The characteristics of NSCLC patients after ICIP treatment

To evaluate the predictive value of pre-treatment TMB, we analyzed data of 600 NSCLC patients with response and survival outcomes after immune checkpoint inhibitors of ICIP treatment. There are a total of 287 patients under 65 years old, and 313 patients over 65 years old. 300 patients (50%) were male (Table 1). The median follow-up duration was recorded at 18 months. The objective response rate was calculated at 24.5%, along with a disease control rate (DCR) of 47.7%.


A
P<0.0001
TOP 10%
Bottom 90%
Survival probability(%)
OS time (Months)
Top 10% 60 41 18 5 1
Bottom 90% 540 229 46 14 2
Number at risk
B
P<0.001
TOP 20%
Bottom 80%
Survival probability(%)
OS time (Months)
Top 20% 120 70 23 7 1
Bottom 80% 480 200 41 12 2
Number at risk
C
P=0.002
TOP 30%
Bottom 70%
Survival probability(%)
OS time (Months)
Top 30% 180 95 27 7 1
Bottom 70% 420 175 37 12 2
Number at risk
D
P=0.007
TOP 40%
Bottom 60%
Survival probability(%)
OS time (Months)
Top 40% 240 121 33 7 1
Bottom 60% 360 149 31 12 2
Number at risk
E
P=0.013
TOP 50%
Bottom 50%
Survival probability(%)
OS time (Months)
Top 50% 300 141 36 9 1
Bottom 50% 300 129 28 10 2
Number at risk
F
P=0.764
TOP 60%
Bottom 40%
Survival probability(%)
OS time (Months)
Top 60% 360 163 40 11 1
Bottom 40% 240 107 24 8 2
Number at risk
G
P=0.936
TOP 70%
Bottom 30%
Survival probability(%)
OS time (Months)
Top 70% 420 190 45 12 2
Bottom 30% 180 80 19 7 1
Number at risk
H
P=0.701
TOP 80%
Bottom 20%
Survival probability(%)
OS time (Months)
Top 80% 480 215 50 13 2
Bottom 20% 120 55 14 6 1
Number at risk
I
P=0.297
TOP 90%
Bottom 10%
Survival probability(%)
OS time (Months)
Top 90% 540 240 56 15 2
Bottom 10% 60 30 8 4 1
Number at risk
J
P<0.0001
TOP 10%
Bottom 90%
Survival probability(%)
PFS time (Months)
Top 10% 60 25 13 2 0
Bottom 90% 540 84 15 5 1
Number at risk
K
P<0.0001
TOP 20%
Bottom 80%
Survival probability(%)
PFS time (Months)
Top 20% 120 39 14 3 0
Bottom 80% 480 70 14 4 1
Number at risk
L
P<0.001
TOP 30%
Bottom 70%
Survival probability(%)
PFS time (Months)
Top 30% 180 51 17 3 0
Bottom 70% 420 58 11 4 1
Number at risk
M
P<0.001
TOP 40%
Bottom 60%
Survival probability(%)
PFS time (Months)
Top 40% 240 63 18 3 0
Bottom 60% 360 46 10 4 1
Number at risk
N
P<0.001
TOP 50%
Bottom 50%
Survival probability(%)
PFS time (Months)
Top 50% 300 73 19 4 0
Bottom 50% 300 36 9 3 1
Number at risk
O
P=0.329
TOP 60%
Bottom 40%
Survival probability(%)
PFS time (Months)
Top 60% 360 77 21 5 0
Bottom 40% 240 32 7 2 0
Number at risk
P
P=0.599
TOP 70%
Bottom 30%
Survival probability(%)
PFS time (Months)
Top 70% 420 85 25 6 1
Bottom 30% 180 24 3 1 0
Number at risk
Q
P=0.921
TOP 80%
Bottom 20%
Survival probability(%)
PFS time (Months)
Top 80% 480 92 25 6 1
Bottom 20% 120 24 3 1 0
Number at risk
R
P=0.456
TOP 90%
Bottom 10%
Survival probability(%)
PFS time (Months)
Top 90% 540 100 26 7 1
Bottom 10% 60 9 2 0 0
Number at risk

◂**Fig. 2** The OS and PFS in ICIP-treated NSCLC patients according to TMB percentile. The Kaplan–Meier curves show the OS (**A**–**I**) and PFS (**J**–**R**) in ICIP-treated NSCLC patients correlated with respective TMB percentiles, with cutoff top 10th, top 20th, top 30th, top 40th, top 50th, top 60th, top 70th, top 80th, and top 90th percentile. P values are calculated by log-rank test. *OS* overall survival, *PFS* progression-free survival, *TMB* tumor mutational burden, *ICIP* immune checkpoint inhibitors of PD-1/PD-L1

TMB is associated with the clinical outcomes of ICIP treatment

To understand the relationship between TMB and the clinical outcomes of ICIP treatment for NSCLC patients, we analyzed a cohort of 600 NSCLC patients. Kaplan–Meier curves showed the OS (Fig. 2A–I) and PFS (Fig. 2J–R) in NSCLC patients of ICIP treatment according to TMB percentile using as cutoff top 10th, top 20th, top 30th, top 40th, top 50th, top 60th, top 70th, top 80th, and top 90th percentile. We found a strong positive correlation between TMB levels and OS, PFS rates when TMB percentile > = 50%.

To further explore the correlation between TMB levels and clinical outcomes of ICIP treatment, the TMB levels ranging from 0 to 77 patients were separated into 0–1.99, 2–3.99, 3–4.99, 4–5.99, 6–7.99, 8–9.99, 10–11.99, 12–15.99, 16–19.99, 20–77. Next, the univariate and multivariate analysis using the Cox regression model showed that a strong positive correlation was revealed between TMB levels and OS and PFS, when TMB > = 16. However, when TMB < 16, any increase in TMB did not exhibit the same trend associations in either OS or PFS rates ($p > 0.05$; Fig. 3A–D), taking the TMB levels of 6–7.99 as the reference.

The correlation between TMB levels and survival in NSCLC patients treated with ICIP

Based on the results, NSCLC patients were classified into TMB > = 16 and TMB < 16. There are a total of 77 patients TMB > = 16, and 523 patients TMB < 16 (Table 2). Next, the Kaplan–Meier analysis showed that the OS and PFS rates of TMB > = 16 were significantly higher than those of TMB < 16 ($p < 0.0001$; Fig. 4A, E). The median months of OS in the TMB levels of > = 16 and < 16 are 35.58, and 10.71 months respectively with average 12.39 months ($p < 0.0001$; Fig. 4B). The median months of PFS in the TMB levels of > = 16 and < 16 are not-obtained, and 2.79 months respectively with an average of 3.32 months ($p < 0.0001$).

Furthermore, the univariate and multivariate analysis utilizing the Cox regression model exhibited the associations of TMB with OS (> = 16: univariate, HR = 0.425; 95% CI 0.295–0.613; $p < 0.0001$; multivariate, HR = 0.412; 95% CI 0.283–0.598; $p < 0.0001$) (Fig. 4C, D) and PFS (> = 16: univariate, HR = 0.424; 95% CI 0.294–0.610; $p < 0.0001$; multivariate, HR = 0.402; 95% CI 0.277–0.583; $p < 0.0001$) (Fig. 4F, G) respectively (Table 3).

The correlation between TMB levels and the best response to ICIP treatment

Subsequently, the optimal treatment responses to ICIP therapy were classified as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). To assess the efficacy of ICIP therapy, the objective response rate (ORR) was employed, reflecting the proportion of patients who achieved a CR or PR, which was calculated at 24.5%. Furthermore, DCR was established to evaluate the effectiveness of the treatment, encompassing the percentage of patients who underwent a CR, PR, or SD, with a total of 47.7% of NSCLC patients demonstrating a positive outcome (Fig. 5A, B). Patients with TMB > = 16 exhibited the ICIP response with an ORR of 49.4% and a DCR of 71.4%, higher than TMB < 16 with an ORR of 20.8% and a DCR of 44.2%.

The variability and reproducibility between TMB and ICIP treatment clinical outcomes

To validate the correlation between TMB and ICIP treatment clinical outcomes, we conduct an internal verification using 400 randomly bootstrap-resampled cohorts (Table 4). There are a total of 77 patients TMB > = 16, and 523 patients TMB < 16. The statistical evaluation involved measurement of clinical outcomes including the median survival times of OS and PFS, and the ORR and DCR (Fig. 6A–C). Patients with TMB > = 16 exhibited the ICIP response with an ORR of 44.4% and a DCR of 68.9%, higher than TMB < 16 with an ORR of 19.4% and a DCR of 41.7% with same trends as the original group (Table 5).

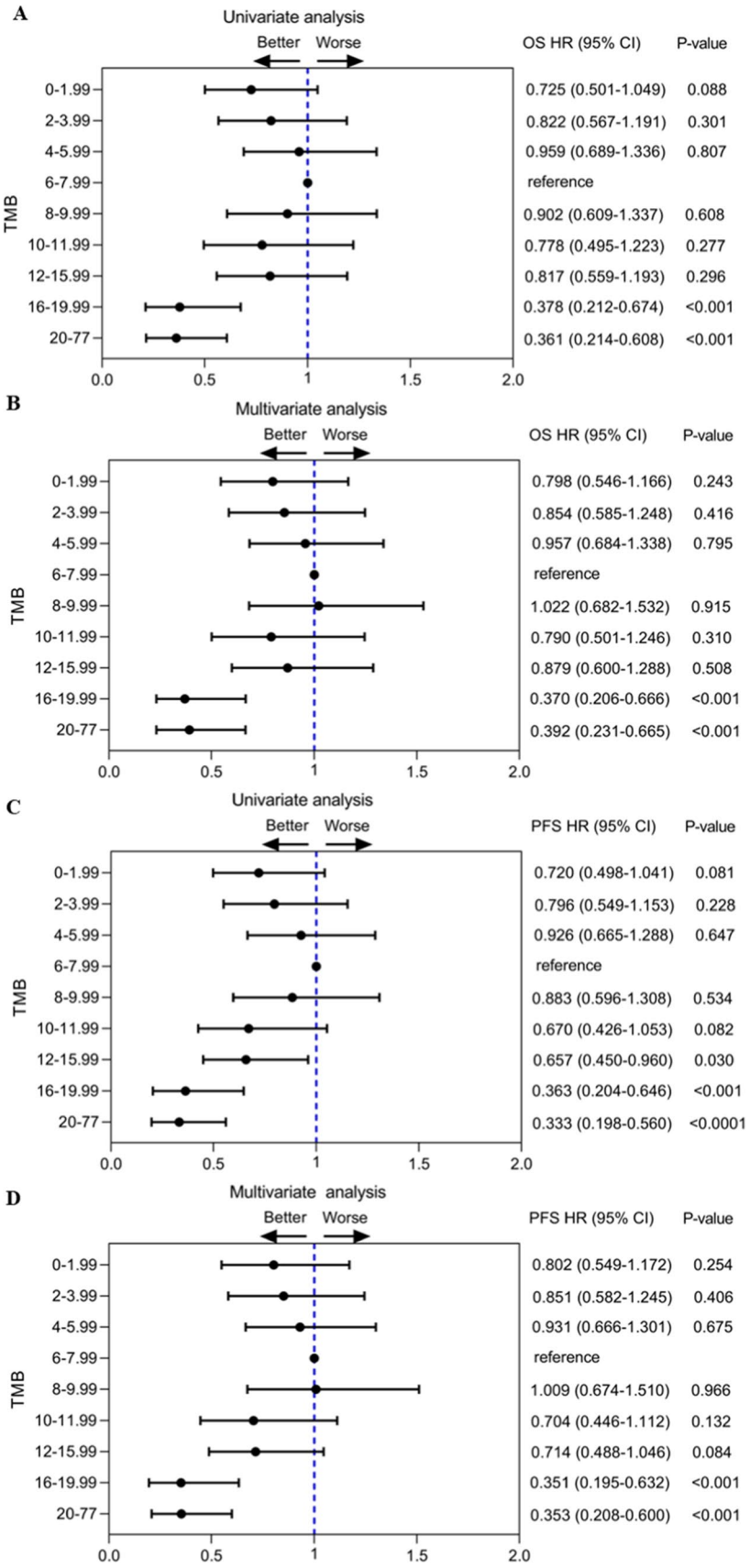


Fig. 3 The OS and PFS in ICIP-treated NSCLC patients based on different TMB levels. The TMB levels ranging from 0.3 to 77 patients were divided into 0–1.99, 2–3.99, 4–5.99, 6–7.99, 8–9.99, 10–11.99, 12–15.99, 16–19.99, 20–77. The forest plot showed the association of TMB levels with OS (**A**, **B**) and PFS (**C**, **D**) following ICIP therapy. HR and 95% CI were calculated by Cox proportional hazards regression analysis, using the TMB level of 6–7.99 as the reference. Multivariate analysis was performed by using the covariates of sex, age, tumor stage, ICI drug class, ICI line of treatment and ECOG score. *ICIP* immune checkpoint inhibitors of PD-1/PD-L1, *OS* overall survival, *PFS* progression-free survival, *TMB* tumor mutational burden

Table 2 Comparison of characteristics between TMB > = 16 and < 16 of patients in the study

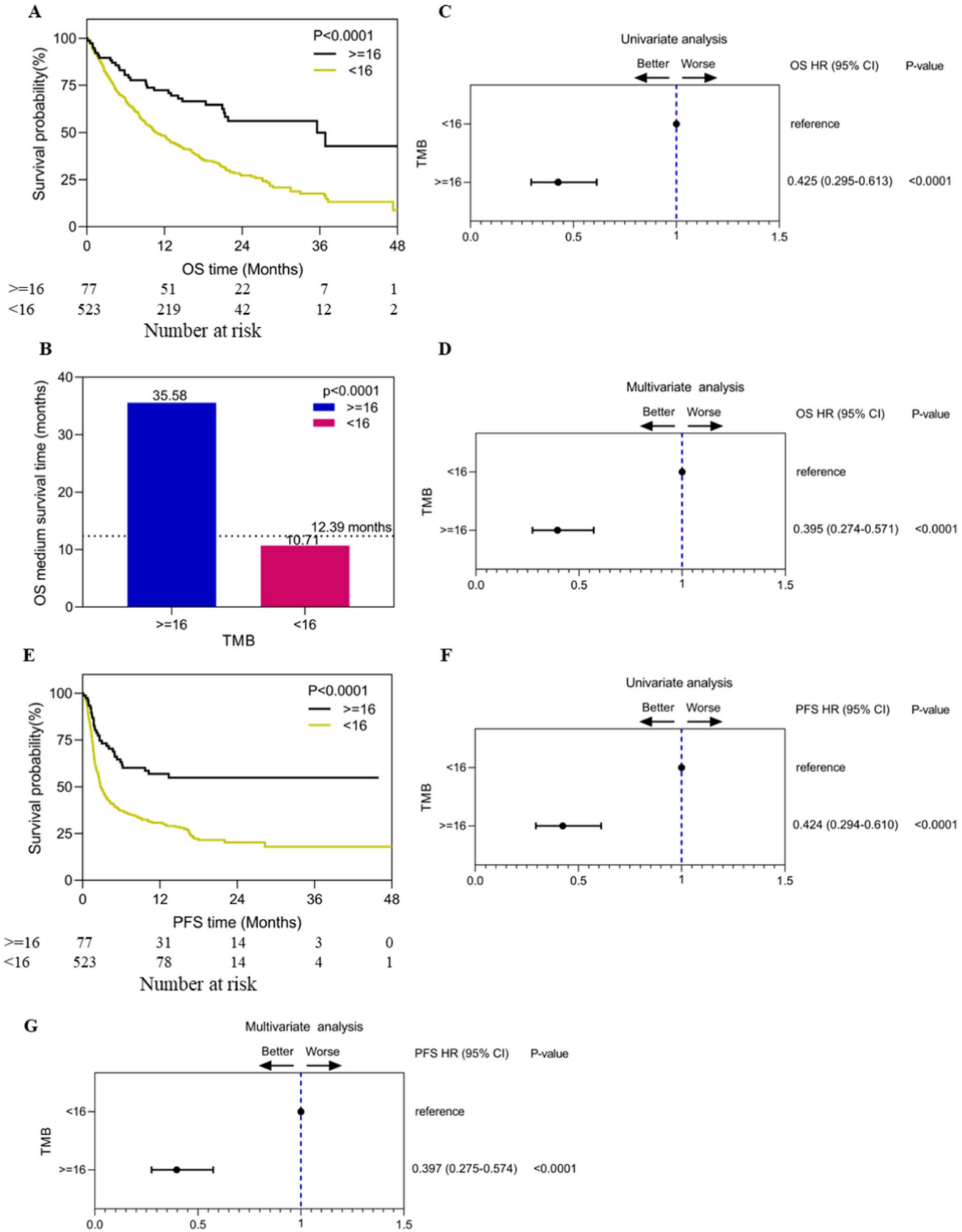
Characteristic	TMB ≥ 16 No. of patients (%)	TMB < 16 No. of patients (%)	*p*
Sex			0.328
Female	43 (55.8)	266 (50.9)	
Male	34 (44.2)	257 (49.1)	
Age, years			0.619
≤ 65	39 (50.6)	274 (52.4)	
> 65	38 (49.4)	249 (47.6)	
ICI line of treatment			0.852
First line	21 (27.3)	148 (28.3)	
Subsequent line	56 (72.7)	375 (71.7)	
Stage			0.416
II + III	5 (6.5)	23 (4.4)	
IV	72 (93.5)	500 (95.6)	
Performance status			0.189
ECOG = 0	13 (16.9)	123 (23.5)	
ECOG = 1	42 (54.5)	288 (55.1)	
ECOG ≥ 2	9 (11.7)	31 (5.9)	
Unknown	13 (16.9)	81 (15.5)	

Discussion

Immunotherapy has high therapeutic efficacy and long-term survival potential in selected patient populations, and its emergence has completely changed the pattern of cancer treatment, making it one of the most promising pillars of modern oncology [17, 18]. Recent approvals of PD-1 and PD-L1 inhibitors, such as nivolumab, pembrolizumab, atezolizumab, and durvalumab for NSCLC patients, underscore the potential of immunotherapy with their ability to deliver safety and significant improvements [19–22]. In routine clinical practice, the expression rate of PD-L1 is often used to guide the selection of appropriate treatments, such as pembrolizumab showing high efficacy in advanced NSCLC patients with PD-L1 ≥ 50% [23, 24]. However, Lung cancer cells have a very high mutation frequency, and clear driving genes can be found in most non-smoking adenocarcinoma, while smoking squamous cell carcinoma mostly has high-frequency mutations [25, 26]. Moreover, the inherent complexity of the tumor microenvironment, including heterogeneity within the tumor, genetic processes controlling PD-L1 expression, and temporal variability of PD-L1 expression between tumors, still have limitations [27]. In immunotherapy, the expression of PD-L1 can guide clinical medication, but it is not yet a perfect biomarker [28]. Given this reality, identifying effective biomarkers that can provide accurate prognostic indicators remains a top priority for optimizing patient prognosis.

High tumor TMB leads to higher tumor immunogenicity and stronger induced T cell responses. There is a significant correlation between high tumor TMB and clinical response to ICIP [12]. Therefore, TMB, as a predictive biomarker for immunotherapy, can help screen out populations more likely to benefit from immunotherapy. In this study, TMB has emerged as a promising biomarker, with its levels potentially indicative of cancer progression and efficacy of immunotherapy. Notably, we found that higher TMB was linked to higher survival outcomes and a greater probability of response to ICIP treatment. However, we also noted an interesting finding: when TMB < 16, there was no significant correlation between TMB and OS and PFS. In summary, our findings add to the growing body of evidence supporting the prognostic significance of TMB in the context of immunotherapeutic checkpoint inhibitors and underscore the potential of TMB as a valuable tool in guiding personalized treatment decisions.

There are several limitations in our study. First, at present, expression of PD-L1 determined by IHC remains the only validated biomarker that has demonstrated strong correlation with ICI response. We have not taken the ability of PD-L1 expression to co-predict with TMB. Previous studies on the relationship between PD-L1 and TMB in NSCLC produced inconsistent results, with some studies failing to find any correlation and others finding a favorable relationship [29, 30]. Yarchoan et al. examined 9887 individual specimens and discovered a small but significant relationship between PD-L1 expression and TMB [31]. Furthermore, Lamberti et al. proposed a significant relationship between high PD-L1 expression and elevated TMB in 421 NSCLC specimens, especially noteworthy in cases with PD-L1 tumor proportion score (TPS) ≥ 90% (N = 133) or < 1% (N = 288) [32]. The ORR to PD-1/PD-L1 inhibition was as high as 57% in patients with high TMB and PD-L1 expression 50% or higher and as low as 8.7% in patients with low TMB and PD-L1 expression less than 1% [33]. Therefore, TMB-High and PD-L1-High can be considered independent populations in NSCLC.


A
P<0.0001
>=16
<16
Survival probability(%)
OS time (Months)
>=16 77 51 22 7 1
<16 523 219 42 12 2
Number at risk
B
p<0.0001
>=16
<16
35.58
12.39 months
10.71
OS medium survival time (months)
TMB
C
Univariate analysis
Better Worse
OS HR (95% CI) P-value
TMB
reference
0.425 (0.295-0.613) <0.0001
D
Multivariate analysis
Better Worse
OS HR (95% CI) P-value
reference
0.395 (0.274-0.571) <0.0001
E
P<0.0001
>=16
<16
Survival probability(%)
PFS time (Months)
>=16 77 31 14 3 0
<16 523 78 14 4 1
Number at risk
F
Univariate analysis
Better Worse
PFS HR (95% CI) P-value
reference
0.424 (0.294-0.610) <0.0001
G
Multivariate analysis
Better Worse
PFS HR (95% CI) P-value
reference
0.397 (0.275-0.574) <0.0001

◂**Fig. 4** The OS and PFS in ICIP-treated patients based on reclassified TMB levels. The Kaplan–Meier curves exhibited the OS (**A**) and PFS (**E**) in ICIP-treated patients with reclassified TMB levels. *p* values are calculated by log-rank test. The median survival times (**B**) has been analyzed by the $\chi 2$ test. TMB levels were divided into > = 16 and < 16. The forest plot shows the association of TMB with OS (**C**, **D**) and PFS (**F**, **G**) following ICIP therapy. HR and 95% CI were calculated by Cox proportional hazards regression analysis, using the TMB level of > = 16 as the reference. Multivariate analysis was performed using the covariates of sex, age, tumor stage, ICI drug class, ICI line of treatment and ECOG score. *ICIP* Immune checkpoint inhibitors of PD-1/PD-L1, *TMB* tumor mutational burden, *OS* overall survival, *PFS* progression-free survival

Second, this study is an observational investigation that was conducted at a solitary center in a relatively small cohort of patients. To this end, we recommend conducting multi-center prospective cohort studies to further validate and expand upon our initial observations. Such studies will be instrumental in enhancing external validity and ultimately maximize their impact on clinical practice. We have attempted to address this concern by performing internal validation, but lack of external verification. In addition, we have carefully stratified patients according to TMB levels and identified that different TMB levels are associated with divergent survival and response outcomes, we recognize that these findings require further validation. The clinical benefits vary among different groups of the ECOG and 15% of patients have not-obtained which is also a disruptive factor. The FDA approved pembrolizumab on June 16, 2020, for the

Table 3 Multivariable analysis for the effect of TMB on OS and PFS (n = 600)

	OS				PFS			
	HR	95% CI		*p* value	HR	95% CI		*p* value
Age at ICI start								
≤ 65								
> 65	0.950	0.772	1.170	0.630	1.059	0.859	1.305	0.592
Sex								
Female	Reference							
Male	0.872	0.710	1.071	0.192	0.870	0.709	1.067	0.181
TMB								
TMB < 16	Reference							
TMB ≥ 16	0.395	0.274	0.571	< 0.0001	0.397	0.275	0.574	< 0.0001
Stage at ICI start								
II + III	Reference							
IV	0.395	0.215	0.725	0.003	0.395	0.215	0.725	0.003
ICI line of treatment								
First line	Reference							
Subsequent line	1.525	1.195	1.946	0.001	1.714	1.343	2.187	< 0.0001
ECOG								
ECOG = 0	Reference							
ECOG = 1	0.615	0.466	0.811	< 0.0001	1.619	1.226	2.139	0.001
ECOG ≥ 2	1.583	1.072	2.338	< 0.0001	2.595	1.666	4.041	< 0.0001
Unknown	0.828	0.616	1.114	0.04	1.175	0.819	1.686	0.380
NLR				< 0.0001				< 0.0001

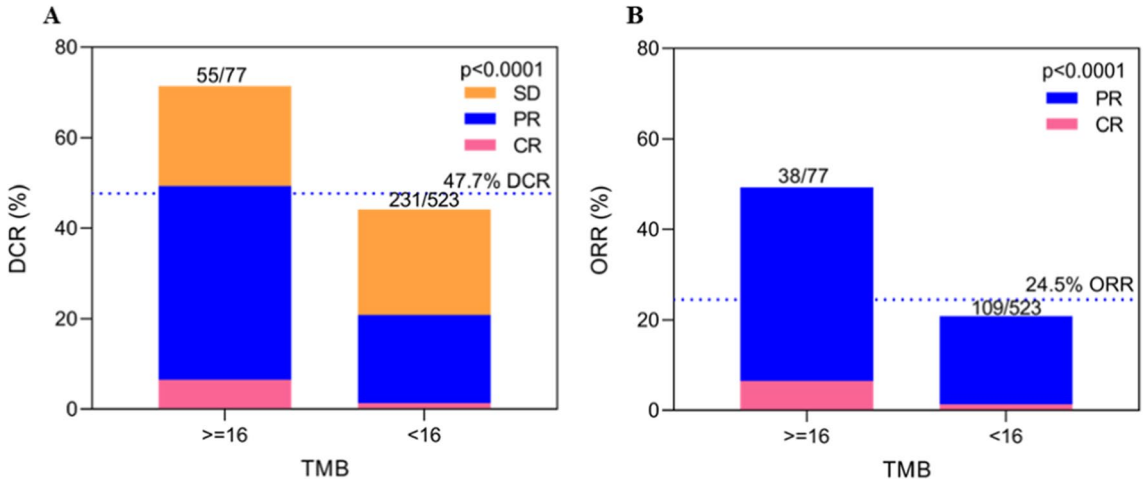


Fig. 5 The correlation between TMB levels and best response to ICIP treatment. The stacked bar plot exhibited the percentage of DCR and ORR in different TMB levels. TMB levels were divided into > = 16 and < 16. *p* values are calculated by the χ^2 test. The blue dashed line represented the average DCR (CR + PR + SD) of 47.7% and ORR (CR + PR) of 24.5% in all NSCLC patients. *ICIP* immune checkpoint inhibitors of PD-1/PD-L1, *TMB* tumor mutational burden, *DCR* disease control rate, *ORR* objective response rate, *CR* complete response, *PR* partial response, *SD* stable disease

treatment of adult and pediatric patients with unresectable or metastatic tumor mutational burden-high [TMB > = 10 (mut/Mb)] solid tumors. In our study, when 10 < TMB < 16, any increase in TMB did not exhibit the trend associations in either OS or PFS rates. These doubts require more clinical cases to verify.

Lastly, although our study highlights the potential of TMB as an inflammatory marker for predicting therapeutic efficacy, further research is still required to unravel the exact mechanisms driving this association.

Conclusion

In this study of 600 NSCLC patients treated with ICIP, the prognostic value of TMB has been confirmed. A strong positive correlation between TMB levels and OS, PFS rates, clinical benefit has been found when TMB > = 16. However, there is no significant difference in this trend when TMB < 16. In summary, TMB can serve as predictive indicators of treatment response and prognosis in NSCLC patients receiving ICIP treatment. These results exhibit great promise for guiding the selection and optimization of ICIP treatment

Table 4 Characteristics of patients in the validation cohort ($n = 400$)

Characteristic	No. of patients (%)
Sex	
Female	192 (48)
Male	208 (52)
Age, years	
≤ 65	187 (46.8)
> 65	213 (53.2)
ICI line of treatment	
First line	112 (28)
Subsequent line	288 (72)
Stage	
II + III	23 (5.8)
IV	377 (94.2)
Performance status	
ECOG = 0	89 (22.3)
ECOG = 1	224 (56)
ECOG ≥ 2	26 (6.5)
Unknown	61 (15.2)
TMB	
TMB ≥ 16	46 (11.5)
TMB < 16	354 (88.5)

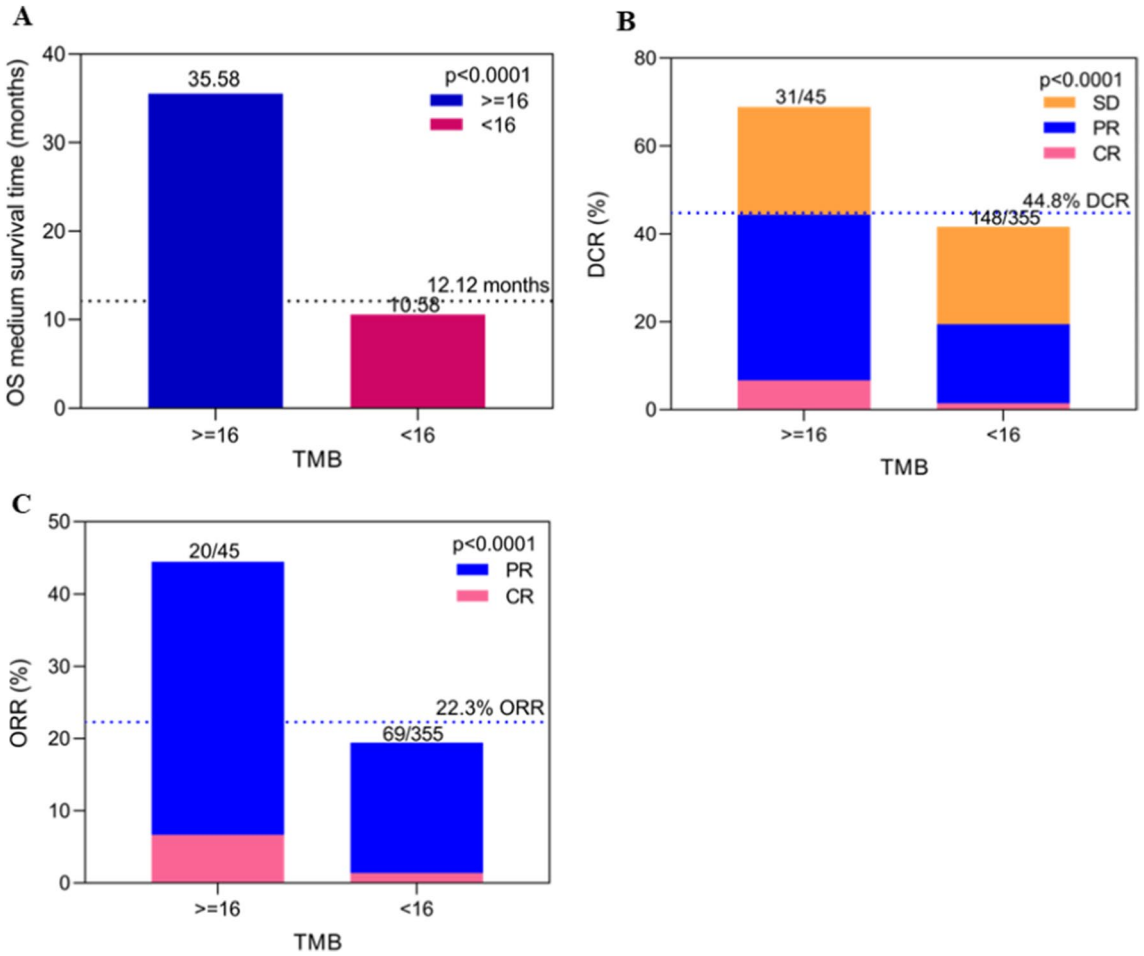


Fig. 6 The correlation between TMB and clinical outcomes to ICIP therapy in the validation cohort ($n=400$). The median survival times of OS (**A**), and DCR (**B**), and ORR (**C**) in different TMB levels has been provided. The blue dashed line represented the average DCR of 44.8% and ORR of 22.3% in the validation cohort. *p* values are calculated by the χ^2 test. *ICIP* immune checkpoint inhibitors of PD-1/PD-L1, *TMB* tumor mutational burden, *DCR* disease control rate, *ORR* objective response rate, *CR* complete response, *PR* partial response, *SD* stable disease

Table 5 Multivariable analysis for the effect of TMB on OS and PFS in validation cohort ($n=400$)

	OS				PFS			
	HR	95% CI		*p* value	HR	95% CI		*p* value
Age at ICI start								
≤65								
>65	0.902	0.697	1.167	0.431	0.985	0.760	1.276	0.906
Sex								
Female	Reference							
Male	0.942	0.733	1.212	0.644	0.905	0.704	1.163	0.435
TMB								
TMB < 16	Reference							
TMB ≥ 16	0.395	0.274	0.571	< 0.0001	0.397	– 0.275	0.574	< 0.0001
Stage at ICI start								
II + III	Reference							
IV	0.516	0.272	0.980	0.043	0.466	0.246	0.884	0.019
ICI line of treatment								
First line	Reference							
Subsequent line	1.374	1.017	1.857	0.039	1.594	1.180	2.153	0.002
ECOG								
ECOG = 0	Reference							
ECOG = 1	1.790	1.282	2.500	0.001	1.643	1.175	2.296	0.004
ECOG ≥ 2	2.543	1.476	4.381	0.001	2.254	1.307	3.887	0.003
Unknown	1.173	0.741	1.856	0.496	0.990	0.625	1.566	0.964
NLR				< 0.0001				< 0.0001

programs for NSCLC patients, providing actionable advice for doctors, and improving clinical decision-making.

Declarations

Competing interests 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 paper.

Ethical approval (Research involving human participants and/or animals), Informed consent No human subjects were directly involved in this study. All the data used in this study were derived from existing biological samples from prior studies.

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