Response to immune checkpoint inhibitor combined therapies in metastatic BRAF mutant lung cancers

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Abstract

The efficacy of immune checkpoint inhibitors (ICIs) combination regimens in BRAF mutant non-small cell lung cancers (NSCLCs) remains poorly explored. Immunotypes, including programmed death-ligand 1 (PD-L1) levels and tumor mutation burden (TMB), were evaluated. Objective response rate (ORR), disease control rate, progression-free survival (PFS), and overall survival (OS) of patients treated with ICI combination therapies were analyzed. A total of 150 patients conducted PD-L1 tests, wherein PD-L1 expression of 22 patients was greater than or equal to 50% (14.7%). Among the 38 TMB-evaluated patients, 28 had fewer than 10 mutations per megabase (73.7%). For patients treated with ICI combined regimens, median PFS was 6 months and median OS was 21.9 months. When stratified by treatment line, OS presented a significant difference (p=0.01), while PFS did not (p=0.40). The ORR for the treated patients was 36.7%. When stratified by BRAF mutation type, no differences were observed in PFS (p=0.74) and OS (p=0.94). Furthermore, no difference was found in PD-L1 expression (p = 0.06) or TMB (p=0.22) between the responders and non-responders. BRAF-mutant NSCLCs is associated with low levels of PD-L1 expression and low/intermediate TMB. ICIs combined therapies present promising activity and could provide an option for BRAF-mutated NSCLCs.

Introduction

Currently, the treatment of non-small cell lung cancer (NSCLC) has entered an era of precision therapy. Benefiting from targeted therapies targeting EGFR mutation, ALK fusion, ROS1 fusion, BRAF V600E mutation, MET exon 14 skipping, RET fusion, HER2 amplification, and NTRK fusion, the overall survival (OS) of patients with advanced NSCLC has greatly extended (1–8). Although agents targeting these gene alterations are highly effective, the development of drug resistance is inevitable.

V-Raf murine sarcoma viral oncogene homolog B (BRAF) kinase, encoded by the BRAF gene, is a member of the mammalian cytosolic serine/threonine kinases and lies downstream of the RAS pathway. BRAF activation results in a cascade of kinase activation, including MEK1/2 and ERK1/2, which plays critical roles in cell signaling, growth, and survival (9–11). BRAF mutations are the most common events that lead to the continuous activation of BRAF. However, BRAF mutations are rare gene alterations in NSCLC, which occur in 2% of lung adenocarcinoma patients and more frequently occur in never-smokers, females, and aggressive histological types (micropapillary) (12). BRAF mutations can be categorized into two classes: BRAF V600E, also known as class I mutation, which is a classical mutation, and non-V600E, including class II and class III, also known as non-classical mutations. BRAF V600E mutation is the most common and targetable mutation in NSCLC. Additionally, BRAF V600E mutations are mostly mutually exclusive with other oncogenic drivers; however, certain BRAF mutations can coexist with KRAS mutations (13, 14).

Previous studies have revealed that platinum doublet chemotherapy shows disappointing efficacy in advanced NSCLC patients with BRAF V600E mutations, wherein a limited objective response rate (ORR) and shorter survival was observed in this population when administered with platinum-based

chemotherapy (15–17). Several reports have revealed that BRAF-mutated NSCLC patients could benefit from BRAF inhibition (18–20). The activities of BRAF blockade monotherapy are still unsatisfactory in BRAF V600E mutated NSCLC with lower ORR ranging from 33–42% and median progression-free survival (PFS) of 5.5–7.3 months (18–20). However, BRAF pathway dual blockade with BRAF inhibitors and MEK inhibitors presented amazing efficacy which achieved an ORR of 63–64% and median PFS of 9.7 months, and extended the OS of this population to 24.6 months when given in the first-line setting (21). In contrast, the majority of BRAF non-V600E mutations do not benefit from BRAF inhibition. Owing to the paucity of data in the Chinese population and high cost, current targeted therapies for BRAF inhibitors are unavailable for most Chinese patients harboring BRAF V600E mutations. Hence, other treatments are urgently needed for BRAF-mutated NSCLC.

Immune checkpoint inhibitors (ICIs) have greatly revolutionized the treatment of NSCLC without driver gene mutations. Previous studies have revealed that the activity of ICIs in EGFR/ALK-altered NSCLC is limited (22, 23), while the efficacy of ICIs in BRAF-mutant NSCLC remains poorly explored. Several small-sample studies have demonstrated that ICI monotherapy presents disappointing results in this specific population; however, the conclusion appears to be controversial (24, 25). A previous case report reported that an NSCLC patient with BRAF V600E mutations treated with ICI combined with chemotherapy displayed a durable response (26). These data suggest that ICI combined regimens might have potential antitumor activity in BRAF-mutated NSCLC. Hence, the optimal sequence of standard systemic options in first-line settings for BRAF-altered NSCLC requires further studies.

Methods

Patients

Patients with histologically confirmed advanced BRAF-mutant NSCLC from January 2019 to December 2021 were screened at the First Affiliated Hospital of Zhengzhou University. Ultimately, 41 patients were included in this study. Patients were divided into two groups: BRAF V600E mutant tumors (group A) and non-V600E BRAF mutant tumors (group B). Eligibility criteria were as follows: (1) patients aged above 18 years; (2) patients histopathologically or cytologically confirmed as stage III/IV NSCLC harboring BRAF mutation; (3) patients who had received ICI combination therapies; (4) patients with at least one measurable target lesion according to the Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1); and (5) patients with OS longer than 3 months. Patients who were administered radiation and other local therapies or those who experienced recurrence after radical mastectomy before inclusion were allowed. Key exclusion criteria were as follows: (1) No history of ICIs combination therapies; (2) Mixed SCLC components.

All the patients provided written informed consent, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

Data collection

Treatment response evaluation was carried out routinely every 6–8 weeks by two independent radiologists based on the RECIST 1.1. Baseline demographics, including sex (male vs. female), age (≥ 65 or < 65 years), smoking status (never, former/current), treatment lines, and gene mutation status, were collected. BRAF status was tested by RT-PCR or next-generation sequencing (NGS). The ORR was defined as the rate of complete response (CR) and partial response (PR). Disease control rate (DCR) included CR, PR, and stable disease (SD) rates. PFS was determined from the time that the first dose of ICI combination therapy was administered until progressive disease (PD) or patient death occurred. Patients were censored at the last follow-up if they were alive or had no recorded disease. OS was calculated from the time of treatment initiation to death.

Programmed death-ligand 1 (PD-L1) expression was tested by immunohistochemistry (IHC) using the DAKO 22C3 PharmDx antibody. Validation of PD-L1 IHC analysis based on the 22C3 PharmDx antibody has been published and has therefore been adopted by the majority of molecular pathology laboratories in Israel (a harmonization study for the use of 22C3 PD-L1 immunohistochemical staining on Ventana's platform). The expression level of PD-L1 was determined by the positive rate of the tumor proportion score (TPS), which is defined as the percentage of partial or complete membrane staining positive tumor cells, and patients based on PD-L1 expression were classified as negative, intermediate, or high (TPS < 1%, 1–49%, and ≥ 50%, respectively). Tumor mutation burden (TMB) was determined by NGS, which covered 425 genes based on the FoundationOne algorithm, as previously published (Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden).

Statistical analysis

All statistical analyses were performed using the SPSS statistical software (version 21.0; IBM Corp., USA). Fisher's exact and Wilcoxon two-sample tests were used to compare the associations between categorical variables. All reported p-values were evaluated with two-sided hypothesis tests, and all p-values smaller than 0.05 were defined as statistically significant. Kaplan-Meier analysis was used to evaluate the median PFS and OS, and survival curves were drawn using a stratified log-rank test. Hazard ratios (HR) and associated 95% confidence intervals (CI) were calculated using Cox regression analysis.

Results

Between July 1, 2014, and December 1, 2021, 304 patients with BRAF-altered NSCLC were identified at the First Affiliated Hospital of Zhengzhou University. Of these, 86, 4, 40, and 171 patients were diagnosed with stages I, II, III, and IV, respectively (Table 1). A total of 29 patients (9.5%) had brain metastases at the time of diagnosis of metastatic disease, and 23 patients (23.3%) presented with liver metastases at initial diagnosis. Most patients (286, 94.1%) were diagnosed with adenocarcinoma, and 224 patients (73.7%) were never smokers or light former smokers (< 10 pack-years). The median age was 64 years (27–98). These characteristics were consistent with the known clinical phenotype of BRAF-altered NSCLCs (16, 24,

27, 28). A total of 21 patients had KRAS mutations and BRAF mutations, congruent with previous studies, which reported that KRAS mutations were the most common with BRAF mutations. In addition, our data revealed that 11 patients had simultaneous EGFR sensitive mutations, including EGFR 19 exon deletion and 21 exon L858R and BRAF mutations, one patient with BRAF mutation and ALK fusion mutation, and one patient with BRAF mutation and CCDC6-RET fusion (Supplemental table 1). A higher proportion of patients in the treatment cohort had a light smoking history (< 10 pack-years) compared with patients in the full BRAF mutation cohort and immunophenotypic cohort (36% versus 17% and 12%, respectively). BRAF mutations were detected by NGS or PCR in 234 patients (77%) and 66 patients (21.7%), respectively, and four patients (1.3%) were tested by NGS and PCR simultaneously. BRAF V600E was the most commonly identified mutation in 196 patients (64.5%). Other common BRAF mutations, BRAF non-V600E mutations including BRAF G469A and BRAF K601E, were also tested (Fig. 1). The treatment regimens and drugs used in this study are summarized in Supplemental table 2.

Table 1
patient demographics

characteristics	total BRAF mutated (n = 304)	Immunophenotypic cohort (n = 263)	Treatment cohort (n = 41)	*p* Value
Age median(range), years	64 (27–98)	64 (27–98)	64 (37–81)	0.95
Sex				0.74
Male	161(53.0)	137(52.1)	24(57.1)	
Female	143(47.0)	126(47.9)	17(42.9)	
Smoking status				0.008
Never smoker	221(72.5)	191(72.3)	30(73.2)	
Light (< 10py)	3(0.9)	2(0.8)	1(2.4)	
Heavy (≥ 10py)	67(22.3)	57(22.0)	10(24.4)	
Not specified	13 (4.3)	13(4.9)	0(0)	
Histology				0.78
Adenocarcinoma	286 (94.1)	249 (94.7)	37(90.2)	
Squamous	7 (2.3)	4 (1.5)	4(9.8)	
Small cell	1(0.3)	1 (0.4)	0(0)	
Adenosquamous	4(1.3)	3 (1.1)	0(0)	
Sarcomatoid	2(0.7)	2 (0.8)	0(0)	
Neurocrine	1(0.3)	1 (0.4)	0(0)	
Large cell	1(0.3)	1 (0.4)	0(0)	
Mucoepidermoid	1(0.3)	1 (0.4)	0(0)	
Not specified	1(0.3)	1 (0.4)	0(0)	
Stage				0.006
I	86(28.3)	86(32.7)	0(0)	
II	4(1.3)	3(1.1)	1(2.4)	
III	40 (13.2)	35(13.3)	5(12.2)	
IV	171(56.3)	136(51.7)	35(85.4)	
NA	3(1.0)	3(1.1)	0(0)	

characteristics	total BRAF mutated (n = 304)	Immunophenotypic cohort (n = 263)	Treatment cohort (n = 41)	*p* Value
Brain metastases				0.004
Yes	28(9.2)	22(8.4)	6(14.6)	
No	182(59.9)	149(56.7)	33(80.5)	
NA	94(30.9)	92(35.0)	2(4.9)	
ICI combined therapy				
First line			25	
Second or later line			16	

Immunophenotype

PD-L1 expression was tested in 150 patients with BRAF-mutated NSCLCs (29 patients in the treatment cohort and 121 in the immunophenotypic cohort). Tumor PDL1 expression was low (< 1%), intermediate (1–49%), and high (≥ 50%) in 70 (46.7%), 58 (38.7%), and 22 (14.6%) patients, respectively (Table 2). TMB data were available for 38 patients with BRAF-mutated NSCLCs (11 in the treatment cohort and 27 in the immunophenotypic cohort). Of these, 28 patients had tumors with less than 10 mut/Mb (73.7%), and 10 patients (26.3%) had tumors ≥ 10 mut/Mb. To avoid comparing TMBs quantified from different NGS panels, we restricted the comparative analysis to the cohort of patients who had undergone NGS and TMB determination using MSK-IMPACT, which is the most frequently used panel. The median TMB of the BRAF-mutated lung cancers was 6.3 mut/Mb (0-27.92).

Table 2
PD-L1 expression and TMB status in BRAF mutated patients

	Total BRAF mutated	Immunophenotypic cohort	ICI treatment cohort	*p* Value
PD-L1 expression TPS (%)	n = 150	n = 121	n = 29	0.01
Negative (< 1)	70(46.7)	65(53.7)	5(17.2)	
Intermediate (1–49)	58(38.7)	42(34.7)	16(55.2)	
High (≥ 50)	22(14.7)	14(11.6)	8(27.6)	
PD-L1 expression TPS (%) median	17.31	15.04	26.58	0.18
TMB (muts/Mb)	n = 38	n = 27	n = 11	0.09
Low (< 5)	17 (44.7)	13 (48.1)	4(36.4)	
Intermediate (5–10)	11 (28.9)	13 (48.1)	6(54.5)	
High (≥ 10)	10 (26.3)	1 (3.7)	1(9.1)	
TMB (muts/Mb) Median (range)	7.3 (0-27.9)	0.7 (0-27.9)	9.2(2.1–20.1)	0.1

Efficacy

A total of 41 patients were administered ICI combinations. Of these, 24 patients were administered ICI combinations in the first-line setting, and 17 patients were administered ICI combinations in the later-line settings. Fifteen (36.6%, 95%CI, 21.2%-52.0%) of 41 patients whose responses were evaluable achieved an objective response at the data cutoff. The ORR in the 1st line was 41.7% (95%CI, 20.4%-62.9%), and the 2nd or later line was 29.4% (95%CI, 5.3%-53.6%); however, no statistical difference was observed in both groups (p = 0.52). The DCR of the 1st line was 75% (95%CI, 56.3%-93.7%) and the 2nd or later line was 58.8% (95%CI, 32.7%-84.9%) (p = 0.32) (Supplemental table 3 and Supplemental Fig. 1A and 1B). The PFS of all patients treated with ICIs is shown in Fig. 2A. The median PFS was 6 months (95%CI, 3.3–8.7) (Fig. 2B). The median OS for all ICI-treated patients was 21.9 months (95%CI, 17.8–26.1) (Fig. 2C). In the subgroup analysis, we found that the median PFS in the first-line treatment was numerically longer than that beyond 1st line (6 m versus 7 m, p = 0.40) (Fig. 3A). In addition, the median OS of patients who received ICI combined therapies in the first-line was significantly longer than that in the 2nd or later line (29 m versus 12m, p = 0.01) (Fig. 3B).

BRAF mutations are classified as BRAF V600E mutations and non-V600E mutations. Patients harboring BRAF V600E mutations could benefit from anti-BRAF targeted therapy. In the present study, the ORR for

patients with V600E mutations was 36.0% (95%CI, 15.8%-56.2%), and the ORR for patients with non-V600E mutations was 37.5% (95%CI, 10.9%-64.1%) (Supplemental Fig. 1C and 1D). In addition, the best changes in the longest diameter of target lesions stratified by mutation status were illustrated in Fig. 3C. The median PFS for BRAF V600E mutations was 5 months (95%CI, 2.26–7.75), and the median PFS for BRAF non-V600E mutations was 10 months (95%CI, 1.56–18.45), however, no significant difference was observed (p = 0.74) (Supplemental Fig. 2A). Additionally, no difference was found in the OS between patients with BRAF V600E mutations and BRAF non-V600E mutations (Supplemental Fig. 2B).

The correlation of ICI efficacy with PD-L1 and TMB

Previous studies have demonstrated that PD-L1 expression and TMB are associated with ICI (29, 30). Hence, in the present report, we also evaluated prognostic biomarkers for the efficacy of ICI combined therapies in BRAF-altered NSCLC. The best changes in the longest diameter of the target lesions stratified by PD-L1 expression levels are depicted in Fig. 3D. No significant correlation was found in activities between PD-L1 expression levels (median 36% versus 18%, p = 0.06, Fig. 4A) or TMB (median 8.5 versus 7.3 mut/mb, p = 0.79, Fig. 4B) between responders (best response PR) and non-responders (SD/PD). In addition, no statistical correlation was observed between the maximum change in the sum of target lesions and TMB (r = 0.17, 95%CI, 0.29–0.83, p = 0.23, Fig. 4C) or PD-L1 levels (r = 0.004,95% CI, -0.46-0.35, p = 0.76, Fig. 4D). However, patients with higher PD-L1 expression appeared to have longer PFS (7 mons (PD-L1 ≥ 1%) versus 1.5 mons (PD-L1 < 1%), p = 0.004). No statistical difference was observed between TMB and PFS (Supplemental Fig. 3A and 3B). Additionally, no significant difference was found between OS and PD-L1 expression levels or TMB (Supplemental Fig. 3C and 3D).

Adverse events

A total of 28 patients treated with ICIs had documented adverse effects when administered ICI combined therapies (Supplemental table 4). Generally, there is a manageable toxicity profile in BRAF-mutated patients who received ICI combined therapies. The most common adverse events were hematologic toxicities, including neutropenia (39.3%), anemia (21.4%), and thrombocytopenia (14.3%); however, the incidence of severe adverse events (≥ grade 3) was 7.1%, 0, and 3.6%, respectively. In addition, nine patients (32.1%) developed mild liver injury. Only 3 patients developed interstitial lung disease, and 1 patient discontinued treatment due to interstitial lung disease. Other adverse effects, including gastrointestinal effects, skin effects, and hypothyroidism, are summarized in Supplemental table 4.

Discussion

To the best of our knowledge, this retrospective study presents the largest series to date of BRAF-mutated lung cancers that describe the immunophenotype and activity of ICI combined therapies in these tumors. Our data suggest that BRAF-mutated lung cancers could benefit from ICI combined therapies; however, owing to the retrospective design and limited sample size, further trials are urgently needed.

Our findings showed that most patients with BRAF mutations displayed lower PD-L1 expression (≤ 50%). These data appear to be inconsistent with documented reports (31–34). Several small retrospective reports have revealed that patients with BRAF mutations tend to show positive PD-L1 expression. Recently, a study including 29 BRAF-altered NSCLC patients found that approximately 69% of patients were PD-L1 positive, and over 40% of patients had PD-L1 expression ≥ 50% (24). However, in our results, the proportion of patients with PD-L1 expression ≥ 50% was only 14.7%, possibly because our sample size was much larger. Furthermore, our data indicated that the TMB in most patients was lower (median TMB = 6.3mut/MB). This was partially consistent with previous data that demonstrated that BRAF-mutated NSCLC were more likely to have low/intermediate TMB and microsatellite-stable status (24). Our reports showed that most patients with BRAF mutations were never (or had a smoking cessation time longer than 20 years) or light (< 10 pack-years) smokers. Previous studies have demonstrated that patients with a history of smoking tend to have a high TMB, which partially explains the low TMB status of NSCLC with BRAF mutations (35). Moreover, low TMB has similarly been found in NSCLC patients with other oncogenic-drivers (36–38). This phenomenon might explain why patients with driver mutations display a modest response to ICIs. Notably, our data showed that patients with BRAF mutations might be accompanied by other oncogenic drivers such as EGFR, ALK, and RET fusion, which contradicts the conclusion that BRAF mutations are common in NSCLC patients without other oncogenic drivers. However, the proportion of co-mutations was low, and BRAF mutations more commonly co-occurred with KRAS mutations.

Previous studies have suggested that NSCLC patients harboring oncogenic drivers have difficulty benefitting from ICIs monotherapy, especially those with sensitizing EGFR/ALK alterations (22, 23, 39, 40). Garon and his collegues revealed that the ORR for EGFR-TKI naïve EGFR-mutant NSCLC (N = 4) treated with pembrolizumb was 50%, wherein the median PFS was 5.3 months, however, the ORR for EGFR-TKI treated EGFR-mutant NSCLC (N = 26) was only 4% and the median PFS was 1.9 months (41). In addition, a larger registry study reported the efficacy of ICI monotherapy in ALK, ROS1, and RET fusion-positive NSCLCs; primary PD was the most common event (41). Hence, the current standard clinical guidelines do not recommend the use of single-agent ICIs in later-line settings for patients with sensitizing EGFR/ALK/ROS1 alterations. The same phenomenon was observed in BRAF-mutated NSCLCs. In a documented separate series, the reported response to single-agent immunotherapy in BRAF V600E-mutant NSCLCs was 25%, and the median PFS was 3.7 months (24). Additionally, several retrospective studies have documented that the ORR to ICI monotherapy in BRAF-mutated NSCLC patients ranged from 10–30%, with a median PFS of 2–4 months, which further confirmed the limited activity of single-agent ICIs in patients harboring BRAF mutations (22, 24, 25, 42, 43). Notably, these data revealed that patients with BRAF non-V600E mutations seemed to have a better response to ICIs than patients with BRAF V600E mutations, although OS was longer in BRAF V600E mutant patients, possibly because patients with BRAF V600E mutations could benefit from BRAF-targeted therapy. Hence, as with other oncogenic drivers, single-drug ICIs are not the first treatment choice for patients with BRAF mutations.

Our findings showed that ICI combined therapy presented encouraging activities in BRAF-mutated NSCLCs. The median PFS was 6 months and the median OS was 21.9 months, although the ORR was 36.6%. Recently, Lu et al. reported a case in which BRAF V600E mutated patients administered chemo-immunotherapy achieved a durable response with a PFS of 20 months (26). To the best of our knowledge, this is the first evidence that patients harboring the BRAF V600E mutation can benefit from ICI plus chemotherapy. These data provide evidence that chemo-immunotherapy may be a promising choice for BRAF V600E mutated NSCLC. In addition, chemo-immunotherapy could improve survival in driver mutation-negative advanced NSCLCs, which has been verified in previous randomized trials (44–48). Hence, our findings suggest that ICI combinations might represent a preferred choice relative to single-agent ICIs in patients with BRAF-mutated NSCLCs. Notably, this did not mean that ICI combination regimens were the priority selections compared to BRAF TKI in treatment-naïve patients with BRAF V600E mutations. BRAF TKI remains the preferred treatment option for BRAF V600E mutated NSCLCs in current guidelines based on a randomized clinical trial that revealed that BRAF TKI therapy achieved prolonged survival (median PFS in 1st line setting = 14.6 months, median PFS in later line = 5-8.6months) (4, 21). However, due to poor availability in China, ICI combined chemotherapy might serve as an option for BRAF-mutated NSCLCs, especially in second-line settings.

Another key point was that only BRAF V600E mutated patients could benefit from BRAF-targeted TKI therapy; patients with BRAF non-V600E mutations responded poorly to BRAF TKIs (18–20). In the present study, we found that patients with BRAF non-V600E mutations presented a better response to ICI combined therapies with a PFS of 10 months; however, the PFS of patients harboring BRAF V600E mutations was 5 months, although the difference was not statistically significant, which might be the reason for the limited sample size. In addition, the ORR for BRAF non-V600E mutant patients was also numerically higher than that for BRAF V600E mutated patients (37.5% versus 36.0%). These results provide evidence that patients with BRAF non-V600E mutations might benefit from ICI combination therapies. These findings are consistent with those of previous studies (22, 24, 42). Hence, our data provided evidence that ICI combined regimens might be an attractive option for BRAF non-V600E mutated NSCLCs and could be used in later line settings for patients with BRAF V600E mutated NSCLCs.

Previous reports have revealed that PD-L1 and TMB may serve as potential biomarkers of response to ICIs (29, 30). Our findings showed that PD-L1 expression level tended to have the ability to predict the efficacy of ICI combined therapies; however, TMB was not correlated with activities with ICIs in BRAF-mutated patients. A possible explanation might be the limited sample size and inappropriate cut-off of TMB. Therefore, further studies are required.

Our study has some notable limitations. First, the limited number of cases limited the ability to observe meaningful differences. Second, PD-L1 testing and TMB status were not routinely assessed in all included patients. Only a small proportion of the patients were administered ICIs. Overall, because of its retrospective nature and potential selection bias, the current data were only hypothesis generating, and further prospective investigations are warranted.

Conclusions

In summary, our findings suggest that BRAF-mutated NSCLC commonly presents a low PD-L1 expression level and low/intermediate TMB status. ICI combined therapies displayed promising antitumor activity and a manageable adverse event profile in both BRAF V600E and BRAF non-V600E mutant tumors; however, BRAF non-V600E mutated patients might be more capable of benefiting from ICI combined regimens. In addition, ICI combinations in the 1st line setting might have favorable OS for NSCLCs with BRAF mutants. Hence, ICI combinations could serve as an effective option for patients with NSCLC with BRAF mutations. However, these results remain unconfirmed.

Declarations

Ethics approval and consent to participate

The study was approved by the independent ethics committee of the First Affiliated Hospital of Zhengzhou University and conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

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Conflict-of-interest

The authors have declared that no conflict of interest exists.

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Figures

Figure 1

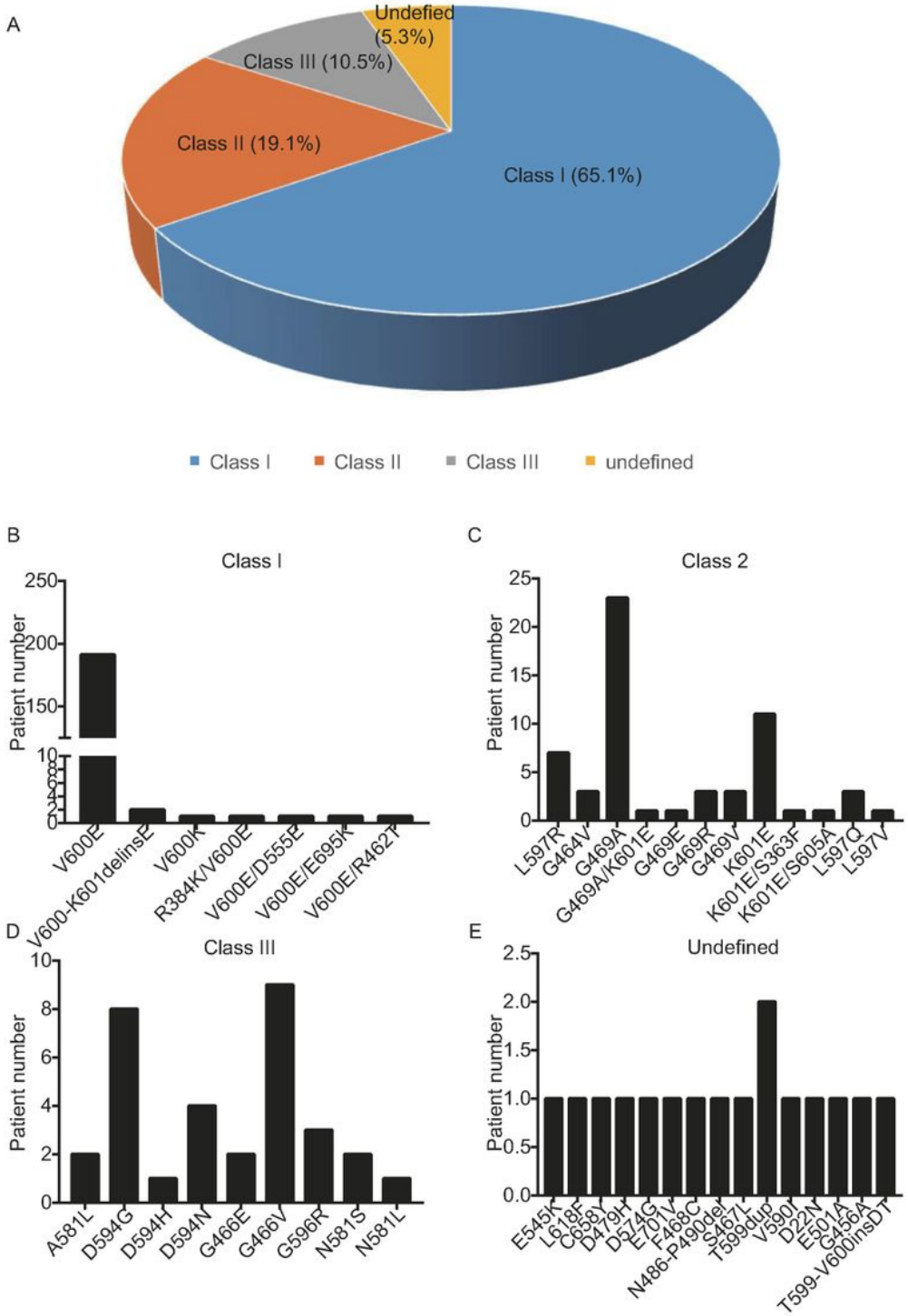

Figure 1

BRAF mutation category. A: BRAF mutations were divided to 3 classes: Class I (65.1%), Class II (19.1%), Class III (10.5%) and Undefined (5.3%). B-E: The occurrence rate of the mutations in our study in class I (B), class II (C), Class III (D) and undefined (E). BRAF mutations were illustrated in X-axis, Y-axis presented the frequencies.

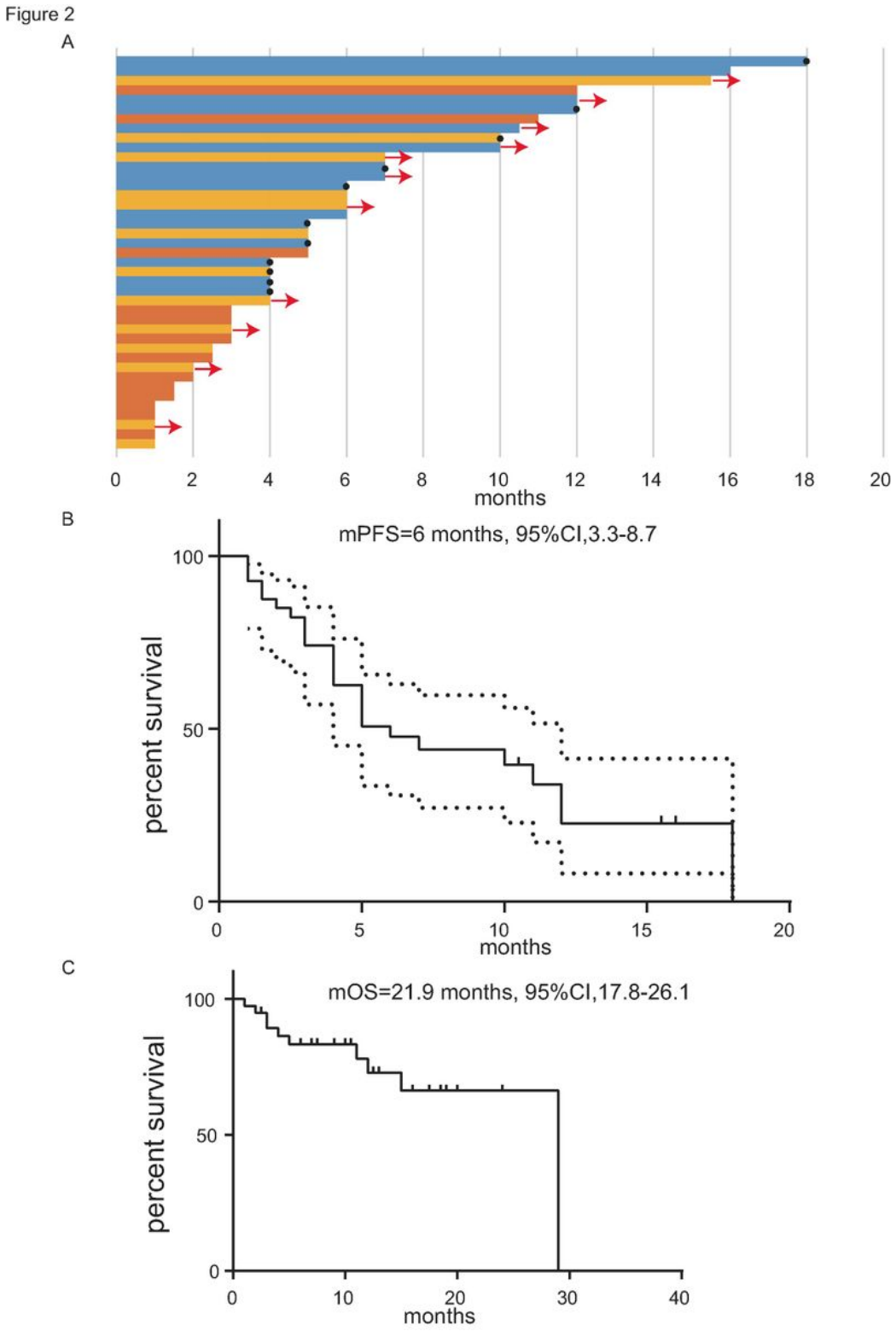

Figure 2

Efficacy and survival analysis in BRAF mutated patients treated with ICI combined therapies. A: Swimmer's plot for 41 patients received ICI combined therapies. Asterisks represents best response determined clinically. B: PFS for patients of treatment cohort with 95% confidence intervals revealed. C: OS for patients of treatment cohort with 95% confidence intervals revealed. Abbreviations: PFS, progression free survival; OS, overall survival.

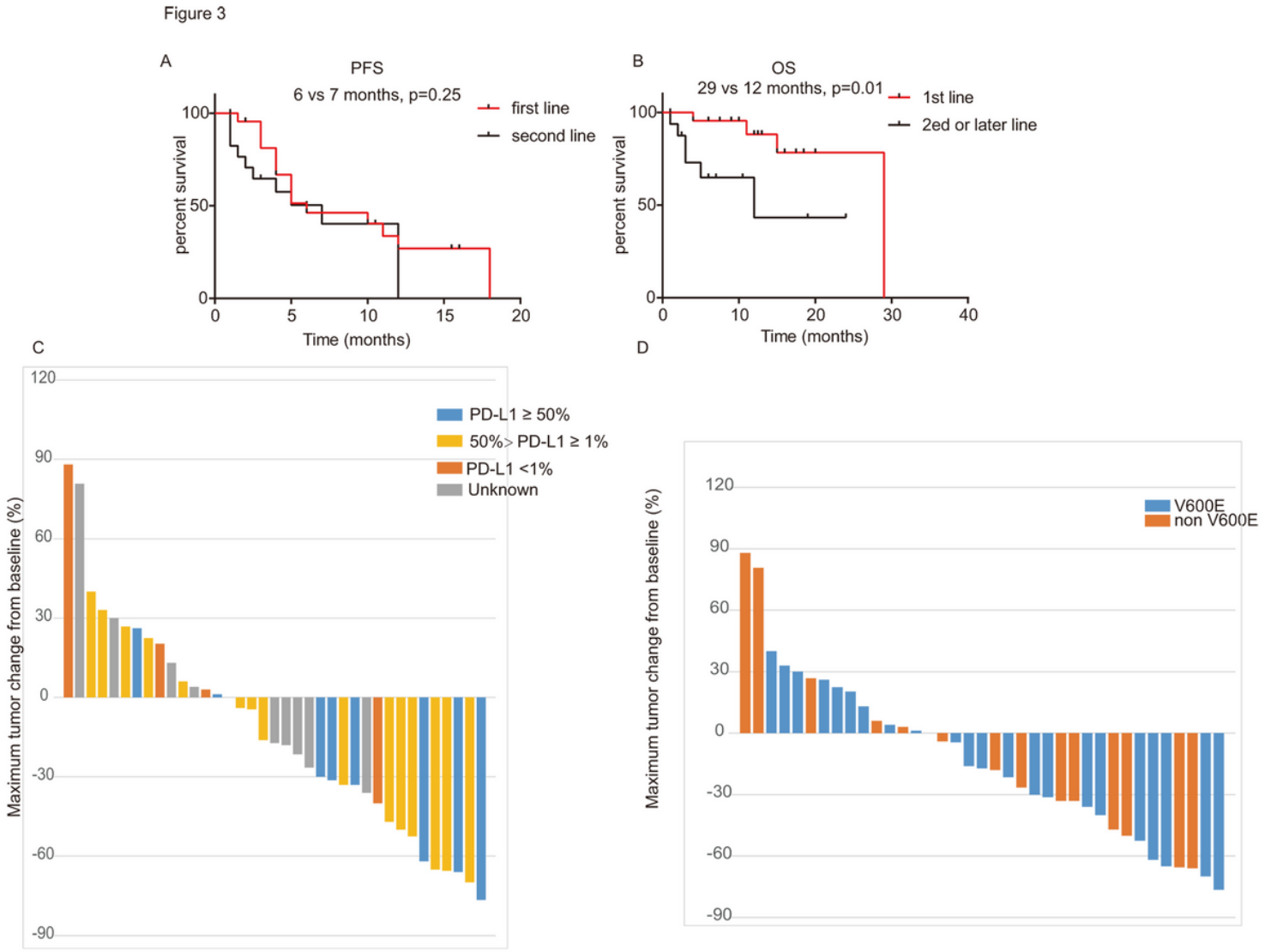

Figure 3

Activities of ICI combined regimens stratified by treatment lines, PD-L1 level and BRAF mutation types. A and B: PFS and OS for patients of treatment cohort stratified by treatment lines. C and D: Changes in the sum of the longest diameters of target lesions in different PD-L1 level or BRAF mutation types. Abbreviations: mPFS, median progression free survival; mOS, median overall survival; OS, overall survival; PFS, progression free survival.

Figure 4

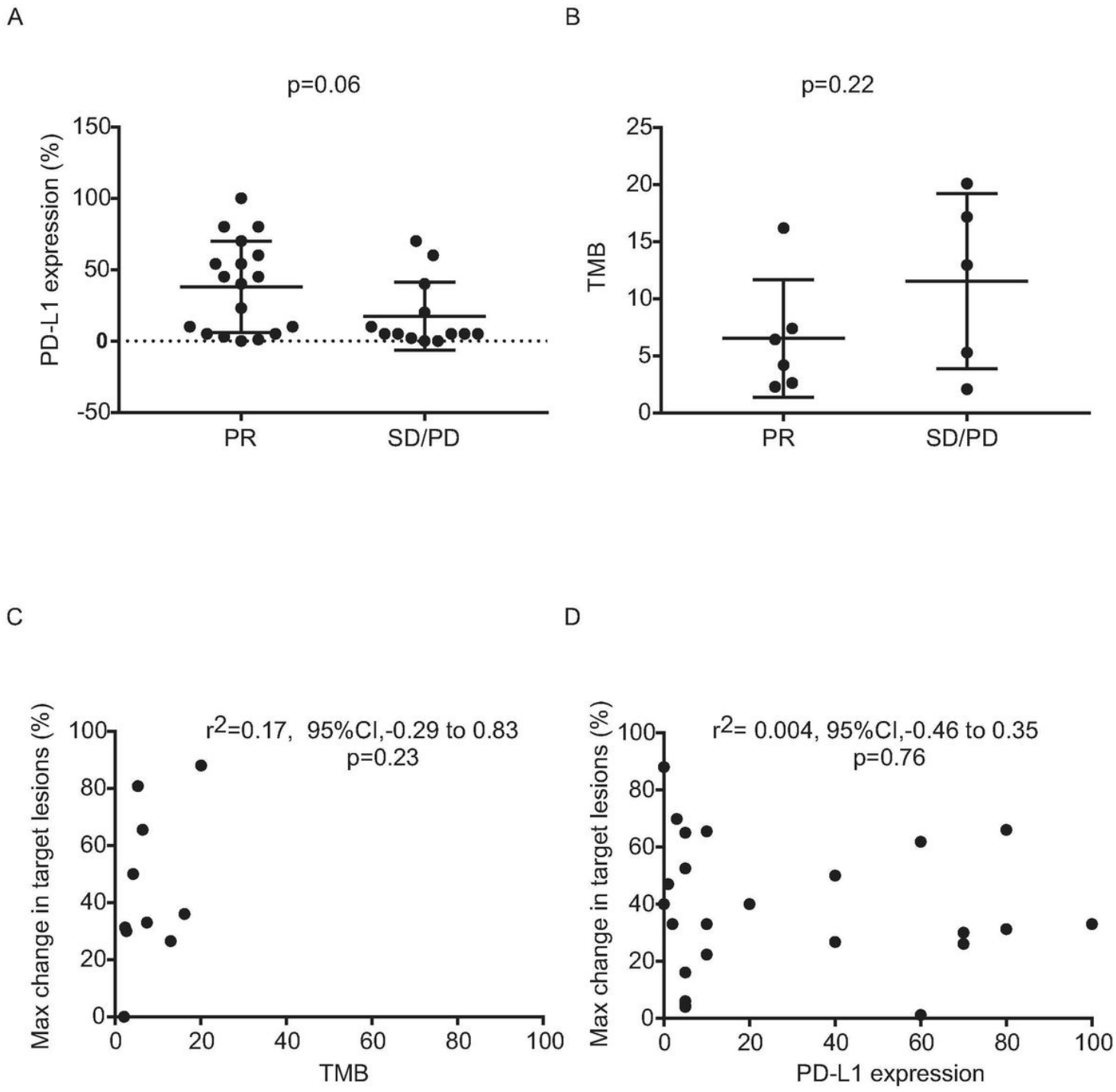

Figure 4

The correlation between efficacy and PD-L1 level and TMB status. A: relationship between PD-L1 expression and efficacy in responders (best response PR) and non-responders (best response SD and PD) (median 36% versus 18%, p =0.06). B: Comparison of TMB in responders and non-responders treated with ICI combined therapies with TMB available (median 8.5 versus 7.3 mut/mb, p = 0.79). C The relationship between PD-L1 level and maximum changes in target lesions. D: Correlation between TMB status and maximum changes in target lesions. Abbreviations: CI, confidence interval; Max, maximum; mut/Mb,

mutation per megabase; SD, stable disease; PD, progressive disease; PR, partial response; PD-L1, programmed death-ligand 1; TMB, tumor mutation burden.

Supplementary Files

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