Original Article

The safety and efficacy of anti-PD-1 inhibitor-based combinational therapy in non-small cell lung cancer patients with oncogenic alterations

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Background: The anti-programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) immunotherapy has been extensively used in patients with non-small cell lung cancer (NSCLC) in which the tumors are negative for oncogenic alterations. However, whether PD-1/PD-L1 blockade therapy could be applicable in patients harboring oncogenic mutations is largely unknown.

Methods: In this retrospective study, we analyzed the safety and efficacy of anti-PD-1 inhibitor-based combinational therapy in a NSCLC cohort of 84 patients who harbored oncogenic alterations in epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), k-Ras, RET, HER2 and BRAF. The patients were followed up till disease progression or death. The adverse effects associated with the treatment were carefully evaluated and timely interrupted.

Results: There were 50 patients harboring EGFR mutations, 17 patients with k-Ras mutation, 2 patients with ALK rearrangement, 6 patients with RET rearrangement, 6 patients with HER2 exon20 insertion and 3 patients with BRAF V600E mutation. About 58.8% of the k-Ras mutant patients responded to the combinational treatment. The median progression-free survival (mPFS) of the k-Ras cohort was 14 months, with the 12-month median overall survival (mOS) ratio and the 24-month OS ratio of 86.7% and 75.8%, respectively. Patients with *EGFR exon21 L858R* mutation or RET rearrangement tended to have a more favorable response, while patients harboring ALK rearrangement, HER2 exon20 insertion and BRAF V600E mutation did not respond well to anti-PD-1 inhibitor-based combinational therapy. The incidence of treatment-related toxicity was 52.3% and the most common immune-related adverse events (irAEs) were PD-1 inhibitors-related hypothyroidism and pneumonitis. The PD-L1 status and lung immune prognostic index (LIPI) could be used as biomarkers dictating therapeutic outcomes of the combinational therapy.

Conclusions: The anti-PD-1 inhibitor-based combinational therapy elicited exciting anti-tumor efficacy and prolonged patient survival with manageable adverse effects in NSCLC patients harboring oncogenic alterations. The PD-L1 status and LIPI could be used as a biomarker predicting response to anti-PD-1 inhibitor-based combinational treatment in these patients.

Keywords: Programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1); non-small cell lung cancer (NSCLC); immunotherapy; oncogenic mutations; immune-related adverse events (irAEs)

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Introduction

The great success of programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) blockade immunotherapy has achieved robust clinical responses in patients with advanced cancer (1-3). However, not all the patients benefit from the PD-1/PD-L1 blockade immunotherapy, in non-small cell lung cancer (NSCLC), the objective response rate (ORR) of anti-PD-1/PD-L1 monotherapy is approximately 20% (4,5). Accumulating evidence from clinical practice indicates a favorable response in NSCLC patients whose tumor possesses a high level of PD-1/PD-L1 (6). The next generation sequence (NGS) testing also suggests a positive correlation between the response rate and tumor mutation burden (TMB) in many types of solid tumors (7,8). Given these considerations, screening PD-1/PD-L1 expression and TMB status are recommended before the initiation of anti-PD-1/PD-L1 immunotherapy.

Another breakthrough for NSCLC in the past decade is the deciphering of oncogenic driver genes. Indeed, *epidermal growth factor receptor (EGFR)*, *anaplastic lymphoma kinase (ALK)*, *k-Ras*, *HER2*, *BRAF*, *ROS1*, *c-MET* and *RET* have been extensively described as potent tumor promoting genes and their mutations contribute to tumorigenesis and resistance to apoptosis in NSCLC (9). Pharmacological targeting these tumor promoting genes by tyrosine kinase inhibitors (TKIs) leads to a rapid tumor regression (10). However, systemic chemotherapy remains the backbone for these patients when they develop resistance to targeted therapy and when TKIs are exhausted. Interestingly, these oncogene-driven NSCLC tends to be mutually exclusive for a positive status of PD-1/PD-L1, and the application of PD-1/PD-L1 blockade immunotherapy in NSCLC patients harboring mutant driven genes remains controversial. For example, pre-clinical evidence from transgenetic engineered mouse model indicates a higher response rate of anti-PD-1 therapy in EGFR mutant NSCLC (11). However, in a phase 3 trial evaluation, EGFR mutant NSCLC patients did not benefit from consolidation Durvalumab therapy and experienced a high frequency of immune-related adverse events (irAEs) (12). As such, most immunotherapy trials exclude patients with druggable oncogenic mutations.

The response rate of anti-PD-1/PD-L1 monotherapy in oncogene-driven tumors is relatively low, and thus, combinational treatment that enhances anti-tumor immunity has been an area of great interest. In the subgroup analysis of IMPOWER150 trial, atezolizumab, an anti-PD-L1 inhibitor, in combination with vascular endothelial growth factor (VEGF) blockade therapy and chemotherapy yielded a median progression-free survival (mPFS) of 9.7 months among treatment-naïve metastatic nonsquamous NSCLC patients with EGFR mutations or ALK rearrangements (13). In consistent with these notions, the Orient-31 trail indicated that anti-PD-1 treatment in combination with anti-VEGF blockade therapy and chemotherapy yielded an ORR of 43.9% and a mPFS of 6.9 months in EGFR-mutated NSCLC patients after targeted therapy, which was significantly higher than that in the chemotherapy cohort (ORR: 25.2%, mPFS: 4.3 months) (14). These lines of evidence prompted potential application of PD-1/PD-L1 blockade therapy in NSCLC patients harboring mutant oncogenic genes, unfortunately, such data in real world is lacking and increasing cautions regarding the incidence of irAEs in these patients have been raised.

In this study, we evaluated therapeutic outcomes of anti-

Highlight box

Key findings

- Anti-programmed cell death 1 (PD-1)-based combinational therapy prolonged patient survival with manageable adverse effects in non-small cell lung cancer (NSCLC) harboring oncogenic alterations.

What is known and what is new?

- The application of PD-1/programmed cell death ligand 1 (PD-L1) blockade therapy in NSCLC patients with oncogenic mutations remains controversial.
- The PD-L1 status and lung immune prognostic index (LIPI) could be biomarkers dictating therapeutic outcomes of the combinational therapy.

What is the implication, and what should change now?

- We recommend anti-PD-1-based combination strategy rather than PD-1 inhibitors alone in NSCLC patients with oncogenic mutations. PD-L1 expression and LIPI score could be used to stratify patients in future prospective studies.

PD-1-based combinational therapy in NSCLC patients with oncogenic mutations. We enrolled a total number of 84 cases of patients and found that anti-PD-1-based combinational therapy elicited exciting anti-tumor efficacy and prolonged patient survival. This combinational strategy was well tolerated with manageable adverse effects. We also identified PD-L1 status and the lung immune prognostic index (LIPI) as biomarkers predicting therapeutic response to combinational immunotherapy in oncogene-driven NSCLC. We present this article in accordance with the STROBE reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1092/rc).

Methods

Study design

A total number of 500 cases of patients with advanced NSCLC who admitted to two independent institutions (Nanjing Jinling Hospital and Jiangsu Cancer Hospital) between January 2017 and June 2021 were analyzed. Patients should have measurable diseases and a positive result of NGS testing or targeted gene assays (EGFR, ALK, k-Ras, RET, HER 2, MET, BRAF, ROS1) performed in their standard-of-care evaluation at baseline. The key exclusion criteria included patients who had received drugs targeting other immune checkpoint pathways or patients with active autoimmune diseases. The protocol of this study was reviewed and approved by the Ethics Committees of Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University (Ethics number: NJJLH 202103265) and Jiangsu Cancer Hospital (Ethics number: JSCH 202205273). The study was conducted in accordance with the provisions of the Declaration of Helsinki (as revised in 2013). Informed consent from individuals was waived based on the retrospective nature of this study.

Patients' baseline demographic, clinical, and pathologic data were obtained from electronic health records. NSCLC histology was classified according to WHO criteria. Disease staging was based on the eighth edition of the American Joint Committee on Cancer and International Union Against Cancer TNM stage classification for NSCLC. The patients' Eastern Cooperative Oncology Group performance status (ECOG PS) at the initiation of immunotherapy was evaluated. The PD-L1 status was ascertained from pathological or molecular sequencing reports by PD-L1 IHC 22C3 pharmDx assay.

Study outcomes

Computer tomography (CT) scan of chest and abdomen, and magnetic resonance imaging (MRI) of the brain were performed every 6 weeks and evaluated by investigator-assessed modified Response Evaluation Criteria in Solid Tumors 1.1. Clinical outcomes were assessed by using ORR, PFS and overall survival (OS). ORR was defined as the percentage of patients who showed complete or partial remission. PFS was measured from the date of immunotherapy initiation to the date of disease recurrence, death from any cause, or last time of follow-up (October 20, 2022). OS was measured from the date of immunotherapy initiation to the date of death from any cause or last time of follow-up. irAEs owing to anti-PD-1 immunotherapy were classified according to the Common Terminology Criteria for Adverse Events version 5.0. The severe irAEs were identified as immunotherapy associated complications that required treatment discontinuation or immunosuppressive agents (such as corticosteroids), and were treated according to standard oncologic guidelines.

Statistical analysis

Baseline characteristics of different groups were compared using Chi-squared test or Fisher's exact test as appropriate. Kaplan-Meier survival curves were generated to evaluate PFS and OS, and were used to estimate the 95% confidence intervals (95% CIs). Log-rank tests were used for subgroup comparisons. The multivariate Cox's proportional hazard regression analysis was conducted by adjusting parameters with P values <0.3 from the univariate analysis. Statistical significance was defined at a two-sided P value <0.05. All statistical analyses were performed using SPSS version 20.0 (IBM Corporation, Armonk, USA) and GraphPad Prism 5.0 (GraphPad Software, San Diego, USA).

Results

Patient baseline characteristics

From January 2017 to June 2021, eighty-four patients who met all inclusion criteria were analyzed (*Figure 1*). Detailed demographic characteristics of the 84 identified patients are listed in *Table 1*. The most frequent genetic alteration was EGFR mutation (n=50, 59.5%), in which EGFR exon19 del/exon21 L858R accounted for nearly 80% of cases. At immunotherapy initiation, ECOG PS was less than two

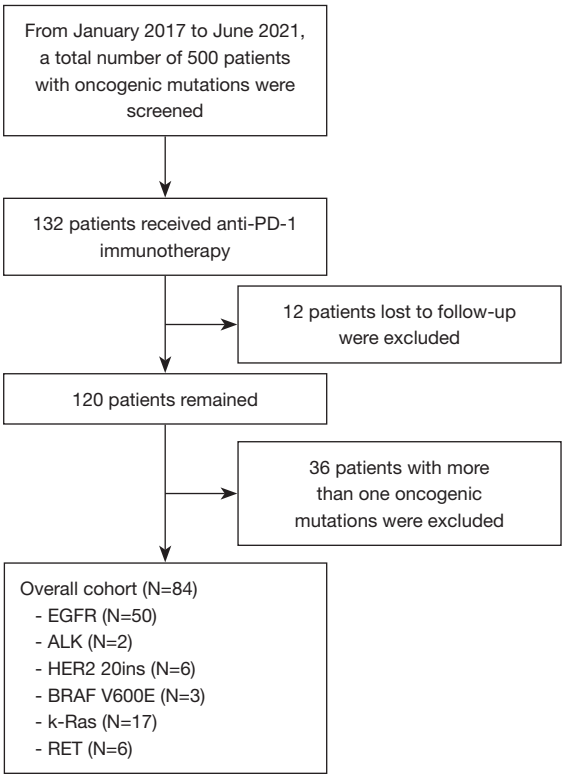

Figure 1 Flow diagram. PD-1, programmed cell death 1; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase.

for 77 patients (92%). PD-L1 status was known for 53 (63.1%) patients. 62.3% was negative (33/53) and 37.7% was positive (20/53), including 9.6% patients with PD-L1 expression over 50% of tumor cells.

Before the initiation of anti-PD-1 immunotherapy, most patients had received at least one line of systemic anti-tumor treatment. The most prevalent anti-PD-1 drug was Sintilimab, which was accounted for 32% (16/50) of the EGFR mutant cases. About 91% patients were treated with PD-1 blockade therapy in combination with chemotherapy or anti-angiogenesis therapy according to laboratory examinations and ECOG PS (Table S1). The main treatment was immunotherapy combined with chemotherapy, accounting for 49%.

Therapeutic outcomes

Survival outcomes of overall cohort

Among the 84 patients with evaluable disease, 33 patients (39.3%) responded to the treatment, with 1 case of complete response (CR), and 32 cases of partial response (PR). The mPFS of the entire cohort was 12 months (95% CI: 7.4–16.6 months), with the 6-month PFS ratio and the 12-month PFS ratio of 66.2% (95% CI: 55.8–76.6%) and 47.2% (95% CI: 35.6–58.8%), respectively. The median OS (mOS) was 32 months (95% CI: 16.4–47.6 months). Intriguingly, more than 70% of patients survived over 12 months, and half of them were still alive after 24 months (*Table 2*). PFS and OS were not statistically significant between chemo-immune-oncology (IO) and antiangiogenesis-IO without cytotoxic chemotherapy (Figure S1).

Survival outcomes of EGFR mutation subgroup

In the EGFR mutant group, 34% responded to PD-1 blockade-based combinational therapy. The EGFR exon21 L858R tumor (47.4%) seemed to preferentially benefit from PD-1 blockade-based combinational therapy, whereas the response rate of the EGFR exon19 del cohort (30%) and the exon20 insertion cohort (20%) tumors was relatively low (*Figure 2A*). The mPFS of EGFR exon19 del/exon21 L858R cohort was 7 months (95% CI: 4.2–9.8 months), while that was only 5 months (95% CI: 2.9–7.1 months) in the EGFR exon20 insertion cohort. In agreement with these findings, patients with the EGFR exon19 del/exon21 L858R mutation elicited a mOS of 20 months (95% CI: 12.2–25.8 months). However, the mOS of the EGFR exon20 insertion patients was 16 months (95% CI: 8.5–23.5 months) (*Table 2*).

Survival outcomes of k-Ras mutation subgroup

Pooled analysis of the k-Ras mutant cohort indicated an ORR of 58.8% (10/17, *Figure 2B*). Interestingly, one of the responders with stage IV disease was treated with first-line chemotherapy (pemetrexed + carboplatin) and immunotherapy (pembrolizumab), after 3 cycles of indicated treatment, MRI examination showed a complete regression of brain metastatic disease. Chest CT scan also revealed a significant resolution of the tumor mass (*Figure 2C*). Surgical resection was done following 21 cycles of treatment. The patient was still alive in our last time follow up without signs of relapse. Notably, four of the 7 nonresponders harbored the co-occurring LKB1 missense mutation, whereas the LKB1 mutation was not detected in the 10 responders (data not shown). The PD-1 blockade-based combinational therapy strategy provided robust clinical benefits to the k-Ras mutant patients and largely extended patient survival with a mPFS of 14 months (95% CI: 10.7–17.3 months). The mOS was not reached at the

Table 1 Clinicopathological characteristics of all participants

Characteristics	All	EGFR exon19 del exon21 L858R	EGFR exon20 insertion	k-Ras	ALK	RET	HER2 exon20 insertion	BRAF V600E
Gender								
Male	46 [55]	18 [45]	7 [70]	14 [82]	0 [0]	2 [33]	4 [67]	1 [33]
Female	38 [45]	22 [55]	3 [30]	3 [18]	2 [100]	4 [67]	2 [33]	2 [67]
Age (years)								
≥60	45 [54]	22 [55]	3 [30]	14 [82]	0 [0]	0 [0]	3 [50]	2 [67]
<60	39 [46]	18 [45]	7 [70]	3 [18]	2 [100]	6 [100]	3 [50]	1 [33]
Stage at diagnosis								
III	7 [8]	2 [5]	0 [0]	3 [18]	0 [0]	0 [0]	0 [0]	2 [67]
IV	77 [92]	38 [95]	10 [100]	14 [82]	2 [100]	6 [100]	6 [100]	1 [33]
Tobacco exposure								
Smoker	50 [60]	8 [20]	6 [60]	12 [71]	0 [0]	2 [33]	4 [67]	1 [33]
Non-smoker	34 [40]	32 [80]	4 [40]	5 [29]	2 [100]	4 [67]	2 [33]	2 [67]
Histology								
Squamous	1 [1]	1 [3]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]
Adenocarcinoma	81 [96]	37 [92]	10 [100]	17 [100]	2 [100]	6 [100]	6 [100]	3 [100]
Adenosquamous	2 [3]	2 [5]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]
ECOG score								
PS ≤2	77 [92]	40 [100]	9 [90]	15 [88]	2 [100]	3 [50]	5 [83]	3 [100]
PS >2	7 [8]	0 [0]	1 [10]	2 [12]	0 [0]	3 [50]	1 [17]	0 [0]
Metastasis								
Bone	37 [45]	19 [48]	6 [60]	5 [31]	2 [100]	2 [33]	2 [33]	1 [33]
Lung	18 [22]	10 [25]	1 [10]	2 [12]	0 [0]	2 [33]	3 [50]	0 [0]
Brain	51 [61]	25 [63]	8 [80]	9 [56]	2 [100]	4 [67]	3 [50]	0 [0]
Pleura	22 [27]	10 [25]	4 [40]	1 [6]	0 [0]	4 [67]	2 [33]	1 [33]
Adrenal glands	10 [12]	3 [8]	4 [40]	2 [12]	0 [0]	1 [17]	0 [0]	0 [0]
Liver	6 [7]	6 [15]	0 [0]	0 [0]	1 [50]	0 [0]	0 [0]	0 [0]
PD-L1 status								
<1%	33 [39]	16 [40]	4 [40]	5 [28]	1 [50]	5 [83]	2 [33]	0 [0]
1–50%	12 [14]	5 [13]	0 [0]	4 [24]	0 [0]	0 [0]	1 [17]	2 [67]
>50%	8 [10]	1 [2]	1 [10]	4 [24]	0 [0]	0 [0]	1 [17]	1 [33]
Unknown	31 [37]	18 [45]	5 [50]	4 [24]	1 [50]	1 [17]	2 [33]	0 [0]

Data are presented as n [%]. EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; HER2, human epidermal growth factor receptor 2; ECOG, Eastern Cooperative Oncology Group; PS, performance status; PD-1, programmed cell death 1.

Table 2 Therapeutic outcomes in the patient cohort according to molecular subgroup

Characteristics	All	EGFR exon19 del exon21 L858R	EGFR exon20 insertion	k-Ras	ALK	RET	HER2 exon20 insertion	BRAF V600E
Best response								
Complete response	1	0	0	0	0	1	0	0
Partial response	33	15	2	10	0	3	2	1
Stable disease	35	13	7	6	1	2	4	2
Progressive disease	15	12	1	1	1	0	0	0
ORR (%)	39.3	37.5	20	58.8	0	66.7	33.3	33.3
mPFS (months)	12	7	5	14			–	
(95% CI)	(7.4–16.6)	(4.2–9.8)	(2.9–7.1)	(10.7–17.3)			–	
6-month PFS (%)	66.2	53.4	40	81.6			–	
(95% CI)	(55.8–76.6)	(37.5–69.3)	(9.7–58.9)	(62.8–100)			–	
12-month PFS (%)	47.2	29.8	0	74.2			–	
(95% CI)	(35.6–58.8)	(14.7–44.9)	0	(52.1–96.4)			–	
mOS (months)	32	20	16	not reported			–	
(95% CI)	(16.4–47.6)	(12.2–25.8)	(8.5–23.5)	–			–	
12-month OS (%)	70.5	64.2	51.9	86.7			–	
(95% CI)	(60.3–80.7)	(49.1–79.3)	(21.1–82.7)	(69.5–100)			–	
24-month OS (%)	50.2	46.4	0	75.8			–	
(95% CI)	(36.3–64.1)	(28.8–64.0)	0	(51.0–100)			–	

EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; HER2, human epidermal growth factor receptor 2; ORR, objective response rate; mPFS, median progression-free survival; mOS, median overall survival.

time of our last follow-up. Intriguingly, 86.7% of k-Ras mutant patients survived over 12 months, and 75.8% of them were still alive after 24 months (*Table 2*).

Survival outcomes of rare mutation subgroup

Four out of 6 RET-rearranged patients (66.7%) responded to PD-1 blockade-based combinational therapy, with one case of CR and three cases of PR. The patient who experienced CR initially received standard chemotherapy (pemetrexed + carboplatin) while disease progressed after six cycles of indicated treatment. Second-line anti-PD-1-based combinational therapy (taxol + sintilimab) was considered, which yielded a rapid anti-tumor response. As shown in *Figure 3*, the tumor mass dramatically shrank following taxol and sintilimab treatment. Notably, this combinational anti-tumor strategy had durable and prolonged efficacy, since we did not observed signs of disease recurrence.

It seemed that there was a slight increase of ORR in patients with HER2 exon20 insertion in comparison with that in the EGFR exon20 insertion group (*Figure 2B*). There were three patients harboring the oncogenic BRAF V600E mutation. One patient received first-line PD-1 blockade combined with chemotherapy and elicited a PR, whereas the remaining two patients had stable disease after indicated treatment. Unfortunately, the two enrolled ALK-positive patients failed to respond to anti-PD-1-based combinational therapy.

The incidence of adverse effect

The overall frequency of treatment-associated adverse effect was 61.9% (52/84) and no patient died due to treatment-related toxicity. Fortunately, most adverse events were mild to moderate and could be well managed. The most common adverse events included bone marrow suppression, gastrointestinal discomfort and hepatic toxicity, which

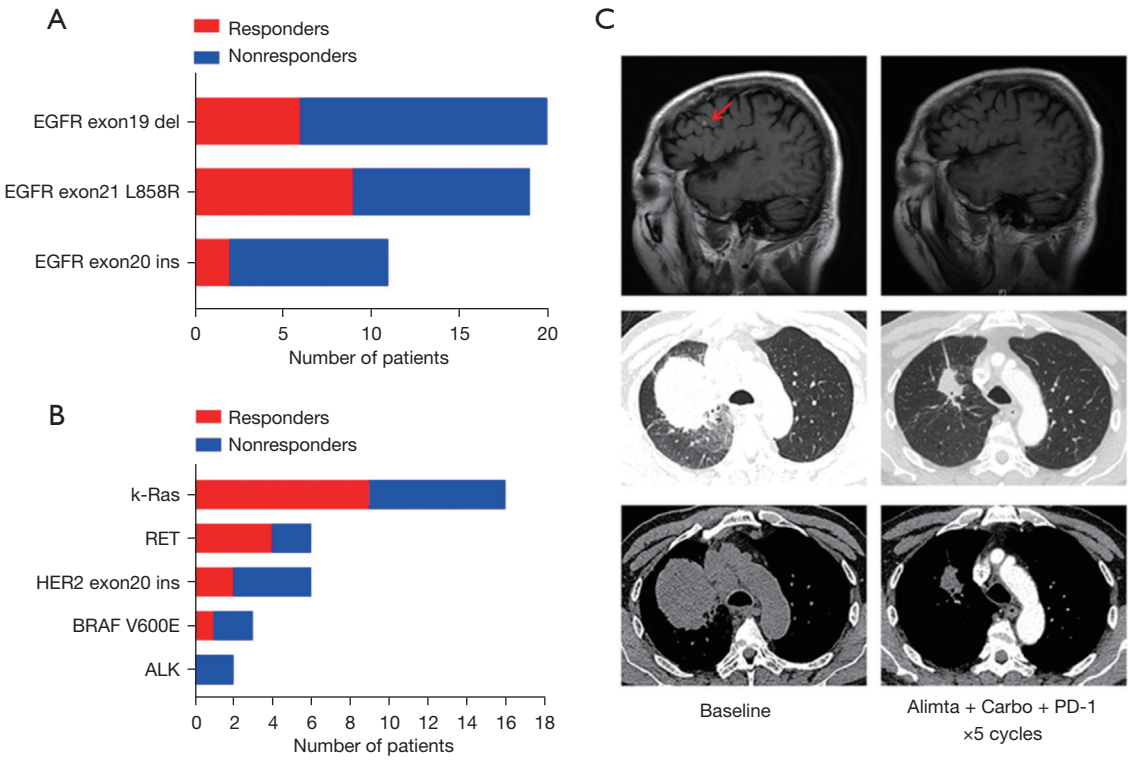

Figure 2 Therapeutic response to PD-1 inhibitors in NSCLC patients harboring oncogenic alterations. (A) Response to PD-1 inhibitors-based combinational therapy in NSCLC with EGFR mutations (n=50). The responders in each group were indicated in red and the nonresponders were in blue. (B) Response in tumors with mutations in k-Ras (n=17), ALK rearranged (n=2), RET rearrangement (n=6), HER2 exon20 insertion (n=6) and BRAF V600E (n=3). The responders in each group were indicated in red and the nonresponders were in blue. (C) Representative radiological images in a k-Ras mutant NSCLC patient receiving chemotherapy and anti-PD-1 immunotherapy. Initial MRI and chest CT examination prior to treatment indicated brain metastatic disease (red arrow) and a tumor mass in the upper lobe of right lung. After 5 cycles of chemotherapy and PD-1 blockade therapy, a repeated MRI examination showed a complete remission in brain metastatic disease. Chest CT examination also revealed a significant resolution of the tumor mass and metastatic lymph nodes. EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; HER2, human epidermal growth factor receptor 2; PD-1, programmed cell death 1; NSCLC, non-small cell lung cancer; MRI, magnetic resonance imaging; CT, computer tomography.

were very likely to be associated with chemotherapy. The irAEs occurred in 25 patients (29.8%), including 8 cases of hypothyroidism, 6 cases of PD-1 inhibitors-related pneumonitis (Table S2).

Two of the pneumonitis patients developed grade 3 disease that required high dose corticosteroid pulse therapy and mechanical ventilation. As such, permanent discontinuation of anti-PD-1 inhibitor was considered (*Figure 4A*). Unfortunately, one patient developed life-threatening PD-1 inhibitors-related myocarditis and admitted to the intensive care unit. The patient received intravenous pulse administration of methylprednisolone. After 4 weeks of indicated treatment, the patient's myocardial enzymes gradually reduced to normal range and he experienced a significant relief of pectoralgia (*Figure 4B*). This patient permanently ceased anti-PD-1 immunotherapy, whereas his disease was very stable and did not show signs of disease recurrence in our last time follow up.

PD-L1 status dictated therapeutic outcomes of anti-PD-1 inhibitor-based combinational therapy

We analyzed the ORR and patient survival in the NSCLC cohort with different level of PD-L1 expression (Table S3). The ORRs of the PD-L1 "unknown" and negative cohort were similar (32.3% *vs.* 39.4%, χ^2=0.354, P>0.05). In contrast, patients with positive status of PD-L1 tended to elicit a more favorable response to PD-1 blockade-based

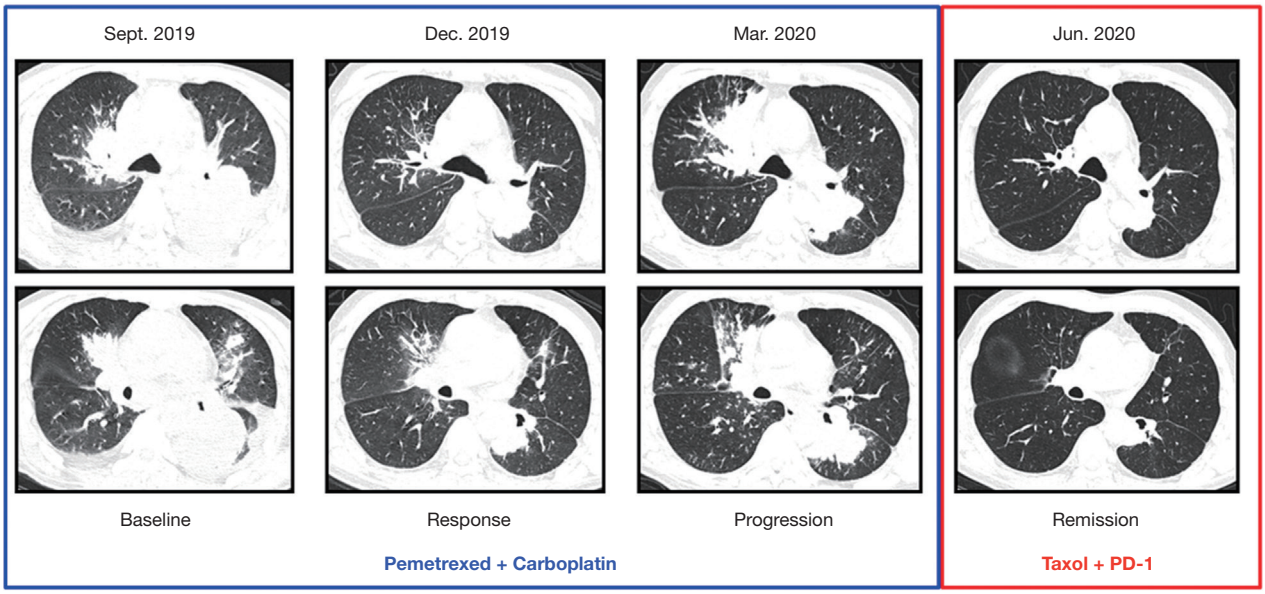

Figure 3 Representative response to PD-1 inhibitors in a patient with RET-rearranged NSCLC. Initial chest CT showed multiple tumor masses in the lower lobe of left lung and in right hilum. After platinum-based standard chemotherapy, a partial response was achieved. However, the patient progressed on chemotherapy in March 2020 and therefore received Taxol in combination with PD-1 inhibitors as a second line treatment. Repeated chest CT scanning in June 2020 showed a complete remission of the tumor mass after four cycles of indicated treatment. PD-1, programmed cell death 1; NSCLC, non-small cell lung cancer; CT, computer tomography.

combinational therapy with an ORR of 50%. The Chi-squared test also revealed a statistic significance over the PD-L1 "unknown" cohort (χ^2=3.912, P=0.048) and PD-L1 negative cohort (χ^2=4.523, P=0.033).

The increased benefit of ORR in the PD-L1 positive cohort could transfer into a survival advantage. As shown in *Figure 5A*, the mPFS of the PD-L1 positive cohort reached 21 months (95% CI: 12.1–29.9 months). The Chi-Squared test indicated markedly prolonged PFS over the PD-L1 "unknown" cohort (χ^2=4.879, P=0.0272) and PD-L1 negative cohort (χ^2=5.801, P=0.016). In agreement with these findings, the estimated mOS data of the PD-L1 positive cohort was significantly longer than that of the PD-L1 negative (χ^2=10.73, P=0.0011) and PD-L1 "unknown" (χ^2=6.271, P=0.0123) cohorts (*Figure 5B*).

LIPI as a novel predictive biomarker of PD-1 blockade-based combinational therapy response

The LIPI that integrates the pre-treatment derived neutrophil-to-lymphocyte ratio (dNLR) and lactate dehydrogenase (LDH) was proposed to be associated with therapeutic outcomes in oncogene-negative NSCLC patients receiving anti-PD-1 immunotherapy (15). Based on these two factors, patients can be characterized as having one of three possible prognosis groups (good LIPI: 0 factors; intermediate LIPI: 1 factor; or poor LIPI: 2 factors). However, whether LIPI could be used to predict the response to PD-1 blockade-based combinational therapy in NSCLC patients with oncogenic mutations remains elusive. In our study, 43 (51.2%) patients had a good LIPI, and the resting 41 (48.8%) patients had a LIPI >0. Interestingly, patients who responded to PD-1 blockade-based combinational therapy were prominently enriched in the LIPI =0 arm, whereas the nonresponders tended to have a higher LIPI (***P<0.001, *Figure 6A*). The association between LIPI and PFS was statistically significant and the mPFS ranged from 5 months for the LIPI >0 group to 21 months for the LIPI =0 group (χ^2=25.94, ***P<0.001, *Figure 6B*). In comparison with patients with a higher LIPI, lower LIPI score dictated more favorable mOS (not reached *vs.* 12 months, χ^2=24.18, ***P<0.0001, *Figure 6C*). These findings raised concerns that LIPI score may inversely confer therapeutic response of PD-1 blockade-based

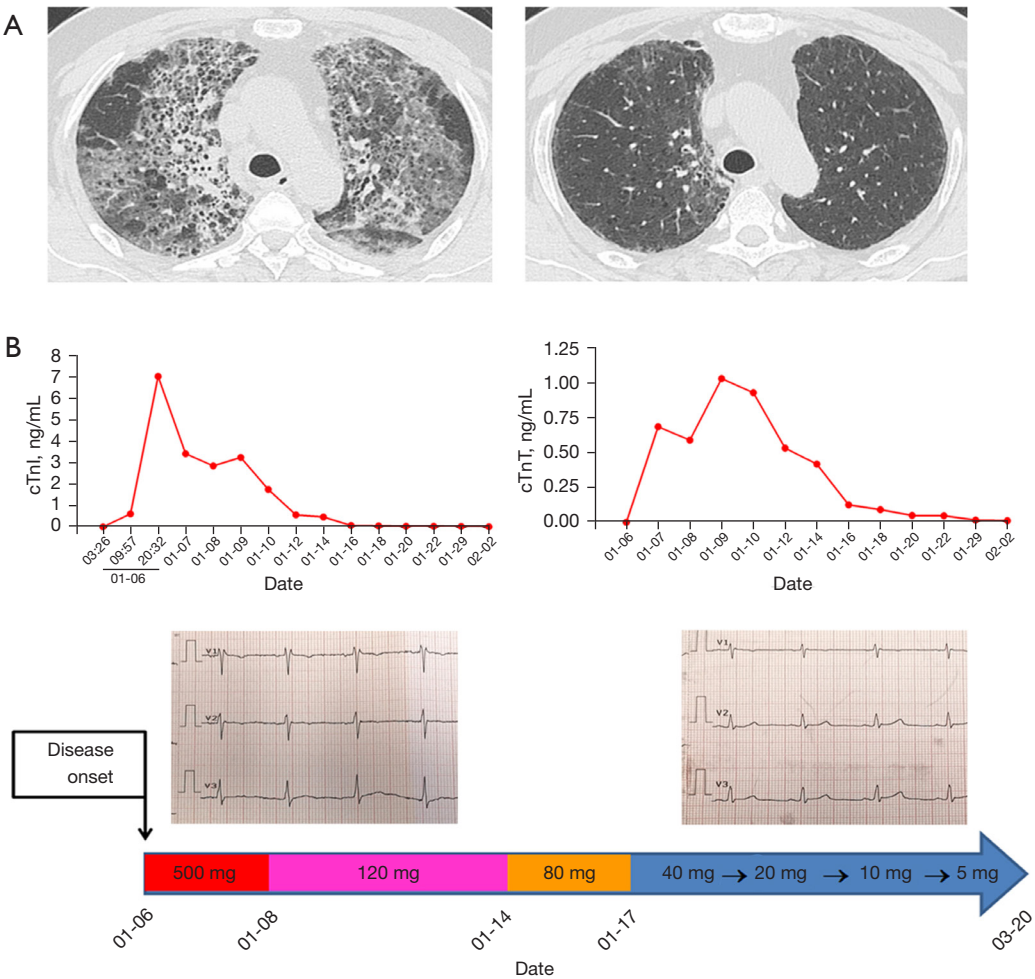

Figure 4 Management of irAEs. (A) Representative chest computer tomography images of PD-1 inhibitors-induced pneumonitis. The patient developed grade 3 pneumonitis and presented as extensive bilateral GGOs. High dose corticosteroid pulse therapy was used and yielded a significant resolution of GGOs. (B) One patient developed myocarditis after receiving anti-PD-1 immunotherapy. The serum myocardial enzymes, including cTnI and cTnT, were monitored during corticosteroid intervention. Representative electrocardiogram indicating an inverse T wave in the onset of disease was shown. Methylprednisolone was gradually tapered from 500 to 5 mg, and repeated ECG testing showed a remission of the inversed T wave. cTnI, cardiac troponin I; cTnT, cardiac troponin T; irAEs, immune-related adverse events; PD-1, programmed cell death 1; GGOs, ground glass opacities; ECG, electrocardiogram.

combinational therapy in NSCLC.

Discussion

In contrast to targeted therapy that is highly effective in NSCLC driven by mutant oncogenes, the efficacy of anti-PD-1 monotherapy in such population is very limited. The IMMUNOTARGET retrospective study evaluating the efficacy of anti-PD-1 monotherapy in NSCLC patients harboring oncogenic alterations showed ORRs ranging from 0% to 26%, with a median PFS of 2.8 months (16). Bodor and colleagues recently reported the real world PFS data of anti-PD-1/PD-L1 monotherapy from a NSCLC cohort of 1,746 patients with EGFR, ALK, BRAF, and k-Ras mutations. The authors found that most patients progressed on PD-1/PD-L1 blockade monotherapy within 4 months (17). Thus, single-agent immunotherapy is not recommended to treat patients whose tumors harbor oncogenic mutations. In this study, we evaluated the safety and efficacy of anti-PD-1-based combinational therapy

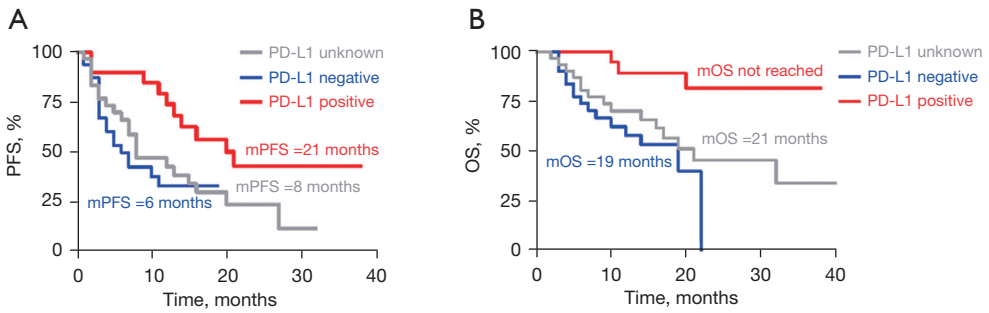

Figure 5 PD-L1 status associated with therapeutic outcomes of oncogenic mutant NSCLC patients. Patients were clarified into PD-L1 "Unknown", Negative and Positive cohorts and followed up until disease progression or death. Kaplan-Meier curves for PFS (A) and OS (B) of patients treated with PD-1 inhibitors-based combinational therapy were shown. PFS, progression-free survival; PD-1, programmed cell death 1; OS, overall survival; NSCLC, non-small cell lung cancer.

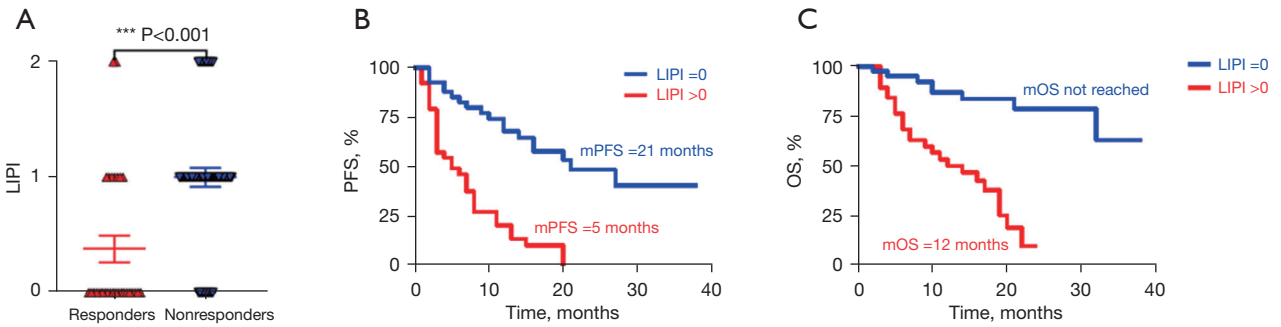

Figure 6 The LIPI as a biomarker predicting response to PD-1 inhibitors in NSCLC patients with oncogenic mutations. (A) The LIPI inversely correlated with response to PD-1 inhibitors-based combinational therapy. Kaplan-Meier curves for PFS (B) and OS (C) of patients treated with PD-1 inhibitors-based combinational therapy. LIPI, lung immune prognostic index; PFS, progression-free survival; OS, overall survival; PD-1, programmed cell death 1; NSCLC, non-small cell lung cancer.

in NSCLC patients with oncogenic alterations and got inspiriting results. We found that the combinational therapy was effective, with manageable adverse events, and largely extended patient survival. Both PD-L1 expression and LIPI could be used as biomarkers predicting therapeutic outcomes of the combinational therapy.

Although small molecular inhibitors targeting mutant k-Ras and EGFR/HER2 exon20 insertion gradually become commercially available, there is still a big gap between the urgent clinical needs and the access to targeted therapeutic agents. For example, NSCLC patients who developed resistance to EGFR TKIs without other reliable therapeutic targets should consider alterative sequential anti-tumor treatment. The combination of targeted therapy and immunotherapy is not feasible due to a lack of response. Specifically, in the CheckMate12 trial, which evaluated the combination of Nivolumab and Erlotinib in EGFR mutant patients, the ORR was only 15% (18). The combination also raises increasing clinical concerns related to treatment-associated toxicity. Based on two phase I studies, grade 3 or higher adverse events were observed in more than 50% of patients receiving the concurrent targeted therapy and immunotherapy, with interstitial lung disease occurring in 38% of patients (19,20). To this end, the combination of chemotherapy and immunotherapy is expected to improve prognosis and extend patient survival. During the preparation of this manuscript, a research team from Beijing evaluated potential benefits of PD-1 inhibitors plus chemotherapy in a total number of 19 patients who failed on targeted therapy against EGFR (n=16), ALK (n=2) and RET (n=1). They found the combinational treatment elicited an ORR of 15.8% in the entire cohort, with mPFS of 4.7 months and mOS of 19.2 months, respectively (21). Our study also provides inspiring data showing the safety

and efficacy of combining PD-1 blockade immunotherapy with chemotherapy in NSCLC patients with distinct genetic alterations. We found that this combinational strategy yielded an ORR of 39.3% in the entire cohort, with mPFS of 12 months and mOS of 32 months. The improved therapeutic outcomes in our study may implicate in the notion that we had enrolled more patients, especially patients with k-Ras mutation who were very likely to respond to the combinational treatment (22). Moreover, the heterogeneity of EGFR mutation subtypes also results in variations in therapeutic efficacy. The patients with EGFR exon19 deletion showed inferior responses compared to those with exon21 L858R mutation, which is in consistent with previous studies showing an ORR of 7% in EGFR exon19 deletion subgroup versus 16% in exon21 L858R subgroup (16,23). Given that randomized controlled trials of chemotherapy combined with PD-1 blockade therapy for EGFR mutant NSCLC are still ongoing (NCT02864251 and NCT03515837), the screening of mutant subtypes of EGFR alterations before the initiation of immunotherapy would be essential.

The efficacy of immunotherapy in patients with HER2 mutation is largely unknown. The ORR of anti-PD-1/PD-L1 monotherapy among the 29 patients with HER2 exon20 mutations in the IMMUNOTARGET study was only 7% (16). The combinational strategy seems to provide more clinical benefits, since we noticed two out of 6 HER2 mutant patients responded to the treatment. The IMMUNOTARGET study also elicited a minimal clinical benefit of PD-1 inhibitors monotherapy in NSCLC patients with RET rearrangement, with an ORR of 6% (16). However, the IMAD2 retrospective multicenter study involving nine cases of RET-rearranged NSCLC showed an ORR of 38% to PD-1 inhibitors monotherapy (24). It is important to note that the higher response rate in the IMAD2 study may be related to the fact that this patient population received immunotherapy as an earlier line of treatment than the IMMUNOTARGET study. As such, it would be reasonable to believe the front-line combinational treatment might be more suitable than posterior-line PD-1/PD-L1 blockade monotherapy for NSCLC patients with RET alterations, or with other oncogenic mutations.

Our study also highlighted the predictive value of PD-L1 expression for anti-PD-1-based combinational therapy in NSCLC harboring distinct genetic alterations, as a high value of this parameter is correlated with more beneficial outcomes. Interestingly, among the 53 patients with reliable PD-L1 data, more than 50% of the patients were negative for PD-L1, which might be a rational explanation for the low response rate to anti-PD-1/PD-L1 monotherapy in previous studies (16-18). In our study, patients with a positive expression of PD-L1 tended to have a higher ORR and increased survival benefits than those without PD-L1 expression. Of noted, NSCLC patients with an "unknown" PD-L1 status elicited a more favorable response to the combinational treatment in comparison to the PD-L1 negative patients, probably because a proportion of the PD-L1 "unknown" patients were actually positive for PD-L1. As such, it is of special importance to explore other biomarkers that are capable of predicting anti-tumor response and patient survival, in particularly for patients without reliable PD-L1 data.

Numerous blood parameters have been investigated as potential inflammatory biomarkers, including elevated neutrophils, LDH, and hypoalbuminemia, all of which are associated with poor outcomes in cancer (25). The LIPI that integrates dNLR and LDH is a specific predictor of therapeutic benefits for immunotherapy, but not chemotherapy (15). Indeed, the neutrophils and lymphocytes shape the tumor microenvironment and their phenotypic and functional polarization elicited either tumor suppressive or tumor promoting effects. Elevated LDH level reflects a pro-inflammatory status, which is also associated with tumor progression. In our study, we found the LIPI score inversely related to anti-tumor response and patient survival. Interestingly, the LIPI reversely correlates with PD-L1 expression (unpublished data), which might mirror the expression of PD-L1 in patients with an "unknown" PD-L1 status. To our knowledge, our study is the first to explore the significance of LIPI in NSCLC patients harboring a panel of oncogenic alterations and receiving PD-1 blockade-based combinational therapy. In comparison to the pooled dataset of 431 NSCLC patients treated with PD-1 /PD-L1 inhibitor alone, which revealed mOS data for the good (18.4 months), intermediate (11.3 months), and poor LIPI (4.5 months) (15), we reported here the mOS for LIPI =0 and LIPI >0 groups extended to not reached and 12 months, respectively.

Given the impressive therapeutic response to combinational treatment in our oncogene mutant patient cohort, we would recommend an anti-PD-1-based combination strategy rather than PD-1/PD-L1 inhibitors alone. Although the incidence of adverse events of the combinational strategy is not high, and in most cases manageable, the occurrence of life-threatening myocarditis certainly raises significant clinical concerns. Thus, the

dynamic monitoring and timely interpretation during the treatment is extremely important. Our study has several limitations that should be acknowledged. First, the sample size included was relatively small, which made it difficult to carry out survival analysis in subgroups with uncommon mutations. Furthermore, in this retrospective study, the baseline treatment plan of the patients was not completely uniform, which would inevitably cause selection bias and confounding factors. Thus, it is critical to conduct validation cohorts in the future.

Conclusions

Despite the limitations above, our study highlights the clinical benefits of anti-PD-1 inhibitor-based combinational therapy in NSCLC patients with oncogenic mutations, in particular for those who failed on standard or salvage therapy. PD-L1 status and LIPI are associated with therapeutic outcomes in oncogene mutant NSCLC patients treated with combinational immunotherapy. We recommend PD-L1 expression and LIPI score for stratification of patients in future prospective randomized studies. Besides, this combinational therapy will probably require more prospective studies that link different multiple genomic features and immunophenotypes with efficacy data to explore effective predictive markers.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was reviewed and approved by the Ethics Committees of Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University (Ethics number: NJJLH 202103265) and Jiangsu Cancer Hospital (Ethics number: JSCH 202205273). Informed consent from individuals was waived based on the retrospective nature of this study.

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