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Optimizing outcomes and addressing adversities of immunotherapy in lung cancer

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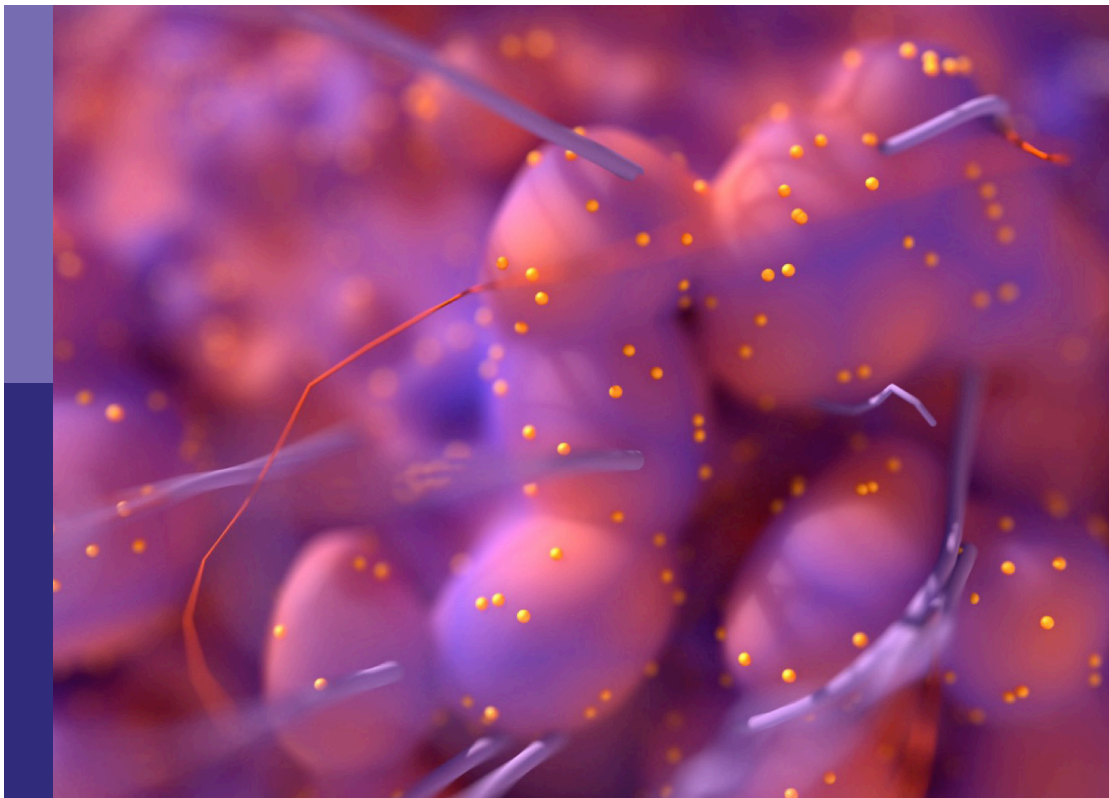

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Optimizing outcomes and addressing adversities of immunotherapy in lung cancer

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Editorial: Optimizing outcomes and addressing adversities of immunotherapy in lung cancer

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Editorial on the Research Topic
Optimizing outcomes and addressing adversities of immunotherapy in lung cancer

Lung cancer is the leading cause of cancer-related death worldwide. Lung cancer is categorized into several histologic subtypes, principally small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), of which NSCLC accounts for 85% of cases. NSCLC is mainly comprised of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (Yang et al.) Because of the poor survival associated with NSCLC, it is imperative to identify efficacious new treatments with the goal of improving outcomes as well as minimizing side effects for all affected patients. Among recent treatments, immune checkpoint inhibitors have been a major class of therapy that has changed how lung cancer is treated- by bolstering the immune response.

This Research Topic in Frontiers in Oncology, "*Optimizing Outcomes and Addressing Adversities of Immunotherapy in Lung Cancer*," is aimed at providing insight into clinical decision making as it applies to the use of immunotherapy for lung cancer. A total of 16 publications are included in this Research Topic. Herein, we aim to summarize these studies and discuss how variables in biology, tumor response, progression, and side effects can potentially influence treatment decisions.

Immune checkpoint inhibitors (ICIs) in the treatment of NSCLC are used to enhance T cell response against cancer cells in the immune system. Programmed cell death protein 1 (PD-1) is a receptor, which is expressed on the surface of activated T cells. If PD-1 binds to its ligand (PD-L1), the cell possessing the ligand may escape its destruction, even if it is cancerous. There are multiple ways to utilize ICIs in the treatment of NSCLC; they can be used as a monotherapy or in combination with another therapy. Each treatment discussed will highlight the benefits of ICIs in patients of various medical conditions and lifestyles. Factors such as age, ethnicity, tumor mutational burden, and comorbidities are possible examples of what can affect the prognosis. Two studies (Huang et al.; Shiotsu et al.) explored the effect of pembrolizumab on NSCLC. Pembrolizumab is an Immune checkpoint inhibitor drug that serves as a humanized IgG4 monoclonal antibody for the PD-1 protein. When evaluated on a patient population who had poor performance status

(PS) or were elderly, pembrolizumab monotherapy was found to be an effective 1st line treatment for those with PD-L1-positive advanced NSCLC (Shiotsu et al.). Huang et al compared pembrolizumab to the angiogenesis inhibitor bevacizumab. Bevacizumab weakens angiogenic behaviors of cancer by promoting the normalization of tumor vessels and reducing the formation of new blood vessels. The results showed that both pembrolizumab and bevacizumab are effective treatment options, especially when combined with another systemic therapy such as chemotherapy. However, in PD-1-positive patients, the results showed that immunotherapy was clearly superior.

ICIs are appealing in that the effect comes with less toxicity when compared to conventional systemic treatments such as chemotherapy. Using meta-analysis, Yang et al's comparison study showed that in the second line setting for advanced/metastatic NSCLC, ICIs were superior to the chemotherapy drug, docetaxel. Docetaxel has less efficacy and more toxicities. ICIs were found to have a better OS and PFS of NSCLC patients when compared to docetaxel (Yang et al.).

Though effective as a monotherapy, ICIs can be more beneficial when used in conjunction with other treatments such as chemotherapy. Two studies investigated the potential of ICIs as a neoadjuvant treatment. Shi et al. confirmed the usefulness of PD-1 inhibitors in the treatment of resectable squamous NSCLC with chemotherapy. Although exploring a relatively small population size (n=63), the majority of the patients in this study (66.7%) demonstrated a major pathologic response (MPR), including 39.7% resulted in pathologic complete response (pCR), with low risk of toxicity when treated with PD-1 inhibitors and chemotherapy. Using another humanized monoclonal PD-1 antibody, camrelizumab, Li et al showcased the potential of camrelizumab in the neoadjuvant setting for resectable IIIA squamous NSCLC, especially in combination with chemotherapy. These studies confirmed the value of using ICIs in the neoadjuvant setting for resectable NSCLC (1).

Though using ICI drugs over other treatments presents the benefit of low toxicity, the emergence of immune-related adverse events (irAEs) can occasionally become life threatening to patients. Because of this, predictive markers for irAEs are greatly needed when ICIs are used. For example, a study by Koh et al. was conducted to evaluate the relation between proteins YTHDF1 and YTHDF2, and ICIs. YTHDF1 and YTHDF2 were found to negatively affect the expression of CD8 and CD4 in T cells, and that groups with low expression of both proteins responded better to PD-1/L1 inhibition. Another study by Lan et al discovered the use of CURB65 scores to predict the incidence of irAEs, primarily the checkpoint inhibitor-associated pneumonitis (CIP) in patients receiving immunotherapy. Among 28 enrolled patients with CIP, they found mortality after onset of CIP was consistently higher in the high-CURB65 group than in the low-CURB65 group, and higher CURB65 score positively correlated with higher grade of CIP. CURB65 therefore could be further evaluated as a potential predictive biomarker for CIP. Another relevant signal for irAEs has been found in cytokines, which are molecules that interact with the immune system. Cytokines' presence in the bloodstream and tendency to appear during response makes them a candidate for potential biomarkers of irAEs or treatment response. A study by Zhao et al searched for positive correlations between a defined cytokine panel (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IFN-α, IFN-γ, TNF-α) and irAEs. A positive association with occurring irAEs was found with cytokines IL-1β and IL-2 levels in peripheral blood. The levels of IL-5, IFN-α and IFN-γ during ICI treatment were also correlated with irAEs. In analyzing clinical response, only levels of IL-6, IL-8, IL-10, and IL-17 levels during treatment were positively associated. A separate case study by Yin et al. extended on the investigation of the role of IL-6 during the incidence of myocarditis. During the patient's treatment, IL-6 rose to thousands of times its normal level while multiple irAEs were present. The level of this cytokine only decreased when steroids were administered to counter the irAEs. These results show that cytokine molecules are immune-related, and a precise understanding of their dynamic composition might be used in predicting treatment response and/or irAEs.

To further characterize biological factors that could impact immunotherapy response, a study by Nakagawa and Kawakami was developed to analyze previous reports on ICI treatment in varying patient populations. They concluded that patients with driver mutations on the EFGR or ALK genes have poorer reactions to ICI therapy, thought to be caused by a lowered tumor mutational burden. Conversely, patients with mutations on the KRAS or BRAF gene received greater benefit from ICI therapy. Finally, co-mutation SKT11/LKB1 with the KRAS mutation has been shown to correlate with lower PD-L1 expression. All in all, driver mutations may have varying effects on treatment depending on the affected gene(s). There are also situations that emerge to affect the treatment of NSCLC, such as metastases in advanced cases. Liver metastases are generally associated with poorer outcomes and have no established optimal treatment. Conversely, brain metastases have a clear treatment decision, and should be treated as soon as possible with radiation. Another emergent effect is pleural effusion, which is associated with worsened outcomes. An article published by Chen et al collected data to correlate the time between neoadjuvant immunotherapy and surgery, known as time-to-surgery (TTS) with treatment outcomes in the early-surgery group, the standard-surgery group, and the delayed-surgery group. They concluded that TTS has no relevant influence on the feasibility and safety of surgery in neoadjuvant immunochemotherapy. It is recommended to combine bevacizumab and ICI therapy to treat pleural effusion, but there is scarce literature published on this topic. A patient's elderly status does not have much correlation with treatment outcomes, but a poorer prognosis often comes with poor PS and comorbidities (Nakagawa & Kawakami). Another study confirmed this, as patients with comorbid burden likely have a weakened physical status from hospitalization. This correlates comorbidities with poorer clinical outcomes (Young et al.). Though often excluded from most studies involving ICIs, patients with interstitial lung disease have worse survival (Nakagawa & Kawakami).

To account for the many variables that may help or hinder the patient's prognosis, optimization of treatments is necessary to discover safer and less strenuous solutions. Combining ICIs with chemotherapy has shown prolonged survival, but other

combination therapies may provide an equally effective result with less toxicity. To extend on this, a study by Martin and Enrico was initiated to investigate other combinations using immunotherapy and discuss the results of multiple therapies. When ICIs were combined with chemotherapy, this combination significantly prolonged the median progression-free survival (PFS) compared with chemotherapy alone. Immunotherapy can also be a main treatment, and in first-line immunotherapy, nivolumab plus ipilimumab significantly improved OS relative to chemotherapy alone. Antiangiogenic agents such as bevacizumab have also been reported to be efficacious when used alongside ICIs. Antiangiogenic agents also synergized with multi-kinase inhibitors such as lenvatinib, cabozantinib, and axitinib. PD-1/L1 blocking agents have been reported to work well with drugs that target LAG3, which is another immune checkpoint expressed with unfavorable clinical outcomes. Martin and Enrico, in their review pointed out that utilizing relatlimab and nivolumab has proven effective in treating metastatic or unresectable melanoma. Other immune checkpoints of T cells exist, such as VISTA and TIM-3, but each have an accompanying drug to be used alongside ICIs for similar results to PD1/L1 blocking. Finally, Oncolytic virus therapy may serve as a novel strategy that uses immunogenic cell death to spur the immune system into a desired response.

A novel area in the field of immunotherapy in which there is no current consensus, is regarding hyperprogressive disease (HPD). Although lacking a precise definition, it was originally described as disease progression at the first evaluation and at least two-fold tumor growth rate increase between pre-immunotherapy and immunotherapy period (2). One study by Britt et al sought to analyze HYD to compile the many speculations on its details. Britt et al described HYD as a rapid acceleration of tumor growth following ICI therapy, where cancer lesions would show an increase of two-fold or higher per RECIST 1.1 criteria, or, 50% or higher increase in tumor burden compared to pretreatment imaging, despite having been treated. The mechanism of such clinical presentation is largely unknown with conflicting accounts (3). To identify a proper biomarker for predicting HYD, the authors concluded that more studies should be devoted to the relation of HYD in T cell regulation, changes in the tumor microenvironment, and genomic changes (Britt et al.).

Finally, two studies in this series explore the pitfalls of immunotherapy across different ethnicities. For example, in comparison to the European and American populations, the Asian population exhibits a unique disease prognosis due to having a differing tumor mutation burden (TMB). There is also a clear difference in the survival between hispanic populations and non-hispanic white populations. Sun et al. defined TMB as a biomarker that can predict the response to ICI therapy, but compared to western populations, it was concluded that the TMB values of Asian populations seem decreased in comparison. Somatic-germline-zygosity is an algorithm to calculate TMB, and by calibrating it to Asian populations, TMB cut-off was found to be seven mut/Mb instead of ten mut/Mb in European and American populations. Having unreliable biomarkers causes a disparity between the two populations. Raez et al reported this disparity of treatment with a different cause. Of the patients with locally advanced stage III NSCLC, non-Hispanic white (NHW) patients had better survival outcomes when compared to Hispanics. As a retrospective study, the explanation could be from multiple differences between Hispanics vs. NHW, including access to optimal second-line therapy or follow-up, which is a crucial part contributing to overall survival.

The combined efforts of these studies map out the ever-expanding effects of immunotherapy on innovating treatment of NSCLC. As new techniques are developed, more information must be gained to each minute detail, or the influence of said treatments cannot be gauged accurately. Novel studies will continue to come out in hopes of discovering combinations with less risk, as well as reasonable counters to the side effects. In the background, algorithms for quantifying biomarkers will also be worked on so members of different populations will have the same access to suitable treatments.

Author contributions

JZ: Supervision, Writing – review & editing. TA-R: Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. JN: Writing – review & editing.

Conflict of interest

JN has had research funded by Merck, AstraZeneca, and Bristol Myers Squibb. JZ has served as a scientific advisor/consultant for: AstraZeneca, Bayer, Biodesix, BMS, Cardinal Health, Daiichi Sankyo, Hengrui, Lilly, Mirati, Nexus Health, Novartis, Novocure, Regeneron, Sanofi, and Takeda. He is on the speakers' bureau for AstraZeneca, Regeneron, Sanofi and MJH Life Sciences. JZ has also received research funding/support from Abbvie, AstraZeneca, BeiGene, Biodesix, Champions Oncology, Genentech, Hengrui, InnoCare Pharma, Janssen, Kahr Medical, Merck, Mirati, Nilogen and Novartis.

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ORIGINAL RESEARCH
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Durvalumab After Chemoradiation for Unresectable Stage III Non-Small Cell Lung Cancer: Inferior Outcomes and Lack of Health Equity in Hispanic Patients Treated With PACIFIC Protocol (LA1-CLICaP)

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Objectives: To compare the rate disparity between outcomes (overall survival (OS), progression-free survival (PFS), and safety) of concurrent chemoradiation (cCRT) followed by durvalumab in two patient cohorts with locally advanced (LA) stage III non-small cell lung cancer (NSCLC), one non-Hispanic White (NHW), and the other Latin-American.

Methods: A multicenter retrospective study was performed, including 80 Hispanic and 45 NHW LA stage III NSCLC patients treated with cCRT followed by durvalumab. Both cohorts were analyzed in terms of main outcomes (OS, PFS, and safety) and compared between them and with the PACIFIC trial population outcomes. The efficacy-effectiveness gap was assessed using an efficacy-effectiveness (EE) factor that was calculated by dividing each cohort median overall survival by the corresponding reference OS from the PACIFIC trial. In both cohorts, results of PD-L1 testing were recorded, and the main outcomes were compared according to PD-1 expression levels (≥50%, 1–49%, and <1%).

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Results: For the entire population (N=125), the overall response rate (ORR) was 57.6% (N=72), and 18.4% (N=25) achieved stable disease. OS was 26.3 months (95%CI 23.9-28.6), and PFS was 20.5 months (95%CI 18.0-23.0). PFS assessed by ethnicity showed a median for the Hispanic population of 19.4 months (95%CI 16.4-22.5) and 21.2 months (95%CI 17.2-23.3; p=0.76) for the NHW group. OS by race showed a significant difference in favor of the NHW group, with a median OS of 27.7 months (95%CI 24.6-30.9) vs. 20.0 months (95%CI 16.4-23.5) for Hispanics. (P=0.032). Unadjusted 12-month and 24-month OS was 86.6% (95%CI 79.9–88.0) and 46.6% (95%CI 40.2–48.3) for NHW compared to 82.5% (95%CI 77.1–84.2) and 17.5% (95%CI 15.6-24.5) in Hispanics. NHW had an EE factor of 0.78 and Hispanics had 0.58, showing a reduction in survival versus NHW and PACIFIC of 20% and 42%, respectively. HR for the OS among NHWs and Hispanics was 1.53 (95%CI 1.12-1.71; P=0.052) and 2.31 (95%CI 1.76-2.49; P=0.004). Fifty-six patients (44.8%) had some degree of pneumonitis due to cCRT plus durvalumab. There was no difference in the proportion of pneumonitis according to race (P=0.95), and the severity of pneumonitis was not significantly different between Hispanics and NHWs (P=0.41).

Conclusions: Among patients with LA stage III NSCLC, NHW had better survival outcomes when compared to Hispanics, with an OS that seems to favor the NHW population and with an EE factor that shows a shorter survival in Hispanics compared with NHW and with the PACIFIC trial group.

Keywords: durvalumab, non-small cell lung cancer, hispanics, survival, health equity, immunotherapy

HIGHLIGHTS

- Chemoradiation followed by durvalumab is the standard of care in locally advanced stage III NSCLC.
- Outcomes of this treatment are not evaluated in Hispanic patients and could be inferior compared with non-Hispanic whites and even more with the results shown in the registry trial (PACIFIC).
- Reasons for inferior results in Hispanic patients must be evaluated and analyzed in prospective trials and could be related to delays in starting durvalumab after chemoradiation treatment.

INTRODUCTION

Lung cancer (LC) has been one of the leading causes of cancer-related deaths during the last years in the United States, and it continues to be one of the leading causes of cancer-related deaths in men and women worldwide (1). Among LC, non-small cell lung cancer (NSCLC) accounts for 85% of all cases, with 1.28 million diagnoses made between 2007 and 2017 (2, 3). In the US, nearly 30% of patients with NSCLC are diagnosed with locally advanced disease (Stage III). This stage represents a complex group of patients with diverse characteristics regarding the extension of the disease, prognosis, and possible management that goes from resectable to unresectable lesions (4, 5). In patients with unresectable disease, platinum-based chemotherapy with concurrent chemotherapy has been the standard of care (6).

However, in 2017, the PACIFIC trial changed the treatment paradigm for locally advanced NSCLC. This study demonstrated a significant improvement in progression-free survival (PFS) and overall survival (OS) among patients who received durvalumab (anti-PD-L1) in addition to concurrent chemoradiotherapy (cCRT) (7). The updated 5-year analysis of the PACIFIC trial remained consistent with the current outcomes and showed a PFS of 33.1% and an OS of 42.9% in the durvalumab arm (8). In addition, durvalumab has been demonstrated to be safe, with pneumonitis as the main adverse effect (4.4%) (9).

Despite the clear evidence of benefits with immunotherapy in locally advanced NSCLC, most clinical trials have been done in Non-Hispanic Whites (NHW), leaving aside other population groups such as Hispanics. There are many disparities in the outcomes of Hispanic patients compared with NHW when they are treated with immunotherapy. These disparities begin with differential access to optimal cancer care and treatment, molecular profiling, or follow-up (10, 11).

To provide some insights into the disparities between Hispanics and NHW in the outcomes of NSCLC treatment, we designed a multicenter retrospective study that included both populations and compared the outcomes after treatment with durvalumab in addition to cCRT.

METHODS

Study Design and Patients

This multicenter retrospective study included 80 Hispanic patients with histologically and/or cytologically confirmed unresectable stage III NSCLC who received at least one cycle of consolidative durvalumab post-cCRT, after reaching stable disease. All were treated in fourth-level centers in Florida (United States), Mexico, Central America, and Colombia between February 2018 and December 2021. To compare the rate of disparity in outcomes, the results of Hispanic patients were compared to a cohort of non-Hispanic white (NHW) patients (N=45) treated in the United States (at Memorial Cancer Institute, part of Memorial Healthcare System, Miami, FL), assuming that their results were homogeneous with those presented in the PACIFIC study (12) (**Supplementary Figure S1**). An independent review board approved the study in Bogotá Colombia (Kayre/FICMAC IRB 2018-14-021), and institutional approval of each linked site was subsequently obtained. In addition, the study was conducted in accordance with the Declaration of Helsinki. In each case, cCRT was administered with curative intent (54-66 Gy) concurrently with platinum-based chemotherapy for at least two cycles, followed by immunotherapy with durvalumab for one year (10 mg/kg intravenously every 2 weeks). Radiotherapy administered to patients was homogeneous, and all patients were treated with radiation in reference centers of main cities in Latin America using intensity-modulated radiation therapy (IMRT) (13). Furthermore, all participating radiotherapy centers have radiation protocols under ASTRO/ESTRO recommendations (14). Treatment patients with EGFR mutations (N=6) or ALK translocations (N=1) were allowed to be included, and each treating physician chose the concurrent chemotherapeutic regimen. The simulation procedure for RT planning and the definitions of target volumes followed previous recommendations and descriptions (15, 16). Follow-up chest CT was performed 1 month after cCRT, positron emission tomography (PET)-CT was done at diagnosis (92% of cases) and 3-4 months after the completion of cCRT (when available), and chest CT was repeated every 3 months after completion of CCRT as follow-up.

Globally, information was collected on tumor status, age, sex, Eastern Cooperative Oncology Group (ECOG) performance status, smoking history, number of pack-year, baseline lung comorbidities (asthma, chronic obstructive pulmonary disease, interstitial lung disease), histology subtype, cancer stage, PD-L1 expression status, platinum type, and time from cCRT completion to ICI start date. Information regarding the main adverse effects, with particular emphasis on post-cCRT pneumonitis, was also collected. The study estimated progression-free survival (PFS), overall response rate (ORR), overall survival (OS), and the primary outcomes obtained with the second line. The analysis of the results was stratified according to the expression of PD-L1 and according to RECIST-1.1 (17).

The PFS was defined as the last date of cCRT until radiographically confirmed progression or death. OS was defined as the time from treatment to death or loss to follow-up. Radiation pneumonitis was diagnosed clinically based on the presence of classic symptoms, timing, history of radiation therapy, imaging findings, and exclusion of alternative causes, such as infection, cardiogenic edema, pulmonary embolism, drug-induced pneumonitis, and other causes. Radiation pneumonitis may be graded using the Common Toxicity Criteria for Adverse Events (Version 5.0) (Common Terminology Criteria for Adverse Events (CTCAE) |, Protocol Development |, CTEP (2000). Retrieved from, https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.) (18).

PD-L1 Testing

PD-L1 expression was determined by immunohistochemistry using the Dako 22C3 pharmDx kit, with more than 100 tumor cells present in the slide section for accurate PD-L1 readings. PD-L1 testing was completed on biopsies taken at diagnoses. Patients were grouped according to PD-L1 status (i.e., ≥50%, 1–49%, and <1% subgroups) for survival analyses. Patients with unknown PD-L1 expression status were also included in this study to reflect real-world durvalumab use.

Statistical Analysis

All analyses were conducted on IBM SPSS Statistics software version 25.0 (SPSS Inc. Chicago, IL, USA). Descriptive analyses were utilized to provide an overview of the characteristics of the study population. Categorical variables were assessed *via* the Chi-Square test or, whenever appropriate, Fisher's Exact test. OS and PFS were reported as Kaplan Meier survival curves. Multivariable Cox regression models were generated to assess potential confounders. Two-sided P-Value was set to determine statistically significant outcomes. There were no adjustments made for multiple comparisons, and in all cases, the significance level was P=0.05. The efficacy-effectiveness gap was assessed using an efficacy-effectiveness (EE) factor that was calculated by dividing each cohort's median overall survival by the corresponding reference OS from the most recent report from PACIFIC (12). This factor was used to estimate the presence of an EE gap and compare the real-world population's survival relative to the clinical trial population. An EE factor of 0.60 indicates that median survival is 40% shorter in clinical practice than in the reference clinical trial (19).

RESULTS

Patients, Tumors, and Treatment Characteristics

Eighty Hispanic patients and 45 NHW were included. Baseline patient and treatment characteristics are summarized in **Table 1**. To establish the comparability of clinical variables between Hispanic patients treated in the US and Latin American countries, a stratified analysis was performed for age (P=0.53), gender (P=0.71), baseline performance status (P=0.22), and place of origin (P=0.57) without finding statistically significant

TABLE 1 | Baseline patient and treatment characteristics.

Variable	All N = 125 (%)	Hispanic N = 80 (%)	Non-Hispanic whites N = 45 (%)	P-value
Age				
Median	66 (41-90)	64 (41-90)	66 (46-90)	0.35
≥65 years	65 (52.0)	39 (48.8)	26 (57.8)	
<65 years	60 (48.0)	41 (51.2)	19 (42.2)	
Gender				
Male	59 (47.2)	38 (47.5)	21 (46.7)	0.92
Female	66 (52.8)	42 (52.5)	24 (53.3)	
Histology				
Adenocarcinoma	100 (80.0)	62 (77.5)	38 (84.8)	0.48
SCC	25 (20.0)	18 (22.5)	7 (15.6)	
ECOG				
0	86 (68.8)	51 (63.7)	35 (77.8)	0.11
1	39 (31.2)	29 (36.3)	10 (22.2)	
Smoking history				
Current	9 (7.2)	6 (7.5)	3 (6.7)	0.71
Former	95 (76.0)	59 (73.8)	36 (80.0)	
Never	21 (16.8)	15 (18.8)	6 (13.3)	
Stage				
IIIA	35 (28.0)	20 (25.0)	15 (33.3)	0.31
IIIB	70 (56.0)	44 (55.1)	26 (57.8)	
IIIC	20 (16.0)	16 (20.0)	4 (8.9)	
Baseline lung				
Comorbidities				
COPD	15 (12.0)	12 (15.0)	3 (6.7)	0.38
ILD	3 (2.4)	2 (2.5)	1 (2.2)	
Other comorbidities				
Yes	43 (34.4)	34 (42.5)	20 (44.4)	0.35
No	82 (65.6)	46 (57.5)	25 (66.6)	
PD-L1 expression				
≥50%	19 (15.2)	15 (18.8)	4 (8.9)	0.097
1-49%	65 (52.0)	38 (47.5)	27 (60.0)	
<1%	35 (28.0)	21 (26.3)	14 (31.1)	
ND	6 (4.8)	6 (7.5)		
EGFR mutation				
Yes	6 (4.8)	4 (5.0)	2 (4.4)	0.43
No	117 (94.4)	76 (95.0)	41 (91.2)	
ND	2 (1.6)		2 (4.4)	
Platinum Type				
Carboplatin	75 (60.0)	53 (66.3)	22 (48.9)	0.072
Cisplatin	50 (40.0)	27 (33.7)	23 (51.1)	
Chemotherapy combination				
Carboplatin/Paclitaxel	50 (40.0)	39 (48.8)	11 (24.4)	0.002
Carboplatin/Pemetrexed	25 (20.0)	14 (17.5)	11 (24.4)	
Cisplatin/Pemetrexed	43 (34.4)	21 (26.2)	1 (2.2)	
Cisplatin/Etoposide	7 (5.6)	6 (7.5)	22 (48.9)	
Type of chemoradiotherapy				
Sequential	8 (6.4)	6 (7.5)	2 (4.4)	0.71
Concurrent	117 (93.6)	74 (92.5)	43 (95.6)	
Time from CRT completion to durvalumab (mean ± SD in days)	37.3 (± 23.7)	39.0 (± 17.3)	29.1 (± 11.1)	0.02
The compliance rate with radiotherapy (%)	90.0	86.0	95.0	0.32

SCC, squamous cell carcinoma; ECOG, Eastern Cooperative Oncology Group; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; ND, no data.

differences. This allowed consideration of a balanced intervention for both populations to carry out a correct analysis of the disparities between groups. At the time of diagnosis, 52% of the patients were ≥65 years old, the majority were women (52.8%), and the most frequent histology was adenocarcinoma (80%). Two-thirds of the non-smoking patients were women (14/21), and six of them had EGFR mutations (four with exon 19 deletions and two with the L858R mutation). Overall, 19 (15.2%), 35 (28.0%), and 65 (52.0%) patients had PD-L1 expression ≥50%, <1%, and between 1%-49%, respectively. Most patients had a good ECOG performance status; 60% received carboplatin as part of the cCRT regimen, especially in combination with paclitaxel (40%) or pemetrexed (20%). The mean time from cCRT completion to durvalumab initiation was 37.3 days (SD ±23.7, range 7-133 days). There were no statistically significant imbalances between PD-L1 subgroups. Still, a significant difference was found in Hispanics regarding using the

cisplatin/pemetrexed combination, while NHW were more exposed to cisplatin/etoposide (P=0.002). Similarly, the mean time interval between cCRT and the start of durvalumab was significantly shorter in NHW (difference of 10 days less, P=0.02). The median follow-up for the entire cohort of included patients was 19.6 months (95%CI 8.1-39.2).

Survival Outcomes

For the 125 patients, the overall response rate (ORR) was 57.6% (N=72), and 18.4% (N=25) achieved stable disease (SD). OS was 26.3 months (95%CI 23.9-28.6) (**Supplementary Figure S2A**), and PFS was 20.5 months (95%CI 18.0-23.0) (**Supplementary Figure S2B**). When PFS was assessed by ethnicity, the median for the Hispanic population was 19.4 months (95%CI 16.4-22.5) and 21.2 months (95%CI 17.2-23.3; P=0.76) for the NHW group (**Figure 1A**). However, analysis of OS showed a significant difference in favor of the NHW group, given the median was 27.7 months (95%CI 24.6-30.9) versus 20.0 months (95%CI 16.4-23.5) for Hispanics. (P=0.032) (**Figure 1B**). Unadjusted 12-month and 24-month OS was 86.6% (95%CI 79.9–88.0) and 46.6% (95%CI 40.2–48.3) for NHW compared to 82.5% (95%CI 77.1–84.2) and 17.5% (95%CI 15.6-24.5) in Hispanics.

Among Hispanics, PFS was higher in those with better ECOG [ECOG 0: 21.4 months (95%CI 17.8-25.1) vs. ECOG 1: 10.2 months (95%CI 4.6-15.7); P=0.19] (**Supplementary Figure S3**), in patients with SCC [25.5 months (95%CI 20.2-30.8) vs. Adenocarcinomas 15.5 (CI95% 12.8-18.3); P=0.06] (**Supplementary Figure S4**) and in those with higher PD-L1 expression [PD-L1 ≥50% PFS NR, PD-L1 1-49% 14.5 months (95%CI 8.8-NR) and PD-L1 <1% 12.3 months (95%CI 6.8-13.6); P=0.001]. Neither history of tobacco exposure (P=0.67), tumor stage (P=0.10), nor presence of pneumonitis (P=0.51) influenced PFS among Hispanics. For the NHW group, the only variable that influenced PFS was the level of PDL-1 expression [PD-L1 ≥50% PFS NR, PD-L1 1%-49% 13.3 months (95%CI 11.9-NR) and PD-L1 <1% 10.4 months (95%CI 9.8-14.6); P=0.018)].

Univariate analysis for OS revealed that overall response to CRT positively impacted the survival in both Hispanics [OS responders 29.2 months (95%CI 25.8-37.7) vs. Non-responders 13.3 months (95%CI 10.3-16.2); P=0.0001] (**Figure 2A**) and NHW groups [OS responders 34.9 months (95%CI 33.6-36.3) vs. Non-responders 14.8 months (95%CI 13.1-16.4); P=0.0001] (**Figure 3A**). Similarly, Hispanic patients [PD-L1 ≥50% OS NR, PD-L1 1%-49% 25.0 months (95%CI 19.2-NR), and PD-L1 <1% 19.0 months (95%CI 13.0-16.1); P=0.0001] (**Figure 2B**) and NHW [PD-L1 ≥50% OS NR, PD-L1 1-49% 24.0 months (95%CI 14.7-NR) and PD-L1 <1% 19.0 months (95%CI 13.0-16.8); P=0.04] (**Figure 3B**) with higher PD-L1 expression had better OS. Neither ECOG, smoking history, tumor staging, histology, nor pneumonitis influenced OS in either group. In the multivariate model for OS, the only predictor of increased mortality was lack of response after CRT (HR 7.8, 95%CI 3.1-19.4). In contrast, the only factor that positively impacted OS among Hispanics and NHW was PD-L1 expression ≥50% compared to the PD-L1 <1% group (HR 0.69, 95%CI 0.50-0.97). 08). In the model for PFS, the only predictor for a better outcome among Hispanics and NHW was PD-L1 expression ≥50% (HR 0.55, 95%CI 0.37-0.81). The OS (P=0.52) and PFS (P=0.40) of patients carrying EGFR mutations did not differ significantly from the *Wt* population.

To compare data derived from the PACIFIC study with real-life Hispanics and NHW treated with CRT and durvalumab in our research, the efficacy-effectiveness factor and hazard ratio for OS (between 24 and 36 months of follow-up) was estimated, comparing both groups to the durvalumab arm in the PACIFIC. NHW had an EE factor of 0.78, indicating that median OS was 22% shorter for those patients treated in clinical practice than median OS from the registered clinical trial receiving the same treatment. In addition, the EE factor for Hispanics was 0.58, showing a reduction in survival versus NHW and PACIFIC of 20% and 42%, respectively. The corresponding HR for the OS among NHW and Hispanics was 1.53 (95%CI 1.12-1.71; P=0.052) and 2.31 (95%CI 1.76-2.49; P=0.004), respectively.

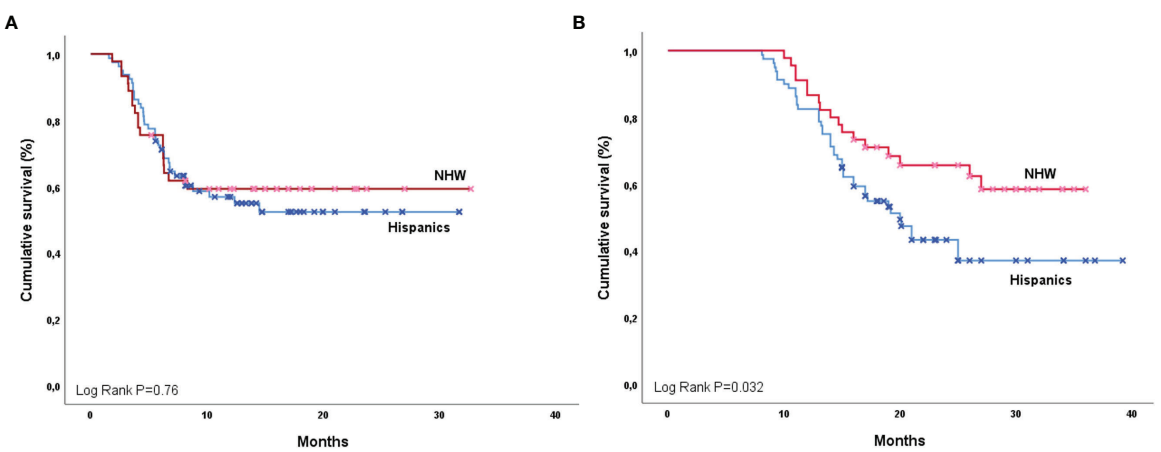

FIGURE 1 | Progression-free survival **(A)** and overall survival **(B)** by ethnicity (Hispanic and NHW).

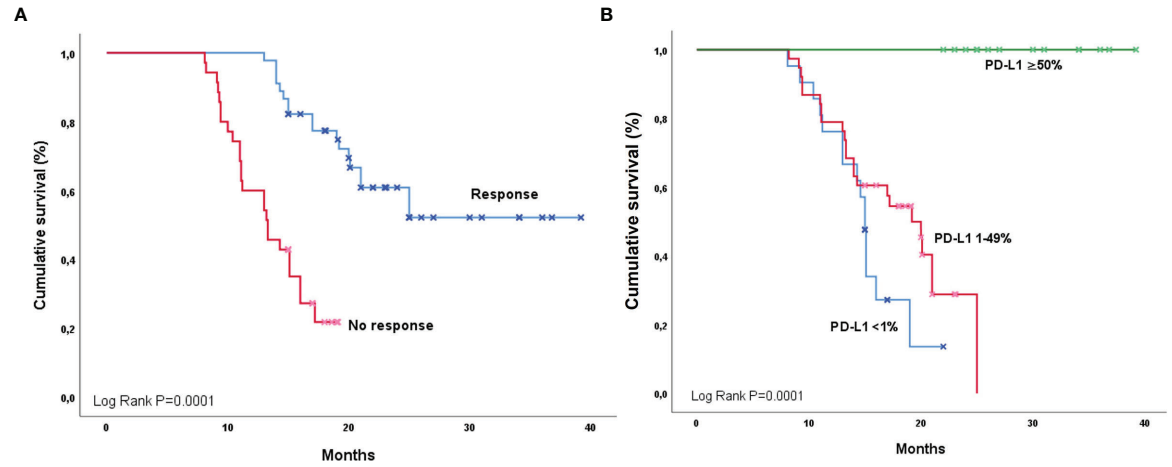

FIGURE 2 | Overall survival among Hispanics according to response **(A)** and PD-L1 expression **(B)**.

Safety Analysis

In the general population, 56 patients (44.8%) had some degree of pneumonitis due to CRT plus durvalumab. Pneumonitis was grade 1, 2, and 3 in 51.8% (N=29), 35.7% (N=20), and 12.5% (N=7), respectively. There was no difference in the proportion of pneumonitis according to race (P=0.95), previous tobacco exposure (P=0.14), type of chemotherapy regimen (P=0.36), or history of pulmonary comorbidity (P=0.55). Similarly, the severity of pneumonitis was not significantly different between Hispanics and NHW (P=0.41) and was not response-dependent (P=0.24).

DISCUSSION

It is essential to do real-world studies with diverse ethnic populations to address cancer disparities, reduce the variability of the results among minorities, and promote global access oncology. In the original PACIFIC trial, a landmark study that changed the standard of care in Stage IIII NSCLC, less than 2% of enrolled patients were documented as a minority, and there was no information about Hispanic ethnicity. In our multicenter retrospective study, we reported the outcomes of cCRT in addition to durvalumab for stage III NSCLC in two populations, Hispanics and NHW. Some studies have shown that the Hispanic population might have worse immunotherapy outcomes, possibly due to a complex interaction of factors such as culture, genomic heritage, or social determinants of health. Compared with NHW, the median PFS among Hispanics was lower but not significant (P=0.76). Nevertheless, when we analyzed OS stratified by race, we found that NHW reached a higher OS than Hispanics. This finding contrasts with previous studies that found no statistical difference in OS between the two ethnic groups (20).

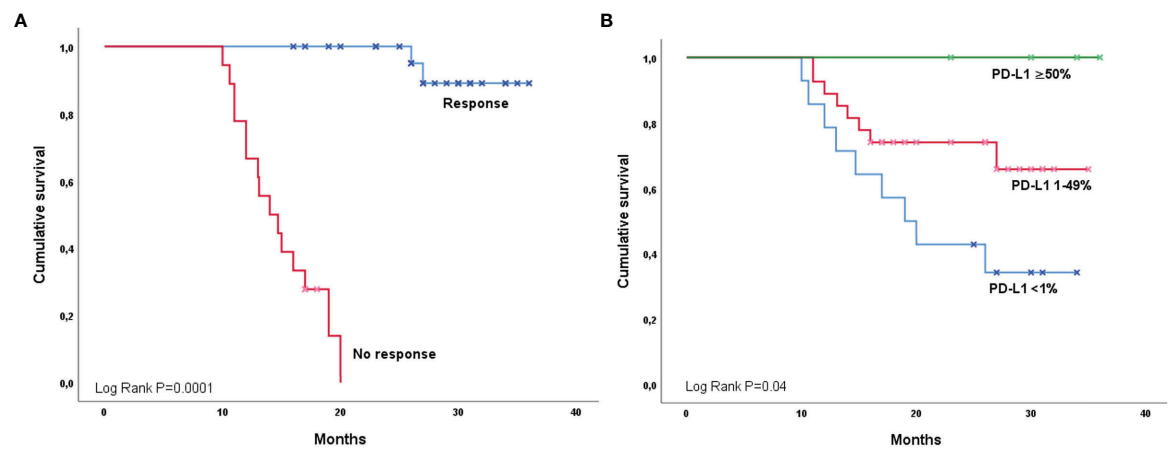

FIGURE 3 | Overall survival among NHWs according to response **(A)** and PD-L1 expression **(B)**.

Interestingly, the PFS and OS were higher in the subgroups with increased expression of PD-L1 in both the Hispanic and NHW groups. This result is consistent with the observations done by Kartolo et al., who found that high expression of PD-L1 was associated with improved survival as an independent prognostic factor (21). However, the most evident benefit was observed in a patient with PD-L1 expression of >50%, with no impact in the groups of PD-L1 expression of 1-49% or <1% (21) and, for our study, the median was not reached for the PDL-1 >50% subgroup. Regardless of the expression, there is evidence that durvalumab has a positive impact on outcomes even with PD-L1 expression higher than 1 to 25% (22, 23). Some evidence suggests that among Hispanics, expression in stage IIIB/IV NSCLC is around 21.7% (24).

Our study reports that overall response to cCRT positively impacts the OS, and the benefit is higher for NHW than for Hispanics (34.9 months vs. 29.2 months, respectively). Previously, some studies documented that complete or partial response to the treatment relates directly to an increased OS (25, 26). In this scenario, the outcome is still better for NHW despite the grade of response.

The results demonstrate that the use of durvalumab consolidation among both Hispanics and NHW is associated with improvement in the OS. When we contrasted the results of our study with those of the PACIFIC trial, we found that among NHW, the OS was slightly inferior in clinical practice (EE gap 0.78). Still, for Hispanics, the median survival was significantly shorter than for NHW (20%) and with the PACIFIC intervention (42%). In real-life scenarios, it has been described that the OS tends to be lower (27). Besides the differences between Hispanics and NHW, the inferior survival in both groups could be attributed to a delayed durvalumab onset and a significantly shorter time in favor of NHW. *Post hoc* analysis of the PACIFIC trial suggests that starting the ICI within 14 days after cCRT is associated with a higher OS (28). Also, the follow-up of the patients was relatively short.

In the general population, 56 patients (44.8%) had some degree of pneumonitis due to cCRT plus durvalumab. Pneumonitis was grade 1, 2, and 3 in 51.8% (N=29), 35.7% (N=20), and 12.5% (N=7), respectively. There was no difference in the proportion of pneumonitis according to race (P=0.95), previous tobacco exposure (P=0.14), type of chemotherapy regimen (P=0.36), or history of pulmonary comorbidity (P=0.55). Similarly, the severity of pneumonitis was not significantly different between Hispanics and NHW (P=0.41) and was not response-dependent (P=0.24).

In terms of safety, pneumonitis represents the most severe and life-threatening adverse effect related to immunotherapy (26). We reported a higher pneumonitis incidence than the PACIFIC trial (44.8% in the general population) (7). However, most cases were mild to moderate, without any patients needing to stop immunotherapy. This study did not find any variables related to a higher incidence of pneumonitis among the subjects. Some studies failed to identify specific risk factors associated with the development of pneumonitis among patients treated with durvalumab (29).

As we exposed earlier, PFS did not differ between the populations; however, the OS did. This finding could be explained in light of multiple differences between Hispanics vs. NHW, including overall access to second-line therapy or follow-up. Unfortunately, our available data related to the treatment approach after initial therapy is scarce and unbalanced between the two groups in our cohort. Further analyses are required to find a possible impact of these new variables in the response to therapy. In addition, we would like to remark that due to the immortality bias, commonly present in lung cancer scenarios, it is frequent that many patients exposed to ICI have a measurable effect on the OS but not in the PFS (30). On the other hand, populations with EGFR mutations (among others) should be analyzed independently (31).

Limitations in our analysis include a relatively short follow-up period for patients; however, in the same period, we were able to distinguish the differences between the Hispanic and NHW groups compared to the PACIFIC trial results. Furthermore, we only considered patients treated with cCRT plus durvalumab, which could create a selection bias in the study because we did not compare our results with a control population. In addition, the analysis of basal characteristics of the Hispanic patients treated in Latin America and those treated in the US did not show any differences, and this could be a risk for the interpretation of the data. On the other hand, the specific dose of durvalumab was not actively recorded, and information about other immuno-mediated side effects besides pneumonitis was not homogeneous.

CONCLUSION

Among patients with stage III NSCLC, NHW have better survival outcomes when compared to Hispanics. With an OS that seems to favor the NHW population and an EE factor that shows a shorter survival in Hispanics in comparison with NHW and with the PACIFIC trial group. Further analyses must be done to identify factors that might lead to these differences between Hispanics and NHW, and large clinical trials must include more representation of Hispanics.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of the Colombian organic law of data protection that limits access to raw genetic information in an open format. Requests to access the datasets should be directed to the corresponding author, who will release it upon formal request to the Ministry of Health of Colombia following the requirements of Law 1581 of 2012, paragraph 201811601170851 of 2018.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Kayre/FICMAC IRB 2018-14-021. The patients/

participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and study design: LER, OA, CR, RR, AC, LC, CM, and LR. Data acquisition: LR, AC, DC, FD, JG-R, EJ, CO, and AR-P. Analysis and Data interpretation: AR-P, AC, LR, OA, DC, CM, MC, SS, GR, LZ-B, and LM. Article Draft: AFC, DFC, LR, OA, LC, CM, MC, SS, GR, LM, LZB, CR, and RR. Manuscript preparation and approval: All authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.904800/full#supplementary-material

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The CURB65 score predicted 180-day mortality of non-small cell lung carcinoma patients with immune checkpoint inhibitor-associated pneumonitis: A pilot retrospective analysis

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Introduction: The immune checkpoint inhibitor-associated pneumonitis (CIP) is a particularly worrisome and potentially lethal form of immune-related adverse events. An objective and evidence-based assessment tool for evaluating the severity of CIP is in urgent need. CURB65 (consciousness, urea nitrogen, respiratory rate, blood pressure, and age) is a potential candidate to meet the need.

Methods: A retrospective study was conducted to explore preliminarily if CURB65 could predict the mortality in non-small cell lung carcinoma (NSCLC) patients with CIP.

Results: A total number of 28 NSCLC patients with CIP were included in the current study and classified into low-CURB65 group (n = 21) and high-CURB65 group (n = 7). Mortality after onset of CIP was consistently higher in the high-CURB65 group than in the low-CURB65 group (30-day: 57.1% vs. 0; 90-day: 71.4% vs. 4.76%; 180-day:71.4% vs. 14.29%). Two patients (9.5%) in the low-CURB65 group had severe CIP, and more than half of patients in the high-CURB65 group had severe CIP (p = 0.0008). The patients in the high-CURB65 group received more aggressive treatment. Both groups showed a predominant organizing pneumonia-like pattern on CT scan. CURB65 was moderately correlated with the American Society of Clinical Oncology (ASCO) grade of CIP, with a Pearson correlation coefficient R of 0.524.

Conclusion: CURB65 accurately stratified the risk of mortality in NSCLC patients with CIP. CURB65 might complement the ASCO grade in the assessment and prediction of mortality in these populations.

KEYWORDS

checkpoint inhibitor-associated pneumonitis, non-small cell lung carcinoma, CURB65, mortality, adverse events - complications

Introduction

Immune checkpoint inhibitors (ICIs) have revolutionized non-small cell lung carcinoma (NSCLC) treatment and became a first-line treatment in advanced and locally advanced NSCLC without driver gene alterations (1–3). With expanded use of ICIs, the unique immune-related adverse events (irAEs) have been increasingly reported (4, 5). As the particularly worrisome and potentially lethal form of irAEs, checkpoint inhibitor-associated pneumonitis (CIP) has drawn increasing attention (6–9). CIP is characterized by the occurrence of respiratory symptoms/signs related to a new emerging infiltration viewed on a chest imaging but excluding new infections or alternative etiologies (10). The reported incidence of CIP in NSCLC ranges from 2% to 38% in clinical trials, and from 4.8% to 39.3% in real-world studies (11).

At present, there is no consensus on the diagnostic evaluation, risk stratification, and optimal management of CIP, which are significant barriers to improved prognosis (12). Because the clinical appearance of CIP varies widely from mild symptoms to severe dyspnea and respiratory failure, it is generally accepted that treatment should be personalized and depend on the severity of CIP (6, 11). Currently, the severity of CIP is usually graded according to clinical symptoms and/or imaging manifestations (13–15). Because clinical symptoms are essential for CIP grade, a considerable level of subjectivity is inevitable. It is possible that patients and their clinicians have different perceptions of the bother caused by different symptoms. Clinicians may have disagreement in the grade for the same CIP and treat the patient differently, which might influence the final outcome. Moreover, all these recommendations are expert consensus based, with benefits outweighing harms, and strengths of recommendations are only moderate. Therefore, an objective and evidence-based assessment tool for evaluating the severity of CIP is in urgent need.

Community-acquired pneumonia (CAP) refers to the infectious inflammation of lung parenchyma acquired outside of hospitals (16, 17). CIP and CAP, while differing in etiologies, present with similar symptoms such as fever, cough, sputum production, chest pain, and dyspnea, which are variable in severity. The evaluation of CAP severity is crucial for the selection of appropriate location of treatment. Among multiple severity assessment tools for CAP, CURB65 (consciousness, urea nitrogen, respiratory rate, blood pressure, and age) stands out for its simplicity and efficacy and has been recommended by major CAP guidelines worldwide (17–20). Moreover, the five components of CURB65 are mostly objective parameters, which make it unlikely to suffer from the subjective interpretation of both patients and clinicians. These features make CURB65 a potential candidate to be applied in the CIP grade. So far, the potential utility of CURB65 in CIP has not been reported yet. Therefore, the aim of the current study is to explore preliminarily if CURB65 could predict the mortality in NSCLC patients with CIP.

Abbreviations: ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung carcinoma; irAEs, immune-related adverse events; CIP, checkpoint inhibitor-associated pneumonitis; CAP, community-acquired pneumonia; CURB65, consciousness, urea nitrogen, respiratory rate, blood pressure and age; PD-1, programmed cell death protein 1; PD-L1, programmed cell death receptor ligand-1; ASCO, American Society of Clinical Oncology; ADL, activities of daily living; OP, organizing pneumonia; NSIP, non-specific interstitial pneumonia; DAD, diffuse alveolar damage; HP, hypersensitivity pneumonitis; SD, standard deviation; IQR, median with interquartile range; BMI, body mass index; IVIG, intravenous immunoglobin; ICU, intensive care unit; COVID-19, Coronavirus Disease 2019; PFS, progression-free survival; OS, overall survival.

Methods

Ethical approval

The present study was a retrospective study conducted in a Chinese hospital (Second Affiliated Hospital of Zhejiang University School of Medicine, China). Ethical approval was sought and granted by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine (Approval Number: 2022-0240). As the non-interventional retrospective study was determined to be no greater than minimal risk, the Ethics Committee of the Second Affiliated

Hospital of Zhejiang University School of Medicine issued a waiver of informed consent. Patient data privacy and confidentiality were maintained as this study was conducted in compliance with the ethical standards of the Declaration of Helsinki.

Study population

The NSCLC patients with CIP were identified from the consultation records of the lung cancer multidisciplinary team in the study hospital from 1 January 2019 to 31 December 2021. The multidisciplinary team consisted of multiple subspecialties, including oncology, pulmonology, radiotherapy, radiology, thoracic surgery, and infectious disease, among others. Before consulting the multidisciplinary team, all patients underwent chest computer tomography (CT) imaging. For identifying CIP patients, the multidisciplinary team considered patients who developed dyspnea or other respiratory symptoms (including fever, cough, sputum production, etc.) after use of ICIs, along with the presence of new radiographic infiltration on CT and lack of evidence of lung infection or other alternative etiologies (tumor progression, radiation pneumonitis, diffuse alveolar hemorrhage, heart failure, etc.). Therapies included programmed cell death protein 1 (PD-1) ICIs and programmed cell death receptor ligand-1 (PD-L1) ICIs with or without additional agents, and tumor types included NSCLC only. The list of lung cancer patients receiving at least one dose of ICIs during the study period was acquired from the Electronic Medical Record System.

CIP was graded by the lung cancer multidisciplinary team according to the irAE guideline published in 2018 by the American Society of Clinical Oncology (ASCO) (13), as follows: G1: asymptomatic, confined to one lobe of the lung or 25% of lung parenchyma, clinical or diagnostic observations only; G2: symptomatic, involves more than one lobe of the lung or 25%–50% of lung parenchyma, medical intervention indicated, limiting instrumental activities of daily living (ADL); G3: severe symptoms, hospitalization required, involves all lung lobes or 50% of lung parenchyma, limiting self-care ADL, oxygen indicated; G4: life-threatening respiratory compromise, urgent intervention indicated (intubation).

CURB65

The CURB65 score was calculated as described before, by a pulmonologist (LXX) who was blinded to patients' ICI treatment history (18). One point was designated for each of confusion, blood urea >7 mmol/l, respiratory rate >30/min, low systolic (<90 mm Hg) or diastolic (≤60 mm Hg) blood pressure, and age ≥65 years. The score ranged from 0 to 5 in this scoring system, with a higher score indicating increasing disease severity.

Data collection

Detailed clinical data were collected retrospectively, including demographic characteristics, tumor history and prior treatment history, types of ICIs, clinical manifestations of CIP, lab test results, results of chest imaging and bronchoscopy, and the treatment outcomes of CIP. For patients who received corticosteroids for CIP treatment, a cumulative hydrocortisone-equivalent dose was calculated. The chest CT scan radiographic patterns were classified by an experienced radiologist (LHW) as described previously (21), including organizing pneumonia (OP)-like pattern, non-specific interstitial pneumonia (NSIP)-like pattern, diffuse alveolar damage (DAD)-like pattern, hypersensitivity pneumonitis (HP)-like pattern, and bronchiolitis-like pattern. Survival status was assessed by medical records and phone call during early April 2022.

Data analysis

The results were analyzed using IBM SPSS Statistics 20. Continuous data were presented as the mean with standard deviation (SD) or median with interquartile range (IQR), depending on the distribution of data. Variables were compared using the unpaired Student's t-test, Welch t-test, or Wilcoxon rank-sum test with continuity correction, depending on data normality and homogeneity of variance. Categorical data were presented as absolute value and percentage and analyzed using chi-square test or Fisher's exact test according to test assumptions. Pearson's correlation analysis was used for analyzing the correlation between variables. Statistical significance was set at $p < 0.05$.

Results

Between 1 January 2019 and 31 December 2021, a total number of 992 lung cancer patients received at least one dose of ICIs in the study hospital. A number of 67 patients suspected of CIP were referred to the multidisciplinary team by their attending doctors. The multidisciplinary team confirmed the diagnosis of CIP in 34 patients and ruled out CIP in 33 patients. Of all 34 patients with CIP, five patients were excluded due to subtype of small cell lung carcinoma, and one patient was excluded due to missing data. Therefore, a final number of 28 NSCLC patients with CIP were included in the current study (Figure 1). Patients were classified into two groups for further analysis according to CURB65: low-CURB65 group (for patients with CURB65 score of 0–1, n = 21) and high-CURB65 group (for patients with CURB65 score ≥2, n = 7).

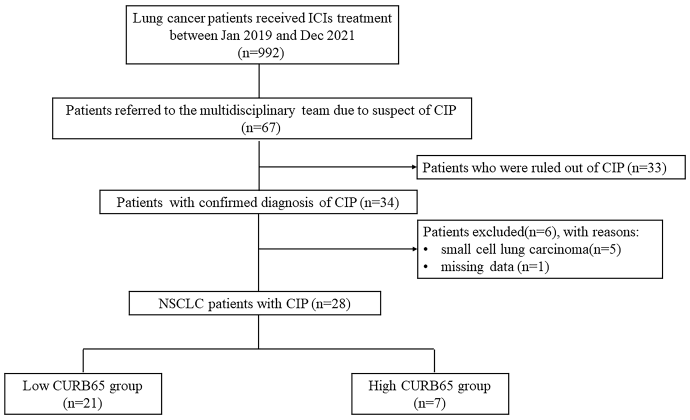

FIGURE 1
Flowchart of the study population. ICIs, immune checkpoint inhibitors; CIP, checkpoint inhibitor-associated pneumonitis; NSCLC, non-small cell lung carcinoma; CURB65, consciousness, urea nitrogen, respiratory rate, blood pressure, and age.

Baseline features

The baseline demographics, comorbidities, and lung function test results between two groups were compared (Table 1). The high-CURB65 group had significantly higher age than the low-CURB65 group (71.29 ± 3.59 vs. 66.14 ± 5.76, p = 0.037). The age difference could be explained by the fact that CURB65 had a component of age ≥65. All the patients in the high-CURB65 group had either ever smoking history (85.7%) or current smoking history (14.3%), which were different to the

TABLE 1 Baseline demographics, comorbidities, and lung function test results.

Variables	Low-CURB65 group (n = 21)	High-CURB65 group (n = 7)	p
Age	66.14 (5.79)	71.29 (3.59)	0.037
Male	17 (81%)	7 (100%)	0.212
BMI	22.71 (3.57)	21.49 (1.84)	0.400
Smoking history			0.051
Ever	7 (33.3%)	6 (85.7%)	
Current	9 (42.9%)	1 (14.3%)	
Never	5 (23.8%)	0	
Pack-years	45 (30.00, 60.00)	50 (30.00, 60.00)	0.824
Comorbidities			
COPD	5 (23.8%)	3 (42.9%)	0.334
Asthma	0	0	
ILD	3 (14.3%)	1 (14.3%)	1.000
Hypertension	7 (33.3%)	3 (42.9%)	0.649
Diabetes mellitus	1 (4.8%)	1 (14.3%)	0.397
Lung function test[#]			
FEV1	1.80 (0.70)	1.75 (0.56)	—
FEV1% predicted	74.03 (18.86)	68.75 (15.71)	—
FVC	2.52 (0.98)	2.52 (0.66)	—
FVC % predicted	81.30 (20.88)	75.80 (11.84)	—
DLCO % predicted	4.04 (1.14)	4.36 (0.99)	—
No spirometry performed	13 (61.9%)	3 (42.9%)	—

All data are presented as no. (%), median (interquartile range), or mean (standard deviation).
BMI, body mass index; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; DLCO, carbon monoxide diffusing capacity.
[#] The statistical analysis was not performed due to a very small sample size.

patients in the low-CURB65 group, although without statistical significance (p = 0.051). The gender, body mass index (BMI), comorbidities, and lung function test results were similar between two groups.

Lung cancer history and ICI treatment

Lung cancer history and ICI treatment were also analyzed (Table 2). The low-CURB65 group had one-third of patients with adenocarcinoma and two-thirds with squamous cell carcinoma, and the high-CURB65 group had three patients (42.9%) with adenocarcinoma, three patients (42.9%) with squamous cell carcinoma, and one patient (14.3%) with large cell carcinoma. The performance status and stage of patients were similar between two groups. ICIs were used predominantly in the second-line setting for both groups, because most patients had received chemotherapy, thoracic radiotherapy, or thoracic surgery before ICI initiation. The commonly used ICIs in the low-CURB65 group were pembrolizumab(19%), camrelizumab (38.1%), and tislelizumab (14.3%). In the high-CURB65 group, the commonly used ICI agents were camrelizumab (57.1%) and tislelizumab (28.6%). The median number of ICI cycles received was 5.0 (2.0–15.0) in the low-CURB65 group and 4.0 (2.0–7.0) in the high-CURB65 group. PD-L1 expression was determined from histologic specimens in nine patients (42.8%) of the low-CURB65 group and two patients (28.5%) of the high-CURB65 group, respectively. ICIs were commonly used in combination with chemotherapy in both groups (90.5% and 85.7%, respectively).

Kaplan–Meier analysis of mortality

Kaplan–Meier analysis identified a significant difference between two groups in all-cause mortality after onset of CIP. Mortality was significantly higher in the high-CURB65 group

TABLE 2 Lung cancer history and ICI treatment.

Variables	Low-CURB65 group (n = 21)	High-CURB65 group (n = 7)	p
Histology			0.163
Adenocarcinoma	7 (33.3%)	3 (42.9%)	
Squamous cell carcinoma	14 (66.7%)	3 (42.9%)	
Large cell carcinoma	0	1 (14.3%)	
Performance status			0.599
0	3 (14.3%)	2(28.6%)	
1	16 (76.2%)	5 (71.4%)	
2	2 (9.5%)	0	
Stage			1.000
III	10 (47.6%)	3 (42.9%)	
IV	11 (52.4%)	4 (57.1%)	
Prior cancer treatment			
Thoracic surgery	10 (47.6%)	4 (57.1%)	0.663
Thoracic radiotherapy	5 (23.8%)	0	0.076
Chemotherapy	7 (33.3%)	2 (28.6%)	0.815
ICIs			
Pembrolizumab	4 (19%)	0	—
Camrelizumab	8 (38.1%)	4 (57.1%)	—
Tislelizumab	6 (28.6%)	2 (28.6%)	—
Others	3 (14.3%)	1 (14.3%)	—
ICI cycles	5.0 (2.0, 15.0)	4.0 (2.0, 7.0)	0.455
PD-L1 expression status[a]			
Positive[b]	5 (23.8%)	0	—
Negative	4 (19.0%)	2 (28.5%)	—
Not assessed	12 (57.2%)	5 (71.5%)	—
Concurrent treatment with ICIs			
Chemotherapy	19 (90.5%)	6 (85.7%)	0.724
None	2 (9.5%)	1 (14.3%)	0.204

All data are presented as no. (%), median (interquartile range), or mean (standard deviation).
ICIs, immune checkpoint inhibitors; PD-L1, programmed cell death-ligand 1.
[a]The statistical analysis was not performed due to the small sample size.
[b]If PD-L1 expression was >1%.

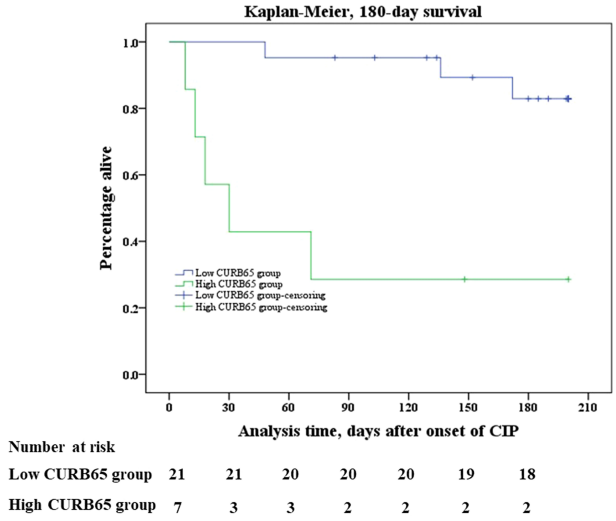

FIGURE 2
Kaplan–Meier survival analysis. Kaplan–Meier analysis of survival in 180 days after onset of CIP showed that mortality was significantly higher in the high-CURB65 group than in the low-CURB65 group (log rank, $p < 0.001$). CURB65, consciousness, urea nitrogen, respiratory rate, blood pressure and age; CIP, checkpoint inhibitor-associated pneumonitis.

than in the low-CURB65 group, up to 180 days after onset of CIP (log rank, $p < 0.001$) (Figure 2). The mortality was consistently higher in the high-CURB65 group (30-day: 57.1% vs. 0; 90-day: 71.4% vs. 4.76%; 180-day:71.4% vs. 14.29%).

The median follow-up time of the study population was 178.5 days (88.0–261.8 days). There were three death events in the low-CURB65 group and five death events in the high-CURB65 group during the follow-up. Six patients died in the hospital, and their death records showed that their cause of death was CIP. One patient died 1 day later after hospital discharge, and the medical records showed that the patient was in critical state due to CIP before discharge. The relatives required the discharge because according to their local custom, people should die at home. The cause of death of the last patient could not be verified. There were 18 censored cases in the low-CURB65 group and the censored cases in the high-CURB65 group, respectively.

CIP characteristics and treatment

The median time to CIP diagnosis from initial ICI treatment was 145.0 days (44.5–333.5 days) for the low-CURB65 group and 139.0 days (45.0–168.0 days) for the high-CURB65 group (Table 3). Two patients (9.5%) in the low-CURB65 group had severe CIP (ASCO grade ≥3), and more than half of patients in the high-CURB65 group had severe CIP ($p = 0.0008$). The symptoms were similar between two groups, although the high-CURB65 group tended to have more patients with fever without statistical significance. The high-CURB65 group had higher C-reactive protein than the low-CURB65 group, but without statistical significance (94.5 ± 83.7 vs. 52.2 ± 45.8, $p = 0.11$). The high-CURB65 group also had significantly higher D-dimer ($p = 0.002$).

The patients in the high-CURB65 group received more aggressive treatment. Corticosteroids were used in 76.2% of patients in the low-CURB65 group and 100% of patients in the high-CURB65 group. The high-CURB65 group tended to have a higher cumulative hydrocortisone-equivalent dose of corticosteroids, daily dose of corticosteroids, and duration of corticosteroid use, but statistical significance was only detected for the daily dose of corticosteroids ($p = 0.042$). The high-CURB65 group was more inclined to receive additional immunosuppressants and respiratory support. In the high-CURB65 group, besides corticosteroids, one patient received both intravenous immunoglobin (IVIG) and non-invasive ventilation, and another patient received both IVIG and invasive ventilation.

Radiographic appearances of CIP

During the evaluation for CIP, all patients underwent chest CT imaging. The low-CURB65 group had 11 patients (52.4%) who presented with bilateral involvement, and the high-CURB65 group had six patients (85.7%) (Table 4). The low-CURB65 group had 3.0 (2.0–4.5) lobes involved, and the high-CURB65 group had 4.0 (4.0–5.0) lobes. The high-CURB65 group had a significantly higher proportion of patients with pleural effusion than the low-CURB65 group (71.8% vs. 14.3%, $p = 0.004$). The overall radiographic pattern

TABLE 3 CIP characteristics and treatment.

Variables	Low-CURB65 group (n = 21)	High-CURB65 group (n = 7)	p
Onset time of CIP	145.0 (44.5, 333.5)	139.0 (45.0, 168.0)	0.490
ASCO grade			0.008
G1–2	19 (90.5%)	3 (42.9%)	
G3–4	2 (9.5%)	4 (57.1%)	
Symptoms			
Fever	4 (19.0%)	4 (57.1%)	0.053
Cough	9 (42.9%)	3 (42.9%)	1.000
Sputum production	8 (36.1%)	3 (42.9%)	0.823
Chest pain	1 (4.8%)	0	0.557
Dyspnea	11 (52.4)	7 (100%)	0.663
Blood test results			
CRP	52.2 (45.8)	94.5 (83.7)	0.110
D-dimer	930.0 (480.0, 1860.0)	3560.0 (2410.0, 8950.0)	0.002
Albumin	34.1 (5.2)	29.7 (7.0)	0.170
White blood cell count	7.02 (2.23)	9.50 (5.93)	0.119
Neutrophil count	5.40 (2.01)	7.67 (5.31)	0.112
Lymphocyte count	0.95 (0.39)	1.02 (0.46)	0.541
Eosinophil count	0.17 (0.27)	0.12 (0.09)	0.613
Hemoglobin	116.20 (18.00)	105.86 (12.47)	0.174
Platelet count	223.40 (69.33)	168.71 (100.63)	0.123
Corticosteroid treatment			
Use of corticosteroids	16 (76.2%)	7 (100%)	0.154
Cumulative dose of corticosteroids	1240.00 (850.00, 2400.00)	3600.00 (750.00, 7000.00)	0.111
Daily dose of corticosteroids	200.00 (200.00, 291.67)	257.14 (148.97, 400.00)	0.042
Duration of corticosteroid use	6.00 (5.00, 10.50)	8.00 (3.00, 23.00)	0.614
Other treatment			
Antibiotics	14 (66.7%)	5 (71.4%)	0.815
IVIG	0	2 (28.6%)	0.056
Non-invasive ventilation	0	1 (14.3%)	0.250
Invasive ventilation	0	1 (14.3%)	0.250

All data are presented as no. (%), median (interquartile range), or mean (standard deviation).
CIP, checkpoint inhibitor-associated pneumonitis; ASCO, American Society of Clinical Oncology; CRP, C-reactive protein; IVIG, intravenous immunoglobins.

TABLE 4 Radiographic appearances of CIP.

Variables	Low-CURB65 group (n = 21)	High-CURB65 group (n = 7)	p
Bilateral involvement	11 (52.4%)	6 (85.7%)	0.118
Number of lobes involved	3.0 (2.0, 4.5)	4.0 (4.0, 5.0)	0.242
Pleural effusion	3 (14.3%)	5 (71.8%)	0.004
Overall pattern of CIP[a]			0.250
OP-like pattern	15 (71.4%)	3 (42.9%)	
NSIP-like pattern	2 (9.5%)	1 (14.3%)	
DAD-like pattern	2 (9.5%)	3 (42.9%)	
HP-like pattern	2 (9.5%)	0	
Bronchiolitis-like pattern	1 (4.8%)	0	

All data are presented as no. (%).
CIP, checkpoint inhibitor-associated pneumonitis; OP, organizing pneumonia; NSIP, nonspecific interstitial pneumonia; DAD, diffuse alveolar damage; HP, hypersensitivity pneumonitis.
[a]One patient in the low-CURB65 group presented both OP-like and NSIP-like patterns.

profile of CIP was similar two groups, which both showed a predominant OP-like pattern.

Correlation between CURB65 score and ASCO grade of CIP

The scatter plots showed a moderate positive linear correlation between CURB65 and ASCO grade of CIP (Figure 3). The Pearson correlation coefficient R between the two variables was 0.524 ($p = 0.004$).

Discussion

There was a lack of objective and evidence-based tool to assess the severity of CIP. To our knowledge, the current study was the first study to explore preliminarily if CURB65 could predict the mortality in NSCLC patients with CIP. Our study showed that CURB65 accurately stratified the risk of mortality in 180 days after onset of CIP. The high-CURB65 group had significantly more severe CIP and received more aggressive treatment. CURB65 was moderately correlated with the ASCO grade of CIP. CURB65 had the potential to be a useful clinical predictive tool, when used in conjunction with ASCO grade, to risk-stratify patients and assist in clinical decision making and personalized medicine approaches in NSCLC patients with CIP. However, further prospective studies were warranted to verify its efficacy.

CURB65 was first derived and validated by Lim *et al.* in 2003 (18). It was based on the modified British Thoracic Society severity assessment tool which used clinical features to identify severe CAP patients at high risk of mortality. They found that CURB65, based on information available at initial hospital assessment, enabled CAP patients to be stratified according to increasing risk of mortality (score 0, 0.7%; score 1, 2.1%; score 2, 9.2%; scores 3–5, 15%–40%). Besides mortality, piling evidence validated the effectiveness of the CURB-65 score in predicting various CAP outcomes including disease complications, hospitalization or intensive care unit (ICU) admission, duration of hospital or ICU stay, intensive respiratory or vasopressor support, mechanical ventilation, and treatment failure (22). The evidence base for the CURB65 score in CAP was robust and continued to increase. Moreover, CURB65 used only five items which required no special tests and was practical for calculations. This simplicity made it easier to be popularized and applied in practice. So CURB65 had been universally recommended by major CAP guidelines to assist the clinical judgment for determination of the site of care (17, 19, 20). To the best of our knowledge, the potential utility of CURB65 in CIP has not been reported yet.

Our study showed for the first time that CURB65 accurately stratified the risk of mortality in NSCLC patients with CIP. Compared with the low-CURB65 group, we found that there was a consistently increased risk of death in the high-CURB65 group. As far as we knew, there was no similar report before. This finding indicated that CURB65 might be used to identify patients at high risk of death, and more aggressive interventions might be warranted for those patients. This finding should be interpreted with caution, because of the small sample size. However, all published studies about CIP in lung cancer had a relatively small sample size, and most studies were case reports or case series. A study by Atchley *et al.* included 30 lung cancer patients with CIP, and another study by Huang *et al.* recruited 32 NSCLC patients with CIP (23, 24). Our sample size was comparable to the previous studies. Besides the small sample size, the low-CURB65 group had more patients than the high-CURB65 group (21 vs. 7). The unbalanced sample size of the two groups may also lead to bias of the analysis results. It was possible that the unbalance groups may cause the

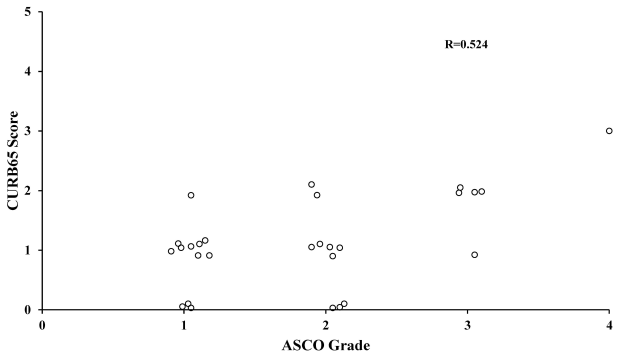

FIGURE 3
Correlation between CURB65 score and ASCO grade of CIP. The Pearson correlation analysis showed a moderately positive linear correlation between CURB65 and ASCO grade of CIP. CURB65, consciousness, urea nitrogen, respiratory rate, blood pressure and age; CIP, checkpoint inhibitor-associated pneumonitis. ASCO, American Society of Clinical Oncology.

overestimation of mortality difference between low-CURB65 and high-CURB65 groups. However, the mortality difference (about fivefold) was so significant that the principal findings of the current study was unlikely to be caused by biased information. Future prospective multicenter studies with a large sample size and more balanced groups were needed to further verify the efficacy of CURB65.

It remained unknown whether CURB65 was a predictor specific to CIP or just a general prognostic factor for lung cancer. On the one hand, our findings tended to support that CURB65 was a predictor specific to CIP. Most clinical studies of CAP used 30-day mortality as a clinical end point, because deaths that occurred within 30 days were most likely attributed to CAP (22). Therefore, it could be plausibly argued that in patients with CIP, deaths that occurred within 30 days after onset of CIP were most likely attributed to CIP. Our study showed that the high-CURB65 group had a significantly higher 30-day mortality than the low-CURB65 group (57.1% vs. 0). Therefore, the high mortality of the high-CURB65 group within 30 days was most likely to be caused by CIP instead of lung cancer. On the other hand, CURB65 used all objective parameters, which were not specific to CIP. It was reported that CURB65 was associated with advanced age, hypertension, overweight/obesity, kidney failure, hypoxemia, requirement for mechanical ventilation, or onset of respiratory distress in patients hospitalized with Coronavirus Disease 2019 (COVID-19) (25).Thus, it was very likely to have some non-CIP patients with high CURB65. In order to answer the abovementioned question, future studies with the aim to explore the predictive value of CURB65 in lung cancer patients without CIP were warranted.

The current study revealed that the high-CURB65 group had significantly more severe CIP (57.1% vs. 9.5%). It was reported that the prognosis of severe CIP was worse than non-severe CIP. A study by Tone *et al.* revealed that patients with severe CIP had significantly shorter progression-free survival (PFS) and overall survival (OS) than patients with non-severe CIP (26). Univariate analysis further confirmed that complication with severe grade CIP was significantly associated with poor PFS and OS. A review by Zhang *et al.* included and analyzed 44 occurrences of CIP in patients with NSCLC, which were all published in case reports and case series (11). Although not powered to detect statistical significance, it was reported that severe CIP had significantly higher mortality than non-severe CIP (57.14%–64.29% vs. 14.29%). Therefore, the high proportion of severe CIP may at least partially explain the high mortality in the high-CURB65 group.

Moreover, the current study found that there was a trend to a higher proportion of ever or current smokers in the high-CURB65 group than the low-CURB65 group, with borderline significance. So far, there were limited reports about the role of smoking history in CIP, which were all from retrospective studies. First, history of smoking may increase the risk of CIP (11). Second, smoking history was a risk factor for severe CIP. In a study conducted by Chen *et al.*, patients with severe pneumonitis had a higher likelihood of being current or former smokers than patients with non-severe pneumonitis (100% vs. 77%, $p = 0.007$) (27). Therefore, our findings were in agreement with previous reports. Future prospective studies were warranted to further explore the role of smoking history in CIP.

Our study found that the patients with high CURB65 received more aggressive treatment. Current guidelines for irAE recommended that management of CIP should be based on CIP grade (13–15). Corticosteroids were recommended as the primary therapy approach, although in mild cases, holding ICIs might suffice. The suggested dose of corticosteroids tended to increase with the grade of CIP, and additional immunosuppressants and empirical antibiotics were recommended for severe CIP. In the current study, corticosteroids were more likely to be used in the high-CURB65 group than in the low-CURB65 group (100% vs. 76.2%). Furthermore, there was a tendency toward a higher dose of corticosteroids and more use of IVIG and respiratory support in the high-CURB65 group. This demonstrated that in the current study, the management was dependent on the severity of CIP according to CURB65. This fact was in agreement with the recommendations by the irAE guidelines that management of CIP should be based on grade.

The current study also revealed that CURB65 was moderately correlated with the ASCO grade of CIP, with a Pearson correlation coefficient R of 0.524. CURB65 evaluates the severity with five objective parameters, and the ASCO grade evaluates the severity by a combination of subjective clinical symptoms and imaging manifestations (13). Therefore, by assessing the severity of CIP from different perspectives, the two scoring systems did not fully agree with each other, which was not unexpected. The moderate correlation indicated that they might complement each other. Of notice, in the current study, there were three patients with a low ASCO grade in the high-CURB65 group. The severity of CIP in these patients might be underestimated, which led to insufficient and inappropriate treatment. This might at least partially contribute to the result that two of the three patients died within 30 days. For these patients, more aggressive interventions might improve the prognosis. Therefore, CURB65 might complement the ASCO grade in the assessment and prediction of mortality. Especially for the patients with a low ASCO grade but high CURB65 score, more aggressive interventions might be warranted.

This study had several limitations. First, the present study was a retrospective study, which came with many inherent limitations. The current retrospective study could not establish a cause–effect relationship between CURB65 and mortality. The retrospective nature of this study was also prone to biases from missing data and reliance on documentation available for review. Second, patients with mild CIP may be under-represented in the current study. Because those patients had no symptom or mild

symptom, the clinicians were less likely to refer these patients to the lung cancer multidisciplinary team for consultation. Third, the paradigm of ICI use had shifted since the initial use of these agents, so our study population could not represent the present profile of patients with ICI treatment.

Conclusion

The current study provided preliminary evidence to support the use of the CURB65 score in predicting mortality in NSCLC patients with CIP for the first time. CURB65 accurately stratified the risk of mortality in 180 days after onset of CIP. The high-CURB65 group had significantly more severe CIP and received more aggressive treatment. CURB65 was moderately correlated with the ASCO grade of CIP. CURB65 might complement the ASCO grade in the assessment and prediction of mortality in NSCLC patients with CIP.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was reviewed and approved by Ethics Committee of Second Affiliated Hospital of Zhejiang University School of Medicine. The ethics committee waived the requirement of written informed consent for participation.

Author contributions

YM and WL contributed to the conception and design of the study. FL contributed to the writing of the manuscript, design of the study, and statistical analysis. BF and LW contributed to the writing of the manuscript, retrieval of data, and organization of the database. LX and TZ contributed to the retrieval of data. All authors contributed to the manuscript revision and read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
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Comparison of Efficacy and Safety Between Immunotherapy and Docetaxel Monotherapy in NSCLC Patients

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Objective: Meta analysis was used to compare the efficacy and safety of immune checkpoint inhibitor and docetaxel in the treatment of non-small cell lung cancer.

Methods: CNKI, CBM, PubMed, EMBASE, Cochrane Library, web of science and other databases were searched by computer, and the randomized controlled trials of immune checkpoint inhibitors and docetaxel in the treatment of NSCLC published as of February 2022 were collected. Two researchers searched independently, screened the literature and extracted the data according to the nanodischarge criteria, and used Revman5.4. The included studies were statistically analyzed, and publication bias was analyzed with Egger test in Stata12.

Results: A total of 8 RCTs were included, including 2444 cases treated with immune checkpoint inhibitors and 2097 cases treated with docetaxel. Compared with docetaxel, the overall survival (HR = 1.40, 95%CI: 1.30-1.50, P < 0.00001) and progression free survival (HR = 1.22, 95%CI: 1.13-1.32, P < 0.00001) of NSCLC treated with ICIs were longer. The risk ratio of any grade of adverse reactions (HR = 0.41, 95%CI: 0.32-0.52, P < 0.00001) and above grade III adverse reactions (HR = 0.27, 95%CI: 0.18-0.41, P < 0.00001) in the treatment of NSCLC with ICIs was lower. There was no publication bias in Egger test.

Conclusion: Compared with docetaxel, immune checkpoint inhibitor treatment can improve the clinical efficacy of NSCLC patients and has a lower incidence of adverse reactions. This treatment may be a promising treatment for NSCLC patients.

Keywords: immune checkpoint inhibitors, docetaxel, non small cell lung cancer, overall survival, progression free survival, security

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INTRODUCTION

Lung cancer is one of the most common malignant tumors in China. Its incidence rate and mortality rate are the first in all tumors. Lung cancer is mainly divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), of which NSCLC accounts for 85%. Non-small cell lung cancer includes squamous cell carcinoma, adenocarcinoma and large cell carcinoma. The etiology of lung cancer is complex. At present, it is considered that it is mainly related to smoking, air pollution, occupational factors and changes in molecular genetics. The early symptoms of lung cancer are not obvious. Later, there are often symptoms such as cough, blood in sputum, chest pain and so on (1, 2). Due to the limited diagnostic tools currently used, 75% of patients were found to be in advanced stage. The prognosis of most patients is poor. Based on the stage of the disease at the time of diagnosis, the 5-year survival rate of patients is 4% - 17% (3). At present, docetaxel chemotherapy is one of the most commonly used second-line treatments for NSCLC (2, 3). Its mechanism is to increase the polymerization of tubulin, and then inhibit the depolymerization of microtubules, and thus inhibit the division and growth of tumor cells (4). However, docetaxel has poor efficacy and high toxicity in the treatment of NSCLC. Therefore, it is necessary to explore new treatment methods to prolong the survival time and improve the quality of life of patients.

In recent years, with the rapid development of tumor immunology, immunotherapy has become another new tumor treatment method besides surgery, chemotherapy and radiotherapy. Great breakthroughs have been made in the research of immune checkpoint inhibitors, and the role of inhibitory antibodies in clinical treatment trials of malignant tumors has also been recognized. PD-1 is a cell surface receptor, which is highly expressed on activated T cells and is considered to be a marker of T cell failure. It can regulate the activity of T cells, activate the apoptosis of tumor specific T cells and inhibit the apoptosis of regulatory T cells, so as to inhibit immune response and promote self tolerance (5). PD-L1 is expressed on some types of tumor cells and antigen-presenting cells and is considered to be a co suppressor of immune response. It can be bind to PD-1, activate PD-1/PD-L1 pathway, inhibit downstream signal transduction and T cell biological function, lead to tumor specific T cell failure and apoptosis, and make tumor cells escape immune surveillance (6). PD-1/PD-L1 pathway induces and maintains immune tolerance in tumor microenvironment and promotes tumor development. In this study, randomized controlled trials (RCTs) of ICIs and docetaxel monotherapy in the treatment of NSCLC were searched and efficacy and safety were evaluated by meta-analysis. The results obtained can provide a reference for clinical treatment.

METHODS AND MATERIALS

Search Strategy

We used computers to search PubMed, EMBASE, Cochrane Library, Web of science database, etc. Chinese search terms: immune checkpoint inhibitor, docetaxel, non-small cell lung cancer, randomized controlled trial; English search terms: ICIs, docetaxel, non small cell lung cancer, NSCLC, randomized controlled trials, RCTs. The search deadline is February 2022.

Study Selection

Inclusion criteria: ① Literature: retrospective study, prospective study; ② The subjects were patients with NSCLC diagnosed by clinicopathological examination; ③ Intervention measures: patients in the experimental group treated with ICI monotherapy and patients in the control group treated with docetaxel monotherapy; ④ The primary clinical outcome measures were overall survival (OS) and progression free survival (PFS). The secondary outcome measures were adverse reactions at any level and adverse reactions above grade 3.

Exclusion criteria: ① repeatedly published literature; ② Documents that cannot obtain original data or contact the author to obtain the original text; ③ Abstract, review, meta-analysis, case report and animal experiment; ④ Non Chinese and English literature.

Literature Screening and Data Extraction

Two researchers independently read the initial literature titles and abstracts according to the inclusion and exclusion criteria, screened the literature that may meet the inclusion criteria by reading the full text, and extracted the data according to the pre-designed table, including the first author, year of publication, number of patients, OS, PFS and adverse reactions. In case of differences, the two researchers shall discuss and solve them.

Bias Risk Assessment

We assessed the quality of inclusion in clinical randomized controlled trials, met the criteria proposed in the Cochrane manual for systematic evaluation of interventions (5.1.0), and evaluated the generation of random sequences, allocation concealment, blinding of participants, blinding of outcome evaluation, and incomplete outcome data to ensure a low incidence of bias.

Statistical Analysis

We use Revman5.4 software to analyze the data of the included studies. Relative risk ratios (RR) and 95% confidence interval (95%CI) were used as effect indexes for counting data, and the difference was statistically significant ($P < 0.05$). I^2 is used to evaluate the heterogeneity. If the heterogeneity test result I^2 is less than 50%, it means that there is no statistical heterogeneity among the research results, and the fixed effect model is used; If the heterogeneity test result $I^2 > 50\%$, analyze the source of heterogeneity. If the heterogeneity still exists, select the random effect model to estimate the combined effect.

RESULTS

Literature Search and Screening

205 literatures (including 86 PubMed, 72 Cochrane, 22 Embase, 20 CNKI, and Wanfang VIP5) were searched by computer, and

55 were selected according to the title and abstract. After full-text analysis and evaluation, 47 literatures with abnormal data, incomplete information or unavailable due to non comparative research were excluded, and finally 8 (7–14) literatures were included for systematic evaluation and meta-analysis. The process of literature screening is shown in **Figure 1**. Among them, 2444 patients were treated with ICIs monotherapy and 2097 patients were treated with docetaxel monotherapy. **Table 1** summarizes the basic characteristics and main evaluation indicators of the included research.

Quality Assessment Results

The quality assessment results are shown in **Figures 2A, B**. All included studies had a low risk of bias.

Meta Analysis Results of Effectiveness of ICIs and Docetaxel

OS comparison 8 RCTs reported the OS of patients, and there were no statistically significant differences between studies. The results of meta-analysis showed that the OS of ICIS treatment group was longer than that of docetaxel chemotherapy group, and the difference was statistically significant (HR = 1.40, 95% CI: 1.30-1.50, P < 0.00001), indicating that the efficacy of ICIs in the treatment of NSCLC was better than that of docetaxel chemotherapy (as shown in **Figure 3**).

PFS comparison Five RCTs reported PFS of patients, and there were no statistically significant differences between studies. The results of meta-analysis showed that the PFS of ICIS treatment group was longer than that of docetaxel chemotherapy group, and the difference was statistically significant (HR = 1.22, 95%CI: 1.13-1.32, P < 0.00001), indicating that the efficacy of ICIs in the treatment of NSCLC was better than that of docetaxel chemotherapy (as shown in **Figure 4**).

Results of Safety Meta-Analysis of ICIs and Docetaxel

Adverse reactions at any level Five RCTs reported typical adverse reactions at any level (including fatigue, nausea, ashenia, diarrhea and anemia). There were no statistically significant differences between studies. The results of meta-analysis showed that the risk of adverse reactions at any level in the ICIs treatment group was lower than that in the docetaxel chemotherapy group, and the difference was statistically significant (HR = 0.41, 95%CI: 0.32-0.52, P < 0.00001), indicating that the safety of ICIs is superior to docetaxel monotherapy in NSCLC (as shown in **Figure 5**).

Adverse reactions above grade III Five RCTs reported typical adverse reactions above grade III (including fatigue, nausea, ashenia, diarrhea and anemia), and there were no statistically significant differences between studies. The results of meta-analysis showed that the risk of grade III and above adverse reactions in the ICIs treatment group was lower than that in the docetaxel chemotherapy group, and the difference was statistically significant (HR = 0.27, 95%CI: 0.18-0.41, P < 0.00001), indicating that the safety of ICIs is superior to docetaxel monotherapy in NSCLC(as shown in **Figure 6**).

Sensitivity Analysis and Publication Bias Assessment

Publication bias assessment was performed only in OS and PFS. Egger test in Stata12 software was used for publication bias test. In a total of 8 studies with OS and PFS as outcome indicators, the results of publication bias test indicated that there was no publication bias OS(P = 0.051) and PFS(P = 0.255), as shown in **Figure 7**.

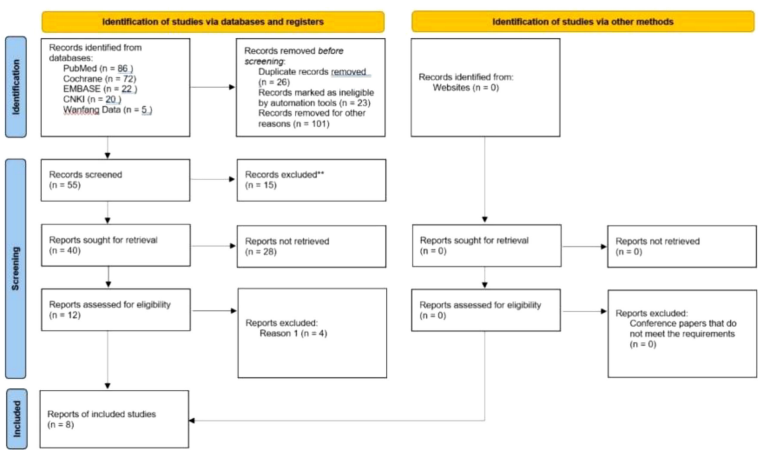

FIGURE 1 | Literature screening process and results.

TABLE 1 | Basic characteristics of included studies and main evaluation indicators (7–14).

First Author	Year	Clinical trial number	Phase	Type of Cancer	NO. of Patients with ICI	NO. of Patients with Docetaxel	HR for OS [95% CI]	p-Value for OS	HR for PFS [95% CI]	p-Value for PFS
Leora Horn (8)	2017	NCT01673867	III	NSCLC	427	427	0.72 [0.62,0.84]	0.001	NA	NA
H. Borghaei (9)	2015	NCT01673867	III	NSCLC	292	290	0.73 [0.59,0.89]	0.002	0.92 [0.77,1.11]	0.39
Julie Brahmer (10)	2015	NCT01642004	III	NSCLC	135	137	0.59 [0.44,0.79]	0.001	0.62 [0.47,0.81]	0.001
Shun Lu (11)	2021	NCT02613507	III	NSCLC	338	166	0.75 [0.61,0.93]	0.001	0.79 [0.65,0.98]	0.001
Yi-Long Wu (12)	2019	NCT02613507	III	NSCLC	338	166	0.68 [0.52,0.90]	0.0006	0.77 [0.62,0.95]	0.0147
Roy S Herbst (13)	2015	NCT01905657	III	NSCLC	345	343	0.71 [0.58,0.88]	0.0008	0.88 [0.74,1.05]	0.07
Louis Fehrenbacher (14)	2016	NCT01903993	II	NSCLC	144	143	0.73 [0.53,0.99]	0.04	NA	NA
Achim Rittmeyer (15)	2016	NCT02008227	III	NSCLC	425	425	0.73 [0.62,0.87]	0.0003	NA	NA

ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer; HR, Hazard ratio; NA, Not available; PFS, Progression free survival; OS, Overall survival.

DISCUSSION

In recent years, the choice of standard treatment for NSCLC patients has gradually changed from routine first-line drugs to immune checkpoint inhibitor (ICIs) therapy, or as a monotherapy, or combined with chemotherapy, anti angiogenic antibodies or other forms of ICIs. ICIs, an antibody against PD-1 or PD-L1, was approved for second-line and third-line treatment in patients with metastatic NSCLC without treatable driver mutations in 2015 (15). Since then, ICIs has been approved for first-line treatment, or for tumors with PD-L1 expression ≥ 50% alone, or in combination with chemotherapy independent of receptor status (16). Some patients treated with ICIs have particularly long-lasting response and survival. The study confirmed that up to 16% of patients with stage IV non-small cell lung cancer received second-line treatment with PD-1 inhibitor nivolumab and 31.9% received first-line treatment with PD-1 inhibitor pembrolizumab, with a survival time of 5 years (17). Another study showed that 4-year OS rates in POPLAR were 14.8% and 8.1% (and those in OAK were 15.5%and 8.7% for atezolizumab and docetaxel, respectively. However, it is worth noting that some patients with low PD-L1 expression may have poor efficacy in the treatment of tumors with immune checkpoint inhibitors. Therefore, it is very important to select biomarkers that can effectively predict the efficacy of PD-1/PD-L1 inhibitors, which is also an urgent problem to be solved in immunotherapy at this stage (18). At the same time, immune checkpoint inhibitors may cause immune related adverse reactions and infusion related reactions in the process of clinical application, which still needs further research (19).

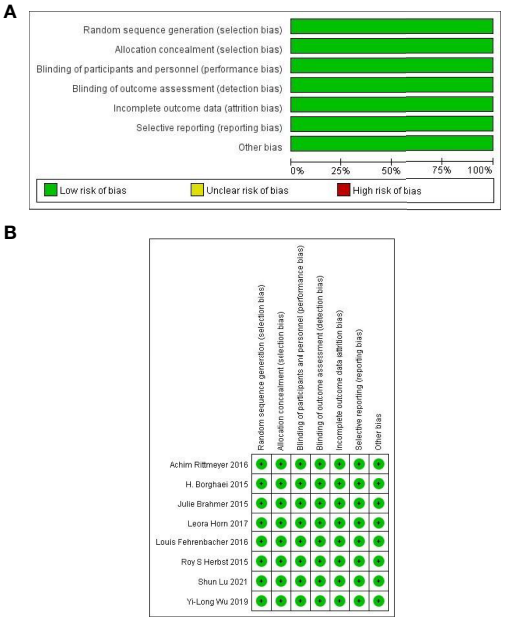

FIGURE 2 | (A, B) Evaluation results of methodology quality of included studies (7–14).

In this meta-analysis, we evaluated the efficacy of ICIs drugs and docetaxel in patients with NSCLC, and selected OS and PFS as the primary outcomes. The results showed that ICIs improved the HR and *p* of OS and PFS in terms of effectiveness, indicating that patients receiving immunotherapy had better OS and PFS than patients receiving docetaxel. In terms of safety, the risk ratio of adverse reactions at any level and above in the ICIs treatment group was significantly lower than that in the docetaxel group, suggesting that the safety of ICIs treatment was higher than that of docetaxel, and it is not easy to produce common and typical adverse reactions (fatigue, nauesa, ashenia, diarrhea, anemia).

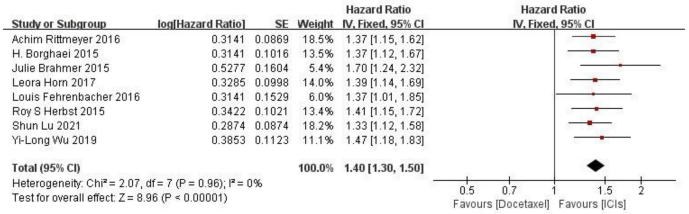

FIGURE 3 | Meta-analysis results of OS between ICIs group and docetaxel group.

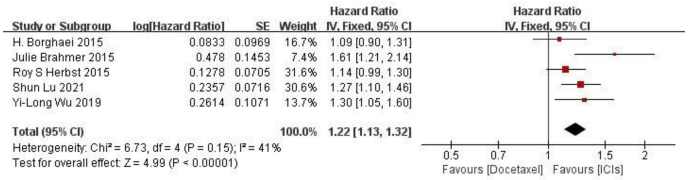

FIGURE 4 | Meta-analysis results of PFS between ICIs group and docetaxel group.

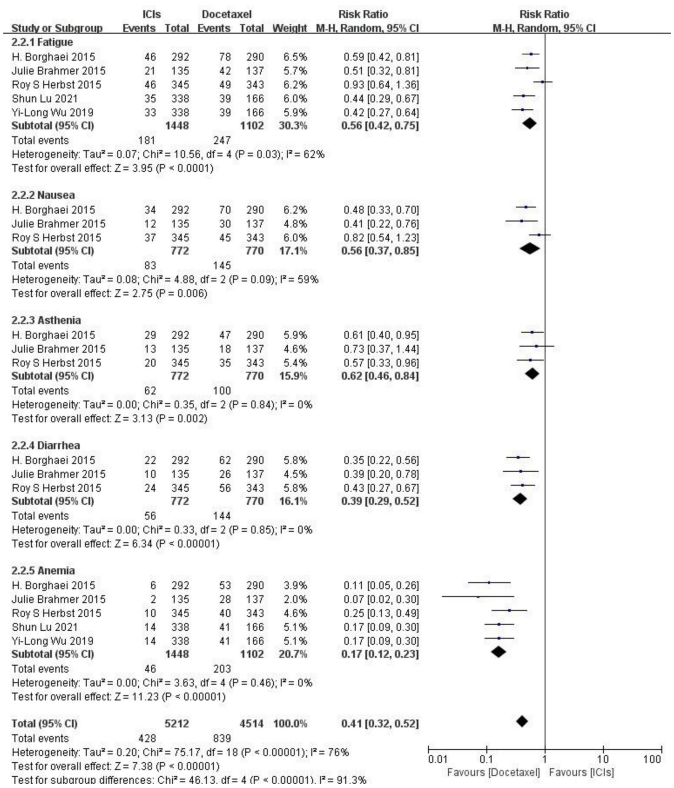

FIGURE 5 | Meta-analysis results of adverse reactions of any grade between ICIs group and docetaxel group.

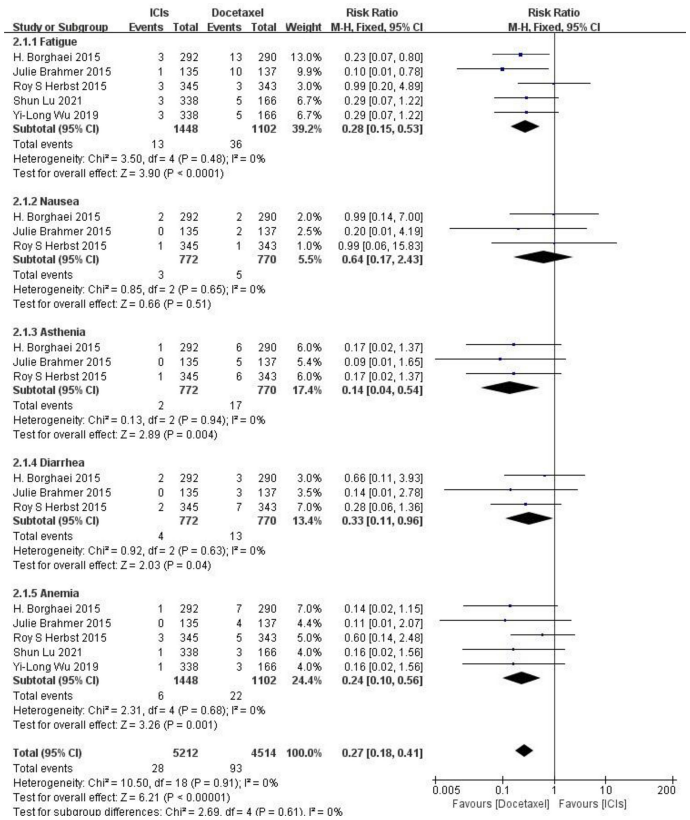

FIGURE 6 | Meta-analysis results of adverse reactions above grade 3 between ICIs group and docetaxel group.

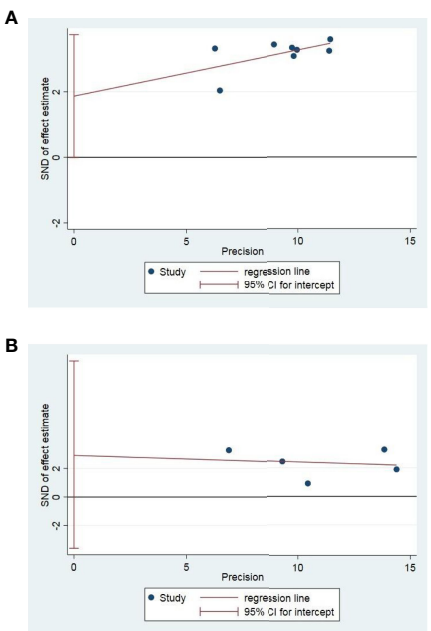

FIGURE 7 | Egger test of OS **(A)** and PFS **(B)**.

Many international researches also show similar results, Khan M et al. (20) shows that compared with chemotherapy drugs, ICI therapy (nivolumab, pembrolizumab, atezolizumab) leads to better OS (HR 0.72 [95% CI 0.63, 0.82; P <. 00001]), PFS (HR 0.84 [95% CI 0.72), 0.97; P <. 02]) and ORR (odds ratio [OR] 1.52 [95% CI 1.08, 2.14; P <. 02]). At the same time, higher safety was observed with ICI therapy (OR 0.31 [95% CI 0.26, 0.38; P <. 00001]). The results of this study are basically consistent with those of previous international studies.

This study also has some limitations: ① after systematic retrieval and screening, only 8 literatures were included for systematic evaluation and meta-analysis, and the sample size is too small; ② The heterogeneity of individual statistical results may affect the credibility of the research results; ③ Different types of NSCLC in different studies may increase heterogeneity and affect the reliability of the results. However, in the study, in order to better reduce the above bias, when implementing retrieval and data consolidation, this study will report scientifically and objectively as much as possible in accordance with the Cochrane system evaluation guidance manual.

In conclusion, compared with docetaxel, ICIs can prolong the OS and PFS of patients with NSCLC, with better clinical efficacy. This therapy may be a promising treatment. However, it still needs to be further confirmed by studies with multiple centers, larger sample size and higher quality.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WY and BX searched the database and analysed the data. MC, XL, JH and HS selected the study and extracted the data. WY and BX wrote the manuscript. YZ reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Choosing the optimal immunotherapeutic strategies for non-small cell lung cancer based on clinical factors

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The treatment landscape of advanced non-small cell lung cancer (NSCLC) has changed dramatically since the emergence of immune checkpoint inhibitors (ICIs). Although some patients achieve long survival with relatively mild toxicities, not all patients experience such benefits from ICI treatment. There are several ways to use ICIs in NSCLC patients, including monotherapy, combination immunotherapy, and combination chemoimmunotherapy. Decision-making in the selection of an ICI treatment regimen for NSCLC is complicated partly because of the absence of head-to-head prospective comparisons. Programmed death-ligand 1 (PD-L1) expression is currently considered a standard biomarker for predicting the efficacy of ICIs, although some limitations exist. In addition to the PD-L1 tumor proportion score, many other clinical factors should also be considered to determine the optimal treatment strategy for each patient, including age, performance status, histological subtypes, comorbidities, status of oncogenic driver mutation, and metastatic sites. Nevertheless, evidence of the efficacy and safety of ICIs with some specific conditions of these factors is insufficient. Indeed, patients with poor performance status, oncogenic driver mutations, or interstitial lung disease have frequently been set as ineligible in randomized clinical trials of NSCLC. ICI use in these patients is controversial and remains to be discussed. It is important to select patients for whom ICIs can benefit the most from these populations. In this article, we review previous reports of clinical trials or experience in using ICIs in NSCLC, focusing on several clinical factors that are associated with treatment outcomes, and then discuss the optimal ICI treatment strategies for NSCLC.

KEYWORDS

aged, interstitial lung disease (ILD), liver metastasis, performance status (PS), pleural effusion

1 Introduction

In the last 10 years, immune-checkpoint inhibitors (ICIs) have changed treatment strategies for non-small cell lung cancer (NSCLC). The benefit of ICIs over previous standard therapy (cytotoxic chemotherapy) has been demonstrated both as monotherapy and as combination therapy, regardless of previous treatment history (1–7). Response duration of ICIs tends to be longer than cytotoxic chemotherapy (1, 2, 5, 6). Survival duration of some patients with advanced NSCLC treated with ICI exceeded 3 years and notably, the 5-years follow-up form KEYNOTE-024 shows an OS rate of 32% (6, 8–13). In clinical trials, the 2-year survival rate of advanced NSCLC patients is 37%–45% when treated with combination therapy of ICI and chemotherapy as the first-line treatment and 23%–29% with ICI monotherapy for previously treated patients, while 18%–29% in treatment-naive patients and 8%–16% in previously-treated patients when treated with chemotherapy (14–16). Despite of these improved treatment outcomes by ICIs in clinical trials, there are still issues to be addressed in daily clinical practice. First, it is difficult to determine which treatment regimen is most suitable for individual cases. Many treatment regimens are available for patients with advanced NSCLC. Most patients with NSCLC experience disease progression as a result of primary or acquired resistance to ICIs (17, 18). In this review, we discuss the clinical factors that could influence the efficacy and safety of drugs including ICIs. Second, many patients in clinical practice do not fulfil the eligibility criteria for clinical trials (19–23). For example, aged patients or patients with poor performance status (PS) are usually considered ineligible for prospective clinical trials. Generally, because of their poor condition, it is difficult to treat these patients with cytotoxic chemotherapy, and their prognosis is worse than that of patients who fulfill the eligibility criteria of clinical trials. ICIs have different toxicity profiles than standard chemotherapy, and their cytotoxicity is usually mild. Therefore, ICIs may be a good treatment option for patients who do not meet the criteria for chemotherapy. It is important to select patients for whom ICIs can benefit the most from this population. In this review, we will summarize previous clinical studies of ICIs used for NSCLC, and then discuss the optimal ICI treatment strategies, focusing on the clinical factors that potentially predict ICI effects.

2 Previous randomized control trials including ICIs for NSCLC

Many studies on ICIs have been conducted in patients with advanced or recurrent NSCLC. Table 1 shows the major clinical trials that tested ICI regimens for treatment-naïve patients in which the primary endpoints were positive and negative, respectively. Tables 1A, C are for squamous NSCLC (Sq-NSCLC), while Tables 1B, D represent non-squamous NSCLC (NSq-NSCLC). The efficacy results were almost the same between Sq and non-Sq patients, except for the KEYNOTE-024 study. It should be noted that the results of KEYNOTE-024, KEYNOTE-042, IMPOWER-110, and KEYNOTE-598 includes both squamous and non-squamous NSCLC patients.

3 Clinical predictive factors for ICI treatment outcomes

3.1 Programmed death-ligand 1 (PD-L1) expression

PD-L1 tumor proportion score (TPS) is widely used to predict ICI effects. In the phase 2 KEYNOTE-001 trial, the objective response rates (ORR) of pembrolizumab for pre-treated NCSLC patients were 45%, 17%, and 11% in the subgroup with PD-L1 TPS score, assessed by the PD-L1 IHC 22C3 pharmDx, with ≥50%, 1%–49%, and <1%, respectively (34). Furthermore, the survival benefit of pembrolizumab was also associated with a high PD-L1 TPS. The superiority of pembrolizumab monotherapy over chemotherapy for treatment-naïve NSCLC patients was observed in both the PD-L1 TPS ≥ 50% and ≥1% groups in KEYNOTE-024 and 042 (2, 25). Subgroup analysis of these studies showed the association of higher PD-L1 TPS with better efficacy of pembrolizumab. This association was confirmed in real-world settings when limited to PD-L1 TPS ≥ 50% (35–37). These data support the notion that PD-L1 TPS assessed by 22C3 assay predicts the outcome of pembrolizumab monotherapy used in the first-line setting. Similar trends have been observed in clinical trials for other cancers (38–40).

The association between PD-L1 TPS and ICI effects was inconsistent when different methods were used to evaluate the TPS. In the Impower110 trial, the efficacy of atezolizumab monotherapy used as a first-line treatment in NSCLC patients was correlated with PD-L1 TPS assessed by SP142 assay, and superiority of atezolizumab over platinum-based chemotherapy was observed in the subgroup with PD-L1 TPS ≥ 50% (5). However, in the CheckMate 026 trial where nivolumab efficacy was tested, no superiority of nivolumab over platinum-based chemotherapy was seen either in the pre-planned group with PD-L1 TPS ≥ 5% or in the exploratory subgroup with PD-L1 TPS ≥ 50% (30). In this study, the PD-L1 TPS was assessed using the 28-8 antibody. This inconsistency among the studies may be attributed from the fact that the assessment assay used to evaluate PD-L1 expression differed in each clinical trial. On the other hand, Impower 110 trial compared PD-L1 scoring methods, SP142, 22C3 and SP263, as an exploratory analysis. Of note, median OS among patients with high PD-L1 TPS assessed by these three assays were similar. In clinical trials, PD-L1 assays often differ among

TABLE 1 Key clinical trials that tested ICI regimens for treatment-naïve patient.

A. Sq-NSCLC, positive study.

Study name	PD-L1 expression	experimental arm	control arm	OS (months, 95% CI)	OS HR [95% CI]	PFS (months, 95% CI)	PFS HR [95% CI]	ORR
KN407 (16, 24)	All	Pembro + Chemo	Placebo + Chemo	17.1 [14.4–19.9]	0.71 [0.58–0.88]	8.0 [6.3–8.4]	0.57 [0.47–0.69]	62.6%
KN024 † (2, 8)	≥ 50%	Pembro	Chemo	26.3 [18.3–40.4]	0.62 [0.48–0.81]	7.7 [6.1–10.2]	0.50 [0.39–0.55]	46.1%
KN042 † (25)	≥ 1%	Pembro	Chemo	16.7 [13.9–19.7]	0.81 [0.71–0.93]	5.4 [4.3–6.2]	1.07 [0.94–1.21]	27%
IM110 † (5, 11)	TC/IC 3 §	Atezo	Chemo	20.2 [17.2–25.6]	0.83 [0.62–1.10]	8.2 [6.8–11.4]	0.59 [0.43–0.81]	40.2%
CM227 ‡ (6, 13)	≥ 1%	Nivo + Ipi	Chemo	15.0 [12.5–18.7] ¶	0.63 [0.49–0.79]	4.1 [2.9–5.6]	0.77 [0.57–1.05]	34.7%
	negative	Nivo + Ipi	Chemo	NA	NA	5.1 [3.5–6.4]	0.74 [0.58–0.94]	27.3%
CM9LA ‡ (7, 10)	All	Nivo + Ipi + Chemo	Chemo	14.5 [13.1–19.3]	0.63 [0.47–0.85]	5.6 [4.3–9.7]	0.60 [0.44–0.81]	48.7%
EMP-L1‡ (26)	≥ 50%	Cemip	Chemo	NA	0.53 [0.36–0.77]	NA	0.53 [0.40–0.70]	NA

B. Nsq-NSCLC, positive study.

KN024 † (2, 8)	≥ 50%	Pembro	Chemo	26.3 [18.3–40.4]	0.62 [0.48–0.81]	7.7 [6.1–10.2]	0.50 [0.39–0.55]	46.1%
KN042 † (25)	≥ 1%	Pembro	Chemo	16.7 [13.9–19.7]	0.81 [0.71–0.93]	5.4 [4.3–6.2]	1.07 [0.94–1.21]	27%
KN189 (12, 14, 27)	All	Pembro + Chemo	Placebo + Chemo	22.0 [19.5–24.5]	0.56 [0.46–0.69]	9.0 [8.1–10.4]	0.49 [0.41–0.59]	48.3%
CM227 ‡ (6, 13)	≥ 1%	Nivo + Ipi	Chemo	19.2 [15.7–21.7] ¶	0.77 [0.66–0.90]	5.5 [4.1–7.6]	0.83 [0.68–1.01]	37.1%
	negative	Nivo + Ipi	Chemo	NA	NA	4.3 [2.9–6.4]	0.81 [0.62–1.07]	24.1%
CM9LA‡ (7, 10)	All	Nivo + Ipi + Chemo	Chemo	17.8 [14.1–20.7]	0.78 [0.63–0.96]	7.0 [5.6–8.3]	0.72 [0.59–0.88]	32.9%
IM150 (4, 9)	All	Atezo + Bev + Chemo	Bev + Chemo	19.5 [17.0–22.2]	0.80 [0.67–0.95]	8.4	0.57 [0.48–0.67]	63.5%
IM130 (28)	All	Atezo + Chemo	Chemo	18.6 [16.0–21.2]	0.79 [0.64–0.98]	7.0 [6.2–7.3]	0.64 [0.54–0.77]	49.2%
IM110 † (5, 11)	TC/IC 3 §	Atezo	Chemo	20.2 [17.2–25.6]	0.83 [0.62–1.10]	8.2 [6.8–11.4]	0.59 [0.43–0.81]	40.2%
EMP-L1 ‡ (26)	≥ 50%	Cemip	Chemo	NA	0.83 [0.59–1.16]	NA	0.65 [0.51–0.84]	NA
TASUKI-52 (29)	All	Nivo + Bev + Chemo	Bev + Chemo	25.4 [21.8–NR]	0.85 [0.63–1.14]	12.1 [9.8–14.0]	0.56 [0.43–0.71]	61.5%

† Both squamous and non-squamous histology are included, ‡ Subgroup analysis based on the histology, § This study met the primary outcome only in a TC/IC 3 population at first analysis, ¶ Histology-based OS was analyzed in the PD-L1 expression ≥1% and <1% combined patient population.
PD-L1, Programmed death-ligand 1; OS, overall survival; CI, confidence interval; PFS, progression free survival; ORR, objective response rate; NA, not available; NR, not reached.

different ICI drugs. Few information is available concerning analysis of the concordance among different PD-L1 assays (41).

In addition to the methods for TPS evaluation, the cut-off levels of PD-L1 expression are not fixed, and they sometimes change even in the middle of ongoing clinical trials (42–44). Another factor that may lead to these inconsistent results is heterogeneity of PD-L1 expression. The spatial and temporal heterogeneity of PD-L1 expression in the same tumor has been previously reported (45–49). PD-L1 expression tends to be high in primary sites, adrenal glands, liver, and lymph nodes, but low in the bone and brain (45, 46). When PD-L1 TPS of lymph node metastatic site was assessed, the association with ICI efficacy was not observed (45). In the clinical trials discussed above, the number of biopsy sites where PD-L1 TPS was evaluated varied among cases.

Inconsistency in the predictive value of PD-L1 expression among clinical trials was also observed in the setting of combination chemoimmunotherapy. PD-L1 expression was positively correlated with progression-free survival (PFS) in the combination of pembrolizumab with platinum plus pemetrexed for NSq-NSCLC; atezolizumab with carboplatin, paclitaxel, and bevacizumab for NSq-NSCLC; and atezolizumab with carboplatin plus nab-paclitaxel for Sq-NSCLC (4, 27, 50). However, this correlation was not proven in the atezolizumab with carboplatin plus nab-paclitaxel combination for NSq-NSCLC and pembrolizumab with carboplatin plus either paclitaxel or nab-paclitaxel for Sq-NSCLC (24, 28). The aforementioned ICIs are inhibitors of the PD-1/PD-L1 checkpoint pathway. In contrast, ipilimumab is a monoclonal antibody for cytotoxic T-lymphocyte associated protein 4 (CTLA-4), which is independent of the PD-1/PD-L1 pathway. It is reasonable that this agent can be effective even in PD-L1-negative population (6, 13). In fact, in the CheckMate 9LA trial, where the combination of nivolumab and ipilimumab with chemotherapy was studied, favorable outcomes were observed regardless of PD-L1 expression for both NSq-NSCLC and Sq-NSCLC (7, 10). This trend is consistent with previous clinical trials involving patients with melanoma and renal-cell carcinoma (51, 52). However, when nivolumab and ipilimumab are used without chemotherapy for patients with NSCLC, median overall survival (OS) was numerically greater in higher

TABLE 1 Key clinical trials that tested ICI regimens for treatment-naïve patient.

C. Sq-NSCLC, negative study.

Study name	PD-L1 expression	experimental arm	control arm	OS (months, 95% CI)	OS HR [95% CI]	PFS (months, 95% CI)	PFS HR [95% CI]	ORR
IM131 (50)	All	Atezo + Chemo	Chemo	14.2 [12.3–16.8]	0.88 [0.73–1.05]	6.3 [5.7–7.1]	0.71 [0.60–0.85]	49.7%
CM026 ‡ (30)	≥ 5%	Nivo	Chemo	10.9 [NA]	0.77 [0.48–1.25]	3.3 [NA]	0.87 [0.53–1.41]	NA
Govidant et al. (53)	≥ 1%	Ipi + Chemo	Placebo + Chemo	13.4 [11.8–14.8]	0.91 [0.77–1.07]	5.6 [5.4–5.9]	0.87 [0.75–1.01]	44%
MYSTIC (31) †	≥ 25%	Durva	Chemo	16.3 [12.2–20.8]	0.76 [0.56–1.02]	4.7 [3.1–6.3]	0.87 [0.59–1.29]	35.6%
		Durva + Treme	Chemo	11.9 [9.0–17.7]	0.85 [0.61–1.17]	3.9 [2.8–5.0]	1.05 [0.72–1.53]	34.4%
KN598 (32) †	≥ 50%	Ipi + Pembro	Placebo + Pembro	21.4 [16.6–NR]	1.08 [0.85–1.37]	8.2 [6.0–10.5]	1.06 [0.86–1.30]	45.4%

D. Nsq-NSCLC, negative study.

CM026 ‡ (30)	≥ 5%	Nivo	Chemo	14.5 [NA]	1.13 [0.84–1.50]	4.2 [NA]	1.24 [0.95–1.62]	NA
MYSTIC (31) †	≥ 25%	Durva	Chemo	16.3 [12.2–20.8]	0.76 [0.56–1.02]	4.7 [3.1–6.3]	0.87 [0.59–1.29]	35.6%
		Durva + Treme	Chemo	11.9 [9.0–17.7]	0.85 [0.61–1.17]	3.9 [2.8–5.0]	1.05 [0.72–1.53]	34.4%
KN598 (32) †	≥ 50%	Ipi + Pembro	Placebo + Pembro	21.4 [16.6–NR]	1.08 [0.85–1.37]	8.2 [6.0–10.5]	1.06 [0.86–1.30]	45.4%
IM132 (33)	All	Atezo + Chemo	Chemo	17.5 [13.2–19.6]	0.86 [0.71–1.06]	7.6 [6.6–8.5]	0.60 [0.49–0.72]	47%

† Both squamous and non-squamous histology are included, ‡ Subgroup analysis based on the histology, § This study met the primary outcome only in a TC/IC 3 population at first analysis, ¶ Histology-based OS was analyzed in the PD-L1 expression ≥1% and <1% combined patient population.
PD-L1, Programmed death-ligand 1; OS, overall survival; CI, confidence interval; PFS, progression free survival; ORR, objective response rate; NA, not available; NR, not reached.

PD-L1 expression population in the CheckMate 227 trial (13). Considering the negative result of the KEYNOTE-598 study, where pembrolizumab plus ipilimumab for metastatic NSCLC with PD-L1 TPS ≥ 50% was tested (Tables 1C, D), the benefit of adding ipilimumab to an anti PD-1 antibody for patients with PD-L1 TPS ≥ 50% should be discussed carefully (32).

Overall, although the results are inconsistent, PD-L1 expression can be used as a predictive biomarker for ICI effects. Recently, a combined positive score has emerged as a new method instead of PD-L1 TPS to evaluate PD-L1 expression (54). A combined positive score is calculated as the proportion of tumor cells, lymphocytes, and macrophages that were positively stained by PD-L1 immunohistochemical staining of total tumor cells. The KEYNOTE-048 trial of pembrolizumab treatment for head and neck cancer demonstrated a positive association of favorable survival with PD-L1 expression level assessed by the combined positive score (43).

3.2 Driver mutation

A correlation between driver mutation subtypes and ICI efficacy has been reported. The ImmunoTarget group retrospectively compared ORR after ICI treatment among NSCLC patients with various driver mutations. It was revealed that the KRAS-driven and BRAF-driven subgroups appreciated a greater benefit from ICI than EGFR-driven or ALK-driven subgroups (55).

Several clinical trials have suggested favorable efficacy of ICIs in NSCLC patients with KRAS mutations (1, 56–60). When pembrolizumab was used as monotherapy in NSq-NSCLC patients with PD-L1 TPS ≥ 50%, KRAS mutation was associated with longer OS, while this association was not observed when pembrolizumab was used as combined chemoimmunotherapy (61, 62). Notably, co-mutation of SKT11/LKB11 with KRAS mutation, which exists in approximately 30% of KRAS-mutated NSCLC, is associated with an unfavorable efficacy of ICIs (63, 64). This mutation was associated with lower PD-L1 expression and fewer tumoricidal immune infiltrates.

Many recent clinical trials of ICIs have excluded those with actionable driver mutations, such as EGFR mutations and ALK fusions. The decision for this exclusion is probably based on the results of a subgroup analysis in large randomized controlled trials conducted in the early days of the ICI era, such as CheckMate 017, CheckMate 057, KEYNOTE-010, and OAK, which compared ICIs and docetaxel for their efficacy and safety as second-line therapy in advanced NSCLC (1, 56, 65, 66). The meta-analysis of these trials demonstrated that the integrated OS hazard ratio of ICIs compared to docetaxel was 1.05 [95% confidence interval [CI]: 0.70–1.55] in the EGFR-mutant subgroup and 0.66 [95% CI: 0.58–0.76] in the EGFR wild-type subgroup (59, 60, 67). Retrospective studies have also shown generally low efficacy of ICIs in driver mutation-positive NSCLC (55, 68, 69). In addition, a recently published phase 2 study comparing nivolumab and carboplatin-pemetrexed for EGFR-mutated NSCLC with resistance to EGFR-

tyrosine kinase inhibitors (TKI) revealed significantly worse survival in patients treated with nivolumab (70). Poor efficacy of ICIs for NSCLC patients with EGFR mutations is thought to be derived from a lower tumor mutation burden and an immunosuppressive tumor microenvironment (71, 72).

However, some prospective studies have shown comparable or superior efficacy of ICIs in NSCLC patients with driver mutations (73–77). An exploratory subgroup analysis of the IMpower150 trial demonstrated that in NSq-NSCLC patients with sensitizing EGFR mutations, OS of atezolizumab, carboplatin, paclitaxel, and bevacizumab combination group was longer than carboplatin, paclitaxel, and bevacizumab combination group. (median OS was26.1 months [95% CI 17.0–41.4] in the atezolizumab arm *vs.* 20.3 months [95% CI 13.4–33.6] in the control arm; hazard ratio [HR] 0.91 [95% CI 0.53–1.59]) (73, 74). Based on these results, two prospective studies are ongoing in Japan to verify the efficacy and safety of combination chemoimmunotherapy with atezolizumab, carboplatin, paclitaxel, and bevacizumab in EGFR-mutant NSCLC patients who were already treated with an EGFR-TKI (78, 79).

Usually, molecular-targeted therapies are more effective than ICIs or cytotoxic agents for NSCLC patients with actionable driver mutations (78–88). Combination therapy with TKIs and ICIs has failed due to severe adverse events (89–91). Based on the idea of "best drug first," there is no doubt that the first-line therapies for NSCLC patients with actionable driver mutations are TKIs (92–94). However, to the best of our knowledge, there is no clear conclusion as to whether ICIs can be a treatment option for these patients at any late treatment line. Some retrospective studies have suggested that PD-L1 expression predicts ICI efficacy, even in EGFR-mutant NSCLC (95). Furthermore, interestingly, PD-L1 expression was upregulated after EGFR-TKI therapy *via* ERK1/2 pathway modulation (47, 48). It has also been reported that EGFR mutations can upregulate PD-L1 expression through the Ras/RAF/MEK/ERK, PI3K/AKT/mTOR, JAK/STAT, NF-kB, and YAP pathways (96–99). Further studies are warranted to clarify the association of driver mutations with PD-L1 expression or ICI efficacies.

3.3 Metastatic site

3.3.1 Liver

Liver metastases have been validated as negative prognostic factors for NSCLC patients (100, 101). More metastases in the liver are correlated with worse survival (102). In addition, the presence of liver metastases predicts poor outcomes after ICI monotherapy (3, 36, 72, 103–106). One possible underlying mechanism is systemic immune tolerance which is mediated by a number of specialized antigen-presenting cells, including dendritic cells, Kupffer cells, liver sinusoidal endothelial cells, and hepatic stellate cells (102, 105, 107–110). From the viewpoint of PD-L1 spatial heterogeneity, PD-L1 expression was relatively higher in liver than other organs in NSCLC patients (45). Conversely, the presence of liver metastases was associated with a lower CD8+ T-cell count at the invasive tumor margin among patients with NSCLC and melanoma who received pembrolizumab (105). This suggests systemic activation of the regulatory immune microenvironment in patients with liver metastases, which results in a poor response to ICI treatments in the presence of liver metastases despite the relatively higher PD-L1 expression.

Currently, there is no consensus regarding the optimal treatment for NSCLC with liver metastases. Although cytotoxic agents and ICIs elicit relatively little efficacy in NSCLC with liver metastases when used alone, one retrospective study showed that combination chemotherapy may be effective (111). Some clinical trials have also suggested that the addition of bevacizumab to ICI treatment is effective for patients with NSCLC with liver metastases (29, 73, 112). Bevacizumab is an anti-vascular endothelial growth factor (VEGF) antibody. Preclinical and clinical data have demonstrated that bevacizumab normalizes vasculature, restores dendritic cell maturation, and reduces T-regulatory cells and myeloid-derived suppressor cells in cancer patients (113–117). Considering these pharmacological effects, treatment regimen containing bevacizumab may be reasonable for patients of NSCLC with liver metastases, where immunosuppressive microenvironment is an issue for ICI treatments as discussed above.

The presence of liver metastases is thought to be associated with the onset of hyperprogressive disease (HPD) (118–120). HPD is characterized by rapid disease progression after initiation of ICIs, often defined as a > 50% increase in tumor size within less than 2 months after initiation of ICIs, although currently there is no widely accepted definition (118, 121). HPD is associated with worse clinical outcomes (118). Other than liver metastases, high LDH levels, low Albumin levels, multiple metastatic sites, poor PS, and a Royal Marsden Hospital prognostic score of ≥ 2 were associated with the risk of HPD occurrence (118, 120). However, underlying mechanisms of HPD are not understood well. Treatment strategies for NSCLC patients at high risk of HPD have not yet been established.

3.3.2 Brain

Radiation therapy is the most important treatment strategy that should be considered first for NSCLC patients with brain metastases (BMs), especially when clinical symptoms derived from BMs are present (122). Thus the role of ICIs, with or without cytotoxic agents, can be discussed only for the regulation of BMs that are asymptomatic or already treated with radiation. The efficacy of ICI in patients with leptomeningeal disease requires further investigation (123).

The survival benefit of ICIs is similar regardless of the presence or absence of BMs based on a subgroup analysis of clinical trials of ICIs with or without cytotoxic agents, as listed in

Table 1 (6, 10, 12, 26, 29, 124, 125). A meta-analysis of 10 clinical trials with ICIs in NSCLC showed an OS HR of 1.25 (95% CI = 1.09–1.44, I2 = 43.8%, P <.001) for BMs compared with those without BMs (104). A retrospective study showed that the presence of BMs and a larger maximum diameter of brain metastases were associated with worse prognosis of NSCLC patients after ICI monotherapy in the second or later treatment line (126). To our knowledge, there are few available data regarding intracranial response rates to ICIs in NSCLC patients. Phase 2 studies on melanoma demonstrated that combination therapy with nivolumab and ipilimumab achieved higher intracranial response rates than treatment with nivolumab alone (127). Considering these data, patients with BMs can be treated in the same way as those without BMs, but combination immunotherapy with anti-PD-1 and anti-CTLA-4 agents, with or without cytotoxic agents, may provide better outcomes (124, 128, 129).

3.3.3 Pleural effusion

Previous studies have reported that malignant pleural effusion is present in 11%–32% of patients with advanced NSCLC (130–132). Even a small amount of pleural effusion (< 10-mm thick on chest computed tomography) is an independent predictor of worse survival (130). This tendency was also observed in cases treated with ICIs, although the available data are limited to retrospective studies. The presence of malignant pleural effusion was associated with worse prognosis in NSCLC patients treated with a single ICI in either first-line or later treatment lines (126, 133–135). Recently reported retrospective study suggests that combination chemoimmunotherapy is more effective than pembrolizumab monotherapy as a first line treatment for NSCLC patients with malignant pleural effusion (136). As observed in liver metastases, malignant pleural effusion induces systemic immunosuppressive microenvironment through several mediators and pathways, including myeloid derived suppressor cells, neutrophils, macrophages, T-regulatory cells, and dysfunctional T cells that might result in low efficacy of ICIs (126, 137). As for safety, existence of pleural effusion before treatment with nivolumab was indicative of poor outcomes of interstitial lung disease (ILD) induced as an immune-related adverse event (irAE) when it occurs (138).

In the setting of combination chemoimmunotherapy, we could not find any studies to assess the effects of the presence of malignant pleural effusion on the efficacy of therapy or to evaluate which combination of drugs is better for use in cases with malignant pleural effusion. VEGF is thought to be one of the key factors that cause malignant plural effusion by increasing vascular and mesothelial permeability and capillary fluid leakage (139). In fact, several studies suggest the efficacy of bevacizumab for the management of malignant pleural effusion in Nsq-NSCLC (117, 140–144). VEGF also plays a principal role in immunosuppressive microenvironment as mentioned in the previous section (113–117). Therefore, the combination of bevacizumab and ICI is potentially a good treatment strategy for patients with malignant pleural effusion, although there is few evidence to support this, thus far.

4 ICIs for the special population

4.1 Elderly

An FDA meta-analysis of four randomized control trials in which ICI monotherapy and docetaxel were compared for patients with disease progression after platinum doublet treatment demonstrated similar survival benefits between these regimens, regardless of age (145). Another meta-analysis of clinical trials for other tumor types also showed a comparable efficacy of ICI monotherapy between patients aged ≥ 65 and < 65 years (19, 146). Furthermore, real-world data supported the evidence for efficacy and safety of ICI monotherapy for the elderly NSCLC patients (147–150). These data suggested that it is the PS or comorbidities rather than age that is associated with the outcome of ICI treatment in the elderly patients (19, 147, 148). It should be noted that the cutoff value for defining elderly varies among studies.

In combination chemoimmunotherapy, more attention should be paid to elderly patients. In the KEYNOTE-189 trials, in which treatment-naïve NSCLC patients were treated with a platinum agent and pemetrexed with or without pembrolizumab, the addition of pembrolizumab was associated with worse survival benefit in the elderly, which was defined as ≥ 75 years old (151). A retrospective study showed a similar result, that is, poor outcome of combination chemoimmunotherapy in the elderly group (152). In general, organ function declines with age, but it is difficult to evaluate these functions sufficiently with clinical examinations that are currently available. Clinical assessment tools for the elderly, such as the comprehensive geriatric assessment and Charlson comorbidity index, have been tested to predict the prognosis of anti-cancer therapy in many clinical trials, but their usefulness has not yet been established (153–157). Currently, there are no clinical assessment tools available to predict which elderly patients can tolerate chemoimmunotherapy well.

4.2 Performance status 2

In the ECOG 1594 study, which revealed almost similar efficacy and safety profiles among four platinum doublet regimens, a subgroup analysis showed that adverse events increased, and prognosis worsened in patients with a PS of 2 compared to those with a PS of 0 or 1 (158, 159). Historically, this is a pivotal study. Thereafter, for more than 10 years, cytotoxic agent monotherapies have been standard therapies for patients with a PS of 2. On the other hand, the advantage of ICIs is their favorable toxicity profiles. Therefore, ICIs may be an alternative treatment option for this population. Many studies have suggested that PS is not associated with the frequency or severity of irAEs (39, 150, 160–162). For

example, in CheckMate 153, a prospective study validating the safety of second-line treatment with nivolumab for NSCLC patients aged ≥ 70 years and with a PS of 2, the incidence rates of grades 3–5 and any grade adverse events were not increased in the population with a PS of 2 (9% and 29%, respectively) compared to the overall population, including a PS of 0–2 (6% and 37%, respectively), and toxicity profiles were comparable between these populations (150). The toxicities of ICIs seem to be acceptable for patients with poor PS.

Regarding prognosis, in both prospective and retrospective studies of ICI monotherapy for NSCLC, patients with a PS ≥ 2 who were treated with ICI monotherapy showed poor survival (150, 161–165). The hazard ratio of PFS ranged from 2.00 to 2.39 and OS ranged from 2.72 to 2.82 in patients with a PS ≥ 2 compared with a PS of 0 or 1. Studies on ICI monotherapy in a relatively large number of patients with a PS ≥ 2 are summarized in Table 2. Unlike PFS and OS, ORR results were inconsistent among the studies. As shown in Table 2, some studies have shown that the ORR of patients with a PS 2 was comparable to that of patients with a PS 0–1 after ICI monotherapy (162, 165). Poor PS of NSCLC patients may result from many different reasons, such as cancer burden, cancer progression rate, comorbidities unrelated to cancer, or a combination of these factors. The analysis of ICI efficacy in patients with a PS 2 based on the reasons for poor PS may help us better understand who is suitable for ICI treatment in this population.

4.3 Interstitial lung disease

Patients with ILD have been excluded from most randomized controlled trials in which ICIs are involved. However, in the real world, ILD is seen frequently (at a rate of 14%) in treatment-naïve patients with NSCLC (166). ILD is an independent risk factor for drug-induced lung injuries, including ICI-related injuries and is associated with poor survival in NSCLC patients treated with ICIs (167–169). Drug-induced lung injuries caused by ICIs are the most common irAE that lead to the discontinuation of ICIs and are associated with worse survival (65, 66, 170). ILD includes a very wide spectrum, and its radiological classification is complex. Radiological assessments of ILD are different, even among radiologists (171, 172). This makes it difficult to stratify the degree of risks of pre-existing ILD for ICI-induced lung toxicities.

Several clinical trials have assessed the efficacy and safety of ICIs in patients with ILD. The AMBITIOUS trial is a prospective study of atezolizumab in NSCLC patients with idiopathic, chronic fibrotic interstitial pneumonia whose %VC was 70% or larger. This study was discontinued early because of the high incidence of pneumonitis (29.4%) (173). In this study, pre-existing honeycomb lung was associated with a high risk of frequency and severity of pneumonitis (57.1% of patients with pre-existing honeycomb lung suffered from drug-induced pneumonitis with a grade greater than or equal to 3). The honeycomb lung has also been reported to be associated with cytotoxic chemotherapy-related exacerbation of ILD (174). Another prospective study to evaluate the efficacy and safety of nivolumab in NSCLC patients with mild idiopathic interstitial pneumonia demonstrated favorable efficacy and a tolerable safety profile, where two out of 18 patients developed grade 2 pneumonitis (175). In this study, patients with mild idiopathic, classified as radiological possible or inconsistent with usual interstitial pneumonia (UIP), were included only when their %VC was 80% or more. Therefore, patients with radiological UIP patterns were excluded. These studies imply

TABLE 2 ICI for PS2.

Author/ year	Trial name	Type of study	Number of patients whose PS > 1	Proportion of PS > 1/ total	Treatment line	Drug	OS (months, 95% CI)	PFS (months, 95% CI)	ORR	Incidence of TRAE of grade3-5
Spigel DR, et al., 2019 (150)	CheckMate 153	prospective	128	9.0%	2nd or later	Nivolumab	4.0 [3.1–6.2]	NA	NA	9%
Felip E, et al., 2020 (161)	CheckMate 171	prospective	103	12.7%	2nd or later	Nivolumab	5.2 [3.0–7.6]	NA	1.6%	6.8%
Middleton G, et al., 2020 (162)	PePS2	prospective	60	100%	1st: 40% Subsequent: 60%	Pembrolizumab	9.8 [7.1–14.6]	4.4 [3.3–9.9]	27%	73%
Matsubara T, et al., 2021 (163)		retrospective	11	8.8%	1st or 2nd: 43.2% 3rd or later: 56.8%	Nivolumab or Pembrolizumab	NA	NA	9.1%	18.2%
Sehgal K, et al., 2021 (165)		retrospective	29	39.2%	1st: 72.4% Subsequent: 27.6%	Pembrolizumab	4.1 [2.1–6.9]	2.3 [1.8–4.8]	17.9%	17.2%

PS, performance status; OS, overall survival; CI, confidence interval; PFS, progression free survival; ORR, objective response rate; TRAE, treatment related adverse events.

that the presence or absence of a honeycomb lung is the principal factor in predicting the safety of ICI treatment.

ILD related to ICIs may occur even in patients without ILD at the initiation of ICI therapy. Several risk factors for the onset and severity of ICI-induced lung toxicities have been suggested, including the primary tumor site of the lung, ICI combination therapy rather than ICI monotherapy, PD-1 inhibitors compared with PD-L1 inhibitors or CTLA-4 inhibitors, and the presence of pleural effusion before treatment (137, 176–179).

5 Discussion and conclusion

ICIs are now indispensable agents for NSCLC treatment and contribute to the extension of survival in NSCLC patients. Considering their relatively mild toxicities, ICIs could provide an opportunity of treatment for patients who cannot tolerate treatment with cytotoxic agents, such as elderly or patients with poor PS. As discussed in this paper, many clinical factors may affect the efficacy and safety of ICI treatment. PD-L1 is currently considered a predictive biomarker of ICI treatment, but clinicians should keep in mind that this is not a perfect biomarker as mentioned above. Emerging biomarkers, including tumor mutational burden, neoantigen load, tumor-infiltrating lymphocytes, immune-regulatory mRNA expression and blood biomarkers, are reported as possibly predictive (180). Further studies are warranted in this area.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Serum cytokine levels for predicting immune-related adverse events and the clinical response in lung cancer treated with immunotherapy

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Background: At present, immunotherapy has become an important treatment for lung cancer. With the widespread use of immune checkpoint inhibitors (ICIs), we must be strict with the emergence of immune related adverse events (irAEs). There are also some patients who do not respond to immunotherapy. However, there was no biomarkers to predict the safety and efficacy of immunotherapy. The selection of immunotherapy beneficiaries contributes to improving the efficacy and safety of lung cancer treatment.

Method: The electronic medical records of 221 lung cancer patients with complete clinical data who received immunotherapy from the First Affiliated Hospital of Xi 'an Jiaotong University from November 2020 to October 2021 were collected and followed up. IBM SPSS Statistic 26.0 and R 4.1.2 software were used for statistical analysis and mapping.

Results: 1.A total of 221 lung cancer patients receiving immunotherapy were included in the study. Higher baseline levels of IL-1β (7.88 vs 16.16pg/mL, *P*=0.041) and IL-2 (1.28 vs 2.48pg/mL, *P*=0.001) were significantly associated with irAEs. Higher levels of IL-5 (2.64 vs 5.68pg/mL, *P*=0.013), IFN-α (1.70 vs 3.56pg/mL, *P*=0.004) and IFN-γ (6.14 vs 21.31pg/mL, *P*=0.022) after the first cycle therapy were associated with irAEs. There was no statistical significance between cytokines and irAEs after the second cycle therapy. Higher IL-5 levels in peripheral blood (9.50 vs 3.57pg/mL, *P*=0.032) were associated with the occurrence of irAEs after the third cycle therapy.2.The efficacy of immunotherapy was assessed in 142 lung cancer patients. There was no statistical significance between baseline cytokine levels and clinical benefit. After the first cycle therapy, the level of serum cytokines had no statistical significance with the occurrence of immunotherapy clinical benefit. Lower serum levels of IL-10 (2.66 vs 1.26pg/mL, *P*=0.016) and IL-17 (8.47 vs 2.81pg/mL, P=0.015) were associated with clinical benefit after the second cycle therapy. Lower serum levels of IL-6 (10.19 vs 41.07pg/mL, *P*=0.013) and IL-8 (8.01 vs 17.22pg/mL, *P*=0.039) were associated with clinical benefit of immunotherapy after the third cycle therapy.

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Conclusion: 1.Baseline IL-1β and IL-2 levels in peripheral blood were associated with the occurrence of irAEs in lung cancer patients. The levels of IL-5, IFN-α and IFN-γ during treatment were associated with irAEs.2. Baseline cytokine levels in peripheral blood were not associated with immunotherapy efficacy. The levels of IL-6, IL-8, IL-10, and IL-17 levels during treatment were associated with immunotherapy efficacy.

KEYWORDS

biomarkers, cytokines, immunotherapy, lung cancer, immune related adverse events

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide (1).Treatment with immune checkpoint inhibitors (ICIs) has led to a shift in the treatment of solid tumors, including lung cancer (2–4). Although recent clinical studies have demonstrated that programed cell death ligand-1 (PD-L1) expression on tumor cells is associated with clinical benefits in the treatment of lung cancer (3, 5), anti ICIs is also effective in some patients whose PD-L1 levels are low in their tumor tissue (2, 4). Moreover, because of the difficulty associated with obtaining tumor tissues, the identification of prognostic biomarkers in circulating blood for patient selection in pragmatic clinical settings would be of considerable value for optimizing and personalizing immunotherapy. Some reports have also suggested that the tumor mutational burden (TMB), the neoantigen burden and the presence of tissue infiltrating lymphocytes are predictive biomarkers in ICI treatment (6, 7). But the sensitivity and specificity of these biomarkers are still insufficient.

Cytokines are the major modulators of the innate and adaptive immune system, mainly involved in maintaining immune homeostasis and mediating immune responses related to infection, autoimmune diseases and cancer. The functions of cytokines are complex and varied. They can protect the body, and excessive activation or severe deficiency can also cause autoimmune diseases or promote the development of cancer (8) (Figure 1). Cytokines involved in cell communication include interleukin, IFN, some members of the TNF superfamily, chemokines and growth factors, etc. Signal transmission is mainly through paracrine and autocrine functions of these cytokines.

An increasing number of preclinical and clinical studies have suggested that infiltrating immune cells within a tumor or the tumor cells themselves produce cytokines and chemokines, leading to modulation of the tumor microenvironment and promoting angiogenesis, growth, invasion and metastasis (9). In addition, cytokines play a functional role in promoting tumor cell growth (pro-tumor factor) or limiting tumor cell growth (anti-tumor factor) (10) (Figure 2). A longitudinal assessment of cytokine profiles in patients with metastatic melanoma receiving immunotherapy had reportedly established their association with irAEs progression and severe irAEs (8). Recent studies had shown that increased IL-1β and IFN-γ during treatment may be positive indicators of efficacy, while increased IL-6 during treatment might be predictive of poorer outcomes in patients with advanced NSCLC recieving immunotherapy (11).

In this study, we explored the biomarkers associated with clinical benefits such as tumor response and onset of irAEs. The aim of our study was to investigate whether a defined cytokine panel (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IFN-α, IFN-γ, TNF-α) can play a prognostic or predictive role in lung cancer patients treated with immune checkpoint inhibitors to assess any potential correlations between their serum levels and clinical safety and the treatment response.

Abbreviations: ICIs, Immune checkpoint inhibitors; IFN-γ, Interferon gamma; IL, Interleukin; IL-1RA, Interleukin 1 receptor antagonist; irAEs, Immune-related adverse events; PD-1, Programmed cell death protein 1; PD-L1, Programmed death-ligand 1; TNF-α, Tumor necrosis factor alpha; TNF-γ, Tumor necrosis factor gamma; CTCAE, Common Terminology Criteria for Adverse Events; RECIST, Response Evaluation Criteria in Solid Tumors; ICIs, Immune checkpoint inhibitors; TMB, Tumor mutational burden CB, Clinical benefit; NCB, No clinical benefit; PR, partial response; SD, stable disease; PD, progressive disease; PFS, Progression-free survival; AE, Adverse events; NAE, Non-adverse events; NK, Natural killer; NSCLC, Non-small cell lung cancer; CTLA-4, Cytotoxic T lymphocyte-associated antigen-4; FDA, Food and Drug Administration; TILs, Tumor-infiltrating lymphocyte; CPI, Checkpoint inhibitor.

Materials and methods

Patients selection

We prospectively analyzed patients treated at the First Affiliated Hospital of Xi'an Jiaotong University from November 2020 to September 2021. Eligible patients were

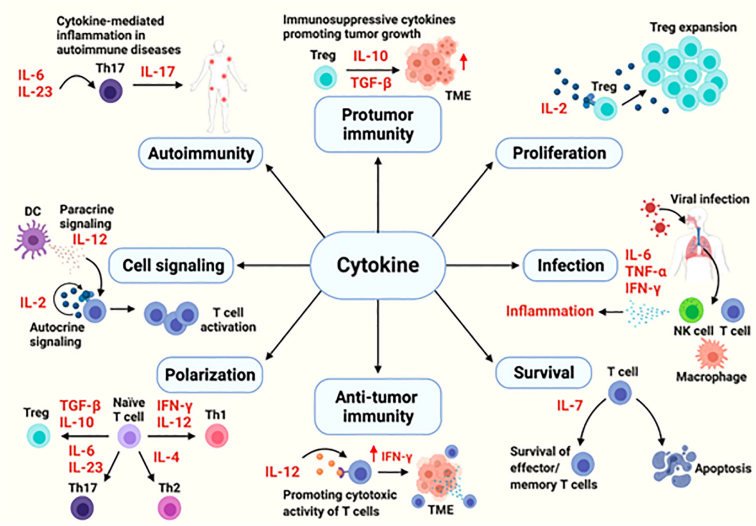

FIGURE 1
The cytokines in immune processes (8).

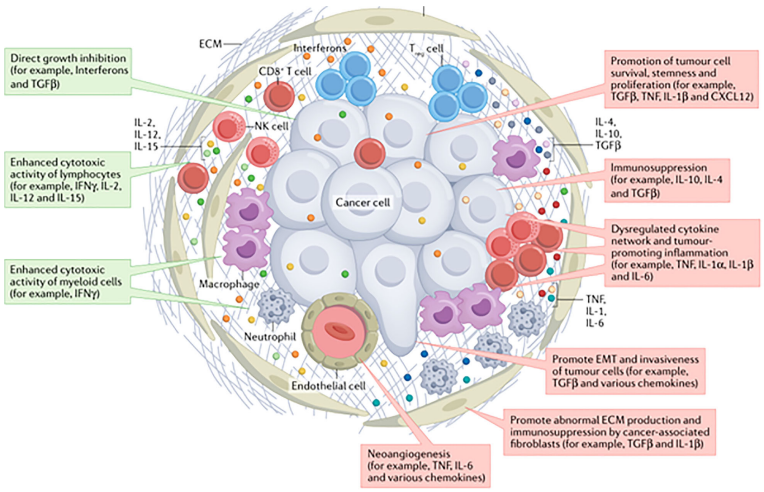

FIGURE 2
The cytokines in tumor environment (10).

adults with histologically confirmed lung cancer. Patients with a previous history of systemic immunosuppressive therapy or active autoimmune disease were excluded (Figure 3). Agent choice was based on PD-L1 status and patients' previous treatment history (first- or second-line setting). 221patients were selected in strict accordance with inclusion and exclusion criteria.

Toxic effects were graded with the use of the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.0). Scheduled computed tomography or magnetic resonance imaging was performed every 9-12 weeks. Immune-related response criteria were carried out using the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1).

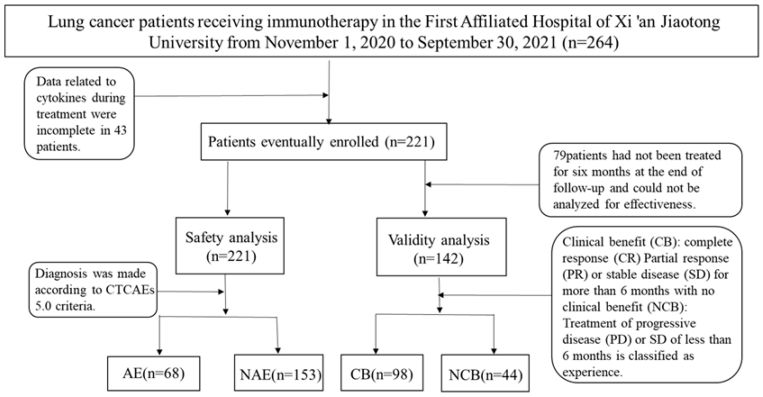

FIGURE 3
Selection process for patients.

Clinical benefit (CB) was classified as a complete response (CR), partial response (PR), or stable disease (SD) in excess of 6 months. Individuals experiencing progressive disease (PD) on therapy or who achieved SD of less than 6 months were classified as experiencing no clinical benefit (NCB).

Cytokine testing in blood by flow cytometry

All patients collected blood samples before starting immunotherapy and the first three cycles (every 3 weeks/1 cycle, a total of four cycles), which is based on the immune system from innate response into adaptive response to determine the necessary time. Serum samples collected and processed the same standardized scheme of detecting serum cytokine IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IFN-α, IFN-γ and TNF-α levels which were worked by pairing biotin-labeled cytokines with antibodies and cytokines in the sample. Then combining with cytokine antibodies coupled with fluorescent-emitting microspheres to form sandwiches. Finally, reaction with phyoglobinin-labeled streptavidin was detected by flow cytometry. Within the detection range, fluorescence intensity was proportional to the cytokine content.

The sample collection and processing

Serum collection:Blood samples were collected using standard tubes. After solidification at room temperature for 30 min, centrifuged at 1000 g for 10 min.The serum was separated and sent for examination (the separated serum could be stored for 72h at -20°C).

Serum or plasma samples generally do not need to be diluted. When the detection limit is exceeded, dilute the sample according to the situation.

Statistical analysis

The data were analyzed using IBM SPSS Statistic 26.0, and the patients were divided into two groups according to the differences between clinical characteristics, and the continuous variables were converted into dichotomous variables. For the high/low (H/L) levels of cytokines, according to the test results, those below the upper limit of the normal range were classified as the low group, and those above the upper limit were classified as the high group. Univariate analysis was conducted by $\chi 2$ test or Fisher's exact probability. Multivariate analysis was conducted by binary logistic regression, and HR and 95% confidence interval (CI) were calculated. We analyzed the relationship between cytokine levels in peripheral blood at baseline and during treatment and the safety and efficacy of immunotherapy, the odds ratio (OR) and 95%CI results were calculated. R 4.1.2 software was used to draw a violin and nomogram to observe the distribution differences of cytokines. The independent risk factors obtained from single-factor analysis were used to construct a line graph and a predictive logistic regression model. All statistical tests were two-side probability tests (α=0.05), Throughout the analysis, P values less than 0.05 were considered statistically significant.

Result

Clinical safety

First, we evaluated baseline clinicopathological characteristics of patients that can be used to assess the safety of immunotherapy. There were significant differences in age, pathological type and PD-L1 expression status ($P < 0.05$) (as shown in Table 1).

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TABLE 1 Relationship of clinicopathological between AE and NAE.

	AE (N=68)	NAE (N=153)	*P*
Age			**0.024**
≤64	35 (15.8%)	84 (38.0%)	
>64	33 (14.9%)	69 (31.2%)	
Sex			0.108
Male	61 (27.6%)	124 (56.1%)	
Female	7 (3.2%)	29 (13.1%)	
Smoke Status			0.579
Current or former	44 (19.9%)	93 (42.1%)	
Never	24 (10.9%)	60 (27.1%)	
Hypertension			0.186
Yes	13 (5.9%)	42 (19.0%)	
No	55 (24.9%)	111 (50.2%)	
Diabetes			0.660
Yes	5 (2.3%)	14 (6.3%)	
No	63 (28.5%)	139 (62.9%)	
Histology			<0.001
NSCLC	58 (26.2%)	113 (51.1%)	
SCLC	10 (4.5%)	40 (18.1%)	
PD-L1 expression			**0.009**
Negative	13 (5.9%)	15 (6.8%)	
Positive	20 (9.0%)	27 (12.2%)	
Unknown	35 (15.8%)	111 (5.0%)	
Metastases Organ*			0.332
Brain metastasis	13 (5.9%)	29 (13.1%)	
Lung metastasis	18 (8.1%)	45 (20.4%)	
Liver metastasis	9 (4.1%)	34 (15.4%)	
Bone metastasis	31 (14.0%)	49 (22.2%)	
Lymph node metastasis	33 (15.0%)	79 (35.7%)	
Metastatic number			0.299
≤2	53 (24.0%)	109 (49.3%)	
>2	15 (6.8%)	44 (20.0%)	
Combined therapy			0.629
Yes	65 (29.4%)	142 (64.2%)	
No	3 (1.3%)	11 (5.0%)	
ICI treatment received			0.154
PD-1	80 (36.2%)	123 (55.7%)	
PD-L1	8 (3.6%)	30 (13.6%)	
Line of therapy			0.413
First line	44 (19.9%)	109 (49.3%)	
≥Second line	24 (10.9%)	44 (19.9%)	
DOT			0.413
≤3	19 (8.6%)	42 (19.0%)	
>3	49 (22.2%)	111 (50.2%)	

*There may be one or more distant migrations at the same time.
The bold values P<0.05.

Relationship between baseline cytokine levels and the irAEs onset

Lung cancer patients receiving immunotherapy were divided into 2 groups according to their serum baseline cytokine levels (those not above the upper limit of the normal range were low groups). Univariate analysis showed that higher baseline IL-1β and IL-2 levels were significantly associated with the occurrence of irAEs ($P \leq 0.05$). In order to exclude the influence of

confounding factors, age, sex, pathological type and PD-L1 expression status were included in the regression model. The results showed that higher baseline levels of IL-1β (IL-1>12.4pg/mL) and IL-2 (IL-2>7.5pg/mL) were independent risk factors for the occurrence of irAEs. AE patients had higher baseline levels of IL-1β and IL-2 (OR=1.012, 95%CI 1.001-1.041, *P*=0.041; OR=1.743, 95% CI 1.237-2.456, *P*=0.001) (Table 2).

Compared with NAE patients, AE patients have higher median baseline IL-1β levels (7.88 vs16.16 pg/mL, *P*=0.041, Figure 4A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 4B). As shown in the nomogram, IL-1β had a greater influence on the occurrence of AE predictions, followed by age and PD-L1 expression state, and finally gender and pathological type had less influence on the prediction of AE.

Compared with NAE patients, AE patients have higher median baseline IL-2 levels (1.28 vs 2.48pg/mL, *P*=0.001, Figure 5A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 5B). As shown in the nomogram, IL-2 had a greater influence on predicting the occurrence of AE, but other factors had less influence on the prediction of AE.

Relationship between cytokine levels after the first cycle therapy and the irAEs onset.

Univariate analysis showed that higher IL-5 and IFN-γ levels were significantly associated with the occurrence of irAEs ($P \leq 0.05$). In order to exclude the influence of confounding factors, age, sex, pathological type and PD-L1 expression status were included in the regression model. The results showed that higher levels of IL-5 (IL-5 > 3.1pg/mL),IFN-α(IFN-α >8.5 pg/ml)and IFN-γ (IFN-γ>8.5pg/mL) after the first cycle therapy were independent risk factors for the occurrence of irAEs. AE patients from had higher levels of IL-5, IFN-α and IFN-γ after the first cycle therapy (OR=1.227, 95% CI 1.044-1.442, *P*=0.013; OR=1.055, 95% CI 1.140-1.986, P=0.004; OR=1.058, 95% CI 1.008-1.110, *P*=0.022) (Table 3).

Compared with NAE patients, AE patients have higher median IL-5 levels after the first cycle therapy (2.64 vs 5.68pg/mL, *P*=0.013, Figure 6A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 6B). As shown in the nomogram, IL-5 had a greater influence on predicting the occurrence of AE, but other factors had less influence on the prediction of AE.

TABLE 2 Univariate and multivariate analysis results of baseline cytokine levels between AE and NAE.

Pretreatment	Univariate analysis *P*	Multivariate analysis	
		OR (95% CI)	*P*
IL-1 (H/L)	**0.022**	**1.021 (1.001-1.041)**	**0.041**
IL-2 (H/L)	**0.029**	**1.743 (1.237-2.456)**	**0.001**
IL-4 (H/L)	–	1.052 (0.660-1.678)	0.831
IL-5 (H/L)	0.145	1.079 (0.994-1.170)	0.068
IL-6 (H/L)	0.527	1.003 (0.989-1.018)	0.658
IL-8 (H/L)	0.862	0.997 (0.977-1.017)	0.775
IL-10 (H/L)	–	1.163 (0.736-1.838)	0.517
IL-12 (H/L)	0.264	0.991 (0.961-1.021)	0.548
IL-17 (H/L)	0.512	1.031 (0.963-1.104)	0.376
IFN-α (H/L)	1.000	1.034 (0.911-1.174)	0.605
IFN-γ (H/L)	0.316	1.009 (0.992-1.026)	0.321
TNF-α (H/L)	0.167	1.081 (0.987-1.185)	0.093

"-"Indicates that a statistic cannot be computed.
The bold values P < 0.05.

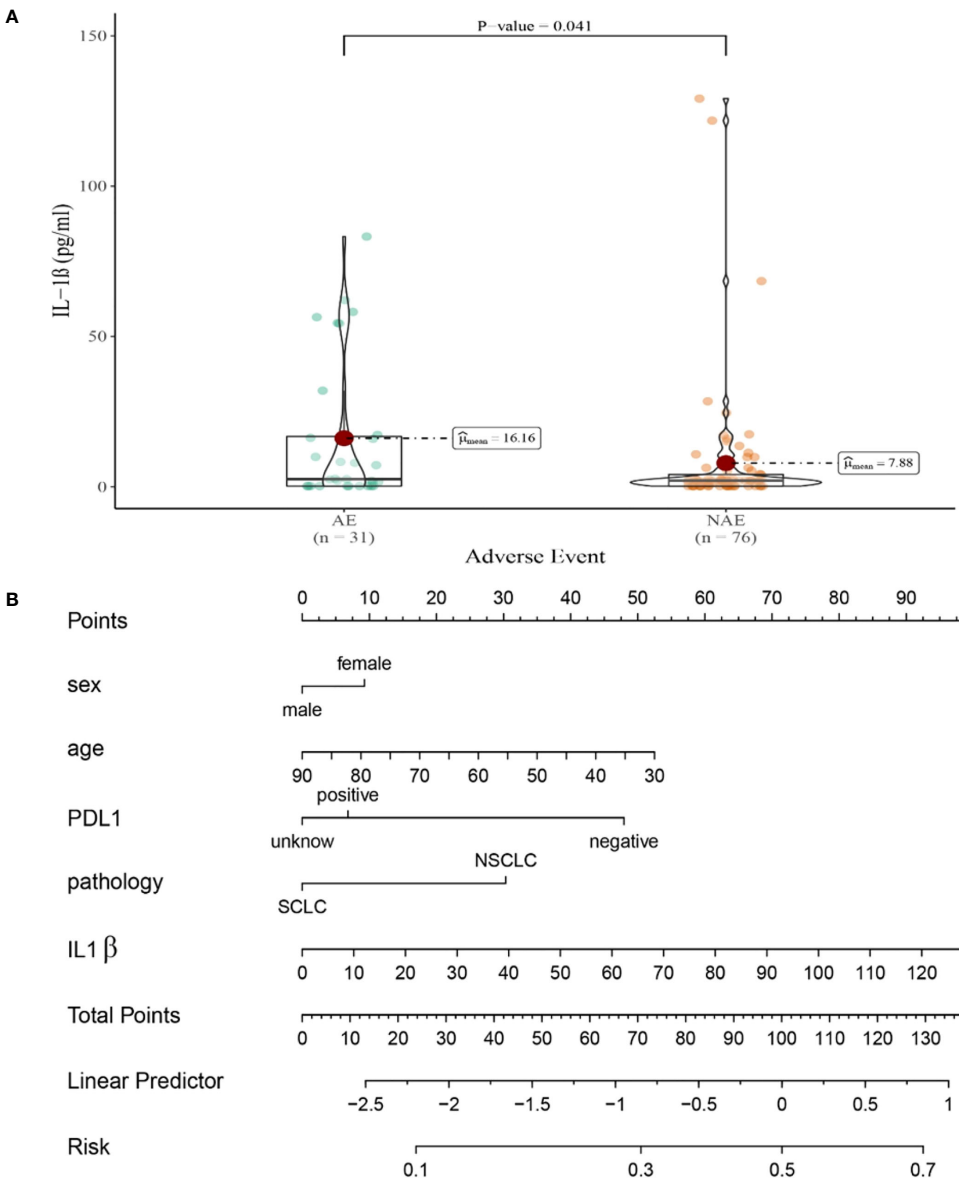

FIGURE 4
(A) Differences in baseline IL-1β levels between AE and NAE; **(B)** The nomogram of irAEs prediction based on logistic multivariate analysis.

Compared with NAE patients, AE patients have higher median IFN-α levels after the first cycle therapy (1.70 vs3.56 pg/mL, *P*=0.004, Figure7A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 7B). As shown in the nomogram, IFN-α had a greater influence on predicting the occurrence of AE, but other factors had less influence on the prediction of AE.

Compared with NAE patients, AE patients have higher median IFN-γ levels after the first cycle therapy (6.14 vs 21.31pg/m, *P*=0.022, Figure 8A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 8B). As shown in the nomogram, IFN-γ had a greater influence on predicting the occurrence of AE, but other factors had less influence on the prediction of AE.

Relationship between cytokine levels after the second cycle therapy and the irAEs onset.

Univariate analysis showed that higher IL-5 and IL-12 levels were significantly associated with the occurrence of irAEs (P ≤

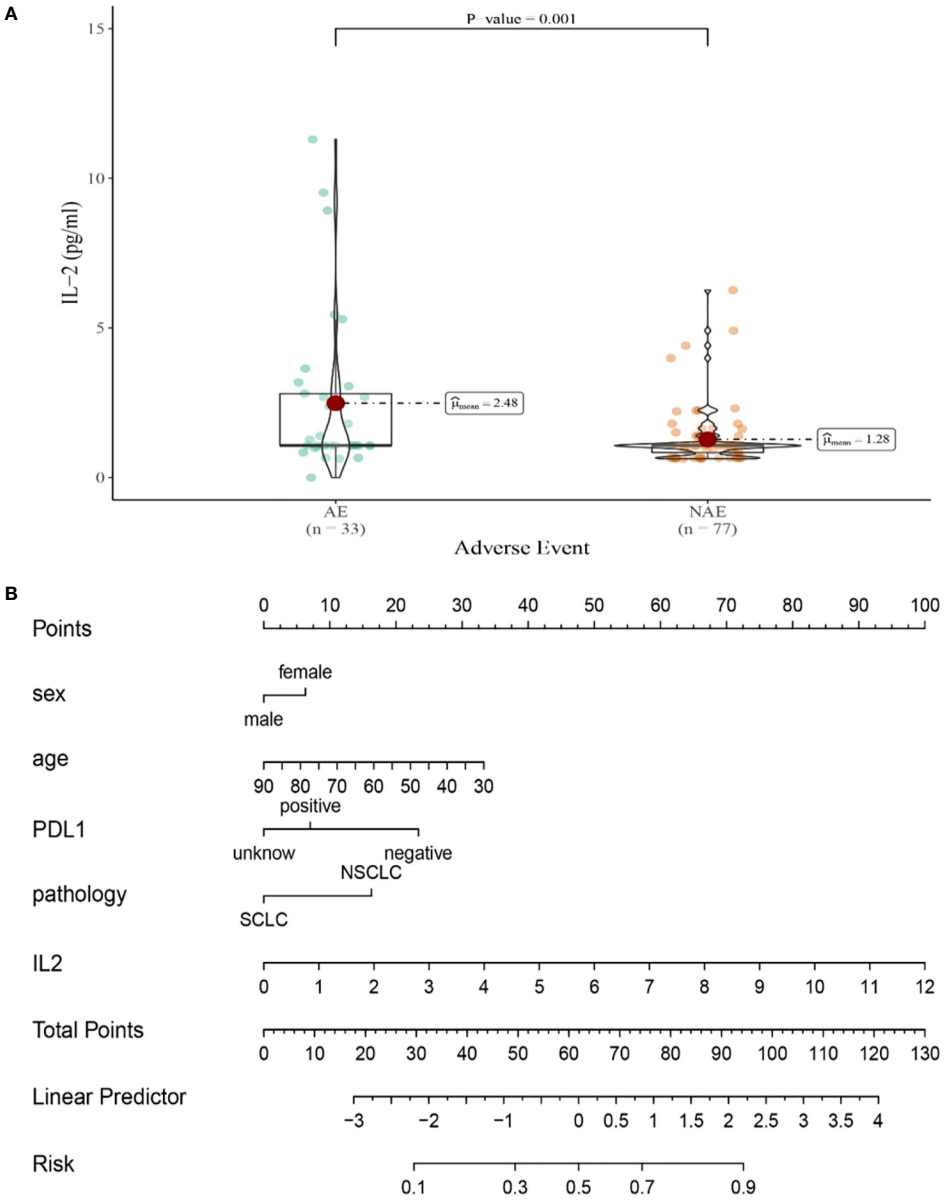

FIGURE 5
(A) Differences in baseline IL-2 levels between AE and NAE; **(B)** The nomogram of irAEs prediction based on logistic multivariate analysis.

0.05). In order to exclude the influence of confounding factors, age, sex, pathological type and PD-L1 expression status were included in the regression model. The results showed that levels of cytokines weren't connected with occurrence of irAEs (Table 4).

3.1.4 Relationship between cytokine levels after the third cycle therapy and the irAEs onset.

Univariate analysis showed that levels of cytokines weren't connected with occurrence of irAEs. Multivariate analysis showed that high levels of IL-5 (IL-5 > 3.1pg/mL) after the third cycle therapy was independent risk factor for the occurrence of irAEs. Patients with AEs from immunotherapy had higher IL-5 levels (OR=1.187, 95% CI 1.015-1.388, P =0.032) (Table 5).

Compared with NAE patients, AE patients have higher median IL-5 levels after the third cycle therapy (9.50 vs 3.57pg/mL, P = 0.032, Figure 9A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 9B). As

TABLE 3 Univariate and multivariate analysis results of cytokine levels after the first cycle therapy between AE and NAE.

After the first cycle therapy	Univariate analysis *P*	Multivariate analysis	
		OR (95% CI)	*P*
IL-1 (H/L)	0.742	1.022 (0.993-1.052)	0.143
IL-2 (H/L)	0.123	1.189 (0.960-1.474)	0.113
IL-4 (H/L)	1.000	0.977 (0.906-1.054)	0.549
IL-5 (H/L)	**0.008**	**1.227 (1.044-1.442)**	**0.013**
IL-6 (H/L)	0.170	0.999 (0.989-1.009)	0.848
IL-8 (H/L)	0.416	1.020 (0.976-1.065)	0.386
IL-10 (H/L)	1.000	0.994 (0.921-1.074)	0.885
IL-12 (H/L)	0.174	0.990 (0.951-1.031)	0.622
IL-17 (H/L)	0.288	1.034 (0.971-1.102)	0.294
IFN-α (H/L)	0.127	**1.505 (1.140-1.986)**	**0.004**
IFN-γ (H/L)	**0.014**	**1.058 (1.008-1.110)**	**0.022**
TNF-α (H/L)	0.282	1.115 (0.983-1.265)	0.091

"-"Indicates that a statistic cannot be computed.
The bold values P < 0.05.

shown in the nomogram, IL-5 had a greater influence on predicting the occurrence of AE, but other factors had less influence on the prediction of AE.

Clinical response efficacy

We evaluated baseline clinicopathological characteristics of 142 patients that can be used to assess the clinical response efficacy of immunotherapy. There were significant differences in PD-L1 expression status and DOT ($P < 0.05$) (as shown in Table 6).

Relationship between baseline cytokine levels and clinical response efficacy of immunotherapy.

Univariate and multivariate analysis showed that the baseline levels of cytokines weren't connected with occurrence of clinical response efficacy (Table 7).

Relationship between cytokine levels after the first cycle therapy and clinical response efficacy of immunotherapy.

Univariate analysis showed that higher IL-6 was significantly associated with clinical response efficacy ($P \leq 0.05$). In order to exclude the influence of confounding factors, PD-L1 expression status and duration of treatment (DOT) were included in the regression model. The results showed that levels of cytokines after the first cycle therapy weren't connected with occurrence of clinical response efficacy (Table 8).

Relationship between cytokine levels after the second cycle therapy and clinical response efficacy of immunotherapy.

Univariate analysis showed that lower IFN-α level was significantly associated with clinical benefit ($P \leq 0.05$). In order to exclude the influence of confounding factors, PD-L1 expression status and DOT were included in the regression model. The results showed that lower levels of IL-10 (IL-10<12.9pg/ml),IL-17(IL-17<21.4pg/ml) after the second cycle therapy were independent risk factors for the clinical benefit. Patients with CB from immunotherapy had lower levels of IL-10 and IL-17 after the second cycle therapy (OR=0.402, 95% CI 0.191-0.848, P=0.016; OR=0.776, 95% CI 0.633-0.951, P=0.015) (Table 9).

Compared with NCB patients, CB patients have lower median IL-10 levels after the second cycle therapy (2.66 vs 1.26pg/mL, P =0.015, Figure 10A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 10B). As shown in the nomogram, PD-L1 had a greater influence on predicting the clinical response efficacy, but DOT and IL-10 had less influence on the prediction of CB.

Compared with NCB patients, CB patients have lower median IL-17 levels after the second cycle therapy (8.47 vs 2.81pg/mL, P=0.015, Figure 11A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 11B). As shown in the nomogram, PD-L1 and IL-17 had a greater influence on predicting the clinical response efficacy, but DOT had less influence on the prediction of CB.

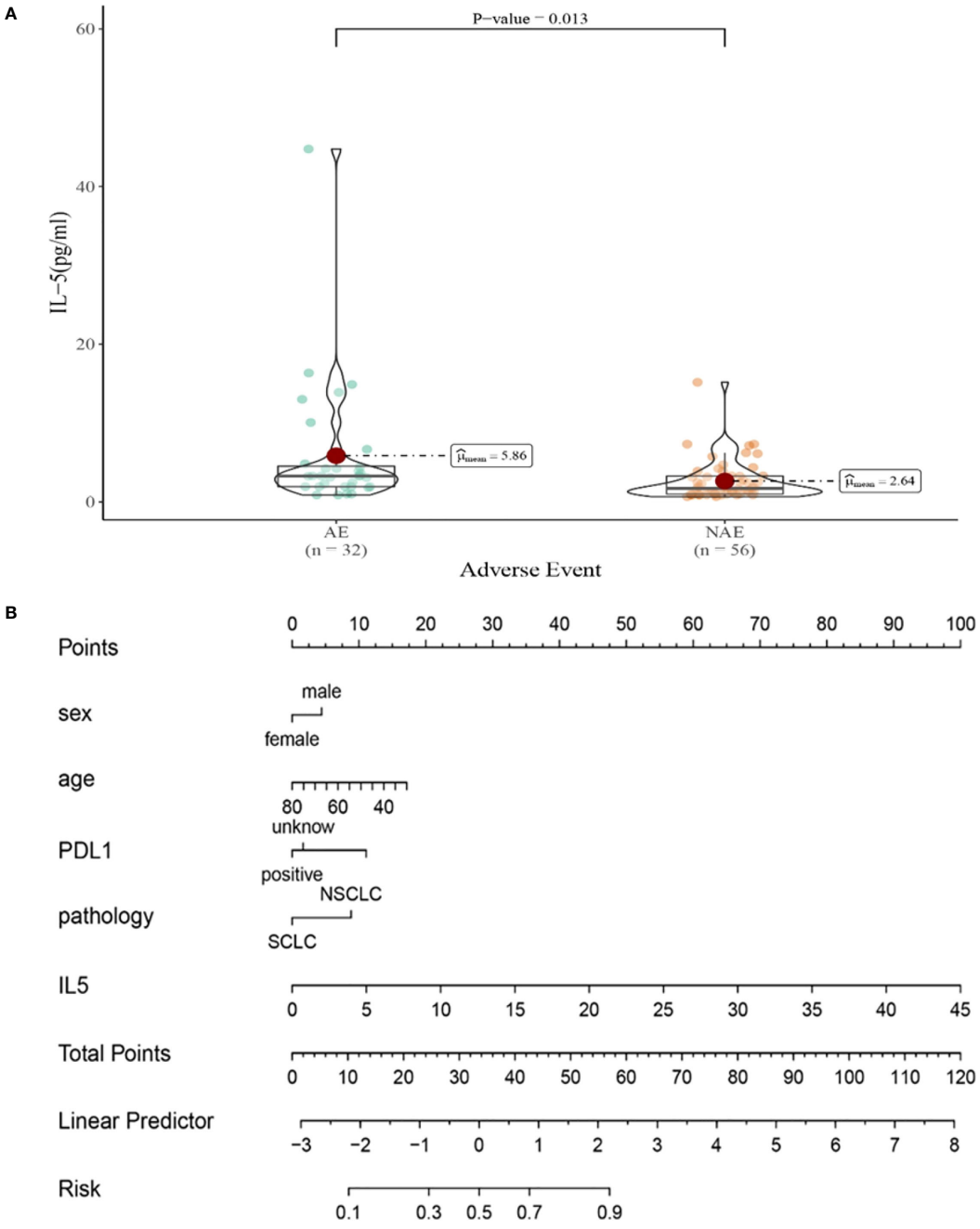

FIGURE 6
(A) Differences in IL-5 levels after the first cycle therapy between AE and NAE; **(B)** The nomogram of irAEs prediction based on logistic multivariate analysis.

Relationship between cytokine levels after the third cycle therapy and clinical response efficacy of immunotherapy.

Univariate analysis showed that higher IFN-α level was significantly associated with clinical benefit ($P \leq 0.05$). In order to exclude the influence of confounding factors, PD-L1 expression status and DOT were included in the regression model. The results showed that lower levels of IL-6 (IL-6<5.4pg/ml),IL-8(IL-8<20.6 pg/ml)after the third cycle therapy were independent risk factors for the clinical benefit. CB patients

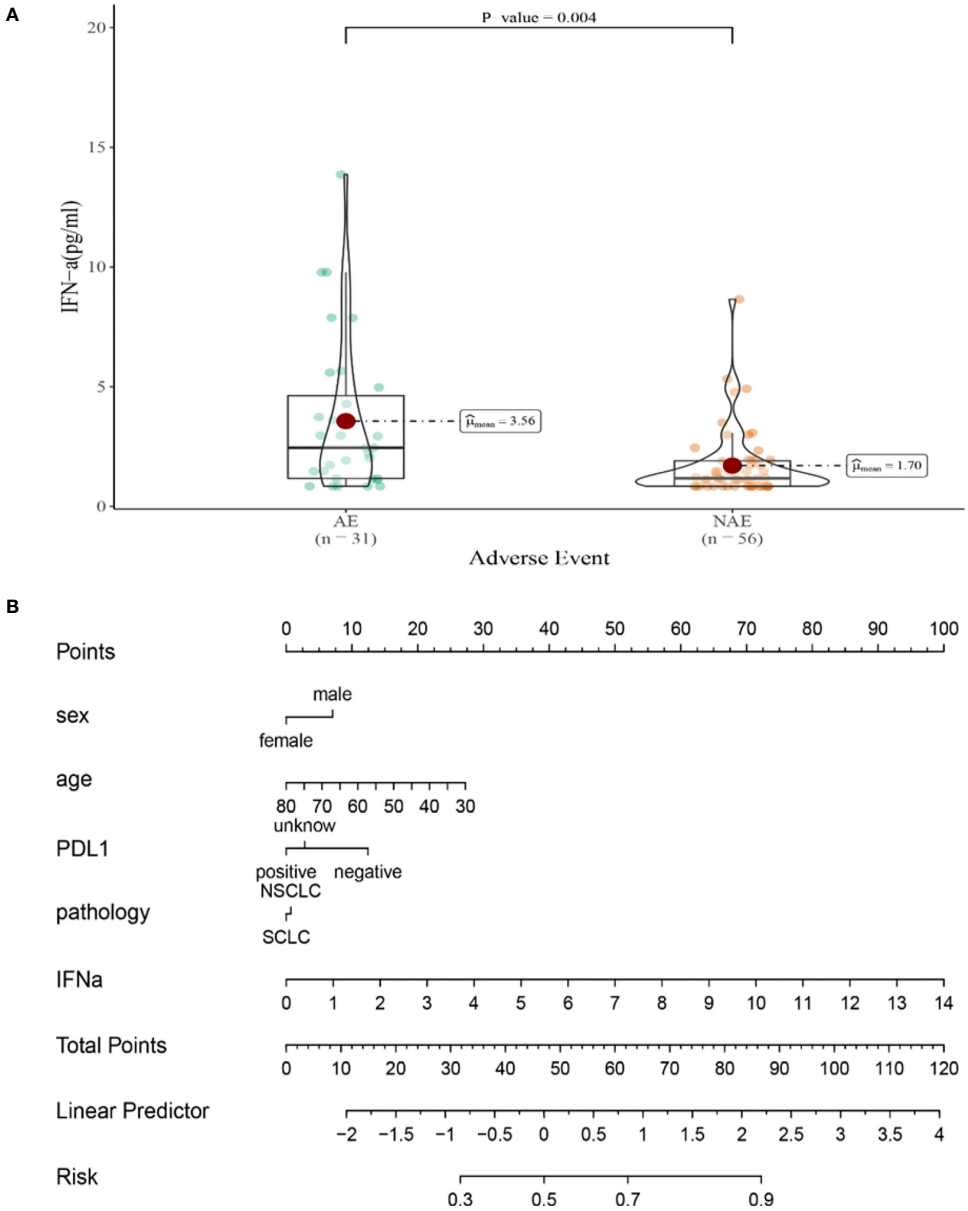

FIGURE 7
(A) Differences in IFN-αlevels after the first cycle therapy between AE and NAE; **(B)** The nomogram of irAEs prediction based on logistic multivariate analysis.

had lower levels of IL-6 and IL-8 after the third cycle therapy (OR=0.402, 95% CI 0.191-0.848, *P*=0.016; OR=0.776, 95% CI 0.633-0.951, *P*=0.015) (Table 10).

Compared with NCB patients, CB patients have lower median IL-6 levels after the third cycle therapy (10.19 vs 41.07pg/mL, *P*=0.013, Figure 12A). Meanwhile, we established a nomogram based on logistic regression analysis (Figure 12B). As shown in the nomogram, PD-L1 and DOT had a greater influence on predicting the clinical response efficacy, but IL-6 had less influence on the prediction of CB.

Compared with NCB patients, CB patients have lower median IL-8 levels after the third cycle therapy (8.01 vs 17.22pg/mL, *P*=0.039, Figure 13A). Meanwhile, we established

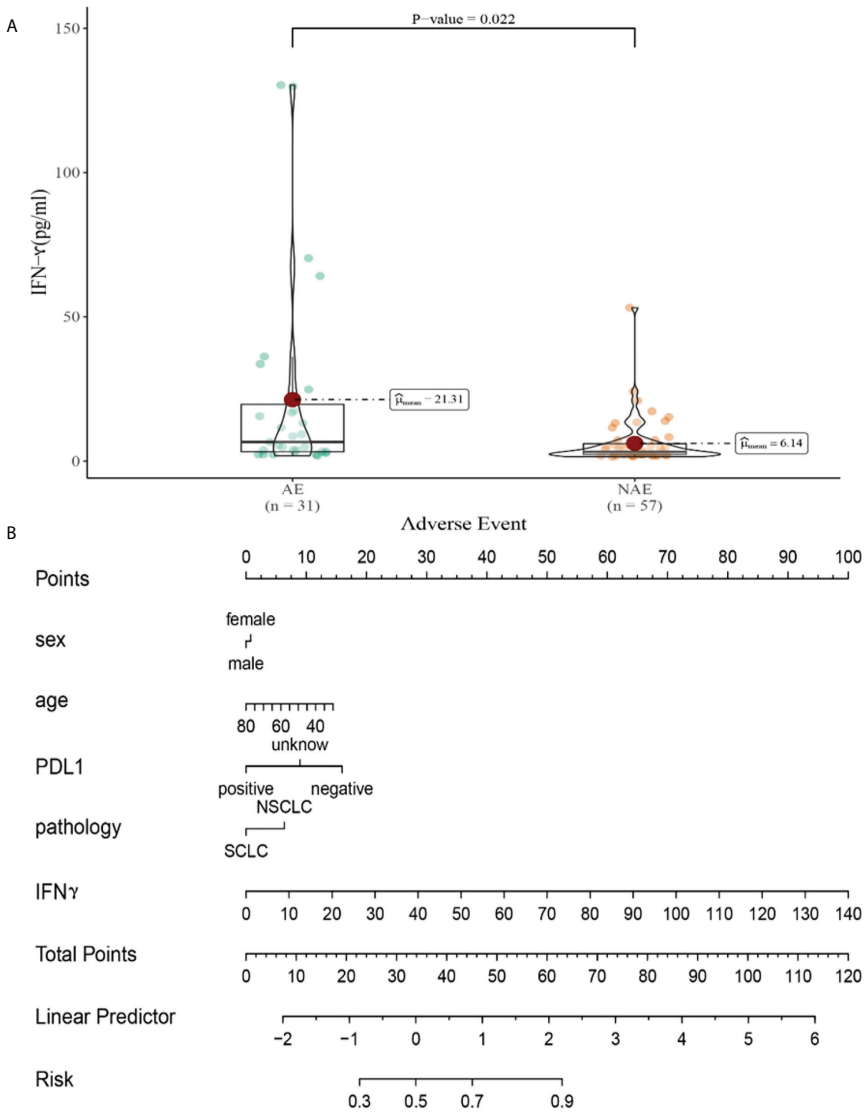

FIGURE 8
(A) Differences in IFN-γ levels after the first cycle therapy between AE and NAE; **(B)** The nomogram of irAEs prediction based on logistic multivariate analysis.

a nomogram based on logistic regression analysis (Figure 13B). As shown in the nomogram, PD-L1 and DOT had a greater influence on predicting the clinical response efficacy, but IL-8 had less influence on the prediction of CB.

Discussion

This is the first retrospective study involving analyses of baseline and on-treatment cytokine concentrations during ICI therapy. We found that baseline levels of IL-1β and IL-2, as well as on-treatment levels of IL-5, IFN-α and IFN-γ were associated with immune-related adverse events. At the same time, on-treatment levels of IL-6, IL-8, IL-10 and IL-17 were related to the clinical response.

IL-1β is a member of the IL-1 family. After IL-1β activates IL-1, it participates in the related immune inflammatory response of lung cancer by activating NF-κB and other pathways (12). Baseline serum cytokine concentrations of IL-1β, IL-2, and GM-CSF were elevated in patients with thyroid

TABLE 4 Univariate and multivariate analysis results of cytokine levels after the second cycle therapy between AE and NAE.

After the second cycle therapy	Univariate analysis *P*	Multivariate analysis	
		OR (95% CI)	*P*
IL-1 (H/L)	0.237	1.019 (0.999-1.039)	0.065
IL-2 (H/L)	0.345	1.043 (0.899-1.211)	0.576
IL-4 (H/L)	0.359	1.864 (0.686-5.066)	0.222
IL-5 (H/L)	**0.043**	1.089 (0.994-1.192)	0.066
IL-6 (H/L)	0.076	1.009 (0.987-1.031)	0.416
IL-8 (H/L)	0.900	1.005 (0.967-1.045)	0.801
IL-10 (H/L)	–	1.408 (0.970-2.042)	0.072
IL-12 (H/L)	**0.020**	1.482 (0.960-2.286)	0.076
IL-17 (H/L)	1.000	1.052 (0.938-1.180)	0.389
IFN-α (H/L)	0.128	1.158 (0.971-1.382)	0.103
IFN-γ (H/L)	0.622	1.017 (0.999-1.036)	0.060
TNF-α (H/L)	0.045	1.096 (0.968-1.240)	0.147

"-"Indicates that a statistic cannot be computed.

related adverse reactions in a study of multiple solid tumors receiving immunotherapy (13). Therefore, higher baseline IL-1β levels are associated with higher levels of pro-inflammatory cytokines. If the use of immune checkpoint inhibitors at this time to activate the body's immune cells, thereby releasing more inflammatory factors, can induce the occurrence of autoimmune response and tissue and organ damage.

Growing evidence indicates that immune-related adverse events can be tied to specific cytokines that can amplify both pro- and anti-inflammatory immunity (8). Among Th2 cytokines, IL-2 is a key cytokine involved in promoting the proliferation of natural killercells and T lymphocytes (14). Constantini (15) showed that a low serum IL-2 concentration measured at nivolumab initiation was associated with grade 3–4 toxicities in patients with advanced NSCLC.

IL-5 is mainly produced by T helper-2 (Th2) lymphocytes and Group 2 innate lymphoid cells. It can increase antibody secretion by promoting the differentiation and growth of B cells and enhance the humoral immune response mediated by Th2 cells. Immunity to tumors is mainly governed by Th1-mediated cellular immunity. A Th1-Th2 drift will lead to immunosuppression and cancer development (16).Therefore, when IL-5 levels are high during immunotherapy, the differentiation and growth of B cells are correspondingly promoted, thus increasing the secretion of antibodies, leading to the over activated humoral immune response which may attack normal tissues and organs of the body.

In cytokine analysis during immunotherapy, we observed a negative correlation between IL-6 concentration and clinical benefit in lung cancer patients after the third cycle of

TABLE 5 Univariate and multivariate analysis results of cytokine levels after the third cycle therapy between AE and NAE.

After the third cycle therapy	Univariate analysis *P*	Multivariate analysis	
		OR (95% CI)	*P*
IL-1 (H/L)	0.413	1.013 (0.991-1.035)	0.252
IL-2 (H/L)	0.298	1.068 (0.871-1.310)	0.527
IL-4 (H/L)	0.418	1.696 (0.718-4.006)	0.229
IL-5 (H/L)	0.268	**1.187 (1.015-1.388)**	**0.032**
IL-6 (H/L)	0.350	1.013 (0.988-1.038)	0.318
IL-8 (H/L)	1.000	1.016 (0.975-1.060)	0.447
IL-10 (H/L)	–	1.512 (0.994-2.298)	0.053
IL-12 (H/L)	0.425	1.100 (0.892-1.357)	0.372
IL-17 (H/L)	0.418	1.143 (0.946-1.387)	0.174
IFN-α (H/L)	0.161	1.210 (0.914-1.603)	0.183
IFN-γ (H/L)	0.113	1.013 (0.990-1.037)	0.279
TNF-α (H/L)	1.000	1.035 (0.956-1.111)	0.334

"-"Indicates that a statistic cannot be computed.

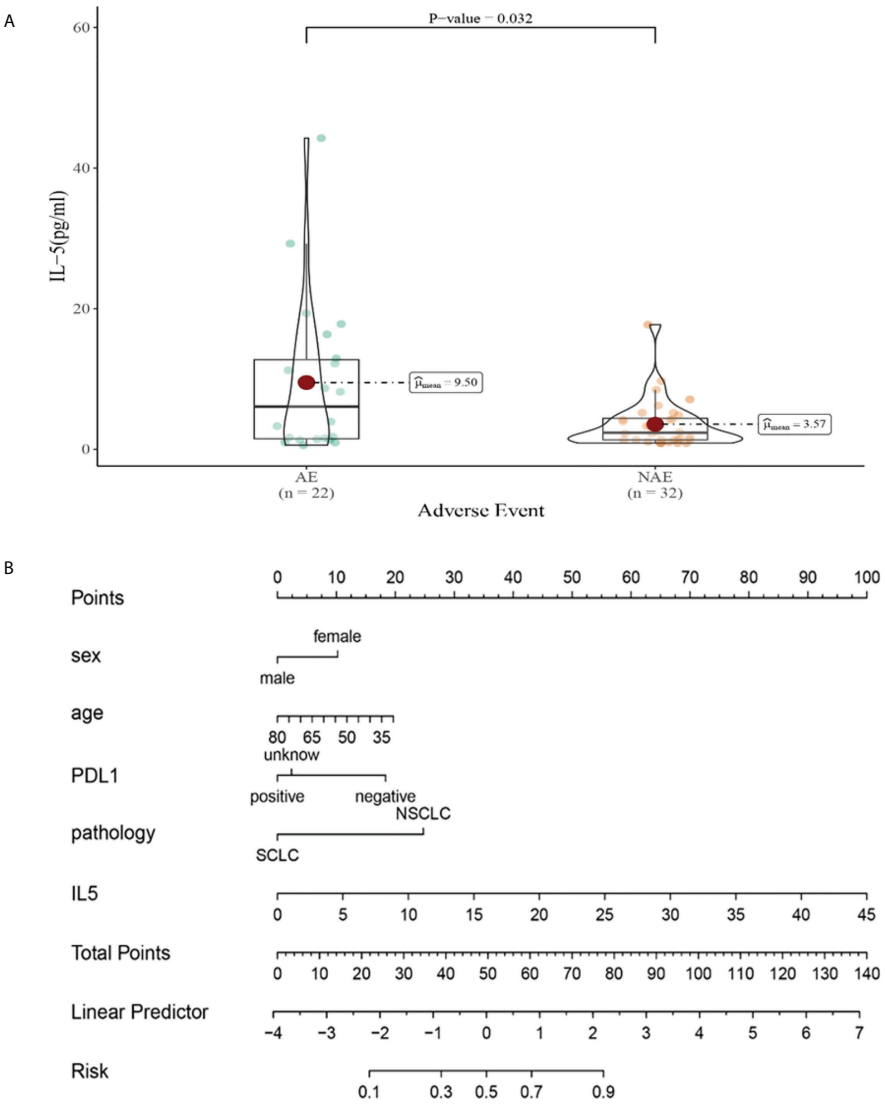

FIGURE 9
(A) Differences in IL-5 levels after the third cycle therapy between AE and NAE; **(B)** The nomogram of irAEs prediction based on logistic multivariate analysis.

immunotherapy. One of the key signaling pathways controlling this phenomenon is the IL-6/JAK/STAT3 axis, which enhances tumor proliferation and cell metabolism by upregulating this signaling pathway (17, 18). Higher IL-6 levels during treatment may be indicative of high tumor cell proliferation and enhanced angiogenesis, and immunotherapy will be less effective in eliminating this state.

Another significant negative correlation with CB found in our study was the concentration of IL-8 in lung cancer patients after the third cycle of immunotherapy. Il-8 is a member of the neutrophil chemokine family (19). Studies have shown that early decreased peripheral blood IL-8 levels are associated with longer overall survival in patients with melanoma (P=0.001) and non-small cell lung cancer (P=0.015) (20). However, further analysis of peripheral blood IL-8 levels in combination with other inflammatory indicators is needed to clearly distinguish between elevated IL-8 caused by cancer progression and elevated IL-8 caused by inflammation.

At the same time, we also find that IL-10 concentration was negatively associated with CB in lung cancer patients after second cycle of immunotherapy. IL-10 is a cytokine that has both anti-inflammatory and pro-tumor/anti-tumor effects. Il-10

TABLE 6 Relationship between clinicopathological characteristics and clinical response efficacy.

	CB(N=98)	NCB(N=44)	*P*
Age			0.802
≤63	53 (37.3%)	27 (19.0%)	
>63	45 (31.7%)	17 (12.0%)	
Sex			0.950
Male	82 (57.7%)	37 (26.1%)	
Female	16 (11.3%)	7 (4.9%)	
Smoke Status			0.477
Current or former	64 (45.1%)	26 (18.3%)	
Never	3 (2.1%)	18 (12.6%)	
Hypertension			0.405
Yes	24 (16.9%)	8 (5.6%)	
No	74 (52.1%)	36 (25.4%)	
Diabetes			0.660
Yes	9 (6.3%)	4 (2.8%)	
No	89 (62.7%)	40 (28.2%)	
Histology			0.790
NSCLC	76 (53.5%)	35 (24.6%)	
SCLC	22 (15.5%)	9 (6.3%)	
PD-L1 expression			**0.050**
Negative	18 (12.7%)	2 (1.4%)	
Positive	16 (11.2%)	12 (8.5%)	
Unknown	64 (45.1%)	30 (2.1%)	
Metastases Organ*			0.859
Brain metastasis	17 (12.0%)	9 (6.3%)	
Lung metastasis	29 (20.4%)	13 (9.2%)	
Liver metastasis	21 (14.8%)	8 (5.6%)	
Bone metastasis	41 (28.9%)	22 (15.5%)	
Lymph node metastasis	54 (38.0%)	20 (14.1%)	
Metastatic number			0.778
≤2	69 (48.6%)	32 (22.5%)	
>2	29 (20.4%)	12 (8.5%)	
Combined therapy			0.724
Yes	90 (63.4%)	42 (29.6%)	
No	8 (5.6%)	2 (1.4%)	
ICI treatment received			0.785
PD-1	82 (57.7%)	36 (25.4%)	
PD-L1	16 (11.3%)	8 (5.6%)	
Line of therapy			0.495
First line	61 (43.0%)	30 (21.1%)	
≥Second line	37 (26.1%)	14 (9.9%)	
DOT			<0.001
≤3	11 (7.7%)	15 (10.6%)	
>3	87 (61.3%)	29 (20.4%)	

*There may be one or more distant migrations at the same time.

binds to the corresponding receptor and initiates transcription of target genes by activating JAK1 and Tyk2, which subsequently leads to phosphorylation of STAT3 (21, 22). Clinically relevant studies have demonstrated that NSCLC patients expressing high levels of IL-10 have poor prognosis (23, 24).However, it has also been reported that insufficient expression of IL-10 in tumors is a negative prognostic factor for early-stage NSCLC (21, 25, 26). These inconsistent studies on IL-10 suggest that the cellular

TABLE 7 Univariate and multivariate analysis results of baseline cytokine levels between CB and NCB.

Pretreatment	Univariate analysis	Multivariate analysis	
	P	OR (95% CI)	*P*
IL-1 (H/L)	0.697	1.001 (0.975-1.028)	0.937
IL-2 (H/L)	–	1.157 (0.803-1.695)	0.418
IL-4 (H/L)	–	0.365 (0.124-1.074)	0.067
IL-5 (H/L)	0.465	0.966 (0.860-1.084)	0.557
IL-6 (H/L)	0.355	0.977 (0.975-1.019)	0.766
IL-8 (H/L)	0.697	0.989 (0.963-1.015)	0.391
IL-10 (H/L)	–	0.801 (0.378-1.698)	0.562
IL-12 (H/L)	0.553	0.684 (0.402-1.164)	0.162
IL-17 (H/L)	1.000	1.066 (0.934-1.217)	0.343
IFN-α (H/L)	–	0.866 (0.599-1.252)	0.444
IFN-γ (H/L)	0.741	0.995 (0.968-1.023)	0.729
TNF-α (H/L)	0.512	1.056 (0.916-1.217)	0.451

"-"Indicates that a statistic cannot be computed.

TABLE 8 Univariate and multivariate analysis results of cytokine levels after the first cycle therapy between CB and NCB.

After the first therapy	Univariate analysis	Multivariate analysis	
	P	OR (95% CI)	*P*
IL-1 (H/L)	1.000	0.979 (0.901-1.063)	0.611
IL-2 (H/L)	1.000	1.147 (0.547-2.407)	0.716
IL-4 (H/L)	0.326	0.957 (0.761-1.203)	0.707
IL-5 (H/L)	0.927	1.160 (0.899-1.497)	0.254
IL-6 (H/L)	**0.047**	0.989 (0.966-1.013)	0.353
IL-8 (H/L)	1.000	1.031 (0.914-1.164)	0.619
IL-10 (H/L)	0.318	0.847 (0.652-1.101)	0.215
IL-12 (H/L)	0.539	1.539 (0.489-4.845)	0.462
IL-17 (H/L)	1.000	1.135 (0.760-1.694)	0.536
IFN-α (H/L)	–	1.012 (0.564-1.817)	0.969
IFN-γ (H/L)	1.000	0.984 (0.952-1.016)	0.325
TNF-α (H/L)	–	0.969 (0.617-1.522)	0.892

"-"Indicates that a statistic cannot be computed.

TABLE 9 Univariate and multivariate analysis results of cytokine levels after the second cycle therapy between CB and NCB.

After the second cycle therapy	Univariate analysis	Multivariate analysis	
	P	OR (95% CI)	*P*
IL-1(H/L)	0.108	0.966(0.933-1.001)	0.054
IL-2(H/L)	0.096	0.917(0.757-1.110)	0.374
IL-4(H/L)	1.000	0.247(0.049-1.237)	0.089
IL-5(H/L)	1.000	0.894(0.784-1.019)	0.093
IL-6(H/L)	0.221	0.977(0.935-1.021)	0.298
IL-8(H/L)	1.000	0.981(0.933-1.031)	0.447

(Continued)

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TABLE 9 Continued

After the second cycle therapy	Univariate analysis	Multivariate analysis	
	P	OR (95% CI)	*P*
IL-10(H/L)	–	**0.402(0.191-0.848)**	**0.016**
IL-12(H/L)	0.172	0.544(0.255-1.161)	0.115
IL-17(H/L)	0.276	**0.776(0.633-0.951)**	**0.015**
IFN-α(H/L)	**0.036**	0.675(0.439-1.038)	0.074
IFN-γ(H/L)	0.257	0.964(0.922-1.007)	0.102
TNF-α(H/L)	0.276	0.805(0.605-1.072)	0.137

"-"Indicates that a statistic cannot be computed.

source of IL-10 and the effects of IL-10 on different cell types are what determine the ultimate role of IL-10 in cancer (27).

Finally, we also observed that IL-17 concentrations in lung cancer patients after the second cycle of immunotherapy were negatively associated with CB. Studies have shown that the IL-17 signaling pathway can increase the immunosuppressive activity of regulatory T cells, leading to tumor growth and development (28).High concentrations of baseline serum IL-17 were identified in ipilimumab-treated metastatic melanoma patients developing severe grade 3 gastrointestinal irAEs and may thus serve as a

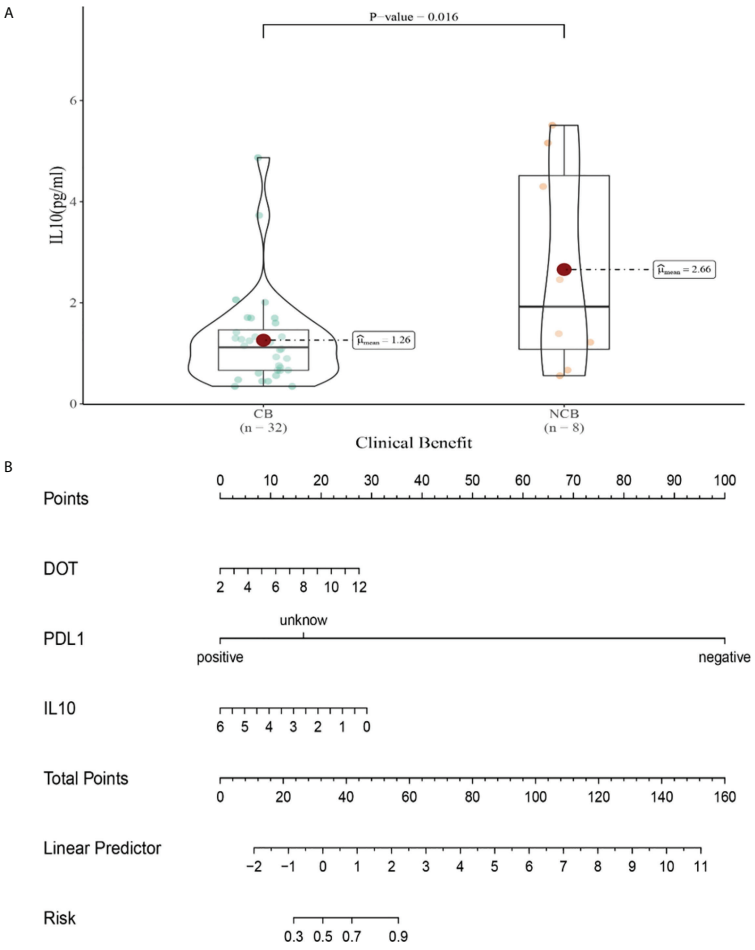

FIGURE 10
(A) Differences in IL-10 levels after the second cycle therapy between CB and UCB; **(B)** The nomogram based on logistic multivariate analysis.

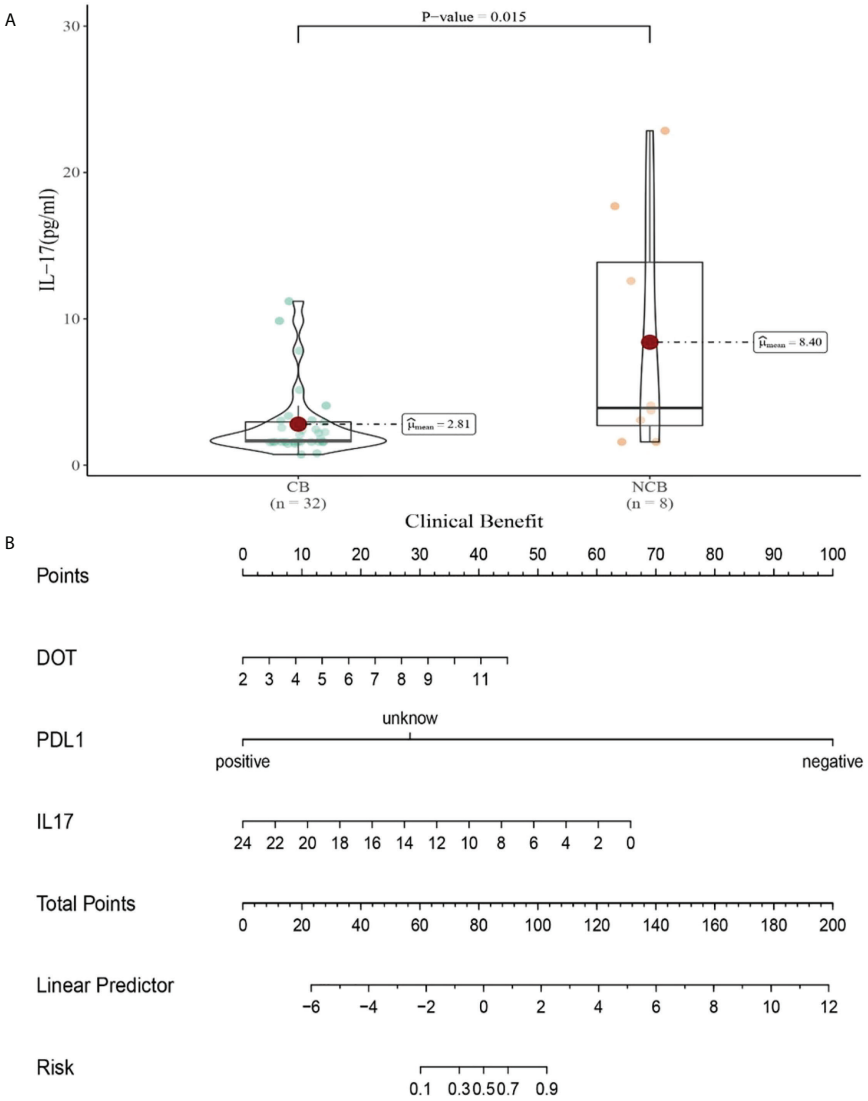

FIGURE 11
(A) Differences in IL-17 levels after the second cycle therapy between CB and UCB; **(B)** The nomogram based on logistic multivariate analysis.

TABLE 10 **Univariate and multivariate analysis results of cytokine levels after the third cycle therapy between CB and NCB.**

After the third cycle therapy	Univariate analysis *P*	Multivariate analysis	
		OR (95% CI)	*P*
IL-1 (H/L)	0.601	0.979 (0.952-1.077)	0.144
IL-2 (H/L)	0.347	0.755 (0.544-1.047)	0.092
IL-4 (H/L)	1.000	0.871 (0.432-1.758)	0.700
IL-5 (H/L)	1.000	0.929 (0.811-1.064)	0.288
IL-6 (H/L)	**0.009**	**0.936 (0.888-0.986)**	**0.013**
IL-8 (H/L)	0.163	**0.919 (0.849-0.996)**	**0.039**

(Continued)

TABLE 10 Continued

After the third cycle therapy	Univariate analysis *P*	Multivariate analysis	
		OR (95% CI)	*P*
IL-10 (H/L)	–	0.800 (0.486-1.315)	0.379
IL-12 (H/L)	0.573	0.711 (0.490-1.033)	0.074
IL-17 (H/L)	0.189	0.862 (0.715-1.040)	0.120
IFN-α (H/L)	0.194	0.807 (0.563-1.155)	0.240
IFN-γ (H/L)	0.073	0.970 (0.932-1.010)	0.136
TNF-α (H/L)	0.086	0.892 (0.774-1.028)	0.113

"-"Indicates that a statistic cannot be computed.

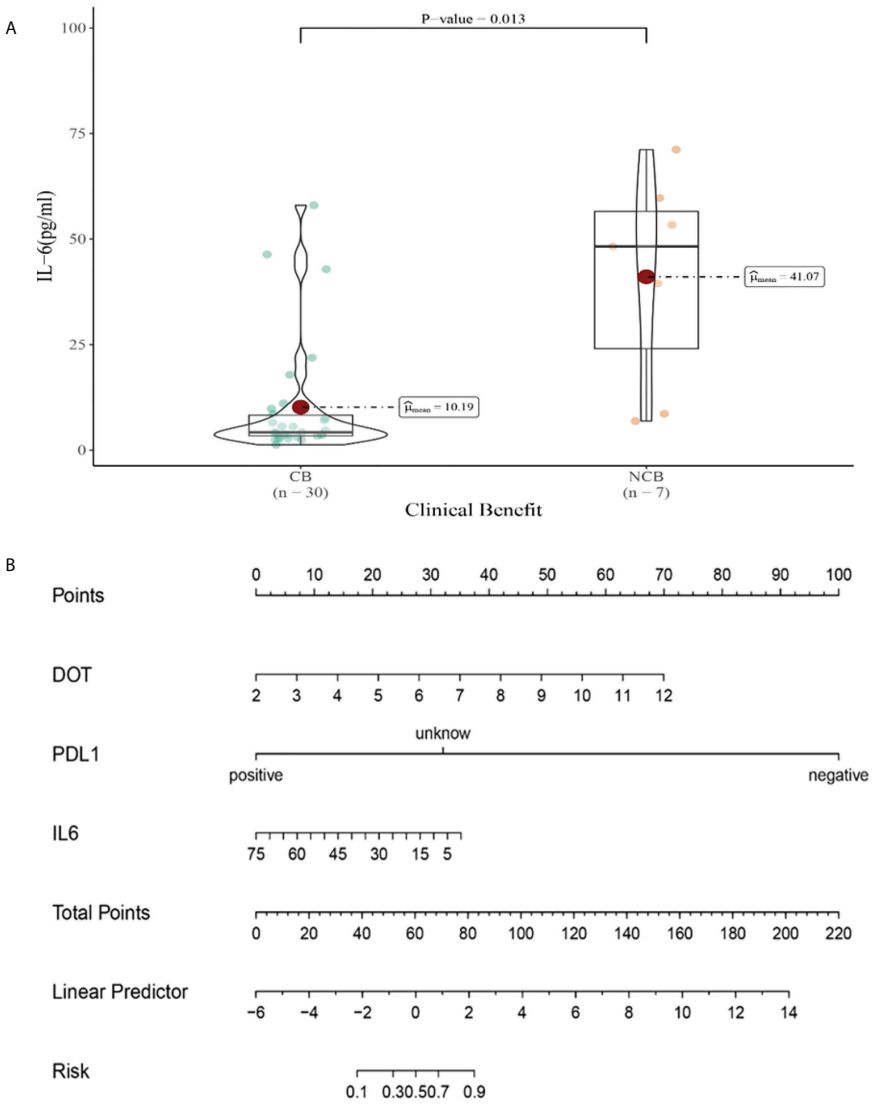

FIGURE 12
(A) Differences in IL-6 levels after the third cycle therapy between CB and UCB; **(B)** The nomogram based on logistic multivariate analysis.

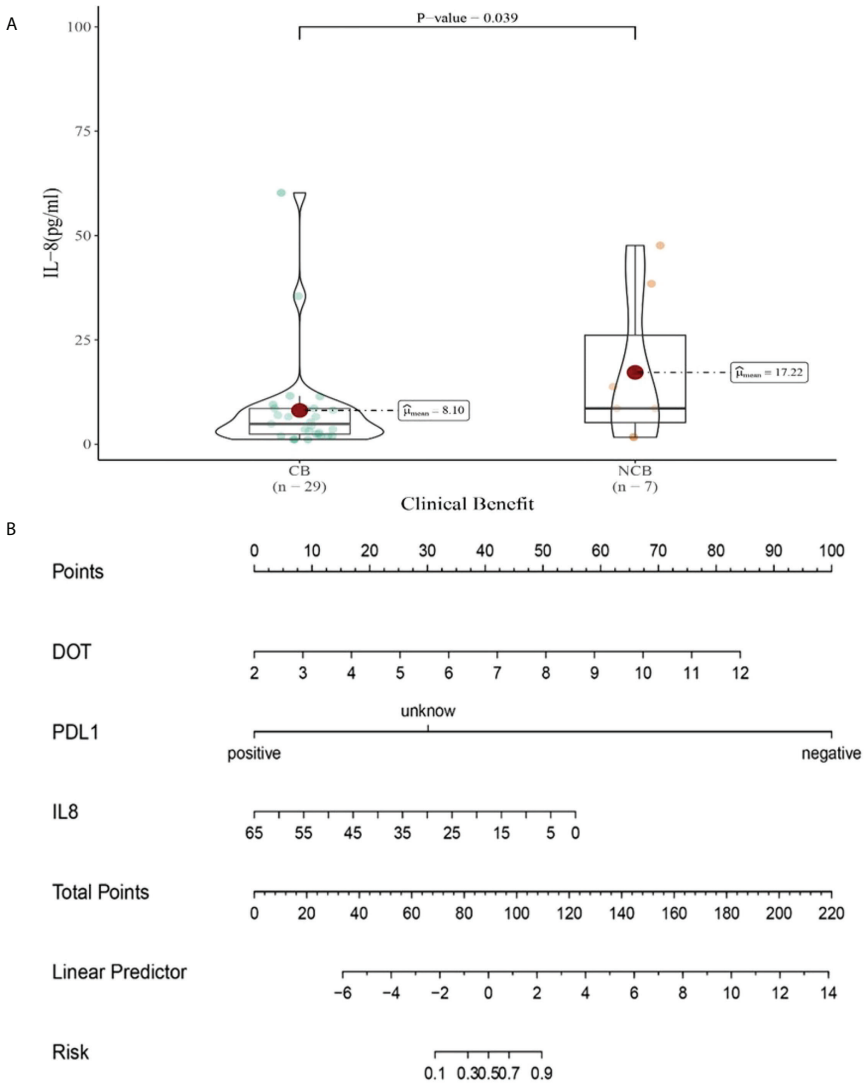

FIGURE 13
(A) Differences in IL-8 levels after the third cycle therapy between CB and UCB; **(B)** The nomogram based on logistic multivariate analysis.

putative biomarker for defining both at-risk patients and the severity of ipilimumab-induced colitis (29).

With close collaborations between academia and industry, recombinant IFNα2 became the first human immunotherapeutic approved by the US Food and Drug Administration (FDA) for cancer and, other than insulin, the first FDA-approved pharmaceutical product produced by recombinant DNA technology (30). IFNα has multiple antitumor properties, including direct tumor cell killing and stimulation of host immune cells, including dendritic cells and CD8+ T cells (31–33). However, no association has been found between the level of IFN-α and immune-related adverse events. According to our results, we can explain why overactivated immune cells can also damage other normal cells, which may lead to immune-related adverse events.

IFN-γ has various roles in immune reactions against tumors, including stimulation of tumor-infiltrating lymphocyte (TIL) proliferation and differentiation and secretion of IFN-γ following activation of T lymphocytes by tumor antigens (34). In contrast, IFN-γ may also promote the production of immunosuppressive molecules, which can have direct negative feedback on effector T cell function (35). During the elimination phase of the immune response against tumor cells, recruited tumor-infiltrating macrophages and NK cells produce various cytokines,

including IFN-γ, to kill tumor cells (36). Therefore, an elevated level of IFN-γ may suggest increased cytotoxic activity against lung cancer tumor cells. However, this mechanism of action can also give rise to autoimmune-like side effects known as irAEs. In a study by Constantini (15) IFN-γ levels at nivolumab initiation and two months later did not show correlations with the objective response rate, clinical benefit, or survival, which is consistent with our study.

The types of inflammatory factors produced by different lung cancer patients receiving immune checkpoint inhibitor therapy and the body's response to the drug treatment, and the activated immune inflammatory pathways are also different. We can further clarify the relationship between cytokine level changes during treatment and the efficacy of immunotherapy by observing the longitudinal cyclical trend of cytokine level changes. In addition, the follow-up period for which clinical data are available is relatively short, and we need to evaluate the significance of these peripheral blood biomarkers in terms of long-term clinical benefit. At the same time, the small sample size may also affect the results of our statistical analysis, which should be addressed in future studies.

In the past decades, cytokines and cytokine receptors have been extensively studied as cancer targets or cancer therapy by enhancing the growth inhibitory and immunostimulatory effects of interferons and interleukins, such as IL-2, IL-7, IL -12 and IL-15, or by inhibiting the inflammatory and tumor-promoting effects of cytokines such as TNF, IL-1β and IL-6 (10). For some cytokines, their ability to initiate pleiotropic immune responses can both increase antitumor immunity and decrease autoimmunity, which may improve their potential for clinical use with immunotherapy, especially in mitigating irAEs. The emergence of immunotherapy and an improved understanding of the tumor microenvironment have provided new approaches for the use of cytokines to treat tumors, including the use of cytokine based therapies to enhance antitumor activity or mitigate immune-related adverse reactions. Many challenges remain, especially due to the pleiotropic and often conflicting roles of many cytokines. The carcinogenic and anticancer mechanisms of cytokines still need to be confirmed by a large number of pre-clinical studies, so their anti-tumor efficacy can only be revealed in the future.

At present, a large number of targeted treatments for irAEs with cytokine antibodies have been reported, suggesting that cytokines are both effector molecules in the anti-cancer effects of immune checkpoint inhibitors and contributors to the mechanism of irAEs development. We found the cytokines as predictive precursors for irAEs. With an increasing number of studies highlight the ability of next-generation immunotherapies to engage individual cytokines in controlling anti-tumor immune responses, more research is needed to determine their impact on irAEs development. Our study showed that IL-1, IL-2, IL-4, IL-5, IL-12, IL-17, IFN-α, IFN-γ, and TNF-α were not associated with the prediction of immunotherapy efficacy, which was related to the relatively short follow-up period for which we could obtain clinical data, and the small sample size may also affect the results of our statistical analysis. This problem should be addressed in future studies.

Conclusion

Cytokine serum levels may provide prognostic information and constitute predictive markers of immunotherapy benefits in patients with lung cancer. Further studies of the predictive effects of these markers in larger populations are warranted.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The First Affiliated Hospital of Xi'an Jiaotong University,2020(G175). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Study concept and design: NZ and CL. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: NZ. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: NZ. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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PD-1 inhibitor therapy causes multisystem immune adverse reactions: a case report and literature review

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Immune checkpoint inhibitors(ICIs), including cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4), programmed cell death protein 1 and its ligand (PD-1/PD-L1) inhibitors, have been shown to have antitumor activity in various solid tumors. Their mechanism of action is to selectively restore and normalize the body's immune reponses by disrupting the immunosuppressive signals mediated by PD-1, PD-L1 and CTLA-4 in the tumor microenvironment. With the increase in clinical applications of ICIs, reports of immune-related adverse events (irAEs) have also increased. This article reports a case of a lung cancer patient who developed multisystemic adverse effects after PD-1 inhibitor application: myocarditis, myositis and thrombocytopenia, and analyzes the role of Interleukin 6(IL-6)in the management of irAEs. Despite the patient's eventual discontinuation of antitumor therapy due to severe irAEs, a significant and durable therapeutic response was observed.

KEYWORDS

immunotherapy, lung cancer, immune checkpoint inhibitor, myocarditis, myositis, thrombocytopenia, IL-6

Introduction

ICI enhances the anti-tumor activity of the host immune system by blocking checkpoint molecules. The results of clinical studies have shown that ICI has clear effects in the treatment of melanoma and advanced non-small cell lung cancer and can also be applied in the treatment of malignant tumors such as breast, head and neck, gastric, uroepithelial, and lymphoma (1, 2). With the increase in clinical use, ICIs have developed immune-related adverse reactions (irAEs) (3, 4), mainly in vital organs such as the skin, gastrointestinal tract, endocrine glands, liver and lungs, and have potential effects on other organs and tissues. Glucocorticoids are the first line of treatment for irAE. If steroid fails, second-line treatment is considered. The main drugs are inhibitors of

T-cell immunity, including mycophenolate mofetil (MMF), azathioprine (AZA), anti-human thymocyte immunoglobulin (ATG) and tacrolimus. Immunoglobulins, plasma replacement and new biologics such as infliximab have also been used to suppress immunity. Esfahani's team noted that a comprehensive assessment of the histological features of the organs involved in irAE, obtaining peripheral blood flow cytology and measuring autoantibody levels and cytokines is needed before treatment. They suggest a more refined classification and treatment of irAE based on individualized features (5).

Among cardiac adverse reactions, myocarditis has a low incidence but can be life-threatening, with a mortality rate of 25% to 50% (6, 7). Here, we report a case of ICI-induced multisystemic adverse effects after the treatment of anti-PD-1 therapy for lung adenocarcinoma in which immune-associated myocarditis can be life-threatening. A 67-year-old gentleman with lung adenocarcinoma was given 5 cycles of chemotherapy combined with immunotherapy as second-line treatment after disease progression from first-line chemotherapy. The patient mainly complained about persistent weakness and myalgia followed by chest pain, dyspnea, and markedly elevated laboratory parameters such as troponin and cytokines. He was diagnosed as ICI-induced myocarditis overlapping with myositis and thrombocytopenia. Then, his symptoms were resolved after prompt therapy with high-dose steroids. Our report aims to raise awareness of the early prediction, early intervention and correct treatments in ICIs-induced rare side effects during the immune checkpoint blockade treatments of common tumor. It also initially analyzes the role of Interleukin 6(IL-6) in the management of irAEs and the relationship between irAE episodes and ICI outcomes, providing clinical evidence for real-world research.

Case presentation

The patient, a 67-year-old male, presented to our hospital in April 2020 with "hoarseness with hemoptysis". He had no history of hypertension, diabetes mellitus, coronary heart disease, asthma, liver disease or other underlying diseases. He also had no family history of tumours. After admission, the patient underwent a lung computed tomography (CT) examination, which revealed a mass-like high-density shadow of about 4.8x4.6x6.1 cm in the upper lobe of the left lung (Figures 1A, B), considering peripheral lung cancer; enlarged lymph node shadow in the mediastinum (Figure 1C), considering metastatic cancer; nodular high-density shadow in both lungs (Figure 1D), considering metastatic cancer. Pathological findings on lung puncture biopsy suggested hypofractionated adenocarcinoma with immunohistochemical results showing CK5/6 (partial +), CK7 (+), Ki-67 (+80%), Napsin A (-), P40 (-), Vimentin (+), CK-pan (+), TTF-1 (+) (Figure 2). A head magnetic resonance imaging (MRI) performed for a systematic evaluation suggested abnormal signal in the right parietal lobe (Figures 1E, F), and metastases were considered. No evidence of metastases was found on abdominal CT and bone scan. Pathologic staging was defined according to the American Joint Committee on Cancer (AJCC) TNM staging system, 8th edition, and combined with imaging and pathologic findings, the clinical diagnosis of this

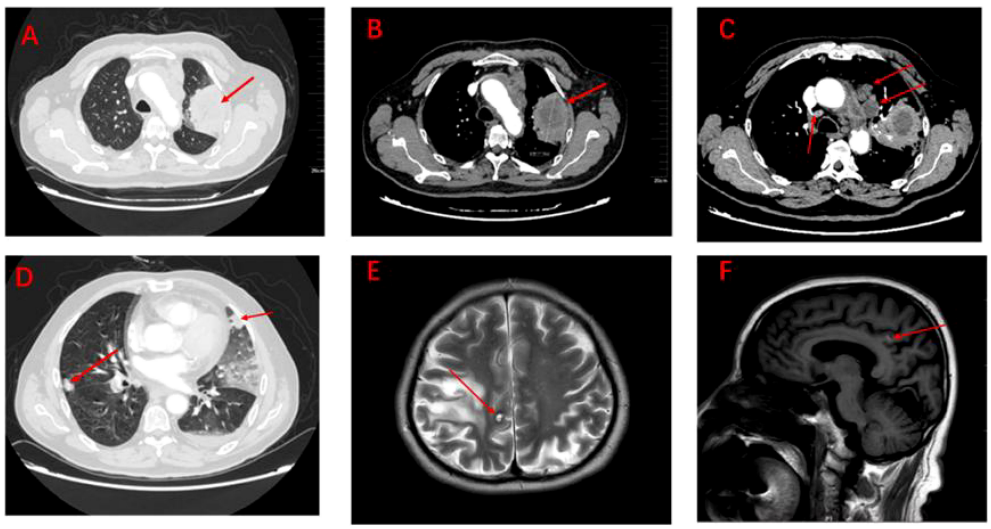


FIGURE 1
Imaging of tumour lesions at baseline **(A–F)**. **(A)** Pulmonary lesions under the lung window. **(B)** Pulmonary lesions under the mediastinum window (measuring 4.8cm x 4.6cm x 6.1cm). **(C)** Lymph node metastatic lesions under the mediastinum window. **(D)** Metastatic lesions in both lungs under the lung window. **(E)** Brain metastases under the coronal plane. **(F)** Brain metastases under the sagittal plane.

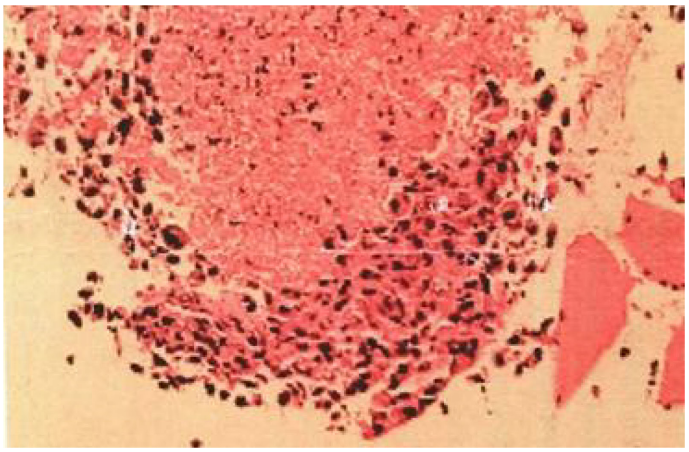


FIGURE 2
Lung tissue sections stained with hematoxylin-e.

patient was left lung adenocarcinoma with bilateral lung and brain metastases (cT4N3M1b stage IV). Genetic testing suggested a positive K-RAS gene. After one cycle of chemotherapy with "pemetrexed + carboplatin", a repeat lung CT showed that the lung lesions, lymph nodes and bilateral lung metastases were larger than before (Figures 3A, B). The second line of treatment was "albumin paclitaxel + nedaplatin + PD-1 inhibitor (Sintilimab)", and the lung lesions (Figures 3C, D) and metastases (Figures 3E–G) were significantly reduced after 2 cycles. The efficacy assessment reached PR (partial response), and chemotherapy combined with immunotherapy was continued for 3 cycles. During the treatment, regular monitoring of routine blood, liver and kidney function, immunological indexes (including cardiac enzymes, thyroid function, pituitary function, etc.) and electrocardiogram and cardiac ultrasound did not reveal any significant abnormalities. The efficacy was assessed as maintenance PR (Figures 3H–K). On September 26, 2020, the patient was admitted to the hospital with "peripheral discomfort with marked malaise and nausea", and laboratory tests showed a decrease in platelets with a minimum value of 27X10^9/L. After symptomatic treatment (thrombopoietin 15000 IU, once per day, subcutaneous injection), platelets were restored to normal, and PD-1 inhibitor (sintilimab) was administered as maintenance therapy. On November 1, 2020, the patient was readmitted to the hospital with "increased malaise with myalgia". In the afternoon of the second day of admission, the patient developed fever with a maximum temperature of 40.1°C, respiratory distress, increased heart rate, decreased oxygen saturation, and drowsiness, accompanied by a decrease in blood pressure (minimum value of 74/43 mmHg). Laboratory tests showed creatine kinase (CK) 1398 U/L (normal range: 50-310 U/L), myoglobin 3346 ng/ml (normal range: 1-121 ng/ml), troponin I 0.153 ng/ml (normal range: 0-0.034 ng/ml), brain natriuretic peptide (BNP) 12300 pg/ml (0- 125 pg/ml), which were significantly elevated, serum IL-6 was 4835.57 pg/ml and IL-10 was 122.18 pg/ml. Electrocardiogram suggested tachycardia, the rightward shift of the cardiac axis, and ischemic-type changes in the ST segment of leads II and V4. Based on medical history and auxiliary examinations, coronary diseases causing ST-segment ischemic manifestations were ruled out. ICI-mediated myocarditis and myositis was highly considered. Based on the above information, steroids were given to the patient according to the changes in troponins, myocardial enzymes and symptoms (methylprednisolone ivvp. 120mg q12h for 10 days, 120mg qd for 3 days, 80mg qd for 4 days, 60mg qd for 6 days, 40mg qd for 5 days). Gradually, the patient's vital signs were relatively stable. Serum IL-6 decreased to 36.17 pg/ml and IL-10 decreased to 8.38 pg/ml. Creatine kinase, myoglobin, troponin and BNP gradually returned to normal (changes in laboratory indicators are shown in the Figures 4A, B). Furthermore, on day 3 of hospitalization, the patient again developed thrombocytopenia with a minimum value of 41X10^9/L. Bone marrow evaluation (Figure 5) showed poor maturation of megakaryocytes, and immune-associated thrombocytopenia was considered. And evidence of metastatic cancer invasion was not present. After excluding chemotherapy, infectious etiology, or other drug-induced thrombocytopenia, we considered a diagnosis of immune thrombocytopenia induced by sintilimab. Patient continued to receive steroids. After discharge, oral prednisone was administered and gradually tapered. His platelets returned to normal on December 12,2020 (changes in platelet levels are shown in Figure 6). No further immune-related adverse events occurred. Regular imaging examinations were performed, the tumor lesions continued to shrink, and the efficacy maintained at PR (Figure 7). At present, the patient is still under regular follow-up. The administration of immune-related toxicity and the effect of treatment are shown on the Table 1. The clinical course of this patient is summarized in Figure 8.

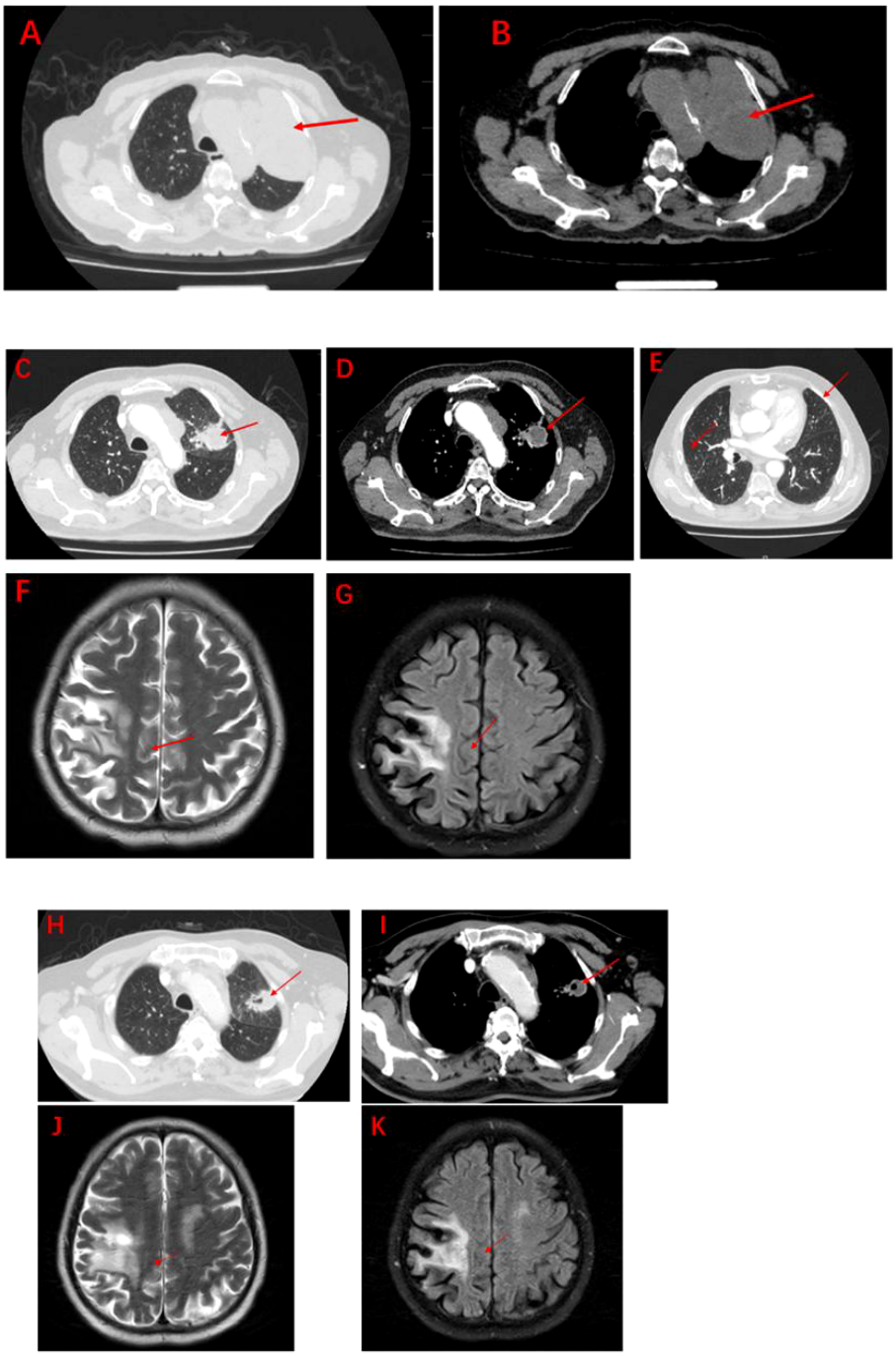
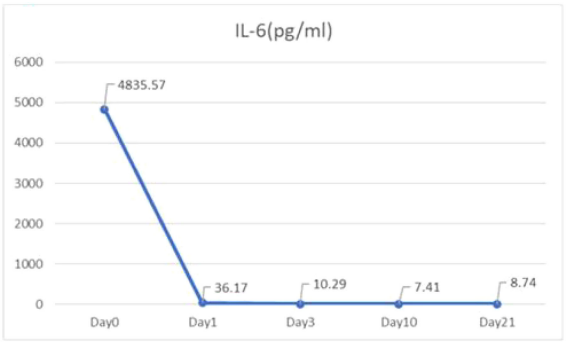


FIGURE 3
Imaging after anti-tumour therapy **(A–K)**. **(A, B):** Significant increase in lung lesions and bilateral lung metastases after 1 course of pemetrexed in combination with carboplatin (measuring 5.5cm x5.7cm x7.1cm). **(C-E):** The second line of treatment was "albumin paclitaxel + nedaplatin + PD-1 inhibitor (Sintilimab)", and the lung lesions and metastases were significantly reduced after 2 courses (measuring 3.3cm x2.8cm x4.6cm). The efficacy assessment reached PR. **(F, G)**: Significant reduction of brain metastases after 2 courses of Sintilimab in combination with chemotherapy. **(H, I):** The lung lesions continue to shrink after 4 courses of Sintilimab in combination with chemotherapy (measuring 2.8cm x 2.4cm x 3.9cm). **(J, K):** Brain metastases barely detectable after 4 courses of Sintilimab in combination with chemotherapy.

A



B

Myosin and cardiac enzymatic indices	Day0	Day1	Day3	Day4	Day5	Day8	Reference range
Creatine kinase	-	1398	320	-	-	65	50-310U/L
Myoglobin	3346	784.1	97	64.6	62.4	75.4	1-121ng/ml
Troponin I	0.015	0	0.153	0.066	0.024	0.015	0-0.034ng/ml
BNP	1720	1510	12300	7960	4320	882	0-125pg/ml

FIGURE 4
Changes in laboratory indicators during the onset of immune-related toxicity in patients **(A, B)**. **(A)**: Changes in IL-6 (Day0 represents the level after 0 days of steroid treatment; Day1 represents the level after 1 days of steroid treatment; Day3 represents the level after 3 days of steroid treatment; Day10 represents the level after 10 days of steroid treatment; Day21 represents the level after 21 days of steroid treatment.) **(B)** Changes in cardiac parameters (Day0 represents levels after 0 day of steroid treatment; Day1 represents levels after 1 day of steroid treatment; Day3 represents levels after 3 days of steroid treatment; Day4 represents levels after 4 days of steroid treatment; Day5 represents levels after 5 days of steroid treatment; Day8 represents levels after 8 days of steroid treatment).

Discussion

Immune-associated myocarditis

Our understanding of the pathophysiological mechanisms of ICI-induced myocarditis comes from animal studies (8), and data from relevant animal model studies suggest that the PD-1/PD-L1 and CTLA-4 signalling pathways downregulate excessive immune responses in cardiomyocytes and have essential protective effects on the myocardium (9). Early on, it has been shown that CTLA-4 and PD-1 deficiency can cause autoimmune myocarditis (10, 11). Both PD-1-CD4+ T cells and PD-1-CD8+ T cells mediate myocardial injury, and both T cell subsets require PD-1 to maintain their tolerance to myocardium (12). PD-L1, the ligand of PD-1, is expressed in the myocardium of both humans and mice. It was found that genetic deletion of both PD-L1 and PD-L2 and the role of ICI can cause transient myocarditis that eventually progresses to fatal disease,

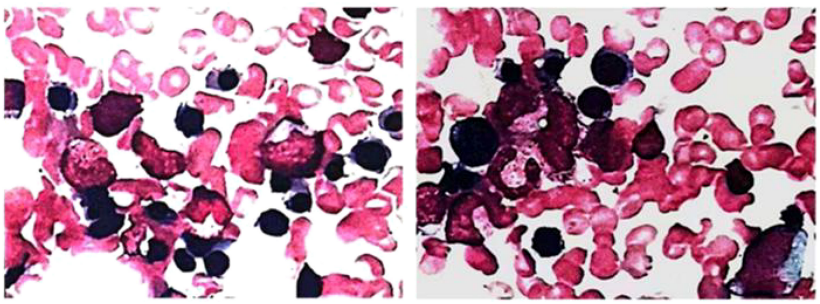


FIGURE 5
Bone marrow evaluation showed poor maturation of megakaryocytes.

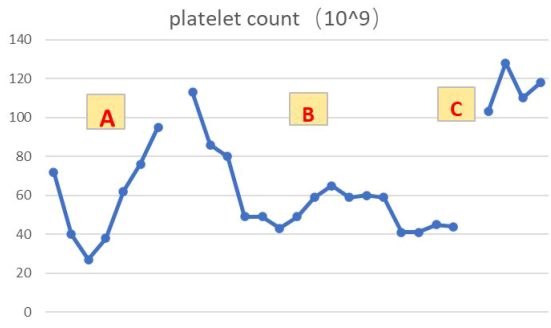


FIGURE 6
Platelet changes during the course of the disease **(A–C)**. **(A):** Reduced platelet levels after 5 courses of chemotherapy combined with immunotherapy, with gradual recovery after symptomatic treatment. **(B):** Platelet levels decreased again after 1 course of immune consolidation therapy. **(C):** After discharge from hospital, oral steroids were administered and platelets recovered and maintained in the normal range.

confirming that PD-1 signalling plays a key role in protecting the myocardium from damage by T-lymphocyte immune responses (13). In addition, recent case reports have found autoantibodies detected in patients with ICI-associated myocarditis, suggesting antibody-mediated myocardial injury (14). Future studies need to clarify further the mechanisms of toxicity and associated risk factors in ICI-associated myocarditis.

Clinical symptoms of immune-related cardiovascular toxicity are varied and may present as mild non-specific symptoms such as malaise and weakness. Typical symptoms associated with cardiac diseases such as dyspnea, chest pain, pulmonary edema, bilateral lower extremity edema, cardiac arrhythmia, and acute heart failure may also be present. Other atypical symptoms include myalgia and syncope. There are no uniform diagnostic criteria for immune-associated myocarditis, and the generally accepted gold standard is endomyocardial biopsy or histopathological findings. Still, its invasive nature, risk of cardiac perforation and time-consuming biopsy limit its application. The sensitivity and specificity of cardiac magnetic resonance T1-weighted and T2-weighted images and late gadolinium enhancement for the diagnosis of immune myocarditis are 76% and 96%, respectively (15). Echo cardiography may suggest abnormal ventricular wall motion, and electrocardiogram may be positive. However, immune myocarditis often occurs insidiously, progresses relatively rapidly, and most patients presenting to the clinic are more severe, making it difficult to obtain definitive diagnostic evidence. According to guideline recommendations (16), cardiac troponin (cTn) and creatine kinase (CK) can be used to guide the diagnosis and management of ICI-associated myocarditis. Therefore, it is particularly important to advise patients to monitor cardiac enzyme profiles (troponin I or T, CK, CK-MB, and natriuretic peptide) at baseline and periodically during drug administration. However, the clinical

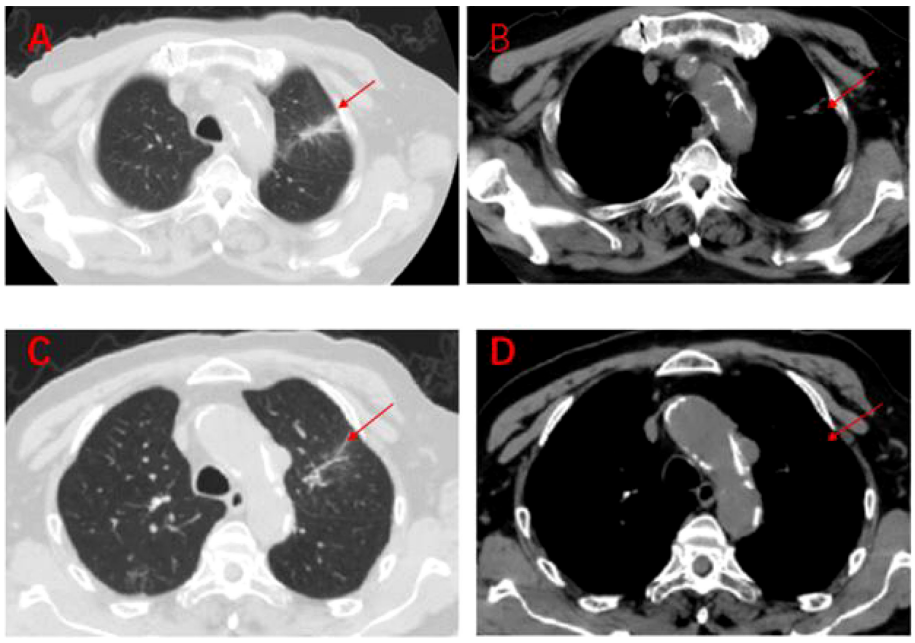


FIGURE 7
Regular imaging at follow-up, tumour lesions continue to shrink, efficacy maintained at PR **(A–D)**. **(A, B)**: On 7 March 2020, a lung CT scan revealed almost no tumour lesions. **(C, D)**: On 3 October 2020, a lung CT scan revealed almost no tumour lesions.

TABLE 1 The administration of immune-related toxicity and the effect of treatment.

IRAE	Treatment	Treatment effects
Myocarditis	**During hospitalization:** Methylprednisolone ivvp. 120mg q12h for 10 days, 120mg qd for 3 days, 80mg qd for 4 days, 60mg qd for 6 days, 40mg qd for 5 days. **After discharge:** oral prednisone was administered and gradually tapered.	Gradually, the patient's vital signs were relatively stable. Serum IL-6 decreased to 36.17 pg/ml and IL-10 decreased to 8.38 pg/ml. Creatine kinase, myoglobin, troponin and BNP gradually returned to normal.
myositis		Weakness and myalgia gradually disappear
thrombocytopenia		Platelets gradually recover and eventually stabilize in the normal range.

value of serum biomarkers (e.g., troponin) for the early detection of ICI-associated myocarditis still needs to be confirmed by further evidence and studies.

The patient had significant chest tightness, palpitations, and precordial discomfort during maintenance treatment with PD-1 inhibitor. Laboratory tests suggested that the cardiac enzyme profile was elevated to 0.153 ng/ml for ultrasensitive troponin I and 12,300 pg/ml for BNP. Electrocardiogram suggested ST-segment ischemic changes, but cardiac ultrasound showed no abnormalities. According to NCCN guidelines, abnormal echoes suggestive of echocardiography without hypotension and cardiac markers >3 times the upper limit of normal were defined as severe ICI cardiovascular toxicity. Heart rate arrhythmia, hemodynamic instability (hypotension/cardiomyopathy), and cardiac markers >3 times the upper limit of average values were defined as life-threatening cardiovascular toxicity (17). Therefore, this patient was diagnosed with ICI-associated myocarditis with reduced blood pressure, classified as severe cardiovascular toxicity and significantly controlled with prompt steroids therapy.

Skeletal and cardiac muscles belong to the same transverse muscle. Some studies (18, 19) have found that anti-transverse muscle antibodies mediate both immune myositis, myocarditis and myasthenia gravis, which may act as biomarkers for these immune-related adverse events. This suggests that autoimmune targets with similar epitopes may exist in cardiac and skeletal muscle. In addition, the PD-1 signaling pathway plays an important role in the autoimmune response of these tissues (12, 20). Usually, the main clinical manifestation of skeletal muscle toxicity after immune checkpoint inhibitor therapy is a weakness with myalgia, characterized by elevated serum creatine kinase and myoglobin, and in severe cases, rhabdomyolysis. In this case, the main manifestation of skeletal muscle toxicity was "weakness with myalgia" before admission to the hospital. After admission, creatine kinase 1398 U/L and myoglobin 3346 U/L were 10 times higher than normal. The patient was considered to have skeletal muscle and cardiac muscle damage, and serum IL-6 was elevated to 4835.57 pg/ml and IL-10 to 122.18 pg/ml, suggesting an immune storm. After steroids treatment, his weakness and myalgia symptoms gradually improved, and creatine kinase and myoglobin levels gradually returned to normal. Therefore, the manifestation of skeletal muscle injury due to ICI may be an early stage of immune-associated myocarditis, and early recognition would be beneficial to improve the patient prognosis.

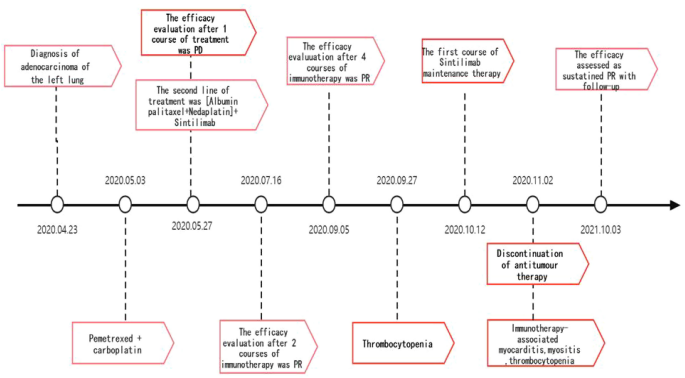


FIGURE 8
A summary of a clinical course.

Immune-related thrombocytopenia

The accepted mechanisms regarding immune-associated thrombocytopenia are antibody-driven and T-cell-mediated. It has been suggested that activation of CD4+ helper T cells and CD8+ cytotoxic T cells in patients treated with ICIs are involved in the immune response, leading to hematopoietic stem cell injury and inducing immune-associated thrombocytopenia and other hematologic complications (21). A single case of non-small cell lung cancer (NSCLC) with nivolumab reported that nivolumab induces or increases the production of platelet auto-specific Ig antibodies, which leads to impaired platelet maturation and reduced platelet production by bone marrow megakaryocytes (22). Thrombocytopenia is associated with the presence of platelet antibodies, autoantibodies, and thyroglobulin antibodies, and is accompanied by a decrease in the number of helper T cells and regulatory T cells (23). In addition, there is evidence that PD-1, Treg pathways may be involved in the development of immune-related thrombocytopenia. Compared to healthy individuals, peripheral blood T cells of immune thrombocytopenic individuals have lower PD-1 expression, and PD-1 levels are lower in acute thrombocytopenic individuals than in chronic individuals (24). Furthermore, bone marrow biopsy revealed that the bone marrow of immune platelet-depleted individuals expressed lower Treg than healthy individuals, suggesting that platelet decline may be associated with Treg (25). Regardless of the mechanism of occurrence or clinical features, immune thrombocytopenia caused by ICIs has similarities to classical immune thrombocytopenia (26), and interference from infection, tumor progression, and other chemotherapeutic agents used in combination with ICIs needs to be excluded.

In this case, the patient developed thrombocytopenia after 5 cycles of chemotherapy combined with immunotherapy. After symptomatic treatment, the platelets returned to normal, at which point the cause of thrombocytopenia would conventionally be considered to be related to chemotherapy. However, the platelets were again reduced after discontinuing chemotherapy drugs and continuing to PD-1 inhibitors. Further observation, we found that there was an overlap between the time points of thrombocytopenia and IL-6 elevation, a phenomenon that indirectly suggests that cytokines further promote immune disorders during irAEs (27). Bone marrow smear examination suggested poor maturation of megakaryocytes and scattered rare platelets, which were considered secondary alterations. After steroids treatment, platelets recovered and were maintained at normal. In this case, we thought thrombocytopenia as a high probability of hematologic toxicity due to ICI, and IL-6 plays an essential role in immune disorders.

Role of IL-6 in irAEs

Interleukin-6 (IL-6) is an inflammatory cytokine which has a critical role in the systemic immune system and is associated with various diseases, including cancer (28). IL-6 signaling is complex. At low levels, IL-6 activates anti-inflammatory pathways *via* classic signaling. However, as observed in CRS (Cytokine Release Sydrome), IL-6 at high levels causes proinflammatory effects *via* trans-signaling (29). Common features of the clinical presentation of irAEs were that of a systemic inflammatory response, with an increase in circulating pro-inflammatory cytokines likely triggered by ICI-induced T-cell stimulation (30, 31). Although the pathogenesis of irAEs remains to be clarified, it is hypothesized that irAEs are related to infiltration of activated CD8+ and CD4+ T-cells in target tissues (30) and elevated serum levels of inflammatory cytokines (including IL-6) (31–33). IL-6 promotes tumor progression and metastasis through multiple mechanisms including feed forward activation of oncogenic pathways, inhibition of dendritic cell differentiation, and myeloid-derived suppressor cells augment (34).

CRS may occur after immunotherapy. Studies suggested IL-6, TNF-α, IFN-γ and CRP as monitoring indicators to avoid severe CRS (35, 36). However, the utility of IL-6 as a biomarker for irAE development is largely unknown. IrAEs effect may induce IL-6, especially against the PD-1/PD-L1 axis (37). Literature summarizing a series of cases and studies has found that baseline levels of some cytokines (including IL-6) may be low in patients with irAEs, but changes that rise abruptly after treatment may be associated with irAEs (38). A case report on immune-associated pneuomonitis also tentatively suggested that elevated IL-6 and CRP (C-reactive protein, as downstream molecular product of IL-6) after PD-L1 inhibitors were associated with the development of irAEs in non-small cell lung cancer (NSCLC) and that IL-6 could be one of the potential mediators of irAEs in NSCLC patients treated with ICIs (39). A case report (37) found that elevated serum IL-6 and CRP were proportional to the severity of ICI-associated colitis, and after receiving steroids, their decreased levels were proportional to the degree of remission of colitis, and the results suggest that IL-6 and CRP may be biomarkers for the diagnosis and prediction of irAEs. Two retrospective case studies exploring the efficacy of tocilizumab(IL-6 receptor antagonist) showed that tocilizumab may be a therapeutic strategy for the management of steroid refractory irAEs secondary to immune checkpoint blockade. Moreover, in most cases in both studies, biomarkers of the inflammatory process (IL-6 or CRP levels) decreased rapidly after tocilizumab treatment with clinical improvement and symptom relief, demonstrating the clinical significance of IL-6 in the pathogenesis and management of these events (40, 41).

In this case, the cytokine IL-6 rose to thousands of times its normal level while multiple irAEs were present, which together indicates T cell hyperactivity. After steroids, IL-6 gradually decreased to near normal. Thus, this case also suggests that IL-6 may be a sensitive indicator of specific immune-related adverse reactions, but its sensitivity still needs to be investigated by a

large amount of data and experiments. Therefore, routine monitoring of IL-6 and CRP in patients treated with ICIs would be helpful in predicting the clinical course of irAEs.

Immunotherapeutic efficacy and immune-related adverse events

Immunotherapy has changed the therapeutic landscape of oncology, and the exploration of effective biomarkers to identify patients most likely to benefit from ICI is one of the hot topics in oncology. To date, predictive biomarker studies have focused on pre-treatment tumor characteristics such as microsatellite instability status, PD-L1 expression and tumor mutational load. Clinical biomarkers in treatment have been less studied. A growing number of studies have found a correlation between the incidence of irAEs and treatment response to ICI (42–46). However, the mechanism between irAE appearance and antitumor effect is not yet apparent. Under molecular mechanisms, irAE may be triggered by a common antigen expressed by tumor and inflamed organ (19, 47–49). Unleashed T cells produce toxicity and response by binding to T cell receptor in target tissues. Besides, study suggests gut microbiome may be a complementary explanation for the relationship between irAE and immune efficacy (50, 51). Gut microbes are diverse and complex in composition, so gut microbiome mechanism still need extensive prospective studies to explore. Unlike the two views above, other studies suggest that tissues which develop autoimmune toxicity after ICIs may express organ-specific antigen independent of antitumor response, i.e., such organ-specific antigen mag be pre-existing (52). Onset of irAE may predict response to PD-1 and PD-L1 antibodies, this correlation has been demonstrated in various advanced malignancies, including melanoma (42, 44), NSCLC (45, 46), and gastrointestinal tumor (53), etc. Most of these studies concluded that patients experiencing irAE show significant improvements in progression-free survival, overall survival, or overall remission rates. Studies on the relationship between immunotoxicity and efficacy of CTLA-4 antibody mainly focus on melanoma. Some studies affirm the predictive role of irAE in response of CTLA-4 antibody (43), but others also questione this hypothesis (54).

Key questions regarding the association between irAE onset and ICI efficacy remain. The primary concerns involve whether irAE site, quantity, severity, timing of onset and management influence ICI efficacy. Most studies favor the perspective that patients cutaneous or endocrine (e.g., thyroiditis) irAE exhibit better PFS, OS benefit (44–46, 55, 56). This correlation may stem from the hypothesis that tumor cells express the same antigens as target organ (57). The number of irAEs may influence irAE versus ICI efficacy. The group experiencing ≥2 irAEs present an unprecedented OS benefit compared to the Nivolumab treatment group experiencing one irAE, which indicate that multiple immunotoxicity can reflect a sustained antitumor response (46). IrAE severity is positively correlated with immune efficacy, deriving from higher T-cell activity and stronger immunosuppressive effects in severe irAE (58). Steroids are usually applied against irAE, but steroids are known to be immune-suppressive. The study found that treatment effect compared with placebo after an irAE onset and after day 30 of steroid use appeared to be lower than the effect after an irAE onset and without steroid or by day 30 of steroid use (42). However, other studies suggested that patients on low-dose steroid show a better survival benefit and that high-dose steroid for irAE may diminish ICI efficacy (59). Another study found two lung cancer patients treated with high-dose steroids after developing autoimmune colitis, yet a sustained tumor remission was still observed (43). It is surprising to note that eliminating the autoimmune adverse effects of anti–CTLA-4 with steroids did not seem to interfere with antitumor activity.

But, in short, a full understanding of the true impact of irAE characteristics on ICI efficacy still needs to be demonstrated in larger prospective studies. The patients in this report achieved a PFS of more than 1 year after the occurrence of irAEs, discontinuation of immunotherapeutic agents, and no further antitumor therapy. In addition, the decision to restart immunotherapy in patients who develop severe ICI-related toxicity needs to be made by a clinical multidisciplinary team after considering the risks and benefits of treatment.

With the boom in immunotherapy, preclinical models related to immunotherapy are at the forefront of the medical field. Animal and *in vitro* models have been used for cancer pathogenesis, signaling pathways, therapeutic screening and translational applications (60–62). Given that, many groups are developing elegant and specific preclinical models to examine irAEs. One study used a syngeneic murine Head and neck squamous cell carcinoma (HNSCC) cell panel to accurately recapitulate the tumor immune microenvironment (TIME)and further explore new immune therapeutic options (63). Using a mouse model of HNSCC, Gilardi M team developed a novel, local delivery strategy based upon an array of soluble microneedles (MN). Local-MN delivery of anti-CTLA-4 *in vivo* can protect animals from irAEs observed, but this process relies on CD8 T cells and conventional dendritic cell type 1 (cDC1) (64). Additionally, the 3D culture models of the tumorpromoting microenvironment *in vitro* will contribute to a comprehensive understanding of the mechanisms of malignant metastasis *in vivo* and facilitate the development of novel anti-tumour drugs (65, 66). The microfluidic technology-based method for lung cancer cell-lines categorization is an efficient and promising model for lung cancer differential diagnosis (67). Microfluidic vascular *in vitro* models are considered an ideal model to replicate and mimic the *in vivo* metastatic progression (68). The complexity of the TIME constitutes a major mechanism of resistance to immunotherapy. Immunotherapy-related toxicity is a major concern. The analysis and modeling of the complexity of the microenvironment should

receive more attention in the field of immuno-oncology. Therefore, given the results obtained in the above studies, future work will extend the framework to predict the occurrence of immunotherapy resistance and irAE.

Conclusion

In summary, clinical vigilance should be increased for rare fatal immunotoxicity caused by PD-1 inhibitors. In this paper, we summarize the diagnosis, treatment, and regression of a case of severe immune myocarditis with myositis and thrombocytopenia caused by PD-1 inhibitors, suggesting the importance of early diagnosis and intervention. This case also provides a preliminary analysis of the predictive value of IL-6 for irAE, as well as real-world clinical evidence for the correlation between irAE and immune efficacy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

NY and XL contributed equally to this work. NY provided case information and contributed to data analysis and manuscript writing. XL drafted the manuscript. XY, WS, and JL performed the clinical management of the patient. XC revised the article critically for important intellectual content and contributed to the project development. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Impact of the interval between neoadjuvant immunochemotherapy and surgery on surgical–pathological outcomes in non-small cell lung cancer

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Introduction: The interval between neoadjuvant immunochemotherapy and surgery in patients with non-small cell lung cancer (NSCLC) has not been well characterized. This study investigated the association between the time-to-surgery (TTS) interval and surgical–pathological outcomes.

Method: Clinical data of patients who received neoadjuvant immunochemotherapy followed by surgery for NSCLC between January 2019 and September 2021 were collected. The patients were divided into three groups based on TTS interval: the early-surgery group (ESG), the standard-surgery group (SSG), and the delayed-surgery group (DSG). The primary outcomes were objective response rate (ORR), major pathological response (MPR), and pathological complete response (pCR). The secondary endpoint was surgical outcome.

Results: Of the 171 patients, 16 (9.4%) received surgery in ≤28 days, 49 (28.7%) received surgery within 29–42 days, and 106 (61.9%) received surgery in ≥43 days after neoadjuvant immunochemotherapy, with a median TTS of 46 days. The postoperative drainage of the ESG group (455.1 ml) was significantly less than that of the SSG group (680.7 ml) and the DSG group (846.5 ml; p = 0.037). However, the TTS interval did not influence the duration of the operation (P = 0.54), the extent of intraoperative bleeding (P = 0.60), or the length of postoperative hospital stay (P = 0.17). The ORR was observed in 69%, 51%, and 56% of patients in the ESG, the SSG, and the DSG, respectively (P = 0.46), and MPR occurred in 50%, 47%, and 58% (P = 0.38) of patients in the ESG, the SSG, and the DSG, respectively. Similarly, no statistically significant difference was found for pCR (ESG: 31%; SSG: 27%; DSG: 42%; P = 0.14).

Conclusion: This retrospective study indicated that TTS exerts no significant effect on the feasibility and safety of surgery in the neoadjuvant immunochemotherapy setting of NSCLC. Analysis of the TTS interval revealed a tendency for delayed surgery to be associated with a pathological response in NSCLC, although this association was not statistically significant.

KEYWORDS

time-to-surgery (TTS) interval, surgery, surgical safety, pathological outcomes, neoadjuvant immunotherapy

Introduction

Lung cancer remains the leading cause of cancer-related death worldwide (1, 2), accounting for 24% and 23% of cancer-related deaths in men and women, respectively. In the past few years, preoperative programmed cell death protein 1 (PD-1) or its ligand, PD-L1, alone or combined chemotherapy, has been investigated in several clinical trials of non-small cell lung cancer (NSCLC) (3–7). This treatment pattern, which can effectively reduce the size of locally advanced tumors and improve their pathological response (8), is recommended for early-stage NSCLC and resectable, locally advanced NSCLC.

Previously, Liu et al. (9) established a spontaneously metastatic cancer model in mice with 4T1.2 and E0771 cancer cell types and demonstrated that a short duration (4–5 days) between the first administration of neoadjuvant immunotherapy and resection of the primary tumor was necessary for optimal efficacy and that extending this duration (≥10 days) or giving neoadjuvant immunotherapy too close to surgery (≤2 days) reduced immunotherapy efficacy. These results suggest that the time-to-surgery (TTS) interval should be carefully considered to achieve a better oncological outcome. However, limited data exist to determine the optimal TTS in NSCLC, particularly in the neoadjuvant immunochemotherapy setting. According to the latest expert consensus (10), it is recommended that surgery be performed 4–6 weeks after the last cycle of neoadjuvant immunochemotherapy. Nevertheless, no research has validated this recommendation or thoroughly investigated the association between the TTS interval and pathological downstaging. Furthermore, whether a long TTS interval increases surgical difficulty has not been established.

We thus conducted a population-based, real-world, retrospective study to evaluate whether TTS impacts surgical and pathological outcomes.

Method

Patients who had biopsy-confirmed, clinical stage II/III NSCLC and who received neoadjuvant immunochemotherapy followed by surgery for NSCLC between January 2019 and September 2021 were identified from the clinical data. The preoperative and postoperative staging were evaluated in accordance with the eighth American Joint Committee on Cancer (AJCC) and lung cancer staging manuals on the tumor, node, and metastasis (TNM) staging systems (11). The TTS interval was defined as the time from the day of the last treatment cycle to the day of surgery. The patients were divided into three groups based on TTS: the early-surgery group (ESG: TTS ≤28 days; n = 16), the standard-surgery group (SSG: TTS 29–42 days; n = 49), and the delayed-surgery group (DSG: TTS ≥43 days; n = 106). The primary outcomes were the objective response rate (ORR), the major pathological response (MPR) rate, and the pathological complete response (pCR) rate. MPR was defined as 10% or fewer viable tumor cells in the resected primary tumor, and the pCR was defined as the removal of carinal tissues and dissected lymph nodes without any viable tumor. The surgical outcomes included operation time, intraoperative bleeding, postoperative drainage, and hospital stay. Multivariable regression analysis was conducted to adjust for confounders such as tumor size, histology, and surgical procedures. Odds ratios for pathological and surgical outcomes were estimated by multivariate regression using robust standard errors. Results are reported as odds ratios with a 95% CI. All statistical analyses were conducted using the SPSS v. 23 software (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics

A total of 171 patients were enrolled in the study, all of whom underwent routine staging, including chest computed tomography (CT) and endoscopy for histological biopsy. Most of the patients (n = 126, 73.68%) had stage IIIA or IIIB disease. The average tumor diameter prior to immunochemotherapy was 5.34 cm (a range of 1.6–15.2 cm). Detailed baseline characteristics and surgical and oncological outcomes are summarized in Table 1. Twenty patients

had delayed administration of neoadjuvant immunotherapy, mostly due to the COVID-19 pandemic and their physical condition. Because of the COVID-19 pandemic, there were 15 patients who had delayed treatment, with five patients having delayed immunochemotherapy due to their physical condition. Moreover, no patient experienced a dose reduction. The TTS intervals after the last cycle of immunochemotherapy ranged from 15 to 107 days, with a median TTS of 46 days. There were 16 (9.4%) patients with TTS ≤28 days, 49 (28.7%) patients with TTS between 29 and 42 days, and 106 (61.9%) patients with TTS ≥43 days.

Surgical outcomes and their relationship between TTS

Of the 171 patients, 145 (85%) received lobectomy, 19 (11%) underwent sleeve lobectomy, and seven were (4%) treated with other types of lung resection. The vast majority of the patients (n = 151, 88.3%) received minimally invasive surgery, and 20 patients were converted to thoracotomy, mostly due to serious pleural adhesions and pulmonary arterial hemorrhage. In the overall cohort, the mean surgical time was 189.3 min (a range of 90–475 min). The mean intraoperative bleeding volume was 172.3 ml (a range of 5–4,000 ml). The average postoperative drainage was 660.8 ml (a range of 10–3,830 ml). The average postoperative hospital stay was 5.4days (a range of 2–21 days). No 90-day surgical-related mortality was recorded. In this analysis, postoperative drainage was associated with the TTS interval. The drainage volume of the ESG group was significantly lower than that of the SSG group and the DSG group (ESG, 455.1 vs. SSG, 680.7 vs. DSG, 846.5; P = 0.037). However, the TTS showed no influence on the duration of operation (P = 0.54), intraoperative bleeding volume (P=0.6), or postoperative hospital (P=0.17).

Pathological response and the relationship with TTS

The percentages of patients who achieved pCR in the SSG, ESG, and DSG were 27%, 31%, and 42%, respectively (P = 0.14), with a similar pattern occurring with MPR (DSG, 58% vs. ESG,

TABLE 1 Baseline characteristics of patients with NSCLC.

Covariate	Full Sample (n = 171)	Time ≤28 d (n = 16)	Time 2,942 d (n =4 9)	Time ≥43 d (n = 106)	*P* value
Age					0.43
Mean (SD)	60.8 (8.7)	58.3 (9)	61.6 (7.6)	60.8 (9.2)	
Median (Min, Max)	62 (25, 84)	57.5 (40, 73)	62 (45, 84)	62 (25, 77)	
Sex					0.91
Female	26 (15)	3 (19)	7 (14)	16 (15)	
Male	145 (85)	13 (81)	42 (86)	90 (85)	
BMI					0.37
Mean (SD)	23 (2.6)	22.1 (1.7)	23.2 (3.4)	23.1 (2.3)	
Median (Min, Max)	23 (16.7, 32.8)	21.3 (19.3, 25.2)	22.7 (16.7, 32.8)	23.1 (18.2, 29.2)	
Cycles of treatment					0.19
≤2	73 (43)	3 (19)	26 (53)	44 (42)	
3	42 (25)	7 (44)	8 (16)	27 (25)	
4	32 (19)	5 (31)	9 (18)	18 (17)	
5	16 (9)	1 (6)	5 (10)	10 (9)	
≥6	8 (5)	0 (0)	1 (2)	7 (7)	
Surgery safety					0.78
Open	20 (12)	2 (12)	7 (14)	11 (10)	
Others	151 (88)	14 (88)	42 (86)	95 (90)	
Surgery type					0.29
Lobectomy	145 (85)	12 (75)	40 (82)	93 (88)	
Sleeve lobectomy	19 (11)	2 (12)	6 (12)	11 (10)	
Others	7 (4)	2 (12)	3 (6)	2 (2)	
Stage					0.33
Before 3	26 (15)	2 (12)	9 (18)	15 (14)	
3a	65 (38)	4 (25)	20 (41)	41 (39)	
3b	61 (36)	9 (56)	12 (24)	40 (38)	
>3b	19 (11)	1 (6)	8 (16)	10 (9)	

50% vs. SSG, 47%; P = 0.38). Although not statistically significant, a slightly higher proportion of patients achieved pCR and MPR in the DSG. ORR showed no difference across the three groups (DSG, 56% vs. ESG, 69% vs. SSG, 51%; P = 0.46; Table 2). Moreover, multivariable regression analysis was performed to adjust for confounders, including tumor size and histology, and surgical procedures (Table 3).

Discussion

The current study indicated that early lung resection within 28 days is safe, as the postoperative morbidity, mortality, safety of surgery, and pathological outcomes were similar across the different study groups.

There is a paucity of data available on optimal TTS after neoadjuvant immunochemotherapy in NSCLC, with more evidence relating to other cancers. Bausys et al. (12) reported that an interval of 30 days or less between the completion of neoadjuvant treatment and surgery significantly correlated with a higher MPR. Omarini et al. (13) and Sanford et al. (14) concluded that a short interval between neoadjuvant chemotherapy (NAC) and surgery might be more effective for breast cancer patients. In contrast, Du et al. (15) demonstrated that a prolonged interval (>8 weeks) contributed to a higher pathological outcome in rectal cancer. Moreover, Terzi (16) reported that extending the interval between neoadjuvant chemoradiation (NCRT) and surgery from 8 to 12 weeks led to a 2-fold increase in the pCR rate. Similarly, a series of studies (17–19) examining the NCRT pattern in esophageal cancer consistently found a prolonged interval between NCRT and esophagectomy to be significantly associated with a higher rate of pCR. The same trend was observed in this study. Although not statistically significant, a tendency toward higher MPR and pCR was also found in patients undergoing delayed surgery.

According to Liu et al. (9), adequate time is necessary for the antitumor response to develop after the administration of immune checkpoint inhibitors. Additionally, patients need time to recover from the short-term side effects of therapy. In the trial by Amaria et al. (20), most of the enrolled patients required a 9-week interval between the three doses of neoadjuvant immunotherapy and surgery due to toxicity issues. In terms of surgical difficulty and safety in the setting of neoadjuvant immunotherapy, Liang et al. (21) found that it is more difficult to perform lung resection after neoadjuvant immunotherapy due to serious tissue edema and increased capillary fragility, which may increase the risk of bleeding and blood loss. Ma et al. (22) indicated that neoadjuvant therapy may increase the chance of structural damage, and in their study, the intraoperative bleeding of cases that received neoadjuvant chemotherapy plus osophagectomy was higher than that for those that received osophagectomy alone. To date, the

TABLE 2 Pathological and surgical outcomes of patients.

Covariate	Time ≤28 d (n = 16)	Time 29–42 d (n = 49)	Time ≥43 d (n = 106)	p-value
Surgery time (min)				0.54
Mean (sd)	202.2 (88.1)	189.2 (91.3)	176.5 (60.2)	
Median (Min, Max)	175 (105, 475)	162.5 (85, 520)	160 (90, 350)	
Blood loss (ml)				0.6
Mean (sd)	199.3 (437.4)	152.9 (275)	164.7 (470.9)	
Median (Min, Max)	40 (20, 1,500)	50 (10, 1,100)	30 (5, 4,000)	
Postoperative drainage (ml)				**0.037**
Mean (sd)	455.1 (345.2)	680.7 (505.8)	846.5 (693)	
Median (Min, Max)	400 (22, 1,120)	505 (10, 2,000)	660 (10, 3,830)	
Postoperative hospital stay (days)				0.17
Mean (sd)	4.8 (1.7)	5.4 (2.5)	6 (2.9)	
Median (Min, Max)	4 (3, 8)	5 (2, 14)	5 (3, 21)	
MPR				0.38
MPR	8 (50)	23 (47)	62 (58)	
Others	8 (50)	26 (53)	44 (42)	
PCR				0.14
pCR	5 (31)	13 (27)	45 (42)	
Others	11 (69)	36 (73)	61 (58)	
ORR				0.46
ORR	11 (69)	25 (51)	59 (56)	
Others	5 (31)	24 (49)	47 (44)	

The bold values meaned the postoperative drainage showed the satistical significance with TTS.

TABLE 3 Multivariable regression analysis was performed to adjust for confounders including tumor size and histology, and surgical procedures.

Outcomes	TTS	OR (95% CI)	*P*-value
ORR	≤28	–	–
	29–42	0.31 (0.05–1.29)	0.13
	≥43	0.37 (0.07–1.47)	0.19
MPR	≤28	–	–
	29–42	0.91 (0.26–3.19)	0.88
	≥43	1.26 (0.39–4.08)	0.69
pCR	≤28	–	–
	29–42	0.45 (0.11–1.85)	0.26
	≥43	0.96 (0.28–3.55)	0.95
Postoperative hospital stay	≤28	–	–
	29–42	1.11 (0.46–2.71)	0.81
	≥43	1.60 (0.70–3.65)	0.26
Operative time	≤28	–	–
	29–42	7.33×10^{-11} (3.33×10^{-21}–1.61×10)	0.06
	≥43	2.25×10^{-4} (4.87×10^{-14}–1.04×10^{6})	0.46
Intraoperative bleeding	≤28	–	–
	29–42	1.38×10^{17} (1.27×10^{-13}–1.50×10^{47})	0.26
	≥43	3.36×10^{9} (1.60×10^{-19}–7.09×10^{37})	0.51
Postoperat ive drainage	≤28	–	–
	29–42	1.43×10^{70} (1.67×10^{-36}–1.23×10^{176})	0.193
	≥43	1.94×10^{74} (1.11×10^{-25}—3.39×10^{173})	0.141

relationship between surgical outcomes and TTS remains unclear. This study showed that, although not statistically significant, a tendency for increased intraoperative bleeding occurred in patients undergoing delayed surgery, likely because a longer TTS usually correlates with structural damage. The COVID-19 pandemic has been responsible for the widespread delay of surgeries. According to several previous studies (23–25), COVID-19 was significantly associated with postoperative complications and a higher rate of mortality. Lei et al. (25) reported that the mortality rate of patients with COVID-19 in their study was 20.5%, and 44.5% of patients required intensive care in an intensive care unit after surgery. Another study (26) indicated that surgery should be delayed after COVID-19 to potentially prevent postoperative complications. Furthermore, during the waves of the current COVID-19 pandemic, the TTS has increased due to deferred surgical resection as a result of operating room closures. Therefore, a considerable number of patients receiving neoadjuvant therapy may experience delayed operations. Although TTS does not affect surgical indicators in the overall population, a few cases suggest that prolonged TTS may still affect the outcome of the operation, as shown in the following patients. First, in this study, there were seven patients with intraoperative bleeding of more than 1,000 ml after neoadjuvant immunochemotherapy. All were treated with surgery in ≥43 days, with mean TTS intervals of 48 days (a range of 43–72 days) after the last cycle of neoadjuvant immunochemotherapy. Of the seven patients with NSCLC, three had stage IIIA, two had stage IIIB, and two had stage IIB. Moreover, five patients received lobectomy and two underwent sleeve lobectomy. Serious pleural adhesion occurred in all of the patients. Thus, randomized controlled trials are required to verify these findings.

This study has several limitations. First, the decisions made for TTS were possibly dependent on personal experience and tumor radiological response. Second, this study was retrospective with a limited sample size. Considering this is a retrospective study, selection bias should be considered.

Conclusion

In this study, the TTS interval showed no significant effect on surgical feasibility or safety in the neoadjuvant immunochemotherapy setting of NSCLC. Pathological outcomes, although not statistically significant, showed a trend in which delayed surgery contributed to a better pathological response. Further studies with larger sample sizes are needed for validation of these findings.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics Statement

Written informed consent was obtained from the individual (s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JC: conception and design of the study and drafting the article. HD: analysis of data, visualization of data, contributing cases who provided administration support. ZW: analysis of data and interpretation of data. JH: revised the article critically. SL: conception and design of the study, revised the article critically, administration support, and general supervision of the research group. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Pembrolizumab monotherapy for untreated PD-L1-Positive non-small cell lung cancer in the elderly or those with poor performance status: A prospective observational study

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Objectives: We investigated the efficacy and safety of pembrolizumab monotherapy as first-line treatment for poor Eastern Cooperative Oncology Group performance status (PS) and elderly patients with programmed cell death-ligand 1 (PD-L1)-positive advanced non-small cell lung cancer (NSCLC). We also investigated clinical prognostic factors for the efficacy of pembrolizumab monotherapy, based on patient characteristics.

Materials and methods: In this prospective observational study, PS-2 and elderly NSCLC patients with PD-L1 tumor proportion score (TPS) ≥1% who received first-line pembrolizumab monotherapy, from October 2019 to March 2021, at 10 institutions in Japan were enrolled. Patients judged eligible by their physicians for combined chemotherapy and PD-1/PD-L1 inhibitors as first-line treatment were excluded. Clinicopathological characteristics and adverse events were investigated for correlation with clinical outcomes.

Results: Forty patients were enrolled in the study. The median progression-free survival (PFS) of patients with PS 2 and those aged ≥ 75 years were 4.4 (95% confidence interval [CI]: 0.9–14.4) months and 5.3 (95% CI 2.9–9.4) months, respectively. The median overall survival (OS) of patients with PS 2 and those aged ≥ 75 years were 11.6 (95% CI: 1.4–not evaluable [NE]) months and 11.6

(95% CI 7.4–18.1) months, respectively. Immune-related adverse events (irAEs) were observed in 19 patients; 6 patients had severe irAEs of Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or higher. Patients who achieved stable disease or better, had a statistically significant increase in PFS ($p < 0.001$) and OS ($p < 0.001$). In the multivariate analysis, the acquisition of disease control with pembrolizumab monotherapy was an independent prognostic factor for PFS and OS.

Conclusion: Pembrolizumab monotherapy was relatively effective and tolerable as a first-line treatment for patients with PD-L1-positive advanced NSCLC who had poor PS or were elderly. Our results suggest that disease control might be an independent prognostic factor for PFS and OS in this population. (UMIN000044052 https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000050176)

KEYWORDS

pembrolizumab, poor performance status, elderly, lung cancer, geriatric 8 (G8)

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide (1). The recent clinical application of immune checkpoint inhibitors (ICIs) has been a paradigm shift in the systemic therapy for patients with advanced lung cancer; also, prolonged prognosis has been observed in long-term follow-up reports (2). Pembrolizumab is a humanized IgG4 monoclonal antibody that binds to programmed cell death-1 (PD-1). It inhibits the binding of PD-1 ligand, programmed cell death-ligand 1(PD-L1) and demonstrates its anti-tumor effects through the activation of tumor-specific cytotoxic T lymphocytes (3). A phase III study (KEYNOTE-024) comparing pembrolizumab monotherapy with platinum-based combination therapy for patients with untreated advanced non-small cell lung cancer (NSCLC), with a PD-L1 tumor proportion score (TPS) ≥ 50%, showed that pembrolizumab monotherapy significantly prolonged progression-free survival (PFS) and overall survival (OS) compared to platinum-based combination therapy (4). Another phase III study (KEYNOTE-042 study) of 1274 patients with unresectable advanced or recurrent NSCLC with PD-L1 TPS ≥ 1% showed that pembrolizumab monotherapy significantly prolonged OS compared to platinum-containing chemotherapy (5). Thus, the current clinical application of pembrolizumab monotherapy was expanded to include the first-line treatment of patients with PD-L1-positive lung cancer cells ≥ 1%, which is recommended in the guidelines of several countries (6, 7). In contrast, this regimen has not been approved and was not recommended for patients with PD-L1 TPS of 1-49% in several countries, because the different clinical outcomes of pembrolizumab monotherapy are related to PD-L1 expression levels ≥ 50% and 1-49% in KEYNOTE-042. Regarding its combination with chemotherapy, a phase III study on non-squamous cell carcinoma (KEYNOTE-189) and a phase III study on squamous cell carcinoma (KEYNOTE-407) showed that pembrolizumab added to chemotherapy significantly prolonged PFS and OS (8, 9). Based on the results of these clinical trials, combination therapy with platinum-doublet chemotherapy and ICIs has been recommended as the first-line treatment for patients with metastatic NSCLC, with a good Eastern Cooperative Oncology Group performance status (PS). However, such combination therapies are difficult to use in vulnerable patients. Therefore, the use of pembrolizumab monotherapy as first-line treatment is expected to increase in vulnerable patients with NSCLC, such as those with poor PS and elderly patients aged > 75 years.

In previous clinical trials of pembrolizumab monotherapy, only patients who met the eligibility criteria of PS 0/1 were enrolled; also, there are few reports on efficacy and safety in patients aged ≥ 75 years. A retrospective study showed that poor PS was an independent poor prognostic factor for PFS and OS in

Abbreviations: PS, performance status; PD-L1, programmed cell death-ligand 1; NSCLC, non-small cell lung cancer; TPS, tumor proportion score; PFS, progression-free survival; OS, overall survival; CI, confidence interval; irAE, immune-related adverse event; CTCAE, Common Terminology Criteria for Adverse Events; ICI, immune checkpoint inhibitor; PD-1, programmed cell death-1; GA, geriatric assessment; G8, geriatric 8 screening tool; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CT, computed tomography; MRI, magnetic resonance imaging; IHC, immunohistochemistry; HR, hazard ratio; ORR, objective response rate; NE, not evaluable.

pembrolizumab monotherapy (10). In a retrospective study of PS 2 NSCLC patients with PD-L1 TPS ≥ 50% receiving first-line pembrolizumab monotherapy, prognosis differed, depending on whether the reason for poor PS was due to cachectic factors or complications (11). In contrast, a recent phase 2 clinical trial, which sought to evaluate the efficacy and safety of pembrolizumab monotherapy in PS 2 patients, reported an equivalent efficacy to that in patients with good PS, and that toxicity was feasible (12). However, there is a lack of real-world data from prospective observational studies examining first-line pembrolizumab monotherapy in patients with advanced NSCLC who are unfit for clinical trials, such as those with poor PS and elderly patients. Facchinetti et al. reported in their meta-analysis of first-line immunotherapy for NSCLC patients with poor PS that prospective evidence supporting the role of immunotherapy in this population is limited, and clinical efforts are needed to improve prognosis, including the definition and factors contributing to poor PS and the development of dedicated treatment strategies (13).

Geriatric assessment (GA) is a multidimensional and multidisciplinary assessment tool that evaluates the identification of functional, nutritional, cognitive, psychological, socially supportive, and comorbid factors (14). The International Society of Geriatric Oncology recommends GA for older cancer patients (15). Instead of the full comprehensive GA, the geriatric 8 screening tool (G8) is easy to use in clinical practice (16) and has been reported as a promising prognostic factor for survival in elderly patients with various cancers (17).

In this prospective study, we investigated the efficacy and safety of pembrolizumab monotherapy as a first-line treatment in patients with advanced NSCLC with PD-L1 TPS positivity who either had PS 2 or were elderly patients aged ≥ 75 years. These patients, judged eligible by their physicians for combination of chemotherapy and PD-1/PD-L1 inhibitors as first-line treatment, were excluded. In addition, we investigated the clinical prognostic factors for pembrolizumab monotherapy efficacy based on patient characteristics, including G8.

Materials and methods

Patients

This multicenter, prospective cohort study was conducted among previously untreated patients with advanced NSCLC without EGFR and ALK gene alterations, with a PS of 2 or age above 75 years (PS 0/1), diagnosed between October 2019 and March 2021 at 10 institutions in Japan. All patients provided written informed consent for participation in this study. The study was conducted in accordance with the Declaration of Helsinki (revised in 2013) and was approved by the independent ethics committees of the Japanese Red Cross Kyoto Daiichi Hospital (no. 846) and each hospital. Patients who concurrently received treatment with other anticancer agents and had a history of treatment with other cancer drug therapies were considered ineligible. Patients judged eligible by their physicians for combined chemotherapy and PD-1/PD-L1 inhibitors as first-line treatment were excluded. The administration of pembrolizumab and the assessment of its efficacy and toxicity, including immune-related adverse events (irAEs), were determined by each investigator. irAEs were graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events version (CTCAE) 5.0. All patients underwent imaging evaluations, including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), using either a conventional computed tomography (CT) or magnetic resonance imaging (MRI) scan, according to the criteria outlined in the Response Evaluation Criteria in Solid Tumors (v.1.1). A CT scan or MRI scan three months after the start of treatment was used as reference to determine the effect of treatment. If non-PR or non-PD was observed on the first imaging evaluation, we determined SD to be non-PR or non-PD on the next imaging evaluation three months later. PFS was defined as the time from initiation of pembrolizumab treatment to the date of objective disease progression or death from pembrolizumab treatment before progression.

Geriatric 8 screening tool analysis

The G8 is an 8-item screening tool that covers the domains of food intake, weight loss, body mass index, exercise capacity, psychological state, number of medications taken, self-perception of health, and age. The G8 scores ranged from 0 (severe disability) to 17 (no disability). The G8 questionnaire is presented in Supplementary Table 1. G8 score was to be obtained by each investigator at the time of diagnosis. A cutoff value of 11 for G8 has been reported as a predictor of prognosis (18, 19). In this study, the cut-off value for G8 was set at 11.

Analysis of PD-L1 expression

PD-L1 expression in tumors was assessed by performing PD-L1 immunohistochemistry (IHC) using the 22C3 pharmDx assay at a commercial clinical laboratory (SRL, Inc., Tokyo, Japan), using pretreatment tumor samples. Tumor PD-L1 expression was expressed as the percentage of at least 100 viable tumor cells with complete or partial membrane staining. Pathologists at commercial vendors interpreted tumor PD-L1 expression according to the assay results. Patients were categorized into the following three groups based on the PD-L1 TPS: high (≥ 50%), low (1–49%), and negative (< 1%).

Treatment

Patients were intravenously administered pembrolizumab at a flat dose of 200 mg on day 1 of a 3-week cycle. In general, these treatments were continued until disease progression, intolerable toxicity, or patient refusal occurred.

Statistical analysis

To analyze PFS and OS, the times to events were estimated using the Kaplan–Meier method and compared using the log-rank test. The hazard ratios (HRs) for PFS and OS were determined using a univariate Cox proportional hazard model. Landmark analyses of PFS and OS at 12 or 24 weeks were performed in patients with disease control or were alive, considering the time-dependence of irAEs. Cox proportional hazard models were used to evaluate several patient factors. To construct the multivariate model, we selected factors related to PFS and OS, which were the most relevant factors identified in the univariate analysis. All statistical analyses were performed using EZR for Windows, version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Statistical significance was set at $p < 0.05$.

Results

Patients' characteristics

A total of 41 patients with advanced NSCLC with PS of 2 or age ≥ 75 years (PS 0/1) were enrolled in this prospective study. One patient was excluded because of withdrawal of consent prior to pembrolizumab administration; the remaining 40 patients were included in the analysis. The median follow-up period was 9.5 (range, 0.3–27.1) months. The median patient age was 78.5 (range, 67.0–87.0) years, and 28 (70.0%) patients were male. Sixteen patients (40.0%) had a PS of 2, and 31 (77.5%) were ≥ 75 years. Among them, 33 (82.5%) patients had a history of smoking and 12 (30.0%) had squamous cell carcinoma. The PD-L1 IHC test was performed for all patients. Twenty-two (55.0%) patients had a PD-L1 TPS of ≥ 50%. For G8, data were collected from 33 of 40 patients. The median G8 was 10.5 (range, 6–15) (Table 1). The proportion of patients who received second-line therapy were 15.0% (n=6) while 7.5% (n=3) received more than third-line therapy (Supplementary Table 2).

Efficacy of pembrolizumab monotherapy in patients with advanced NSCLC

In this prospective study, the objective response rate (ORR) of all patients was 40.5% (95% confidence interval (CI): 24.8–57.9%) and the disease control rate was 62.2% (95% CI: 44.8–77.5%). Median PFS and OS for patients aged ≥ 75 years were 5.3 (95% CI: 2.9–9.4) months and 11.6 (95% CI: 7.4–18.1) months, respectively; those for PS 2 patients were 4.4 (95% CI: 0.9–14.4) months and 11.6 months (95% CI: 1.4 months– not evaluable [NE]), respectively (Figures 1A-D). There was no significant difference in PFS and OS based on age (≥ 75 years versus < 75 years) or PS status (PS 0 and 1 versus PS 2) (Supplementary Figures 1A-D). The median PFS and OS for PS 2 patients, excluding the elderly population (≥ 75 years of age), was 1.6 (95% CI: 0.3–NE) months and NE (95% CI: 0.3M–NE), respectively. Median PFS in PS 2 patients < 75 years of age was shorter than that in PS 2 patients ≥ 75 years of age, although this difference was not statistically significant (Supplementary Figures 2A, B). Although patients with a PD-L1 TPS of ≥ 50% did not show significant difference in PFS compared to patients with a TPS of 1–49% ($p = 0.812$), those with a PD-L1 TPS of ≥ 90% showed a trend of prolonged PFS compared to those with a TPS of 1–89% ($p = 0.098$). In addition, patients with a PD-L1 TPS of ≥ 90% showed a trend of prolonged PFS compared to those with TPS of 1-49% ($p = 0.174$) and 50–89% ($p = 0.116$) (Figure 2A, Supplementary Figure 3A). There was no significant difference in OS between the two groups, regardless of PD-L1 expression (Figure 2B, Supplementary Figure 3B).

Patients who achieved PR with pembrolizumab monotherapy had a statistically significant increase in PFS and OS compared to those who did not ($p < 0.001$ and $p < 0.001$, respectively). In addition, there was a statistically significant increase in PFS and OS in patients who achieved SD or better ($p < 0.001$ and $p < 0.001$, respectively) (Table 2 and Figures 2C, D). There was no significant difference between PFS/OS and the presence/absence of irAEs (Supplementary Figure 4). Regarding G8, there was a trend toward longer OS in the G8 ≥ 11 group when a G8 score of 11 was used as the cut-off value ($p = 0.058$). In contrast, PFS was divided into groups with a cut-off value of 11 points; however, no significant difference was observed (Table 2; Figures 2E, F).

In the univariate analysis, achieving a response of SD or better was a prognostic factor for PFS; a response of SD or better was a prognostic factor for OS (Table 2A). Multivariate analysis demonstrated that a response of SD or better was an independent prognostic factor for prolonged PFS (HR: 0.04; 95% CI: 0.01–0.16, $p < 0.001$) and OS (HR: 0.20; 95% CI: 0.08–0.51, $p < 0.001$) in pembrolizumab monotherapy (Table 2B).

Toxicity of pembrolizumab monotherapy

Subsequently, we examined the impact of irAEs on pembrolizumab monotherapy in 40 patients with NSCLC. Of these, 19 (47.5%) patients developed irAEs. The most frequent irAE was skin rash, which occurred in six patients, followed by interstitial pneumonia in four patients. Severe grade 3 or higher irAEs included skin rash (1 case of grade 4; pemphigoid),

TABLE 1 Patients' characteristics.

		N = 40
Median age, years (range)		78.5 (67.0–87.0)
Age categorization, years, n (%)	<75	9 (22.5)
	≥75	31 (77.5)
Sex, n (%)	Male	28 (70.0)
	Female	12 (30.0)
ECOG PS, n (%)	0, 1	24 (60.0)
	2	16 (40.0)
Disease stage, n (%)	III	2 (5.0)
	IV	30 (75.0)
	Postoperative relapse	8 (20.0)
Histology, n (%)	Squamous	12 (30.0)
	Non-squamous	28 (70.0)
Brain metastasis, n (%)	Positive	6 (15.0)
	Negative	34 (85.0)
Liver metastasis, n (%)	Positive	5 (7.5)
	Negative	35 (92.5)
Smoking status, n (%)	Current or former	33 (82.5)
	Never	17 (17.5)
PD-L1 TPS, n (%)	1-49%	18 (45.0)
	50-89%	12 (30.0)
	≧90%	10 (25.0)
IrAE	With	19 (47.5)
	Without	21 (52.5)
G8, median (range)		10.5 (6.0-15.0)
Response, n (%)	PR	15 (37.5)
	SD	8 (20.0)
	PD	14 (35.0)
	NE	3 (7.5)
	ORR (95% CI)	40.5% (24.8–57.9%)
	DCR (95% CI)	62.2% (44.8–77.5%)

ECOG PS, Eastern Cooperative Oncology Groups Performance Status; PD-L1, programmed death-ligand 1; TPS, total proportion score; irAE, immune-related adverse event; G8, Geriatric 8; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; ORR, objective response rate; DCR, disease control rate.

interstitial pneumonia (1 case of grade 3), central adrenal insufficiency (1 case of grade 3), and brain infarction (1 case of grade 4). Furthermore, myocarditis was observed in two patients (1 case each of grades 4 and 5). Of the 19 patients who developed irAEs, 10 discontinued treatments, including 1 case of myocarditis (grade 5), 4 cases of interstitial pneumonia (3 of grade 1 and 1 of grade 3), 1 case each of arthritis (grade 2), skin rash (grade 4), central adrenal insufficiency, renal failure (grade 3), and brain infarction (grade 4). The observed irAEs and their frequencies are listed in Table 3. There was no statistically significant difference in the rate of treatment discontinuation according to age or PS. None of the patients were able to resume treatment. A review of the clinical background of the 33 patients for whom G8 was available for evaluation, with and without irAEs, significantly showed that more patients with G8 ≥11 were in the group with irAEs ($p = 0.038$). In addition, the frequency of irAEs was higher in women and patients without PD ($p = 0.038$ and $p = 0.002$, respectively) (Table 4).

Discussion

Immune senescence is associated with age-related remodeling of immune function. In addition, various effects on host immunity, including increased vulnerability to infectious diseases, are also influenced (20). Therefore, it is important to determine whether the efficacy and safety of immunotherapy can be applied not only to patients with good PS but also to those with poor PS and the elderly, who are unfit, or minor populations, in clinical trials; however, they form the majority of patients seen in daily clinical practice. In this prospective study, we investigated whether first-line treatment with pembrolizumab monotherapy can be used as

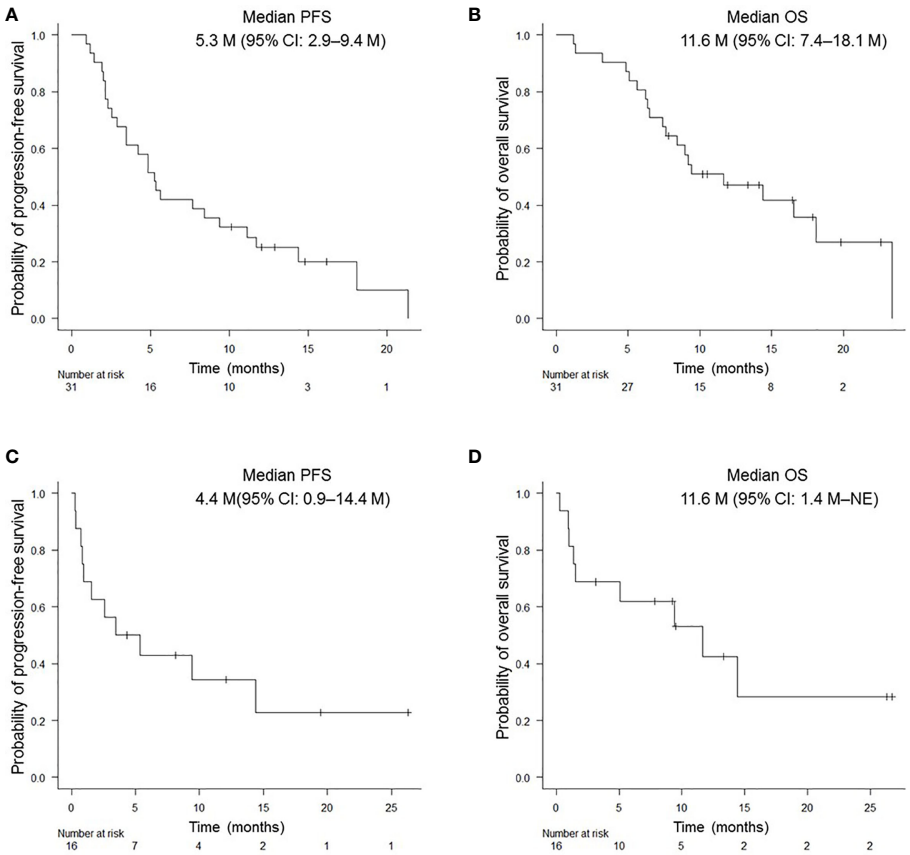

FIGURE 1
Kaplan-Meier survival curves for PFS and OS in NSCLC patients. PFS **(A)** and OS **(B)** of patients aged ≥ 75 years who received pembrolizumab monotherapy. PFS **(C)** and OS **(D)** of patients with PS of 2. PFS, progression-free survival; OS, overall survival.

a treatment option for patients aged ≥ 75 years or those with a PS of 2.

Our observational study showed that the median PFS was 4.4 (95% CI: 0.9–14.4) months and median OS was 11.6 months (95% CI: 1.4 months–NE) for NSCLC patients with PS of 2, which was consistent with a previous prospective study in patients with poor PS (12). These results suggest that first-line treatment with pembrolizumab monotherapy may be effective for patients with PD-L1-expressed NSCLC with poor PS.

Age-related decline affects the activation of CD8+ T cells, which are key elements involved in the PD-1/PD-L1 pathway (21). In this study, 31 (77.5%) patients aged ≥ 75 years were evaluated, resulting in a median PFS of 5.3 (95% CI: 2.9–9.4) months and median OS of 11.6 (95% CI: 7.4–18.1) months. In Elderly NSCLC patients with good PS, the response to pembrolizumab monotherapy may have been boosted. Accumulating evidence has revealed that tumor PD-L1 expression of ≥ 50% is a predictive biomarker of good response to pembrolizumab monotherapy (2, 4, 5). A retrospective cohort study reported that the best survival benefit was shown in patients with PD-L1 > 90% among those with NSCLC, including those with PS 2 status (22). In this study, a trend of prolonged PFS was observed in the PS 2 and elderly groups of patients with NSCLC and PD-L1 ≥ 90%. Clinically, it is worth highlighting that a survival benefit was shown in NSCLC patients with very high PD-L1 expression treated with pembrolizumab monotherapy, even in those with poor PS and the elderly.

It is important to carefully select the first-line therapeutic strategy for NSCLC patients with poor PS and those who are elderly because the next treatment option is not often readily available when the disease worsens due to continued ineffective treatment. This prospective study revealed that patients who demonstrated a treatment effect of SD or better had statistically significant prolonged PFS and OS compared to those who did not, regardless of PS status. A previous meta-analysis of 13

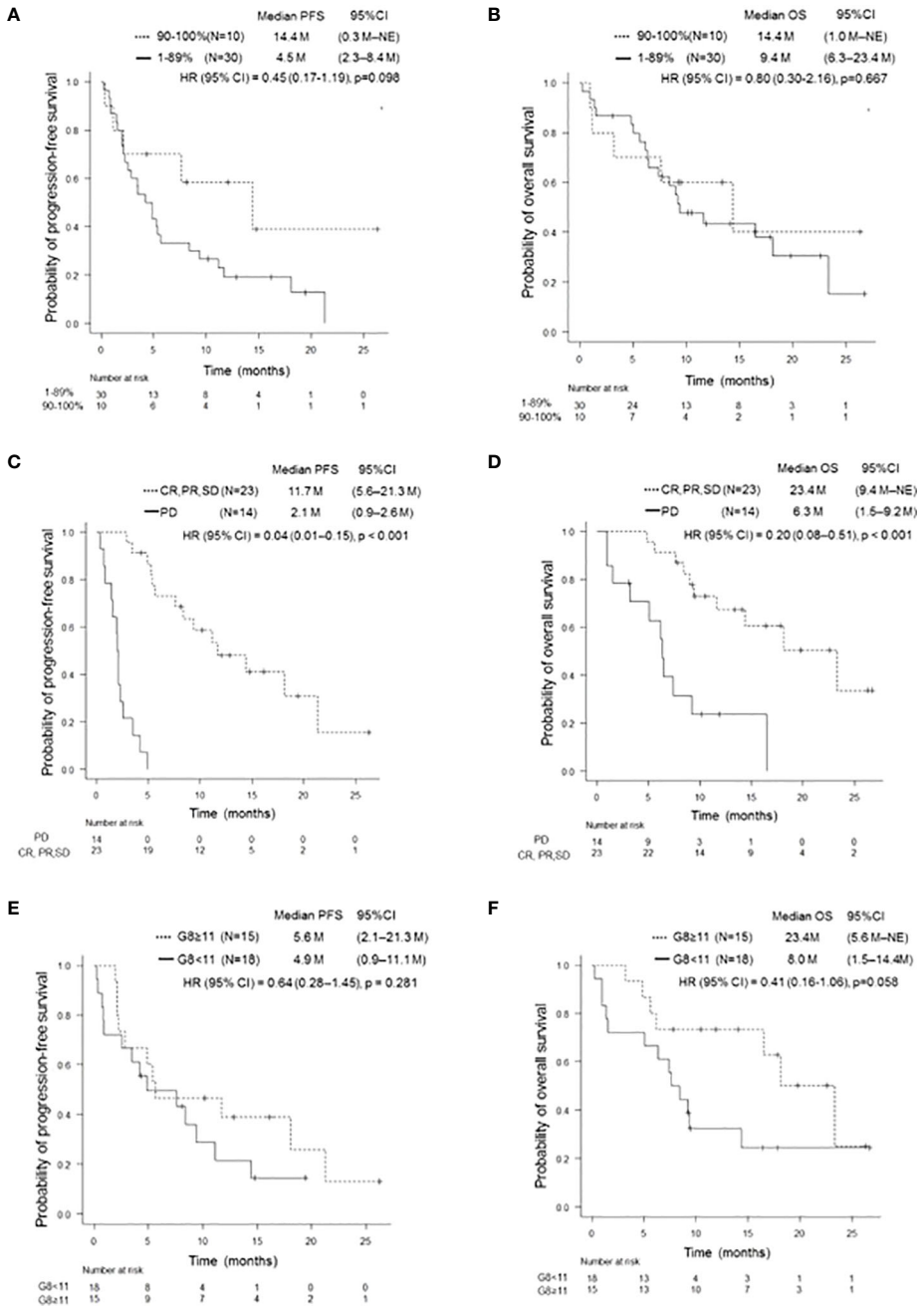

FIGURE 2
Kaplan-Meier survival curves for PFS and OS according to several clinical features. PFS **(A)** and OS **(B)** of patients with PD-L1 TPS ≥ 90% and 1–89%, respectively. Patients with a PD-L1 TPS of ≥ 90% showed a trend of prolonged PFS compared to those with a PD-L1 TPS of 1–89% (p = 0.098). There was no significant difference in OS (p = 0.667). PFS, progression-free survival; OS, overall survival; PD-L1, programmed cell-death Ligand 1; TPS, tumor proportion score. PFS **(C)** and OS **(D)** of patients on and not on pembrolizumab treatment who achieved SD or better. Patients who achieved SD or better had significantly longer PFS and OS than those who did not (p < 0.001, p < 0.001). PFS, progression-free survival; OS, overall survival; SD, stable disease. PFS **(E)** and OS **(F)** of patients with and without G8 ≥11. There was no significant difference in PFS (p = 0.281). Patients with G8 ≥11 tended to have longer OS than those with G8 <11 (p = 0.058). PFS, progression-free survival; OS, overall survival; G8, geriatric 8 screening tool.

TABLE 2 Univariate analysis (A) and multivariate analysis (B) for PFS and OS.

(A)			Patient's No.	Median PFS (95% CI), months	*P* value	Median OS (95% CI), months	*P* value
	Age categorization (years)	<75	9	1.6 (0.3–NE)	0.717	NE (0.3–NE)	0.743
		≧75	31	5.3 (2.9–9.4)		11.6 (7.4–18.1)	
	Sex	Male	28	3.5 (2.1-11.1)	0.411	14.4 (6.2–NE)	0.507
		Female	12	8.0 (1.2–11.7)		9.1 (1.2–23.4)	
	ECOG PS	0, 1	24	5.1 (2.3–11.1)	0.907	12.9 (6.5–NE)	0.797
		2	16	4.4 (0.9–14.4)		11.6 (1.4–NE)	
	Disease stage	III	2	1.5 (1.5–NE)	0.793	NE (NE–NE)	0.200
		IV	30	5.4 (2.1–11.1)		16.5 (5.6–NE)	
		Postoperative relapse	8	4.5 (1.2–NE)		9.1 (1.2–NE)	
	Brain metastasis, n (%)	Positive	6	3.8 (0.3–NE)	0.258	5.8 (0.3–NE)	0.112
		Negative	34	4.9 (2.6–11.1)		14.4 (7.4–23.4)	
	Liver metastasis, n (%)	Positive	6	4.8 (0.3–NE)	0.966	7.1 (0.3–NE)	0.756
		Negative	34	4.9 (2.6–9.4)		11.6 (7.4–23.4)	
	Cell type, n (%)	Squamous	12	10.3 (1.5–18.1)	0.326	14.4 (3.2–NE)	0.604
		Non-squamous	28	4.5 (2.1–7.6)		9.2 (6.3–23.4)	
	Smoking status, n (%)	Current or former smoker	33	5.4 (2.9–11.1)	0.256	11.6 (7.4–NE)	0.356
		Never smoker	7	2.0 (0.8–11.7)		9.2 (1.0–NE)	
	PD-L1 TPS, n (%)	1-49%	18	4.9 (2.9–9.4)	0.812	9.3 (6.3–NE)	0.802
		50-100%	22	5.4 (1.5–14.4)		14.4 (3.2–NE)	
	PD-L1 TPS, n (%)	1-89%	30	4.5 (2.3–8.4)	0.098	9.4 (6.3–23.4)	0.667
		90-100%	10	14.4 (0.3–NE)		14.4 (1.0–NE)	
	IrAEs	With	19	9.4 (5.3–18.1)	0.027	14.4 (8.4–23.4)	0.280
		Without	21	2.6 (1.5–4.9)		7.4 (4.9–NE)	
	G8	<11	18	4.9 (0.9–11.1)	0.281	8.0 (1.5–14.4)	0.058
		≥11	15	5.6 (2.1–21.3)		23.4 (5.6–NE)	
	Response	PR	15	18.1 (9.4–NE)	< 0.001	23.4 (14.4–NE)	< 0.001
		Non-PR	22	2.7 (1.9–4.9)		7.4 (5.1–11.6)	
	Response	Non-PD	23	11.7 (5.6–21.3)	< 0.001	23.4 (9.4–NE)	< 0.001
		PD	14	2.1 (0.9–2.6)		6.3 (1.5–9.2)	

(B)	Items	PFS hazard ratio (95% CI)	*P* value	OS hazard ratio (95% CI)	*P* value
	PD-L1 TPS 90–100%	0.58 (0.19–1.77)	0.330		
	With irAEs	1.39 (0.49–3.92)	0.540		
	Non-PD	0.04 (0.01–0.16)	< 0.001	0.20 (0.08–0.51)	< 0.001

PFS, progression-free survival; OS, overall survival; CI, confidential interval; NE, not evaluable; ECOG PS, Eastern Cooperative Oncology Groups Performance Status; PD-L1, programmed death-ligand 1; TPS, total proportion score; irAEs, immune-related adverse events; G8, geriatric 8; PR, partial response; PD, progression disease.

clinical trials, including immunotherapy, showed that ORR and PFS can be surrogate indicators of OS (23), which is in line with the results of our study. Therefore, much attention should have been paid to the clinical outcomes of NSCLC patients with poor PS or those who were elderly, when assessing the responsiveness of pembrolizumab monotherapy as a first-line therapy.

Recently, the results of an International Expert Panel Meeting supported the safety of immunotherapy, but not immunochemotherapy, in NSCLC patients with PS 2, based on clinical evidence (24). In the KEYNOTE-042 study, irAEs were reported to be 63% at any grade and 18% at grade 3 or higher in the pembrolizumab group of NSCLC patients with good PS (5). In this study, there was no increase in the frequency of irAEs of any grade (47.5%) and grade 3 or higher (15%), compared to those of the KEYNOTE-042 study, which indicated that pembrolizumab monotherapy is a tolerable regimen for NSCLC patients with poor PS. In addition, a retrospective study evaluating first-line pembrolizumab in patients with poor PS with PD-L1 ≥ 50%, found

TABLE 3 Adverse events and immune-related adverse events in all NSCLC patients.

Category	Number of patients, (%)		
	Total	Grade 1, 2	Grade 3-5
Any irAEs	19 (47.5)	13 (32.5)	6 (15.0)
Pneumonitis	4 (10.0)	3 (7.5)	1 (2.5)
Rash	6 (15.0)	5 (12.5)	1 (2.5)
Hypothyroidism	1 (2.5)	1 (2.5)	0 (0.0)
Adrenal insufficiency	1 (2.5)	0 (0.0)	1 (2.5)
Carditis	2 (5.0)	0 (0.0)	2 (5.0)
Nephritis	1 (2.5)	1 (2.5)	0 (0.0)
Colitis	2 (5.0)	2 (5.0)	0 (0.0)
Arthritis	2 (5.0)	2 (5.0)	0 (0.0)
Brain infarction	1 (2.5)	0 (0.0)	1 (2.5)

NSCLC, non-small cell lung cancer; irAEs, immune-related adverse events.

no increase in toxicity (11). A prospective study evaluating the efficacy and safety of pembrolizumab monotherapy in patients with PS 2 (PePS2) also concluded that the safety was acceptable (12). In our study, myocarditis of grade 3 or higher was observed in 5% (2) of patients, although previous reports showed less than 1% in the KEYNOTE-042 study, 1.14% by Mahmood et al., and 0% in a prospective study of 140 patients (5, 25, 26). The reason for the increased severity of myocarditis may not be because of the vulnerability of the patients; however, it might be due to the fact that severe myocarditis occurred in approximately half of the patients (25). However, a retrospective study on the safety of single-agent ICIs in patients older than 80 years also reported an increase in irAEs with increasing age (26). From these observations, further verification of specific adverse effects is required in determining whether myocarditis occurs more frequently in vulnerable patients.

TABLE 4 Patient characteristics in the "with irAEs" and "without irAEs" groups (N = 40).

		With irAEs (%)	Without irAEs (%)	*P* value
		N = 19	N = 21	
Age categorization	<75	2 (10.5)	7 (33.3)	0.133
	≧75	17 (89.5)	14 (66.7)	
Sex	Male	10 (52.6)	18 (85.7)	0.038
	Female	9 (47.4)	3 (14.3)	
ECOG PS	0, 1	12 (63.2)	12 (57.1)	0.755
	2	7 (36.8)	9 (42.9)	
Disease stage	III	0 (0.0)	2 (9.5)	0.464
	IV	14 (73.7)	16 (76.2)	
	Postoperative relapse	5 (26.3)	3 (14.3)	
Brain metastasis, n (%)	Positive	3 (15.8)	3 (14.3)	1
	Negative	16 (84.2)	18 (85.7)	
Liver metastasis, n (%)	Positive	2 (10.5)	4 (19.0)	0.664
	Negative	17 (89.5)	17 (81.0)	
Cell type, n (%)	Squamous	7 (36.8)	5 (23.8)	0.494
	Non-squamous	12 (63.2)	16 (76.2)	
Smoking status, n (%)	Current or former smoker	17 (89.5)	16 (76.2)	0.412
	Never smoker	2 (10.5)	5 (23.8)	
PD-L1 TPS, n (%)	1-49%	9 (47.4)	9 (42.9)	1

(Continued)

TABLE 4 Continued

		With irAEs (%)	Without irAEs (%)	*P* value
		N = 19	N = 21	
	50-100%	10 (52.6)	12 (57.1)	
PD-L1 TPS, n (%)	1-89%	7 (36.8)	3 (14.3)	0.148
	90-100%	12 (63.2)	18 (85.7)	
G8	<11	10 (66.7)	5 (27.8)	0.038
	≥11	5 (33.3)	13 (72.2)	
Response	PR	9 (50.0)	6 (31.6)	0.325
	Non-PR	9 (50.0)	13 (68.4)	
Response	Non-PD	16 (88.9)	7 (36.8)	0.002
	PD	2 (11.1)	12 (63.2)	

irAEs, immune-related adverse events; ECOG PS, Eastern Cooperative Oncology Groups Performance Status; PD-L1, programmed death-ligand 1; TPS, total proportion score; G8, geriatric 8; PR, partial response; PD, progression disease.

The G8 was developed as a tool to validate the need for GA in elderly cancer patients; it is known to be a prognostic factor of many cancer types (16, 27). A report of G8 as a prognostic factor in elderly patients with lung cancer and a prospective study of G8 as a predictor of adverse events in an elderly cohort of patients with lung cancer and malignant melanoma showed no significant difference in the increase in adverse events compared to the younger cohort (28). However, there was a significant increase in the risk of death and hospital admissions in patients with low G8 (26). In this study, there was a trend toward higher OS in the group with higher G8 levels, although the difference was not significant. Therefore, the G8 score is expected to be a potentially useful tool for determining prognosis in vulnerable patients with NSCLC receiving ICIs. Further large-cohort investigations are warranted for confirming the impact of the G8 score on the clinical benefit of pembrolizumab monotherapy in these cohorts.

This study had several limitations. First, the sample size was small even though this was a prospective study. Second, in the eligibility criteria, PS 0/1 included only those aged ≥ 75 years, which makes it difficult to interpret the influence of PS status. Third, this was an observational study, and there was a bias in patient selection and assessment of treatment effect. Fourth, patients with diverse backgrounds, poor PS, and older age were included in the analysis.

In conclusion, our prospective study showed that pembrolizumab monotherapy as first-line treatment for patients with advanced NSCLC who had poor PS or were elderly was relatively effective and tolerable. However, further large-cohort investigations are needed to confirm our observations in patients with NSCLC, such as the emergence of irAEs and the impact of the high expression of tumor PD-L1.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the independent ethics committees of the Japanese Red Cross Kyoto Daiichi Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SS, TdY, and KT contributed to the study conception and design. SS, KM, MT, HY, OH, YC, TkY, IH, TO, TT, NH and KT obtained the clinical data. Data were interpreted by SS, AY, TdY, and KT. The manuscript was prepared by SS, AY, and TdY. All authors contributed to the article and approved the submitted version.

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Conflict of interest

TY received commercial research grants from Pfizer, Ono Pharmaceutical, Chugai Pharmaceutical, Janssen Pharmaceutical K.K., and Takeda Pharmaceutical Company Limited., and honoraria for lecture fee from Eli Lilly. HY received research expenses from MSD, Delta-Fly Pharma, Novartis, Boehringer Ingelheim, and honoraria for lecture fee from Boehringer Ingelheim, Chugai pharmaceutical, BMS, Eli Lilly, Nippon Kayaku, Taiho pharmaceutical. KT received research grants from Chugai-Roche and Ono Pharmaceutical, and honoraria for

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.904644/full#supplementary-material

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Neoadjuvant camrelizumab and chemotherapy in patients with resectable stage IIIA squamous non-small-cell lung cancer: Clinical experience of three cases

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Neoadjuvant immunochemotherapy has attracted much attention as a treatment for locally advanced non-small-cell lung cancer. However, there is scarce evidence of the safety and efficacy of camrelizumab as neoadjuvant in lung cancer. Here, we present three patients who were diagnosed with IIIA squamous non-small-cell lung cancer from September to December in 2020 and received two cycles of neoadjuvant camrelizumab plus nab-paclitaxel and nedaplatin, followed by surgical resection. All three patients had a reduction in the tumor size on CT image and not delayed planned surgery. We did not observe grade 3 or 4 adverse events. Two of the three patients achieved a major pathological response (MPR), including one complete tumor regression of the primary lung tumor. Multiplex fluorescent immunohistochemistry revealed that CD8+ T cells, FoxP3+ regulatory T cells, and PD-L1 expression on immune cells in the surgical specimen were much higher than in the pretreatment biopsy sample in patients with MPR. This was not observed in the patient without MPR. Camrelizumab plus chemotherapy could potentially be a neoadjuvant regimen for resectable IIIA squamous non-small-cell lung cancer, with a high MPR proportion, and did not compromise surgical procedure. Our findings should be validated in a future randomized clinical trial.

KEYWORDS

neoadjuvant immunotherapy, non-small cell lung cancer (NSCLC), camrelizumab, major pathological response (MPR), surgery

Introduction

Neoadjuvant therapy is one of the many approaches to locally advanced non-small cell lung cancer (NSCLC) (1). However, traditional platinum-based chemotherapy either before or after resection provides only 5% higher of overall survival (OS) than surgery alone for treating patients with stage IB–IIIA NSCLC (2–4). Recently, checkpoint inhibitors targeting PD-1 and PD-L1 have revolutionized the treatment paradigm for several cancers. On the basis of previous success, several studies have focused on the utility of immune checkpoint inhibitors as neoadjuvant therapy for treating patients with surgically resectable NSCLC (5–7). Camrelizumab, a humanized monoclonal antibody against PD-1, has proved to be effective and safe in multiple tumor types including advanced NSCLC in phase 1, 2, and 3 studies (8–11). However, there has been no report regarding the efficacy and safety of the combination of camrelizumab with chemotherapy as a neoadjuvant treatment in patients with resectable lung cancer to date. Hence, we evaluated the safety and feasibility of the use of neoadjuvant camrelizumab in a small group of patients with resectable stage IIIA squamous lung cancer.

Methods

This single-group study was developed by the author's medical center. Three patients who were diagnosed with stage IIIA squamous lung cancer between September and December in 2020 were evaluated to undergo lobectomy surgery. Patients received the following drugs intravenously: camrelizumab (200 mg) on day 1; nab-paclitaxel (100 mg/m^2) on days 1, 8, and 15; and nedaplatin (80 mg/m^2) on day 1 of every 21 days for 4–6 cycles. Surgery was performed after the first two treatment cycles with an interval of 3–6 weeks (12). All the patients underwent baseline tumor assessment, including pretreatment pathological diagnosis by means of bronchoscopy or percutaneous core needle lung biopsy, contrast-enhanced CT of chest and abdomen, single photon emission computed tomography (SPECT) of bone, and magnetic resonance imaging (MRI) of brain; chest CT was repeated within 1 week before surgery. The changes in tumor size were judged according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Major pathological response (MPR) was defined as the presence of 10% or less residues of cancer cells in the primary tumor surgical specimen (13).

Results

All three patients received the two planned cycles and underwent complete tumor resection. The clinical characteristics of these patients are presented in Table 1. No grade 3 or 4 adverse events or treatment-related deaths occurred. Neoadjuvant camrelizumab and chemotherapy were not associated with any toxic effects, which was not previously reported. There were no treatment-related surgeries that were postponed or canceled. The intervals between the administration of the second dose of camrelizumab and surgery were 32, 39, and 41 days, respectively. Of the three patients, two received the six planned treatment cycles; one patient (patient B) was diagnosed with Alzheimer's disease while on study and terminated the therapy after one postoperative treatment cycle. The median follow-up was 10 months (10, 11, and 12 months, respectively). None of the patients died or experienced disease recurrence during the follow-up.

TABLE 1 Characteristics of the three patients.

Pathological response	Patient A Major pathological response	Patient B Major pathological response	Patient C Incomplete pathological response
Age	68	78	68
Sex	Male	Male	Male
Smoking status	Current smoker	Current smoker	Current smoker
Histologic diagnosis	Squamous-cell carcinoma	Squamous-cell carcinoma	Squamous-cell carcinoma
Pathological stage	T2bN2M0, IIIA	T2bN2M0, IIIA	T2bN2M0, IIIA
Downstaging of nodal status	N2 to N1	N2 to N2	N2 to N2
Interval between the last dose of Camrelizumab and surgery	41	39	32
Operation time (min)	130	90	115
Bleeding (ml)	Minimal	Minimal	Minimal
Chest tube stay (days)	1	2	1
Intensive care unit stay (days)	0	0	0
Hospitalization stay following the surgery (days)	3	4	5

All three patients were operated by video thoracoscopy approach and had an R0 surgical resection. The durations of the surgery were 90, 115, and 130 min, respectively. No significant hemorrhaging occurred during surgery. The length of postoperative hospital stay was 3, 4, and 5 days, respectively, without stay in the intensive care unit (ICU). No postoperative complications, such as pneumothorax, hemoptysis, chylothorax, and pneumonia, occurred.

Two of the three patients (patient A and patient B) achieved MPR, including one complete tumor regression of the primary lung tumor but had residual lymph-node metastases (patient A). One patient (patient C) had an incomplete pathological response to the neoadjuvant treatment. Reduction in the tumor size was noted in all patients' CT images after two cycles of treatment (Figure 1).

Hematoxylin–eosin staining (HE staining) revealed that residual non-viable tumor and lymph nodes of patients with MPR composed of extensive necrosis and a large amount of inflammatory cell infiltration, foamy histiocytes, and multinucleated giant cells can be seen locally. These features were not evident in areas distant from the tumor bed, which was consistent with immunological response, but this response was barely noticeable in the patient without MPR (Figure 2). To further explore these cases, multiplex immunofluorescence analysis which contained PD-1-positive, PD-L1-positive, CD8+ T cells, CD68+ macrophages, and FoxP3+ regulatory T cells was performed in both pretreatment and surgical specimens (Figure 3). In two patients with MPR, CD8+ T cell, FoxP3+ regulatory T cell, and PD-L1 expression on immune cells in the surgical specimen was much higher than in the pretreatment biopsy sample, whereas this immunoreactive intensity was faint in the patient without MPR. Table S1 shows the changes of tumor cells and immune cells in lesions before and after neoadjuvant treatment.

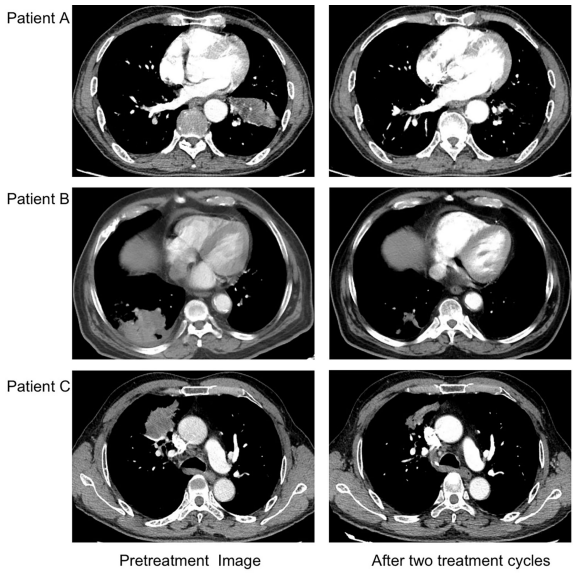

FIGURE 1
CT images of lesions before and after two cycles of neoadjuvant camrelizumab plus chemotherapy. After two doses of camrelizumab plus chemotherapy, the lesions of three patients were significantly reduced.

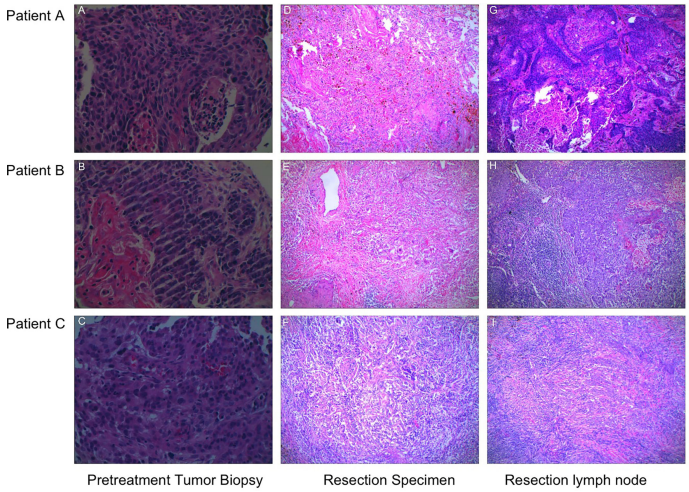

FIGURE 2
The representative sections of tumor specimens from three patients before and after the administration of camrelizumab plus chemotherapy. **(A–C)** Pretreatment tumor biopsy, HE staining (×400). **(D–E)** The resection specimens were infiltrated by lymphocytes and macrophages, and there were over 90% tumor tissue regression, HE staining (×100). **(F)** Over 50% residual tumor cells were present, and a little fibrous tissue can be observed, HE staining (×100). The presence of necrosis, fibrosis, and macrophages was observed in the metastatic lymph nodes of patients with MPR **(G, H)**, whereas this founding was not observed in the patient without MPR **(I)**, HE staining (×100).

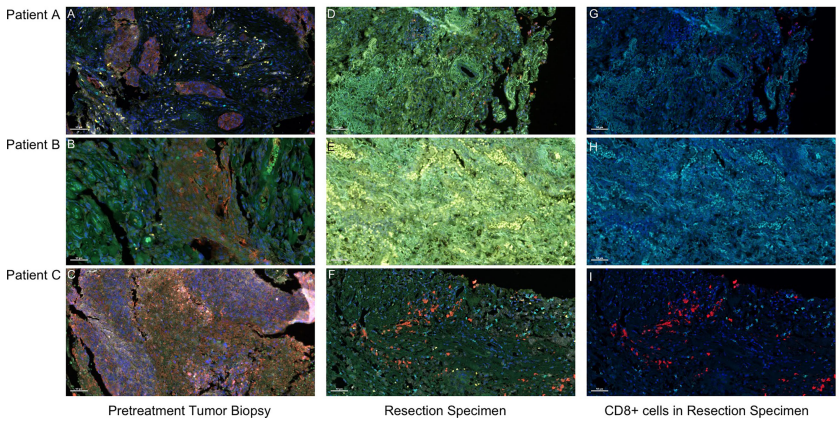

FIGURE 3
Presence of CD 68+ macrophages, CD8+ T cells, and FoxP3+ Treg cells in pretreatment and surgical specimen detected by multiplex fluorescent immunohistochemistry. Visible structures include cytokeratin-positive tumor cells (red), PD-1+ cells (orange), PD-L1+ cells (green), CD68+ macrophages (white), CD8+ T cells (cyan), and FoxP3+ regulatory T cells (yellow). **(A–C)** Pretreatment tumor biopsy tissues. After two doses of camrelizumab plus chemotherapy, the surgical specimens of patients A and B who achieved MPR contained an influx of CD8+ T cells **(G, H)**, and the presence of macrophages, Treg cells, and PD-1 and PD-L1+ immune cells were more common in the tumor area **(D, E)**. By contrast, in patient C who had no MPR, the tumor immune response was not obvious, and the amount of PD-1 and PD-L1+ immune cells was reduced after treatment **(F, I)**. Scale bar, 50 μm.

Discussion

In our study, we observed that neoadjuvant administration of two cycles of camrelizumab plus nab-paclitaxel and nedaplatin in patients with stage IIIA squamous NSCLC was not associated with additional adverse events than in previous studies (8–11). There was no delay of the planned surgery in all three patients. In addition, a radiological reduction in the tumor size was noted in all patients, which made the surgery easier to perform. Among them, two patients had MPR. The adverse events in this study were grade 1 or 2 and consistent with those of the individual drugs. The reactive cutaneous capillary endothelial proliferation that was most commonly reported related to camrelizumab was not observed in our study, which might be contributed to the combination with chemotherapy (14, 15).

Limited to traditional cytotoxic chemotherapy, advanced squamous NSCLC was associated with shorter survival than non-squamous NSCLC (16). Recently, several studies have shown that anti-PD-1 or anti-PD-L1 plus chemotherapy could improve progression-free survival and overall survival compared with chemotherapy alone in advanced squamous NSCLC (17, 18). Furthermore, MPR was more frequently observed in squamous cell carcinoma than in adenocarcinoma in neoadjuvant immunotherapy (5), which correlated with overall survival (13). The CheckMate 816 study, a phase 3 trial of neoadjuvant anti-PD-1 immunotherapy (nivolumab) plus chemotherapy versus chemotherapy alone in resectable NSCLC, reported that neoadjuvant nivolumab plus chemotherapy did not delay surgery and achieved a remarkable higher of pathologic complete response (pCR) than chemotherapy alone (24% *vs.* 2.2%). The study also showed that, compared with chemotherapy, the improvement of nivolumab plus chemotherapy on pCR was consistent in key subgroups, including disease stage (IB/II [26.2% *vs.* 4.8%]; ≥IIIA [23.0% *vs.* 0.9%]), PD-L1 tumor proportion score (<1% [16.7% *vs.* 2.6%]; ≥1% [32.6% *vs.* 2.2%]), and tumor mutational burden (low [22.4% *vs.* 1.9%]); high [30.8% *vs.* 2.7%]) (19). In terms of safety, neoadjuvant therapy with nivolumab combined with chemotherapy did not increase postoperative complications. The CheckMate 816 study reported that 11% of the patients encountered grade 3–4 surgery-relate adverse events in the nivolumab plus chemotherapy group, compared with 15% in the chemotherapy group (19). The heterogeneous ethnicities may lead to different responses to therapies. In previous studies for advanced NSCLC, the effect of camrelizumab was not inferior to nivolumab or pembrolizumab in Chinese populations (10, 11). Therefore, camrelizumab might be more suitable and economical for Chinese patients with NSCLC (10, 11). We chose nab-paclitaxel to avoid the need for steroid. Because of the concerns about serious side effects and effectiveness, patients in our study received two cycles of camrelizumab plus nab-paclitaxel and nedaplatin followed by surgical removal of the tumor, which was less than in most studies. Our results demonstrated that this shorter cycle was also very effective.

Compared to adjuvant therapy, neoadjuvant immunotherapy may improve efficacy by reducing metastasis or recurrence in early-stage NSCLC (20). PD-1 blocking enhances T-cell-mediated antitumor activity not only directly

killing tumor cells but also increasing tumor antigen-specific T-cell priming (6, 21). The activated tumor-specific T cells circulate in the body to eradicate micrometastatic tumor deposits that might otherwise drive postsurgical relapse (20). Platinum-based chemotherapy could induce immunogenic tumor elimination by increasing antigen presentation, following T-cell priming (22). In our study, more infiltration of CD8+ T cells and of PD-L1 on immune cells was observed in patients with MPR after PD-1 blockade, which was consistent with an adaptive PD-L1 upregulation mechanism (23, 24). It was unexpected that patient A with complete tumor regression of the primary lung tumor had a 1.1% tumor cell PD-L1 expression in the pretreatment specimen, which was the lowest among the three patients. However, the high expression of his PD-1 on immune cells was the highest after treatment, which may contribute to the treatment response. Furthermore, the CD8+ T cells of his surgical specimen were lower than those of another patient with MPR, but his FoxP3+ regulatory T cells were also lower, which may increase immune response. By contrast, in patient C who was without MPR, the presence of PD-1 and PD-L1+ immune cells was reduced after treatment (Table S1). The limitations of our study include the small patient numbers, the absence of a randomized control group, and the short postoperative follow-up period. Larger studies are needed to correlate the pathological response resulting from neoadjuvant therapy with overall survival.

In conclusion, all three patients in this study had a radiographic response, and two of them reached a major pathological response. There were no new safety signals identified. Neoadjuvant camrelizumab plus chemotherapy could potentially be a therapeutic option for patients with stage IIIA squamous NSCLC, which requires confirmation in future prospective multicenter randomized studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was reviewed and approved by Review Board of Tianjin Medical University General Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XL, CX, ML, HL, and JC wrote the manuscript. XL, CX, ML, JL, MD, HZ, SX, SW, DW, ZS, and GC took care of the patients, collected, and analyzed the data. JC and HL supervised the research. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Hospitalized cancer patients with comorbidities and low lymphocyte counts had poor clinical outcomes to immune checkpoint inhibitors

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Background: Immune checkpoint inhibitor (ICI) therapy has improved survivals with a favorable toxicity profile in a variety of cancer patients. We hypothesized that hospitalized cancer patients who have acute or chronic comorbidities may have suppressed immune systems and poor clinical outcomes to ICIs. The objective of this study was to explore clinical outcomes and predictive factors of hospitalized cancer patients who received ICI therapy at an NCI-designated Comprehensive Cancer Center.

Methods: A retrospective review of electronic medical records was conducted for adult cancer patients who received an FDA-approved ICI during admission from 08/2016 to 01/2022. For each patient we extracted demographics, cancer histology, comorbidities, reasons for hospitalization, ICI administered, time from treatment to discharge, time from treatment to progression or death, and complete blood counts. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method and compared using the log-rank test. The 95% confidence interval for survival was calculated using the exact binomial distribution. Statistical significance was defined as 2-sided $p<0.05$.

Results: Of 37 patients identified, 2 were excluded due to lack of complete blood counts on admission. Average hospital stay was 24.2 (95% CI 16.5, 31.9) days. Ten (27.0%) patients died during the same hospitalization as treatment. Of those who followed up, 22 (59.5%) died within 90 days of inpatient therapy. The median PFS was 0.86 (95% CI 0.43, 1.74) months and median OS was 1.55 (95% CI 0.76, 3.72) months. Patients with ≥3 comorbidities had poorer PFS (2.4 vs. 0.4 months; p=0.0029) and OS (5.5 vs. 0.6 months; $p=0.0006$). Pre-treatment absolute lymphocyte counts (ALC) <600 cells/μL were associated with poor

PFS (0.33 vs. 1.35 months; p=0.0053) and poor OS (0.33 vs. 2.34 months; p=0.0236). Pre-treatment derived neutrophil to lymphocyte ratio (dNLR) <4 was associated with good median PFS (1.6 vs. 0.4 months; p=0.0157) and OS (2.8 vs. 0.9 months; p=0.0375).

Conclusions: Administration of ICI therapy was associated with poor clinical outcomes and high rates of both inpatient mortality and 90-day mortality after inpatient ICI therapy. The presence of ≥3 comorbidities, ALC <600/μL, or dNLR >4 in hospitalized patients was associated with poor survival outcomes.

KEYWORDS

immune checkpoint inhibitor (ICI), inpatient, survival outcome, comorbidities, absolute lymphocyte count (ALC), derived neutrophil to lymphocyte ratio (dNLR), hospitalized adult patients

Introduction

The advent of immune checkpoint inhibitor (ICI) therapy has revolutionized cancer treatment and improved survival outcomes for a variety of cancers globally (1–4). Since the deployment of these agents in 2011, the field of cancer therapy has witnessed an ever-expanding landscape of biomarker-driven precision oncology and novel treatments (5–7). Because of promising outcome data and favorable toxicity profiles, ICI has increasingly been integrated into the treatment of a variety of cancer types across all clinical settings. However, ICIs only work in subsets of cancer patients for each cancer type and can be associated with severe or even fatal immune related adverse effects (irAEs) (8). In the current era of precision oncology, it is critical to select the appropriate cancer patients who are most likely to benefit from ICI therapy (9). Historically, the focus of much research has been on the predicted value of PD-L1 expression in tissue and host biomarkers as a means to determine clinical response to ICI therapy (10–12). Currently, there are limited studies focused on understanding the impact of clinical factors on patient selection for ICI treatment, and the choice of whom to treat can sometimes represent a difficult question (13). For patients receiving chemotherapy, the presence of poor performance status (PS) and/or concurrent high comorbid burden are associated with low rates of disease control, progression-free survival (PFS) and overall survival (OS) in cancer populations (14). Performance status (ECOG) of >2 (15, 16), active autoimmune diseases (17), and concurrent use of high dose steroids (17, 18) have been associated with poor clinical response and/or high irAEs to ICI therapy.

In hospitalized cancer patients, ICI treatment is often deferred due to the uncovered cost and efficacy in this population is unclear. Intuitively, hospitalized cancer patients often have worse performance status (PS) and in the elderly these functional losses can often be irreversible (19). In patients with solid tumors and poor PS, inpatient chemotherapy is associated with high mortality (14). However, little is known for the clinical outcomes in hospitalized cancer patients with high comorbid burden who receive ICI treatment (20). ICI therapy sometimes represents the last treatment option for patients with advanced solid tumors. Our study explored the clinical factors and predictive biomarkers that can be used to select cancer patients who may derive long term clinical benefit from ICI treatment during admission at an NCI designated comprehensive cancer center.

Materials and methods

We conducted a retrospective review of all adult (≥18 years old) cancer patients who received a FDA-approved ICI, either alone or in combination with chemotherapy, while admitted to inpatient services from August of 2016 through January of 2022 (i.e., 5 years) through a pharmacy database under an Institutional Review Board (IRB) approval protocol (University of California, Davis Protocol No. 937274). The ICIs used included ipilimumab, nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab and cemiplimab. Anonymized data were extracted from electronic medical records for age, gender, ethnicity, cancer histology, comorbidities, reasons for hospitalization, ICI administered, time from treatment to discharge, complete blood counts with differential, and clinical response to ICI treatment. Cancer types were classified by site of origin and defined as lung, melanoma, lymphoma, genitourinary, and "other." Tumor histology, metastatic status, and date of diagnosis were obtained from outpatient records when applicable. Performance status (PS) assessment using the Eastern Cooperative Oncology Group (ECOG) criteria was provided by the treating oncologist (21). Comorbid burden was captured for each

patient based on inpatient and outpatient documentation and evaluated by the Charlson Comorbidity Index (22, 23). Comorbid conditions that were evaluated in our study included evidence of any major organ failure (including heart, lung, kidney, and liver), thromboembolic disease (including pulmonary embolism or deep venous thrombosis), stroke, infection during inpatient stay requiring use of intravenous antibiotics, and malnutrition or failure to thrive recorded from either hospital notes (including history and physician notes, discharge summaries and inpatient progress notes) or from the problem list observed in the electronic medical record (EMR). The number of prior lines of therapy, type of therapy, and reason for treatment discontinuation were also recorded. Additionally, information on length of stay (LOS), time from treatment to discharge, time from ICI treatment to progression (PFS) or death (OS), absolute lymphocyte count (ALC) <600 cells/μL, derived neutrophil to lymphocyte ratio (dNLR) ≥4 prior to therapy on admission were computed and analyzed. Charlson Comorbidity Indices were independently calculated by two investigators (HP and RBY). Indications for ICI use were verified independently by at least two investigators (HP, RBY and TL). ICI expenditure data was calculated using wholesale average cost (WAC) which is the acquisition cost paid for drugs administered in the inpatient setting (AI). Equivalent data was obtained for the same medication administration in the outpatient setting using 340b costs. When relevant, information regarding immune-related adverse events (irAEs) was procured *via* review of inpatient progress notes, discharge summaries, and follow up oncology clinic notes. Last known follow-up and date of death were established by EMR review through January 26, 2022.

Data were summarized according to frequency and percentage for qualitative variables, and by mean ± standard deviation, median, and range for quantitative variables. PFS and OS were estimated using the Kaplan–Meier method along with their medians and relevant confidence intervals and compared using the log-rank test between groups. Cox proportional hazards models were used to estimate hazard ratios (HRs). Statistical significance was defined as 2-sided $p<0.05$.

Results

Patient characteristics

Between August 1, 2016 and January 26, 2022, 37 cancer patients who received ICI therapy while admitted to the hospital were identified through the institutional pharmacy database (Figure 1). The majority of patients were male (78.4%) and of Caucasian descent (59.4%). The median age was 53.5 years with a range of 21 to 79 years of age. All patients had the FDA-approved indications to receive an ICI which is usually given in the outpatient setting. The known information of companion and complemental biomarkers for ICI is provided in Column H in Supplemental Table 1. Lung cancer was the most common cancer type (13, 35.1%) to receive inpatient ICI therapy, followed by melanoma (8, 21.6%), genitourinary (8, 21.6%), and lymphoma (4, 10.8%). Reasons for admission were variable, but frequently included infection (5, 13.5%) and initiation of cancer-directed therapy (14, 37.8%). Indications for inpatient ICI use were initiation of new treatment (13, 35.1%), emergent use for tumor progression (10, 27.0%), need for therapy while awaiting disposition (6, 16.2%), and convenience to the patient

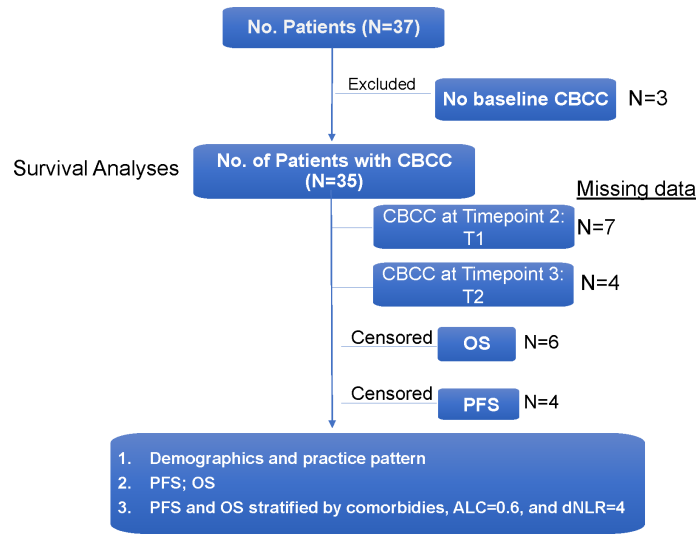

FIGURE 1
Summary of Study Patients.

(6, 16.2%). Full patient demographic information is summarized in Table 1.

Hospitalized cancer patients had poor clinical outcomes to ICI therapy

The average length of hospital stays was 24.2 (95% CI 16.5, 31.9) days. With a median (range) follow-up of 1.3 (0.1-60.4) months, most patients died during the study period, and only 5 (13.5%) were alive at the time of data analysis. On review of both inpatient and outpatient records, the average number of comorbid conditions present in our population was 2.24. Charlson Comorbidity Index ranged from 2-18 points, with an average score of 8.5 points. The majority of patient ECOG functional assessments were rated by the treating oncologist as 1 (48.6%) or 2 (21.6%). Ten cases (27.0%) had an ECOG score of 3 and 1 case (2.7%) had an ECOG score of 4. Of all study patients, 10 (27.0%) patients died during the same hospitalization they received treatment. Amongst the patients who had follow-up data, 22 died within 90 days of inpatient ICI therapy. For the entire patient population, the median PFS was 0.86 (95% CI 0.43, 1.74) months and median OS was 1.55 (95% CI 0.76, 3.72) months (Figure 2).

TABLE 1 Hospitalized patient demographics and characteristics.

No. Patients: N (%)	**N=37**
Male	29 (78.4%)
Female	8 (21.6%)
Age: mean (range), yo	53.5 (21-79)
Race/Ethnicity:	
White	22 (59.4%)
African American	5 (13.5%)
Hispanic or Latino	3 (8.1%)
Other	7 (18.5%)
Type of cancer:	
Lung	13 (35.1%)
Melanoma	8 (21.6%)
Lymphoma	4 (10.8%)
Genitourinary	8 (21.6%)
Others	4 (10.8%)
Reason for admission:	
Infection	5 (13.5%)
Initiate treatment	14 (37.8%)
Other	18 (48.6%)
Reason for inpatient therapy:	
Convenience to patient	6 (16.2%)
Assist with hospital disposition	6 (16.2%)
Initiation of new treatment	13 (35.1%)
Emergent for tumor progression	10 (27%)
Other	2 (5.4%%)

Based on WAC pricing the total cost of therapy was noted to be $466,040 and average cost per dose was $10,592. Many patients received just one dose of ICI therapy during admission. Six patients received two doses of ICI during admission, three patients received three doses during admission, and one patient received four doses (two cycles of nivolumab and ipilmumab).

Pretreatment clinical and blood biomarkers were correlated with poor clinical outcomes to ICIs

When evaluating the effect of comorbid burden on prognosis, subjects with 0 to 2 comorbidities had a better prognosis (both PFS and OS) than those with 3 or greater comorbid conditions (2.4 vs. 0.4 months; HR 3.4, 95% CI 1.5-7.9, p=0.0029 and 5.5 vs. 0.6 months; HR 4.5, 95% CI 1.9-10.5, p=0.0006) (Figure 3). Two of the 37 identified cases were excluded from the ALC analysis due to incomplete blood count records at the time of admission. Evaluation of the remaining 35 patients demonstrated that pre-treatment ALC values of less than 600 cells/μL were associated with poor PFS (0.33 vs. 1.35 months; HR 6.9, 95% CI 10.8-25.9, p=0.0053) and poor OS (0.33 vs. 2.34 months; HR 4.66, 95% CI 1.2-17.5, p=0.0236) (Figure 4). Furthermore, patients with ALC <600 were less likely to receive a subsequent ICI dose than their counterparts (28.6% vs 36.4%). Table 2 summarizes observed hospitalization duration, ECOG assessments, and ALC characteristics with survival data. Furthermore, when compared to those patients with high dNLR, a low dNLR (defined as less than 4) was associated with a better median PFS (1.6 vs. 0.4 months; HR 2.9, 95% CI 1.2-7.0, p=0.0157) and OS (2.8 vs. 0.9 months; HR 2.4, 95% CI 1.05-5.56, p=0.0375), respectively (Figure 5). Notably, grade 3 and 4 immune mediated adverse events are summarized in Table 3, of which two patients required upgrading care to intensive care unit.

Discussion

Our institutional review of patients who received ICI therapy while admitted revealed inpatient ICI treatment is associated with a poor clinical prognosis and high cost of therapy. In our study, the most common type of cancer based on site of origin was lung, which included both small cell and non-small cell lung cancer (NSCLC). As seen in the IMPOWER133 study, patients with extensive stage small cell lung cancer who received first line atezolizumab plus chemotherapy demonstrated a median OS of 12.3 months (24). Similarly, the KEYNOTE-189 investigators showed that in patients with metastatic NSCLC who were treated with first line pembrolizumab plus chemotherapy achieved a median OS of 12 months, and median PFS of 8.8

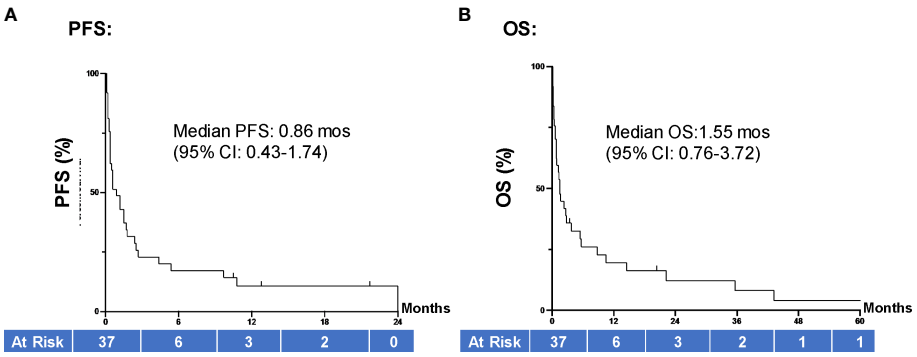

FIGURE 2
PFS and OS of all hospitalized cancer pts. **(A)** Median PFS for all patients in our study (N=37) was 0.86 months (95% CI 0.43, 1.74) and **(B)** median OS was 1.55 (95% CI 0.76, 3.72) months. Groups were compared using the log-rank test. Tick marks indicate censored data. P<0.05 indicates statistical significance. PFS, progression free survival; OS, overall survival.

months (25). The results observed in our sample population (median PFS of 0.86 months and median OS of 1.55 months) are significantly worse than would be customarily anticipated.

Hospitalized ICI therapy candidates represent a minority of patients with a dearth of information available to guide clinical practice. The use of chemotherapy in patients who are admitted and in those with poor functional status has been observed and generally accepted as being associated with worse clinical outcomes (14, 26). Performance status as determined by the use of ECOG or similar physical status assessment is an integral part of pretreatment evaluation for the survival of outpatients with advanced cancer (27). Similarly, studies evaluating use of ICI therapy in NSCLC patients with poor functional status has demonstrated similarly worse prognosis than functionally "fit" patients (28–30). Because of the comparatively more tolerable toxicity profile, and aforementioned lack of clinical data, the role of ICI treatment while hospitalized is less clear. Our investigation led to the identification of several predictive factors that can assist decision making in this population. As shown in Figure 3, the presence of increasing comorbidities (greater than three comorbid conditions) was associated with a statistically significant worse survival (PFS and OS) compared to those with a lower number of chronic illnesses.

The safety and efficacy of ICI therapy has been demonstrated in numerous clinical trials for a variety of cancers (31–33). However, many patients were excluded from these trials due to concurrent significant medical comorbidities. In practice, patients are given these therapies despite being excluded from the seminal trials. There are few studies to date that study the safety and efficacy of ICI therapy in patients with significant medical comorbid burden. A retrospective review of outpatients who received ICI therapy who had major organ (renal, cardiac,

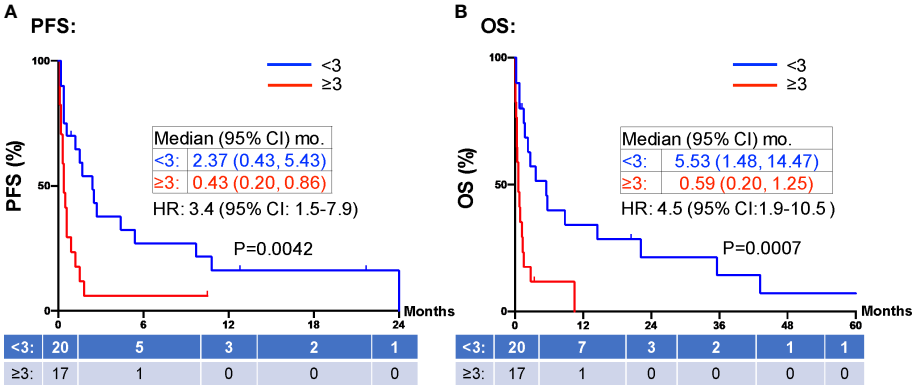

FIGURE 3
PFS and OS stratified by comorbidities. Patients with greater than 3 comorbid conditions (≥3 red) were associated with shorter PFS **(A)** and OS **(B)** compared to those patients with fewer than 3 comorbid conditions (<3, blue). Groups were compared using the log-rank test. Tick marks indicate censored data. P<0.05 for statistical significance. PFS, progression free survival; OS, overall survival.

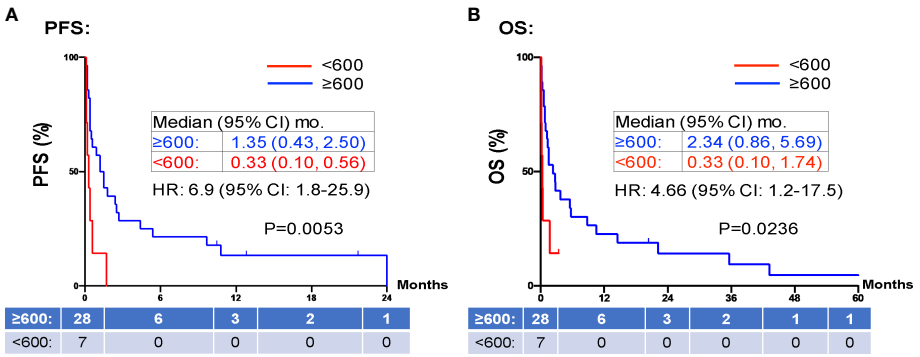

FIGURE 4
PFS and OS stratified by ALC. Patients with high ALCs (≥ 600 K/mm^3 shown in red) prior to inpatient treatment was associated with shorter PFS **(A)** and OS **(B)** compared to those patients with low ALCs (<600 K/mm^3 shown in blue). Groups were compared using the log-rank test. Tick marks indicate censored data. P<0.05 for statistical significance. ALCs, absolute lymphocyte counts; dNLR, derived-neutrophil-to-lymphocyte ratio, PFS, progression free survival; OS, overall survival.

or hepatic) dysfunction showed that these patients did not experience a higher incidence of irAE's and had durable response rates (34). However, this is in the setting of preserved performance status and functional reserve. In contrast, hospitalized patients with comorbidities often have a reduced physical status simply by definition of being hospitalized. Our study demonstrates that a high comorbid burden (i.e., major organ dysfunction) and a loss of functional reserve (by being hospitalized) is associated with poorer clinical outcomes (Figure 3). Due to the ease of use and our findings herein, the continued deployment of routine functional status assessments, assessment of comorbid conditions, and comprehensive medical history taking remain important tools in determining treatment candidacy.

TABLE 2 Patient clinical and laboratory treatment characteristics.

No. Patients: N (%)	N=37
Average comorbidities:	2.24
Average PS (ECOG):	
1	18 (48.6%)
2	8 (21.6%)
3	10 (27.0%)
4	1 (2.7%)
Duration of admission (Days)	24.2 (23.1)
Median ALC (± SD) (N=36)	1.2, 1.32 (1.08)
ALCs:	
ALCs ≥ 0.6	28
ALCs < 0.6	7
Patients who received a follow-up ICI dose	
ALCs ≥ 0.6	10 (35.7%)
ALCs < 0.6	0 (0%)
Median (95% CI) PFS (N=35, in months)	0.86 months
≥ 0.6 (N= 28)	1.35 (0.43, 2.50)
< 0.6 (N= 7)	0.33 (0.10, 0.56)

PS, performance status; ECOG, Eastern cooperative oncology group; ALCs, absolute lymphocyte counts; SD, standard deviation; ICI, immune checkpoint inhibitor; PFS, progression free survival; CI, confidence interval.

PD-1 and PD-L1 inhibitors up-regulate T-cell mediated anti-tumor activity, and thus rely on the presence of functional lymphocytes (35). Therefore, it is reasonable to hypothesize that a low pre-treatment ALC is associated with a poor response to ICI therapy. Our study confirms this finding in line with previous reports (36, 37), showing pre-treatment ALC <600 cells/μL were associated with poor PFS (0.33 vs. 1.35 months; HR 6.9, 95% CI 10.8-25.9, p=0.0053) and poor OS (0.33 vs. 2.34 months; HR 4.66, 95% CI 1.2-17.5, p=0.0236) (Figure 4). Furthermore, patients with ALC <600 cells/μL were less likely to receive a subsequent ICI dose than their counterparts (28.6% vs 36.4%). dNLR is thought to represent a systemic inflammatory state. Inflammation is one mechanism of immune resistance which can lead to activation of tumor growth signaling pathways (38). In recent years, dNLR has been used as a novel biomarker to predict response to immunotherapy in various cancers including NSCLC, melanoma and head and neck cancers (39, 40). It has been shown that a high pre-treatment dNLR (indicating a high inflammatory state) is associated with poor OS and PFS in a variety of cancers (37, 39–42). For instance, Bongiovanni et al, observed a positive association between OS and a NLR ≤ 5 (42). Moreover, NLR < 4 at week 8 of treatment is associated with objective response to treatment (43, 44). In our study, we confirmed pre-treatment dNLR <4 was associated with good median PFS (1.6 vs. 0.4 months; HR 2.9, 95% CI 1.2-7.0, p=0.0157) and OS (2.8 vs. 0.9 months; HR 2.4, 95% CI 1.05-5.56, p=0.0375) (Figure 5). The use of both dNLR and ALC may be useful in assessing a patient's "immune fitness" prior to the initiation of immune checkpoint therapy and may be helpful in

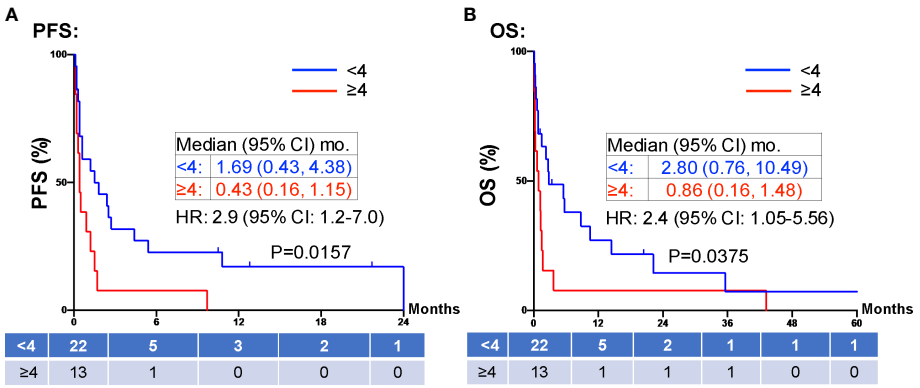

FIGURE 5
PFS and OS stratified by dNLR. Patients with high dNLR (≥4, red) at prior to inpatient treatment was associated with shorter PFS **(A)** and OS **(B)** compared to those patients with low dNLR (<4, blue). Groups were compared using the log-rank test. Tick marks indicate censored data. P<0.05 for statistical significance. ALCs, absolute lymphocyte counts; dNLR, derived-neutrophil-to-lymphocyte ratio. PFS, progression free survival; OS, overall survival.

predicting response to therapy. Nevertheless, the median PFS of 1.35 months and median OS of 2.34 months in our study patients with pre-treatment ALC ≥600 cells/μL, and the median PFS of 1.6 months and median OS of 2.8 months in patients with dNLR <4, respectively, argues against the use of ICI in hospitalized cancer patients. For hospitalized cancer patients, systemic chemotherapy is frequently used to elicit rapid reduction of tumor burden and symptomatic improvement in patients with chemotherapy-naïve or -sensitive solid tumors (45). However, compared to outpatient chemotherapy, urgent inpatient chemotherapy was associated with higher cost, increased mortality, worse clinical response, and higher mortality rates. These hospitalized cancer patients also had higher comorbidities, longer length of stay, higher discharge rates to skilled nursing, and increased inpatient mortality (46). Despite the hope that ICI might induce significant, durable tumor response with favorable toxicity profile, our study does not support inpatient use of ICI due to the low clinical response. Due to the high cost, ICI uses in hospitalized patients is not cost effective compared to chemotherapy.

Although elderly (≥65 years old) patients consist of over 50% of cancer patients, they are underrepresented in the clinical trials leading to the FDA approval of ICI trials. Currently data suggest age does not significantly affect the tolerability and clinical response to ICI monotherapy (47). However, aging is associated with "immunosenescence", which includes dysregulation of both cellular and humoral immunity; and is associated with lymphocyte depletion, fewer CD4+ and CD8+ T cells, decreased diversity of regulatory and memory T cells, defective DNA repair response pathway, and metabolic changes. In addition, aging is associated with "inflammaging", which has an overall increased pro-inflammatory state. All these factors were associated with decreased response to ICI therapy (47). In our study, the mean age was 53.5 years, and 13 (35%) of cancer patients were ≥65 years old. Although ALC was lower in elderly patients compared to younger pts (900 vs 1200/μL), dNLR was lower in elderly patients compared to younger patients (2.9 vs 3.2). It is likely that our hospitalized patients had more inflammatory changes from acute factors other than aging. Further studies are needed to evaluate the role of these easily accessible blood biomarkers to evaluate the immune fitness in predicting prognosis and ICI response in elderly patients.

Lastly, ICI therapy can incur significant costs to both the patient and healthcare system. This is particularly true in the inpatient setting where the cost is not reimbursable and valuable

TABLE 3 Immune related severe adverse events (grade 3 or 4).

Severe Adverse Events	Number of cases	Cancer type	Type of ICI treatment
Acute interstitial nephritis	1 (2.7%)	Genitourinary carcinoma	Pembrolizumab
Acute kidney injury	2 (5.4%)	Adenocarcinoma of lung	Pembrolizumab
Acute kidney injury	1 (2.7%)	Adenocarcinoma of prostate	Pembrolizumab
Pneumonitis	1 (2.7%)	Squamous cell carcinoma of lung	Pembrolizumab
Hypersensitivity reaction	1 (2.7%)	Adenocarcinoma of lung	Pembrolizumab

discounts, such as utilization of a 340b pharmacy program, are not applicable. While qualification for 340b (or similar) programs does require certain regulatory and institutional standards to be met, outpatient payments in general treat ICI treatment as a per line charge. This is in stark contrast to inpatient payments that are almost universally bundled into a daily charge without specific treatment carved out in the billing. In this study, we show that the high overall cost and cost per dose does not necessarily lead to significant overall survival. In addition, when taking into account toxicities associated with ICI therapy, the cost can increase exponentially (for instance, one patient in our review developed pneumonitis after ICI administration necessitating intensive care unit admission). Future studies include assessing data from the Surveillance, Epidemiology, and Results (SEER) program database to compare costs nationally and between institutions.

While our study offers new insights into clarifying hospitalized patients have poor clinical outcomes to ICI treatment, significant limitations exist. Most notably our review represents only a single institution that is geographically confined to northern California. Furthermore, we were only able to identify small sample population over the course of 5 years that received ICI treatment while hospitalized. As a result of this small sample size our pooled population represents a diverse group of malignancies with different histologic groups. Additionally, by design our study only offers observational data. As it is based on institutional pharmacy review, further investigation *via* retrospective cohort study, or prospectively with the inclusion of a control group that would allow recruitment of diverse populations which our study was unable to by nature of being observational, would offer superior information to draw conclusions from.

Conclusion

The results of our investigation suggest that in general ICI therapy offered to hospitalized patients should be provided cautiously. Clinical assessment tools such performance status, assessment of comorbid conditions, and thorough history taking continue to offer benefit in guiding treatment decision making. Furthermore, utilization of simple blood tests for pre-treatment ALC and dNLR may help to assess the "immune fitness" and identify appropriate candidates for inpatient therapy. Further studies are needed to assess the "immune fitness" of cancer patients receiving ICI treatment, especially in the inpatient setting.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by University of California, Davis Protocol No. 937274). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

TL contributed to the conception and design of the study. All authors contributed to the acquisition, analysis, or interpretation of data. RY, HP, and TL drafted and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.980181/full#supplementary-material

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Pathologic response and safety to neoadjuvant PD-1 inhibitors and chemotherapy in resectable squamous non-small-cell Lung cancer

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Background: Several randomized studies have shown that the combination of programmed cell death 1 (PD-1) inhibitor and chemotherapy is efficacious as a treatment for advanced non-small-cell lung cancer (NSCLC). However, in the neoadjuvant setting, there is scarce evidence of the effectiveness and safety of the combinations in squamous NSCLC. We conducted a retrospective study to evaluate neoadjuvant PD-1 inhibitor plus chemotherapy in resectable squamous NSCLC.

Methods: Patients from Beijing Chest Hospital, Capital Medical University, between October 2019 and October 2021, treated with PD-1 inhibitors and chemotherapy for resectable squamous NSCLC were retrospectively studied. The primary objectives were to assess the pathological tumor response and safety of neoadjuvant PD-1 inhibitors and chemotherapy.

Results: 63 patients with resectable squamous NSCLC stage IIA-IIIB were included. Two to four cycles of PD-1 inhibitors (37 cases with camrelizumab, 11 cases with toripalimab, 8 cases with tislelizumab, and 7 cases with sintilimab) and chemotherapy were administered prior to surgery. 42 patients (66.7%) achieved a major pathologic response (MPR), including 25 (39.7%) with a pathologic complete response (pCR). Twenty-one patients (33.3%) experienced grade 3 neoadjuvant treatment-related adverse events (TRAEs), and no patient had grade 4 or 5 TRAE.

Conclusion: Neoadjuvant PD-1 inhibitors and chemotherapy are feasible therapies for resectable squamous NSCLC. It was associated with a 66.7% MPR rate, 39.7% pCR rate, and tolerable toxicity.

KEYWORDS

resectable non-small-cell lung cancer, squamous cell carcinoma, neoadjuvant chemoimmunotherapy, programmed death-1 inhibitors, pathologic response

Introduction

In non-small-cell lung cancer (NSCLC), squamous NSCLC (sqNSCLC) represents approximately 25% to 30% (1), and it is associated with a shorter survival time than nonsquamous NSCLC (2, 3). Squamous NSCLC has historically been treated almost exclusively with cytotoxic chemotherapy due to the lack of targetable aberrations (4).

Patients with resectable NSCLC at high recurrence risk may benefit from neoadjuvant or adjuvant chemotherapy; however, the 5-year overall survival (OS) gain is only 5% (5, 6). Inhibitors of programmed death receptor 1 (PD-1) and its ligand programmed death-ligand 1 (PD-L1) are effective in the treatment of advanced squamous and nonsquamous NSCLC (7–11). These PD-1/PD-L1 inhibitors are evaluated in multiple clinical trials, rapidly moving from advanced NSCLC to resectable stages and from palliative to curative strategies.

Single-arm phase 2 studies with immunotherapy agents as monotherapy or in combination have recently shown encouraging outcomes (pathologic complete response, event-free survival, and OS) in the neoadjuvant setting (12–16). CheckMate 816 is a randomized, phase 3, open-label study evaluating nivolumab-plus-chemotherapy versus chemotherapy as neoadjuvant treatment for resectable NSCLC, The CheckMate 816 showed statistically significant improvements in the primary endpoints of event-free survival (EFS, median EFS was 31.6 months in the nivolumab-plus-chemotherapy arm and 20.8 months in the chemotherapy-alone arm; hazard ratio, 0.63; 97.38% CI, 0.43 to 0.91), and the pathologic complete response (pCR, pCR rate was 24% in the nivolumab-plus-chemotherapy arm and 2.2% in the chemotherapy-alone arm, odds ratio, 13.94; 99% CI, 3.49 to 55.75) (17). As a result of CheckMate 816, the FDA approved using nivolumab in combination with platinum-doublet chemotherapy for resectable NSCLC patients in the neoadjuvant setting, but in China, this strategy has not yet been approved.

However, in neoadjuvant therapy for NSCLC, few clinical studies on neoadjuvant treatment are designed for squamous cell carcinoma. Therefore, there is scarce evidence of the effectiveness and safety of the neoadjuvant chemoimmunotherapy in squamous NSCLC, especially in several ones approved in China for first-line treatment in advanced sqNSCLC (18–20). In addition, the pathological response of neoadjuvant chemoimmunotherapy in squamous cell carcinoma is not clear. Therefore, we conducted a retrospective study to evaluate pathological response and safety of neoadjuvant PD-1 inhibitor plus chemotherapy in resectable squamous NSCLC.

Materials and methods

Patient population

Patients from Beijing Chest Hospital, Capital Medical University, treated with PD-1 inhibitors and chemotherapy for resectable sqNSCLC between October 2019 and October 2021, were retrospectively studied. The inclusion criteria were as follows: age 18 years or older, confirmed histological diagnosis of sqNSCLC, Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2, clinical stage IIA-IIIB before the treatment, and ≥2 neoadjuvant treatment cycles, adequate organ function and undergone surgical resection. The exclusion criteria were as follows: had previous treatment before diagnosis or lacked completed radiological or pathological data. The resectable criteria were followed by defining the resectability status of the National Comprehensive Cancer Network (NCCN) Guidelines for all stage IIA-IIIA cases. In terms of stage IIIB patients, the cases including tumor T3/T4 with single-station non-bulky N2 disease of mediastinal lymph nodes, excluding tumor T3/T4 with multi-station N2 disease or bulky N2 disease, were judged as potentially resectable or marginal resectable. Therefore, only cases that met the resectable criteria were administrated for neoadjuvant chemoimmunotherapy. Finally, 63 patients were included in the study (Figure 1). The study was carried out following the Declaration of Helsinki (as revised in 2013). It was reviewed and approved by the institutional review board (IRB)/ethics committee of Beijing Chest Hospital, Capital Medical University. In the neoadjuvant chemoimmunotherapy for operable NSCLC of this study, all PD-1 inhibitors were given for off-label use. All patients

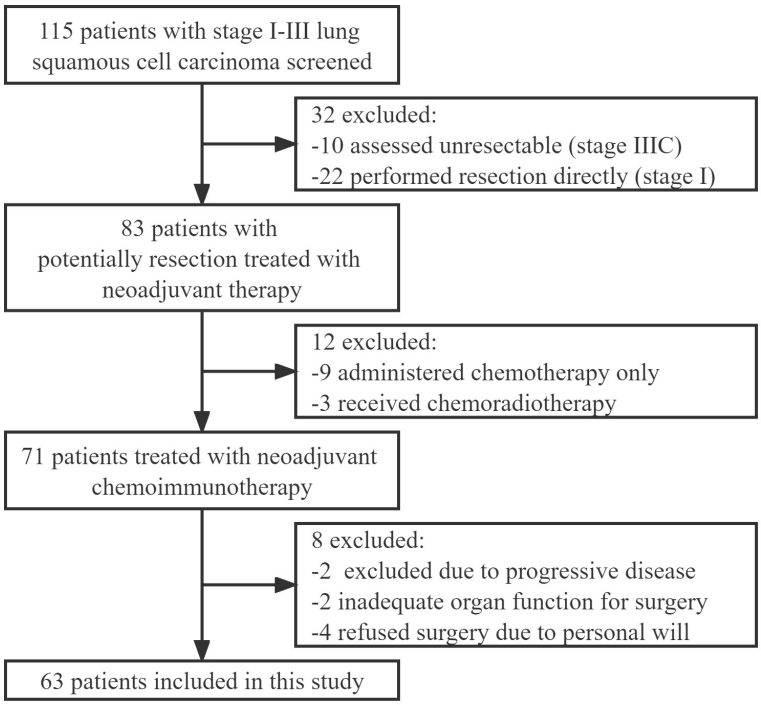

FIGURE 1
Flow diagram of patients included in this study.

were fully informed and signed informed consent before starting treatment.

The collected clinicopathologic data of the patients included sex, age, smoking history, ECOG PS, PD-L1 expression (22C3 PD-L1 antibody, Dako, Denmark), clinical TNM (cTNM) stage, neoadjuvant treatment regimen, treatment cycle, surgical treatment, radiological and pathological efficacy evaluation, and treatment-related adverse events (TRAEs). In addition, clinical TNM was determined according to the 8th edition of the lung cancer staging system of the American Joint Committee on Cancer (21).

Treatment regimen and surgery

All of the included patients were scheduled to receive surgery within 4-6 weeks after neoadjuvant chemoimmunotherapy that consisted of 2-4 cycles of a conventional platinum-based doublet chemotherapy regimen with PD-1 inhibitor on day 1 of each 21-day cycle. Patients received one of the following PD-1 inhibitors intravenously as neoadjuvant immunotherapy: camrelizumab (200 mg), toripalimab (240 mg), tislelizumab (200 mg), or sintilimab (200 mg).

As per standard institutional procedures, all surgical resections were performed with thoracotomy or video-assisted thoracoscopic surgery.

Treatment evaluation

The primary objectives were to assess the pathological tumor response of neoadjuvant PD-1 inhibitors and chemotherapy. The pathological tumor response endpoints were MPR, defined as ≤10% residual viable tumor cells in the primary tumor and sampled lymph nodes, and pCR, defined as the complete absence of residual viable tumor cells in the primary tumor and sampled lymph nodes (22).

Secondary endpoints were the imaging response and safety profile of the combination.

Contrast-enhanced CT scans were repeated to assess objective imaging response within seven days before surgery. The imaging responses were evaluated for all patients per the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (23), and the therapeutic response was considered as complete response (CR), partial response (PR), stable disease (SD), or progression disease (PD). The safety endpoints included

treatment-related adverse events according to the Common Terminology Criteria for Adverse Events (CTCAE, v.5.0).

Statistical analysis

All statistical analyses were performed with Stata version 17.0 (StataCorp, TX, USA) or GraphPad Prism version 9.0 (GraphPad Software Inc., CA, USA). Frequency tabulation and summary statistics for the patient's baseline characteristics, surgical outcomes, and safety evaluation provided data distribution characteristics. Continuous variables were expressed as medians with ranges. Categorical variables were expressed as numbers with percentages. The association of baseline characteristics and pathological response were conducted with the Fisher's exact test. The association between the clinical response and the pathological response was performed with Pearson correlation coefficient analysis. A two-sided p value less than 0.05 was considered statistically significant.

Results

Patient characteristics

Sixty-three patients with resectable squamous NSCLC stage IIA-IIIB were included (Table 1). Of these patients, eight were females and 55 males aged from 47 to 75 years old (median age of 63 years old). Most patients (73.0%) had stage IIIA to IIIB disease, according to the IASLC eighth edition of the TNM Classification for Lung Cancer. PD-L1 expression before treatment was detected by the PD-L1 IHC 22C3 pharmDx assay. For the 40 patients with available PD-L1 data, 32 patients (50.8%) had a PD-L1 tumor proportion score of 1% or higher.

Neoadjuvant treatment and imaging efficacy

Two to four cycles of PD-1 inhibitors (37 cases with camrelizumab, 11 cases with toripalimab, 8 cases with tislelizumab, and 7 cases with sintilimab) and chemotherapy were administered prior to surgery (Table 1). The clinical activity of the chemoimmunotherapy neoadjuvant combination was evaluated according to the RECIST v.1.1 criteria. In particular, 43 out of the 63 cases achieved a partial response (PR, 68.3%), while 20 patients presented a stable disease (SD, 31.7%).

Surgical treatment and pathological efficacy

All 63 patients received R0 surgical resection. The results for surgical treatment are shown in Table 2. Surgical methods included video-assisted thoracoscopic surgery (VATS) (n=32) and thoracotomy (n=31), including 47 (74.6%) lobectomy, 9 (14.3%) bilobectomy and 7 (11.1%) pneumonectomy. The median days of hospitalization after surgery operations was 10 (range, 1–68), the median operation time was 154 (range, 85–310) minutes, and the median amount of estimated blood loss was 150 mL (50–1100 mL). One patient died within 48 hours of lobectomy. He had a clinical T3N2 primary tumor. Radiographic SD was observed after two cycles of neoadjuvant chemoimmunotherapy, which resulted in a technically challenging resection. The patient developed severe hypoxemia, required ventilator support, and died 48 hours postoperatively.

In total, 42 patients (66.7%) achieved a major pathologic response (MPR), including 25 (39.7%) with a pathologic complete response (pCR) in the primary tumor and sampled lymph nodes. In two patients, the primary tumor disappeared, but the regional lymph node involvement persisted, achieving an MPR in the final overall evaluation.

The waterfall plot shows pathological regression in the resected primary lung tumor after neoadjuvant administration, according to the subgroup of sex, smoking status, clinical TNM stage, PD-L1 expression, PD-1 inhibitor regimen, and RECIST response (Figure 2). There was correlation between the imaging regression and pathological regression (Spearman correlation coefficient = 0.43; P = 0.0004; Figure 3). The MPR was related to the clinical lymph nodal stage (Fisher's exact test P = 0.009) and clinical TNM stage (Fisher's exact test P = 0.027). The pCR was only related to the clinical TNM stage (Fisher's exact test P = 0.047, Table 3). The Sankey diagram shows the degree of relationship between the pathological response of neoadjuvant therapy in different clinical stages (Figure 4).

Safety

Treatment-related adverse events were reported for 62 (98.4%) patients treated with neoadjuvant PD-1 inhibitors plus chemotherapy. Most of the adverse events were in grades 1-2. Grade 3 treatment-related adverse events occurred in 21 (33.3%) patients, including decreased neutrophil count 11 (17.5%) patients, pneumonia 7 (11.1%), and decreased white blood cell count 5 (7.9%), (Table 4). No grade 4-5 toxicities occurred during the neoadjuvant treatment phase.

TABLE 1 Clinicopathologic characteristics of 63 patients.

Characteristic	Value or No. of Patients	%
Patients	63	
Age, years		
Median	63	
Range	47-75	
Sex		
Female	8	12.7%
Male	55	87.3%
Smoking status		
Never	17	27.0%
Current/Former	46	73.0%
ECOG PS		
0	39	61.9%
1	24	38.1%
Clinical T stage		
T1	3	4.8%
T2	20	31.7%
T3	22	34.9%
T4	18	28.6%
Clinical N stage		
N0	18	28.6%
N1	17	27.0%
N2	28	44.4%
Clinical stage (8th edition)		
IIA	3	4.8%
IIB	14	22.2%
IIIA	31	49.2%
IIIB	15	23.8%
PD-L1 expression		
Positive (≥1%)	32	50.8%
≥1%-49%	13	20.6%
≥50%	19	30.2%
Negative (<1%)	8	12.7%
NA	23	36.5%
Neoadjuvant PD-1 inhibitor regimen		
Camrelizumab	37	58.7%
Toripalimab	11	17.5%
Tislelizumab	8	12.7%
Sintilimab	7	11.1%
Neoadjuvant treatment cycles		
2	41	65.1%
3	18	28.6%
4	4	6.3%

ECOG PS, Eastern Cooperative Oncology Group performance score; NA, not applicable; PD-1, programmed death 1; PD-L1, programmed death-ligand 1.

TABLE 2 Surgical outcomes.

Surgical outcomes	Patients (n=63)
Operation time (minutes)	
Median	154
Range	85-310
Hospitalization after surgery (days)	
Median	10
Range	1-68
Estimated blood loss (mL)	
Median	150
Range	50-1100
R0 resection, n (%)	
Yes	63 (100%)
No	0 (0%)
Extent of resection, n (%)	
Lobectomy	47 (74.6%)
Bilobectomy	9 (14.3%)
Pneumonectomy	7 (11.1%)
Surgical methods, n (%)	
Video-assisted thoracoscopic surgery	32 (50.8%)
thoracotomy	31 (49.2%)
Perioperative death, n (%)	1 (1.6%)

Discussion

Resectable sqNSCLC is usually treated with a combination of surgery, radiation, and systemic chemotherapy. However, it has been proven that immunotherapy is a very effective front-line treatment for advanced sqNSCLC (8, 11, 18–20). Additionally, perioperative immunotherapy has been proven successful in NSCLC (13, 17, 24), but the effect of chemoimmunotherapy in resectable sqNSCLC has rarely been reported. In this study, we retrospectively analyzed 63 squamous NSCLC with stage II-IIIB treated with neoadjuvant chemoimmunotherapy. Our study revealed that PD-1 inhibitors plus chemotherapy were prescribed preoperatively, thus resulting in 66.7% (42/63) of patients achieving an MPR and 39.7% (25/63) cases achieving a pCR. Meanwhile, no unexpected adverse reactions were observed.

NSCLC is classified into squamous cell carcinomas and non-squamous cell carcinomas based on their unique biological behavior, clinical molecular characteristics, and therapeutic responses (25). The study found that compared with adenocarcinoma, the expression of PD-L1 in squamous cell carcinoma is more common, and the infiltration of macrophages and other immune cells is more prominent, which brings an opportunity for the treatment of patients with advanced squamous cell carcinoma, and also leads to the different response of squamous cell carcinoma and nonsquamous cell carcinoma to immunotherapy (26).

Notably, our study only included patients with squamous cell carcinoma. After neoadjuvant immunotherapy combined with chemotherapy, we achieved an excellent pathological response from a numerical point of view. Two-thirds of the patients obtained MPR, and nearly 40% of the cases achieved

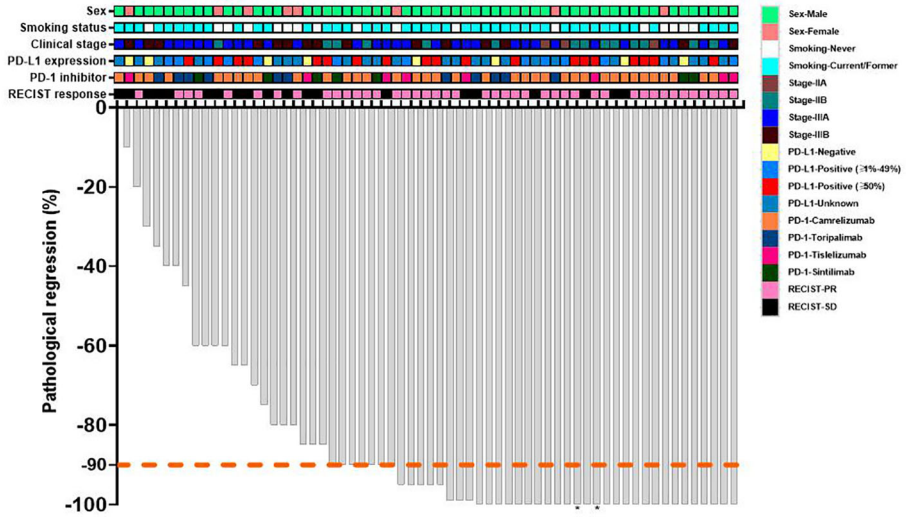

FIGURE 2
Waterfall plot of pathological response of neoadjuvant therapy. Each bar represents one patient. The upper rows show clinical characteristics and radiological responses. PD-L1, programmed death-ligand 1; PD-1, programmed death 1; RECIST, response evaluation criteria in solid tumors; PR, partial response; SD, stable disease. *The regional lymph node of dissection involvement persisted.

pCR. Our findings further confirmed the findings of previous small samples of neoadjuvant immunotherapy for lung squamous carcinoma (27, 28).

A major pathological response is more likely to be observed in patients with squamous cell carcinoma (26%) than in those with adenocarcinoma (12%) following neoadjuvant chemotherapy studies, possibly because of greater baseline tumor necrosis in squamous cell carcinomas (29). However, the pCR rates of squamous and nonsquamous NSCLC to neoadjuvant nivolumab plus chemotherapy were similar in the CheckMate 816 study, with 25.3% in squamous and 22.8% in nonsquamous. Therefore, more studies are needed to investigate whether the efficacy of neoadjuvant immunotherapy varies against squamous and nonsquamous NSCLC.

In terms of treatment course before surgery, most previous studies choose 2 to 4 cycles. The neoadjuvant single-agent

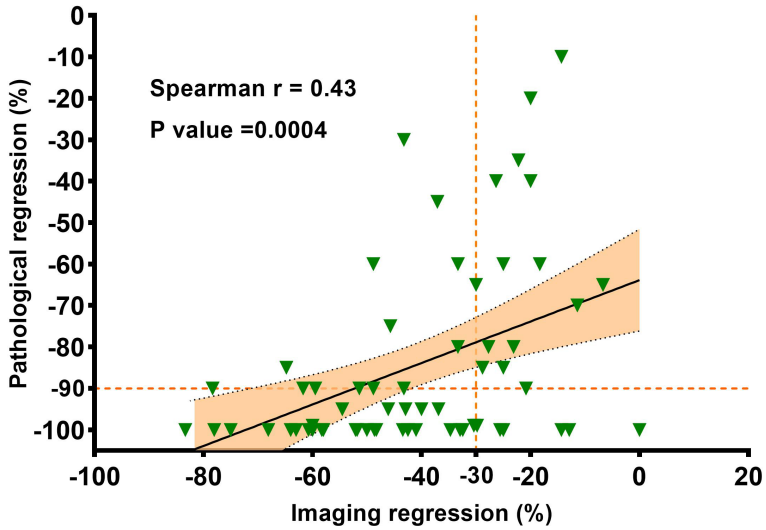

FIGURE 3
The correlation between the imaging regression and the pathologic regression. Pearson correlation coefficient and two-sided P value are shown.

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TABLE 3 Association between clinical characteristics and pathological response.

Characteristic	MPR/pCR (n=42)	Non-MPR (n=21)	P Value	pCR (n=25)	Non-pCR (n=38)	P Value
Age, years						
<65	21 (50%)	14 (67%)	0.21	13 (52%)	22 (58%)	0.65
≥65	21 (50%)	7 (33%)		12 (48%)	16 (42%)	
Sex						
Female	3 (7%)	5 (24%)	0.061	2 (8%)	6 (16%)	0.36
Male	39 (93%)	16 (76%)		23 (92%)	32 (84%)	
ECOG PS						
0	9 (21%)	8 (38%)	0.16	6 (24%)	11 (29%)	0.67
1	33 (79%)	13 (62%)		19 (76%)	27 (71%)	
Smoking status						
Never	28 (67%)	11 (52%)	0.27	18 (72%)	21 (55%)	0.18
Current/Former	14 (33%)	10 (48%)		7 (28%)	17 (45%)	
Clinical T stage						
T1	3 (7%)	0 (0%)	0.78	3 (12%)	0 (0%)	0.22
T2	13 (31%)	7 (33%)		8 (32%)	12 (32%)	
T3	15 (36%)	7 (33%)		8 (32%)	14 (37%)	
T4	11 (26%)	7 (33%)		6 (24%)	12 (32%)	
Clinical N stage						
N0	14 (33%)	4 (19%)	0.009	10 (40%)	8 (21%)	0.095
N1	15 (36%)	2 (10%)		8 (32%)	9 (24%)	
N2	13 (31%)	15 (71%)		7 (28%)	21 (55%)	
Clinical stage (8th edition)						
IIA	3 (7%)	0 (0%)	0.027	3 (12%)	0 (0%)	0.047
IIB	13 (31%)	1 (5%)		8 (32%)	6 (16%)	
IIIA	19 (45%)	12 (57%)		10 (40%)	21 (55%)	
IIIB	7 (17%)	8 (38%)		4 (16%)	11 (29%)	
PD-L1 expression						
Negative (<1%)	4 (10%)	4 (19%)	0.65	3 (12%)	5 (13%)	0.84
Positive (≥1%-49%)	8 (19%)	5 (24%)		6 (24%)	7 (18%)	
Positive (≥50%)	14 (33%)	5 (24%)		6 (24%)	13 (34%)	
NA	16 (38%)	7 (33%)		10 (40%)	13 (34%)	
Neoadjuvant PD-1 inhibitor regimen						
Camrelizumab	27 (64%)	10 (48%)	0.34	17 (68%)	20 (53%)	0.69
Toripalimab	5 (12%)	6 (29%)		4 (16%)	7 (18%)	
Tislelizumab	6 (14%)	2 (10%)		2 (8%)	6 (16%)	
Sintilimab	4 (10%)	3 (14%)		2 (8%)	5 (13%)	
Neoadjuvant treatment cycles						
2	29 (69%)	12 (57%)	0.61	15 (60%)	26 (68%)	0.57
3	11 (26%)	7 (33%)		9 (36%)	9 (24%)	
4	2 (5%)	2 (10%)		1 (4%)	3 (8%)	

MPR, major pathologic response; pCR, pathologic complete response; ECOG PS, Eastern Cooperative Oncology Group performance score; NA, not applicable; PD-1, programmed death 1; PD-L1, programmed death-ligand 1.

immunotherapy in CheckMate159 and LCMC3, was performed for two cycles (12, 30). Neoadjuvant immunotherapy combined with chemotherapy in NADIM and CheckMate 816, or a combination of two checkpoint inhibitors in NEOSTAR, was performed for three to four cycles (13, 17, 31). In our study, 41 (65.1%) patients received two cycles of preoperative treatment, 18 (28.6%) patients received three cycles, and only four patients received four cycles of treatment (Table 1). In terms of efficacy (Table 3), further comparing the difference between 2 cycles treatment and 3-4 cycles treatment, we found no statistical correlation (data not shown). In determining the best neoadjuvant treatment course, various factors are taken into

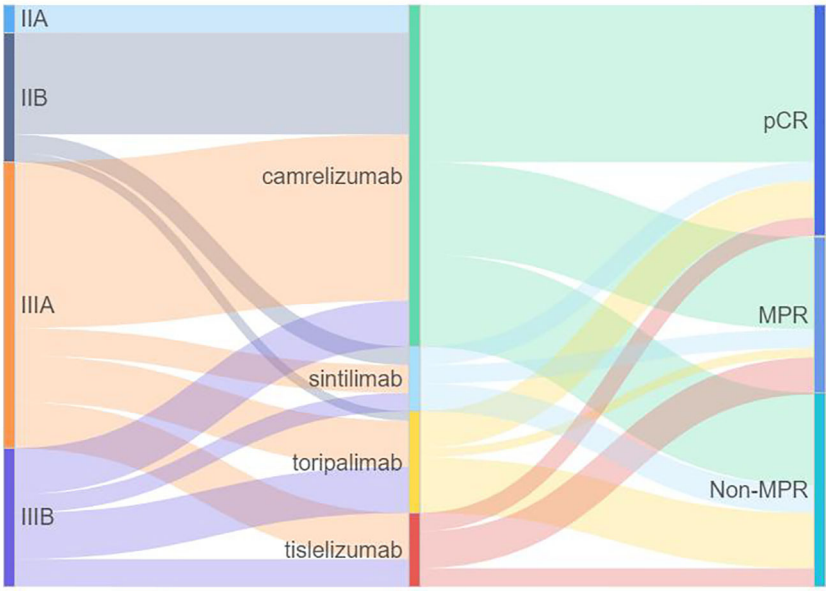

FIGURE 4
Sankey diagram of the relationship between pathological response of neoadjuvant therapy in different clinical stages. PD-1, programmed death 1; MPR, major pathologic response; pCR, pathologic complete response.

TABLE 4 Treatment-related adverse events during neoadjuvant treatment (n=63).

	Any grade, n (%)	Grade 1-2, n (%)	Grade 3, n (%)
Any treatment-related adverse event	62 (98.4)	62 (98.4)	21 (33.3)
Hematological toxicities			
Anemia	45 (71.4)	45 (71.4)	0
Neutrophil count decreased	30 (47.6)	19 (30.2)	11 (17.5)
White blood cell decreased	20 (31.7)	15 (23.8)	5 (7.9)
Platelet count decreased	7 (11.1)	7 (11.1)	0
Nonhematological toxicities			
ALT/AST increased	26 (41.3)	26 (41.3)	0
Vomiting	23 (36.5)	23 (36.5)	0
Hypoalbuminemia	22 (34.9)	22 (34.9)	0
Blood bilirubin increased	16 (25.4)	16 (25.4)	0
TSH increased	13 (20.6)	13 (20.6)	0
Pneumonia	12 (19.0)	5 (7.9)	7 (11.1)
Hyponatremia	11 (17.5)	11 (17.5)	0
Serum amylase increased	10 (15.9)	10 (15.9)	0
Hyperuricemia	9 (14.3)	9 (14.3)	0
Constipation	8 (12.7)	8 (12.7)	0
Rash	7 (11.1)	7 (11.1)	0
Hypokalemia	6 (9.5)	6 (9.5)	0
Creatinine increased	6 (9.5)	6 (9.5)	0
Anorexia	5 (7.9)	5 (7.9)	0
Fatigue	3 (4.8)	3 (4.8)	0
Alopecia	2 (3.2)	2 (3.2)	0
Diarrhea	2 (3.2)	2 (3.2)	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TSH, thyroid stimulating hormone.

account, including efficacy, timing of surgery, and patient compliance. In order to determine the optimal course of treatment, there is a need for more clinical evidence.

Of the 63 patients included in our study, 43 achieved radiological PR, of which 35 (81.4%) achieved pathological MPR or pCR, we found that there was a positive correlation between the imaging regression and pathological regression (Spearman correlation coefficient = 0.43; P = 0.0004; Figure 3). However, 20 patients were evaluated as radiological SD, with seven (35.0%) achieving pathological MPR or pCR. The primary role of immunotherapy promotes the immune cells to infiltrate the tumor and then kill the tumor cells. Patients may benefit from neoadjuvant immunotherapy without initial tumor shrinkage, which is likely to contribute to immune cells infiltrating the tumor (32).

A long-standing method of evaluating neoadjuvant therapy is to examine the pathological changes after surgery. Major pathological response to neoadjuvant treatment is a potential surrogate endpoint for survival (33). Several studies in NSCLC suggest an association between pCR and survival (HR, 0.49; 95% CI, 0.42-0.57) (34). Of note, resectable NSCLC treated with neoadjuvant chemotherapy shows low rates of pCR (median, 4%; range, 0-16%) (33). In CheckMate 816 of neoadjuvant chemoimmunotherapy, the pCR rate was 24% in the nivolumab-plus-chemotherapy arm and 2.2% in the control arm (odds ratio, 13.94; 99% CI, 3.49 to 55.75), the event-free survival appeared to be longer in patients who had a pCR than those who did not (17). Our study found that neoadjuvant PD-1 inhibitors and chemotherapy resulted in a 66.7% MPR rate and a 39.7% pCR rate. Patients who achieved either an MPR or a pCR might benefit long-term survival. In the future follow-up period, this point will be clarified further. For the 40 patients with available PD-L1 data in our study, There was no correlation between the PD-L1 expression of the primary baseline tumor and pathological regression (Spearman correlation coefficient = -0.131; P = 0.42; Supplementary Figure 1).

In advanced NSCLC, PD-L1 expression is a critical marker to guide treatment selection. Among patients with PD-L1 expression ≥ 50%, PD-1 or PD-L1 inhibitor monotherapy can be selected for first-line treatment (9, 35, 36), and patients with high PD-L1 expression may benefit more from the combined immunotherapy (18, 37). However, in a chemoimmunotherapy neoadjuvant setting, PD-L1 expression is not an ideal therapeutic or prognostic marker, and the results differ in different studies. A benefit with nivolumab plus chemotherapy was seen across PD-L1 subgroups in CheckMate 816 study, with a greater event-free survival benefit in patients with a tumor PD-L1 expression level of 1% or more than in those with a level of less than 1% (18, 37). There was a significant difference in PD-L1 tumor proportion score between patients who had a complete pathological response and those who had an incomplete pathological response in the NADIM study (p=0.042) (13), but PD-L1 staining was not predictive of survival (38).

The association of PD-L1 expression in tumor tissues with the efficacy and prognosis of neoadjuvant immunotherapy is unclear and requires continued studies with a larger sample size. Neoadjuvant immunotherapy for NSCLC requires biomarkers that accurately predict efficacy to select people who benefit (13, 38). A single biomarker may be challenging to meet the clinical needs of the published clinical studies. Combining multiple biomarkers is the future trend, and the best biomarkers to predict the efficacy also need to be explored.

The limitations of our study include, but are not limited to, the bias of a retrospective single cohort study, the small number of patients who were included, and the lack of survival follow-up. Therefore, larger randomized control studies are needed to reduce bias and determine the most effective PD-1 blockades of neoadjuvant therapy. Furthermore, long-term follow-up of these studies will be necessary to define the role of neoadjuvant PD-1 blockade in reducing recurrences and curing resectable cancers. In addition, PD-L1 was detected in some but not all patients. At the same time, the ctDNA and tumor mutational burden were not recorded in our study, and adequate biomarker studies are needed to identify the best predictive biomarkers of response and to correlate the pathologic response of neoadjuvant chemoimmunotherapy.

Neoadjuvant PD-1 inhibitors and chemotherapy are feasible therapies for resectable squamous NSCLC. It was associated with a 66.7% MPR rate, 39.7% pCR rate, and tolerable toxicity.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the institutional review board (IRB)/ethics committee of Beijing Chest Hospital, Capital Medical University. All patients were fully informed and signed informed consent before starting treatment.

Author contributions

ZL and LS conceived the study. LS, QM, LT, HL, YD, and CS collected the data. LS, HL, and ZL analyzed the data. LS, QM, LT, HL, YD, CS, and ZL interpreted the data. LS and ZL wrote the first draft of the manuscript. All authors read and contributed to the final version of the manuscript and approved its submission for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JN declared a shared parent affiliation with the authors to the handling editor at the time of review.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.956755/full#supplementary-material

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Systematic assessment and optimizing algorithm of tumor mutational burden calculation and their implications in clinical decision-making

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Tumor mutation burden (TMB) has been validated as a biomarker to predict the response of immune checkpoint inhibitors (ICIs) treatment in various cancers. However, the effects of different sequencing platforms, cancer types, and calculation algorithms on TMB as well as its cut-off value for predicting immunotherapy efficacy in the East Asian population still need to be further investigated. In this study, the data of 4126 samples generated by targeted panel sequencing or whole-exome sequencing (WES) in different platforms and public sequencing data from 3680 samples that contained targeted panel sequencing, WES and whole-genome sequencing (WGS) were obtained. The impact of different sequencing platforms and methods on TMB calculation was assessed. No significant bias was found in TMB calculated by different platforms. However, TMB calculated from WGS was significantly lower than those calculated from targeted panel sequencing and WES. The distribution of TMB at different sequencing depths and tumor purity were analyzed. There was no significant difference in the distribution of TMB when the sequencing depth was greater than 500, the tumor purity estimated by hematoxylin-eosin (HE) staining was between 0.1-1.0 or estimated by next-generation sequencing (NGS) was greater than 0.4. In addition, the somatic-germline-zygosity (SGZ) algorithm was optimized to calculate TMB from tumor-only sequencing samples in the East Asian population. The correlation coefficient of TMB calculated with the optimized SGZ algorithm and paired normal-tumor sequencing is 0.951. Furthermore, the optimal cut-off value of TMB in East Asian lung cancer patients treated with ICIs was determined to be 7 mut/Mb instead of 10 mut/Mb through the ROC curve and Log-rank analysis in the training cohort and validated in the test cohort. Patients with TMB ≥ 7 mut/Mb had better outcomes than patients with TMB<7 mut/Mb. In conclusion, this study systematically analyzed the factors that influenced the TMB calculation and optimized the SGZ algorithm to calculate TMB from tumor-only

sequencing samples in the East Asian population. More importantly, the cut-off value of TMB for predicting immunotherapy efficacy was determined to be 7 mut/Mb instead of 10 mut/Mb in East Asian lung cancer patients, which can help in clinical decision-making.

KEYWORDS

immunotherapy, tumor mutation burden, East Asian populations, method optimizing, clinical decision-making

Introduction

The advent of immune checkpoint inhibitors (ICIs), including anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA4), have revolutionized cancer therapy (1–4). Several ICIs have been approved by Food and Drug Administration (FDA) in multiple tumor types (5). However, only a subset of patients achieved durable clinical responses, and some may even suffer from unique immune-related toxicities or even hyperprogression (6–10). Therefore, predictive biomarkers were urgently required to optimize the treatment of ICIs.

The expression of PD-L1 in tumor and/or tumor-infiltrating lymphocytes assessed by immunohistochemistry (IHC) is an established biomarker to predict efficacy in the treatment of ICIs across many cancer types including melanoma, non-small cell lung cancer (NSCLC) and colorectal cancer (9, 11). However, on the one hand, PD-L1 alone as a biomarker is insufficient to distinguish responders (12); on the other hand, the detection methods and thresholds for PD-L1 expression are variable (13, 14). Therefore, new biomarkers are required to improve the treatment decision-making and identify potential responders from ICIs therapy.

Tumor mutation burden (TMB), which was defined as the number of all non-synonymous somatic mutations per megabase based on the genome examined, has been reported to predict the efficacy of ICIs therapy in multiple tumor types (15–17). The more mutations, the more neoantigens are produced, which ultimately activate the stronger antitumor immune response (18). Based on the results from the phase 2 KEYNOTE-158 trial, pembrolizumab was approved by the FDA for patients with TMB ≥10 mut/Mb. Patients with a TMB ≥10 mut/Mb were defined as TMB-high and associated with better response rates (19). However, there were racial differences in TMB across multiple cancer types (20). Compared with European and American populations, the TMB is lower in East Asian populations. The cut-off of 10 mut/Mb may lead to fewer East Asian populations meeting eligibility for the treatment of ICIs. Therefore, the association of TMB cut-off in East Asian populations with ICIs treatment outcomes needs to be further investigated.

Currently, multiple platforms and sequencing methods have been used for next-generation sequencing (NGS) (21). However, the effect of different sequencing platforms and methods on TMB calculation has not been systematically evaluated. Compared with sequencing both tumor and matched normal specimens, tumor-only sequencing could reduce time and cost. In addition, many clinical tumor samples lack matching normal tissue, which requires the development of algorithms to calculate TMB for these samples. However, the current algorithms, including somatic-germline-zygosity (SGZ), were designed based on European and American populations. For the East Asian population, the corresponding algorithm is lacking (22–24). Therefore, an algorithm for TMB calculation from tumor-only sequencing samples in the East Asian population was urgently needed.

In this study, the effect of different sequencing platforms and methods on the calculation of TMB has been systematically evaluated. To calculate TMB for Asian patients with tumor-only sequencing, we optimized the SGZ algorithm and demonstrated the reliability of calculating TMB from tumor-only sequencing samples. Furthermore, the cut-off of TMB in Asian patients was determined and its efficacy in the treatment of ICIs has been investigated.

Materials and methods

Samples and datasets

Data for 4126 samples sequenced with different sequencing platforms and methods were obtained from a CAP-accredited laboratory (YuceBio Technology Co., Ltd, China) (Table S1). To investigate the racial differences in TMB value, 3680 genomic data of European and American populations were collected from cBioPortal (25–28).

Sixty-two samples with matched control were retrospectively obtained to analyze the correlation coefficient of TMB calculated from methods of tumor-only sequencing and paired normal-

tumor sequencing (Tables S2–S5). To determine the cut-off of TMB for predicting immunotherapy efficacy in the East Asian population, tumor samples of sixty-six lung patients treated with ICIs between July 2019 to September 2020 were retrospectively collected as a training cohort and sequenced without normal control (Table S6). Furthermore, genomic and clinical data of Sixty-nine East Asian NSCLC patients subjected to ICIs treatments were obtained as a test cohort to validate the cut-off of TMB (29). Durable clinical benefit (DCB) was defined as complete response, partial response, or stable disease (SD) that lasted for ≥ 24 weeks, and non-durable benefit (NDB) was defined as SD that lasted for< 24 weeks or progressive disease.

Next-generation sequencing (NGS) and mutation analysis

Genomic profiling was implemented on tumor tissues and matched peripheral blood samples. The GeneReadDNA FFPE kit (Qiagen) and Qiagen DNA blood mini kit (Qiagen) were used to extract DNA from tumor specimens and blood, respectively. For tumor-only sequencing, DNA from the tumor sample was extracted with a GeneReadDNA FFPE kit (Qiagen). DNA quantification was performed with the dsDNA HS Assay Kit (ThermoFisher Scientific, USA). For the platform of Illumina, sequencing libraries were built by SureSelect XT Human All Exon V6 (Agilent) for WES or a customized next-generation sequencing panel targeting exons of 1267 genes for panel sequencing, respectively. Sequencing procedures were utilized by the NextSeq 550AR platform with 150-bp paired-end reads. For the platform of MGI, sequencing libraries were built by Exome Plus Panel V1.0 (IDT, USA) for WES or a customized next-generation sequencing panel targeting exons of 1267 genes for panel sequencing, respectively. Sequencing procedures were utilized by the MGISEQ-T7 platform with 100-bp paired-end reads.

Sequencing reads with > 10% N rate and/or > 10% bases with a quality score of< 20 were filtered using SOAPnuke (Version 1.5.6) (30). Somatic single nucleotide variants and insertions and deletions (indels) were detected using VarScan (Version 2.4) (31). Next, Bcftools (1.14) was utilized to filter possible false-positive mutations with the parameter set as follow: "basicfilter = ""'(STRLEN(REF)>50 || STRLEN(ALT)>50) || INFO/STATUS! ~"Somatic"""" hotspotfilter = """"INFO/HOTSPOT!="." && ((INFO/SOR!=0 && INFO/SOR<3) || INFO/VD<5 || INFO/AF<0.007 || INFO/SSF>0.05)'""" fpdbfilter = """'INFO/HOTSPOT="." && ((INFO/FPDB!="0" && INFO/FPDB!=".") || (INFO/GERMLINE!="0" && INFO/GERMLINE!="."))""" normalfilter = """'INFO/HOTSPOT="." && ((INFO/GERMLINE)!="." || (FORMAT/PMEAN [0]<20)||((INFO/SOR!=0 && INFO/SOR<5) || INFO/AF<0.02 || INFO/SSF>0.01)||(INFO/AF<0.05 && FORMAT/MQ[0]<50)||(FORMAT/MQ[0]<30)||(INFO/AF<0.05 && FORMAT/QUAL[0]<30) || ((INFO/MSI>10||(INFO/MSILEN>1 && INFO/MSI>4)) && INFO/AF<0.3)||(type!="snp" && INFO/MSI>3 && ((INFO/MSILEN=(strlen(REF)-1))||(INFO/MSILEN=(strlen(ALT[0])-1))) && INFO/AF<0.1) || (FORMAT/NM[0]>2 && FORMAT/MQ[0]<60 && INFO/AF<0.2) || (FORMAT/NM[0]>3 && (FORMAT/MQ[0]<55||FORMAT/NM[1]>3)) || (FORMAT/DP[0]<30 || FORMAT/DP[1]<30)|| INFO/VD<10 || (FORMAT/BIAS[0:0]="2" && FORMAT/BIAS[0:1]="1") || (FORMAT/SBF[0]< 0.05 && FORMAT/VD[0]<50) || ((INFO/SOR!=0 && INFO/SOR<10) && FORMAT/MQ[0]<60))' """" (32). Finally, SnpEff (Version 4.3) was used to functionally annotate the mutations detected in the tumor samples (33).

TMB was determined as the number of all nonsynonymous mutations and indels per megabase of the genome examined.

Tumor purity estimation

To estimate the tumor purity by hematoxylin-eosin (HE) staining, the sample was fixed in the 10% formalin solution, embedded in paraffin. Then the 5 μm slide was stained with HE. The tumor purity is the value of tumor cells divided by all cells. To estimate the tumor purity by NGS, the sequencing reads were quality controlled using SOAPnuke (Version 1.5.6) (30), then aligned to the reference genome using BWA (v0.7.12). The tumor purity was estimated by Ascatngs (v3.1.0) (34).

SGZ optimization and mutation analysis

Mutations from tumor-only sequencing samples were identified by the somatic-germline-zygosity (SGZ) algorithm. For each sample, massively parallel sequencing (MPS) variant analysis was executed to create a genome-wide copy number profile, which is segmented and modeled to estimate the ploidy (Ψ) and overall tumor purity (p), as well as per segment copy number (C) and minor allele count (M). The log-ratio of variants was defined by the following formula:

$$Logratio = log_2\left(\frac{P*C+2*(1-P)}{P*\Psi+2*(1-P)}\right)$$

For each variant, the error log ratio was obtained by calculating the absolute value of the difference in log ratio between variant and segments. Finally, the germline variant or somatic variant was identified mainly by frequency, purity and error log ratio. However, the cut-off value of the above parameter was fit to European and American populations, which resulted in a high false-positive rate in the East Asian population (23). In order to calculate mutation from tumor-only sequencing in the East Asian population, the SGZ algorithm was optimized as followed: (1) generating a mutation background library based on the East Asian population, (2) analyzing mutations with SGZ, (3)

filtering out variants that appear more than 5 times in background library, while variants with a frequency higher than 0.9 were retained.

Statistical methods

All statistical analyses were implemented in Python (3.10.1). An independent *t*-test was used to compare TMB values between different groups. Correlation analysis was performed using the Pearson correlation analysis. Roc-curve and Log-rank test analyses were conducted to determine the cutoff of TMB. Categorical variables were evaluated with the Fisher-exact test. Kaplan-Meier curve, Log-rank test, and Cox regression were used to determine the significance of TMB on overall survival (OS) and Progression-Free-Survival (PFS). Statistical significance was set at p-value< 0.05.

Results

Effects of different sequencing platforms, sequencing methods and races on TMB values

To study the effect of different sequencing platforms and methods on TMB calculation, data of 4126 tumor samples sequenced with different platforms and methods were obtained. As shown in Figure 1A, no significant difference in TMB calculation from different sequencing platforms, including Illumina and MGI, was found. TMB from panel sequencing was higher than whole-exome sequencing (WES), however, there was no significant difference. To further verify the effect of different sequencing methods on TMB calculation, public sequencing data from 3680 samples performed with different methods were analyzed (Figure 1B). TMB values calculated from whole-genome sequencing (WGS) were significantly lower than those calculated from WES and panel sequencing. Furthermore, TMB in different races was analyzed. As shown in Figure 1C, TMB values of the East Asian populations were significantly lower than that of European and American populations in both WES sequencing and panel sequencing. The similar tendency was found in lung cancer (Figure 1D).

TMB calculation was affected by the sequencing depth and tumor purity

To investigate the effect of sequencing depth on TMB calculation, TMB calculated from lung cancer at different panel sequencing depths were analyzed. As shown in Figure 2A, the TMB calculated from sequencing depths ≥ 500 was significantly lower than that calculated from sequencing depths< 500. To determine the effect of tumor purities on TMB calculation, the distribution of TMB values with different tumor purities was analyzed. As shown in Figure 2B, the TMB values

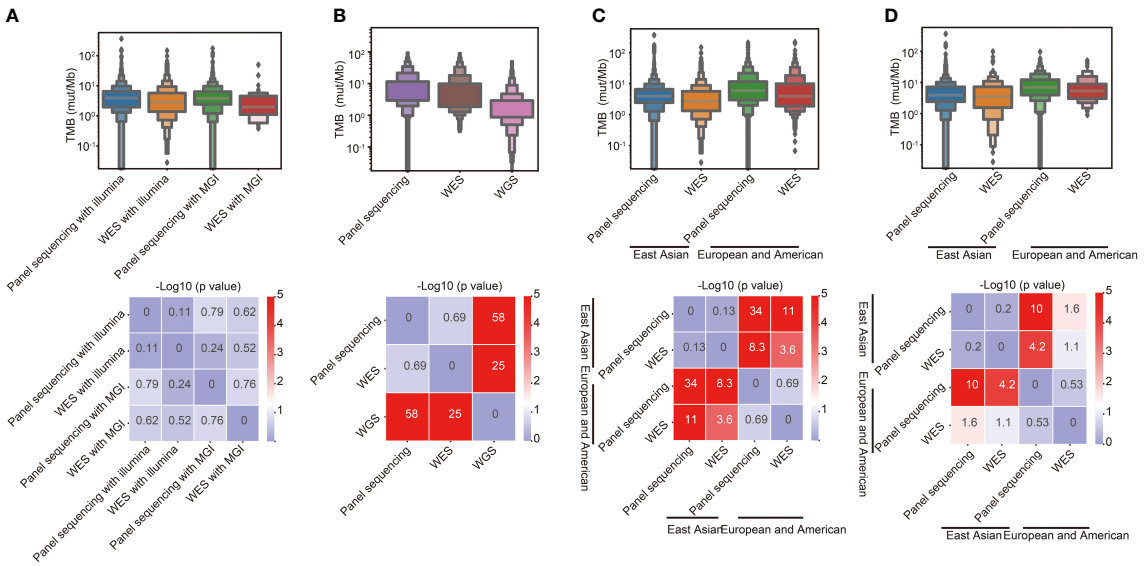

FIGURE 1
Effects of different sequencing platforms, sequencing methods and races on TMB. **(A)** Comparison of TMB between different sequencing platforms and methods in East Asian populations. **(B)** Comparison of TMB between different sequencing methods in European and American populations. **(C)** Comparison of TMB between different racial groups. **(D)** Comparison of TMB between different racial groups in lung cancer.

were higher than others for NGS purity between 0.0-0.1. As the NGS purity increased, the TMB values also tended to increase. However, the values of TMB were more stable with tumor purity ≥ 0.4. Compared with NGS tumor purity, The TMB calculation was less affected by the HE purity. There was no significantly difference with HE purity between 0.1-1.0 (Figure 2C).

High correlation of TMB calculated with the optimized SGZ algorithm and paired normal-tumor sequencing in East Asian populations

TMB calculation requires paired normal samples to remove germline mutations, which increases the cost of sequencing. In addition, in terms of clinical accessibility, paired normal samples are sometimes unavailable, which limits the clinical application of TMB. The SGZ algorithm for TMB calculation with tumor-only sequencing samples was designed based on European and American populations. To evaluate the accuracy of the SGZ algorithm for TMB calculation in East Asian populations, tumor tissues and matched peripheral blood samples from 62 patients including 43 lung cancer were collected and performed with targeted panel sequencing. The mean depths of tumor tissues and matched peripheral blood samples were 1027× and 455×, respectively (Table S2). As shown in Figure 3A, the TMB calculated by the SGZ algorithm had a low correlation with the TMB calculated by the method of paired normal-tumor sequencing in the East Asian populations. In order to calculate TMB from tumor-only sequencing samples in Asian populations, the SGZ algorithm was optimized. As shown in Figure 3B, we added the mutation filtering step, and constructed a background library with normal samples from East Asian patients to filter germline mutations, which ultimately reduced the false positives of TMB. To verify the accuracy of TMB calculation with an optimized algorithm, TMB calculated from the methods of the optimized algorithm and paired normal-tumor sequencing in sixty-two samples were compared. As shown in Figures 3C, D, their correlation coefficient is 0.95 and 82.7% of the mutations identified from the method of paired normal-tumor sequencing could be identified with the optimized SZG algorithm. These results demonstrate the accuracy of TMB calculation from the tumor-only sequencing with the optimized SGZ algorithm in East Asian populations.

Identification of the TMB cut-off for predicting immunotherapy efficacy in the training cohort

The cut-off of TMB for predicting the efficacy of immunotherapy in European and American populations is 10 mut/Mb (19). However, since the TMB of East Asian populations is lower than that of European and American populations, a TMB cut-off of 10 mut/Mb may not be suitable for East Asian populations. To determine the cut-off of TMB for predicting immunotherapy efficacy in the East Asian population, tumor samples from sixty-six lung patients treated with ICIs were retrospectively collected as a training cohort. TMB was calculated with the optimized SGZ algorithm. The performance of TMB for predicting patient durable clinical benefit was analyzed with a ROC curve and Log-rank test. As shown in Figure 4A, the optimal cut-off of TMB was 7 mut/Mb with AUC = 0.74, and validated with Log-rank analysis (Figure 4B). Furthermore, the response rate and survival period were higher in patients with TMB ≥ 7 mut/Mb than in those with TMB< 7 mut/Mb, and the TMB cut-off of 7 mut/Mb is better than the TMB cut-off of 10 mut/Mb in East Asian populations (Figures 4C–F). To further investigate the role of TMB in predicting the efficacy of immunotherapy, the effects of TMB, medication type, tumor type and age group on patient survival were analyzed through multi-factor cox-regression. It was found that TMB was a favorable factor for patient survival, while other factors had no significant effect (Figure 4G).

Validation of TMB cut-off in the test cohort

To further validate the TMB cut-off of 7 mut/Mb, genomic and clinical data of sixty-nine East Asian NSCLC patients treated with ICIs were collected (29). Consistent with the above results, the survival period of patients with TMB ≥ 7 mut/Mb was longer than those with TMB< 7 mut/Mb (Figure 5A). Furthermore, the predicting efficacy with the TMB cut-off of mut/Mb is better than the TMB cut-off of 10 mut/Mb (Figure 5B).

Discussion

Currently, different platforms and sequencing methods have been used to calculate TMB (21). However, the effect of different platforms and sequencing methods on the calculation of TMB is unclear. For the East Asian population, there are no methods to accurately calculate TMB from tumor-only sequencing samples and the optimal cut-off of TMB for predicting response to ICIs treatment is lacking. In this study, we have demonstrated that TMB calculation was not affected by different platforms, but was affected by different sequencing methods. Calculated TMB were more accurate and stable with sequencing depths ≥ 500, NGS purity ≥ 0.4 or HE purity between 0.1-1.0. After optimizing the SGZ algorithm, the correlation coefficient between TMB calculated from tumor-only sequencing samples and paired sequencing samples is 0.95. Through ROC curve and Log-rank test analysis, the cut-off for TMB was determined to be 7 mut/Mb in the training cohort. The TMB cut-off of 7 mut/Mb can

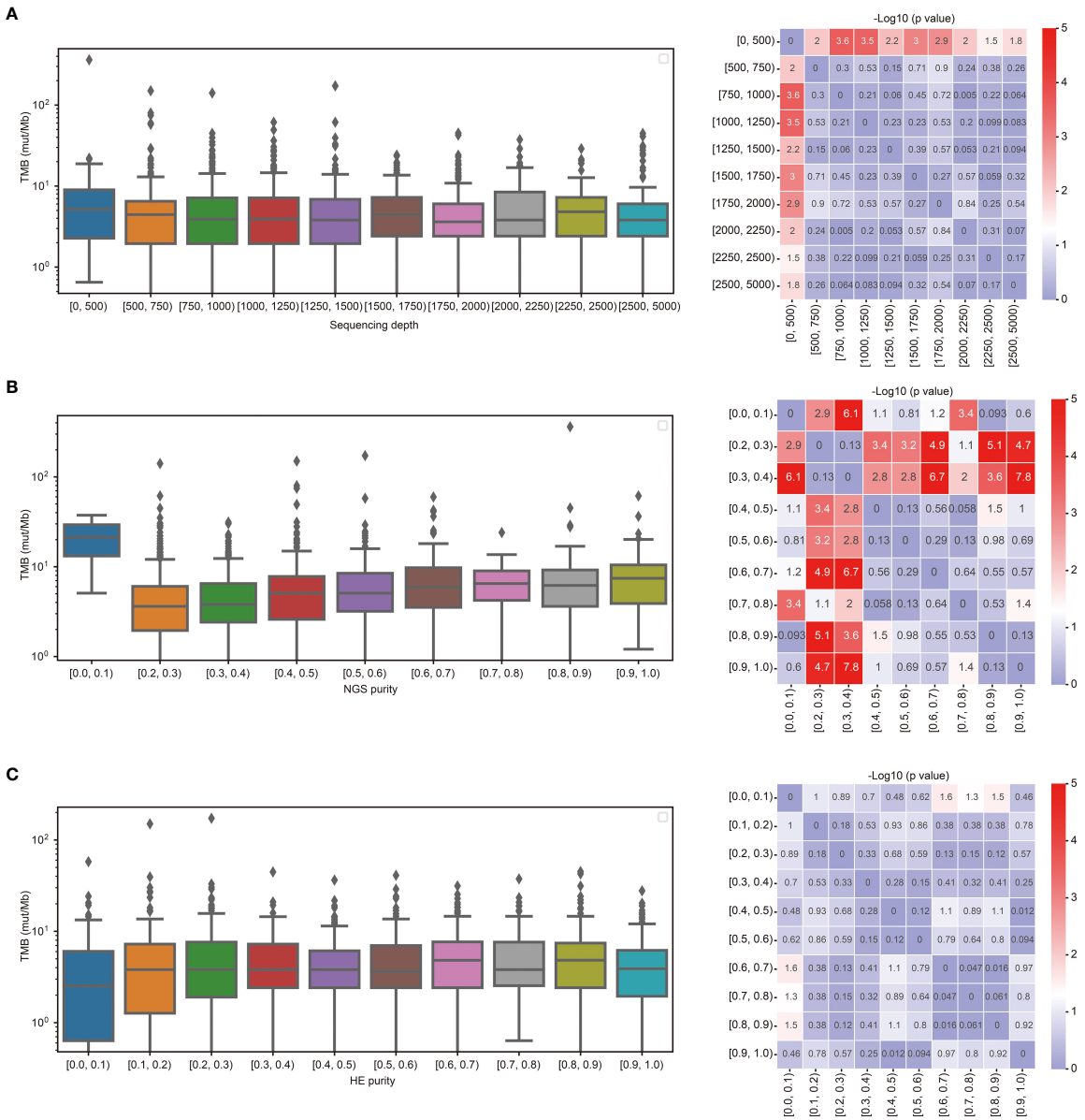

FIGURE 2
TMB calculation was affected by the sequencing depth and tumor purity. **(A)** Comparison of TMB between different sequencing depths. **(B)** Comparison of TMB between different purities estimated by NGS. **(C)** Comparison of TMB between different purities estimated by HE staining.

better distinguish responders from non-responders than the TMB cut-off of 10 mut/Mb. Patients with TMB ≥ 7 mut/Mb experienced a higher response rate and survival period than those with TMB< 7 mut/Mb. Furthermore, genomic and clinical data of sixty-nine East Asian NSCLC patients treated with ICIs were applied to validate the TMB cut-off of 7 mut/Mb, and the same results were obtained.

Multiple studies have demonstrated that TMB is a predictive biomarker for immunotherapy in several types of cancers (15–17). However, consensus on how to measure TMB has not been reached. WES was considered the gold standard for TMB calculation. Compared with WES, panel sequencing has a shorter turnaround time and lower cost, thus increasing its clinical accessibility. However, whether the TMB calculated from panel sequencing could represent the TMB calculated from WES was unclear. Previous studies have shown a high concordance rate (R2 = 0.887) between TMB calculated from panel sequencing and WES, however, the samples measured were limited (35). In our study, no significant difference between TMB calculated from panel sequencing and WES was found.

When sequencing depth increases, mutations with low variant allele frequency (VAF) will be identified, which

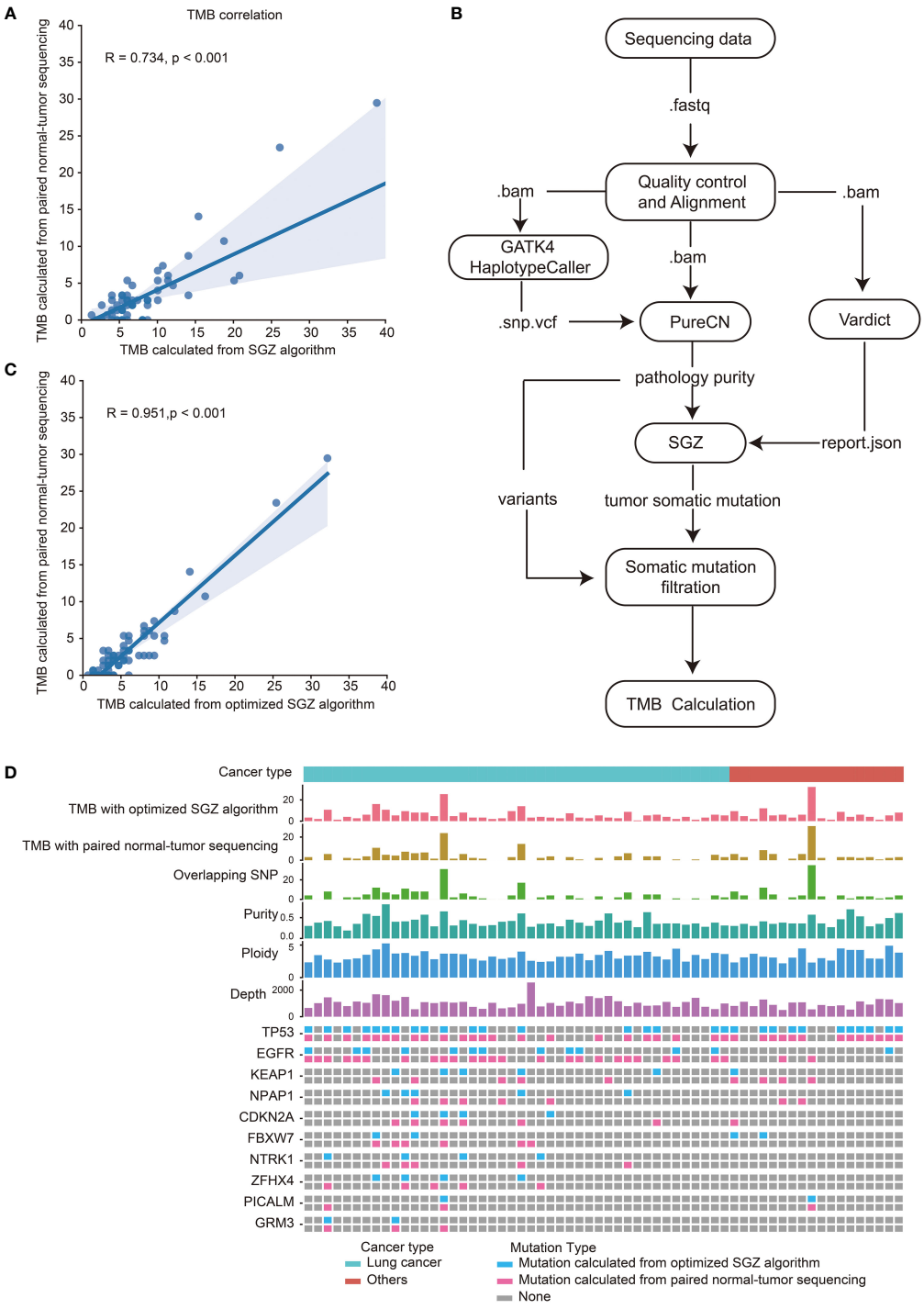

FIGURE 3
High correlation of TMB calculated with optimized SGZ algorithm and paired normal-tumor sequencing in East Asian populations. **(A)** Correlation of TMB calculated with the SGZ algorithm and the method of paired normal-tumor sequencing. **(B)** The process of TMB calculation from tumor-only sequencing samples with the optimized SGZ algorithm. **(C)** Correlation of TMB calculated with optimized SGZ algorithm and the method of paired normal-tumor sequencing. **(D)** The mutation landscape of sixty-two patients calculated with methods of optimized SGZ algorithm and paired normal-tumor sequencing. The top three histograms are the values of TMB calculated with the optimized SGZ algorithm, TMB calculated with the method of paired normal-tumor sequencing and overlapping SNPs calculated by both methods. Center three histograms are purity, ploidy and sequencing depth of samples. The mutation spectrum of each patient is shown under the value of sequencing depth. The upper row is the mutation detected by the method of paired normal-tumor sequencing, and the lower row is the mutation detected by the method of the optimized SGZ algorithm.

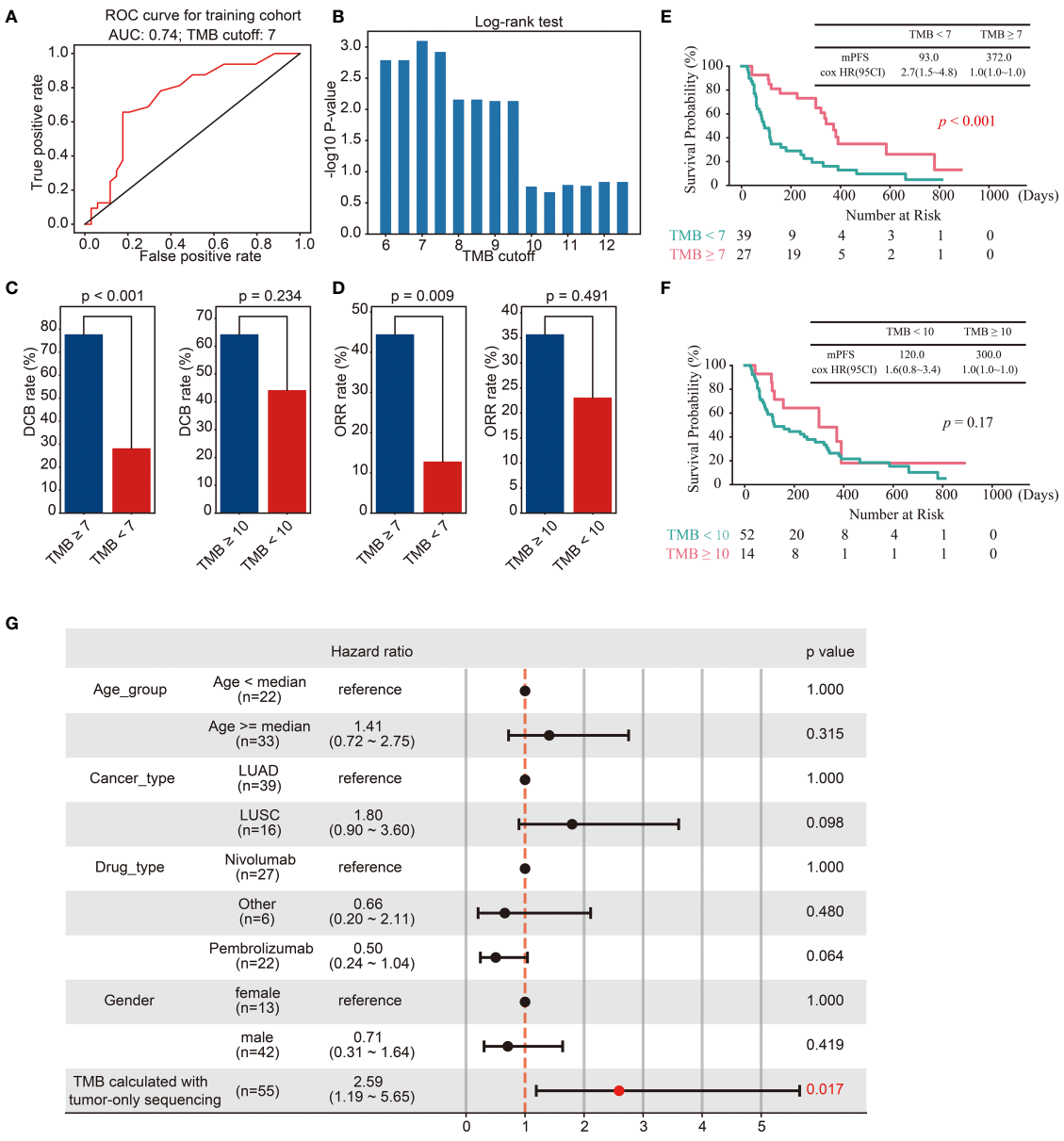

FIGURE 4
Identification of the cut-off of TMB for predicting immunotherapy efficacy in the training cohort. **(A, B)** ROC curves **(A)** and Log-rank test **(B)** for the identification of the TMB cut-off. **(C, D)** Barplots of DCB rate **(C)** and ORR rate **(D)** between different groups of TMB cut off 7 and 10. **(E)** Kaplan–Meier curves of OS comparing TMB ≥ 7 group and TMB< 7 group. **(F)** Kaplan–Meier curves of OS comparing TMB ≥ 10 group and TMB< 10 group. **(G)** The multivariate Cox regression analyses of the TMB, gender, age, drug, and cancer type.

suggests that sequencing depth may have an impact on TMB calculation. A previous study has reported that multiple mutations were missed when the sequencing depth was between 100× to 200× (35). In our study, TMB calculated from WGS was significantly lower than those calculated from panel sequencing and WES, suggesting that the value of TMB would be affected by sequencing depth. Therefore, the effect of different sequencing depths on TMB calculation was systematically analyzed. It was found the calculated TMB was more stable with sequencing depths ≥ 500.

Due to the costs of sequencing and lack of matched normal samples, many clinical samples are tumor-only sequenced. At present, several algorithms were developed to calculate TMB from tumor-only sequencing samples (22–24). However, these algorithms were developed based on European and American populations, and are not suitable for East Asian people. In this

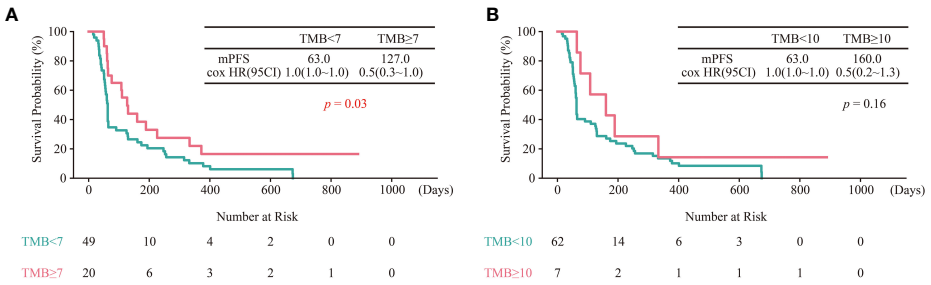

FIGURE 5
Validation of TMB cut-off of 7 mut/Mb in the test cohort. **(A)** Kaplan–Meier curves of OS comparing TMB ≥ 7 group and TMB< 7 group. **(B)** Kaplan–Meier curves of OS comparing TMB ≥ 10 group and TMB< 10 group.

study, the SGZ algorithm was optimized and a high concordance rate between TMB calculated from the methods of optimized SGZ algorithm and paired normal-tumor sequencing was found.

A previous study has investigated the racial differences in TMB and found that TMB cutoffs less than 10 mut/Mb may be more suitable for predicting response to ICIs in Asian populations (20). However, the optimal cut-off of TMB for predicting the efficacy of ICIs in the East Asian population is currently unclear. In this study, the TMB cut-off of 7 mut/Mb was identified in the East Asian population through the ROC curve and Log-rank analysis, which is less than 10 mut/Mb. Furthermore, this cut-off value was validated in another independent cohort.

There were several limitations in the study. First, the sample size used to correlate TMB calculated from tumor-only sequencing and paired sequencing was not very large, and further studies are needed to validate our optimized SGZ algorithm. Second, due to there was no other cohort that contained sufficient genomic and clinical data for patients with lung cancer in East Asian populations receiving ICIs, more researches are needed to further validate the cut-off value of TMB.

In summary, we have systematically evaluated the effect of different sequencing platforms and methods on the calculation of TMB, and optimized the SGZ algorithm. Furthermore, the cut-off of TMB to predict the efficacy of ICIs in the East Asian population has been identified and validated in another independent cohort. Ongoing intense work is needed to further validate and optimize the cut-off of TMB in the East Asian population who are treated with ICIs.

Data availability statement

The datasets presented in this study can be found in online repositories via the following link: https://ngdc.cncb.ac.cn/gsa-human/browse/HRA003220.

Ethics statement

The study involving human participants was reviewed and approved by YuceBio Ethics Committee (2021-003-04). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception and design: DS and HL. Acquisition of Data: DS, MX, and CP. Analysis and interpretation of data: MX, CP, HT, PW, and DW. Wrote the manuscript: MX, CP, and HT. Revised the manuscript: DS and HL. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

Authors CP, HT, PW, DW, and HL were employed by the company YuceBio Technology Co.

The remaining authors declare that the research was conducted in the absence of any commercial or financial

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Supplementary material

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PD-1 inhibitor versus bevacizumab in combination with platinum-based chemotherapy for first-line treatment of advanced lung adenocarcinoma: A retrospective-real world study

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Background: Chemotherapy combined with immunotherapy or anti-vascular therapy is both recommended by guidelines for first-line treatment of lung adenocarcinoma. However, no head-to-head clinical trial has ever compared which strategy is the optimal choice. This real-world retrospective study was done to compare the efficacy and treatment-related adverse events of immunotherapy and bevacizumab in combination with chemotherapy.

Patients and methods: From January 2018 to March 2021, we retrospectively collected 276 patients with advanced lung adenocarcinoma managed with chemotherapy combined with bevacizumab or PD-1 inhibitors at our center. Among them, 139 patients were treated with chemotherapy combined with bevacizumab, while 137 patients were treated with chemotherapy combined with PD-1 inhibitors. After receiving four cycles of combination therapy, all patients received maintenance therapy until disease progression. Progression-free survival (PFS), overall response rate (ORR), overall survival (OS), disease control rate (DCR), and adverse events (AE) were analyzed between the two groups.

Results: Compared to patients who received anti-vascular therapy, patients who underwent immunotherapy achieved better PFS (7.3 months vs. 10 months, p = 0.002) while ORR (40.9% vs. 51.1%, p = 0.093), as well as OS (18 months vs. 24 months, p = 0.060), had no statistical difference between the two groups. In the PD-L1-negative population, there was no statistical

difference in PFS and OS between the two groups. (8.0 months VS. 6.0 months, p = 0.738; and 19 months vs. 13 months, p = 0.274). In the PD-L1-positive population, there was a significant benefit in PFS in the population receiving immunotherapy (7.0 months vs. 10.0 months, p = 0.009). Proteinuria and hypertension occurred more frequently in the bevacizumab-treated group (p = 0.001 and p = 0.002), whereas immune-related pneumonia and hypothyroidism occurred more frequently in the immunotherapy-treated group (p = 0.007 and p = 0.030).

Conclusions: The addition of a PD-1 inhibitor was superior to bevacizumab in terms of PFS among patients with advanced lung adenocarcinoma. PD-L1-positive patients appeared to exhibit better PFS, OS, and ORR. Toxic reactions were manageable in both groups.

KEYWORDS

bevacizumab, chemotherapy, immune checkpoint inhibitors, NSCLC, over-all survival

Introduction

Lung cancer is the highest incidence of cancers in men and the leading cause of cancer-related deaths worldwide (1, 2). The treatment landscape for advanced, unresectable, and/or metastatic non-small cell lung cancer (NSCLC) is evolving. The standard of care for patients with driver mutation-negative metastatic lung adenocarcinoma included the combination of platinum-doublet chemotherapy with bevacizumab or immune checkpoint inhibitors (ICIs) (3). Bevacizumab exerts an effective antitumor effect by targeting and inhibiting human vascular endothelial growth factor, promoting the normalization of tumor vessels, and reducing the formation of new blood vessels. The combination of bevacizumab plus chemotherapy (B + C) is a formally approved intervention in unselected patients except those with treatment-related contraindications (4). The anti-angiogenic therapy has greatly improved and to a certain extent, prolonged the survival time of patients and improved their quality of life (5–8).

The monotherapy of ICIs (anti-programmed death 1 PD-1) has been shown to provide an overall survival benefit for selected NSCLC patients who have programmed death ligand 1 (PD-L1) expression on at least 50% of tumor cells (9, 10). The combination of chemotherapy and ICIs (I + C) improves survival regardless of PD-L1 status and results in a higher ORR than monotherapy (11, 12). Although integrating immunotherapy into a treatment plan for NSCLC improved survival and quality of life for some patients, predictive biomarkers for ICIs are still under investigation. What is certain is that some oncogenetic alterations in tumors, such as EGFR or ALK, show poor response to ICI treatment and are associated with an increased occurrence of toxic effects (13, 14). Therefore, the initial treatment for NSCLC patients with EGFR or ALK genetic alterations should be target therapy. The ICI agents (PD-1/PD-L1 inhibitors) are recommended by the NCCN guidelines for first-line treatment of driver mutation-negative advanced NSCLC (15–17).

However, it is inconclusive whether chemotherapy combined with bevacizumab (B + C) or chemotherapy combined with immunotherapy (I + C) is optimal for patients with negative driver mutations in lung adenocarcinoma because of a lack of head-to-head trials. In the IMpower150 study, bevacizumab in combination with chemotherapy showed significant efficacy, and the overall survival benefit was not significantly inferior to atezolizumab plus chemotherapy but was significantly inferior to the addition of atezolizumab to bevacizumab and chemotherapy (ABC) (18). Moreover, in a number of network meta-analyses, B + C can be an optimal strategy as an initial first-line treatment for PD-1 positive advanced non-squamous NSCLC, while there is no detailed disadvantage compared with pembrolizumab treatment (19).

This retrospective cohort study aims to explore the efficacy and safety of chemotherapy combined with either bevacizumab or immunotherapy for first-line treatment of lung adenocarcinoma in a real-world setting to fill the gap in this regard.

Patients and methods

Patients

We retrospectively analyzed 2,522 treatment-naïve patients who were diagnosed with advanced lung adenocarcinoma from January 2018 to January 2022 at the Hunan Cancer Hospital. Patients with EGFR mutations, ALK fusions, or ROS-1 fusions were excluded. A total of 276 patients who were eligible for

inclusion and received chemotherapy combined with bevacizumab or ICI were analyzed (Figure S1). All patients were ≥18 years old and histologically diagnosed with lung adenocarcinoma with stages III–IV. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2. The characteristics of the patients, including sex, age, smoking history, brain metastasis, liver metastasis, bone metastasis, and gene mutation status, are summarized in Table 1. For the classification of concomitant gene mutations, we referred to the results of the BENEFIT study by Jie et al. (20). All procedures in our study were performed in accordance with the ethical standards of the institutional and national research committees and 2013 revised Declaration of Helsinki. It was approved by the Ethics Committee of Hunan Cancer Hospital (approval number: 2017YYQ-SSB-026).

Treatment

For this retrospective study, all patients who received induction treatment were administered on day 1 of each 21-day period: the regimen of cisplatin (75 mg/m^2)/carboplatin (area under the curve, AUC 6), pemetrexed (500 mg/m^2) and (7.5 or 15 mg/kg) bevacizumab (B + C) or cisplatin/carboplatin plus pemetrexed and PD-1 inhibitors (I + C). The prescription of PD-1 inhibitors in this study included pembrolizumab (n = 65) and sintilimab (n = 74), with a fixed dose of 200 mg. Induction chemotherapy was repeated every 3 weeks for a maximum of four cycles. After completion of at least three cycles of induction chemotherapy, patients received maintenance chemotherapy on day 1 of the 21-day cycle comprising pemetrexed with either bevacizumab or ICIs until the occurrence of unmanageable toxic effects or disease progression.

Assessment

Chemotherapy response was evaluated after every two treatment cycles by computed tomography (CT). They were evaluated as complete response (CR), partial response (PR), stable disease (SD), and progression disease (PD) according to the Response Evaluation Criteria in Solid Tumor Criteria 1.1.9. The objective remission rate (ORR) was defined as the sum of

TABLE 1 Characteristics of patients in this study.

	Chemo + BEV (n = 137)	Chemo + ICIs (n = 139)	p-value
Age [median (range), year)]	60 (37–74)	59 (33-79)	0.109
Gender			0.657
Male	107 (78.1)	112 (80.6)	
Female	30 (21.9)	27 (19.4)	
Smoking			0.692
Non-smoker	42 (30.7)	39 (28.1)	
Former smoker	95 (69.3)	100 (71.9)	
ECOG PS			1.000
Low (0–1)	133 (97.1)	134 (96.4)	
High (2–3)	4 (2.9)	5 (3.6)	
Stage			0.521
IIIB–C	13 (9.5)	10 (7.2)	
IV	124 (90.5)	129 (92.8)	
Brain metastasis at baseline			0.514
With	24 (17.5)	20 (14.4)	
Without	113 (82.5)	119 (85.6)	
Liver metastasis at baseline			0.174
With	24 (17.5)	16 (11.5)	
Without	113 (82.5)	123 (88.5)	
Bone metastasis at baseline			0.549
With	61 (44.5)	67 (48.2)	
Without	76 (55.5)	72 (51.8)	
Gene mutation			0.282
None	66 (48.2)	54 (38.8)	
Multi-drive mutation	57 (41.6)	66 (47.5)	
Tumor-suppress mutation	14 (10.2)	19 (13.7)	

CR and PR. The disease control rate (DCR) was defined as the sum of CR, PR, and SD. Toxicities were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0. The primary endpoints were PFS and ORR. Secondary endpoints were overall survival (OS), DCR, and adverse effects (AEs).

Statistics analysis

Descriptive summaries were created for demographic and clinical variables. The chi-squared test was used to compare subset variables and toxicities. All *p*-values were two-tailed. Kaplan–Meier curves were generated for progression-free survival and overall survival. Log-rank tests were used to compare the survival between groups. All statistical analyses were performed using the SPSS 26.0 software for Windows (SPSS Corp., Armonk, NY, USA); $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics

A retrospective analysis was performed on 276 lung adenocarcinoma patients who had received first-line treatment. A total of 137 patients received chemotherapy combined with bevacizumab, and 139 patients received chemotherapy with PD-1 inhibitors. All the patients were without driver mutations. The characteristics of the patients, including sex, age, smoking history, brain metastasis, liver metastasis, bone metastasis, and gene mutation status, are summarized in Table 1. There were no significant differences in the baseline characteristics. According to the TNM classification for NSCLC patients (AJCC 7th). All patients had locally advanced or advanced lung adenocarcinoma.

Clinical efficacy

Patients who received B + C achieved an mPFS of 7.3 months, while patients who received I + C achieved an mPFS of 10.0 months. The I + C group's progression-free survival was longer (HR = 0.62, 95% CI: 0.47–0.80, $p = 0.002$, Figure 1A). The mOS was 18.0 months in the B + C group and 24.0 months in the I + C group. There was a prolonged OS observed in patients in the I + C group, although the difference was not statistically significant (HR = 0.75, 95% CI: 0.55–1.01, $p = 0.060$, Figure 1B).

The treatment responses are listed in Table 2. There was no patient who achieved CR in the whole population. Of the 137 patients in the treatment of the B + C group, 56 (40.9%) achieved PR, 68 (49.6%) achieved SD, and six (9.3%) showed PD, resulting in an ORR of 40.9% and a DCR of 90.5%. Of the 139 patients in the treatment of the I + C group, 71 (51.1%) achieved PR, 52 (37.4%) achieved SD, and 16 (11.5%) showed PD, resulting in an ORR of 51.1% and a DCR of 88.5%. There is no significant difference in ORR (p = 0.093) and DCR (p = 0.695) between the two groups.

Considering that tumor PD-L1 expression is an important biomarker for immunotherapy, we further analyzed the relationship between PD-L1 expression and prognosis in the population. In the B + C group, only 36 patients were tested for PD-L1, including 15 PD-L1-negative patients, 15 low-expressing patients, and six high-expressing patients. In the I + C group, 91 patients' PD-L1 expression status was available, including 27 negative patients, 33 patients with low expression, and 31 patients with high expression (Figure 2A). Next, we further divided the population by PD-L1 expression level to analyze the treatment effect in different populations. We found that there was no statistical difference between PFS and OS in the two groups in the PD-L1-negative population (8.0 months vs. 6.0 months, p = 0.738; and 19 months vs. 13 months, p = 0.274) (Figures 2B, C). However, in the PD-L1-positive population, the I + C group achieved a significantly better PFS (7.0 months vs.

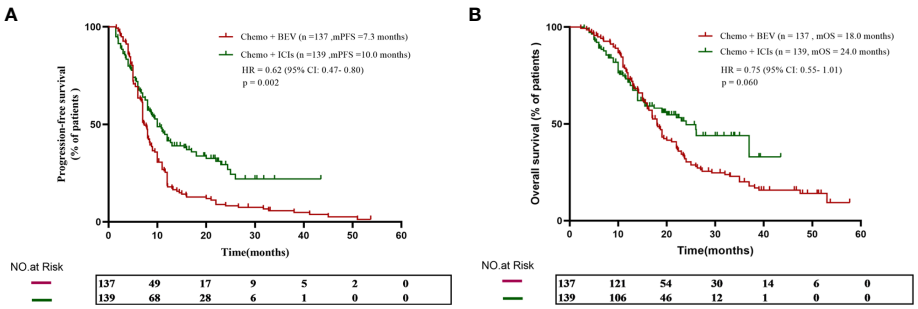


FIGURE 1
(A) The progression-free survival curve of patients who received chemotherapy plus either bevacizumab or ICIs. **(B)** The overall survival curve of patients who received chemotherapy plus either bevacizumab or ICIs.

TABLE 2 Treatment response.

	Chemo + BEV (n = 137)	Chemo + ICIs (n = 139)	p-value
CR	0	0	
PR	56 (40.9)	71 (51.1)	
SD	68 (49.6)	52 (37.4)	
PD	13 (9.5)	16 (11.5)	
ORR	56 (40.9)	71 (51.1)	0.093
DCR	124 (90.5)	123 (88.5)	0.695

CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; ICI, immune checkpoint inhibitor; BEV, bevacizumab.

10.0 months, p = 0.009) (Figure 2D). Although there was no statistical difference in OS between the two groups, there was still a sustained benefit for patients in the I + C group (19 months vs. 26 months, p = 0.170) (Figure 2E).

An exploratory subgroup analysis of survival time was conducted, which was based on patients' initial different characteristics. We found that in most patients, immunotherapy achieved better PFS. Consistent with previous results, OS was not

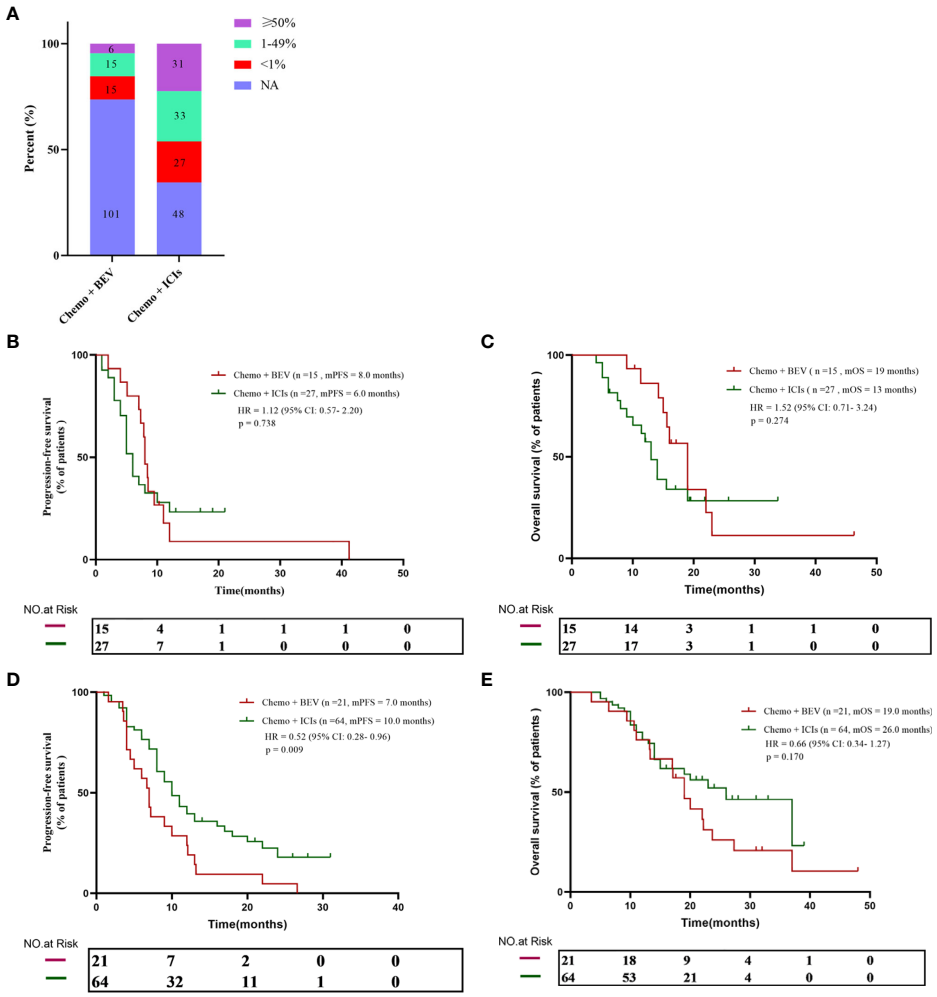


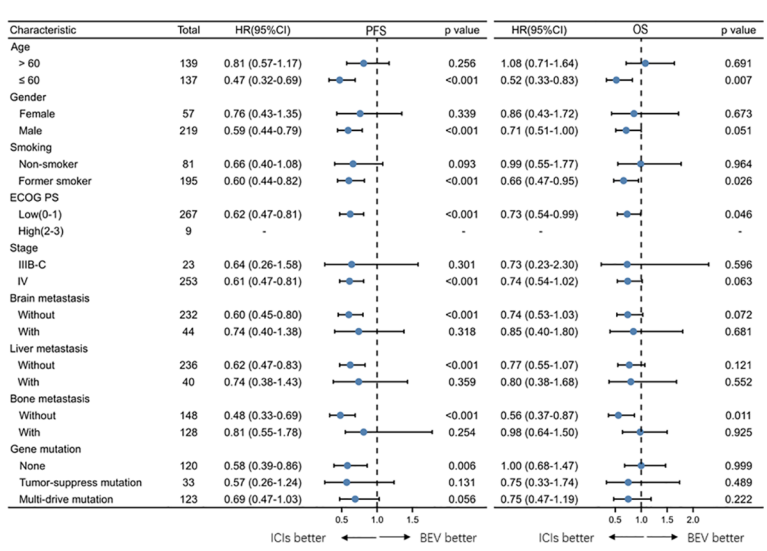
FIGURE 2
(A) PD-L1 expression in all patients. **(B, C)** The progression-free survival and overall survival curve of PD-L1 negative patients received chemotherapy plus either bevacizumab or ICIs. **(D, E)** The progression-free survival and overall survival curve of PD-L1 positive patients who received chemotherapy plus either bevacizumab or ICIs.

statistically different between the two treatment modes for most patients. Immunotherapy has a better OS in patients younger than 60 years old, with a PS score of 0–1, smoking, as well as in patients without initial brain metastases (Figure 3A). In univariate and multivariate Cox regression analyses of PFS, the addition of ICI was a protective factor, whereas in patients with initial brain metastases it was a poor prognostic factor (Figure 3B).

Toxicity

The most common grade I/II adverse events in the B + C group were leukopenia (n = 14, 10.2%) and liver injury (n = 14, 10.2%). In the I + C group, it was liver injury (transaminases increased) (n = 16, 11.5%). We found that these adverse events were mostly related to chemotherapy, resulting in no statistical difference between the two groups. Proteinuria occurred in 10 patients (7.3%) and hypertension in nine patients (6.6%) in the B + C group, which did not occur in the I + C group (p = 0.001 and p = 0.002) and was considered to be a bevacizumab-specific adverse event. In the immunotherapy group, immune pneumonitis occurred in eight patients (5.8%) and hypothyroidism in six patients (4.3%), which were not present in the bevacizumab treatment group, considering the unique adverse events of immunotherapy (P = 0.007 and p = 0.030). Similarly, there was no statistical difference in the incidence of

A



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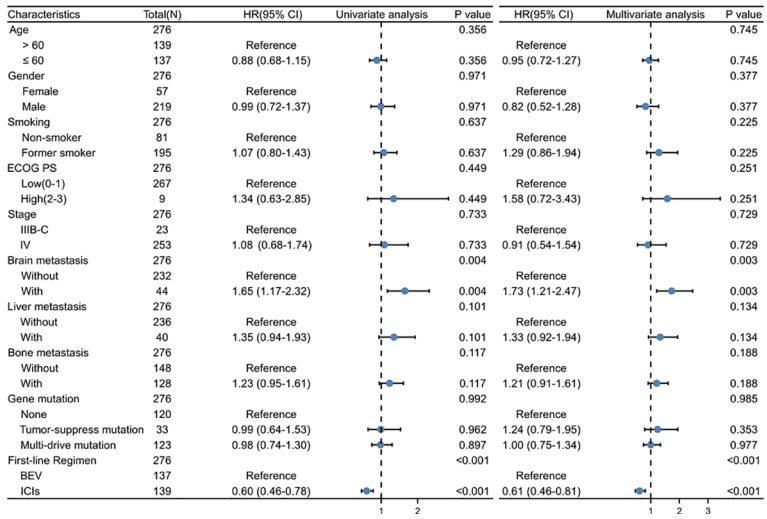

FIGURE 3
(A) Forest plots of hazard ratios for progression-free survival by subgroup for BEV + Chemotherapy and ICI + Chemotherapy group.
(B) Univariate and multivariate Cox regression analysis of clinical characteristics of all patients.

grade III/IV adverse events between the two groups (Table 3, Figure S2).

Discussion

Immunotherapy has become an important therapy for advanced cell lung cancer, and a variety of immune checkpoint inhibitors have been approved for the first-line treatment of lung cancer (11, 15, 16, 18, 21). Especially in patients with driver gene-negative non-squamous NSCLC, multiple clinical trials have confirmed that immunotherapy not only improves the disease response rate but also prolongs survival compared with chemotherapy, largely improving the treatment outcome of advanced lung cancer. Meanwhile, bevacizumab combined with platinum-based doublet chemotherapy is the recommended regimen for first-line treatment of non-squamous non-small cell lung cancer and prolongs the survival time of patients compared with chemotherapy (7, 22–25). There is no study comparing the efficacy of the addition of PD-1/L1 inhibitors or bevacizumab to chemotherapy, and the question of whether PD-L1 negative patients should receive B + C remains controversial. There is rapidly evolving evidence showing the data of different combination strategies. In the IMpower150 study, the overall survival of chemotherapy combined with bevacizumab was not significantly inferior to atezolizumab combined with chemotherapy (19 months vs. 15 months, p = 0.07) (18). In the final overall survival analysis of IMpower150, in the PD-L1-negative subgroups, no difference in OS was observed with each combination subgroup (26). With meta-analyses, we have demonstrated that in non-squamous NSCLC with PD-L1 ≥50%, B + C was similar to pembrolizumab alone in terms of PFS. With PD-L1 <50%, the ICIs plus chemotherapy performed only marginally better than B + C (19). As far as we know, there are few real-world studies for comparison of the first-line PD-1 inhibitor versus bevacizumab in combination with chemotherapy directly.

The population of our study was patients with advanced or locally advanced lung adenocarcinoma, and the pathological types

TABLE 3 Adverse events.

	Grades 1–2		p value	Grades 3–4		p value
	Chemo + BEV (n = 137)	Chemo + ICIs (n = 139)		Chemo + BEV (n = 137)	Chemo + ICIs (n = 139)	
Leukopenia	14 (10.2)	1 (0.7)	<0.001	7 (5.1)	2 (1.4)	0.102
Pneumonia	0	8 (5.8)	0.007	0	0	–
Transaminases increased	14 (10.2)	16 (11.5)	0.847	0	1 (0.7)	1.000
Enteritis	0	0	–	0	1 (0.7)	1.000
Fatigue	4 (2.9)	1 (0.7)	0.212	0	0	–
Appetite Decreased	3 (2.2)	2 (1.4)	0.683	0	0	–
Rash	0	1 (0.7)	1.000	0	1 (0.7)	1.000
Hypothyroidism	0	6 (4.3)	0.030	0	0	–
Vomiting	2 (1.5)	0	0.245	6 (1.4)	2 (1.4)	0.171
Myositis	0	1 (0.7)	1.000	0	0	–
Bilirubin increased	0	3 (2.2)	0.247	0	0	–
Hepatitis	0	1 (0.7)	1.000	0	1 (0.7)	1.000
Anemia	8 (5.8)	2 (1.4)	0.059	2 (1.5)	2 (1.4)	1.000
Thrombocytopenia	2 (1.5)	1 (0.7)	0.621	1 (0.7)	2 (1.4)	1.000
Hypopituitarism	0	1 (0.7)	1.000	0	0	–
Myocarditis	0	0	–	0	1 (0.7)	1.000
Proteinuria	10 (7.3)	0	0.001	0	0	–
Hemoptysis	2 (1.5)	0	0.245	0	0	–
Hypertension	9 (6.6)	0	0.002	3 (2.2)	0	0.121
Epistaxis	1 (0.7)	0	0.496	0	0	–
Insomnia	2 (1.5)	0	0.245	0	0	–
Thrombosis	1 (0.7)	0	0.496	0	0	–
Constipation	4 (2.9)	0	0.059	0	0	–
Hematochezia	1 (0.7)	0	0.496	1 (0.7)	0	0.496

were consistent. We observed a significant PFS benefit in IC, somewhat different from the results of the previous IMpower150 study. This may be relevant to our population of patients selected for lung adenocarcinoma alone and without driver mutations. In addition, the PD-1 inhibitor we used for immunotherapy may be somewhat different from atezolizumab, while the chemotherapeutic drugs pemetrexed and paclitaxel may also be somewhat different. In this real-world study, time-to-event outcomes for each group were consistent with most published data on similar treatment strategies in clinical trials (12, 27, 28). Although there was no statistically significant difference in OS between the two groups, the benefit of the immunotherapy group was evident, and the conclusion was consistent with the study results of IMpower150 (26). More interestingly, we found an association between the expression level of PD-L1 and treatment modalities. The ORR for Bev with PEM/CARBP (40.9%) in our study was higher than that of the POINTBREAK study (34%). This may have reduced the magnitude of the benefit of ICI+ chemo (29). Compared with PFS, treatment beyond first-line progression had an impact on the analysis of OS. More interestingly, we found an association between the expression level of PD-L1 and treatment modalities. In the PD-L1-negative population, there was no significant difference in PFS and OS between the two groups. In PD-L1-positive patients, PFS was beneficial in patients receiving immunotherapy. This result was consistent with Impower 150 analysis data (26). However, due to the limited number of patients receiving PD-L1 testing in the B + C group (36/137), we did not observe the OS benefit in the I + C group. Table S1 shows the results of comparing the use of chemo + ici *vs.* chemo + bev, which were cited from three meta-analyses and IMpower150.

In our study, there was no statistically significant difference in adverse events between groups. However, adverse events like those specific to bevacizumab, such as the occurrence of proteinuria, were not balanced between groups (p = 0.001). Similarly, such as rash, pneumonia, and enteritis, these phenomena were only observed in the ICI group. Fortunately, numerous treatment-related adverse events were controlled after certain management.

The limitations of our study include its retrospective nature and small sample size. A larger multi-center prospective study is needed to further confirm our findings. Moreover, among patients in the B + C group, the population for PD-L1 testing was too small, which affected the analysis results. In addition, OS was not reached in the immunotherapy arm due to the length of follow-up.

In conclusion, our study provides clinical evidence for the effectiveness of ICIs and bevacizumab in treating patients with advanced lung adenocarcinoma. In our study, ICI therapy resulted in a higher PFS, OS, and ORR. In PD-L1-negative patients, chemotherapy combined with bevacizumab was not inferior to immunotherapy, and in PD-L1-positive patients, immunotherapy was clearly superior. Toxicities were manageable in both groups.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Hunan Cancer Hospital (approval number: 2017YYQ-SSB-026). Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

(I) Conception and design: ZW and NY. (II) Administrative support: ZW, CZ, and NY. (III) Provision of study materials or patients: ZH, FY, and CZ. (IV) Collection and assembly of data: ZH, FY, YZ, YX, FZ, LL, WJ, and HY. (V) Data analysis and interpretation: ZW, ZH, and YZ. (VI) Manuscript writing and editing: ZW and ZH. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.909721/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Flow diagram of the study design. BEV: bevacizumab. ICIs: immune checkpoint inhibitors

SUPPLEMENTARY FIGURE 2
Comparison of Adverse Events in B+C group and I+ C group

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Prognostic and predictive value of YTHDF1 and YTHDF2 and their correlation with tumor-infiltrating immune cells in non-small cell carcinoma

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Background: YTH domain-containing family protein 1 (YTHDF1) or YTHDF2 play crucial roles in cancer immunotherapy. We examine the expression of YTHDF1, YTHDF2, CD8, CD4, and FOXP3 to identify their prognostic or predictive role for PD-1/PD-L1 inhibitor in non-small cell lung cancer (NSCLC).

Methods: Immunohistochemical expression of YTHDF1, YTHDF2, CD8, CD4, and FOXP3 was investigated in 266 patients not receiving PD-1/PD-L1 inhibitors and in 59 patients receiving PD-1/PD-L1 inhibitors. Immunohistochemical results were verified using mRNA dataset obtained from The Cancer Genome Atlas (TCGA) database.

Results: Immunohistochemical expression of YTHDF1 or YTHDF2 was negatively associated with CD8- and CD4-positive T cells; however, the same expression was positively associated with FOXP3-positive T cells. YTHDF1 or YTHDF2 mRNA expression was also negatively associated with CD8- and CD4-positive T cells. Gene set enrichment analysis revealed that low YTHDF1 was related to immune hot tumor gene sets. Expression of YTHDF1 or YTHDF2 was negatively associated with expression of most immune checkpoints. YTHDF1 and YTHDF2 were predictive markers of response to PD-1/PD-L1 inhibitors. YTHDF1 or YTHDF2 expression was associated with better prognosis. YTHDF1 has an immune hot profile in both cell types, whereas YTHDF2 is only seen in adenocarcinoma.

Conclusion: Low YTHDF1 or YTHDF2 reflects an immune hot tumor signature and may serve as a predictor or prognostic marker.

KEYWORDS

non-small cell lung cancer, YTHDF1, YTHDF2, CD8, CD4, FOXP3

Introduction

Anti-programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) drugs have been approved for treatment of patients with advanced non-small cell lung cancer (NSCLC) (1–3). The expression of PD-L1 by tumor cells has been focused on as the best marker of sensitivity to PD-1/PD-L1 inhibitors (4). However, durable response to anti-PD-1/PD-L1 inhibitor have also been reported in PD-L1-negative patients (5). Various predictors, including tumor mutational burden (6), tumor-infiltrating lymphocytes (7), and immune-related gene signatures (8), are also candidate biomarkers; however, these biomarkers have not been validated. Furthermore, in the clinic, the evaluation of tumor mutational burden or immune-related gene signatures is difficult because it requires expensive techniques, including next-generation sequencing or nanostring technology.

N6-methyl adenosine (m^6A), is responsible for post-transcriptional modification of mRNA in most eukaryotes (9). The m^6A pathway components play important roles in oncogene-mediated cell transformation (10), cell proliferation and tumorigenicity (11, 12), and tumor progression (13). The YTH domain-containing family protein 1 (YTHDF1), a component of the m^6A pathway, affects mRNA translation efficiency (14). Recently, Han et al. reported an important effect of YTHDF1 in the antitumor immunity (15). In melanoma and colon cancer models, YTHDF1 knockout mice showed favorable outcomes and increased CD8 positive T cells and NK cells (15). Furthermore, in a melanoma cancer model, the frequency of tumor regression to anti-PD-L1-treatment was increased in YTHDF1 knockout mice than in wild-type mice (15). YTHDF2 induces NSCLC growth by enhancing mRNA translation of 6-phosphogluconate dehydrogenase (16). YTHDF2 also promotes tumor proliferation by increasing CDKN1B mRNA degradation in intrahepatic cholangiocarcinoma (17). YTHDF2 expression was negatively associated with PD-L1 in esophageal cancer (18). Tsuchiya et al. revealed that YTHDF1 or YTHDF2 expression showed better clinical outcomes in NSCLC (19). Previous findings suggest that YTHDF1 or YTHDF2 may be a therapeutic target for cancer immunotherapy or a predictive biomarker predicting the response to anti-PD-1/PD-L1. However, there are no studies on the predictive role of YTHDF1 or YTHDF2 in NSCLC patients receiving PD-1/PD-L1 inhibitor. Although the role of YTHDF1 or YTHDF2 in the tumor immune microenvironment may differ depending on cell type, no such study has been performed.

Our study investigated the prognostic significance of YTHDF1 or YTHDF2 expression in a cohort of 266 patients who did not receive PD-1/PD-L1 inhibitor. We further investigated whether expression of YTHDF1 or YTHDF2 affected the response in a group of 59 patients treated with PD-1/PD-L1 inhibitor. Correlation analyses of YTHDF1, YTHDF2, and tumor infiltrating lymphocytes (CD4- and CD8-positive T cells and FOP3-positive T regulatory cells (Treg)) were performed on immunohistochemical and gene expression data. We also performed gene set enrichment analysis (GSEA) using The Cancer Genome Atlas (TCGA) to identify overexpressed gene classes based on YTHDF1 or YTHDF2 expression. In order to identify the tumor immune microenvironment associated with the expression of YTHDF1 or YTHDF2, the association between such expression and immune checkpoints other than PD-1/PD-L1 was investigated. All experiments were performed in two cell types (adenocarcinoma and squamous cell carcinoma).

Materials and methods

Study population and patient characteristics

Our study included a cohort of 266 patients not receiving PD-1/PD-L1 inhibitors and a group of 59 patients receiving PD-1/PD-L1 inhibitors. PD-1/PD-L1 inhibitor blockade was used in all patients from 2016 to 2022 and their drug responses were evaluated. The ethical approval was approved by the Institutional Review Board of Ajou University School of Medicine (AJIRB-BMR-KSP-19-416 and 2019-11-11). Complete response, partial response, or stable disease was defined as the responder group, and disease progression was defined as the non-responder group (20). Patient characteristics are summarized (Table 1). In the group not receiving PD-1/PD-L1 inhibitor treatment, 64% had adenocarcinoma and 29% had advanced stage. In the group treated with PD-1/PD-L1 inhibitor, 61% had adenocarcinoma and all were advanced stage. All patients receiving PD-1/PD-L1 inhibitor were previously refractory to chemotherapy, radiation therapy, or targeted agents. Twenty-five patients (42.4%) were responders

Immunohistochemistry of YTHDF1, YTHDF2, CD8, CD4 and FOXP3

Antibodies YTHDF1 (polyclonal, Proteintech), YTHDF2 (polyclonal, Proteintech), CD8 (clone C8/144B, DAKO), CD4 (clone SP35, Cell Marque), and FOXP3 (clone 236A/E7, Abcam) were used. The intensity of YTHDF1 or YTHDF2 staining was defined in four categories: 0, 1, 2, 3. The percentages of cytoplasmic or membranous expression were also evaluated. H-scores were applied to examine the YTHDF1 or YTHDF2 stains (21). For interpretation of CD4, CD8, or FOXP3 cells, membrane-positive CD4 or CD8 cells or nuclear-positive FOXP3 cells were measured at three locations at 400x magnification in the tumor area and averaged.

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TABLE 1 Demographic and clinical characteristics of patients.

Variable	Non-treatment group of PD-1/PD-L1 Inhibitors (n = 266)	Treatment group of PD-1/PD-L1 Inhibitors (n = 59)
Age, median (range) (years)	63 (31–86)	67 (32–81)
Male sex	188 (70.7%)	51 (86.4%)
Smoking history	167 (67.3%)	35 (83.3%)
Histologic subtype		
Adenocarcinoma	171 (64.3%)	36 (61%)
Squamous cell carcinoma	95 (35.7%)615	20 (33.9%)
Not otherwise specified	0 (0%)	3 (5.1%)
pTNM 8th edition		
Unclassified	7 (2.6%)	0 (0%)
Stage I	107 (40.2%)	0 (0%)
Stage II	74 (27.8%)	0 (0%)
Stage III	78 (29.3%)	18 (30.5%)
Stage IV	0 (0%)	41 (69.5%)
Type of PD-1 blockade		
Nivolumab	–	23 (39%)
Pembrolizumab	–	13 (22%)
Atezolizumab		23 (39%)
Response to PD-1 blockade		
Responder	–	25 (42.4%)
Non-responder	–	34 (57.6%)

Gene expression analysis

mRNA data of 1018 NSCLCs (517 lung adenocarcinoma and 501 squamous cell carcinoma) obtained from TCGA cBioportal were used. (http://cbioportal.org) (22).

GSEA is a method to analyze underlying biological processes using mRNA expression. We performed GSEA using GSEA version 4.0.3 (23). We analyzed data based on the median value of YTHDF1 or YTHDF2 expression. The Hallmark gene set was used as the gene set database. If $p < 0.05$ and false discovery rate (FDR) < 0.25, it was defined as statistically significant. Web-based Kaplan Meier plotter tool was used for survival analyses (24). Survival analysis was performed using mRNA data from 719 adenocarcinomas and 524 squamous cell carcinomas.

Statistical analyses

Correlation between quantitative variables was determined using Spearman's method. Logistic regression analysis was performed to identify predictive biomarker for anti-PD-1/PD-L1. The cutoffs of YTHDF1 and YTHDF2 were determined using receiver operating curve (ROC) analysis. Kaplan–Meier estimator was used for survival analysis. A cox proportional hazard model was used for survival multivariate analysis. IBM SPSS Statistics for Windows (Version 25.0. Armonk, NY, USA) was used, and a p-value less than 0.05 was defined as statistically significant.

Results

Correlation among YTHDF1, YTHDF2, CD4, CD8, and FOXP3 analyzed by mRNA expression and immunohistochemistry

The correlation analysis of YTHDF1, YTHDF2 and tumor infiltrating lymphocytes in the non-treatment group is summarized in Figure 1 In the adenocarcinoma group not receiving PD-1/PD-L1 inhibitor, the immunohistochemical expression of YTHDF1 was significantly negatively associated with CD4 and CD8 and positively correlated with FOXP3 expression. The immunohistochemical expression of YTHDF2 was also significantly negatively associated with CD4 and positively correlated with FOXP3 expression. In the squamous cell carcinoma group not receiving PD-1/PD-L1 inhibitor, the immunohistochemical expression of YTHDF1 was significantly negatively associated with CD4 and CD8 expression. The immunohistochemical expression of YTHDF2 was also significantly negatively associated with CD8 expression.

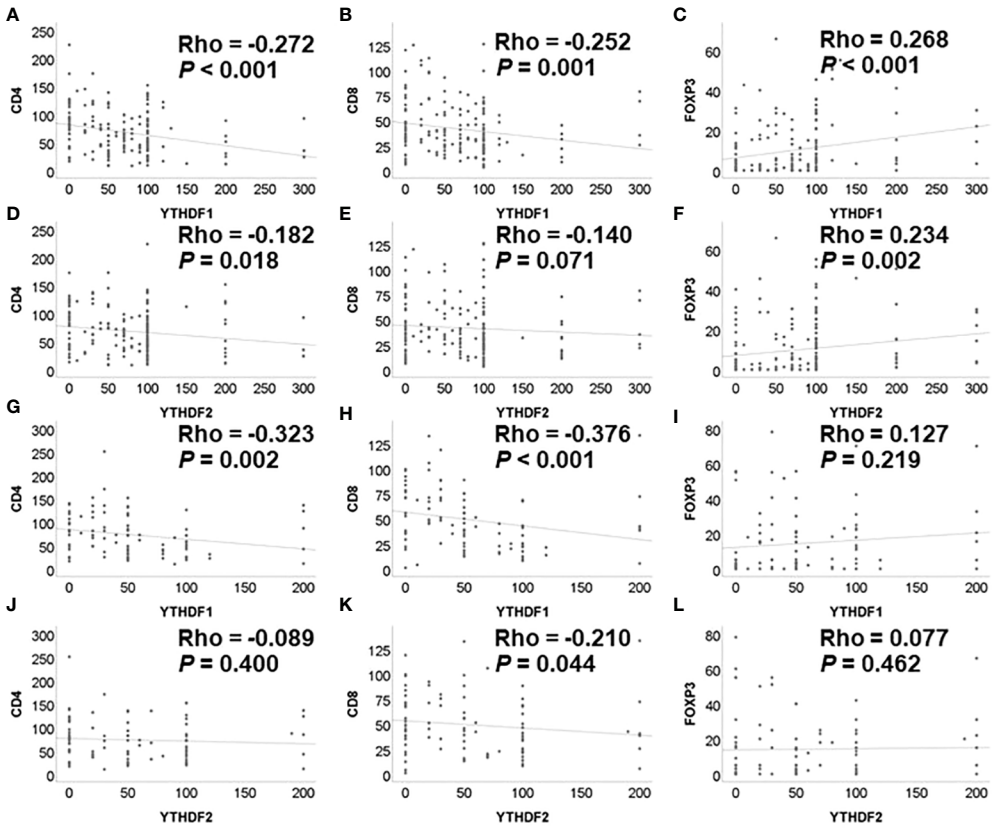

FIGURE 1
Correlation among YTHDF1, YTHDF2, CD4, CD8, and FOXP3 analyzed by immunohistochemistry in PD-1 inhibitor non-treatment groups. **(A)** YTHDF1 and CD4 in adenocarcinoma. **(B)** YTHDF1 and CD8 in adenocarcinoma. **(C)** YTHDF1 and FOXP3 in adenocarcinoma. **(D)** YTHDF2 and CD4 in adenocarcinoma. **(E)** YTHDF2 and CD8 in adenocarcinoma. **(F)** YTHDF2 and FOXP3 in adenocarcinoma. **(G)** YTHDF1 and CD4 in squamous cell carcinoma. **(H)** YTHDF1 and CD8 in squamous cell carcinoma. **(I)** YTHDF1 and FOXP3 in squamous cell carcinoma. **(J)** YTHDF2 and CD4 in squamous cell carcinoma. **(K)** YTHDF2 and CD8 in squamous cell carcinoma. **(L)** YTHDF2 and FOXP3 in squamous cell carcinoma.

In the adenocarcinoma group receiving PD-1/PD-L1 inhibitor treatment, the immunohistochemical expression of YTHDF1 was significantly negatively associated with CD4 expression (Supplementary Figure 1).

Correlation analyses among YTHDF1, YTHDF2, and tumor infiltrating lymphocytes were performed using the mRNA expression data of TCGA. The YTHDF1 or YTHDF2 mRNA expression was significantly negatively associated with CD4, CD8, and FOXP3 mRNA expression in adenocarcinoma (Figure 2). The YTHDF1 mRNA expression was also significantly negatively associated with CD4, CD8, and FOXP3 mRNA expression in squamous cell carcinoma (Figure 2). The YTHDF2 mRNA expression was not associated with CD4, CD8, and FOXP3 mRNA expression in squamous cell carcinoma (Figure 2). The YTHDF2 mRNA expression was not associated with CD4, CD8, and FOXP3 mRNA expression in squamous cell carcinoma (Figure 2). Representative figures of immunohistochemistry in adenocarcinoma show that high YTHDF1 or YTHDF2 cases are associated with low CD4, CD8 and high FOXP3 expression in both PD-1 inhibitor treatment and non-treatment groups (Figure 3). However, low YTHDF1 or YTHDF2 cases are associated with high CD4, CD8 and low FOXP3 expression in both PD-1 inhibitor treatment and non-treatment groups (Figure 3).

Prognostic or predictive role of YTHDF1 or YTHDF2

In the adenocarcinoma group not receiving PD-1/PD-L1 inhibitor, the cutoff values of YTHDF1 and YTHDF2 were 60 and 75, respectively. In the squamous cell carcinoma group not receiving PD-1/PD-L1 inhibitor, the cutoff values of YTHDF1 and YTHDF2 were 45 and 40, respectively. Because the sample size of the PD-1/PD-L1 inhibitor-treated group was small, all cell types were combined for survival analysis. In the group receiving PD-1/PD-L1 inhibitor treatment, the cutoff values of YTHDF1 and YTHDF2 were 30 and 20, respectively.

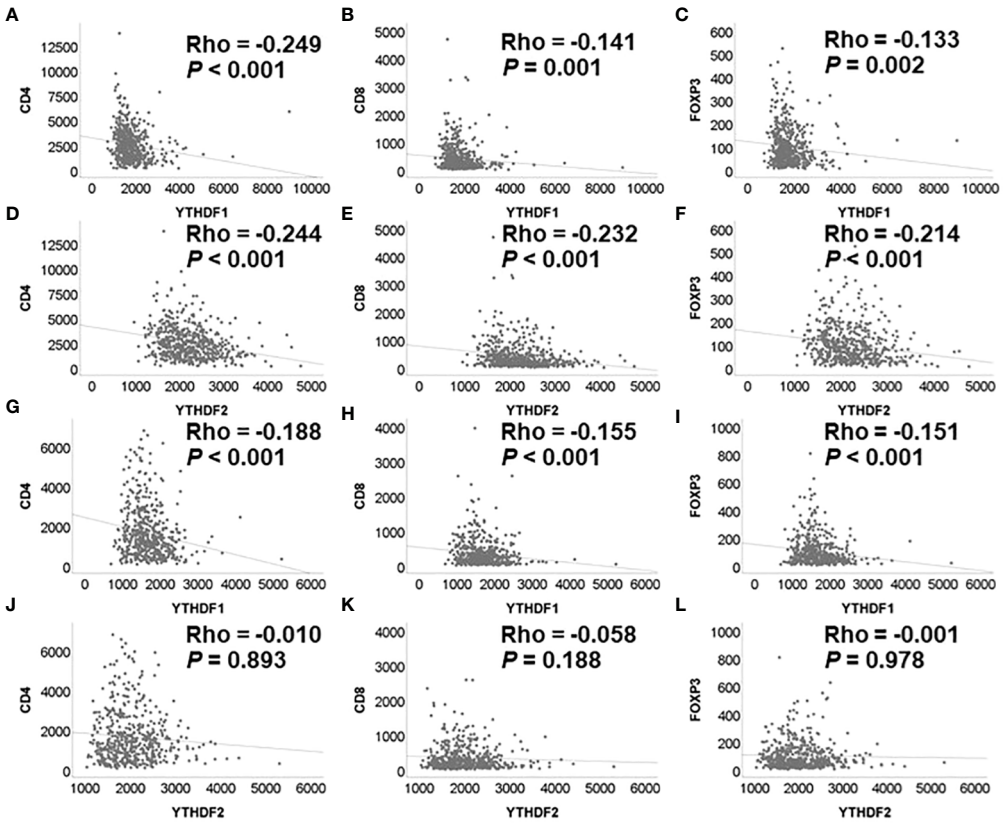

FIGURE 2
Correlation among YTHDF1, YTHDF2, CD4, CD8, and FOXP3 analyzed by mRNA expression in PD-1 inhibitor non-treatment groups. **(A)** YTHDF1 and CD4 in adenocarcinoma. **(B)** YTHDF1 and CD8 in adenocarcinoma. **(C)** YTHDF1 and FOXP3 in adenocarcinoma. **(D)** YTHDF2 and CD4 in adenocarcinoma. **(E)** YTHDF2 and CD8 in adenocarcinoma. **(F)** YTHDF2 and FOXP3 in adenocarcinoma. **(G)** YTHDF1 and CD4 in squamous cell carcinoma. **(H)** YTHDF1 and CD8 in squamous cell carcinoma. **(I)** YTHDF1 and FOXP3 in squamous cell carcinoma. **(J)** YTHDF2 and CD4 in squamous cell carcinoma. **(K)** YTHDF2 and CD8 in squamous cell carcinoma. **(L)** YTHDF2 and FOXP3 in squamous cell carcinoma.

In the adenocarcinoma group not receiving PD-1/PD-L1 inhibitor treatment, the immunohistochemical expression of YTHDF1 or YTHDF2 was correlated with better overall survival (p = 0.023, Figure 4A and p = 0.023, Figure 4C, respectively). In the squamous cell carcinoma group not receiving PD-1/PD-L1 inhibitor treatment, the immunohistochemical expression of YTHDF1 or YTHDF2 showed a trend toward better overall survival but was not statistically significant (p = 0.062, Figure 4B and p = 0.097, Figure 4D, respectively). In multivariate analysis, YTHDF1 or YTHDF2 immunohistochemical expression was an independent favorable prognostic marker for overall survival in adenocarcinoma patients (hazard ratio (HR) = 0.418, p = 0.001 and HR = 0.449, p = 0.001, respectively; Table 2). In Kaplan Meier plotter analysis, the group with high YTHDF1 mRNA expression showed better overall survival than the group with low YTHDF1 mRNA expression from adenocarcinoma or squamous cell carcinoma (p < 0.01, Figure 4E and p = 0.037, Figure 4F, respectively). The group with high YTHDF2 mRNA expression was also correlated with better overall survival in adenocarcinoma (p < 0.01, Figure 4G), although there was no difference in survival rate according to the level of YTHDF2 mRNA in squamous cell carcinoma (p = 0.89, Figure 4H).

We evaluated the predictive roles of YTHDF1, YTHDF2, and clinicopathologic variables on the response to PD-1/PD-L1 blockade. In univariate analysis, the group with low YTHDF1 expression was statistically more likely to respond to the PD-1/PD-L1 inhibitor than the group with high YTHDF1 expression (p = 0.003, Table 3). In multivariate analysis, the expression of YTHDF1 was an independent predictor for PD-1/PD-L1 blockade (p = 0.024, odd ratio (OR) = 0.189). Low expression of YTHDF2 was also statistically more likely to respond to PD-1/PD-L1 inhibitors in univariate analysis (p = 0.013, Table 3). In multivariate analysis, the expression of YTHDF2 was an independent predictor for PD-1/PD-L1 blockade (p = 0.031, OR = 0.196). We then performed survival analyses in the groups receiving PD-1/PD-L1 inhibitor treatment. The group with low YTHDF1 immunohistochemical

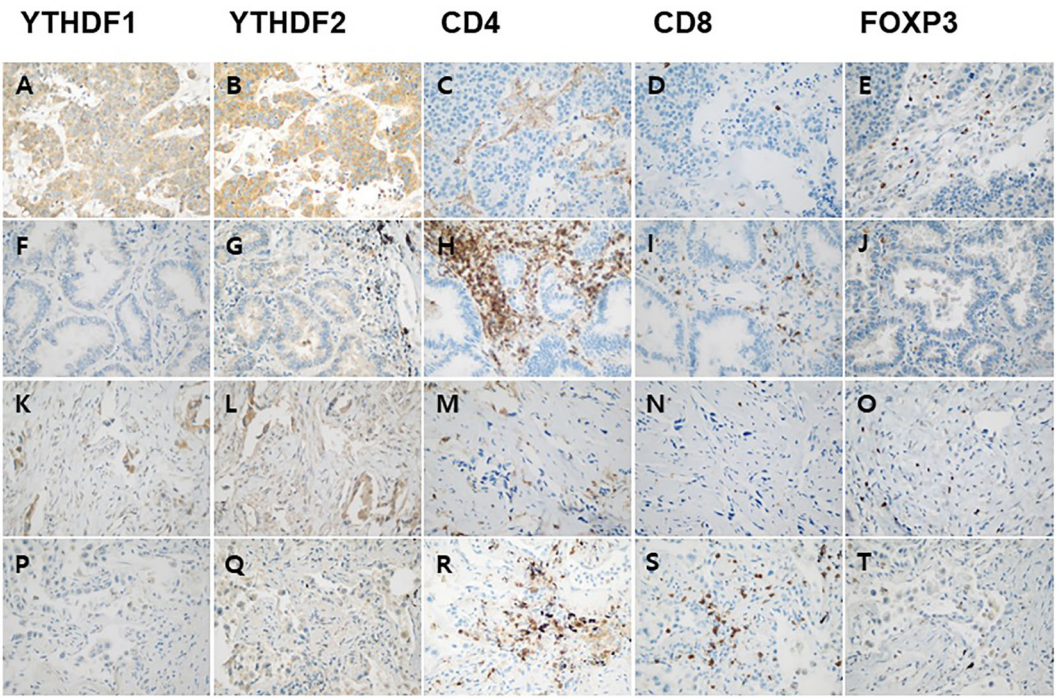

FIGURE 3
Representative immunohistochemical images of YTHDF1, YTHDF2, CD4, CD8, and FOXP3 expression in adenocarcinoma. High YTHDF1 **(A)** or YTHDF2 **(B)** case is associated with low CD4 **(C)**, CD8 **(D)** and high FOXP3 **(E)** expression in PD-1 inhibitor non-treatment group. Low YTHDF1 **(F)** or YTHDF2 **(G)** case is associated with high CD4 **(H)**, CD8 **(I)** and low FOXP3 **(J)** expression in PD-1 inhibitor non-treatment group. High YTHDF1 **(K)** or YTHDF2 **(L)** case is associated with low CD4 **(M)**, CD8 **(N)** and high FOXP3 **(O)** expression in PD-1 inhibitor treatment group. Low YTHDF1 **(P)** or YTHDF2 **(Q)** case is associated with high CD4 **(R)**, CD8 **(S)** and low FOXP3 **(T)** expression in PD-1 inhibitor treatment group.

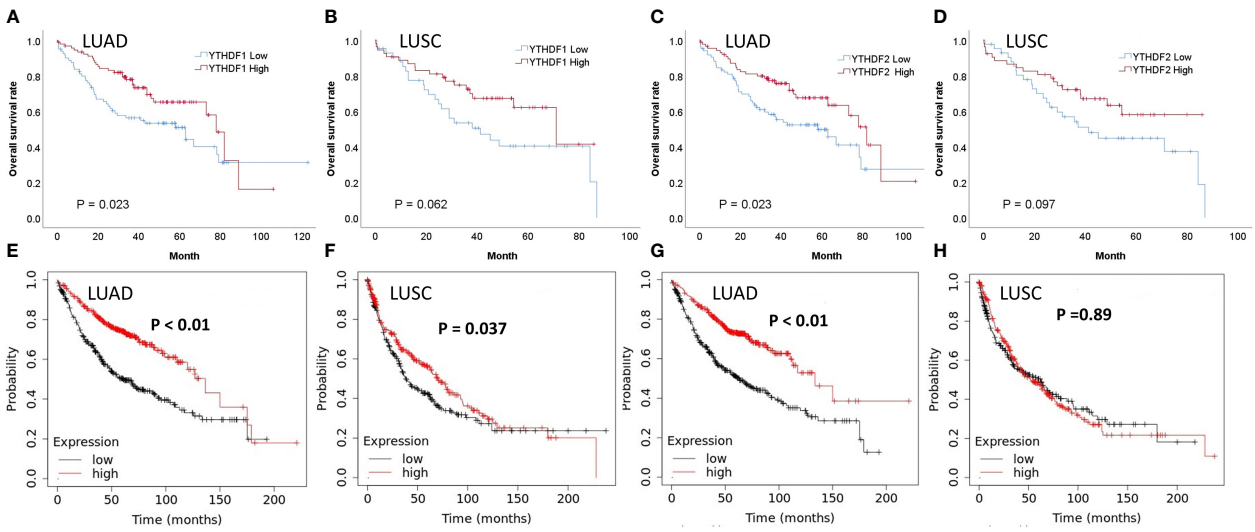

FIGURE 4
Survival analyses according to YTHDF1 and YTHDF2 expression in patients not receiving PD-1/PD-L1 inhibitors. **(A)** Overall survival (OS) and immunohistochemical expression of YTHDF1 in lung adenocarcinoma (LUAD). **(B)** OS and immunohistochemical expression of YTHDF1 in lung squamous cell carcinoma (LUSC). **(C)** OS and immunohistochemical expression of YTHDF2 in LUAD. **(D)** OS and immunohistochemical expression of YTHDF2 in LUSC. **(E)** OS and mRNA expression of YTHDF1 in LUAD. **(F)** OS and mRNA expression of YTHDF1 in LUSC. **(G)** OS and mRNA expression of YTHDF2 in LUAD. **(H)** OS and mRNA expression of YTHDF2 in LUSC.

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TABLE 2 Univariate and multivariate analyses of overall survival in immunohistochemical data of non-treatment group of PD1/PDL1 Inhibitors.

Univariate analysis

covariate	Adenocarcinoma			Squamous cell carcinoma		
	HR	95%CI	P-value†	HR	95%CI	*P*-value†
Age (≥65 y vs. <65 y)	1.326	0.828-2.125	0.240	1.217	0.764-1.939	0.409
Sex (male vs. female)	2.245	1.327-3.797	0.003	1.232	0.782-1.941	0.368
Stage (III–IV vs. I–II)	2.385	1.486-3.830	<0.001	2.665	1.701-4.178	<0.001
Smoking history (+ vs. -)	1.628	0.978-2.708	0.061	1.428	0.872-2.341	0.157
YTHDF1 (low vs. high)	0.583	0.364-0.934	0.025	0.704	0.434-1.141	0.154
YTHDF2 (low vs. high)	0.565	0.353-0.907	0.018	0.461	0.272-0.783	0.004
			Multivariate analysis			
Covariate		Adenocarcinoma			Squamous cell carcinoma	
	HR	95%CI	P-value	HR	95%CI	*P*-value
Sex (male vs. female)	2.449	1.425-4.211	0.001	2.064	1.279-3.330	0.003
Stage (III–IV vs. I–II)	2.458	1.523-3.965	<0.001	2.064	1.279-3.330	0.003
YTHDF1 (low vs. high)	0.418	0.255-0.685	0.001	0.704	0.434-1.141	0.154
Sex (male vs. female)	2.132	1.248-3.642	0.006	2.064	1.279-3.330	0.003
Stage (III–IV vs. I–II)	2.330	1.445-3.755	0.001	2.064	1.279-3.330	0.003
YTHDF2 (low vs. high)	0.449	0.274-0.736	0.001	0.704	0.434-1.141	0.154

CI, confidence interval; HR, hazard ratio; LVI, lymphovascular invasion.
†cox proportional hazard model analysis.

expression had better progression-free survival and overall survival than the group with high YTHDF1 expression; however, there were not statistically significant ($p = 0.154$, Supplementary Figure 2A and $p = 0.494$, Supplementary Figure 2B, respectively). The immunohistochemical expression of YTHDF2 was also not correlated with progression-free survival or overall survival rate ($p = 0.9$, Supplementary Figure 2C and $p = 0.967$, Supplementary Figure 2D, respectively). We provided the immunhistochemical data of the PD-1 inhibitor treatment group and non-treatment group in the form of Supplementary material Datasheet 1 (treatment group) and Supplementary material Datasheet 2 (non-treatment group).

TABLE 3 Univariate and multivariate logistic regression analysis for predicting clinical response to PD-1/PD-L1 blockade.

Univariate analysis

covariate	OR	95%CI	P-value†
Age (≥65 years vs.<65 years)	2.125	0.724-6.233	0.170
Sex (male vs. female)	6.222	0.713-54.29	0.098
Histology (ADC vs. non-ADC)	0.929	0.322-2.674	0.891
PD-L1 (≥50% vs. <50%)	3.030	0.991-9.268	0.052
YTHDF1 (high vs. low)	0.159	0.046-0.545	0.003
YTHDF2 (high vs. low)	0.187	0.050-0.700	0.013
	Multivariate analysis		
Covariate	OR	95%CI	P-value†
Age (≥65 years vs.<65 years)	1.032	0.261-4.086	0.964
Sex (male vs. female)	9.066	0.769-106.8	0.080
PD-L1 (≥50% vs. <50%)	4.281	1.110-16.51	0.035
YTHDF1 (high vs. low)	0.189	0.045-0.805	0.024
Age (≥65 years vs.<65 years)	2.138	0.574-7.959	0.257
Sex (male vs. female)	5.784	0.526-63.61	0.151
PD-L1 (≥50% vs. <50%)	5.094	1.282-20.23	0.021
YTHDF2 (high vs. low)	0.196	0.045-0.865	0.031

ADC, adenocarcinoma; CI, confidence interval; OR, odd ratio; PD-L1, programmed Death-Ligand 1.
†Logistic regression analysis.

GSEA and correlation analysis between YTHDF1, YTHDF2, and immune checkpoints

GSEA was performed using mRNA data obtained from TCGA. In lung adenocarcinoma, the low YTHDF1 group was mainly enriched in immunity-related signaling pathways (allograft rejection, IL6-JAK-STAT3 signaling, inflammatory response, and IL2-STAT5 signaling) (Table 4). In lung squamous cell carcinoma, the low YTHDF1 group was also mainly enriched in immunity-related signaling pathways (allograft rejection, IL2-STAT5 signaling, inflammatory response, IL6-JAK-STAT3 signaling, and TNFA signaling *via* NFKB and interferon gamma response). In lung adenocarcinoma, the low YTHDF2 group was mainly enriched in immunity-related signaling pathways (inflammatory response, IL6-JAK-STAT3 signaling, allograft rejection, and IL2-STAT5 signaling). In lung squamous cell carcinoma, there was no immune-related gene set related to YTHDF2.

We then performed correlation analysis between YTHDF1, YTHDF2, and immune checkpoints using mRNA expression data. In lung adenocarcinoma, YTHDF1 is significantly negatively correlated with PD-L1, PD-1, PD-L2, CTLA-4, TIGIT, VISTA, and TIM3 (Table 5). In lung squamous cell carcinoma, YTHDF1 is significantly negatively correlated with PD-L1, PD-1, PD-L2, CTLA-4, TIGIT, VISTA, and TIM3. YTHDF2 is significantly negatively correlated with PD-L1, PD-1, PD-L2, CTLA-4, TIGIT, LAG3, VISTA, and TIM3 in lung adenocarcinoma. In lung squamous cell carcinoma, YTHDF2 is significantly negatively associated with PD-L1 and PD-L2.

Discussion

Protein expression of YTHDF1 or YTHDF2 was negatively correlated with CD8- and CD4-positive T cells, but positively correlated with Treg cells. The mRNA data also showed that the level of YTHDF1 was negatively correlated with CD8 and CD4 expression. In GSEA, low YTHDF1 mRNA expression was confirmed to be closely related to the immune-related pathway. The expression of YTHDF1 showed a negative correlation with most immune checkpoints. High YTHDF1 expression was associated with better prognosis. However, groups with low YTHDF1 or YTHDF2 expression were more likely to respond to PD-1/PD-L1 inhibitors than groups with high YTHDF1 expression. These results indicate that the low YTHDF1 and YTHDF2 groups are immune-inflamed tumors, also named "hot tumors." Hot tumors are generally known to respond better to immunotherapy (25, 26). As expected, the low YTHDF1 and low YTHDF2 groups responded better to PD-1/PD-L1 inhibitor treatment. The expression of YTHDF1 or YTHDF2 in NSCLC can be a good predictive biomarker for PD-1/PD-L1 inhibitor.

m^6A methylation plays important roles in regulating mRNA splicing, export, localization, translation, and stability (9). Only a few previous studies have reported on the relationship between YTHDF1 and cancer. Zhao et al. reported that YTHDF1

TABLE 4 Immune-related gene sets in GSEA.

NAME	SIZE	ES	NES	Nominal p-val	FDR q-val	FWER p-val
Gene sets related to low YTHDF1 in adenocarcinoma patients						
HALLMARK_ALLOGRAFT_REJECTION	164	0.609	1.884	0.014	0.102	0.057
HALLMARK_IL6_JAK_STAT3_SIGNALING	74	0.556	1.823	0.018	0.051	0.105
HALLMARK_INFLAMMATORY_RESPONSE	172	0.493	1.727	0.022	0.089	0.203
HALLMARK_IL2_STAT5_SIGNALING	166	0.419	1.689	0.006	0.089	0.268
Gene sets related to low YTHDF1 in squamous cell carcinoma patients						
HALLMARK_ALLOGRAFT_REJECTION	164	0.705	2.172	0.000	0.000	0.000
HALLMARK_IL2_STAT5_SIGNALING	166	0.519	2.024	0.000	0.011	0.025
HALLMARK_INFLAMMATORY_RESPONSE	172	0.594	1.947	0.000	0.019	0.048
HALLMARK_IL6_JAK_STAT3_SIGNALING	74	0.633	1.939	0.000	0.017	0.051
HALLMARK_TNFA_SIGNALING_VIA_NFKB	166	0.551	1.813	0.018	0.033	0.137
HALLMARK_INTERFERON_GAMMA_RESPONSE	166	0.614	1.771	0.023	0.042	0.190
Gene sets related to low YTHDF2 in adenocarcinoma patients						
HALLMARK_INFLAMMATORY_RESPONSE	172	0.549	1.917	0.005	0.052	0.037
HALLMARK_IL6_JAK_STAT3_SIGNALING	74	0.558	1.803	0.021	0.084	0.139
HALLMARK_ALLOGRAFT_REJECTION	164	0.583	1.796	0.016	0.056	0.149
HALLMARK_IL2_STAT5_SIGNALING	166	0.430	1.733	0.002	0.057	0.213

ES, enrichment score; FDR, false discovery rate; FWER, family-wise error rate, NES, normalized enrichment score.

TABLE 5 Correlations between YTHDF1, YTHDF2 and immune checkpoints in mRNA expression data.

	Adenocarcinoma (n = 517)				Squamous cell carcinoma (n = 501)			
	YTHDF1†	*p*	YTHDF2†	*p*	YTHDF1†	*p*	YTHDF2†	*p*
PD-L1	-0.087	0.048	-0.243	<0.001	-0.115	0.009	-0.268	<0.001
PD-1	-0.090	0.039	-0.302	<0.001	-0.118	0.007	-0.067	0.134
PD-L2	-0.194	0.001	-0.291	<0.001	-0.213	<0.001	-0.205	<0.001
CTLA-4	-0.130	0.002	-0.257	<0.001	-0.154	<0.001	-0.076	0.088
TIGIT	-0.130	0.003	-0.222	<0.001	-0.125	0.005	-0.016	0.720
LAG3	0.039	0.366	-0.242	<0.001	-0.055	0.217	-0.060	0.179
VISTA	-0.267	<0.001	-0.278	<0.001	-0.216	<0.001	-0.001	0.978
TIM3	-0.226	<0.001	-0.242	<0.001	-0.206	<0.001	-0.051	0.253

† Spearman's correlation test

expression was associated with poor clinical outcomes in patients with hepatocellular carcinoma (27). Nishizawa et al. reported that the c-Myc oncogene promoted YTHDF1 expression and the knockdown of YTHDF1 resulted in the suppression of cell proliferation and sensitization to anticancer drugs in colorectal cancer (28). YTHDF2 is also a reader protein and plays an important role in regulating mRNA stability (29). High expression of YTHDF2 in ovarian cancer induces tumor progression (30). YTHDF2 is known to inhibit hepatocellular carcinoma cell proliferation and growth by inhibiting EGFR mRNA stability (31).

Han et al. reported that knockout of YTHDF1 resulted in higher levels of CD8+ T-cells and NK cells in melanoma and colon cancer mouse models (15). The knockout of YTHDF1 induced an increase in PD-L1 expression (15). In a melanoma cancer mouse model, tumor regression was found more frequently in anti-PD-L1-treated YTHDF1 knockout mice than in anti-PD-L1-treated wild-type mice (15). Our study also revealed that low expression of YTHDF1 was correlated with CD8 and CD4 protein or mRNA expression. Previous studies have shown that high CD4+ or CD8+ cells are associated with better responses to PD-1/PD-L1 blocking therapy. Before PD-1/PD-L1 blockade treatment, high level of peripheral blood CD4+ cells was associated with long-term survival (32). The transcriptome signature of PD-1 high CD8+ T cells showed a better prognosis in multiple cancers that underwent immune checkpoint inhibitor therapy (33). In our study, the expression of YTHDF1 was positively correlated with FOXP3. Treg cells are immunosuppressive and downregulate the induction and proliferation of effector T cells (34). Treg cells also play an important role in PD-1/PD-L1 therapy. Because Treg cells proliferate after PD-1/PD-L1 blockade, hyperprogression occurs during PD-1/PD-L1 blockade (35). Non-responders to PD-1/PD-L1 blocking therapy usually show an increase in PD-1 in Treg (36). The response was better when the ratio of tumor-infiltrating PD-1 +CD8+T cells was higher than that of PD-1+Treg cells (36). The CD8 and CD4 high and FOXP3 low profile seen in the low YTHDF1 group indicates immune hot tumors and is a key factor in the response to PD-1/PD-L1 inhibitor.

In our study, YTHDF1 showed no difference in immune profile and prognosis according to cell type, although YTHDF2 showed a significant difference. In adenocarcinoma, YTHDF2 was negatively correlated with CD4 and CD8 and positively correlated with FOXP3 in protein and mRNA analysis. In squamous cell carcinoma, YTHDF2 showed a negative correlation with CD8 in protein analysis, but there were no correlations among YTHDF2, CD3, CD8, and FOXP3 in mRNA analysis. In GSEA of squamous cell carcinoma, there were no immune-related gene sets associated with YTHDF2. However, four immune-associated gene sets related to YTHDF2 were found in adenocarcinoma. In adenocarcinoma, all eight immune checkpoints showed a negative relationship with YTHDF2, but only two immune checkpoints were negatively correlated in squamous cell carcinoma. In Kaplan Meier plotter analysis, high YTHDF2 is associated with a better prognosis in adenocarcinoma, but YTHDF2 is not associated with prognosis in squamous cell carcinoma. Because YTHDF2 expression does not affect the immune profile of squamous cell carcinoma, there is no difference in survival rate.

In GSEA, the low YTHDF1 group was correlated with several immune-related pathways including IL2-STAT5 signaling, IL6-JAK-STAT3 signaling, and TNFA signaling *via* NFKB and interferon gamma response. The low YTHDF2 group was also associated with IL2-STAT5 and IL6-JAK-STAT3 signaling pathways. The association between immune-related pathways and PD-1/PD-L1 inhibitors has been reported several times in the past. IL2-STAT5 immune signatures are known to predict reactivity to PD-1/PD-L1 inhibitors (37). The IL-6/JAK1 pathway induces PD-L1 Y112 phosphorylation, leading to cancer immune evasion (38). TNF-α promotes PD-L1 expression in human prostate and colon cancer cells (39). The IFN-γ-related mRNA profile is a biomarker for PD-1 inhibitors that are currently attracting attention (40, 41).

High YTHDF1 or YTHDF2 expression was associated with better prognosis in immunohistochemistry and mRNA data sets.

High YTHDF1 or YTHDF2 expression groups showed low immune checkpoint expression. Because immune checkpoints expressed on tumor cells protect tumor cells from attack by local immunity, the higher is the expression of immune checkpoints, the worse is the prognosis (42, 43). When treating with PD-1/PD-L1 inhibitor, the higher is the expression of immune checkpoints, the better is the expected response to treatment. In our low YTHDF1 or YTHDF2 expression group, immune checkpoint expression is high, indicating a good response to the PD-1/PD-L1 inhibitor. Similar to YTHDF1 and YTHDF2, PD-L1 expression is a poor prognostic factor in NSCLC (44, 45); however, the higher is the expression of PD-L1, the higher is the response rate to PD-1/PD-L1 inhibitor (4).

Our study had some limitations. First, ours was a retrospective observational study with a relatively small sample size. Second, we used an immunohistochemical method. However, immunohistochemistry has limitations regarding standardization, reliability, and reproducibility (46). Third, YTHDF1 or YTHDF2 expression was a predictive marker of response to PD-1/PD-L1 inhibitor but had no correlation with prognosis. Because the number of patients receiving PD-1/PD-L1 inhibitor was small (59 patients), our results need to be verified in a larger study. Forth, we performed immunohistochemical studies and mRNA studies on samples from different groups. Therefore, because protein or mRNA expression in the same sample is not compared, there is a limit to the analysis of protein and mRNA expression. Fifth, our study only confirmed the relationship between YTHDF1, YTHDF2, CD4, CD8, and FOXP3, however did not reveal which pathway YTHDF1, YTHDF2 affects on the tumor immune profile. Thereafter, experiments such as *in vivo* mouse models need to confirm our results and additional studies also determine how the YTHDF1 and YTHDF2 pathways affect immune profiles.

Conclusion

Low YTHDF1 or YTHDF2 expression shows an immune hot profile of high CD8, high CD4, and low FOXP3. GSEA confirmed that low YTHDF1 or YTHDF2 tumor expression reflects the gene set of immune hot tumors. Low YTHDF1 or YTHDF2 showed higher expression of immune checkpoints than high YTHDF1 or YTHDF2. YTHDF1 or YTHDF2 was a predictive marker of response to PD-1/PD-L1 inhibitors. The expression of YTHDF1 or YTHDF2 was associated with prognosis. YTHDF1 has an immune hot profile in both lung adenocarcinoma and squamous cell carcinoma, whereas YTHDF2 is only seen in adenocarcinoma.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Ajou University School of Medicine (AJIRB-BMR-KSP-19-416 and 2019-11-11). The ethics committee waived the requirement of written informed consent for participation.

Author contributions

Conception/design: YK. Provision of study material or patients: YK, J-HH, SH and HWL. Data analysis and interpretation: YK, J-HH, SH and HL. Manuscript writing: YK, J-HH, SH and HL. Final approval of manuscript: YK, J-HH, SH and HL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.996634/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Correlation among YTHDF1, YTHDF2, CD4, CD8, and FOXP3 analyzed by immunohistochemistry in PD-1 inhibitor treatment groups. (A) YTHDF1 and CD4 in adenocarcinoma. (B) YTHDF1 and CD8 in adenocarcinoma. (C) YTHDF1 and FOXP3 in adenocarcinoma. (D) YTHDF2 and CD4 in adenocarcinoma. (E) YTHDF2 and CD8 in adenocarcinoma. (F) YTHDF2 and FOXP3 in adenocarcinoma. (G) YTHDF1 and CD4 in squamous cell carcinoma. (H) YTHDF1 and CD8 in squamous cell carcinoma. (I) YTHDF1 and FOXP3 in squamous cell carcinoma. (J) YTHDF2 and CD4 in squamous cell carcinoma. (K) YTHDF2 and CD8 in squamous cell carcinoma. (L) YTHDF2 and FOXP3 in squamous cell carcinoma.

SUPPLEMENTARY FIGURE 2
Survival analyses according to YTHDF1 and YTHDF2 expression in patients receiving PD-1/PD-L1 inhibitors. (A) Progression-free survival and expression of YTHDF1. (B) Overall survival and expression of YTHDF1. (C) Progression-free survival and expression of YTHDF2. (D) Overall survival and expression of YTHDF2.

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Hyperprogressive disease in non-small cell lung cancer treated with immune checkpoint inhibitor therapy, fact or myth?

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The therapeutic landscape for patients with non-small cell lung cancer (NSCLC) has dramatically evolved with the development and adoption of immune checkpoint inhibitors (ICI) as front-line therapy. These novel antibodies target the interactions in immunoregulatory pathways, between programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1), or cytotoxic T-lymphocyte antigen 4 (CTLA-4) and B7, resulting in the activation of T cells and cytotoxic response to induce an immunologic response. ICIs have demonstrated significant survival benefits and sustained responses in the treatment of NSCLC leading to the long-term survival of up to 5 year. One unusual response to ICI is a phenomenon termed Hyperprogressive Disease (HYD), which occurs in a subset of patients for whom ICI therapy can induce rapid disease growth, which ultimately leads to poorer outcomes with an incidence rate ranging from 5 to 37% in NSCLC patients. Prior reviews demonstrated that HYD can be defined by rapid tumor progression, deterioration of patient's symptoms or new onset of disease. The mechanism of HYD could be related to genomic and tumor microenvironment changes and altered immune response. It will be important to establish a common definition of HYD for future research and clinical care.

KEYWORDS

checkpoint inhibition therapy, hyperprogression, non-small cell lung cancer, definition, mechanism

Introduction

The therapeutic landscape for patients with non-small cell lung cancer (NSCLC) has dramatically evolved in the last several years with the development and adoption of immune checkpoint inhibitors (ICI) as front-line therapy. These novel antibodies target the interactions in immunoregulatory pathways, such as those between programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1), or cytotoxic T-lymphocyte antigen 4 (CTLA-4) and B7, resulting in the activation of T cells and cytotoxic response to induce an immunologic response in several solid tumor types (1). ICIs have demonstrated significant survival benefits and sustained responses in the treatment of NSCLC leading to the long-term survival of up to 5 years (2–4).

One unusual response to ICI is a phenomenon termed Hyperprogressive Disease (HYD), which occurs in a subset of patients for whom ICI therapy can induce rapid disease growth, which ultimately leads to poorer outcomes (5). Existing data suggest an incidence rate ranging from 5 to 37% in NSCLC patients (6–8). Despite this known entity, a consensus definition for the diagnosis of HYD has not been determined, and the explication of underlying pathophysiologic mechanisms has remained elusive. In this review, we will evaluate recent data on HYD in the NSCLC population, as well as discuss the proposed mechanisms, predictors, and biomarkers potentially implicated in the process.

Case illustration

61-year-old white female presented on 3/17/2022 with several months of cognitive changes (including confusion) and gait instability. CT of the head done on 3/17/2022 showed a large frontal lobe mass measuring 3.2 x 2.6 cm with extensive adjacent edema of the left frontal and parietal lobes, midline shift of 7 mm, and marked compression/distortion of the left frontal horn of the left lateral ventricle. The patient was admitted to the neurosurgery service and CT chest/abdomen/pelvis on 3/18/2022 showed a 2.1 cm nodule in the medial azygos lobe of the right upper lobe, compatible with primary lung ca. There were several smaller irregular ground glass and nodular opacities in the left lower lobe (indeterminate or synchronous malignancies or metastases). She also had mild mediastinal and right hilar lymphadenopathy, but no abdominal pelvic metastatic disease. The patient underwent endobronchial ultrasound biopsy and bronchioalveolar lavage on 3/21/2022. Fine Needle Aspiration of the right hilar mass showed poorly differentiated carcinoma pulmonary non-small cell carcinoma. PD-L1 TPS was 40%. MRI of the brain done on 3/22/2022 showed enhancing anteromedial left frontal cerebral cortical nodule, indicating solitary cerebral metastasis, marked associated left anterior cerebral vasogenic edema, and mild rightward frontal midline shift. The patient underwent a left frontal craniotomy on 3/23/2022 and pathology showed poorly differentiated carcinoma consistent with metastasis from lung adenocarcinoma primary. PD-L1 TPS was 1 to 2%. NGS testing showed mutation of TP53, BRAF L597Q (not V660E), STK-11, PDGFRA, KMT2D, ZNF217, and RNA testing was negative for an actionable mutation. She was discharged on 3/25/22 and underwent post-operative radiation to the surgical bed from 4/12/22 to 4/25/22. She tapered off Decadron and enrolled in a trial randomized to Pembrolizumab single agent which started on 5/13/22. After 2 cycles of therapy, the patient developed deterioration of her performance status, and required hospital admission 6/30/22. The CT scan after 2 cycles done on 6/23/22 showed significant progression of her disease (Figure 1).

The definition and diagnosis of HYD

HYD is generally described as unexpected, accelerated tumor growth after treatment with ICI therapy (9). Early after governing approval and real-world application of ICI in therapies for solid tumor patients, this phenomenon was often reported anecdotally as a disease flare, with an increased size of cancer lesions noted on imaging noted shortly after initiation of such treatment (10). Although this can be attributed to the natural progression of the disease, the course of HYD is punctuated by the unproportioned growth of the disease compared to the course of the disease before therapy and significant deterioration of the patient's condition. It should be noted that while the literature on HYD more recently has been centered around single-agent ICI therapy. The shape of progression-free survival curve in Checkmate 277 and Mystical trial, suggests that this also occurs when using dual ICI (anti-PD-1 with anti-CTL4 combination) (11, 12). HYD has been described in chemotherapy, in about 5% (6), and tyrosine kinase inhibitor therapies, varying up to 25% (13), as well. However, these studies are not definitive that HYD exists in non-immunotherapy treated patients since both the population and definition criteria were very heterogeneous (5, 6).

Despite increased recognition of the hyperprogressive phenomenon, there is a lack of a unifying definition of this process. Several previous studies have sought to define HYD across a broad range of tumor types. Definitions thus far largely have been categorized into time-dependent criteria or size/clinically dependent criteria.

Examples of time-dependent criteria employed in the literature include tumor growth rate (TGR) and tumor growth kinetics (TGK). TGR calculation involves the difference (or ratio) of 3-dimensional tumor volume per month, related to the sum of the target lesion(s) diameter(s) as well as the time between imaging evaluations (14). Tumor growth kinetics (TGK) on the other hand is a function of 2-dimensional tumor diameter over time.

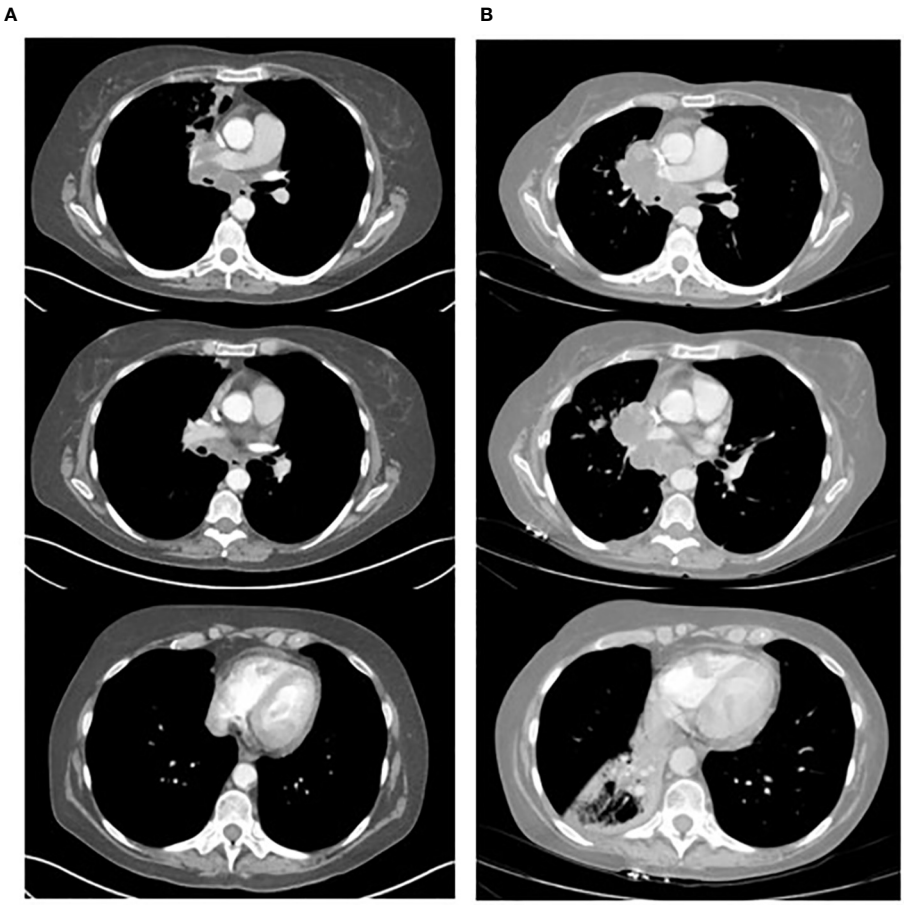

FIGURE 1
Shows the CT scans of a 61-year-old patient with NSCLC. **(A)** shows 3 axial CT scan images of the tumor located in the right para-mediastinal area and lymph node enlargement at level 7 and 10. **(B)** corresponding axial CT scan images after 2 cycles of Pembrolizumab monotheraphy (49 days later) showing clear increase in tumor mass and lymph nodes at level 7 and 10 and atelectasis of right lower lobe.

Champiat et al. (2017) were the first to collectively describe and define HYD in solid-tumor patients who were treated with immunotherapy (5). This retrospective analysis of 131 patients evaluated the prevalence of hyperproliferative disease in those treated in phase I clinical trials with immunotherapy. The authors defined the HYD as progression at first evaluation with a TGR ratio increase of two-fold or higher by RECIST 1.1 criteria. Of note, this study included only 13 patients with lung cancer, none of whom developed HYD (5, 6).

A later study by Singavi et al. incorporated a similar definition to the criteria set out by Champiat, including a TGR increase of two-fold or higher per RECIST 1.1 criteria, with an additional requirement of RECIST 1.1 tumor size increase of 50% or higher (15). Eventually, data from Ferrara et al. in 2018 would evaluate HYD in NSCLC patients using a definition of progression per RECIST 1.1 criteria as well as TGR difference (rather than a ratio) of 50% or higher (6).

Additional data from Kato et al. (2017) reviewed 155 patients with advanced solid tumors who received immunotherapies and had their tumors evaluated by next-generation sequencing. This study defined HYD as a TTF of fewer than 2 months, a 50% or higher increase in tumor burden compared to pretreatment imaging, and a 2-fold or higher increase in progression pace (16). Notably, this study had a total of 38 NSCLC patients included, with 18 of these patients experiencing a TTF in less than 2 months. Saâda-Bouzid et al. (2017) evaluated HYD in 34 patients with squamous cell carcinoma of the head and neck, with the definition of HYD relying on TGK (17, 18).

The heterogeneity in definitions of HYD has real-world implications in the current diagnosis of HYD. A recent retrospective cohort study evaluated 406 patients with NSCLC, analyzing the incidence and outcomes in a single population of patients with HYD as defined by five different, established

definitions per previous trials. The data revealed a variance in reported incidence (5.4%-18.5%) of HYD, with concordance between definitions ranging from 33.3% to 69.3% (19). Indeed, a previous meta-analysis and systematic review of 3109 patients across 24 studies suggest that despite being a distinct outcome, the lack of a standardized, validated definition of HYD leads to significant variability in reported incidence (20). Given the implications of HYD on survival outcomes, it is of great interest to oncologic physicians to standardize definitions of this phenomenon in the future.

Beyond HYD, other patterns of progression on ICI have been described in the literature. Gandara et al. described fast progression (FP) and early death (ED) in a retrospective evaluation of the OAK study. FP was defined on size-based criteria (50% or greater increase in the sum of largest diameters of target lesions per RECIST 1.1 criteria) and did not require pre-baseline assessment. ED was defined as death due to disease progression within 12 weeks from baseline in patients without a response assessment (21). Further evaluation has suggested that these are distinct patterns of progression with limited overlap between the groups (22).

The use of parameters such as the TGR or TGK allows for the evaluation of tumor kinetics as guided by tumor size. Of note, the TGK does not involve a three-dimensional evaluation of tumor size, which may lead to some overestimation of the incidence of HYD (5, 6, 15, 18). These time-based criteria require at least three radiologic examinations (pre-baseline, baseline, and post-treatment) to allow for a dynamic assessment of tumor growth momentum (5). This allows for differentiation of the natural course of the disease (in which tumor growth curves would largely remain similar before and after treatment) versus true HYD, in which tumor growth speed would increase after initiation of ICI. Unfortunately, time-dependent criteria cannot be readily applied to all patients in a first-line setting, as often these patients do not have pre-baseline imaging.

Size or clinically dependent criteria require pre-baseline imaging but do require dynamic data regarding tumor momentum in growth, i.e., RECIST criteria measuring size (13) or reliant on the changes in the patient's clinical condition (23). Another criterion is time to treatment failure (TTF), defined as the time from the start of treatment with ICI to its discontinuation, increase in the sum of target lesions from baseline imaging to current radiologic evaluation, the appearance of new lesions from baseline imaging, or clinical deterioration.

Matos et al. used RECIST and defined HYD as a progression of disease within the first 8 weeks after treatment with ICI, an increase of a minimum of 10mm and addition to increasing≥ 40% in the sum of target lesions compared with baseline (double of the RECIST 1.1 definition of progression) and/or increase of ≥ 20% in the sum of target lesions compared with baseline and the appearance of new lesions in at least 2 different organs. In this study, they analyzed 287 patients treated with ICI monotherapy or in combination. HYD by RECIST definition occurred in 10.7% of patients representing 27.1% of patients with disease progression. Their outcome was worse with median overall survival (mOS) of 5.23 months vs. 7.3 months without HYD (13).

Furthermore, size- or clinical-dependent criteria may be easier to implement in the real-world setting and possibly in clinical trials. However, these evaluations cannot describe the rates or speed of tumor growth inherently associated with time-based evaluations, and thus distinguishing between natural disease progression and HYD remains difficult (24). A limitation of size-dependent criteria like RECIST could be potentially overestimating HYD when the disease has rapid TGR, but even with this limitation patients with rapid TGR are also likely to have a worse outcome and are of clinical significance (13).

Future implementation of early disease assessments and integrating time-based tumor kinetic evaluation will be crucial in identifying those with HYD. A proposed set of parameters as the definition of HYD based on the review of the literature is shown in Table 1.

Proposed mechanisms of HYD

While the process of HYD in NSCLC with ICI therapy has been increasingly documented, the mechanisms responsible remain relatively unknown. Several proposed hypotheses and mechanisms have been suggested, including factors involving expansion of PD-1 expression and T regulatory cell, changes in the immunosuppressive tumor microenvironment, the diminished response of anti-tumor immune cells to ICIs, and the involvement of alternative signaling networks *via* oncogenic driver mutations (25). A summary of the proposed mechanisms is shown in Table 2.

It has been suggested that the use of ICI can lead to the expansion of regulatory T cells, which are immunosuppressive cells that may proliferate in the setting of PD-1 or PD-L1 blockade. A study by Kamada et al. showed that patients without HYD showed a markedly decreased ratio of regulatory T cells to $CD8^+$ T cells, whereas those with HYD showed no significant change to maybe a slight increase ratio of regulatory T cells (26). This may lead to increased immunosuppression and tumor hyperprogression. T cell exhaustion, or T cell dysfunction, may also be implicated in ICI therapy, possibly as a result of upregulation of alternate inhibitory receptors such as T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3), T cell immunoglobulin and ITIM domain (TIGIT), and Lymphocyte activation gene-3 (LAG3) (27, 28). Additionally, highly differentiated, circulating senescent T cells may have implications in the role of HYD, as it has been identified that those with HYD NSCLC (and those that did not respond to anti-PD1/PD-L1 therapy) have an increase in this T cell population

after ICI therapy (29). Recent data have further supported the hypothesis that circulating T cell immunosenescence plays a role in ICI responsiveness. Ferrara et al. reported that 28% of 83 advanced non-small cell lung cancer patients were observed to have circulating senescent T cells. Among them, 4 patients had HYD with a delta of TGR>50 and all of them had between 47% to 63% of circulating CD8 T cells with a senescent immunophenotype (CD28-CD57+ killer-cell lectin-like receptor G1 (KLRG1+)). None of them had a response compared to 30% in patients without T cell immunosenescence markers (30).

The tumor microenvironment plays a significant role in responses to ICI therapy, and it has been proposed as a potential mechanism in the development of HYD as well. ICI-induced upregulation of immunosuppressive cytokines, including interleukin 10 and interferon-gamma (IFN-γ), may lead to IFN-γ-dependent recruitment of immunosuppressive myeloid-derived suppressor cells (31). Inflammatory cell presence in the tumor microenvironment can lead to tumor escape from ICI in a variety of mechanisms including local inflammation, modifying metabolism, and increased angiogenesis. A study by Lo Russo et al. analyzed 152 patients with NSCLC who underwent treatment with immunotherapy, and in patients with HYD there was an increased population of tumor-associated macrophages, and it has been theorized that this relationship may be due to increased interaction between the macrophages and the Fc fragment of the ICI antibodies (23).

Specific genomic mutations have also been posited as driver events for HYD. The study by Kato et al. (16) noted an association between HYD and MDM2/MDM4 amplification. This may be related to dysregulation of p53 and resultant downstream Vascular Endothelial Growth Factor (VEGF) upregulation, as MDM2 directly leads to p53 degradation *via* proteasome (32). ICI therapy leads to increased JAK-STAT signaling, with a resultant increase in interferon-regulatory factor (IRF)-8 expression, leading to downstream MDM2 expression (33). Epidermal Growth Factor Receptor (EGFR) activation is also associated with the upregulation of tumor immune escape markers (PD-1/PD-L1, CTLA4), and is associated with a slight increase in the risk of developing HYD (15, 16, 34).

Predictive features and outcomes of HYD

With the accelerated tumor growth noted in this subset of patients, a focus on potential predictive factors has been highlighted in previous data. These data sets span several different solid tumor subtypes, but more recent studies have highlighted specific risk factors in the NSCLC patient population.

The association between HYD and age is not entirely clear. Several studies have shown that patients who are older when treated with ICI have a higher risk of developing HYD (5, 35). This could be due to noted declines in T cell immunity as patients age (36). However, other studies have not shown an association between HYD and age. In the 2018 data from Ferrara et al, the first study to specifically address HYD in an NSCLC population, this association with age was not seen, although the definition of hyperprogression did differ (6).

Some studies have found a correlation between metastatic burden, locoregional recurrence, and risk of HYD. Head and neck cancer patients in one study were found to have a higher incidence of hyperprogression in those with metastatic cervical nodes versus those without, as well as a higher rate of regional

TABLE 1 Proposed criteria of Hyperprogressive disease.

Tumor measurement criteria

1- Increase of two-fold or higher per RECIST 1.1 criteria OR 50% or higher increase in tumor burden compared to pretreatment imaging

2- Time to progression less than 3 cycles of therapy (2 months)

3- 2-fold or higher increase in progression pace

4- Progression of new lesions

Patient symptoms criteria

5- Rapid decrease in baseline performance status or worsening of symptoms related to the disease progression

6- New onset of complications related to disease progression i.e. SVC, increase pleural effusion

Laboratory Criteria

7- **LDH > upper limit of normal**

Measurement Methods

Tumor Growth Rate (TGR) 3 D difference in tumor volume per month, related to the sum of the target lesion(s) diameter(s) as well as the time between imaging evaluations.

Tumor Growth Kinetics (TGK) 2D as a function of tumor diameter over time.

Potential Factors associated with HYD

1. **Increased Age**
2. **Higher tumor burden with 2 or more metastatic sites with one of the liver**
3. **High LDH**

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TABLE 2 Mechanism of hyperprogression.

Genomic changes	Changes in tumor microenvironment	Altered immune response
MDM/MD4 (↑ VEGF)	↑ VEGF	Activation of Fcy receptor
Deletion Mutations	↑ M2 Microphage	Change Ratio of Effector/Regulatory T cells
JAK-STAT activation	↑ IFN-γ	↑PD-1 expression
EGFR activation	↑T-Reg	Activation of alternative inhibitory receptors:
DNMT3A mutation		TIM3, TIGIT, and LAG3
↓ Antigen processing genes		Immunesenescense: T cells (CD28-CD57+KLRG1+).
	Resulting effect	
Abnormal signaling	Immunosuppression	↓ Anti-tumor response to ICI

ICI, Immune-checkpoint inhibitors; VEGF, Vascular Endothelial Growth Factor; EGFR, Epidermal Growth Factor Receptor; DNMTA3A, DNA methyltransferase 3A T-cell; TIM3, T-cell immunoglobulin and mucin domain-containing protein 3; TIGIT, T cell immunoglobulin and ITIM domain; LAG3, Lymphocyte activation gene-3; KLRG1, killer-cell lectin like receptor G1. ↑, Increase; ↓, Decrease.

recurrence noted in those who had developed HYD (18). In NSCLC patients, those with a higher metastatic burden at the time of treatment were more likely to develop HYD, although the mechanism behind this is unclear (6).

As previous data have indicated, amplification of MDM2 and alterations of EGFR are associated with an increased risk of HYD. NGS evaluation of patients with hyperprogression revealed MDM2/MDM4 amplification in 6 different patients (16). Additional data support the association between copy number alterations in MDM2/MDM4, as well as EGFR and several chromosome 11 alterations, and HYD (15). The study by Kato et al. also noted DNA methyltransferase 3A (DNMT3A) alterations as an independent predictor of poorer clinical outcomes with ICI therapy (16). Additionally, previous studies seem to suggest a possible role for other markers such as lactate dehydrogenase (LDH) and derived neutrophil to lymphocyte ratio (37), although this has not been reliably replicated in all studies.

While previous data has largely included multiple solid tumor subtypes in the analysis of hyperprogression, more data specific to NSCLC patients has been elucidated. A recent systematic review and meta-analysis compared 6 studies with 1389 NSCLC patients and identified five different factors significantly associated with the risk of HYD, including an Eastern Cooperative Oncology Group (ECOG) score greater than 1, Royal Marsden Hospital (RMH) score of two or higher, serum LDH greater than the upper limit of normal, more than two metastatic sites, and presence of liver metastasis (38). Ferrara et al. demonstrated, as previously stated, an increased risk of HYD in patients with a higher number of metastatic sites, but no correlation between age, LDH, neutrophil to lymphocyte ratio, or MDM2 or EGFR mutations (6).

The development of HYD is largely associated with a poorer prognosis in the available literature. Early data from Champiat et al. revealed an mOS of 4.6 months in patients with hyperprogression (vs. mOS of 7.6 months in those without), with another study by Kim et al. showing an mOS of 50 days in patients with HYD (vs. 205 days in those without) (5, 8). In the NSCLC-specific population in the data by Ferrara et al, HYD was associated with a particularly poor survival if it developed within the first 6 weeks after starting ICI therapy (3.4 months vs 6.2 months) (6).

Conclusion

Since the advent and adoption of ICI therapy in the treatment of advanced NSCLC, multiple studies have shown significant improvements in outcomes for these patients (3, 4), but occasionally patients can develop a paradoxical rapid acceleration of tumor growth labeled as HYP. HYP remains a challenge in patient management for the oncology physician due to variable definition, lack of an easily measurable biomarker, and HYD's implications for therapeutic choice and outcomes for patients. The debate about which criteria should be adopted among time-dependent or size-related variables is ongoing. A selected combination of these criteria may be used in a universal definition of HYD in the future. Further research into the mechanism of HYD in T cell regulation, changes in the tumor microenvironment, and genomic changes could eventually lead to the identification of a potential biomarker of HYD. This could complement subjective criteria like clinical parameters and settle cases that are in doubt. While the body of literature is increasing, there is a relative dearth of high-quality data related to hyperprogression, as the majority of studies are limited to retrospective reviews. Therefore, the development of universal HYD definition criteria and identification of a reliable biomarker will be paramount to establish HYD as a formal entity recognized by academic oncologists and governing agencies and allow for uniform diagnosis to be applied in prospective clinical trials. This will spur the design of therapeutic investigations that will guide the future management of HYD and change the trajectory of HYD in the field of immune-oncology.

Author contributions

CHH review and editing of manuscript, conceptualization of the review, tables and figures, and literature review. AB literature

review, draft of manuscript and literature review. CH review and editing, creation of figures. All authors contributed to the article and approved the submitted version.

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Current and novel therapeutic strategies for optimizing immunotherapy outcomes in advanced non-small cell lung cancer

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During the past decade, immunotherapy has dramatically improved the outcomes of patients with non-small cell lung cancer (NSCLC). The development of specific antibodies against the programmed death (PD1) receptor and its ligand PD-L1 (programmed death ligand-1) has demonstrated substantial efficacy in advanced NSCLC either in the first or in the second line. However, the success of immune checkpoint inhibitors (ICIs) as monotherapy did not reach all patients and long-term responders still represent a small subset of cases. Under these circumstances, different strategies have been and are being tested to optimize clinical outcomes. Here, we reviewed the current evidence and the more promising perspectives of ICI combination approaches, such as the addition of chemotherapy, antiangiogenic agents, other co-inhibitory or co-stimulatory checkpoints, and targeted therapies.

KEYWORDS

non-small-cell lung cancer, immunotherapy, immune checkpoint inhibitors, combinations, chemotherapy, antiangiogenic, co-inhibitory

Introduction

During the past decade, the advent of immunotherapy has dramatically changed the outcomes of patients with non-small cell lung cancer (NSCLC) (1). The growing understanding of the environment in which tumor and immune cells interact led to the discovery of immune checkpoint inhibitors (ICIs) that block inhibitory pathways that physiologically control the immune response driving to restore and sustain the immune system against cancer cells (2).

Under this circumstance, the development of specific antibodies against the programmed death (PD1) receptor and its ligand PD-L1 (programmed death ligand-1) has led to a change of paradigm in the therapeutic strategies of advanced NSCLC either in the first- or in the second-line setting. Importantly, these drugs have unprecedented prolonged survival for a substantial proportion of these patients (3). However, not all NSCLCs respond appropriately to ICI as monotherapy, and long-term responders still represent a limited group that is challenging to find and predict. The objective response rate when using first-line single-agent ICI treatment is below 45% in highly biomarker-selected NSCLC patients such as PD-L1 expression (4). Furthermore, 40% to 60% of patients experienced disease progression within the first 6 months of treatment. Of note, this situation differs substantially from those reported for the efficacy of targeted therapy in oncogene-addicted NSCLC (5).

In this context, we are now in a race to find different strategies to optimize the efficacy of immunotherapy in lung cancer. The recent understanding of *de novo* or adaptive resistance, as well as the mechanisms involved in the induction of an effective antitumor immune response, provides the rationale for several established and novel ICI combination approaches such as the addition of chemotherapy, antiangiogenic agents, other immunotherapy, or targeted therapies. Here, we reviewed the current evidence and the more promising perspectives in this field.

First-line combinations with chemotherapy

It has been demonstrated that modulation of the immune response through PD-1 inhibition may be enhanced by the synergistic immunogenic effects of cytotoxic chemotherapy by different mechanisms, including increasing the potential for antigen cross-presentation by dendritic cells after the destruction of tumor cells, induction of proinflammatory cytokines, inhibition of myeloid-derived suppressor cells, and induction of PD-L1 expression on tumor cells (6–10). Following this rationale, the combination of chemotherapy plus ICI has been tested in several NSCLC phase III clinical trials in the first-line setting. Notably, this approach has shown substantial efficacy when compared with platinum-based chemotherapy in unselected PD-L1 expression for both histology tumors among phase III clinical trials in the first-line scenario (Figure 1) (11–22). The addition of chemotherapy to ICI reported global overall response rates (ORRs) between 45% and 75%. Across all the trials, the immune-chemotherapy strategy significantly prolonged the median progression-free survival (PFS) compared with chemotherapy, showing safety and a generally manageable toxicity profile. However, overall survival (OS) improvement was not consistent in all the studies. Impower-131 and Impower-132 trials did not demonstrate a statistically significant difference in the intention-to-treat OS analysis,

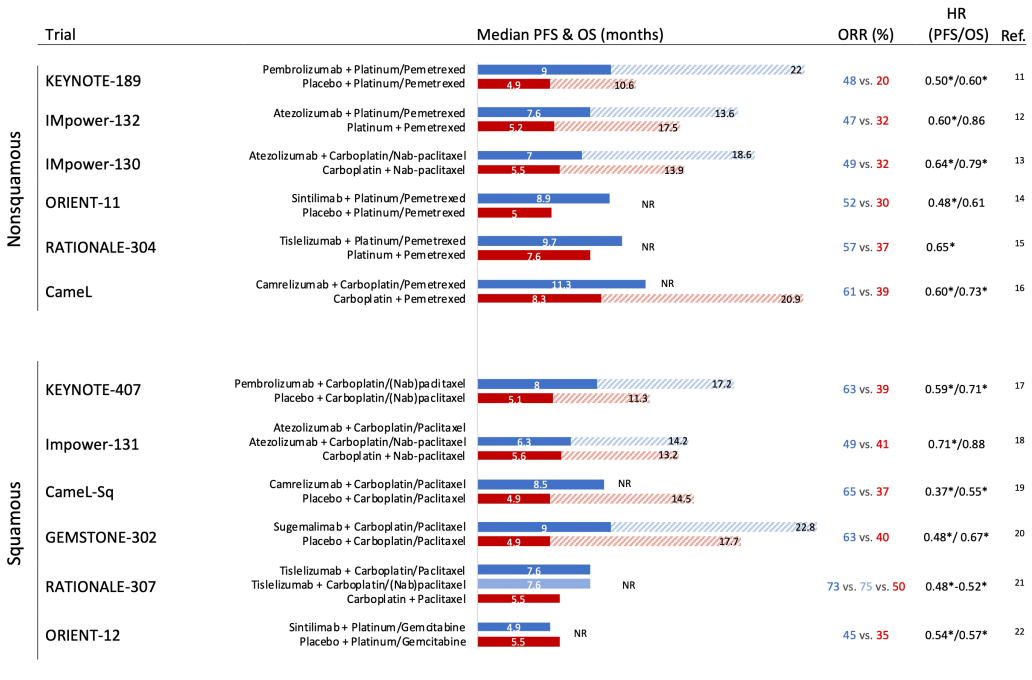

FIGURE 1
Phase III trials assessing an immune checkpoint inhibitor + chemotherapy strategies in the first-line setting in nonsquamous and squamous non-small cell lung cancer with outcomes. HR, hazard ratio; OS, overall survival; PFS, progression-free survival; ORR, overall response rate; NR, not reached (overall survival). * Significant improvement.

potentially explained by subsequent second-line treatments, percentage of PD-L1 tumor expression, patient population selection, overperformance of comparators arms, and possible differences across PD-1 and PD-L1 treatments.

First-line immunotherapy combinations

PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are complementary coinhibitory receptors that modulate T-cell responses (23). Thus, using antibodies to blockade both receptors simultaneously has been fruitful in many tumor types, including melanoma, renal cell carcinoma, malignant pleural mesothelioma, esophageal squamous cell carcinoma, microsatellite instability-high colorectal cancer, hepatocellular carcinoma, and NSCLC (24–30).

The promising results in phase I and II trials using anti-PD-1 plus anti-CTL-4 antibodies led to the evaluation of this dual strategy alone or in combination with chemotherapy in the advanced NSCLC first-line scenario (Figure 2). Phase III Checkmate 227 investigated the efficacy of nivolumab alone or in combination with chemotherapy or ipilimumab as first-line therapy in stage IV or recurrent patients with NSCLC. The randomization was performed according to PD-L1-positive or -negative. In both groups, nivolumab plus ipilimumab significantly improved OS compared with chemotherapy alone. Of note, nivolumab plus ipilimumab showed numerically better efficacy compared with nivolumab monotherapy in patients with tumors with PD-L1 expression ≥ 1% and PD-L1 ≥ 50% (30). In this specific exploratory analysis, tumors with PD-L1 ≥ 50% presented 4-year OS rates of 37%, 26%, and 20% with nivolumab plus ipilimumab, nivolumab alone, and chemotherapy alone, respectively.

Notably, in the phase III MYSTIC trial, the combination of durvalumab (anti-PD-L1) plus tremelimumab (anti-CTLA-4) could not improve OS against chemotherapy in PD-L1 ≥ 25% first-line advanced NSCLC (31).

To mitigate the inferior outcomes during the first months when using PD-1 plus CTLA-4 blockade, two trials evaluated the addition of chemotherapy to this regimen. The phase III CheckMate-9LA tested nivolumab plus ipilimumab plus two cycles of chemotherapy demonstrating a significant PFS and OS improvement versus chemotherapy alone in treatment naïve, stage IV, or recurrent NSCLC (Figure 2) (32). Similarly, the POSEIDON trial also reported superiority in terms of OS and PFS with first-line durvalumab plus tremelimumab plus chemotherapy versus chemotherapy alone in a recent press release announced (33).

Combinations with antiangiogenics

Angiogenesis and immunosuppression are both physiological mechanisms involved in nonpathological tissue repair that can be

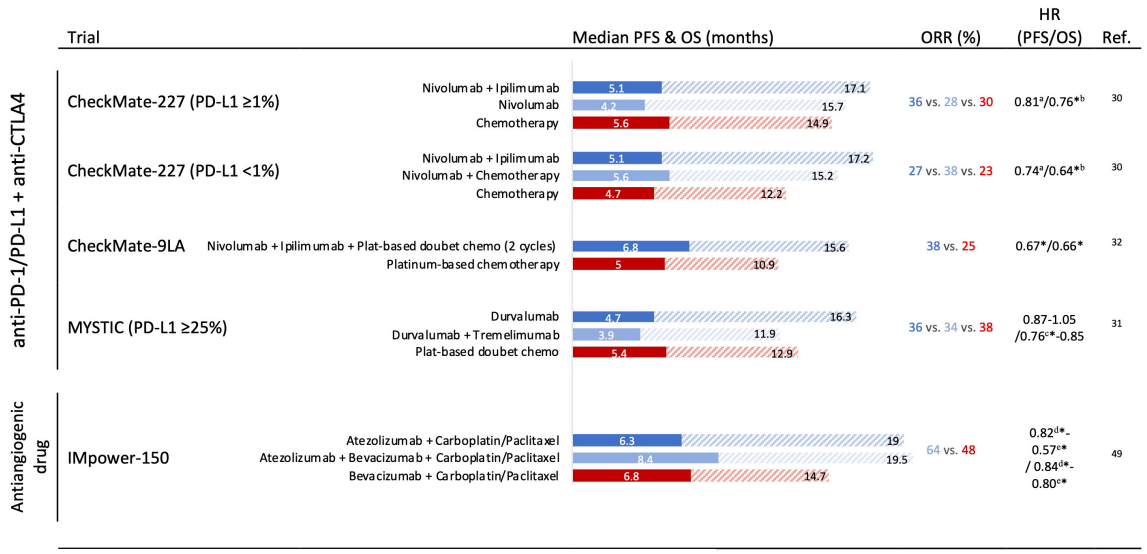

FIGURE 2
Phase III trials assessing immune checkpoint inhibitor combination and antiangiogenic drug combination strategies in the first-line setting in non-small cell lung cancer with outcomes. HR, hazard ratio; OS, overall survival; PFS, progression-free survival; ORR, overall response rate. * Significant improvement. [a] Significantly improvement of PFS in patients with a high tumor mutational burden (≥10 mutations per megabase). [b] Nivolumab + ipilimumab vs. chemotherapy. [c] Durvalumab vs. chemotherapy. [d] Atezolizumab + carboplatin + paclitaxel *vs.* bevacizumab + carboplatin + paclitaxel. [e] Atezolizumab + bevacizumab + carboplatin + paclitaxel vs. bevacizumab + carboplatin + paclitaxel.

taken advantage of by cancer development and progression (34). Several pro-angiogenic molecules, such as the vascular endothelial growth factor (VEGF), have been linked to a range of immunosuppressive effects at successive steps in the cancer immunity cycle, such as antigen presentation, T-cell priming, T-cell trafficking, and T-cell tumor infiltration (35).

Although blood vessel formation within solid tumors is necessary for cancer survival, tumor abnormal vasculature is characterized by dilated and fragile vessels, which result in leaking, hypoxia, acidosis, and high interstitial pressure. The normalization of this vasculature by specific therapies, such as chemotherapy, irradiation, or especially anti-VEGF antibody, leads to increased T-cell infiltration and therefore enhances tumor immunogenicity (36).

Otherwise, multi-kinase inhibitors such as lenvatinib, cabozantinib, and axitinib, with a preferential antiangiogenic activity, have reported efficacy in combination with anti-PD-1/L1 ICI in some tumor models including renal cell carcinoma and endometrial cancer (37–41). Additionally, bevacizumab plus atezolizumab resulted in positive outcomes in systemic treatment-naive and unresectable hepatocellular carcinoma (42). Although all this evidence supports the combination of ICI and antiangiogenic agents as a successful strategy for some tumor models, previous limited phase I and II trials using this approach reported modest activity in NSCLC (43, 44).

In NSCLC, some trials such as the phase III LEAP-006 evaluate the combination of chemotherapy plus pembrolizumab and lenvatinib in first-line nonsquamous tumors. Preliminary results of the open-label safety run-in (part 1) showed a promising ORR of 69.2% among 13 evaluated patients (45). Additionally, the phase II WJOG @Be study reported encouraging results when testing atezolizumab with bevacizumab for advanced treatment-naive nonsquamous NSCLC with PD-L1 expression ≥50%. In this trial, ORR was 64.1% and median PFS was 15.9 months (46).

Moreover, the phase II Lung-MAP S1800A study testing ramucirumab plus pembrolizumab versus standard of care chemotherapy ± ramucirumab for advanced NSCLC previously treated with immunotherapy demonstrated a significant OS improvement with the combination, whereas no differences were observed in PFS and ORR (22% vs. 28% in combination and standard of care, respectively) (47). Similarly, results from the phase Ib COSMIC-021 were modest when comparing cabozantinib plus atezolizumab (cohort 7) or cabozantinib alone (cohort 20) in patients with advanced NSCLC previously treated with ICIs. In this study, ORR and median PFS were respectively 19% and 4.5 months with the combination, versus 6% and 3.4 months with cabozantinib alone (48).

To date, the most promising was the combination of ICI with antiangiogenic agents and doublet chemotherapy (Figure 2). The phase III Impower-150 compared atezolizumab–bevacizumab carboplatin–paclitaxel (ABCP) or atezolizumab–carboplatin–paclitaxel (ACP) versus bevacizumab–carboplatin–paclitaxel (BCP) in nonsquamous metastatic NSCLC. In the intention-to-treat populations, ABCP showed superior PFS and OS compared to BCP (HR 0.57 [0.48–0.67]) and OS (19.5 months vs. 14.7 months; HR 0.80 [0.67–0.95]) (49). However, no differences were observed between ACP and BCP arms. Interestingly, an exploratory analysis showed an OS improvement with ABCP versus BCP in special subgroups with low benefit from ICI monotherapies, such as sensitizing *EGFR* mutations (HR 0.60 [0.31–1.14]), and patients with baseline liver metastases (HR 0.52 [0.33–0.82]) (50).

Newly emerging co-inhibitory and co-stimulatory checkpoints

The positive clinical impact when using the combination of anti-CTLA-4 and anti-PD-L1 has driven the investigation of other promissory ICI combinations that may increase efficacy. Importantly, resistance to immunotherapy is associated with loss of immunogenic neoantigens, an increase of immunosuppressive cells, and upregulation of alternate immune checkpoint receptors (51). As a consequence, this provides a potential opportunity for novel emerging co-inhibitory and co-stimulatory immune checkpoints.

TIGIT

T-cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) is an encouraging new target for cancer immunotherapy. TIGIT is upregulated by immune cells, including activated T cells, natural killer cells, and regulatory T cells. TIGIT binds to two ligands (CD155 and CD112) that are expressed by tumor cells and antigen-presenting cells in the tumor microenvironment (52). Furthermore, TIGIT is coexpressed with PD-1 on exhausted T cells supporting a strong rationale for the dual blockade in restoring T-cell immunity (53). This double inhibition synergizes the proliferation and function of antitumor CD8 T cells, resulting in protective memory T cells and complete tumor rejection (53–55).

Several anti-TIGIT candidate drugs are in development in clinical trials, but tiragolumab is the most advanced. The phase II CITYSCAPE study evaluated tiragolumab plus atezolizumab versus placebo plus atezolizumab as first-line treatment in patients with PD-L1-positive EGFR/ALK wild-type locally advanced or metastatic NSCLC. A higher efficacy was shown with the combination compared with atezolizumab monotherapy (ORR 37% versus 21%, and PFS HR 0.58 [0.39 to 0.88]) (56). A particular benefit was observed in those tumors with PD-L1 ≥ 50% (ORR 66% for combination versus 24% for atezolizumab alone). These findings supported the ongoing phase III SKYSCRAPER-01 with a similar drug arms design,

for patients with PD-L1-high locally advanced or metastatic NSCLC. Unfortunately, a recent press release revealed that this trial did not meet the co-primary PFS end point (57).

In addition, a phase I study testing vibostolimab (other anti-TIGIT) showed an ORR of 26% when combined with pembrolizumab in anti-PD-1/PD-L1-naive patients with NSCLC, but minimal efficacy in the anti-PD-1/PD-L1 refractory cohort (ORR 3%) (58).

These results highlight that single anti-TIGIT agents seem not to be an effective strategy, whereas the coadministration with an anti-PD-1/PD-L1 or especially with chemotherapy may be useful and needs to be tested in ongoing clinical trials (NCT04619797, NCT04513925, NCT0495881, NCT04738487, NCT04725188, NCT05226598, NCT05298423, and NCT04165070).

LAG-3

The transmembrane protein Lymphocyte-activation gene 3 (LAG3, CD223) is an immune inhibitory checkpoint and is expressed on the surface of lymphocytes, such as $CD4^+$ T cells, $CD8^+$ T cells, natural killer (NK) cells, NK T (NKT) cells, and regulatory T (Treg) cells, which appear when T cells are activated (59–62). The intracellular signaling pathways of LAG3 play a role in the regulation of immune cell function as the coexpression of LAG3 with other inhibitory molecules, including PD-1, TIGIT, TIM-3, 2B4, and CD160, inhibits the tumor immune microenvironment by accelerating T-cell exhaustion and blocking T-cell proliferation (63). The high expression of LAG3 has been associated with unfavorable clinical outcomes in various tumor types including NSCLC (64–66). Furthermore, ICIs can induce resistance through the activation of additional immune checkpoints such as LAG-3 (67).

Since LAG-3 and PD-1 are complementary inhibitory immune checkpoints, dual LAG-3/PD-1 blockade provided a consistent rationale for predicting clinical benefits. In this sense, the combination of the LAG-3-blocking antibody relatlimab and nivolumab has recently revealed a greater benefit in metastatic or unresectable melanoma in the phase II to III RELATIVITY-047 trial (68).

In lung cancer, the combination of eftilagimod alpha, a soluble LAG-3 protein that mediates antigen-presenting cell and CD8 T-cell activation, with pembrolizumab was tested in PD-L1 unselected metastatic NSCLC in the first-line setting (phase II TACTI-002 trial). Among the 36 patients included, response rates by different PD-L1 subgroups were 27% for patients with tumor proportion score (TPS) <1%, 39% for TPS ≥1%, and 54% for ≥50% TPS. Median PFS was 8.2 months while the median OS was not yet reached (69).

Following the favorable evidence in melanoma, current ongoing clinical trials are investigating safety and efficacy of anti-LAG3 drugs in NSCLC (NCT04623775, NCT04205552, NCT04140500, NCT03219268, NCT03365791, NCAGN02385, NCT03849469, NCT02750514, NCT02465060, NCT03780725, NCT03516981, NCT02460224, NCT03250832, NCT01968109, NCT03005782, NCT02966548, and NCT03459222).

VISTA

V-domain Ig suppressor of T-cell activation (VISTA) is a protein capable of acting as both a ligand and a receptor. VISTA suppresses T-cell proliferation and reduces cytokine production, including IL-10, TNF-α, and IFN-γ (70). Therefore, VISTA blockade can potentially enhance antitumor immune responses. In a phase II pan tumor trial, an oral dual blocker anti-VISTA and PD-L1 agent (CA-170) showed a clinical benefit of 75% and a median PFS of 19.5 weeks among eight previously treated nonsquamous NSCLC patients (71). Of note, several VISTA-targeting inhibitors are being tested in phase I and II trials in patients with metastatic or unresectable solid tumor malignancy including NSCLC (NCT05082610, NCT02671955, and NCT02812875).

TIM-3

TIM-3 is another inhibitory immune checkpoint molecule similar to CTLA-4 and PD-1. Interaction of TIM-3 with its ligands has been shown to induce T-cell inhibition (72, 73). Interestingly, TIM-3 overexpression has been associated as a negative prognostic marker in NSCLC patients (74). Since the discovery of the negative impact on the immune system by upregulated TIM-3 and PD-L1 coexpression in melanoma, a combination blockade strategy was proposed to restore the T-cell exhaustion (75). The only current clinical data available are a preliminary analysis from the phase I AMBER trial, which included 39 patients with NSCLC who had progressed following initial anti-PD-1 treatment and were tested to receive the anti-TIM-3 antibody cobolimab alone, and in combination with the anti-PD-1 dostarlimab. Of the 20 patients who received the higher dose of cobolimab and were evaluable for response, 3 (15%) had confirmed partial responses and 8 (40%) had stable disease. Notably, all objective responses were among patients with PD-L1 TPS ≥1 (76). Other investigational agents targeting TIM-3 are presently being evaluated in ongoing phase I and II clinical trials enrolling NSCLC patients (NCT03708328, NCT04931654, NCT03652077, NCT03307785, NCT02608268, NCT03099109, NCT03744468, and NCT02817633).

Co-stimulation

Co-stimulatory immune molecules promote T-cell activation and antitumor immunity. Agonist antibodies against

co-stimulatory molecules such as 4-1BB (CD137), OX40 (CD134), and ICOS (CD278) are being investigated in combination with anti-PD-1 agents. However, to date, prohibitive toxicity profiles and modest responses were observed in phase I multi-tumor trials including advanced NSCLC patients (77–82).

Oncolytic viruses

Oncolytic virus therapy is a novel strategy that promotes immune activation *via* targeted immunogenic cell death. The most developed oncolytic virus T-VEC demonstrated interesting efficacy by injecting intratumorally in patients with melanoma in a phase III study, which led to FDA approval in 2015 (83). However, limited studies evaluated this strategy in lung cancer. Phase Ib KEYNOTE-200 investigated the intravenously delivered oncolytic virus Coxsackievirus A21 (CVA21, CAVATAK) in combination with pembrolizumab in advanced NSCLC and bladder cancer, demonstrating encouraging overall responses of 23% and 33% in 31 ICI-naïve and 21 EGFR/ALK mutation-negative NSCLC patients, respectively (84).

Targeted therapy

Primarily, the presence of specific oncogene-addicted driver mutations and co-mutations, such as *STK11* and *KEAP1*, has been previously linked to a negative impact on ICI efficacy in NSCLC (85–87).

Preclinical data demonstrated that KRAS-G12C inhibition drives antitumor immunity by enhancing the tumor microenvironment with CD8 T cells, macrophages, and CD103 cross-presenting dendritic cells (88). Consequently, the recent development of direct KRAS-G12C inhibitors has gained interest in the utility of combining KRAS inhibition with immunotherapy, especially for PD-1 refractory *KRAS-STK11* and *KRAS-KEAP1* co-mutated advanced NSCLC. As a consequence, multiple ongoing clinical trials are evaluating KRAS-G12C inhibitors in combination with ICI (NCT03600883, NCT04613596, NCT04449874, NCT04699188, and NCT03785249).

Moreover, based on data from The Cancer Genome Atlas, lung cancer exhibits high levels of homologous recombination deficiency associated with particular mutational signatures. Given these findings, several studies are evaluating PARP inhibitors in combination with chemotherapy and PD-1 blockade in first-line NSCLC (NCT03976323, NCT03976362, and NCT04475939) (89). However, the toxicity profile may still represent a limitation for these combinations since grade ≥3 treatment-emergent adverse events occurred in 88.2% of cases in the phase II JASPER trial evaluating first-line niraparib plus pembrolizumab in patients with advanced NSCLC (90).

Discussion

ICIs have opened a new era in cancer treatment and particularly for lung cancer. The unprecedented efficacy in NSCLC has begun to resonate with the question of whether the possibility of a cure, at least for a still small subset of patients with advanced disease, is closer. Strong progress has been made in this field, and new challenges for the coming years will be the focus on improving efficacy through a long-term durable response for a larger group of patients. In the course of optimizing the clinical outcomes of ICI in NSCLC, some important steps have substantially impacted patients' survival, such as the combination of anti-PD-1/L1 with chemotherapy, another ICI, and antiangiogenic agents. Today, multiple strategies are being tested with promising results, from adding different co-inhibitory and co-stimulatory checkpoints, to the combination of ICI with targeted therapy to synergize the anticancer effect.

Altogether this progress was led by a deeper understanding of the defects or alterations in the complex biological relationship processes between the tumor, the microenvironment, and the host, as well as broader insights into the mechanism underlying the resistance of ICI. Regarding the tumor cell-intrinsic features, some areas are of crucial interest beyond the PD-L1 expression as the most studied biomarker in the immunotherapy field. In this context, the study of somatic mutations in the cancer genome that increase tumor mutational and neoantigen burdens has been strongly related to the efficacy of ICI (91). Additionally, multiple efforts are being made to properly characterize the deficiency in neoantigen presentation, aberrations in oncogenes and tumor suppressor genes that regulate immune response (e.g., KRAS, STK11/KEAP1), and the study of genetic alterations in DNA replication and repair genes, epigenetic modulation, and alterations in the interferon-gamma (INF-g) signaling cascade (92). Furthermore, the feature of the tumor microenvironment is now of remarkable interest and is being associated with ICI activity, including the investigation of the phenotype of T-infiltrating lymphocytes, tumor-infiltrating B cells, tertiary lymphoid structures, tumor-associated macrophages, cancer-associated fibroblasts, and endothelial cells. Finally, active investigations are focusing on a comprehensive understanding of the host-related characteristics. Multiple studies have associated the gut microbiome, patient concomitant medications, and autoimmunity with ICI response and/or toxicity (92).

Certainly, as research grows rapidly in this field, the challenge of designing rational and synergistic ICI combination approaches will lead to a lower risk of resistance and prolonged benefits for patient outcomes.

Author contributions

All authors contributed to the article and approved the submitted version.

Conflict of interest

CM works as a speaker in Roche, MSD, BMS, Boehringer Ingelheim, Astra Zeneca, and Pfizer.

The remaining author declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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