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Advancements and cutting-edge approaches to counteract the inefficacy of immune checkpoint inhibitor therapies in lung cancer

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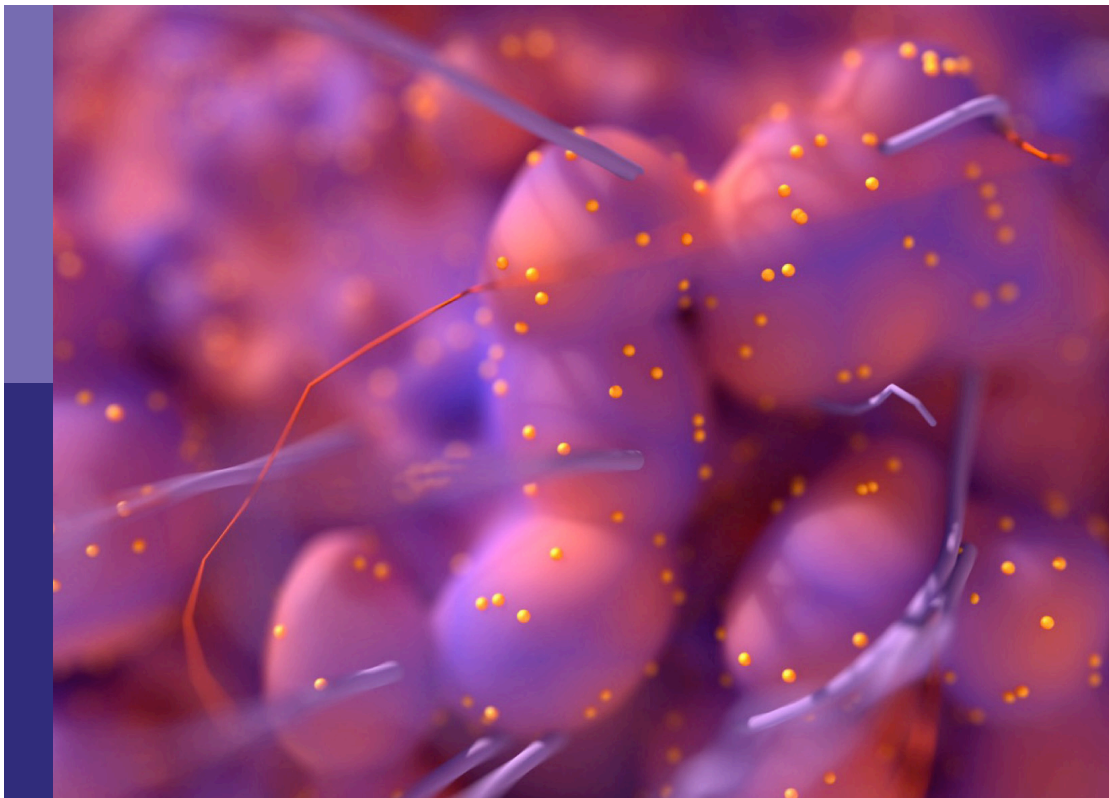
Published in
Frontiers in Oncology
Frontiers in Immunology



ISSN 1664-8714
ISBN 978-2-8325-6279-6
DOI 10.3389/978-2-8325-6279-6

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Advancements and cutting-edge approaches to counteract the inefficacy of immune checkpoint inhibitor therapies in lung cancer

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Citation

Han, G., Pei, G., Wang, Z., eds. (2025). *Advancements and cutting-edge approaches to counteract the inefficacy of immune checkpoint inhibitor therapies in lung cancer*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6279-6

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frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 22 September 2023
DOI 10.3389/fonc.2023.1273478

OPEN ACCESS

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RECEIVED 06 August 2023
ACCEPTED 07 September 2023
PUBLISHED 22 September 2023

CITATION
Althoff FC, Schäfer LV, Acker F, Aguinarte L, Heinzen S, Rost M, Atmaca A, Rosery V, Alt J, Waller CF, Reinmuth N, Rohde G, Saalfeld FC, Becker von Rose A, Möller M, Frost N, Sebastian M and Stratmann JA (2023) Survival benefit with checkpoint inhibitors versus chemotherapy is modified by brain metastases in patients with recurrent small cell lung cancer. *Front. Oncol.* 13:1273478. doi: 10.3389/fonc.2023.1273478

Survival benefit with checkpoint inhibitors versus chemotherapy is modified by brain metastases in patients with recurrent small cell lung cancer

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Introduction: Small cell lung cancer (SCLC) is a rapidly growing malignancy with early distant metastases. Up to 70% will develop brain metastases, and the poor prognosis of these patients has not changed considerably. The potential of checkpoint inhibitors (CPI) in treating recurrent (r/r) SCLC and their effect on brain metastases remain unclear.

Methods: In this retrospective multicenter study, we analyzed r/r SCLC patients receiving second or further-line CPI versus chemotherapy between 2010 and 2020. We applied multivariable-adjusted Cox regression analysis to test for differences in 1-year mortality and real-world progression. We then used interaction analysis to evaluate whether brain metastases (BM) and/or cranial radiotherapy (CRT) modified the effect of CPI versus chemotherapy on overall survival.

Results: Among 285 patients, 99 (35%) received CPI and 186 (65%) patients received chemotherapy. Most patients (93%) in the CPI group received nivolumab/ipilimumab. Chemotherapy patients were entirely CPI-naïve and only one CPI patient had received atezolizumab for first-line treatment. CPI was associated with a lower risk of 1-year mortality (adjusted Hazard Ratio [HR_{adj}]

0.59, 95% CI 0.42 to 0.82, p=0.002). This benefit was modified by BM and CRT, indicating a pronounced effect in patients without BM (with CRT: HR_{adj} 0.34, p=0.003; no CRT: HR_{adj} 0.50, p=0.05), while there was no effect in patients with BM who received CRT (HR_{adj} 0.85, p=0.59).

Conclusion: CPI was associated with a lower risk of 1-year mortality compared to chemotherapy. However, the effect on OS was significantly modified by intracranial disease and radiotherapy, suggesting the benefit was driven by patients without BM.

KEYWORDS

small cell lung cancer, recurrent disease, metastatic disease, brain metastases, systemic treatment, checkpoint inhibitors, brain irradiation

1 Introduction

Small cell lung cancer (SCLC) is an aggressive and rapidly growing malignancy with early metastases. Among the 70% of patients presenting with extensive disease at initial diagnosis, the 5-year overall survival remains less than 5% (1). While response rates to first-line platinum plus etoposide chemotherapy are as high as 60 to 70% in patients with extensive disease, data have demonstrated early disease recurrence (2). For refractory or relapsed (r/r) SCLC, treatment options are scarce (3).

Over the last decade, clinical trials have evaluated a variety of novel agents for the treatment of SCLC. In the IMpower-133 and CASPIAN phase 3 trials, the addition of checkpoint inhibitors (CPI) to platinum-based chemotherapy modestly improved overall survival (OS), leading to an approval of atezolizumab and durvalumab for first-line treatment in combination with platinum and etoposide (4, 5). Moreover, the Chinese phase 3 trials ASTRUM and CAPSTONE-1 have confirmed an OS benefit by adding the CPI serplulimab and adebrelimab, respectively, to first-line platinum/etoposide (6, 7). For patients with r/r SCLC, further trials such as CheckMate032, KeyNote158, and KeyNote028 evaluated the use of CPI in second or further-line treatment regimen (8–10). In this pre-treated, CPI-naïve setting, results have been inconclusive, and a potential survival benefit of CPI over chemotherapy could not be demonstrated. As a consequence, temporary FDA approvals of nivolumab and pembrolizumab for pre-treated SCLC patients were withdrawn in early 2021.

Importantly, every fifth patient presents with brain metastases (BM) at disease onset and an additional 50% will develop BM during the course of their disease (1, 11). BM are particularly challenging due to often detrimental effects on the patient's performance status and their poor response to systemic agents with limited penetration of the blood-brain barrier, resulting in a significant shorter median OS of 8.5 versus 12.6 months (5). Whole-brain radiotherapy (WBRT) still remains the standard treatment in these patients but is associated with a worsening quality of life and neurocognitive function (12). The potential of CPI in treating patients with r/r SCLC and their effect on BM remain unclear. In an exploratory analysis of the CASPIAN trial, the authors suggested the OS benefit was maintained irrespective of the presence or absence of BM (13).

In this retrospective multicenter cohort study, we hypothesized that treatment with CPI versus chemotherapy improved overall survival and real-world progression-free survival in r/r SCLC. We then evaluated whether brain metastases and/or cranial radiotherapy modified the effect of CPI versus chemotherapy on survival in this hard-to-treat patient population.

2 Materials and methods

2.1 Study design

We conducted a multicenter, retrospective cohort study to analyze the effect of second or further-line (≥2L) CPI versus chemotherapy on survival in adult patients with r/r SCLC. Patient data were obtained between 2010 and 2020 at 11 academic healthcare institutions across Germany, including university hospitals and specialized treatment centers. Patients were eligible for inclusion if they received treatment for refractory/recurrent, incurable, extensive disease SCLC. We included patients in the CPI group if they were treated with single or double CPI regimen. All patients had received at least one previous non-curative treatment line. Patients who received CPI within a clinical trial were excluded. Since CPI had not been approved by the European Medical Agency for the treatment of r/r SCLC, their therapeutic use was limited to cases where a funding request to cover the costs had been accepted by the health insurance provider. However, due to the limited treatment options available, requests for reimbursement were made on a regular basis as an individual therapeutic trial, as described in detail previously (14). The study was approved by the local ethics committee at the University Hospital Frankfurt, and a data use agreement was established between institutions (protocol number UCT-2-2020). Data were collected from electronic hospital-registry

databases and merged into a combined dataset after strict de-identification within the respective hospital network. This manuscript adheres to the STROBE guidelines for reporting observational studies (Supplemental Digital Content; Table S1) (15).

2.2 Primary and secondary analysis

We used a multivariable-adjusted Cox proportional hazards regression to investigate the effect of CPI versus chemotherapy on 1-year OS and real-world progression-free survival (rwPFS), respectively. Analyses started on the first day that the patient received the treatment (day 1 of the first cycle of CPI/chemotherapy) to avoid immortal time bias and ensure that all time intervals during which patients may have experienced the outcome were captured in the analysis. Analyses were adjusted for confounding variables based on literature review and clinical plausibility. Confounding variables included age (quintiles), sex, progressive disease within 180 days of first-line treatment, prior cranial radiotherapy (CRT), a history of brain metastases (BM), and liver metastases. Regarding the tumor staging at the time of this investigation, we present a homogenous cohort of patients with incurable, extensive, stage IV disease as all patients had previously shown tumor progression (r/r SCLC). We provide the UICC tumor staging at the time of initial diagnosis (Table 1), albeit this initial staging was considered to have no impact on the outcome of the recurrent disease. We did not assess co-existing malignancies as the SCLC and its metastases were judged as the major determinants of the prognosis even when multiple cancers exist. We tested for violation of the proportional hazards assumption and utilized the Cox regression model to estimate hazard ratios with 95% confidence intervals. Additionally, we performed univariate Kaplan-Meier analysis using logrank-test.

2.3 Effect modification by brain metastases and/or cranial radiotherapy

To investigate whether the effect of CPI versus chemotherapy on 1-year OS was modified by a patient's history of BM and/or cranial radiotherapy (CRT), we included an interaction term between the primary exposure and the individual patient's "CNS category" in the Cox regression model. For the interaction term "CNS category", patients were divided into eight groups by ≥2L treatment (CPI versus chemotherapy), BM (binary), and CRT (binary). Interaction analyses were performed across groups of the interaction term. Comparisons were made with the baseline group of patients who received chemotherapy, had no history of BM, and did not receive CRT. Subgroups are displayed in Table 1.

In addition, we analyzed a subgroup of patients where brain imaging was available within six weeks before treatment initiation to provide further information on intracranial progression.

2.4 Sensitivity analyses

In sensitivity analyses, we used a multivariable-adjusted standard logistic regression and marginal effects to estimate the adjusted risk of 1-year mortality per 100 patients across groups. In addition, we applied propensity score analyses to address the possibility of unbalanced confounding between patients receiving CPI versus chemotherapy for ≥2L treatment. Both inverse probability of treatment weighting and 1:1 propensity score matching was used to assess the robustness of the primary association to analytic approach. The propensity score for a patient was defined as the probability of receiving CPI versus chemotherapy, conditional on all covariates described for the primary analysis. Based on the estimated propensity score, patients were matched on a 1:1 basis using an algorithm with a caliper of 0.1 without replacement (16). This algorithm identifies matched pairs within a closeness range of 0.00001 of the propensity score. Only if no more patients are identified for matching, the program then selects pairs in a range of 0.0001, 0.001, 0.01, up to a range of 0.1. Variables were examined for residual imbalances. Matching effectiveness was evaluated by calculating standardized differences of confounding variables after propensity score adjustment. In the propensity score matched cohort, we used a logistic regression model on the primary outcome and included confounding variables with a standardized difference of more than 0.1 (17, 18). Additionally, we performed Cox regression and Kaplan-Meier survival analyses in the matched cohort. Moreover, we used propensity score estimates in an inverse probability of treatment weighting model (19). We further included additional confounding variables into the primary model such as best response to first line treatment (complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD)), the Eastern Cooperative Oncology Group (ECOG) performance status at start of ≥2L treatment, and the UICC staging at the time of initial diagnosis, respectively, to test for robustness of the primary analysis. Finally, we provide data on three-year survival along with a "number at risk"-table using Kaplan Meier analysis.

2.5 Statistical analyses

The primary outcome was 1-year overall survival (OS) following initiation of ≥2L treatment with CPI versus chemotherapy. The secondary outcome was 1-year rwPFS. Tumor response assessments were obtained in clinical routine and were performed without an independent review. The assumption of linearity between the outcome and continuous covariates was tested using scatter plots (Supplemental Digital Content; Figure S2). To adjust for non-linear relationships, continuous confounding variables were divided into quintiles. Cases with missing data required for statistical analyses were excluded using the complete-case approach. A two-sided p-value of <0.05 was considered statistically significant. Data analyses were performed using Stata (StataCorp LP, version 13.0).

10.3389/fonc.2023.1273478

TABLE 1 Baseline patient characteristics of the full study cohort across treatment groups.

Variables	Chemotherapy N=186 (65%)	Checkpoint inhibitor N=99 (35%)	P-value
Age (y), mean ± SD	62.6 ± 8.6	61.0 ± 9.3	0.16
Sex, female, n (%)	63 (33.9%)	37 (37.4%)	0.56
Smoking, n (%)			<0.001
Never smoker	2 (1.1%)	3 (3.0%)	
Smoker	77 (41.4%)	40 (40.4%)	
Ex-smoker	56 (30.1%)	47 (47.5%)	
n.a.	51 (27.4%)	9 (9.1%)	
Pack years, median (IQR)	40 (30, 50)	31 (20, 42)	0.009
Pathology, n (%)			0.37
SCLC	179 (96.2%)	95 (96.0%)	
LCNEC	7 (3.8%)	3 (3.0%)	
Not otherwise specified (nos)	0 (0%)	1 (1.0%)	
UICC staging at the time of initial diagnosis, n (%)			<0.001
IA	0 (0%)	1 (1.0%)	
IB	0 (0%)	2 (1.7%)	
IIA	2 (1.1%)	1 (1.0%)	
IIB	0 (0%)	1 (1.0%)	
III, nos	0 (0%)	3 (3.0%)	
IIIA	5 (2.7%)	13 (13.1%)	
IIIB	2 (1.1%)	9 (9.1%)	
IIIC	1 (0.5%)	5 (5.1%)	
IV, nos	147 (79.0%)	27 (27.3%)	
IVA	7 (3.8%)	10 (10.1%)	
IVB	22 (11.8%)	26 (22.3%)	
Extensive disease at the time of initial diagnosis, n (%)	175 (94.1%)	63 (63.6%)	<0.001
Drugs of first-line (1L), n (%)			0.008
Cisplatin/etoposide	108 (58.1%)	42 (42.4%)	
Carboplatin/etoposide	73 (39.2%)	56 (56.6%)	
Carboplatin/etoposide/atezolizumab	0 (0%)	1 (1%)	
n.a.	5 (2.7%)	0 (0%)	
Brain imaging prior to start of 1L, n (%)			<0.001
No brain imaging	65 (34.9%)	33 (33.3%)	
MRI	88 (47.3%)	64 (64.6%)	
CT	33 (17.7%)	2 (2.0%)	
Best response to 1L treatment, n (%)			<0.001
CR	2 (1.3%)	3 (3.4%)	
PR	16 (10.5%)	15 (16.9%)	
SD	52 (34.2%)	9 (10.1%)	

(Continued)

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TABLE 1 Continued

Variables	Chemotherapy N=186 (65%)	Checkpoint inhibitor N=99 (35%)	P-value
PD	82 (53.9%)	62 (69.7%)	
n.a.	34 (18.3%)	10 (10.1%)	
Progression on 1L within 365 days, n (%)	128 (68.8%)	75 (75.8%)	0.22
Progression on 1L within 180 days, n (%)	62 (33.5%)	32 (32.3%)	0.57
ECOG at start of ≥2L treatment, median (IQR)	1 (0, 1)	1 (1, 2)	0.10
Metastases at start of ≥2L treatment, n (%)			
Lung	26 (14%)	47 (47.5%)	<0.001
Liver	80 (43%)	43 (43.4%)	0.95
Adrenal glands	45 (24.2%)	23 (23.2%)	0.86
Bone	64 (34.4%)	25 (25.3%)	0.11
Brain	75 (40.3%)	41 (41.4%)	0.86
CNS category at start of ≥2L treatment, n (%)			0.79
No CRT, no BM	52 (28%)	30 (30.3%)	
No CRT, with BM	12 (6.5%)	9 (9.1%)	
With CRT, no BM	59 (31.7%)	28 (28.3%)	
With CRT, with BM	63 (33.9%)	32 (32.3%)	
Drugs of ≥2L treatment, n (%)			<0.001
Topotecan	110 (59.1%)	0 (0%)	
Carboplatin/etoposide	43 (23.1%)	0 (0%)	
Cisplatin/etoposide	10 (5.4%)	0 (0%)	
Adriamycin/cyclophosphamide/vincristin	12 (6.5%)	0 (0%)	
Epirubicin/cyclophosphamide/vincristin	4 (2.2%)	0 (0%)	
Docetaxel	2 (1.1%)	0 (0%)	
Mitomycin/gemcitabine/cisplatin	1 (0.5%)	0 (0%)	
Etoposide	1 (0.5%)	0 (0%)	
Alisertib/paclitaxel	1 (0.5%)	0 (0%)	
Other	1 (0.5%)	0 (0%)	
Nivolumab/ipilimumab	0 (0%)	92 (93.0%)	
Nivolumab	0 (0%)	7 (7.0%)	

BM, brain metastases; CPI, checkpoint inhibitor; CR, complete remission; CRT, cranial radiotherapy; ECOG, Eastern Cooperative Oncology Group; LCNEC, large cell neuroendocrine carcinoma; n.a., not available; nos, not otherwise specified; PD, progressive disease; PR, partial remission; SCLC, small cell lung cancer; SD, stable disease; 1L, first line of treatment; ≥2L, second or further-line treatment.
The UICC tumor staging refers to the time of initial diagnosis, while all included patients had r/r SCLC with incurable, extensive, stage IV disease at the time of this study.

3 Results

3.1 Study cohort

In total, 1703 patients with r/r SCLC were considered for inclusion, of which only 309 (18%) patients received second-line treatment. After application of the exclusion criteria, the final cohort consisted of 285 patients (Figure 1). 186 (65%) patients received chemotherapy and 99 (35%) received CPI for second or further-line treatment (≥2L) of r/r SCLC. In the CPI group, 26% of patients received CPI as second-line, 46% as third-line, 16% as fourth-line, 11% as fifth-line, and 1% as sixth-line treatment. The median number of prior treatment lines was 2 (IQR 1 to 3). No patient in the chemotherapy group had previously received a checkpoint inhibitor, while one patient in the CPI group had received atezolizumab in combination with carboplatin and etoposide for first-line treatment. Most patients in the CPI group received double-CPI, combining the PD-1 inhibitor nivolumab with the CTLA-4 inhibitor ipilimumab in

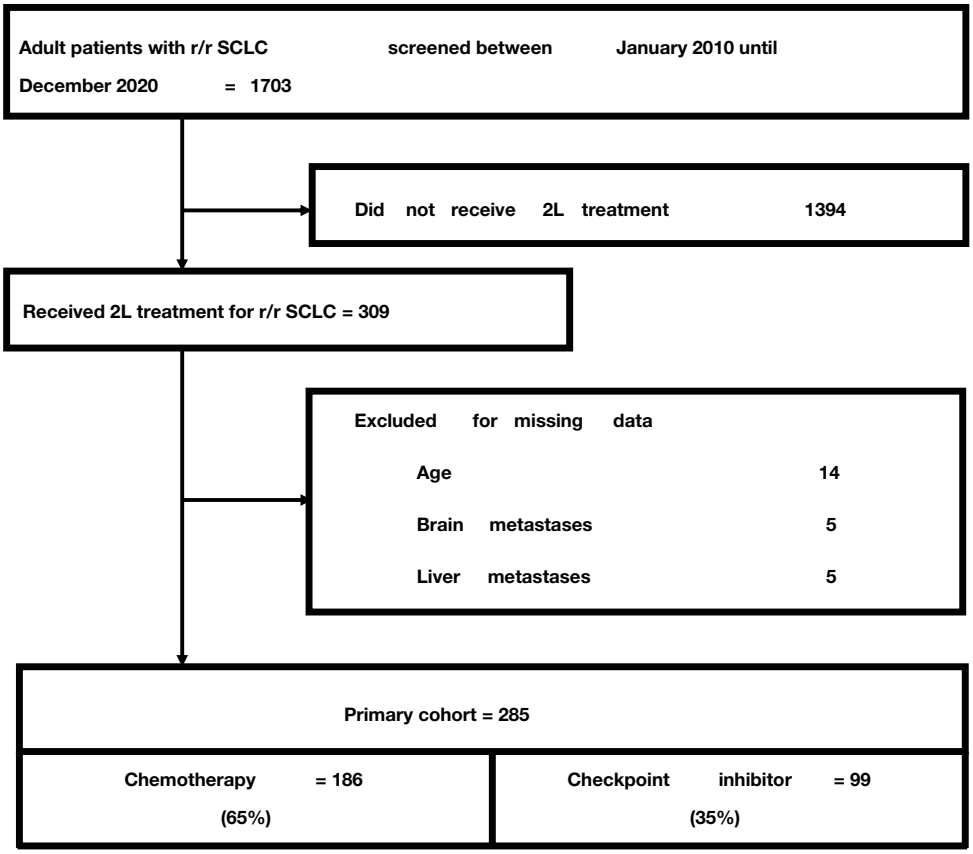

FIGURE 1
Study flow chart.

93% of cases, some patients received nivolumab monotherapy (7%). Treatment with chemotherapy most often included topotecan in 59.1% of patients, followed by carboplatin or cisplatin plus etoposide in 23.1% and 5.4%, respectively, ACO (adriamycin, cyclophosphamide, and vincristine) in 6.5%, and other chemotherapy regimen (Table 1). Patient characteristics and distribution of confounding variables by treatment groups are provided in Table 1. The median follow-up time was 30.8 months (95% confidence interval (CI) 23.3 to 38.3 months) according to the method provided by Schemper & Smith (20). The total range was 1 to 1404 days.

3.2 One-year overall survival

In total, 187 (65.6%) patients died within one year of initiation of ≥2L treatment, 138 (74.2%) with chemotherapy and 49 (49.5%) with CPI. The median OS was 6.3 months (95% confidence interval (CI) 5.4 to 7.9). CPI versus chemotherapy was associated with an improved 1-year overall survival in unadjusted (HR 0.60, 95% CI 0.44 to 0.84, p=0.003) as well as adjusted analyses (adjusted HR [HR_{adj}] 0.59, 95% CI 0.42 to 0.82, p=0.002). Survival curves from Kaplan Meier estimates (Log-rank test p=0.002) and Cox regression analysis are shown in Figures 2A, B. In the primary confounder model, brain metastases (HR 1.92, 95% CI 1.36 to 2.69, p<0.001) and liver metastases (HR 1.40, 95% CI 1.04 to 1.90, p=0.026) were independent risk factors of mortality, respectively, while a prior cranial radiotherapy was associated with a lower risk (HR 0.66, 95% CI 0.47 to 0.94, p=0.021).

3.3 One-year real-world progression-free survival

The median rwPFS was 2.9 months (95% CI 2.6 to 3.6). There was no difference in 1-year rwPFS between patients receiving CPI versus chemotherapy in unadjusted (HR 1.01, 95% CI 0.78 to 1.32, p=0.922) and adjusted analyses (HR_{adj} 1.02, 95% CI 0.78 to 1.34, p=0.884). Cox regression and Kaplan-Meier survival curves are displayed in Figures 2C, D.

3.4 Analyses on the role of brain metastases and/or cranial radiotherapy

The effect of CPI versus chemotherapy on 1-year OS was significantly modified by a patient's history of BM and/or prior CRT towards a more pronounced effect among patients without brain metastases. The strongest effect was observed in patients without BM who received CPI and a prior CRT (HR_{adj} 0.34, 95%

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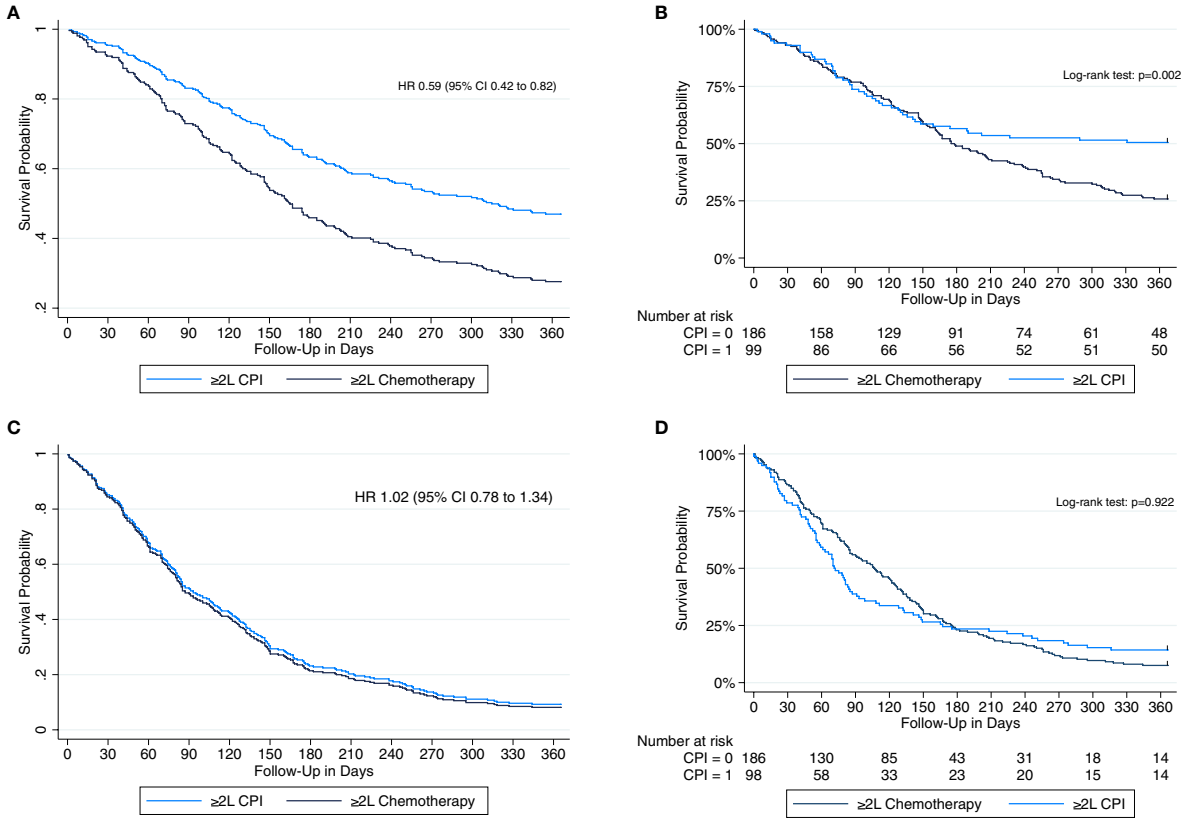

FIGURE 2
Multivariable-adjusted cox proportional hazards regression to estimate hazard ratios (HR) with 95% confidence intervals (CI) and univariate Kaplan-Meier survival analysis including a 'number at risk' table and a logrank test to analyze 1-year overall survival **(A, B)** and 1-year real-world progression-free survival **(C, D)**, respectively, among patients receiving second or further-line (≥2L) treatment with checkpoint inhibitors (CPI) versus chemotherapy.

CI 0.17 to 0.69, p=0.003), followed by patients without BM who received CPI but no prior CRT (HR_{adj} 0.50, 95% CI 0.25 to 0.99, p=0.05). Results suggested a trend for an OS improvement in patients with BM who received CPI and a prior CRT (HR_{adj} 0.85, 95% CI 0.47 to 1.54, p=0.59), however, there was no significant difference compared to baseline. In a small sub-cohort of only nine patients with BM who received CPI but no CRT, OS was significantly worse compared to baseline (HR_{adj} 2.89, 95% CI 1.20 to 6.98, p=0.02, Table 2).

In a subgroup of 79 patients where brain imaging was available within six weeks before treatment initiation, 16/26 (64%) patients who received CPI and 52/53 (98%) patients who received chemotherapy had brain metastases. Whole brain irradiation had already been performed in 21/26 (81%) and 48/53 (91%) patients

TABLE 2 Results of interaction analysis demonstrating a modification of the primary effect of CPI versus chemotherapy on 1-year OS by a patient's history of brain metastases and/or CRT.

Subgroup	N of pts.	HR (95% CI)	P-value
Baseline=CT, no CRT, no BM	52	-	-
CT, no CRT, with BM	12	1.77 (0.88-3.56)	0.109
CT, with CRT, no BM	59	0.68 (0.43-1.08)	0.104
CT, with CRT, with BM	63	1.12 (0.73-1.73)	0.601
CPI, no CRT, no BM	30	0.50 (0.25-0.99)	0.05
CPI, no CRT, with BM	9	2.89 (1.20-6.98)	0.018
CPI, with CRT, no BM	28	0.34 (0.17-0.69)	0.003
CPI, with CRT, with BM	32	0.85 (0.47-1.54)	0.602

The aRD is the absolute risk difference in the observed risk between the two groups. The negative value (minus symbol) means that CPI were associated with a decreased risk of 1y-mortality (by 26%).

who received CPI versus chemotherapy, respectively. The median (IQR) time to intracranial real-world progression was 71 (31, 144) days after initiation of CPI and 97 (71, 172) days after chemotherapy. There was no difference in 1-year intracranial rwPFS (HR 1.49, 95% CI 0.78 to 2.86, p=0.226), adjusting for prior brain irradiation.

3.5 Sensitivity analyses

In standard logistic regression analysis, 1-year mortality was significantly lower in patients receiving CPI versus chemotherapy (OR_{adj} 0.31, 95% CI 0.18 to 0.53, p<0.001; Table 3). The adjusted risk of 1-year mortality was 74 deaths (95% CI 69 to 82) per 100 patients treated with chemotherapy and 49 deaths (95% CI 39 to 59) per 100 patients treated with CPI (p<0.001). In the 1:1 propensity-score matched cohort including 198 patients, we compared 99 patients receiving chemotherapy with 99 patients receiving CPI. Patient characteristics in the propensity score matched cohort were well balanced between treatment groups and are provided in the Supplemental Digital Content; Table S2. Propensity score matching confirmed a lower risk of 1-year mortality in patients with CPI versus chemotherapy (OR_{adj} 0.32, 95% CI 0.17 to 0.58, p<0.001), with an adjusted absolute risk difference of -25.9% (95% CI -39% to -13%, p=0.0003; Table 3). The median OS in the PSM cohort was 6.5 months (95% CI 5.5 to 8.4). When applying the Cox model to the PSM cohort, CPI versus chemotherapy was associated with an improved 1-year OS (HR 0.61, 95% CI 0.44 to 0.88, p=0.008; Table 3). Kaplan Meier estimates using logrank testing confirmed a significant difference (p=0.0072). Following inverse probability of treatment weighting with confounders of the primary analysis, treatment with CPI was significantly associated with a lower risk of 1-year mortality (OR_{adj} 0.24, 95%CI 0.20 to 0.27, p<0.001; Table 3). Results were robust when including additional confounding variables into the primary Cox regression model (Table 3). Kaplan Meier estimates on three-year OS confirmed a significant benefit in the CPI group (p<0.001) and are provided in Figure S1 in the Supplemental Digital Content.

4 Discussion

In this retrospective real-world multicenter study of more than 280 patients with refractory or recurrent small cell lung cancer, second or further-line treatment with checkpoint inhibitors was associated with a lower risk of 1-year mortality compared with chemotherapy. However, the effect on overall survival was significantly modified by a patient's history of brain metastases and/or cranial radiotherapy. The benefit was magnified in patients without brain metastases (with or without radiotherapy), while there was no difference between CPI and chemotherapy in patients with brain metastases who received radiotherapy. Our data suggest the overall survival benefit with CPI was driven by patients without brain metastases.

In line with epidemiological data, our real-world cohort of pre-treated patients included 40.7% with brain metastases and 64% of all patients had received brain irradiation. In the CASPIAN trial on the use of first-line durvalumab plus carboplatin/etoposide (4), 10.2% of patients had BM that were asymptomatic or treated and stable, and 23% of all patients received radiotherapy to the brain. Of note, 90% of those patients with BM had not received a prior brain radiation at study entry. The authors performed *post-hoc* subgroup analyses to evaluate the role of intracranial disease, and concluded an improved overall survival by the addition of CPI was irrespective of whether or not patients presented with BM (13). However, in the CASPIAN subgroups, a potential trend towards an improved OS did not reach significance among patients with BM (HR 0.79, 95% CI 0.44 to 1.41), while there was a clear benefit among those without BM (HR 0.76, 95% CI 0.62 to 0.92). In the present study, we performed an interaction analysis to test for significant differences across groups. Our data demonstrated that the effect of CPI versus chemotherapy on OS was significantly modified by a patient's history of BM and cranial radiotherapy, indicating a pronounced benefit of CPI among patients without BM, while there was no difference between CPI versus chemotherapy in patients with prior BM who received CRT. Similarly, when looking at data from the first-line setting, the IMpower133-study and the KeyNote604-study demonstrated that patients with BM did not benefit from the

TABLE 3 Primary outcome across analyses.

Analysis	N of pts.	Effect measure (95% CI)	P-value
Cox proportional hazards regression (HR)	285	0.59 (0.42 to 0.82)	=0.002
Standard logistic regression (OR)	285	0.31 (0.18 to 0.53)	<0.001
Inverse probability weighting (OR)	285	0.24 (0.20 to 0.27)	<0.001
Propensity score matching (aRD)	198	-25.9% (-39% to -13%)	=0.0003
Cox proportional hazards regression in the PSM cohort (HR)	198	0.61 (0.44 to 0.88)	=0.008
Additional confounding variables (HR):			
Best response to 1L treatment	258	0.60 (0.41 to 0.89)	=0.011
ECOG at start of ≥2L treatment	235	0.65 (0.45 to 0.93)	=0.018
UICC staging at the time of initial diagnosis (UICC IB to IIIB versus IIIC to IVB)	281	0.60 (0.42 to 0.86)	=0.006

Association between ≥2L treatment with CPI versus chemotherapy and 1-year overall survival obtained from multivariable-adjusted Cox proportional hazards regression, standard logistic regression, inverse probability of treatment weighting, propensity score matching (PSM) analysis, and when including additional confounding variables into the primary Cox regression model.

addition of atezolizumab or pembrolizumab, respectively, to standard chemotherapy with platinum and etoposide (5, 21).

Overall, there is limited evidence on the intracerebral efficacy of CPI-based therapies as the majority of trials included only patients with asymptomatic or treated brain metastases. Among non-small cell lung cancer (NSCLC) patients, intracranial response rates were high when treated with first-line combined chemoimmunotherapy, such as camrelizumab with carboplatin/pemetrexed from the CAP-BRAIN trial (22) (intracranial ORR 46.7%) and atezolizumab with carboplatin/pemetrexed from the ATEZO-BRAIN trial (intracranial ORR 40%) (23). In contrast, intracranial response was lower in a phase II trial that used a single-CPI regimen (29.7% with pembrolizumab monotherapy) in patients with or without previous systemic treatment but naïve to PD-1 and PD-L1 inhibitors (24). In NSCLC, discussions have started more recently as to whether radiotherapy to the brain (especially whole brain radiotherapy, WBRT) can be initially omitted in some patients to reduce the associated risk of neurocognitive deterioration, while still maintaining local tumor control by improved systemic treatment options. In SCLC, from our perspective irradiation remains the important standard of care to treat brain metastases in every patient, including WBRT and stereotactic radiosurgery where possible. Our data indicate superior survival in patients who received brain radiation, irrespective of whether CPI or chemotherapy were used as systemic treatment, which is also acknowledged by current guidelines (1, 25).

The presentation of real-world SCLC patients not selected by strict inclusion criteria may provide a more generalisable conclusion on high-risk subgroups such as patients with brain metastases. Nevertheless, this study was retrospective in nature with several limitations. Tumor response assessments were obtained in clinical routine not following standardized criteria and without an independent review, which may limit direct comparability to prospective trials. For instance, unpublished data from the real-world, prospective, multicenter clinical research platform into molecular testing, treatment, and outcome data on lung carcinoma patients (CRISP) in Germany between 2019 and 2021 suggest higher rates of patients receiving second-line treatment (40%) for stage IV SCLC, while 31% died prior to second-line treatment. In contrast to our patients, 73% of that more current cohort received chemotherapy with CPI for first-line treatment. One concern in the present study is that patients who received a chemotherapy-based treatment may have had a higher need of a fast remission-induction than those who received CPI. The two groups were similar with respect to metastatic sites that were independent risk factors of mortality, such as brain (40.3 versus 41.4%) and liver (43 versus 43.3%). More patients in the chemotherapy group had extensive disease at the time of disease onset (94 versus 64%). However, rates of primary progressive disease as best response upon first-line chemotherapy were higher in the CPI group (11 versus 30%). Predominant use of the doublet of nivolumab/ipilimumab in the CPI-group may indicate a good and perhaps better general condition in these patients. Importantly, the improved overall survival with CPI versus chemotherapy remained robust across several sensitivity analyses. We conducted a propensity score matching as well as an inverse probability weighting, both statistical methods that have been designed to partly reduce bias due to unbalanced confounding. Patient characteristics across groups were well-balanced after using propensity score matching, and both statistical approaches yielded similar results, which strengthened our confidence in the finding. However, these methods can only address bias due to measured covariates, while residual unmeasured confounding also exists. To inform decision-making in the treatment choice for r/r SCLC especially for patients with BM, future research on the effectiveness of CPI should investigate differences by molecular subtypes of SCLC, ideally in a prospective setting. Further tumor-related factors predictive of the effectiveness of CPI in SCLC are still largely unknown (14, 26).

Finally, our real-world study in recurrent SCLC only partly supports the primary hypothesis that CPI compared with chemotherapy are associated with an improved overall survival. Interaction analysis revealed that this benefit was driven by patients without brain metastases, while no difference could be observed among patients with BM. Patients with BM represent a risk group where CPI do not seem to add any benefit to standard chemotherapy and may even bring additional risks. This may also be true in the first-line setting, where *post-hoc* subgroup analyses of clinical trials did not demonstrate a survival benefit by the addition of CPI in patients with BM. Instead of the current practice of treating all SCLC patients with first-line combined chemoimmunotherapy, it would be clinically relevant to identify whether certain subsets of patients with BM do benefit from CPI. Only a prospective randomized comparison between chemoimmunotherapy and chemotherapy for first-line treatment of patients with SCLC and BM could answer this question. Importantly, future studies of newer agents such as antibody drug conjugates and bispecific T-cell engagers targeting DLL3 as well as inhibitors of EHZ2 or PARP should evaluate the intracranial efficacy to finally address the unmet need in SCLC patients with brain metastases.

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 humans were approved by The Ethics Committee at the University Hospital Frankfurt. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the retrospective study design using de-identified data.

Author contributions

FCA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft. LS: Writing – review & editing, Conceptualization, Data curation, Investigation, Project administration, Visualization. FA: Methodology, Validation, Visualization, Writing – review & editing. LA: Methodology, Validation, Visualization, Writing – review & editing. SH: Methodology, Validation, Visualization, Writing – review & editing. MR: Methodology, Validation, Visualization, Writing – review & editing. AA: Data curation, Project administration, Resources, Validation, Writing – review & editing. VR: Data curation, Project administration, Resources, Writing – review & editing. JA: Data curation, Project administration, Resources, Validation, Writing – review & editing. CW: Data curation, Project administration, Resources, Validation, Writing – review & editing. NR: Data curation, Project administration, Resources, Validation, Writing – review & editing. GR: Data curation, Project administration, Resources, Validation, Writing – review & editing. FS: Data curation, Project administration, Resources, Validation, Writing – review & editing. AB: Data curation, Project administration, Resources, Validation, Writing – review & editing. MM: Data curation, Project administration, Resources, Validation, Writing – review & editing. NF: Data curation, Project administration, Resources, Validation, Writing – review & editing. MS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. JAS: Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Conflict of interest

Outside of the submitted work, the following authors report personal fees, research funding, and travel grants as detailed below: AA reports personal fees from BMS, MSD, Roche, Takeda, Pfizer, Novartis, Astra Zeneca, Sanofi, Amgen, Biontech, reports travel grants from BMS, Roche. CW reports personal fees by Amgen, Astra Zeneca, Boehringer Ingelheim, BMS, Chugai, Lilly, Merck, MSD, Novartis, Pfizer, Roche and Takeda. Consultancy fees by Viatris, Avotech and Roche. Travel grants by Amgen, BMS, Janssen and Lilly. FS reports personal fees from Janssen, personal fees from Takeda, personal fees from Pfizer, personal fees from Novartis, personal fees from AstraZeneca, personal fees from Lilly, personal fees from Thieme, research funding from Roche. GR reports personal fees from Astra Zeneca, Atriva, Boehringer Ingelheim, GSK, Insmed, MSD, Sanofi, Novartis, Pfizer, Berlin Chemie, BMS, Chiesi, Essex Pharma, Grifols, Roche, Solvay, Takeda, and Vertex. JA reports personal fees from AstraZeneca, BMS, Roche, Boehringer-Ingelheim, participates in advisory councils or committees for AstraZeneca, MSD, Novartis, Roche, BMS, Janssen, Merck. JAS reports personal fees from Boehringer Ingelheim, personal fees from AstraZeneca, personal fees from Roche, personal fees from BMS, personal fees from Amgen, personal fees from LEO pharma, personal fees from Novartis, personal fees from Takeda, outside of the submitted work. MS reports personal fees from Lilly, personal fees from Astra-Zeneca, personal fees from Bristol-Myers & Squibb, personal fees from Merck Sharp & Dohme, personal fees from Pfizer, personal fees from Takeda, personal fees from Roche, personal fees from AbbVie, personal fees from Boehringer-Ingelheim, personal fees from Celgene, personal fees from Novartis, outside the submitted work.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1273478/full#supplementary-material

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frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 24 October 2023
DOI 10.3389/fimmu.2023.1297588

OPEN ACCESS

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RECEIVED 20 September 2023
ACCEPTED 12 October 2023
PUBLISHED 24 October 2023

CITATION
Wang Q, Tang Z, Li C, Li X and Su C (2023) Evaluating distinct KRAS subtypes as potential biomarkers for immune checkpoint inhibitor efficacy in lung adenocarcinoma.
Front. Immunol. 14:1297588.
doi: 10.3389/fimmu.2023.1297588

Evaluating distinct KRAS subtypes as potential biomarkers for immune checkpoint inhibitor efficacy in lung adenocarcinoma

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Background: Despite the acknowledged predictive value of KRAS in immune checkpoint inhibitor (ICI) responses, the heterogeneous behavior of its mutations in this sphere remains largely unexplored. As of now, no studies have definitively categorized KRAS subtype variations as independent prognostic indicators for ICI responses in lung cancer patients.

Methods: We analyzed a cohort of 103 patients, all harboring different KRAS mutation subtypes, and complemented this data with information from TCGA and GEO databases. Our research focused on delineating the relationships between KRAS mutation subtypes and factors like immunotherapy markers and immune cell composition, in addition to examining survival rates, drug sensitivity, and PD-L1 responses corresponding to distinct KRAS subtypes.

Results: We found that the G12V and G12D subtypes demonstrated elevated expressions of immunotherapy markers, implying a potentially enhanced benefit from immunotherapy. Significant variations were identified in the distribution of naive B cells, activated CD4+ memory T cells, and regulatory T cells (Tregs) across different KRAS mutant subtypes. A notable difference was observed in the Tumor Mutation Burden (TMB) levels across the four KRAS subtypes, with the G12D subtype displaying the lowest TMB level. Furthermore, G12C subtype showcased the worst prognosis in terms of progression-free intervals (PFI), in stark contrast to the more favorable outcomes associated with the G12A subtype.

Conclusion: Our study reveals that KRAS mutations exhibit considerable variability in predicting outcomes for LUAD patients undergoing ICI treatment. Thus, the evaluation of KRAS as a biomarker for ICIs necessitates recognizing the potential diversity inherent in KRAS mutations.

KEYWORDS

KRAS, subtype, immune checkpoint inhibitors (ICIs), lung adenocarcinoma (LUAD), tumor immune microenvironment

1 Introduction

Immune checkpoint inhibitors (ICIs) have emerged as a potent frontier in cancer treatment, demonstrating promising potential in combatting various malignancies (1, 2). Particularly in the context of lung adenocarcinoma, ICIs herald a new era of therapeutic possibilities (3). Despite the enthusiasm surrounding the clinical impact of ICIs, it is imperative to acknowledge that a significant portion of patients remain unresponsive to this form of treatment, underscoring the pressing need for efficacious biomarkers.

Current research indicates that PD-L1, TMB, and IFN-γ stand as credible biomarkers in predicting ICI responses (4, 5). However, the reliance on expensive panels for the accurate detection of TMB and immune signatures presents a significant obstacle. Consequently, the scientific community is pivoting towards more accessible methodologies, like next-generation sequencing (NGS), to facilitate the identification of genomic alterations that could potentially dictate patient responsiveness to ICIs (2). This approach aims to streamline the process of pinpointing individuals who are likely to benefit from ICI treatments, fostering a more targeted and cost-effective therapeutic strategy.

Numerous genetic variations have been identified as having a correlation with the efficacy of immune checkpoint inhibitors (ICIs), encompassing mutations found in genes such as EGFR, ALK, KRAS, TP53, STK11, JAK2, and ATM (4, 6). Within this array, the KRAS gene, a member of the ras gene family, stands as one of the most frequently mutated oncogenes in non-small cell lung cancer (NSCLC). Traditionally, KRAS has been dubbed an "undruggable target," evading the grasp of effective targeted therapies, thereby necessitating a focus on driver gene negative NSCLC for the long-term treatment of patients harboring KRAS mutations (7).

Recent primary and clinical research endeavors have embarked on a detailed exploration of the immune microenvironment characteristics and the clinical outcomes of immunotherapy in patients with KRAS mutations (8–10). Analyses of clinical samples from this patient demographic revealed heightened levels of Tumor Mutation Burden (TMB), PD-L1 expression, and tumor-infiltrating lymphocytes (11).

While existing literature hints at a significant association between KRAS mutations and a heightened immunogenicity within the tumor and inflammatory microenvironment, suggesting a potential favorable response to ICI therapy, the precise impact on prognosis remains inadequately elucidated. Furthermore, the predictive value of these mutations concerning patient survival outcomes is yet to be firmly established (10, 12).

Existing research indicates that while KRAS mutations as a whole might not be robust indicators of patient outcomes, a deeper analysis into the distinct subtypes of this mutation — as well as their coexistence with mutations in other genes — could potentially offer a richer insight into patient prognosis and inform subsequent immunotherapy strategies (13). It is plausible that NSCLC featuring KRAS mutations constitutes a heterogeneous spectrum of diseases, each harboring unique molecular subtypes. This underlines the necessity for a comprehensive appraisal of these subtypes in the context of clinical treatments. At this juncture, KRAS mutation subtypes are not recognized as standalone predictors for responses to ICIs. Given the functional diversity inherent to KRAS mutations, we propose the hypothesis that their predictive power concerning ICI efficacy may also be distinctly varied. Consequently, a structured and detailed categorization of the diverse mutant KRAS variants is imperative to leverage their full potential as predictive biomarkers in clinical settings.

2 Method

2.1 mRNA expression profiling and analysis from public datasets

RNA-seq data was available for 563 LUAD patients within the TCGA database. We utilized resources like the cBioPortal and the TCPA from the Cancer Genome Atlas to obtain protein array data pertinent to cancer studies. For the purpose of correlation analysis, gene expression data was extracted employing the appropriate R package. Furthermore, the Java GSEA Desktop Application can be accessed at http://software.broadinstitute.org/gsea/index.jsp to facilitate the use of GSEA in linking genetic markers to KRAS mutations. To illustrate the functional pathways effectively, a point plot was generated using the ClusterProfiler tool within the R programming environment.

2.2 Data sources

RNA-seq data, somatic mutation information, and immunotherapy data specific to lung adenocarcinoma were retrieved from the TCGA subpopulation and the MSK cohort pertaining to lung cancer. From the extracted somatic mutation data, seven distinct KRAS mutation subtypes were identified. This included limited sample subsets such as five samples exclusively identifying with G12S, five with G13C, and three with G13D. Given the insufficient sample sizes of these three subtypes, they were deemed unsuitable for subsequent statistical analysis. Consequently, the study focused on the four primary mutation subtypes: G12A, G12C, G12D, and G12V, which presented a more substantial data pool for comprehensive investigation.

2.3 Drug sensitivity analysis

The R package was used to predict drug IC50 values for TCGA RNA-seq samples. It mainly indicates IC50 values of samples using two drug databases, the cancer therapeutics response portal (CTRP) and genomics of drug sensitivity in cancer (GDSC). The IC50 values of 148 lung cancer drugs were analyzed using the database of GDSC version V2.0.

2.4 NGS-based assay

Following the protocol, DNA was isolated from FFPE tumor samples utilizing the QIAamp DNA FFPE Tissue Kit and the Tissue

Extraction Kit from Qiagen. To guarantee a tumor content exceeding 70%, expert pathologists meticulously examined the FFPE tissue specimens. Meanwhile, DNA from peripheral blood was obtained using Qiagen's DNEasy Blood and the QIAamp DNA Blood Mini Kit, facilitating the purification of genomic DNA from 2 ml of peripheral blood samples.

The integrity and quality of the extracted genomic DNA, both from tumor tissues and peripheral blood, were assessed through agarose gel electrophoresis. The Agilent 2100 Bioanalyzer, equipped with a DNA 1000 Kit, facilitated the evaluation of the size dispersion of the circulating DNA fragments. To determine the purity and concentration levels of the DNA samples, instruments such as the NanoDrop2000 spectrophotometer and the Qubit 2.0 fluorometer were employed in conjunction with a dsDNA HS Assay Kit supplied by Yeasen China.

To maintain rigorous quality control throughout the testing phase, each assay incorporated a minimum of one positive control, one negative control, and one blank control. Concurrently, routine samples were processed, establishing and adhering to stringent quality control standards.

2.5 Patients and clinical information

We conducted a retrospective study, in which a total of 103 individuals diagnosed with stage III-IV and KRAS mutation NSCLC at Shanghai Pulmonary Hospital from January 2018 to November 2022, their tissue and peripheral blood samples were routinely subjected to next-generation sequencing (NGS) to check for specific genomic alterations before mono-immunotherapy. Only individuals with measurable diseases, and subsequent image studies available for response assessment, were selected for this research. This study was a retrospective single-center study to explore the response and recurrence after PD-1/PD-L1 monoclonal antibody treatment. In addition to overall response rate (ORR), progression-free survival (PFS), and overall survival (OS). Oncology Group Performance Status (ECOG PS) 0 or 1. Clinical data were extracted from the electronic patient record system. Information, including patients' age, gender, ethnicity, pathology, and tumor stage, was collected (Table 1). The hospital's Ethics Committee granted its approval for this study.

2.6 Statistical analysis

Data analysis was conducted utilizing GraphPad Prism 9 (GraphPad Software, Inc.) and R version 4.0 (R Foundation for Statistical Computing). Statistical significance was determined using Fisher's exact test. To compare progression-free survival rates, we utilized the log-rank test available in GraphPad Prism 9, facilitating the creation of Kaplan-Meier survival curves. In this study, a p-value less than 0.05 was deemed statistically significant, with all tests being two-sided. A p-value less than 0.1 was considered to be marginally significant.

TABLE 1 Baseline patient characteristics.

Characteristic	N (%)
Gender	
Male	47 (45.6%)
Female	56 (54.4%)
Age (years)	
≥50	83 (80.6%)
<50	20 (19.4%)
TNM stage	
I-IIIA	11 (10.7%)
IIIB-IV	92 (89.3%)
Smoking Status	
No	49 (47.6%)
Yes	54 (52.4%)
KRAS Subtype	
G12A	12 (11.7%)
G12V	22 (21.4%)
G12C	46 (44.7%)
G12D	23 (22.2%)
ECOG	
1	98 (95.1%)
2	5 (4.9%)

2.7 Single cell RNA-seq data analysis

In this study, we sourced public single-cell RNA sequencing data of colorectal cancer from the NCBI Gene Expression Omnibus (GEO) database, under the accession code GSE132465. Following quality control procedures, we proceeded with standard normalization and unsupervised clustering utilizing Seurat V4. This involved the application of functions such as 'NormalizeData', 'FindVariableFeatures', and 'ScaleData'. Dimensionality reduction analyses were facilitated through Principal Component Analysis (PCA) and Uniform Manifold Approximation and Projection (UMAP). We executed cell clustering utilizing the 'FindClusters' function, which adopts the shared nearest neighbor modularity optimization-based clustering algorithm at a resolution of 0.2. We then conducted differential gene expression (DEG) analysis between varying cell clusters, utilizing the Wilcoxon rank-sum test as implemented in the 'FindAllMarkers' function within Seurat. Criteria for determining DEGs were genes exhibited in over 25% of cells, a log2 fold change exceeding 0.25 compared to the background, and a false discovery rate below 0.05. Upon identifying the top DEGs for each cluster, we annotated the cell types using the deCS package. Additionally, KRAS mutation data was extracted from its corresponding whole-

exome sequencing (WES) data present in the original study. To scrutinize each cell type further, we analyzed the fold change of immune checkpoint genes using the Wilcoxon test.

3 Results

3.1 Association of KRAS mutation subtypes with immunotherapy and immune checkpoint expression metrics

RNA-seq data, somatic mutation details, and immunotherapy information specific to lung adenocarcinoma were extracted from the TCGA subpopulation and the MSK lung cancer cohort. KRAS mutation subtypes were analyzed for associations with common immunotherapy indicators, including PD-L1, PD-L2, PD-1, CYT, and GEP. Previous studies suggest that GEP and CYT of T cell inflammation are emerging as predictive biomarkers for PD-1 blockade therapy. Our study found that the expression differences of immunotherapy indicators were not particularly significant among the overall four subtypes. However, from the results of our analysis, higher expression of immunotherapy indicators was observed in the G12V, and G12D subtypes, which illustrates the potential for the greater clinical benefit of immunotherapy targeting patients with KRAS mutations in both subtypes (Supplementary Figures 1A–E).

Expression heat maps of immune checkpoint genes were plotted for different subtypes of KRAS mutations (Figure 1). The results showed that G12C overall immune checkpoint expression was lower and that each KRAS isoform was differentially expressed with high immune checkpoint expression. The above results, illustrate that different therapeutic targets should be selected for different KRAS subtypes.

3.2 Single-cell analysis of immune checkpoint gene expression in KRAS mutant vs. wildtype patients

While bulk RNA-seq has indicated that various KRAS mutants may influence the response to immune therapy, a more detailed analysis is necessary to substantiate this, ideally at the single-cell level. In Figure 2A, we present an overview representation of scRNA-seq based on publicly available data of colorectal cancer. A total of 10 cell types were detected, including two distinct fibroblast clusters. The UMAP analysis confirmed that KRAS mutation demonstrate certain level of impact for most cell type. We observed from the UMAP plot that KRAS mutations have a certain degree of impact on specific cell types, especially epithelial (malignant tumor) and fibroblast populations (Figure 2B). Next, we assessed the proportions of major cell types across between WT and KRAS mutant group. As showed in Figure 2C, we observed the increase of CD4+ T cell and myeloid cells in KRAS mutant group. In contrast, we notice the plasma cells, as well as fibroblast cluster 2 (fibroblast c2) where this particular subpopulation appeared to decrease, even diminish in KRAS mutant group.

To further explore the impact of KRAS mutation for different cell types, we conducted a comprehensive DEG analysis on immune

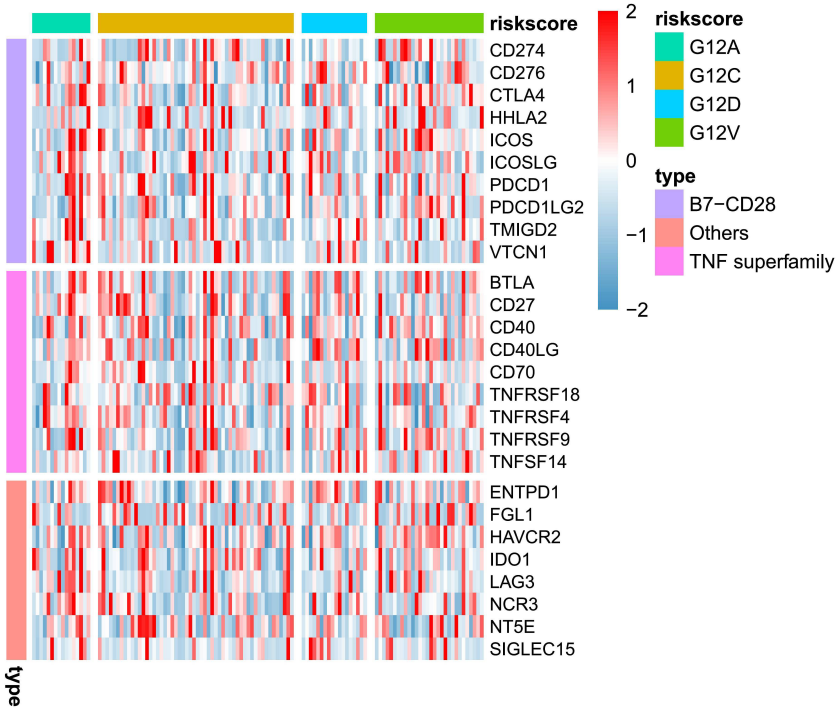

FIGURE 1
Heatmap illustrates the distribution of immune checkpoint gene expressions across various KRAS mutation subtypes.

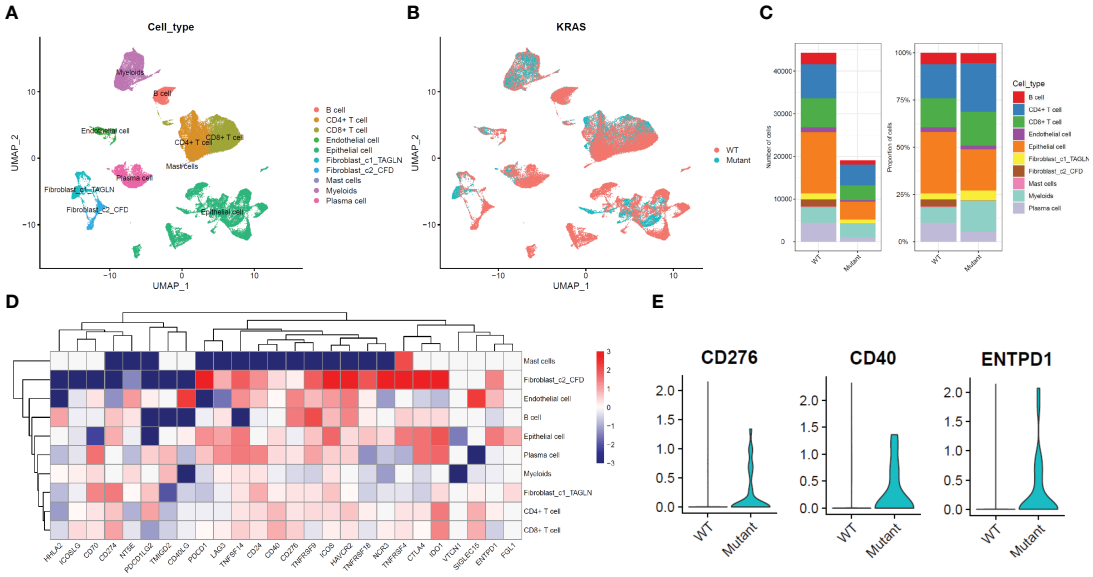

FIGURE 2
Expression Changes of Immune Checkpoint Genes at the Single-Cell Level in Colorectal Cancer with KRAS mutation. **(A)** UMAP plot displaying single-cell RNA sequencing data, categorized by cell type. **(B)** UMAP plot categorizing single-cell RNA sequencing data based on KRAS mutation status. **(C)** Comparison of total cell count and cell type composition between WT (Wild-Type) and KRAS mutant patient groups. **(D)** Differential gene expression analysis of immune checkpoint genes at the cell type level. **(E)** Violin plot illustrating significantly higher expression levels of CD276, CD40, and ENTPD1 in the fibroblast_c2_CFD group. For the comparison of those three genes, a student's t-test was conducted with a P-value < 0.0001.

checkpoint genes (The selection of these genes is same in Figure 1). As shown in Figure 2D, we noticed most checkpoint genes were up-regulated in fibroblast c2 and epithelial (malignant tumor). For example, CD276, CD40, ENTPD1 demonstrated significant up regulated in KRAS mutant group (Figure 2E).

3.3 Differences in the distribution of 22 immune cell types across different subtypes of KRAS

To further explore the differences between the different mutational subtypes of KRAS, we utilized the R package cluster profiler (v3.16.1) to perform GO, KEGG, and GSEA functional enrichment analyses on the KRAS mutation subtype data. Used the R package Mitch to analyze differences in the hallmark functions of KRAS subtypes. We found that the G12A and G12V subtypes were more enriched in interferon related response, and that G12V was enriched in inflammatory response, TNF-α signaling via NF-kB (Supplementary Figures 2A–G).

TILs (tumor-infiltrating lymphocytes) exhibit pro- and anti-tumor properties. Tregs, tumor-associated macrophages (TAMs; M2), and other cells linked to immunotherapy side effects and pre-tumor function. We wanted to find out how immune cells were distributed among the various KRAS mutant populations in LUAD tumors. Based on extensive RNA expression data, a deconvolution approach for cell type enrichment analysis was used to calculate the immune cell level (Figures 3A, B). B cells are Naive, T cells have CD4 $^{+}$ memory activation, Tregs and other immune cells differ significantly between the KRAS mutant subtypes (Figures 3C–F). The findings additionally suggest that tumor tissues harboring distinct KRAS mutations exhibit variations in immune cell composition.

3.4 Differences in TMB, DNA damage repair defects between different mutational subtypes of KRAS

The cellular stress response caused by DNA damage is essential for ensuring the stability of genetic material, inhibiting the generation of gene mutations, and maintaining the life span of cells. As in other malignancies, the development of NSCLC is a multifactorial, multistage, and multistep complex process. The primary mechanism result from various factors leading to proto-oncogene activation or tumor suppressor gene inactivation, hypofunction or deletion of DNA damage repair genes, and the joint participation of some signaling pathways. DNA damage repair defects included DNA mutations (Nonsilent mutation rate, SNV), copy number variations (Aneurysm Score, number of segments, fraction altered, homologous repair deficiency), loss of heterozygosity (number of SEGS with LOH, number of SEGS with LOH). We aimed to investigate if distinct KRAS mutation subtypes correspond to differing mutation burdens. Notably, patients harboring the G12C mutation demonstrate a significantly elevated overall mutation rate (Figures 4A, B), while The G12D subtype has the lowest TMB level. Those mutations are all defined as nonsilent mutations through our analysis (Figures 4C). We next

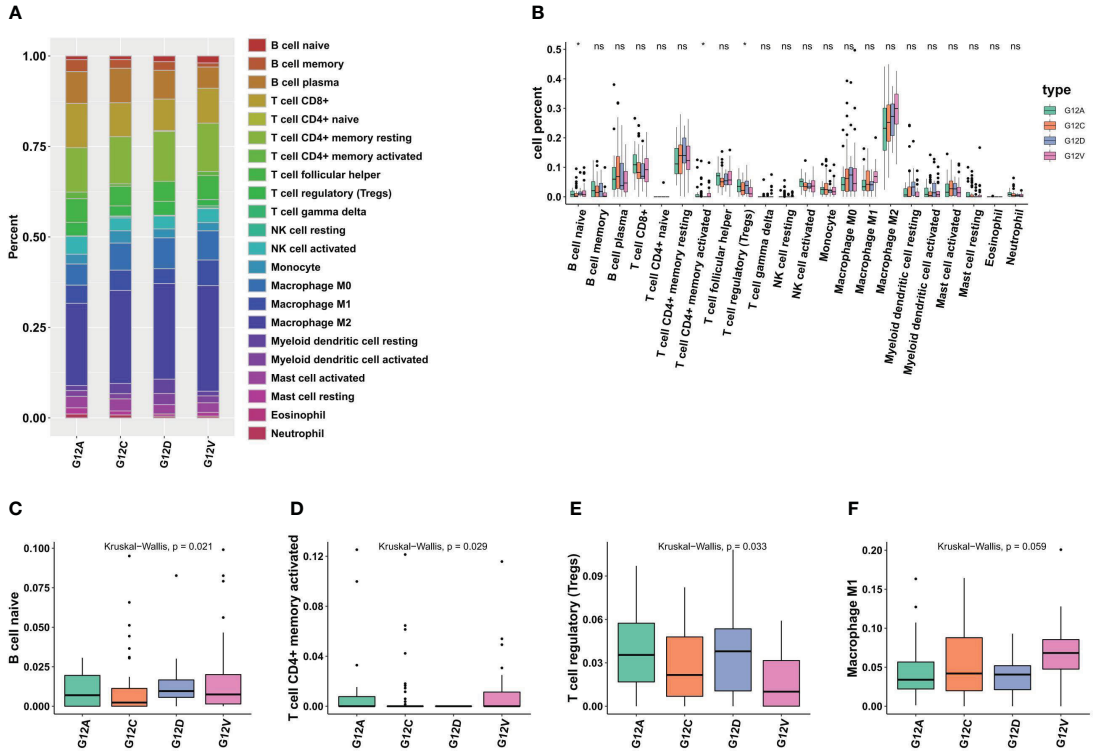

FIGURE 3
Different KRAS mutation subtypes are associated with different immune cell composition and tumor microenvironment. **(A)** The bar plots illustrate the varying cell composition percentages among different KRAS mutation subtypes. **(B)** Statistical analysis reveals the extent of significant differences in the Tumor Microenvironment (TME) among four KRAS mutation subtypes. **(C–E)** Boxplots illustrate significant variations in the proportions of naive B cells, CD4+ memory cells, Tregs, and M1 macrophages across the four KRAS mutations. *, $p<0.05$; ns, not significant.

also determined which DDR pathway was associated with KRAS mutation status and whether DDR deletion led to significant increases in TMB and neoantigen levels in different KRAS states. The analysis results showed that there were significant differences in SNV neoantigen counts among the four KRAS mutant subtypes (Figure 4D).

3.5 Prognostic differences among different KRAS mutation subtypes in the TCGA lung cancer database

We conducted an analysis of the prognostic disparities among various subtypes of KRAS mutations utilizing the "survival" package in R. Subsequently, Kaplan-Meier survival curves were constructed with the assistance of the "survminer" package in R. The analysis of Progression-Free Intervals (PFI) revealed that individuals with the G12C mutation faced the poorest prognosis, while those with the G12A mutation exhibited the most favorable outcomes (Figures 5A–E). Moreover, the statistical analysis underscored a significant difference in PFI results when G12C was compared with other subtypes. In terms of overall survival (OS), the trend persisted with G12C representing the worst prognosis and G12A denoting the best. However, it is noteworthy that the differences in OS prognosis among the various subtypes were not statistically significant, as depicted in Figures 5F–J.

3.6 LUAD cases with the KRAS G12A mutation demonstrate a more favorable response to anti-PD-1/L1 therapy compared to those possessing the KRAS G12C mutation

Utilizing the R package, we estimated the IC50 values for TCGA RNAseq samples, leveraging two prominent drug databases: the Cancer Therapeutics Response Portal (CTRP) and the Genomics of Drug Sensitivity in Cancer (GDSC). For our analysis, we opted for the GDSC database's Version 2 to scrutinize the IC50 values associated with 148 lung cancer pharmaceuticals. Notably, AZ6102 stands out as a potent inhibitor of TNKS1/2, exhibiting a hundredfold selectivity over other enzymes in the PARP family. Statistical analysis revealed significant differences between the IC50 values obtained for different KRAS mutant subtypes targeting AZ6102 (Supplementary Figures 3A–C).

Our previous analyses have highlighted the distinct impacts of variances and similarities in factors such as PD-L1 expression, TMB, and TME within the KRAS subgroup on the efficacy of ICI therapy. Consequently, we sought to determine whether LUAD cases exhibiting different KRAS mutations respond differently to anti-PD-1/L1 therapy. In this study, we included 103 well-balanced patients who were free of EGFR and ALK gene variants and had received first-line immunotherapy. The patient cohort, which received ICI treatment between June 2018 and November 2022,

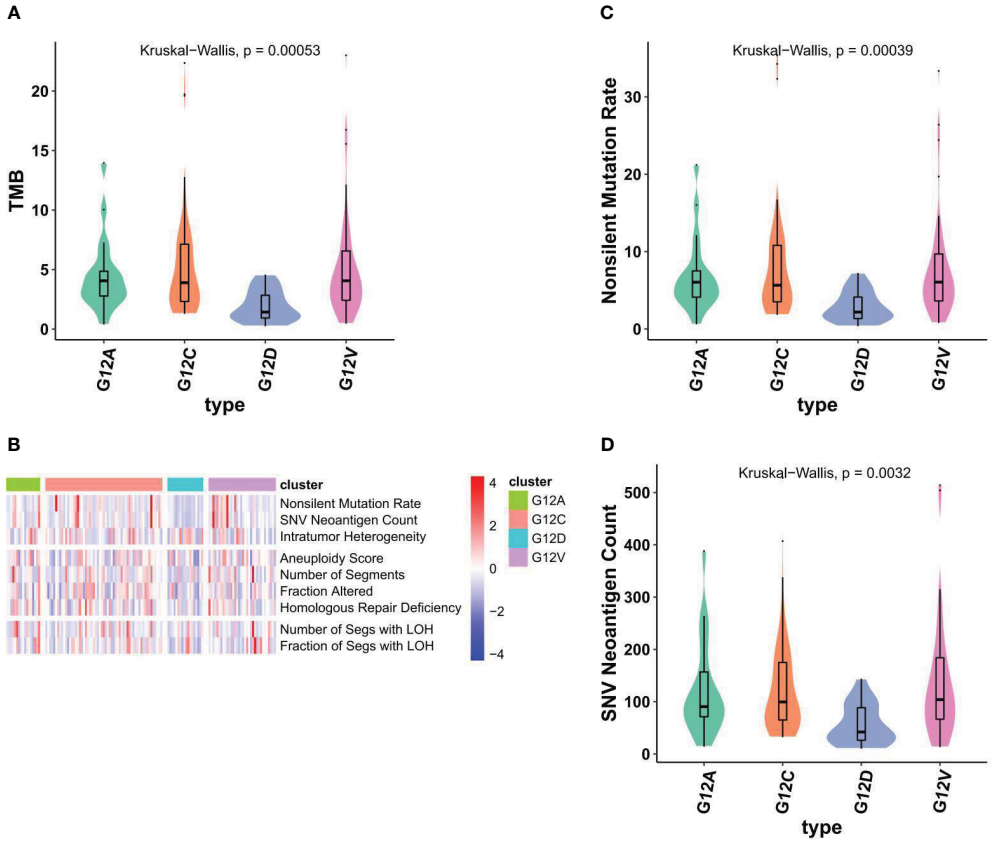

FIGURE 4
Different KRAS mutation subtypes are associated with TMB and nonsilent mutation rate. **(A)** Violin plots showed TMBs are associated with KRAS subtypes. **(B)** The comprehensive heatmap displays the variations in DNA alterations across individual KRAS subtypes. **(C, D)** violin plots showed nonsilent mutation rate **(C)** and SNV neoantigen counts across individual KRAS subtypes **(D)**.

was characterized by well-confirmed KRAS mutant subtypes as determined through NGS sequencing. Initially, the breakdown of patients with measurable lung tumors was as follows: 12 with KRAS G12A, 22 with KRAS G12V, 46 with KRAS G12C, and 23 with KRAS G12D.

Upon evaluation for treatment efficacy, data revealed a notable divergence in progression-free survival (PFS) rates between groups. Specifically, the KRAS G12A group exhibited a significantly extended PFS—6.5 months—compared to the 4.7 months observed in the KRAS G12C group, a difference substantiated by a log-rank p-value of 0.003 (Figure 6). In conclusion, our clinical data analysis indicates that the KRAS subtype serves as a distinct marker in forecasting the responsiveness to ICI therapy. Specifically, LUADs harboring the KRAS G12C mutation did not exhibit any enhanced clinical benefits from ICI treatment.

4 Discussion

Tumor immunity is a new pillar in cancer treatment today, and it plays a revolutionary role in the treatment of cancer. The ICI is composed of PD-1 and PD-L1, and its function is to unleash the tumor-suppressive immune system (14–16). Although there have been some breakthroughs, ICI treatment is not entirely without side effects, and it is unacceptable to every patient. Therefore, it is necessary to find biomarkers that can effectively identify the therapeutic effects of ICIs. The clinically applied biomarkers mainly include PD-L1 immunohistochemistry and high instability-high, MSI-H or error repair (dMMR) (17–19). Pembrolizumab(anti-PD-1) was approved by the U.S. Food and Drug Administration (FDA) in 2017 to treat advanced MSI-H/dMMR solid tumors that have progressed after prior treatment, regardless of tumor type. The FDA has never before approved a molecular biomarker regardless of the type of malignancy. Even though PD-L1 and MSI-H/dMMR are both regarded as indicators of ICI response (17, 20). They still lack stripes, however, and they have limited sensitivity and specificity. As a result, there is a need to keep looking for biomarkers that can more accurately predict how well an ICI will respond to treatment. One such indicator is tumor mutation burden (TMB). High TMB is linked to ICI responsiveness in several tumor types (21). For instance, in patients treated with nivolumab and ipilimumab, high TMB was associated with significantly improved progression-free survival in non-small cell lung cancer (NSCLC) regardless of PD-L1 expression (22).

There were significant differences in the expression of TMB and PD-L1 in tumor genes. A systematic meta-analysis showed that patients with KRAS gene variants benefited from anti-PD-1/PD-L1 immunotherapy (23). The immunogenicity of cancer is usually caused

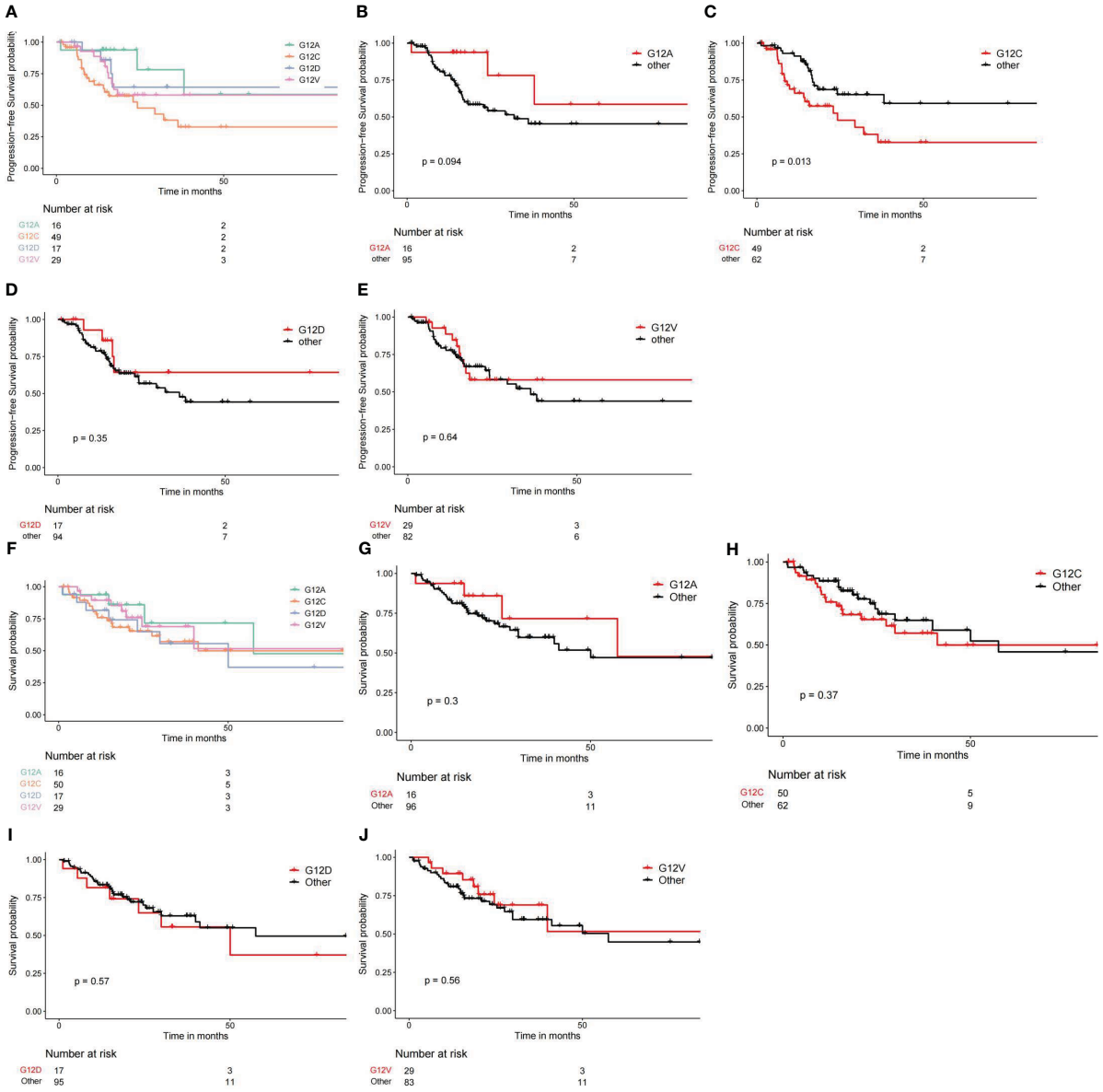

FIGURE 5
Different KRAS mutation subtypes lead to different survival outcomes. **(A–E)** Comparing PFI or PFS from different KRAS subtypes. **(F–J)** Comparing overall survival outcomes across different KRAS subtypes.

by gene mutation, and the greater the amount of mutation of TMB, the stronger its immunogenicity. However, dMMR can also contribute to tumor immunogenicity, and both genes may be favorable populations for immunotherapy. Mutations in the KRAS gene are related to the microenvironment of inflammatory tumors and the immunogenicity of tumors, making patients respond better to PD-1 inhibitors (12). Different types of KRAS variants were associated with treatment outcomes, while specific gene variants were destined not to benefit from immunotherapy. The expression levels of PD-L1 and TMB genes are still unknown in different KRAS gene mutation subtype.

Our results showed that TMBs of four common KRAS variants significantly differed in TMB expression. TMB content was lowest in the G12D subtype. Several recent studies have shown that TMB can act as a surrogate to replace the entire new antigen load, and disruption of DNA damage repair pathways causes an increase in TMB. In non-small cell lung cancer, TMB is the most effective biomarker for predicting immune-checkpoint blockade (ICB) response. Although TMB has good application prospects in the ICB treatment of other solid tumors, TMB still has some limitations. Many studies have turned to the development of other biomarkers closely related to TMB statuses, such as gene variation in the DNA damage response pathway and TP53/KRAS.

At present, integrating information about KRAS status and concurrent mutations into a comprehensive predictive model is a promising strategy to identify patients who might significantly benefit from, or possibly remain unresponsive to, immune checkpoint inhibitors (ICIs). However, the current body of data is yet insufficient to substantially influence treatment decisions. As it stands, definitive evidence delineating varied clinical outcomes with ICIs, contingent on the presence or absence of KRAS mutations, remains elusive.

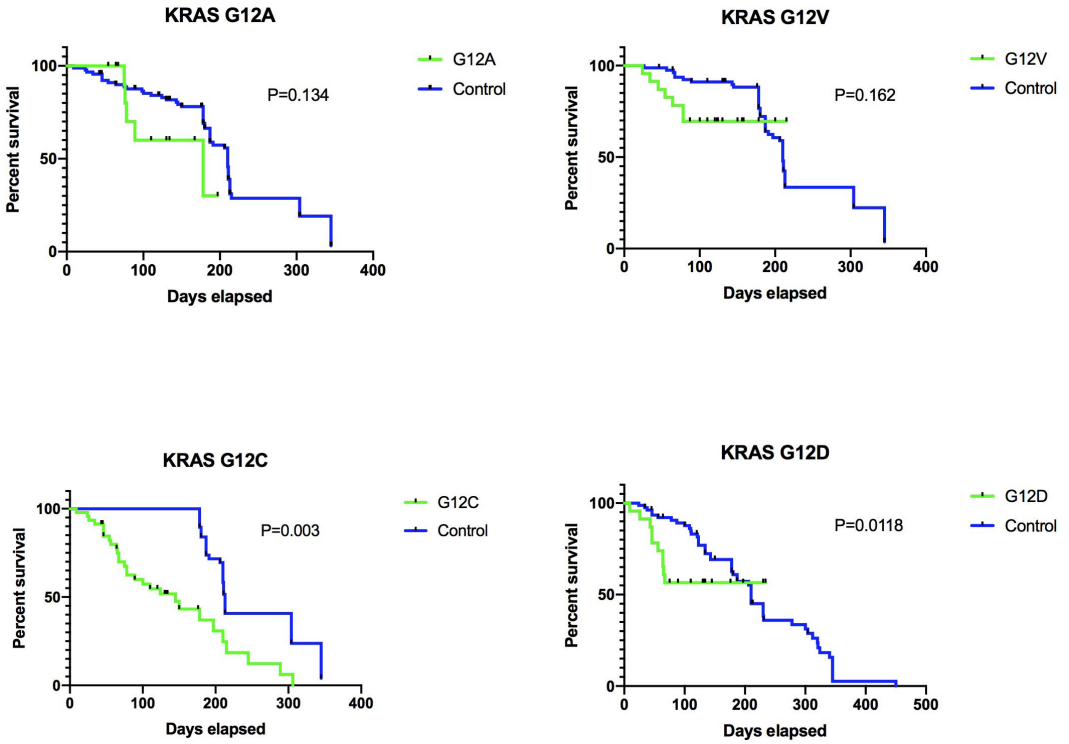

FIGURE 6
Various KRAS mutation subtypes result in distinct PD-L1 responses and affect different cohorts. Our data is derived from an in-house cohort of 103 individuals. The breakdown of these mutation subtypes is as follows: G12A, n=12 (11.7%); G12V, n=22 (21.4%); G12C, n=46 (44.7%); G12D, n=23 (22.2%). The Kaplan-Meier analysis was employed to evaluate the survival outcomes between the two groups.

Nonetheless, our research underscores the potential predictive value of KRAS mutations in determining responses to ICIs in cases of non-small cell lung cancer (NSCLC). Moving forward, a thorough investigation into the roles of KRAS mutations and their subtypes, alongside an analysis of drug response patterns and impacts on the immune system, will be pivotal in shaping the design of forthcoming trials, geared towards addressing the nuanced needs of this diverse patient population. In addition, the composition of the TME, including TILs, Tregs, and TAMs, are crucial for the immune response (24, 25). Furthermore, our data showed that B cell naive, T cell CD4 + memory activated, T cell regulatory, and other immune cells differ significantly among different KRAS mutated subtypes, which are associated with a suppressive immune environment for ICI therapy. Among them, Treg cells suppress antitumor immune responses, but the performance of Treg cells in the metabolically abnormal tumor microenvironment remains unknown (26). Regulatory T cells were capable of spontaneous and PD-L1 binding to block T cell-mediated anti-tumor immune responses before undergoing death (27).

Limitations of this study encompass: While our patient cohort consists of 103 individuals diagnosed with stage III-IV NSCLC and bearing KRAS mutations, additional cohorts from diverse centers would further validate our findings. To deepen our understanding of the intricate relationship between the KRAS mutant subtype and the tumor microenvironment (TME), more detailed mechanistic studies on how this mutation influences the TME are warranted.

In conclusion, we demonstrate that not all KRAS mutations are equivalent in predicting the efficacy of ICIs in patients with non-small cell lung cancer. At the same time, our data showed that different subtypes of KRAS mutations were significantly different in their association with TMB and TME compositions and the distribution of DNA damage repair defects. Our study suggests that selecting appropriate treatment modalities according to the subtype of patients with KRAS mutations may be a more desirable treatment selection strategy. In addition, the potential heterogeneity of KRAS mutations should be considered when evaluating KRAS as a biomarker for ICIs.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

QW: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. ZT: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. CL: Supervision, Validation,

Writing – review & editing. XL: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. CS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by grants from the "Dream Tutor" New Person Cultivation Program of Shanghai Pulmonary Hospital (No.fkxr1907), National Natural Science Foundation of China (No.81803101 and No.81972169), Science and Technology Commission of Shanghai Municipality (No.19411950300), Shanghai Innovative Collaboration Project (No.2020CXJQ02).

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/fimmu.2023.1297588/full#supplementary-material

SUPPLEMENTARY FIGURE 1
(A–E) Violin plots illustrating variations in the expression levels of different immune checkpoint markers across distinct KRAS subtypes.

SUPPLEMENTARY FIGURE 2
(A–G) Gene Set Enrichment Analysis (GSEA) showcasing the distribution of differentially expressed genes across four distinct KRAS mutation subtypes.

SUPPLEMENTARY FIGURE 3
Graphical representation of IC50 drug response, showing the disparate responses of different KRAS mutation subtypes to various pharmaceutical agents.

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frontiers | Frontiers in Oncology

TYPE Brief Research Report
PUBLISHED 24 October 2023
DOI 10.3389/fonc.2023.1283360

OPEN ACCESS

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RECEIVED 30 August 2023
ACCEPTED 10 October 2023
PUBLISHED 24 October 2023

CITATION
Allen E, Umoru G, Ajewole V and Bernicker E (2023) Incidence and outcome of immune checkpoint-induced pneumonitis in oncology patients with history of pulmonary disease.
Front. Oncol. 13:1283360.
doi: 10.3389/fonc.2023.1283360

Incidence and outcome of immune checkpoint-induced pneumonitis in oncology patients with history of pulmonary disease

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Background: Immune checkpoint-induced pneumonitis (ICIP) is one of the most fatal adverse events caused by immune checkpoint inhibitors (ICI) and accounts for 35% of anti-PD-[L]1-related deaths. Risk factors including thoracic radiation and use of EGFR tyrosine kinase inhibitors have been identified as contributors to ICIP development. However, there has been very limited information on obstructive pulmonary disease as a risk factor.

Objective: The purpose of this study is to evaluate the incidence and management of ICIP in a cohort of patients with pre-existing obstructive pulmonary disease.

Methods: This retrospective, descriptive study, includes data from 139 patients between January 1, 2017 and August 31, 2022. Patients included were adult patients 18 years or older, received at least 2 cycles of an immune checkpoint inhibitor, and had a history of an obstructive pulmonary disorder prior to administration. Patients were excluded if they had literature-established risk factors for pneumonitis.

Results: The incidence of ICIP was 7.19% (10 out of 139 patients). From a management perspective, 90% of patients had immunotherapy held, 40% received oral steroids, and 70% received intravenous steroids at the time of ICIP identification. After receiving treatment for the initial episode of ICIP, 6 patients restarted immunotherapy and 3 (50%) subsequently experienced a recurrent episode. One patient experienced grade 4 ICIP event and subsequently died from respiratory failure attributed to ICIP.

Conclusion: These findings indicate that a pre-existing history of an obstructive pulmonary disorder may be a risk factor for the development of ICIP and subsequent recurrence of ICIP when rechallenged.

KEYWORDS

immune checkpoint-induced pneumonitis, immunotherapy, pulmonary disease, immune checkpoint inhibitors, PD-1/PD-L1 inhibitors

Introduction

The use of immune checkpoint inhibitors (ICI) such as pembrolizumab, nivolumab, ipilimumab, durvalumab, atezolizumab, and cemiplimab has been increasing over the past several years. These agents continue to receive FDA approval across a variety of cancer types and function by downregulating inhibitory pathways on T cells, leading to increased immune system activation and T-cell recognition and attack of tumor cells. Since the introduction of these agents, pneumonitis has proven to be one of the most common fatal adverse effects seen and account for 35% of anti-PD-[L]1-related deaths (1, 2). The incidence in literature has been reported to be 2.5-5% with monotherapy immune checkpoint inhibitor use (mean onset of 2.8 months) but recent data have suggested that the overall incidence and time to onset may be higher outside of clinical trial settings (3–6). However, there is paucity of data available on how to stratify patients at significant risk for development of ICI-pneumonitis (ICIP) and whether specific tumor characteristics, histology, or combination treatments increase the incidence. A subgroup analysis of KEYNOTE-001 study which investigated utilization of pembrolizumab for the treatment of metastatic non-small cell lung cancer (NSCLC) found that pneumonitis occurred more frequently in patients with a history of asthma and chronic obstructive pulmonary disease (COPD) than in those without this history (5.4 vs. 3.1%) (4). However, given the underrepresentation of patients with underlying lung diseases in clinical trials, it is unknown if certain pre-existing obstructive lung diseases impact the risk for developing ICIP.

Furthermore, from a management standpoint, the optimal dose, duration, and type of immunosuppressive treatment for steroid refractory ICIP have not been clearly elucidated in literature. For instance, in patients who achieve clinical resolution from ICIP, there is limited data on pre-disposing factors for recurrence of ICIP upon rechallenge with immune checkpoint inhibitors. Moreover, we do not have consensus across various guidelines on the efficacy and timing of steroid-sparing agents (e.g. infliximab, cyclophosphamide, IVIG) in patients who develop steroid-refractory ICIP.

The purpose of this retrospective, single-center descriptive study is to describe the real-world incidence of ICIP and management strategies in oncology patients with a past medical history of pulmonary disease.

Methods

This single-institution retrospective chart review was conducted within the Houston Methodist Hospital system on patients with cancer that received nivolumab, ipilimumab, pembrolizumab, cemiplimab, or atezolizumab for an FDA approved indication from January 1st, 2017 to August 31st, 2022. Electronic medical records along with other institution sources (databases, pathology reports, and admission logs) were reviewed to identify potential participants. Patients were excluded if: a patient's care was transferred to another institution, patient received durvalumab with radiation for early-stage NSCLC, had clinical suspicion of pneumonitis within three months of receiving thoracic radiation, or had previously received an EGFR tyrosine kinase inhibitor (TKI). Patients included in this study met the following inclusion criteria: adult patients 18 years or older diagnosed with any malignancy, received at least two cycles of an immune checkpoint inhibitor, and had a history of an obstructive pulmonary disease prior to introduction of an immune checkpoint inhibitor. A patient's history of obstructive pulmonary disease was identified using ICD-10 codes and verified through chart review that focused on physician documentation of the disease state, pulmonary function tests (PFTs), medication records, and CT scan records, if available. The primary objective was to evaluate the incidence and management of immune checkpoint inhibitor-induced pneumonitis. Additional data points characterized the pneumonitis events observed and what management strategies were employed to achieve resolution of symptoms. For statistical analysis, descriptive statistics including median, interquartile range, and percentage were used to analyze the baseline characteristics along with the primary and secondary endpoints. Patients were determined to have a diagnosis of immune-induced pneumonitis based on a combination of the following factors: ICIP was indicated on imaging, physicians' notes indicated diagnosis of ICIP, and management approach indicated a suspicion for ICIP.

Results

A total of 626 cancer patients were identified to have a history of an obstructive pulmonary disease prior to introduction of an ICI based on ICD-10 codes and initial ICI administration dates. After applying the exclusion criteria, we reviewed the charts of 139 evaluable patients to identify cases of immune-induced pneumonitis (Figure 1). The baseline characteristics for both the overall population and the population that experienced an ICIP event are summarized in Table 1. In the overall population, these patients received one of the following immunotherapy agents: pembrolizumab (n=80), nivolumab (n=40), atezolizumab (n=18) or cemiplimab (n=1). The median age, depending on the immunotherapy agent received, is as follows: nivolumab 70 (IQR:13), pembrolizumab 70 (IQR:14), atezolizumab 75 (IQR:12), and cemiplimab 55 (IQR:0), with approximately 50 to 60% of the population being male. The majority (46%) of the population had a history of COPD identified as their underlying obstructive pulmonary

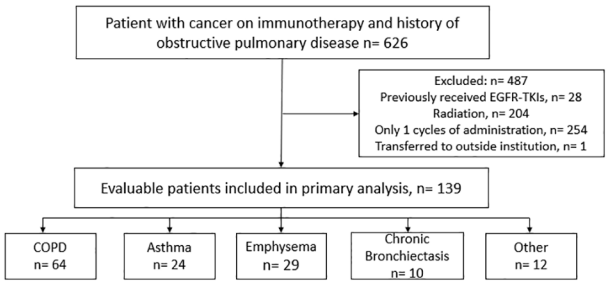

FIGURE 1
Consort flow diagram.

10.3389/fonc.2023.1283360

TABLE 1 Baseline demographics.

Baseline Demographics for Evaluable Patients				
Metric	Pembrolizumab	Nivolumab	Atezolizumab	Cemiplimab
Immunotherapy – no. (%)	80 (57)	40 (29)	18 (12.9)	1 (0.7)
Age (yr) – median (IQR)	70 (14)	70 (13)	75 (12)	55 (0)
Male Sex – no. (%)	37 (46.3)	22 (53.6)	12 (66.7)	1 (100)
Smoking status – no. (%)				
Current smoker	11 (13.7)	3 (7.5)	3 (16.7)	0 (0)
Former smoker	56 (70)	31 (77.5)	11 (61.1)	1 (100)
Never smoked	13 (16.3)	6 (15)	4 (22.2)	0 (0)
Cancer Staging – no. (%)				
Stage I	10 (12.7)	5 (12.2)	0 (0)	0 (0)
Stage II	2 (2.5)	5 (12.2)	1 (5.6)	0 (0)
Stage III	21 (26.6)	11 (26.8)	6 (3.3)	0 (0)
Stage IV	47 (58.2)	20 (48.8)	11 (61.1)	1 (100)
History of pulmonary disease – no. (%)				
COPD	38 (47.5)	20 (48.8)	6 (33.3)	1 (100)
Asthma	15 (19)	8 (19.5)	1 (5.6)	0 (0)
Emphysema	14 (17.7)	8 (19.5)	7 (38.9)	0 (0)
Chronic Bronchitis	4 (5.1)	1 (2.4)	1 (5.6)	0 (0)
Chronic Bronchiectasis	5 (6.3)	3 (7.3)	2 (11.1)	0 (0)
Chronic Bronchiolitis	1 (1.3)	0 (0)	1 (5.6)	0 (0)
Obstructive Sleep Apnea	3 (3.8)	0 (0)	0 (0)	0 (0)
IPD	0 (0)	1 (2.4)	0 (0)	0 (0)
Median Number of Cycles	5	9	10	9
Cancer Type – no. (%)				
Non-small cell lung cancer	43 (53.8)	17 (42.5)	7 (38.9)	0 (0)
Hepatocellular carcinoma	1 (1.3)	1 (2.4)	6 (33.3)	0 (0)
Bladder cancer	9 (11.4)	4 (9.8)	1 (5.6)	0 (0)
Renal cell carcinoma	1 (1.3)	6 (14.6)	1 (5.6)	0 (0)
Small cell lung cancer	1 (1.3)	3 (7.3)	2 (11.1)	0 (0)
Other	25 (31.6)	9 (21.9)	1 (5.6)	1 (100)

Baseline Demographics of Patients with Pneumonitis		
Metric	**Pembrolizumab**	**Nivolumab**
Immunotherapy – no. (%)	6 (60)	4 (40)
Cancer Type – no. (%)		
Non-small cell lung cancer	6 (100)	2 (50)
Small cell lung cancer	0 (0)	1 (25)
Mesothelioma	0 (0)	1 (25)
Cancer Staging – no. (%)		
Stage III	0 (0)	1 (25)
Stage IV	6 (100)	3 (75)
Smoking status		
Current smoker	0 (0)	0 (0)
Former smoker	5 (83.3)	4 (100)
Never smoked	1 (16.7)	0 (0)
History of pulmonary disease – no. (%)		
COPD	2 (33.3)	3 (75)
Asthma	1 (16.7)	1 (25)
Emphysema	2 (33.3)	0
Chronic Bronchiectasis	1 (16.7)	0
Combination therapy – no. (%)		
Yes	4 (66.7)	2 (50)
No	2 (33.3)	2 (50)

disease, followed by asthma and emphysema. The most common oncologic diagnosis in this population was non-small cell lung cancer followed by bladder cancer. Ten patients (7.19%) out of the 139 patients reviewed experienced an ICIP event. Patients who experienced an ICIP event had received either pembrolizumab (n=6; 60%) or nivolumab (n=4; 40%). All of the patients with ICIP had a primary pulmonary lesion (NSCLC, n=8; SCLC, n=1; Mesothelioma, n=1) with the majority having received immunotherapy in combination with chemotherapy (n=6; 60%).

Table 2 describes the initial immune-induced pneumonitis events that occurred in this population. The severity of the events was graded based on the common terminology criteria for adverse events with the majority meeting criteria for grade 2 pneumonitis (n= 6, 60%). Signs and symptoms of pneumonitis were discovered after a median of 6 cycles in the NSCLC group, 1 cycle in the SCLC group, and 4 cycles in the mesothelioma group. At the time of ICIP identification, 90% of patients had immunotherapy held and were started on either oral or intravenous steroids. Upon initiation of treatment, 60% of patients experienced complete improvement of symptoms and 30% had a partial improvement. One patient showed no improvement in symptoms and was determined to have grade 4 pneumonitis on initial presentation that ultimately proved to be steroid refractory. Once proven to be steroid refractory, this patient received cyclophosphamide and infliximab with no response. Ultimately, the patient succumbed to pneumonitis raising the grade to 5. One additional patient (10%) experienced disease progression while immunotherapy was held for management of ICIP.

Of the 10 patients who experienced an ICIP event, 6 (60%) were re-started on immunotherapy as described in Table 3. Unfortunately, 3 (50%) of the patients re-started on immunotherapy had a subsequent recurrence of ICIP with the breakdown as follows: nivolumab (n= 2, 33%) and pembrolizumab (n= 1, 17%). All 3 of these patients received higher doses of steroids compared to their initial pneumonitis treatment and 2 (67%) patients required the introduction of intravenous steroids. The median number of cycles in between ICIP events varied widely among the nivolumab and pembrolizumab groups. The nivolumab group displayed a longer median number of cycles at 22 cycles compared to the pembrolizumab group which displayed an almost immediate recurrence after just 1 cycle.

TABLE 2 Characterization of patients who developed pneumonitis.

Metric		NSCLC (n=8)	SCLC (n=1)	Mesothelioma (n=1)
Pneumonitis Grade – no. (%)	Grade 1	1 (12.5)	0 (0)	0 (0)
	Grade 2	4 (50)	1 (100)	1 (100)
	Grade 3	2 (25)	0 (0)	0 (0)
	Grade 4	0 (0)	0 (0)	0 (0)
	Grade 5	1 (12.5)	0 (0)	0 (0)
Pre-pneumonitis Immunotherapy Cycles – Median (IQR)		6 (5.25)	1 (0)	4 (0)
Pneumonitis Recurrence – no. (%)		3 (37.5)	0 (0)	0 (0)
Pneumonitis Management – no. (%)	Immunotherapy held	7 (87.5)	1 (100)	1 (100)
	Oral steroids	4 (50)	0 (0)	0 (0)
	Intravenous steroids	6 (75)	0 (0)	1 (100)
	Cyclophosphamide	1 (12.5)	0 (0)	0 (0)
	Infliximab	1 (12.5)	0 (0)	0 (0)
Immunotherapy restarted – no. (%)	Yes	5 (62.5)	1 (100)	0 (0)
	No	3 (37.5)	0 (0)	1 (100)
Post-pneumonitis Immunotherapy Cycles – Median (IQR)		3 (20)	46 (0)	0 (0)

TABLE 3 Characterization of patients who developed recurrence of pneumonitis.

Metric		Nivolumab (n=2)	Pembrolizumab (n=1)
Combination therapy – no. (%)	Yes	1 (50)	1 (100)
	No	1 (50)	0 (0)
Pre-pneumonitis Immunotherapy Cycles – Median		2.5	4
Pneumonitis Initial Management – no. (%)	Immunotherapy held	2 (100)	1 (100)
	Oral steroids	2 (100)	0 (0)
Pneumonitis Recurrence Management – no. (%)	Immunotherapy held	1 (50)	0 (0)
	Oral steroids	2 (100)	1 (100)
	Intravenous steroids	1 (50)	1 (100)
Immunotherapy restarted – no. (%)	Yes	2 (100)	1 (100)
	No	0 (0)	0 (0)
Immunotherapy Cycles Post-Initial Pneumonitis Event – Median		22	1

Discussion

Since the introduction of immunotherapy agents, randomized controlled trials have reported the incidence of immune-induced pneumonitis at about 2 to 2.5%. With little real-world data to support pneumonitis incidence rates, Tiu et al. explored the impact of real-world variability on the incidence of ICIP in the lung cancer population (7). This retrospective cohort study explored the impact of PD-1 and PD-L1 inhibitors on the incidence of pneumonitis and pneumonia as a composite endpoint. The results of this study showed only a marginal increase of 2.49% in risk of pneumonitis and pneumonia-like conditions in the ICI treated versus untreated group (7). However, only about 50% of the ICI treated patients had a prior history of asthma, COPD, or pleural disease at baseline (7). The relatively low representation of patients with a history of pulmonary disease coupled with the use of a composite endpoint makes it difficult to discern the impact pneumonitis events had on the ICI treated population with a prior pulmonary disease history. In our cohort from our hospital system, the incidence of pneumonitis was shown to be higher at 7.19% compared to previous reports from randomized controlled trials and the real-world data reported in the Tiu et al. study. This could be due to our focus on a potentially more vulnerable patient population with an obstructive pulmonary disease history and indicates a potential need to monitor these patients more closely. The higher incidence rate in our cohort is supported by results from the KEYNOTE-001 subgroup analysis which found that pneumonitis occurred more frequently in patients with a history of asthma and chronic obstructive pulmonary disease (COPD) compared to those without (5.4 vs. 3.1%) (3). Our data not only suggests a higher real-world incidence of ICIP events, but it also indicates that any form of obstructive pulmonary disease could potentially be a risk factor for increased risk of ICIP.

Identifying risk factors for the development of pneumonitis has been an ongoing process since the widespread use of immunotherapy began with factors such as recent radiation, use of EGFR TKIs, and combination durvalumab plus radiation clouding the picture. To determine the role of COPD and asthma on the development of ICIP, several studies have investigated these risk factors with mixed results. Chao et al. retrospectively assessed NSCLC patients receiving ICIs to identify risk factors for the development of ICIP and found that COPD was independently associated with a higher ICIP incidence with an odds ratio of 7.194 (CI= 1.130 to 45.798; P-value= 0.037) (8). However, the Zeng et al. study, that collected data from a very similar population to the Chao et al. study, did not see a link between co-existing COPD and a higher risk of ICIP but did support a higher incidence of pneumonitis in their subgroup of patients with a history of pulmonary fibrosis (8, 9). The lack of support for the hypothesis that history of COPD contributes to higher incidences of ICIP development may be explained by the smaller population size evaluated in that trial. Nonetheless, our finding within our population who possessed a variety of pulmonary disorders corroborates this trial's implication of pulmonary disease as a potential contributor to ICIP occurrence and establishes a history of obstructive pulmonary disease as a risk factor for pneumonitis that clinicians should be wary of prior to initiating ICI. The top 3 pulmonary diseases represented in our study were COPD (n= 64, 46%), emphysema (n= 29, 21%), and asthma (n= 24, 17%). In addition to our findings, Galant et al. studied 187 patients, 26 of which had an asthma diagnosis (10). Out of those 26 patients, 3 patients developed ICIP which accounted for 11.5% of the asthma patients in the population (10). All 3 asthma patients presented with severe pneumonitis that were classified as grade 3 and 4 reactions (10). The severity of the presentation coupled with the high percentage of asthma patients found to have developed pneumonitis implicates asthma as a potential serious risk factor to consider in the setting ICIP. Our data supports both COPD and asthma as risk factors for ICIP due to our high percentage of both pulmonary diseases in our base population. In addition, our findings also highlight that patients with an underlying diagnosis of emphysema and chronic bronchiectasis are also at risk for ICIP. These findings necessitate closer monitoring and follow-up after initiation of ICI treatment in patients with an underlying obstructive pulmonary disease.

Tiu et al. investigated the real-world impact of pneumonitis in lung cancer in which glucocorticoids were frequently administered to their patients with suspected ICIP. 212 (83.5%) of their 254 patients that experienced an ICIP event received oral prednisone within 30 days following diagnosis (7). Intravenous methylprednisolone was administered to 145 patients (57.1%) with high-grade adverse effects in the same 30-day interval post-diagnosis (7). Of the 225 patients that received an ICI, 158 (70.2%) discontinued treatment within 90 days of pneumonitis diagnosis (7). In the initial management of ICIP for our population, 4 patients (40%) received oral prednisone tapering over twelve to fifteen days and 6 patients (60%) received intravenous methylprednisolone with one patient receiving both oral and intravenous steroids in the management of their first pneumonitis event. Additionally, 9 patients (90%) had immunotherapy held quickly after ICIP diagnosis. Compared to the Tiu et al. study, our population was more likely to have their immunotherapy agent held and intravenous steroids started which resulted in a complete resolution of symptoms in 6 patients (60%) and partial resolution of symptoms in 3 patients (30%). Based on the data from both studies, there is continued evidence of the effectiveness of steroids for the management of initial ICIP episodes. Although holding immunotherapy and initiating steroids assisted in resolving ICIP symptoms for some patients, Tiu et al. reported a high rate of mortality at 32.7% in their population within 90 days of ICIP diagnosis due to a combination of disease progression and high-grade ICIP (7). At completion of the chart review time frame, 1 patient (10%) had died due to respiratory failure attributed to ICIP after treatment with steroids, cyclophosphamide, and infliximab.

In our study, 6 patients were re-started on immunotherapy after resolution of their initial ICIP symptoms. However, 3 patients (50%) experienced a recurrent episode of ICIP upon re-initiation requiring longer, higher doses of steroids along with more frequent use of intravenous steroids. The timing of these recurrent episodes varied from a median of 1 month to a little over one-year post-ICI therapy re-introduction. The wide time frame for recurrence can be attributed to the small number of patients in our population that experience a recurrence. Two of the 3 patients who experienced a recurrence received only 1 to 3 cycles of immunotherapy while one

patient received 41 cycles prior to a subsequent ICIP event. In a study conducted by Tao et al., the authors reported a median ICIP recurrence onset of 2.78 months which most closely aligns with the timeframe in which 2 of our patients presented with recurrence (11). This indicates that the majority of recurrence cases have the tendency to occur within a 2-to-3-month time interval of treatment re-initiation. Another study by Dolladille et al. looked at a total of 6,123 cases of immune-induced adverse effects (irAEs) and found that 452 (7.4%) of irAEs were associated with ICI rechallenges with 28.8% of irAE recurrences involving the same organ as the initial irAE identified (12). Additionally, pneumonitis was specifically associated with a higher recurrence rate compared with other irAEs (12). This further supports our findings of a higher pneumonitis recurrence rate among the patients in our population. Additionally, two patients (20%) experienced disease progression while immunotherapy was held for management of ICIP. Unfortunately, due to the evidence that supports high levels of recurrence and risk for disease progression while holding immunotherapy further investigations are needed into how to safely re-introduce ICI agents in this patient population.

Our study was limited by the small population size and therefore the small number of ICIP events reported which prohibits us from drawing statistically significant conclusions from the data presented. However, the data collected in this study does show trends toward pre-existing obstructive pulmonary disease having an impact on ICIP events rates, presentation, and management which necessitates a need for future prospective large-scale studies to further our knowledge of ICIP management. Although steroids and holding immunotherapy have been proven strategies for resolving ICIP for most patients, further research needs to elucidate management strategies that would be effective in the setting of rechallenge, steroid refractory ICIP, and ultimately reduce the risk of recurrence associated with holding ICI therapy and utilizing immunosuppressive doses of steroids. Our identification of ICIP cases was also dependent on manual chart review due to the retrospective nature of this study, which relied heavily on physician diagnosis documentation, medication administration records and imaging interpretation which may not be as reliable as data collected in a prospective manner. Future studies could evaluate the effects of ICIP on survival outcomes and the contribution of all obstructive pulmonary disease on incidence of ICIP.

Conclusion

Based on our findings, the real-world incidence of ICIP in patients with an underlying obstructive pulmonary disease history is higher than previously described in literature. Due to this increased concern for the development of ICIP in this population, more frequent monitoring and follow-up may be warranted to catch the development of ICIP at an earlier grade. Furthermore, a history of an obstructive pulmonary disorder should be a part of the risk versus benefit discussion surrounding use of an immunotherapy agent prior to patient initiation. Lastly, the high rates of ICIP recurrence reported coupled with the lack of effective management options for steroid refractory ICIP should be considered prior to re-introduction of ICIP therapy.

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 humans were approved by Houston Methodist Research Institute IRB. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

EA: Conceptualization, Data curation, Formal Analysis, Writing – original draft. GU: Conceptualization, Supervision, Writing – review & editing. VA: Supervision, Writing – review & editing. EB: Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Funding for publication fees provided by Texas Southern University Grant.

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 12 January 2024
DOI 10.3389/fimmu.2023.1309531

OPEN ACCESS

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RECEIVED 08 October 2023
ACCEPTED 20 December 2023
PUBLISHED 12 January 2024

CITATION
Pan S, Xie H, Wang L, Wang Y, Zou M, Xu Y, Tian X, Fan J and Wang J (2024) Case report: Checkpoint inhibitor pneumonitis with positive anti-melanoma differentiation-associated gene 5 antibodies in a patient with lung cancer.
Front. Immunol. 14:1309531.
doi: 10.3389/fimmu.2023.1309531

Case report: Checkpoint inhibitor pneumonitis with positive anti-melanoma differentiation-associated gene 5 antibodies in a patient with lung cancer

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With the widespread use of immune checkpoint inhibitors to treat various cancers, pulmonary toxicity has become a topic of increasing concern. Anti-melanoma differentiation-associated gene 5 (anti-MDA5) antibodies are strongly associated with rapidly progressive interstitial lung disease (RP-ILD) in patients with clinically amyopathic dermatomyositis. However, anti-MDA5 antibody expression has not been reported in patients with immune-related adverse events. We present the case of a 74-year-old man with lung adenocarcinoma who developed RP-ILD after treatment with immune checkpoint inhibitors. Further investigation revealed multiple autoantibodies, including anti-MDA5 antibodies. He initially responded to systemic glucocorticoids, immunosuppressants, and tocilizumab but eventually died from worsening pneumomediastinum. This case is the first one to suggest that checkpoint inhibitor pneumonitis can present as RP-ILD with positive anti-MDA5 antibodies, which may be predictive of a poor prognosis.

KEYWORDS

immune checkpoint inhibitors, immune-related adverse events, pneumonitis, rapidly progressive interstitial lung disease, anti-MDA5 antibodies

1 Introduction

Immune checkpoint inhibitors (ICIs) can have various adverse effects, including checkpoint inhibitor pneumonitis (CIP), which can be life-threatening. Myositis and dermatomyositis (DM) are considered part of the spectrum of immune-related adverse events (irAEs). The relationship between anti-melanoma differentiation-associated gene 5 (anti-MDA5) antibodies and clinically amyopathic dermatomyositis was first described by Sato et al. in a Japanese cohort (1). Anti-MDA5 antibodies are considered markers of poor prognosis for rapidly progressive interstitial lung disease (RP-ILD) which refers to a course with measurable progression within a short period of time since onset of interstitial lung disease (ILD). However, anti-MDA5 antibodies have not been reported in patients with irAEs until now. Herein, we report the first case of a patient with CIP who tested positive for anti-MDA5 antibody.

2 Case presentation

A 74-year-old man was admitted to the Peking Union Medical College Hospital's respiratory intensive care unit for a rash that lasted for one month and dyspnea that lasted for 2 weeks. He had been diagnosed with lung adenocarcinoma (Figures 1A, B) four months before admission. He had declined surgery due to severe comorbidities. Four weeks before admission, he was started on camrelizumab (200 mg, day 1), an ICI, combined with chemotherapy with pemetrexed (700 mg, day 1) and carboplatin (260 mg, day 1). He developed a pruritic maculopapular rash on his neck, chest wall, and sacrum. Two weeks before reporting to the hospital, he started experiencing shortness of breath on exertion, which gradually progressed. Chest computed tomography (CT) showed new bilateral subpleural opacities, which were more severe in the right lung (Figures 1C, D). CIP was suspected. Intravenous methylprednisolone 80 mg/day was administered for 5 days, tapered to 60 mg/day for 5 days, and maintained at 40 mg/day until admission. His rash improved but the dyspnea worsened. His medical history included hypertension, hyperlipidemia, cerebral infarction, and myocardial infarction. The patient had no history of ILD.

On physical examination, his body temperature was 36.5°C, blood pressure was 147/56 mmHg, heart rate was 97 beats per minute, respiratory rate was 25 per minute, and oxygen saturation was 92% while he was receiving supplementary oxygen via a non-rebreathing mask at a flow rate of 12 liters per minute. Inspiratory tri-concave signs and scattered desquamated rashes were observed on inspection, and Velcro rales were heard bilaterally, but were more prominent in the right lung. Arterial blood gas (fraction of inspired oxygen was 70%) showed elevated pH (7.506), partial pressure of oxygen (76.6 mmHg; 83–108 mmHg), partial pressure of carbon dioxide (27

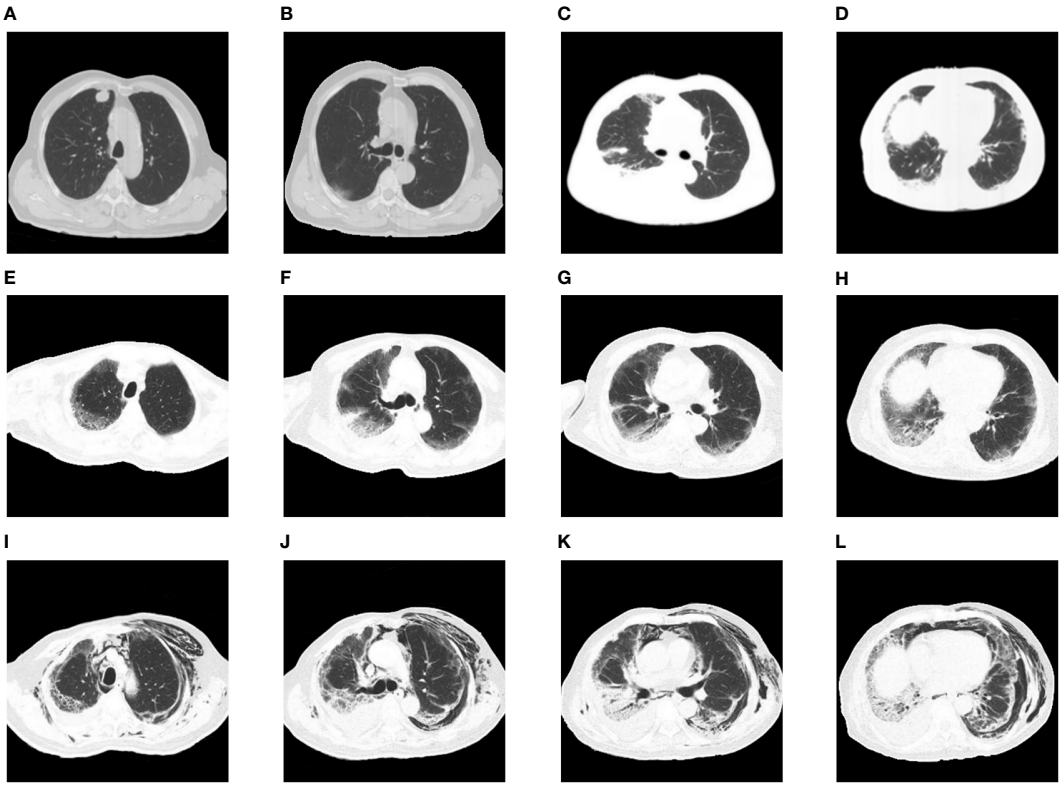

FIGURE 1
Chest images during the clinical episode. **(A, B)** The baseline chest CT before initiating therapy showed a nodule in the right upper lobe without any interstitial change. **(C, D)** Chest CT after onset of dyspnea showed new bilateral subpleural opacities, which were more severe in the right lung. **(E–H)** Chest CT on intubation day showed bilateral ground-glass opacities and consolidation with reticulation. **(I–L)** Chest CT after extubation revealed severe subcutaneous and mediastinal empysema, along with bilateral opacities consistent with interstitial pneumonitis.

10.3389/fimmu.2023.1309531

mmHg; 35–45 mmHg), and lactose concentration (2.3 mmol/L; 0.5–1.6 mmol/L). Elevated concentrations of C-reactive protein (69.8 mg/L; 0–3 mg/L), ferritin (1124 ng/ml; 24–336 ng/ml), and interleukin-6 (77 pg/ml; 0–5.9 pg/ml) were also documented. The rheumatology panel showed an elevated rheumatoid factor concentration of 57 IU/ml (0–20 IU/ml) and weak positivity for anti-nuclear antibody (1:160; normal range< 1:80 by ELISA), anti-mitochondrial M2 antibody (17; normal range< 15 by Western Blot), anti-MDA5 antibody (+; "+++" represents the strongest by Western Blot), and anti-Ro 52 antibody (+; "+++" represents the strongest by Western Blot). The other laboratory findings are presented in Table 1.

CIP (grade 4) was diagnosed, and the methylprednisolone dose was increased to 80 mg twice daily for 3 days before being tapered slowly. Tocilizumab (480 mg/week) was administered for 7 weeks, intravenous immunoglobulin was administered at 20 g/day for 3 days. Cyclophosphamide and tacrolimus were also administered. The patient was intubated and mechanically ventilated due to worsening respiratory distress. Chest CT (Figures 1E–H) showed bilateral ground-glass opacities and consolidation with reticulation. The patient improved and was successfully weaned from ventilation to a high-flow nasal cannula 11 days after intubation. However, he received a second round of intravenous methylprednisolone and intravenous immunoglobulin (20 g/day) due to exacerbation of his chest CT findings. Anti-MDA5 antibodies turned negative 20 days after the first report. His respiratory support level stabilized with the high-flow nasal cannula (flow rate of 30 L/min; fraction of inspired oxygen, 35–50%) until severe mediastinal and subcutaneous emphysema developed, which involved the neck and chest and extended to the scrotum and lower extremities (Figures 1I–L). Two months after admission, he strongly insisted on returning home. He died 2 days later due to severe dyspnea. The details of his treatment are summarized in Figure 2.

TABLE 1 Laboratory data.

Variable	Reference Range, Adults, This Hospital	On Admission
White-cell Count (10^9/L)	3.50-9.50	7.46
Lymphocytes Count (10^9/L)	0.80-4.00	0.33
Hemoglobin (g/L)	120-160	134
Platelet Count (10^9/L)	100-350	251
Alanine Transferase (U/L)	9-50	41
Total Bilirubin (μmol/L)	5.1-22.2	7.3
Direct Bilirubin (μmol/L)	≤6.8	2.4
Albumin (g/L)	35-52	31
Urea (mmol/L)	2.78-7.14	7.18
Creatinine (μmol/L)	59-104	50
Lactate dehydrogenase (U/L)	0-250	437
Creatine Kinase (U/L)	24-195	27
Cardiac Troponin I (μg/L)	0-0.056	<0.017

Reference values are influenced by a wide range of factors, such as the patient group and the types of laboratory techniques. The ranges used at Peking Union Medical College Hospital are intended for adults without any health issues that might have an impact on the outcomes. They may not be appropriate for all patients.

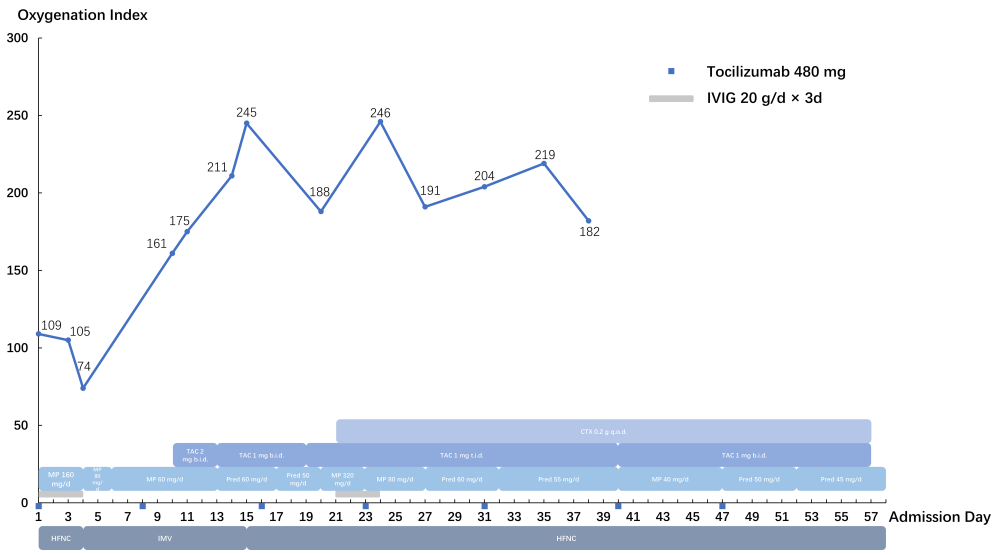

FIGURE 2
Summary of the clinical course. IVIG, intravenous immunoglobulin; HFNC, high-flow nasal cannula; IMV, invasive mechanical ventilation; MP, methylprednisolone; Pred, prednisone; TAC, tacrolimus; CTX, cyclophosphamide.

10.3389/fimmu.2023.1309531

3 Discussion

ICIs are immunomodulatory antibodies that boost the immune system. Their main targets are programmed cell death receptor 1, programmed cell death ligand 1, and cytotoxic lymphocyte-associated antigen 4. ICIs have significantly improved the prognosis of patients with various advanced malignancies, including lung cancer. Immune-related adverse events (irAEs) are inflammatory responses caused by ICIs. They can involve the skin, gastrointestinal tract, endocrine system, lungs, and other organs (2). A study by Naidoo et al. showed that the overall incidence of CIP caused by anti-programmed cell death receptor 1 or programmed cell death ligand 1 treatment was 5%. The duration of treatment before pneumonitis onset varied with a median of 2.8 months (9 days to 19 months) (3). The clinical manifestations and imaging findings of CIP are nonspecific. Cough and dyspnea are the most common symptoms, but some patients may be asymptomatic. The signs and patterns on chest imaging include ground-glass opacity, consolidation, and diffuse alveolar damage. CIP is usually sensitive to glucocorticoid therapy (4). This patient developed a rash and pulmonary lesion soon after the administration of ICIs. Although his onset was rapid, immunotherapy-related skin and pulmonary toxicities should be considered first. Antineoplastic agent like pemetrexed can infrequently cause lung toxicities including ILD, with ground glass opacities the predominate CT pattern and good response to steroids, which should be differentiated in this case (5). However, pemetrexed is unlikely to cause the autoantibodies.

Anti-MDA5 antibodies are associated with clinically amyopathic dermatomyositis, which usually manifests as a characteristic rash and RP-ILD with high mortality. RP-ILD in anti-MDA5-positive DM is suggested to be defined as either worsening of dyspnea and CT progression within 1 month, or deterioration to respiratory failure within 3 months since respiratory symptom onset (6). According to the consensus of the 239th European Neuromuscular Centre meeting, DM can be diagnosed with the following criteria: typical DM-related rash, dermatopathological evidence of interface dermatitis, evidence of myositis, or positive DM-specific autoantibodies (7). Although this patient tested positive for anti-MDA5 antibodies, he did not have a typical DM-related rash or any evidence of myositis such as creatine kinase elevation; hence, DM could not be diagnosed. Moreover, he showed no signs of ILD on chest CT besides lung cancer at baseline. Anti-MDA5 and other autoantibodies before ICI therapy were not measured, which is a limitation of this report, but we disregarded them as a marker of newly developed or exacerbated anti-MDA5-positive DM. Additionally, the pathophysiology was different from that of typical anti-MDA5-positive DM in the following respects. First, the pulmonary lesion was more prominent on the tumor side. Second, the patient responded better to large doses of glucocorticoids and immunosuppressants and was successfully weaned from invasive mechanical ventilation, which is rare for patients with anti-MDA5-positive DM. Third, the titer of anti-MDA5 antibodies was mismatched with the severity of lung disease, and the patient tested negative for them immediately after therapy. A detailed comparison of anti-MDA5-positive DM, typical CIP, and CIP in this patient is shown in Table 2.

ICIs can lead to various rheumatic irAEs such as arthritis and myositis, as well as presence of new autoantibodies. The incidence of ICI-induced myositis is approximately 0.6% (8). Ghosh et al. systematically reviewed the incidence of autoantibodies in patients with irAEs and found that 67 patients tested positive for myositis-associated antibodies, and 27% tested positive for at least one antibody, such as anti-Mi-2 antibody, anti-PM/Scl antibody, and anti-signal recognition particle (SRP) antibody (9). However, anti-MDA5 antibodies, which represent a type of myositis-associated antibodies, have not been previously reported after the administration of ICIs. In short, it is more reasonable to consider anti-MDA5 antibodies and other autoantibodies in this patient as byproducts of immunotherapy.

Anti-MDA5 antibodies are not found exclusively in patients with DM. Wang et al. reported that 48.2% of patients with coronavirus disease 2019 (COVID-19) tested positive for anti-

TABLE 2 Comparison of anti-MDA5-positive DM, typical CIP, and our patient's condition.

	Anti-MDA5-Positive DM	Typical CIP	Our Patient's condition
Rash Manifestation	Extensor articular erythema, periorbital erythema, typical DM-related rashes such as V sign, Gottron sign, and mechanic's hands	Variable	Maculopapular rash
RP-ILD Manifestation	Always	Seldom	Yes
Mediastinal emphysema	Common	Seldom	Yes
Anti-MDA5 antibody and its titer	Positive, titer decreasing after effective treatment	No report so far	Positive, titer decreasing after effective treatment
Other autoantibodies	Anti-Ro 52 antibody	Seldom	ANA, AMA-M2, anti-Ro 52 antibody
Chest CT imaging manifestation	Interstitial changes in the bibasilar subpleural lung, manifesting as mass, reticulum, GGO	Variable	Bilateral subpleural GGO, the right side is prominent
Response to systemic glucocorticoids and immunosuppressants	Poor	Variable	Partial response
Prognosis	Poor	Depending on severity and grading	Poor

MDA5, melanoma differentiation-associated gene 5; DM, dermatomyositis; CIP, checkpoint inhibitor pneumonitis; RP-ILD, rapidly progressive interstitial lung disease; ANA, anti-nuclear antibody; AMA-M2, anti-mitochondrial M2 antibody; GGO, ground glass opacity.

MDA5 antibodies, and the non-survival group had a greater prevalence (10). Based on the shared features between this case and classical anti-MDA5-positive DM patients, we speculate that the anti-MDA5 antibodies may have partially accounted for the clinical characteristics justifying the use of immunosuppressive medication, and might be predictive of a poor prognosis. Tocilizumab, an interleukin-6 receptor monoclonal antibody, is licensed for the treatment of rheumatoid arthritis, systemic juvenile idiopathic arthritis, and COVID-19. It has been reported to improve the outcomes of patients with irAEs (11) and is utilized as salvage treatment for anti-MDA5-positive DM with RP-ILD (12). In our case, the administration of tocilizumab may have contributed to clinical remission.

4 Conclusion

We report the first case of a patient with CIP with anti-MDA5 antibody positivity, who transiently responded to treatment with glucocorticoids, immunosuppressants, and tocilizumab. However, he eventually died from dyspnea. Therefore, screening for anti-MDA5 antibodies is warranted in patients with CIP who present with RP-ILD.

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 authors.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements. 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

SP: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. HX: Data curation, Validation, Writing – review & editing. LW: Data curation, Validation, Writing – review & editing. YW: Validation, Writing – review & editing. MZ: Data curation, Resources, Writing – review & editing. YX: Writing – review & editing. XT: Supervision, Validation, Writing – review & editing. JF: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. JW: Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National High Level Hospital Clinical Research Funding (grant numbers 2022-PUMCH-A-129, 2022-PUMCH-C-054). These funding sources had no role in the study design or execution, analyses, interpretation of the data, or decision to submit results.

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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frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 26 January 2024
DOI 10.3389/fonc.2024.1303543

OPEN ACCESS

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RECEIVED 28 September 2023
ACCEPTED 12 January 2024
PUBLISHED 26 January 2024

CITATION
Yoshimura A, Takeda T, Kataoka N, Tanimura K, Fukui M, Chihara Y, Takei S, Kawachi H, Nakanishi K, Yamanaka Y, Tamiya N, Honda R, Okura N, Yamada T, Uryu K, Murai J, Shiotsu S, Yoshioka H, Yamada T, Kurata T and Takayama K (2024) Impact of the response to platinum-based chemotherapy on the second-line immune checkpoint inhibitor monotherapy in non-small cell lung cancer with PD-L1 expression ≤49%: a multicenter retrospective study. *Front. Oncol.* 14:1303543. doi: 10.3389/fonc.2024.1303543

Impact of the response to platinum-based chemotherapy on the second-line immune checkpoint inhibitor monotherapy in non-small cell lung cancer with PD-L1 expression ≤49%: a multicenter retrospective study

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Introduction: The efficacy of second-line immune checkpoint inhibitor (ICI) therapy is limited in non-small cell lung cancer (NSCLC) patients with ≤ 49% PD-L1 expression. Although chemoimmunotherapy is a promising strategy, platinum-based chemotherapy followed by ICI monotherapy is often used to avoid synergistic adverse events. However, predictors of the efficacy of ICI monotherapy after platinum-based chemotherapy in NSCLC with ≤ 49% PD-L1 expression remain scarce.

Methods: This multicenter retrospective study evaluated 54 advanced or recurrent NSCLC patients with ≤ 49% PD-L1 expression who were treated with second-line ICI monotherapy following disease progression on first-line platinum-based chemotherapy at nine hospitals in Japan. The impact of response to platinum-based chemotherapy on the efficacy of subsequent ICI monotherapy was investigated.

Results: The response to first-line platinum-based chemotherapy was divided into two groups: the non-progressive disease (PD) group, which included

patients who did not experience disease progression after four cycles of chemotherapy, and the PD group, which included patients who showed initial PD or could not maintain disease control during the four cycles of chemotherapy and switched to second-line ICI monotherapy. Among the 54 patients, 32 and 22 were classified into the non-PD and PD groups, respectively. The non-PD group showed better response rates (p = 0.038) and longer overall survival (OS) with ICI monotherapy (p = 0.023) than the PD group. Multivariate analysis identified that maintaining a non-PD status after four cycles of chemotherapy was an independent prognostic factor for ICI monotherapy (p = 0.046). Moreover, patients with a modified Glasgow Prognostic Score (mGPS) of 0 showed a tendency for longer OS with ICI monotherapy (p = 0.079), and there was a significant correlation between maintaining non-PD after four cycles of chemotherapy and an mGPS of 0 (p = 0.045).

Conclusion: Maintaining a non-PD status after four cycles of platinum-based chemotherapy was a predictor of OS after second-line ICI monotherapy. These findings will help physicians select the most suitable treatment option for NSCLC patients who were treated with platinum-based chemotherapy and switched to second-line treatment. Those who experienced early PD during platinum-based chemotherapy should not be treated with ICI monotherapy in the second-line setting.

KEYWORDS

immune checkpoint inhibitor monotherapy, modified Glasgow prognostic score, non-small cell lung cancer, platinum-based chemotherapy, predictive marker

1 Introduction

Lung cancer is the most common cause of cancer-related death worldwide (1), with non-small cell lung cancer (NSCLC) comprising approximately 85% of cases (2). The introduction of immune checkpoint inhibitors (ICIs) has dramatically altered treatment strategies for several cancers, including melanoma, lung cancer, and renal cell carcinoma (3). Programmed death-ligand 1 (PD-L1) expression in tumor cells serves as a positive predictive biomarker during ICI treatment in patients with advanced NSCLC (4). This is attributable to the fact that increased PD-L1 expression in tumor cells suppresses T-cell activation and proliferation by inducing effector T-cell apoptosis, resulting in an escape from immune responses (5, 6).

In the first-line setting, ICI monotherapy does not provide longer overall survival (OS) than platinum-based chemotherapy in patients with advanced NSCLC with low (1–49%) PD-L1 expression (7–9) compared to those with high (≥ 50%) PD-L1 expression on tumor cells (4). In contrast, chemoimmunotherapy (CIT) demonstrated superiority in OS over platinum-based chemotherapy as the first-line treatment for NSCLC, irrespective of PD-L1 expression status (10–13). Although an increase in adverse events associated with first-line CIT was shown in a network meta-analysis of 16 randomized controlled trials (14), CIT was adopted in patients with low or negative PD-L1 expression and a low rate of treatment failure. In contrast, sequential administration of first-line platinum-based chemotherapy followed by ICI monotherapy is sometimes selected to avoid the synergistic adverse events of CIT. This strategy is based on phase III trials that demonstrated the superiority of second-line ICI monotherapy over docetaxel (15–18).

CD8-positive tumor-infiltrating lymphocytes (TILs), which are representative markers of the tumor microenvironment (TME), also serve as predictors of anti-programmed cell death-1 (PD-1) treatment in NSCLC (19). CD8-positive TILs are known to increase after neoadjuvant chemotherapy in resected NSCLC specimens, suggesting that cytotoxic chemotherapy promotes antitumor immunity through T- and B-cell recruitment in the immune microenvironment (20). CD8-positive TILs are significantly increased in patients with advanced gastric cancer who respond to cytotoxic chemotherapy compared to those who do not (21). Thus, the response to first-lineplatinum-based chemotherapy in advanced NSCLC with PD-L1 expression of ≤49% could affect the efficacy of second-line ICI monotherapy; however, this has never been investigated.

In addition to the TME, cancer cachexia is an important host condition that affects the response to tumor cells (22). The modified Glasgow Prognostic Score (mGPS) is defined by serum C-reactive

protein (CRP) and albumin levels (23, 24). Cancer cachexia can be assessed by mGPS which focuses on nutrition and systemic inflammation (25). Since neutrophil and platelet are known to have pro-inflammatory role in patients with cancer, while lymphocyte lead to tumor suppression, the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) are considered as useful immunological and nutritional markers in predicting the outcomes (26, 27).

In this multicenter retrospective study, the impact of response to platinum-based chemotherapy on the efficacy of subsequent ICI monotherapy was investigated. The differences among the subgroups with PD-L1 expression of 1–49% and <1% and the influence of mGPS values, NLR, and PLR on OS were also evaluated.

2 Patients and methods

2.1 Study population

We analyzed the electronic medical records of consecutive patients with advanced or recurrent NSCLC with PD-L1 expression ≤49% between January 1, 2016, and September 30, 2021, at nine hospitals in Japan. The study protocol was approved by the Ethics Committees of the Japanese Red Cross Kyoto Daini Hospital (February 2, 2022; S2021-43) and each participating hospital. The requirement for consent was waived due to the retrospective nature of the study and its anonymity. Patients were allowed to withdraw their data and relevant information, which were available on each hospital's website.

Inclusion criteria were as follows: (a) patients aged 20 years or older; (b) those with pathologically diagnosed NSCLC without driver gene alteration; (c) those with metastatic NSCLC or NSCLC with postoperative recurrence; (d) PD-L1 expression on tumor cells ≤49%; e) patients with evaluable lesions by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.; (f) patients treated with first-line platinum-based chemotherapy followed by second-line ICI monotherapy during the study period. Adjuvant chemotherapy after surgery was not considered platinum-based chemotherapy.

2.2 Data collection

The following clinical data were obtained from electronic medical records: age, sex, smoking status, Eastern Cooperative Oncology Group performance status (ECOG-PS), clinical stage, histological subtype, PD-L1 expression in tumor cells, and pretreatment serum CRP and albumin levels at the time of ICI monotherapy administration. Patients with missing data were excluded from the analysis.

2.3 Clinical outcomes

Either computed tomography scan or magnetic resonance imaging was performed to determine complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), and not evaluable (NE) status based on the RECIST version 1.1. Objective response rate (ORR) and disease control rate (DCR) were defined as "the percentage of patients in the study or treatment group who achieved CR or PR after the treatment" and "the percentage of patients in the study or treatment group who achieved CR, PR, and SD", respectively (28). Progression-free survival (PFS) was defined as the duration from the initiation of ICI monotherapy to the date of disease progression or death, whichever came first. Patients who remained alive without disease progression were censored at the date of their last imaging examination. OS was defined as the duration from the initiation of ICI monotherapy to death. Patients who were still alive at the time of data acquisition were censored at the date of the last visit.

2.4 PD-L1 testing

PD-L1 expression was evaluated in pretreatment samples by PD-L1 immunohistochemistry (IHC) using the 22C3 pharmDx assay (Dako North America, USA). Patients were categorized into two groups based on their PD-L1 expression status: low (1–49%) and negative (< 1%).

2.5 Modified Glasgow prognostic score

The mGPS was determined as previously described (24). Patients with neither elevated CRP levels (> 1 mg/dl) nor hypoalbuminemia (< 3.5 g/dl) were assigned a score of 0; those with either of these biochemical abnormalities were assigned a score of 1; and those with both abnormalities were assigned a score of 2.

2.6 Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio

NLR was the ratio of absolute neutrophil count (/μL) divided by absolute lymphocyte count (/μL). PLR was the ratio of absolute platelet count (/μL) divided by absolute lymphocyte count. Based on the previous reports (26, 27), the cut-off values for NLR and PLR were set at < 3.5 or ≥ 3.5 and < 200 or ≥ 200, respectively.

2.7 Statistical analysis

PFS and OS curves were plotted using the Kaplan–Meier method. The log-rank test was used to evaluate the PFS and OS. The hazard ratios (HRs) for PFS and OS were determined using a univariate Cox proportional hazards model. Cox proportional hazard models were used to evaluate the patients' background factors. To construct the multivariate model, we selected factors associated with OS that were most relevant to the univariate analysis results and previous reports. All statistical analyses were performed using the GraphPad Prism software (v.9.41; GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

3 Results

3.1 Characteristics of patients before immune checkpoint inhibitor monotherapy

Among the 54 patients enrolled in this study with advanced or postoperative recurrent NSCLC with low (1–49%) or negative (< 1%) PD-L1 expression, the median age was 72.5 years (range: 33.0–85.0). Of these patients, 49 (90.7%) were males, 49 (90.7%) were current or former smokers, and all (100.0%) had an ECOG-PS of 0 or 1 (Table 1). Nine patients (16.7%) experienced postoperative recurrence, with adenocarcinoma being the most prevalent type (55.6%). PD-L1 expression in tumor cells was low in 43 patients and negative in 11 patients.

The objective responses to the first-line platinum-based chemotherapy were as follows: CR in 0, PR in 23 (42.6%), SD in 22 (40.7%), PD in nine (16.7%), and NE in no patients. ORR was 42.6% (95% confidence interval [CI]: 29.2-56.8) and DCR was 83.3% (95% CI: 70.7-92.1).

TABLE 1 Patients characteristics.

		n = 54
Median age, years (range)		72.5 (33.0–85.0)
Age categorization, n (%)	<75	36 (66.7)
	≥75	18 (33.3)
Sex, n (%)	Male	49 (90.7)
	Female	5 (9.3)
Smoking status, n (%)	Current or former	49 (90.7)
	Never	5 (9.3)
PS, n (%)	0	9 (16.7)
	1	45 (83.3)
Disease stage, n (%)	III	4 (7.4)
	IV	41 (75.9)
	Postoperative relapse	9 (16.7)
Histology, n (%)	Adenocarcinoma	30 (55.6)
	Others	24 (44.4)
PD-L1 TPS, n (%)	≥50%	0 (0.0)
	1-49%	43 (79.6)
	<1%	11 (20.4)
Response of platinum doublet, n (%)	PR	23 (42.6)
	SD	22 (40.7)
	PD	9 (16.7)
	NE	0 (0.0)
	ORR (95% CI)	42.6% (29.2–56.8%)
	DCR (95% CI)	83.3% (70.7–92.1%)
Disease control after 4 cycles of platinum doublet, n (%)	Achieved	32 (59.3)
	Not achieved	22 (40.7)
Response of ICIs monotherapy, n (%)	PR	4 (7.4)
	SD	18 (33.3)
	PD	29 (53.7)
	NE	3 (5.6)
	ORR (95% CI)	7.8% (2.2–18.9%)
	DCR (95% CI)	43.1% (29.3–57.8%)

PS, performance status; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; ORR, objective response rate; CI, confidence interval; DCR, disease control rate; PD-L1, programmed death-ligand 1; TPS, tumor proportion score; ICI, immune-checkpoint inhibitor.

3.2 Relationship between the response to platinum-based chemotherapy and clinicopathological features

The patients were divided into two groups based on the response to first-line platinum-based chemotherapy: the non-PD group, which included patients who did not experience disease progression after four cycles of induction chemotherapy, and the PD group, which included patients who showed initial PD or could not maintain disease control during the four cycles of induction chemotherapy and switched to second-line ICI monotherapy. Among the 54 patients, 32 and 22 were classified into the non-PD and PD groups, respectively (Supplementary Table 1). There was no significant difference between the two groups in terms of clinicopathological features, except for adenocarcinoma histology, which showed better disease control than non-adenocarcinoma ($p = 0.027$). Among the 32 patients in the non-PD group, 25 and seven patients had low and negative PD-L1 expression, respectively. Among the 22 patients in the PD group, 18 and four patients had low and negative PD-L1 expression, respectively (Supplementary Table 1).

3.3 Significance of the response to platinum-based chemotherapy and the efficacy of immune checkpoint inhibitor monotherapy

Among 54 patients, the objective responses to the second-line ICI monotherapy were as follows: CR in 0, PR in 4 (7.4%), SD in 18 (33.3%), PD in 29 (53.7%), and NE in 3 (5.6%) (Table 1). The ORR and DCR of the second-line ICI monotherapy were 7.8% (95% CI:2.2-18.9) and 43.1% (95% CI:29.3-57.8), respectively (Table 1; Figure 1A), showing lower ORR and DCR compared to those of platinum-based chemotherapy (42.6% and 83.3%, respectively) (Table 1).

The effect of the response to platinum-based chemotherapy on the efficacy of ICI monotherapy was evaluated. The ORR for ICI monotherapy was significantly higher in the non-PD group than in the PD group (13.8% vs. 0.0%, p = 0.038) (Figure 1B).

3.4 Predictor for the progression-free and overall survival of immune checkpoint inhibitor monotherapy

Subsequently, the predictors of PFS and OS of second-line ICI monotherapy for NSCLC with PD-L1 expression ≤49% were investigated. The median follow-up period was 11.0 months (range: 1.6–66.5). The median PFS and OS of ICI monotherapy were 2.0 months (95% CI: 1.6–3.0) and 11.7 months (95% CI: 8.2–13.5), respectively (Figures 2A, B).

Univariate analysis identified non-PD group (maintaining disease control after 4 cycles of first-line platinum-based chemotherapy) as a predictor for longer OS with ICI monotherapy; median OS in the non-PD group (13.5 months [95% CI, 7.7–23.6]) and in the PD group (8.6 months [95% CI, 5.3–12.1]) (p = 0.023) (Table 2; Figure 2D). In contrast, there was no significant difference in the PFS between the non-PD and PD groups (P = 0.304) (Figure 2C). There was no significant difference between tumor PD-L1 expression of 1–49% and that of <1% in PFS (p = 0.441) and OS (p = 0.485) (Table 2).

Multivariate analysis showed that the non-PD group was an independent predictor for OS of ICI monotherapy (HR: 0.49, 95% CI: 0.24–0.99, p = 0.046) (Table 3).

Furthermore, in NSCLC with PD-L1 expression of 1–49%, the median OS of ICI monotherapy was significantly longer in the non-PD group (13.5 months [95% CI, 7.5–24.2]) than in the PD group (8.3 months [95% CI, 5.1–9.5]), with a p-value of 0.003 (Supplementary Figure 1B), while there was no significant difference in the PFS between the two groups (p = 0.473) (Supplementary Figure 1A). However, there was no significant difference in PFS (p = 0.519) and OS (p = 0.555) based on PD-L1 expression in the < 1% subgroup between the non-PD and PD groups (Supplementary Figures 1C, D).

3.5 Influence of immunological and nutritional markers and the response to platinum-based chemotherapy on the efficacy of immune checkpoint inhibitor monotherapy

Serum CRP and albumin levels were available at the start of ICI monotherapy in 43 patients, among whom 13, 16, and 14 patients were categorized as having an mGPS of 0, 1, and 2, respectively. Neutrophil, lymphocyte, and platelet counts at the start of ICI monotherapy were available among 44 patients. Among 44 patients, 20 and 24 patients showed NLR < 3.5 and ≥ 3.5 and < 3.5, while 14 and 30 patients showed PLR < 200 and ≥ 200, respectively. The relationship between the response to first-line platinum-based chemotherapy and the mGPS, NLR and PLR values at the start of ICI monotherapy was assessed in 43 patients. Although there was no significant difference between the effect of platinum-based chemotherapy and the NLR or PLR values, patients with an mGPS score of 0 were significantly more prevalent in the non-PD group, which maintained disease control after four cycles of induction chemotherapy (42.3%), compared to the PD group (11.8%), with a p-value = 0.045 (Supplementary Table 2). In contrast to the NLR and PLR showing no significant difference in PFS and OS (Supplementary Figure 2), the median OS of ICI monotherapy was relatively longer in patients with mGPS of 0 (16.1 months [95% CI: 6.5–32.3]) than in patients with mGPS of 1–2 (10.9 months [95% CI: 6.9–13.0]), with a p-value = 0.079 (Table 2; Figure 2F). In contrast, there was no significant difference in the PFS after ICIs monotherapy between patients with an mGPS of 0 and those with an mGPS of 1–2 (p = 0.768) (Table 2; Figure 2E).

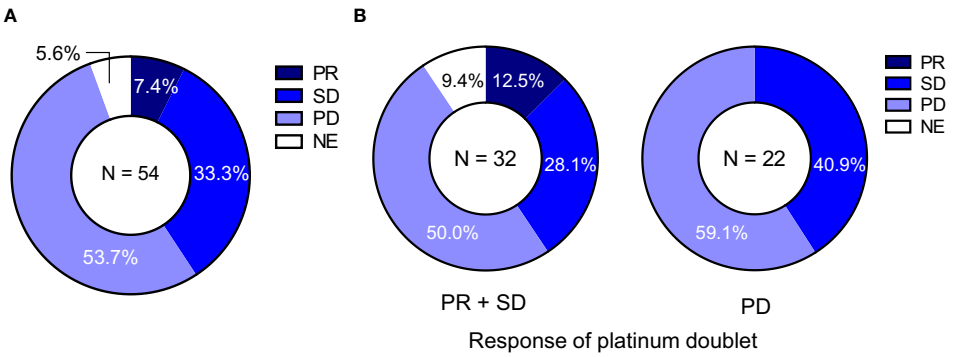

FIGURE 1
Second-line ICI monotherapy efficacy according to the response to the first-line platinum-based chemotherapy. **(A)** The response to second-line ICI monotherapy in 54 patients with programmed death ligand 1 (PD-L1) expression ≤49%. **(B)** The response to second-line ICI monotherapy in patients with NSCLC and PD-L1 expression ≤49% stratified according to the response (non-PD vs. PD) to the first-line platinum-based chemotherapy. There was a significant relationship in ORR of second-line ICI monotherapy between the response (non-PD and PD) to the first-line platinum-based chemotherapy (13.8% vs. 0.0%, p = 0.038). ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.

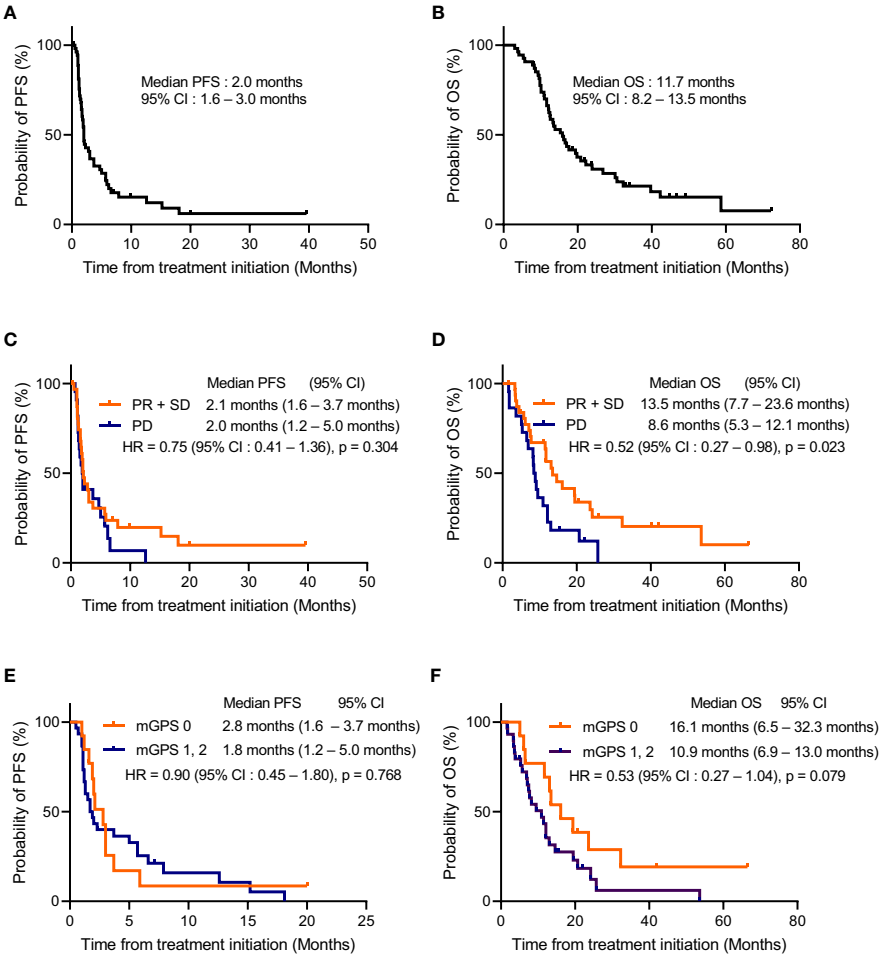

FIGURE 2
Kaplan-Meier estimates of progression-free survival and overall survival of second-line immune checkpoint inhibitor monotherapy. Kaplan–Meier estimates for progression-free survival [PFS: **(A)**] and overall survival [OS: **(B)**] in patients receiving immune checkpoint inhibitor (ICI) monotherapy after disease progression on platinum-based chemotherapy (n = 54). Kaplan–Meier estimates for PFS **(C)** and OS **(D)** of second-line ICI monotherapy were classified according to the response to first-line platinum-based chemotherapy (non-progressive disease [PD] vs. PD). The median PFS was 2.1 months in the non-PD group (95% confidence interval [CI]: 1.6–3.7 months) and 2.0 months in the PD group (95% CI: 1.2–5.0 months) with a p-value = 0.304, and the median OS was 13.5 months in the non-PD group (95% CI:7.7–23.6 months) and 8.6 months in the PD group (95% CI:5.3–12.1 months) with a p-value = 0.023. Kaplan–Meier estimates for PFS **(E)** and OS **(F)** of second-line ICI monotherapy were classified using the modified Glasgow Prognostic Score (mGPS; 0 vs. 1–2). The median PFS of ICIs monotherapy was 2.8 months in the subgroup with mGPS of 0 (95% CI: 1.6–3.7 months) and 1.8 months in the subgroup with mGPS of 1–2 (95% CI: 1.2–5.0 months) with a p-value = 0.768, and the median OS was 16.1 months in the subgroup with mGPS of 0 (95% CI: 6.5–32.3 months) and 10.9 months in the subgroup with mGPS of 1–2 (95% CI: 6.9–13.0 months) with a p-value = 0.079. PFS, progression-free survival; OS, overall survival; NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1; ICI, immune checkpoint inhibitor; PR, partial response; SD, stable disease; PD, progression disease; CI, confidence interval; mGPS, modified Glasgow Prognostic Score; NE, not evaluable.

4 Discussion

This study elucidated the impact of the response to first-line platinum-based chemotherapy on the efficacy of second-line ICI monotherapy for NSCLC with low or negative PD-L1 expression. The maintenance of non-PD after four cycles of platinum-based chemotherapy showed a strong relationship with the longer OS associated with subsequent ICI monotherapy for patients with NSCLC with PD-L1 expression of 1–49%. In contrast, this phenomenon was not observed in patients with NSCLC and PD-L1 expression <1%.

The median OS of the second-line ICI monotherapy among the subgroup with PD-L1 expression 1–49% who experienced PD before 4 cycles of platinum-based chemotherapy in this study (8.6 months) was shorter than that of the standard second-line treatment with docetaxel in a phase III trial in Japan (13.6 months) (29). ICI monotherapy was superior to docetaxel in phase III trials (15–18); therefore, identification of a population

TABLE 2 Cox proportional hazard models for PFS and OS in patients with non-small cell lung cancer who received ICIs monotherapy, univariate analysis.

Characteristics		Patient's No.	Median PFS (95% CI), months	*P* value	Median OS (95% CI), months	*P* value
Age categorization	<75	36	2 (1.5–3.7)	0.975	11.5 (7.1–16.1)	0.784
	≥75	18	2.3 (1.2–6.2)		11.7 (6.1–13.5)	
Sex	Male	49	2.0 (1.6–3.7)	0.413	11.7 (8.2–13.5)	0.849
	Female	5	1.9 (1.1–NE)		10.9 (5.3–NE)	
Smoking status	Current or former smoker	5	1.9 (1.0–NE)	0.246	7.7 (3.8–NE)	0.728
	Never smoker	49	2.0 (1.6–3.7)		11.7 (8.3–13.5)	
PS	0	9	2.0 (1.0–4.7)	0.870	19.4 (3.8–NE)	0.200
	1	45	2.0 (1.5–3.7)		11.5 (7.5–13.1)	
Disease stage	III	4	3.6 (2.1–NE)	0.903	12.4 (9.5–NE)	0.502
	IV	41	2.0 (1.3–3.7)		8.8 (6.5–13.5)	
	Postoperative relapse	9	1.9 (1.1–15.2)		16.1 (7.7–32.3)	
Histology	Adenocarcinoma	30	2.0 (1.3–5.0)	0.772	13.5 (7.7–20.7)	0.211
	Others	24	2.0 (1.5–3.7)		9.0 (6.9–11.7)	
PD-L1 TPS	1–49%	43	2.0 (1.6–3.0)	0.441	10.9 (7.5–12.1)	0.485
	< 1%	11	2.1 (1.1–7.9)		14.6 (3.4–NE)	
Disease control after 4 cycles of platinum-based doublet chemotherapy	Achieved	32	2.1 (1.6–3.7)	0.304	13.5 (7.7–23.6)	0.023
	Not achieved	22	2.0 (1.2–5.0)		8.6 (5.3–12.1)	
Modified Glasgow Prognositc Score	0	13	2.8 (1.6–3.7)	0.768	16.1 (6.5–32.3)	0.079
	1, 2	30	1.8 (1.2–5.0)		10.9 (6.9–13.0)	
Neutrophil-to-lymphocyte ratio	<3.5	24	3.0 (1.7–5.0)	0.143	13.0 (9.5–23.6)	0.145
	≥3.5	20	1.4 (1.1–2.1)		7.1 (3.5–13.1)	
Platelet-to-lymphocyte ratio	<200	30	2.0 (1.6–3.0)	0.596	12.1 (7.1–19.4)	0.139
	≥200	14	1.7 (1.1–5.7)		9.5 (4.4–13.5)	

PFS, progression-free survival; OS, overall survival; ICI, immune-checkpoint inhibitor; CI, confidence interval; PS, performance status; NE, not evaluable; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

that would not benefit from ICI monotherapy is crucial. The results of this study suggest that patients who experience PD before 4 cycles of first-line platinum-based chemotherapy would not benefit from second-line ICI monotherapy, which would help physicians select docetaxel or nanoparticle albumin-bound (nab-) paclitaxel as the second-line treatment for this population (29).

TABLE 3 Cox proportional hazard models for OS in patients with non-small cell lung cancer who received ICIs monotherapy, multivariate analysis.

Items	Hazard ratio (95% CI)	*P* value
Age ≥ 75	0.81 (0.40-1.65)	0.560
Adenocarcinoma	0.83 (0.43–1.59)	0.580
Achievement of disease control after 4 cycles of platinum-based doublet chemotherapy	0.49 (0.24-0.99)	0.046

OS, overall survival; ICI, immune-checkpoint inhibitor; CI, confidence interval.

In order to predict the responses to ICI-based treatment, monitoring quantified circulating cell-free DNA (cfDNA) is effective, which reflects longitudinal tumor dynamics in advance to the radiographic response (30). However, monitoring cfDNA has problem in its accessibility and cost.

In the current study, we aimed to find out the easily evaluable predictive makers. Thus, the relationship between mGPS and OS or PFS after ICI monotherapy was also investigated, considering the impact of cachexia, which is a poor prognostic factor for immunotherapy. A significant relationship was observed between the maintenance of disease control during the four cycles of platinum-based chemotherapy and the mGPS score at the start of ICI monotherapy (Supplementary Table 2). This is the first study to show the impact of disease control with first-line platinum-based chemotherapy on subsequent ICI monotherapy in patients with

NSCLC with PD-L1 expression ≤49%. Although a significant correlation was not observed between mGPS at the start of ICI monotherapy and the median OS (Table 2), this finding suggests the significance of TME in ICI treatment.

The TME status is important for obtaining adequate effects from ICIs. Tumors with low or negative PD-L1 expression and scarce TILs are called "immune-desert" which are resistant to ICI monotherapy and need the activation of priming phase. In contrast, tumors with high PD-L1 expression and abundant TILs are called "immune-inflamed" which are sensitive to immunotherapy (31). To achieve the optimal "immune-inflamed" status by immunogenic cell death (32) and to obtain the most effective outcome, CIT was established as a new strategy in patients with NSCLC (15–18). Although CIT is effective compared to ICI monotherapy for NSCLC with PD-L1 expression ≤49%, the efficacy is not satisfactory compared to that with PD-L1 expression ≥50%. Furthermore, the increase in serious adverse events during CIT (14) is an obstacle in adopting CIT for NSCLC with PD-L1 expression ≤49%.

The median OS of CIT for NSCLC with PD-L1 expression ≤49% in updated 5-year follow-up of phase III trials remains at 15–21 months (33, 34). Since the OS of the non-PD group in the current study was comparable to that of the CIT group, the treatment strategy for NSCLC with PD-L1 expression ≤49% should be reconsidered.

Although NSCLC with low or negative PD-L1 expression is considered to show poor response to immunotherapy, the change in TME from "immune-desert" to "immune-inflamed" status with increased CD8-positive TILs prior to immunotherapy would lead to a good response to immunotherapy (31). An increase in CD8-positive TILs was observed in patients with resectable NSCLC who received neoadjuvant chemotherapy (20), showing the effect of platinum-based chemotherapy on the TME in NSCLC. When tumor cells are attacked by chemotherapy, the release of tumor-derived neoantigens into the blood facilitates the migration and functioning of antigen-presenting cells and augments antigen presentation, tumor recognition, and TIL activity (31, 35). The altered PD-L1 expression after neoadjuvant chemotherapy in patients with squamous NSCLC (36) should be also taken into account when treating patients with NSCLC with PD-L1 ≤49%, because underestimation of the expected outcome of ICI monotherapy in this population would lead to avoidance of the ICI treatment.

The TME status after disease progression with first-line chemotherapy should be re-evaluated to determine the most appropriate second-line treatment regimen; however, it is difficult to perform a re-biopsy and re-evaluate the immune status in all patients. Focusing on the impact of the TME on the development of cancer cachexia (37), immunological and nutritional indices such as mGPS, neutrophil-to-lymphocyte ratio, systemic immune-inflammation index, and platelet-to-lymphocyte ratio are surrogate markers in immunotherapy for NSCLC (38–41). However, there was only a slight correlation between mGPS and OS with ICI monotherapy in the current study, suggesting that mGPS is not an adequate predictor. In contrast, maintaining a non-PD status after four cycles of platinum-based chemotherapy was a predictor of the efficacy of second-line ICI monotherapy. Disease progression during the four cycles of induction chemotherapy indicates insufficient antitumor activity, failing to induce the activation of the priming phase, and failure to improve the TME for subsequent ICI monotherapy. The observed relationship between maintaining disease control and mGPS supports this speculation. This is consistent with the correlation between the prevalence of CD8-positive TILs and response to chemotherapy in advanced gastric cancer (21).

This study had several limitations. First, this retrospective study had a limited sample size and was susceptible to a selection bias. The enrollment of patients with advanced NSCLC with low or negative PD-L1 expression who were treated with platinum-based chemotherapy followed by ICI monotherapy was susceptible to bias. Second, all patients enrolled in this study were Japanese. Because the efficacy of the treatment for NSCLC has ethnic differences, this also led to bias. Thus, patients with a relatively favorable prognosis were included in this study. Despite these limitations, the novel findings of this study are useful for decision-making in patients with NSCLC with low or negative PD-L1 expression. Larger real-world clinical studies evaluating the predictive role of the response to first-line platinum-based chemotherapy are warranted.

5 Conclusion

Maintaining disease control (i.e., non-PD) after four cycles of platinum-based chemotherapy was a predictor of OS after second-line ICI monotherapy. These findings will help physicians select the most suitable treatment option for patients with NSCLC who were treated with platinum-based chemotherapy and subsequently with second-line treatment. Those who experienced early PD during platinum-based chemotherapy should not be treated with second-line ICI monotherapy, but with docetaxel or nab-paclitaxel. Further investigations are required to validate 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

The studies involving humans were approved by the Ethics Committees of the Japanese Red Cross Kyoto Daini Hospital (February 2, 2022; S2021-43). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because of the retrospective study.

Author contributions

AY: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Writing – original draft. TT:

Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. NK: Data curation, Resources, Writing – review & editing. KKT: Data curation, Formal analysis, Validation, Writing – review & editing. MF: Conceptualization, Resources, Validation, Writing – review & editing. YC: Methodology, Resources, Validation, Writing – review & editing. ST: Data curation, Resources, Writing – review & editing. HK: Data curation, Resources, Validation, Writing – review & editing. KN: Data curation, Resources, Writing – review & editing. YY: Data curation, Resources, Validation, Writing – review & editing. NT: Resources, Validation, Writing – review & editing. RH: Conceptualization, Data curation, Resources, Validation, Writing – review & editing. NO: Data curation, Resources, Writing – review & editing. TY: Resources, Validation, Writing – review & editing. KU: Methodology, Resources, Validation, Writing – review & editing. JM: Data curation, Resources, Writing – review & editing. SS: Resources, Validation, Writing – review & editing. HY: Data curation, Validation, Writing – review & editing. TY: Data curation, Methodology, Validation, Writing – review & editing. TK: Formal analysis, Supervision, Validation, Visualization, Writing – review & editing. KCT: Formal analysis, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We would like to thank Editage (www.editage.com) for English language editing.

Conflict of interest

HK has received personal fees from Ono Pharmaceutical, Chugai Pharmaceutical, AstraZeneca, Taiho Pharmaceutical, Eli Lilly, and MSD. HY has received honoraria for lecture fees from Boehringer Ingelheim, Chugai Pharmaceutical, Nippon Kayaku, Taiho Pharmaceutical, Eli Lilly, Takeda Pharmaceutical, and Bristol Meyers Squibb. TY has received grants from Pfizer, Ono Pharmaceutical, Janssen Pharmaceutical, AstraZeneca, Takeda Pharmaceutical, and honoraria from Eli Lily. TK has received grants from AstraZeneca, MSD, Takeda Pharmaceutical, Janssen Pharmaceutical, Bristol Myers Squibb, Daiichi Sankyo Pharmaceutical, and honoraria for lectures from AstraZeneca, Eli Lilly, MSD, Ono Pharmaceutical, Bristol Myers Squibb, Chugai Pharmaceutical, and Nippon Kayaku. KoT has received research grants from Chugai Pharmaceutical and Ono Pharmaceutical and personal fees from AstraZeneca, Chugai Pharmaceutical, MSD-Merck, Eli Lilly, Boehringer-Ingelheim, and Daiichi-Sankyo, outside the purview of the submitted work.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1303543/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Kaplan-Meier estimates for progression-free survival and overall survival of second-line immune checkpoint inhibitor monotherapy according to programmed death-ligand 1 expression on tumor cells. Kaplan–Meier estimates for progression-free survival [PFS: **(A)**] and overall survival [OS: **(B)**] of immune checkpoint inhibitor (ICI) monotherapy in patients with programmed death-ligand 1 (PD-L1) expression of 1-49%, compared according to the response to first-line platinum-based chemotherapy (non-progressive disease [PD] vs. PD). The median PFS in the non-PD and PD subgroups were 2.0 months (95% confidence interval [CI]: 1.6–3.0 months) and 2.0 months (95% CI: 1.2–4.7 months), respectively (p = 0.473). The median OS in the non-PD and PD subgroups were 13.5 months (95% CI: 7.5–24.2 months) and 8.3 months (95% CI: 5.1–9.5 months), respectively (p = 0.003). Kaplan–Meier estimates for PFS **(C)** and OS **(D)** of ICI monotherapy in patients with PD-L1 expression <1%, compared according to the response to first-line platinum-based chemotherapy (non-PD vs. PD). The median PFS in the non-PD and PD subgroups were 2.1 months (95% CI: 1.0–18.1 months) and 3.7 months (95% CI: 1.4 months–not evaluable [NE]), respectively (p = 0.519). The median OS in the non-PD and PD subgroups were 14.6 months (95% CI: 3.3 months–NE) and 13.0 months (95% CI: 8.4 months–NE), respectively (p = 0.555). PFS, progression-free survival; OS, overall survival; PD-L1, programmed death-ligand 1; ICI, immune checkpoint inhibitor; PR, partial response; SD, stable disease; PD, progression disease; CI, confidence interval; NE, not evaluable.

SUPPLEMENTARY FIGURE 2
Kaplan-Meier estimates for progression-free survival and overall survival of second-line immune checkpoint inhibitor monotherapy according to neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio. Kaplan–Meier estimates for progression-free survival [PFS: **(A)**] and overall survival [OS: **(B)**] of immune checkpoint inhibitor (ICI) monotherapy according to neutrophil-to-lymphocyte ratio (NLR) (NLR < 3.5 vs. ≥ 3.5). The median PFS in patients with NLR of <3.5 and ≥3.5 were 3.0 months (95% confidence interval [CI]: 1.7–5.0 months) and 1.4 months (95% CI: 1.1–2.1 months), respectively (p = 0.143). The median OS in patients with NLR of < 3.5 and ≥ 3.5 were 13.0 months (95% CI: 9.5–23.6 months) and 7.1 months (95% CI: 3.5–13.1 months), respectively (p = 0.145). Kaplan–Meier estimates for PFS **(C)** and OS **(D)** of ICI monotherapy according to platelet-to-lymphocyte ratio (PLR) (PLR < 200 vs. ≥ 200). The median PFS in patients with PLR of < 200 and ≥ 200 were 2.0 months (95% CI: 1.6–3.0 months) and 1.7 months (95% CI: 1.1–5.7 months), respectively (p = 0.596). The median OS in patients with PLR of < 200 and ≥ 200 were 12.1 months (95% CI: 7.1–19.4 months) and 9.5 months (95% CI: 4.4–13.5 months), respectively (p = 0.139). PFS, progression-free survival; OS, overall survival; ICI, immune checkpoint inhibitor; NLR, neutrophil-to-lymphocyte ratio; CI, confidence interval; PLR, platelet-to-lymphocyte ratio.

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frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 26 February 2024
DOI 10.3389/fimmu.2024.1344858

OPEN ACCESS

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RECEIVED 26 November 2023
ACCEPTED 07 February 2024
PUBLISHED 26 February 2024

CITATION
Grenda A, Kuźnar-Kamińska B, Kalinka E, Krawczyk P, Sawicki M, Filip A, Chmielewska I, Frąk M, Krzyżanowska N and Milanowski J (2024) MicroRNA-126 selected with broad-spectrum analysis of microRNAs – a new predictive factor for the effectiveness of immunotherapy or chemoimmunotherapy in advanced NSCLC patients?
Front. Immunol. 15:1344858.
doi: 10.3389/fimmu.2024.1344858

MicroRNA-126 selected with broad-spectrum analysis of microRNAs – a new predictive factor for the effectiveness of immunotherapy or chemoimmunotherapy in advanced NSCLC patients?

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Introduction: Expression of PD-L1 on cancer cells is the only validated predictive factor for immunotherapy in NSCLC (Non-Small Cell Lung Cancer) patients. However, on this basis, it is difficult to predict the occurrence of resistance to immune checkpoint inhibitors (ICIs). MicroRNAs are widely studied as biomarkers of cancers. Our study was designed to determine whether microRNAs can be sensitive predictive factors in the qualification of NSCLC patients to first-line immunotherapy or chemoimmunotherapy.

Material and methods: The two-stage research on validation group (n=20) and study group (n=35) of patients with advanced NSCLC was conducted. Analysis of microRNAs expression by qPCR in plasma collected prior to the start of immunotherapy (pembrolizumab) or chemoimmunotherapy (combination of pembrolizumab with chemotherapy) was made. Broad-spectrum analysis of microRNAs expression was used in the studied group. Three microRNAs selected in that group as important for the effectiveness of ICIs were then examined in the validation group.

Results: In the studied group, significantly higher expression of miRNA-126-3p, miR-144-3p and miR-146-5p was observed in patients with long PFS compared to those with short PFS. In the validation group, low miRNA-126 expression indicated lower median progression-free survival and overall survival (2.3 vs. 5.0 months and 5.2 vs 11.2, respectively). These patients had a significantly higher risk of progression (HR= 2.92, 95% CI: 1.01 to 8.40, p=0.04) and death (HR=3.64, 95% CI: 1.22 to 10.84, p=0.02).

Conclusion: Our study showed that the expression of miR-126 in blood plasma may be a predictive factor for the effectiveness of first-line immunotherapy or chemoimmunotherapy in advanced NSCLC patients.

KEYWORDS

microRNA, immunotherapy, anti-PD-1, NSCLC, miRNA

Introduction

The percentage of tumor cells (TC) with PD-L1 (Programmed Cell Death Ligand 1) expression is determined during the qualification of patients with advanced non-small cell lung cancer (NSCLC) to therapy with immune checkpoint inhibitors (ICIs) in monotherapy or combination with chemotherapy. Atezolizumab, pembrolizumab or cemiplimab can be used in first-line monotherapy when PD-L1 expression is observed on ≥50% of TC. Immunotherapy combined with chemotherapy can be considered for first-line therapy if PD-L1 expression is found on less than 50% of tumor cells. For advanced NSCLC patients with PD-L1 expression on less than 1% of TC or regardless of this expression, pembrolizumab or nivolumab or atezolizumab could be used in second-line therapy in patients who have not previously received immunotherapy (1–14).

PD-L1 is the only validated predictor of immunotherapy efficacy, but it is not perfect. The probability of disease progression and resistance to ICIs therapy cannot be accurately determined based on PD-L1 expression. Approximately 40% of patients with high PD-L1 expression have primary resistance to immunotherapy and show disease progression. Another 30% of patients achieve disease stabilization with a short progression-free survival (PFS) of approximately 6 months. These patients develop an acquired resistance to immunotherapy. However, in the remaining patients, the response to immunotherapy is long-lasting (11–13). Moreover, immunotherapy may be highly beneficial in patients with low or no expression of PD-L1 protein on TCs. The mechanism of resistance to immunotherapies is not fully understood (1, 14). Resistance to immunotherapy may be influenced by intrinsic factors of the cancer cell, such as epigenetic factors and disruption in gene expression, as well as mutations that compose the molecular landscape of the cancer cells (15–17). The action of immunosuppressive cytokines or growth factors, neoangiogenesis associated with the formation of abnormal blood vessels, expression of molecules on T cells that send signals to silence the immune system, tumor-infiltrating lymphocyte (TIL) status, or the composition of the gut microbiome belong to the external factors, independent of the tumor cells (17, 18).

Our attention was drawn to epigenetic factors which are microRNA molecules. They are short in length (~21nt), stable, and present in plasma/serum, which ensures easy availability of material for testing, without the need for invasive methods. MicroRNAs affect almost all cellular processes, by regulating gene expression at the post-transcriptional level. Expression of microRNAs changes under pathological conditions, including cancer development. Thus, they may be related to all molecular and immunological mechanisms of resistance to immunotherapy. MicroRNAs are widely studied as precise biomarkers in the context of early cancer diagnosis. Our present study was designed to determine whether they can be sensitive predictive factors for the effectiveness of immunotherapy or chemoimmunotherapy in patients with advanced NSCLC.

Materials and methods

Patients characteristic

The study consisted of two stages. The first involved the selection of microRNAs from a panel of miRCURY LNA Human Serum/Plasma Focus PCR Panels (Qiagen, Venlo, Netherlands) and was conducted on a group of 20 NSCLC patients (in stage IV) treated with immunotherapy (10 patients with PD-L1 expression on ≥50% of TC) or chemoimmunotherapy (10 patients with PD-L1 expression on <50% of TC).

Patients were divided based on the length of progression-free survival. Ten patients were characterized by short disease stabilization or disease progression with a PFS of less than 6 months and 10 patients had a PFS longer than 6 months. There were 6 (30%) women and 14 (70%) men. 13 (65%) patients were over 65 years of age and 7 (35%) patients were under 65 years of age. All patients were in stage IV according to 8th TNM classification. There were 7 patients diagnosed with squamous cell carcinoma and 13 patients with adenocarcinoma.

In the second stage, we performed assays on an independent, validation group of patients with selected micoRNAs (from the first stage of the study) that were classified as potential predictors of immunotherapy or chemoimmunotherapy efficacy. The group consisted of 35 patients. Thirty-four patients were in stage IV and one patient in stage IIIB of the disease. *EGFR* mutations (*Epidermal Growth Factor Receptor*), *ALK* (*Anaplastic Lymphoma Kinase Tyrosine Kinase Receptor*) and *ROS1* (*ROS Proto-Oncogene 1, Tyrosine Kinase Receptor*) rearrangement were excluded in all

patients. Responses to immunotherapy, progression-free survival, and overall survival were calculated from the start of therapy in all 35 patients.

Demographic and clinical characteristics of the entire study group are presented in Table 1.

The research was approved by the bioethics committee at the Medical University of Lublin (KE-0254/95/2018). Informed consent was obtained from all patients.

Sample collection

The material for the study consisted of plasma samples taken from patients before the treatment. The blood was collected in EDTA (ethylenediaminetetraacetic acid) tubes and centrifuged for 10 min at 2000 x g. The plasma was pipetted in equal amounts into eppendorf tubes. Plasma samples were stored at -80°C until isolation of RNA was carried out.

TABLE 1 Clinical and demographic characteristics of patients in the validation group (n=35).

Characteristic	miRNA-126 n (%)		miRNA-144 n (%)		miRNA-146 n (%)	
	Below the median	Above the median	Below the median	Above the median	Below the median	Above the median
Age (median=69 years, min-max: 48-77, SD=6.4 **<69 n=13** **≥69 n=22**	 1 (8) 8 (36)	 12 (92) 14 (64)	 5 (38) 12 (55)	 8 (62) 10 (45)	 4 (31) 12 (55)	 9 (69) 10 (45)
X^2 *p*	3.51 0.06		0.84 0.36		1.86 0.17	
Gender Male n=20 Female n=15	 6 (30) 3 (20)	 14 (70) 12 (80)	 10 (50) 7 (47)	 10 (50) 8 (53)	 9 (45) 7 (47)	 11 (55) 8 (53)
X^2 *p*	0.45 0.50		0.04 0.85		0.01 0.92	
Histopathology Non-SqC, n=24 SqC n=11	 8 (33) 1 (9)	 16 (67) 10 (91)	 11 (46) 6 (55)	 13 (54) 5 (45)	 10 (42) 6 (55)	 14 (58) 5 (45)
X^2 *p*	2.32 0.13		0.23 0.63		0.50 0.48	
PD-L1 IHC <50% n=12 ≥50% n=23	 4 (33) 5 (22)	 8 (67) 18 (78)	 6 (50) 11 (48)	 6 (50) 12 (52)	 7 (58) 9 (39)	 5 (42) 14 (61)
X^2 *p*	0.56 0.46		0.015 0.90		1.17 0.28	
Response to immunotherapy PD n=14 SD+ PR n=21	 6 (43) 3 (14)	 8 (57) 18 (86)	 8 (57) 9 (43)	 6 (43) 12 (57)	 6 (43) 10 (48)	 8 (57) 11 (52)
X^2 *p*	3.59 0.06		0.68 0.41		0.078 0.78	
PFS <6onths n=25 ≥6months n=10	 8 (32) 1 (10)	 17 (68) 9 (90)	 14 (56) 3 (70)	 11 (44) 7 (70)	 14 (56) 2 (20)	 11 (44) 8 (80)
X^2 *p*	1.81 0.18		1.93 0.16		3.73 0.05	
OS <6onths n=19 ≥6months n=16	 6 (32) 3 (19)	 13 (68) 13 (81)	 11 (58) 6 (37)	 8 (42) 10 (63)	 9 (47) 7 (44)	 10 (53) 9 (56)
X^2 *p*	0.74 0.39		1.45 0.23		0.05 0.83	

10.3389/fimmu.2024.1344858

MicroRNA expression testing in the experimental group

Isolation of free-circulating microRNAs was performed using the miRNeasy Serum/Plasma Kit (Qiagen, Venlo, Netherlands). The isolated RNA was stored at -80°C until the reverse transcription reaction was performed. Reverse transcription reactions were performed using miRCURY® LNA® RT Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions on the T Personal instrument (Analitik Jena, Jena, Germany). MicroRNAs expression was evaluated by qPCR in 20 patients from the experimental group using the miRCURY LNA Human Serum/Plasma Focus PCR Panels (Qiagen, Venlo, Netherlands) kit on the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, USA). The 10-microliter reaction was prepared according to the manufacturer's instructions. qPCR was performed according to the following time and temperature conditions: PCR initial heat activation: 2 min, 95°C, and next 40 cycles: denaturation 10 s, 95°C, annealing/extension 60 s, 56°C. A melting curve analysis was attached to each run. The obtained Ct values were used for calculations using the method $2^{-\Delta Ct}$. MiRNA-484 and cel-miR-39-3p spike-in were used as controls.

MicroRNA expression testing in the validation group

Isolation of free-circulating miRNAs was performed using miRNeasy Serum/Plasma Kit (Qiagen, Venlo, Netherlands). The isolated RNA was stored at -80°C until the reverse transcription reaction was performed. For cDNA synthesis TaqMan™ Advanced miRNA cDNA Synthesis Kit (ThermoFisher Scientific, Waltham, Massachusetts, USA) was used.

Three microRNAs were selected for further validation analyses among the microRNAs tested in the experimental group. These microRNAs showed significantly different expression in patients with short and long PFS in the experimental group. The tests were performed using TaqMan probes. Expression of miRNA-126-3p (cat. A25576 477887_mir), miR-144-3p (cat. A25576 477913_mir), miR-146a-5p (cat. A25576 478399_mir) were examined. Expression of miRNA-484 (A25576 478308_mir) and cel-miR-39-3p (cat. A25576 478293_mir) spike-in were used as controls. Reactions were performed on the illumina Eco Real-Time PCR system. The 20 microliter reaction contained: 10 µl TaqMan™ Fast Advanced Master Mix (ThermoFisher Scientific, Waltham, Massachusetts, USA), 1 µl TaqMan® Advanced miRNA Assay, 4 µl nuclease free water and 5 µL of the diluted cDNA (1:10) template. Temperature conditions were used as follows: enzyme activation 95°C by 20 seconds and then 40 cycles: denature 95°C by 3 seconds and anneal/extend 62°C by 30 seconds. The obtained Ct values were used for calculations using the $2^{-\Delta Ct}$ method.

Statistical analysis

Statistical analysis was performed using Statistica 13.3 (TIBCO Software Inc, Palo Alto, USA) and MedCalc (MedCalc Software Ltd, Ostend Belgium) software. The U-Mann–Whitney test was used to assess differences in miRNA expression between groups of patients. Kaplan-Meier survival analysis were used for the calculation of PFS and OS. The results are presented as medians and maximum and minimum values (min-max). A p-value below 0.05 was considered statistically significant.

Results

The median PFS in the experimental group was 1.9 months (95%CI: 1.5 to 11.6, min-max: 1.0-23.6). The median overall survival calculated from the start of immunotherapy was 6.6 months (95%CI: 1.7 to 13.8, min-max: 1.0-23.6).

Based on the analysis of microRNAs expression in experimental group, significantly higher expression of miRNA-126-3p (p=0.007), miR-144-3p (p=0.04) and miR-146-5p (p=0.03) was observed in patients with long PFS compared to those with short PFS (Figures 1A–C respectively).

Therefore, further studies in the validation group focused on these three microRNA molecules. In an independent validation group, miRNA-126 expression was non-significantly higher in patients with PFS over 6 months (p=0.07, Figure 2A) in comparison to patients with shorter PFS. Significantly higher

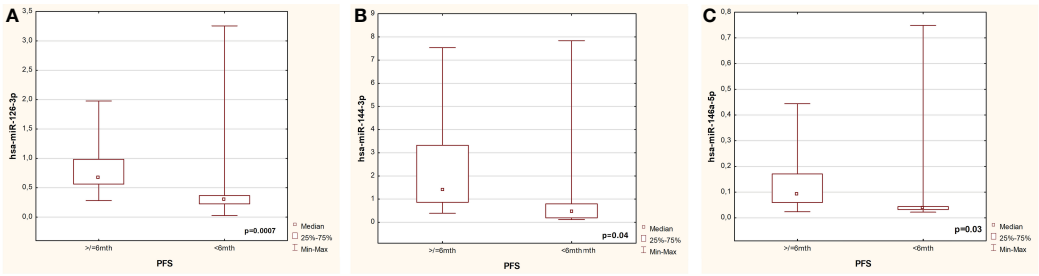

FIGURE 1
Differences in expression of miRNA-126 **(A)**, miR-144 **(B)**, miR-146 **(C)** in patients with short and long PFS from the experimental group.

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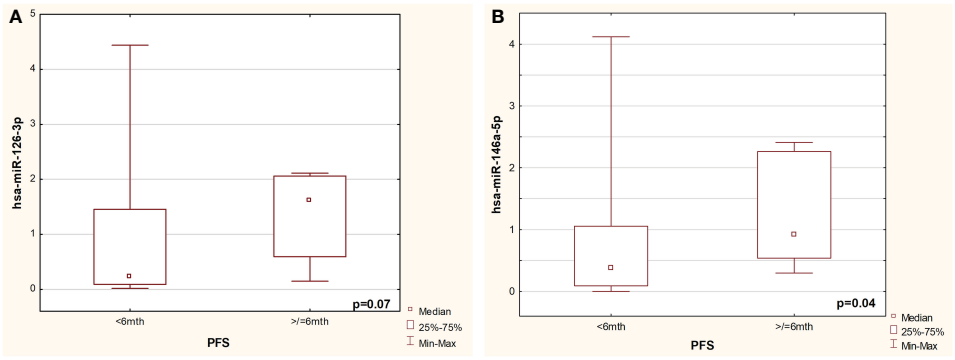

FIGURE 2
Comparison of expression of miRNA-126-3p **(A)** and miR-146-5p **(B)** in patients with short and long PFS from validated group.

expression of miRNA-146 (p=0.04, Figure 2B) was found in patients with long PFS than in patients with short PFS. No such differences were observed during analysis of the miRNA-144 expression in these groups (p=0.5).

Kaplan-Meier analysis showed that the median PFS was lower in patients with low expression of miRNA-126 compared to patients with high expression of this molecule (2.3 vs. 5.0 months). The risk of progression was almost three times higher in the group of patients with low expression of the tested microRNA compared to patients with high expression of this molecule (HR= 2.92, 95% CI: 1.01 to 8.40, p=0.04, Figure 3A). Moreover, median OS was lower in patients with lower miRNA-126 expression than in patients with higher miRNA-126 expression (5.2 vs. 11.2 months). Lower expression of miRNA-126 indicated almost four times higher risk of OS shortening (HR=3.64, 95% CI: 1.22 to 10.84, p=0.02, Figure 3B).

Discussion

We selected three microRNAs which expression could be a predictive factor for the efficacy of immunotherapy with anti-PD-1 antibodies. For this purpose, we used an analysis of a broad panel of miRNA molecules in the plasma of NSCLC patients treated with first-line immunotherapy or chemoimmunotherapy. Expression of miRNA-126-3p, miRNA-144-3p and miRNA-146a-5p have been indicated as potentially useful factors in the qualification for immunotherapy. In further studies in an independent validation group, we found that high miRNA-126-3p expression could be a predictive factor for first-line immunotherapy efficacy in non-small cell lung cancer patients. It should be mentioned that the expression of miRNAs, including miRNA-126, may be influenced by chemotherapy or other method of treatment. Our goal was to evaluate whether any miRNA molecules expression could be a universal biomarker of response to immune checkpoints inhibitors used alone or in combination with chemotherapy in advanced NSCLC patients. Therefore, we analyzed these potential predictive factors before starting treatment.

Other authors' research indicated that miRNA-126 expression is significantly reduced in adenocarcinoma of the lung compared to the normal tissue. Therefore, reduction of miRNA-126 expression is associated with the development of lung adenocarcinoma (LUAD) (19). Moreover, lower expression of miRNA-126-3p and miRNA126-5p promotes vascular invasion, and lymph node metastasis, and occurs in higher stages (III-IV) of adenocarcinoma patients.

The target transcripts for miRNA-126 activity were identified: *IGF2BP1* (*Insulin Like Growth Factor 2 MRNA Binding Protein 1*), *TRPM8* (*Transient Receptor Potential Cation Channel Subfamily M*

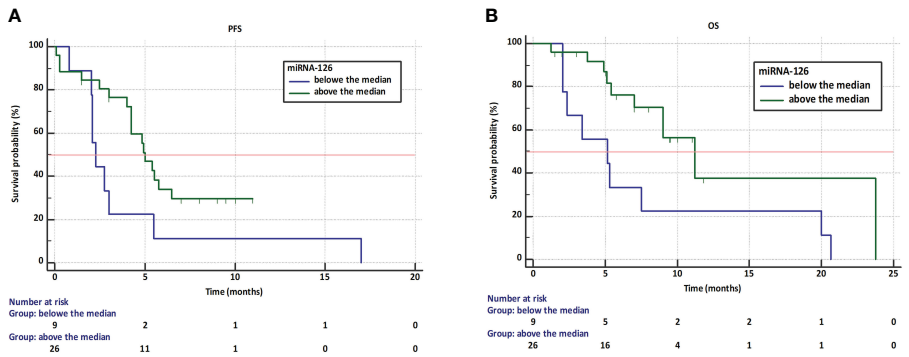

FIGURE 3
Kaplan-Meier curves showing progression-free survival **(A)** and overall survival **(B)** in NSCLC patients treated with first-line immunotherapy or chemoimmunotherapy with different expression of miRNA-126-3p.

Member 8), *DUSP4* (*Dual Specificity Phosphatase 4*), *SOX11* (*SRY-Box Transcription Factor 11*), *PLOD2* (*Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 2*), *LIN28A* (*Lin-28 Homolog A*), *LIN28B* (*Lin-28 Homolog B*), *SLC7A11* (*Solute Carrier Family 7 Member 11*), *mTOR* (*Mechanistic Target Of Rapamycin Kinase*), *PIK3R2* (*Phosphoinositide-3-Kinase Regulatory Subunit 2*) (19–21). MiRNA-126 has tumor suppressor properties. MiRNA-126 has an inhibitory effect on NSCLC cell invasion by silencing oncogenes: *VEGFA* (*Vascular Endothelial Growth Factor A), AKT1 (AKT Serine/Threonine Kinase 1*) and *KRAS* (*Kirsten Rat Sarcoma Virus Proto-Oncogene, GTPase*) (22). Moreover, it has been found that miR-126-3p inhibits the growth, migration, and invasion of NSCLC by targeting *CCR1* (*C-C Motif Chemokine Receptor 1*) in NSCLC cells (23). However, it was demonstrated that the over-expression of CCR1 molecule rescued the inhibitory effects of miR-126-3p on NSCLC cell growth, migration and invasion. Further, the knocked-down of CCR1 was able to mimic the inhibitory effects of miR-126-3p on the progression of NSCLC cells (23).

Di Paolo et al. identified in a group of 38 NSCLC patients that the expression of miR-126-3p and miR-221-3p was significantly changed in tumor tissue compared to healthy tissue (24). They found that concomitant miR-126-3p activation and miR-221-3p inhibition reduced lung cancer cell viability by inhibiting AKT, PIK3R2 and PTEN (Phosphatase And Tensin Homolog) signaling pathways (24). *PIK3R2* was a target for the action of miR-126-3p and *PTEN* for miR-221-3p. Researchers proved that the simultaneous interaction of these molecules reduced metastatic dissemination of lung cancer cells both *in vitro* and *in vivo* through CXCR4 (C-X-C Motif Chemokine Receptor 4) inhibition. Further, Ichikawa et al. showed miR–126–3p could inhibit cell migration and invasion and induce apoptosis by regulating the PI3K/PDK1/AKT pathway in HeLa cells (25). A meta-analysis of Sun et al. showed that generally high expression of miR-126 is associated with better prognosis in NSCLC patients (26). In research by Yang et al., expression of miRNA-126 was decreased in NSCLC lines and tumor tissues. The patients with low expression of miRNA-126 had significantly poorer overall survival than those with high miRNA-126 expression (means OS reached 24.4 vs. 29.3 months, respectively) (27).

It has also been shown that miRNA-126-3p down-regulation contributes to dabrafenib-acquired resistance in melanoma patients by up-regulating *ADAM9* (*ADAM Metallopeptidase Domain 9*) and *VEGFA* (28). This is consistent with the observation that decreased *ADAM9* mRNA expression correlated with a better response to nivolumab therapy in hepatocellular cancer (29). Liu et al. reported that miR-126 suppressed esophageal cancer cell proliferation and migration by interacting with *ADAM9* mRNA 3′UTR (Untranslated Region) (30). They indicated that expression of miR-126 was reduced in esophageal cancer tissues, which was correlated with shorter overall survival of patients, implying their potential function as a prognostic factor (30). In contrast, in colorectal cancer, high expression of miR-126 in tumor and stroma was associated with increased overall survival. In multivariate analyses, high miR-126 expression in tumor remained a significant independent predictor of improved survival (31). The authors postulate that this factor may help in the qualification of patients for adjuvant chemotherapy (31).

In turn, Schmittnaegel et al. postulated that the blockade of Ang2 (angiopoietin-2) and VEGFA induces antitumor immunity enhanced by PD-1 checkpoint blockade (32). These are indications that the expression of the miRNA-126-3p molecule is a beneficial factor for NSCLC patients receiving immunotherapy. Researchers indicated that the miR-126 molecule is associated with the functioning of T lymphocytes, especially Treg cells. Chen et al. postulated that investigation of the role of miR-126 in lung cancer development and progression, including in activation of the immune response, may be valuable for the estimation of immunotherapy efficacy (22).

The previous considerations and our results indicate that higher miR-126 expression may be a favorable predictive factor for immunotherapy. Qin et al. found that miRNA-126 was expressed in mouse and human Treg cells (33). It has been shown that miRNA-126 regulates the activity of the PI3K-AKT signaling pathway, crucial for Foxp3 (Forkhead Box P3) expression, and limited activation of PI3K/AKT pathway was necessary for Tregs development and function. Researchers in further studies showed that silencing of miRNA-126 using antisense oligonucleotides (ASO) could significantly reduce the induction of Treg cells *in vitro*. Furthermore, miR-126 silencing could reduce the expression of Foxp3 on Treg cells, which was accompanied by decreased expression of CTLA-4 (Cytotoxic T-Lymphocyte Associated Protein 4) and GITR (TNF Receptor Superfamily Member 18), as well as IL-10 and TGF-β production. Therefore, high expression of miRNA-126 in lymphocytes may be associated with the activation, differentiation and suppressive activity of Treg cells. Moreover, Fortunato et al. found that high level of miRNA-126-3p in plasma may be related to the induction and activation of Treg cells, which enhance the metabolism and secretion of exosomes containing microRNAs, including miRNA-126-3p (34). In turn, high activity of Treg cells in the tumor microenvironment and lymph nodes is associated with immunosuppression and poorer effectiveness of immunotherapy. However, the function of Treg lymphocytes is only one of many immunological factors influencing the efficacy of immunotherapy. One of them may be the function of monocytes on the spreading of cancer. Zhang et al. indicated that miRNA-126 independently suppress the sequential recruitment of mesenchymal stem cells and inflammatory monocytes into the tumor stroma. The lack of these cells in the microenvironment may be favorable for metastases development in the breast cancer mouse xenograft model (35).

Conclusion

Our study shows that miR-126 may be a predictive factor of the effectiveness of first-line immunotherapy or chemoimmunotherapy in NSCLC patients. We are aware that our study groups were not very large. We postulate that further research should be carried out in a larger group and in patients treated with second-line immunotherapy to investigate whether the expression of this molecule has predictive properties in these patients.

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 humans were approved by Bioethics Committee at the Medical University of Lublin. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AG: Conceptualization, Formal analysis, Methodology, Project administration, Writing – original draft. BK: Conceptualization, Formal analysis, Investigation, Resources, Writing – original draft. EK: Data curation, Formal analysis, Methodology, Writing – review & editing. PK: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. MS: Data curation, Investigation, Supervision, Writing – review & editing. AF: Software, Supervision, Writing – review & editing. IC: Data curation, Formal analysis, Investigation, Resources, Visualization, Writing – original draft. MF: Data curation, Formal analysis, Writing – original draft. NK: Data curation, Writing – review & editing. JM: Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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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frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 18 April 2024
DOI 10.3389/fimmu.2024.1332303

OPEN ACCESS

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RECEIVED 02 November 2023
ACCEPTED 05 April 2024
PUBLISHED 18 April 2024

CITATION
Lou J, Gong B, Li Y, Guo Y, Li L, Wang J, Liu W, You Z, Zhang H, Pan F, Liang B, Yang L and Zhou G (2024) Bone mineral density as an individual prognostic biomarker in NSCLC patients treated with immune checkpoint inhibitors. *Front. Immunol.* 15:1332303. doi: 10.3389/fimmu.2024.1332303

Bone mineral density as an individual prognostic biomarker in NSCLC patients treated with immune checkpoint inhibitors

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Background: Immune checkpoint inhibitors (ICIs) have left a deep impression in the treatment of non-small cell lung cancer (NSCLC), however, not all patients benefit from it. The purpose of this study was to investigate the prognostic value of baseline bone mineral density (BMD) derived from chest computed tomography (CT) scans in NSCLC patients treated with ICIs.

Methods: This study included patients with advanced NSCLC who underwent ICI treatment at the Wuhan Union Hospital from March 2020 to October 2022. Baseline BMD was evaluated at non-contrast chest CT at the level of first lumbar vertebra. Patients were divided into BMD-lower group and BMD-higher group according to the optimal cutoff value calculated by X-tile software. Baseline characteristics of the two groups were compared and variables between the two groups were balanced by propensity score matching (PSM) analysis. We calculated the objective response rate (ORR) and disease control rate (DCR) of the two groups and analyzed overall survival (OS) and progression-free survival (PFS) using BMD and other clinical indexes through Cox regression models and Kaplan-Meier survival curves.

Results: A total of 479 patients were included in this study, and all patients were divided into BMD-lower group (n=270) and BMD-higher group (n=209). After PSM analysis, each group consisted of 150 patients. ORR (43.3% vs. 43.5% before PSM, P = 0.964; 44.7% vs. 44.7% after PSM, P = 1.000) and DCR (91.1% vs. 94.3% before PSM, P = 0.195; 93.3% vs. 96.7% after PSM, P =0.190) were similar in two groups. There was no statistically significant relationship between BMD degree and PFS before (16.0 months vs. 18.0 months, P = 0.067) and after PSM analysis (17.0 months vs. 19.0 months, P = 0.095). However, lower BMD was associated with shorter OS both before (20.5 months vs. 23.0 months, P< 0.001) and after PSM analysis (20.0 months vs. 23.0 months, P = 0.008).

Conclusion: Lower baseline BMD is associated with worse clinical outcomes in NSCLC patients treated with ICIs. As a reliable and easily obtained individual prognostic biomarker, BMD can become a routine detection indicator before immunotherapy.

KEYWORDS

bone mineral density, osteoporosis, immunotherapy, immune checkpoint inhibitors, non-small cell lung cancer, prognosis

Introduction

Lung cancer is the leading cause of cancer death worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers (1). Unprecedented advances have been made in the treatment of lung cancer, such as new targeted therapies and immunotherapies (2). Immune Checkpoint Inhibitors (ICIs), including anti-programmed cell death protein 1 (PD-1), anti-programmed death-ligand 1 (PD-L1), and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), could block inhibitory signals of T cell activation to promote anti-tumor immune response (3). ICIs have greatly prolonged survival for patients with advanced or metastatic NSCLC (4). However, a subset of patients do not derive clinical benefit and may even develop disease progression with ICIs due to systemic factors (5). Therefore, there is an urgent need for reliable predictive biomarkers to identify patients who are suitable for immune checkpoint therapy.

As a chronic wasting disease, malignant tumors cause the prevalence of osteopenia and sarcopenia to be significantly higher than that in people of the same age (6, 7). As a result of malnutrition, these protracted musculoskeletal disorders negatively influence the quality of life ultimately resulting in a poor prognosis. Notably, a number of studies have reported that sarcopenia was significantly associated with increased mortality in patients with NSCLC (8, 9), however, the association between osteopenia and the prognosis of NSCLC has been less frequently reported, especially among patients receiving immunotherapy. T-score evaluated by dual-energy X-ray absorptiometry (DXA) was the gold standard for osteoporosis diagnosis (10). Currently, computed tomography (CT)-derived bone mineral density (BMD) was reported to be correlated with T-score and has been widely used to evaluate preoperative osteopenia in patients with digestive tract cancers (11). Patients with lung cancer routinely receive chest CT scans, and BMD can be obtained non-invasively through measuring the HU value at the level of the first lumbar vertebra.

To the best of our knowledge, there are no studies reporting the impact of baseline BMD on the efficacy of immunotherapy and clinical prognosis in patients with NSCLC. Therefore, we evaluated the objective response rate (ORR) and disease control rate (DCR) after the treatment of ICIs in NSCLC patients with low baseline BMD and high baseline BMD and tried to analyze overall survival (OS) and progression-free survival (PFS) using BMD and other clinical indexes through Cox regression models and Kaplan-Meier survival curves.

Abbreviations: ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer; BMD, baseline bone mineral density; CT, computed tomography; PD-1, programmed cell death 1; PD-L1, programmed cell death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PSM, propensity score matching; ORR, objective response rate; DCR, disease control rate; OS, overall survival; PFS, progression-free survival; COPD, chronic obstructive pulmonary disease; SRE, skeletal-related events; DXA, X-ray absorptiometry; ROIs, regions of interest; CR, complete response; PR, partial response; SD, stable disease; BM, brain metastasis.

Materials and methods

The local ethics committee and the institutional review board of the Tongji Medical College have approved this retrospective cohort study (Institutional Review Board No. S054), and they waived the requirement for informed consent. Clinical data were analyzed retrospectively and anonymously.

Study design and patients selection

Consecutive advanced NSCLC patients treated with ICIs between March 2020 to October 2022 at Wuhan Union Hospital were reviewed retrospectively. The diagnosis of NSCLC was based on radiological imaging, medical history, and/or lung biopsy.

Inclusion criteria were as follows: (1) Patients diagnosed with advanced NSCLC according to the NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer (2); (2) Patients older than 18 years; (3) The follow-up duration was more than 12 months; (4) Performing non-contrast chest CT before initial ICIs; (5) Patient received ICI treatment for the first time and for more than 4 cycles. Exclusion criteria were as follows: (1) Patients who did not undergo baseline CT; (2) Patients combined with other malignant tumors; (3) Patients with incomplete clinical information.

Procedures

Covariates of interest were collected retrospectively, including patient demographics (age, sex, body mass index, ECOG status,

diabetes, hypertension, smoking, hyperlipidemia, COPD), biochemical data (alkaline phosphatase, lactic dehydrogenase, Ca, blood urea nitrogen, hemoglobin, albumin-globulin ratio, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio), tumor-related information (pathological types, stages) and further disease specific information (type of ICIs, prior radiation therapy, occurrence of vertebral bone metastasis, corticosteroid application and osteopenia treatment). All baseline data are derived from the first admission and discharge medical records of patients.

Bone mineral density measurement and assessment

The CT examinations were performed on the 128-section CT scanner (SIEMENS SOMATOM Definition AS+, Siemens Healthcare Erlangen, Germany) using the same parameters. Tube voltage: 120kVp. Tube current: automatically adjusted. Reconstruction method: standard soft convolution kernel. Slice thickness: 1 mm. Slice interval: 1 mm. Two independent radiologists (L.B. and P.F. with 26 and 15 years of thoracic imaging experience, respectively) analyzed images and calculated the BMD independently on the Phillips Intelli Space Portal workstation (version 10.1, Best, the Netherlands), blinded to the clinical data. The average BMD from two independent radiologists was calculated for subsequent analysis and any disagreements were resolved by consensus. BMD was calculated as the average pixel density (HU) within a circle in the mid vertebral core at the bottom of the first lumbar vertebra on non-contrast chest CT (Figure 1). Draw three regions of interest (ROIs) repeatedly and average them to reduce errors. If the difference in HU values among them is greater than 30, another observer would repeat the drawing and calculation. Evaluations were repeated using the same method after two weeks, and intraobserver and interobserver agreements were 0.94 (95% CI, 0.89 to 0.97) and 0.90 (95% CI, 0.84 to 0.93), respectively. Using the X-tile software (version 3.6.1) to obtain the optimal cutoff value, all patients were divided into the BMD-lower group and BMD-higher group. To demonstrate osteopenia, this study also measured BMD at the tenth thoracic vertebra and performed correlation analysis between two BMD measurements from the first lumbar vertebra and the tenth thoracic vertebra. Age-adjusted standard BMD was calculated by the following formulae (12):

$$\text{BMD (HU) for men} = 308.82 - 2.49 \times \text{Age in years}$$

$$\text{BMD (HU) for women} = 311.84 - 2.41 \times \text{Age in years}$$

Definition and evaluation of data

All patients underwent follow-up until October 2023. Follow-up chest CT was compared to the baseline imaging to determine the time of PFS and the ratio of ORR and DCR between the two groups based on the Response Evaluation Criteria in Solid Tumors, version 1.1 (13). ORR and DCR were calculated based on the number of complete response (CR), partial response (PR), and stable disease (SD). PFS was defined as the time elapsed between initial ICI treatment and the onset of tumor progression or patient death. OS was defined as the period from the initial ICI treatment to the last follow-up or patient death.

In addition, we calculated the incidences of skeletal-related events (SRE) in the BMD-lower group and BMD-higher group. SRE include pathologic fracture, need for surgery/radiation therapy to bone and spinal cord compression (14, 15).

Statistical analysis

The mean and standard deviation of continuous variables were compared using the paired or independent student's t-test, and the percentages of discrete variables were calculated using the Chi-square test. Correlation was performed using Pearson's correlation analysis and Spearman's correlation analysis. The cut-off values (BMD-related indexes) were determined using the X-tile software (Yale University School of Medicine, New Haven, Connecticut, USA). This software provided a comprehensive approach to dividing a cohort into low-level and high-level marker expressions based on survival curves (16). Propensity score matching (PSM) analysis was performed with a caliper value of 0.05 to reduce patient selection bias and to balance the

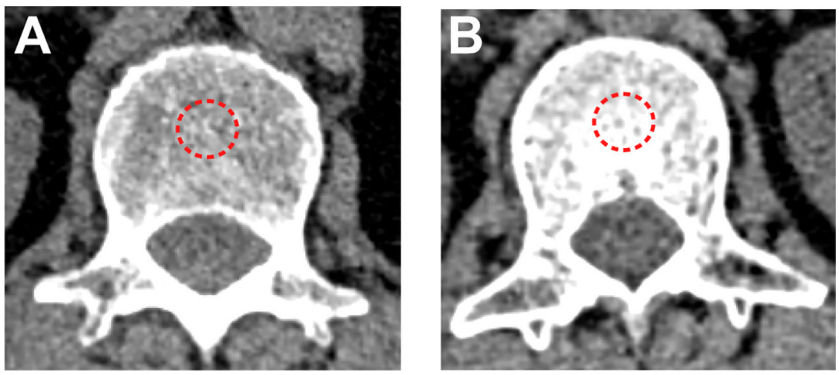

FIGURE 1
BMD measured by non-contrast chest CT scan. **(A)** A patient in the BMD-lower group (BMD=83 HU). **(B)** A patient in the BMD-higher group (BMD=190 HU). The red dotted line represents the outlined regions of interest.

variables between the BMD-lower and BMD-higher groups. One-to-one matching based on baseline characteristics of patients. The Kaplan-Meier method and the log-rank test were used to compare the differences in PFS, and OS in two groups. In the Cox regression analysis, variables with a univariate *P* value less than 0.1 were included in the multivariable Cox regression model. PFS and OS hazard ratios for each subgroup were calculated using unstratified univariate Cox models and presented as forest plots. All the tests were two-tailed; a *P*-value of less than 0.05 denoted statistical significance. All analyses were performed with SPSS version 26.0 software (IBM, Armonk, NY, USA), and R version 4.3.0 (R Foundation).

Results

Patient characteristics

This study included 479 patients with advanced NSCLC (270 in the BMD-lower group and 209 in the BMD-higher group), and all of these underwent the treatment of ICIs. Table 1 shows the baseline demographic and clinical characteristics of patients before and after PSM analysis. Compared with the BMD-higher group, the BMD-lower group had a higher proportion of patients older than 65 years old and a higher prevalence of hypertension. In addition, the BMD-lower group had a higher proportion of patients with ECOG ≥1. These differences were reduced after PSM analysis and reached balance. After PSM analysis, both the BMD-lower group and the BMD-higher group consist of 150 patients (Table 1). Baseline characteristics of patients excluded by PSM are shown in Supplementary Table 1. Histogram of propensity scores showed a closer distribution of propensity scores between the BMD-lower and BMD-higher groups after matching (Supplementary Figures 1A, B).

The optimum cutoff value of BMD

The X-tile software was used to determine the optimal cutoff value for BMD classification (Supplementary Figures 2A, B). Specifically, we used the OS outcome as a reference, and on the basis of ensuring that the OS of the two groups of patients were in the same trend at all cutoff points, we selected the point with the most significant difference in the outcomes of the two groups of

TABLE 1 Baseline characteristics of patients before and after PSM analysis.

Characteristics	Before PSM			After PSM		
	BMD-lower	BMD-higher	*P* value	BMD-lower	BMD-higher	*P* value
Patients, n	270	209		150	150	
Sex, n (%)			0.107			0.854
Male	231 (85.6%)	189 (90.4%)		133 (88.7%)	134 (89.3%)	
Female	39 (14.4%)	20 (9.6%)		17 (11.3%)	16 (10.7%)	
Age, n (%)			<0.001			0.401
<65	132 (48.9%)	158 (75.6%)		92 (61.3%)	99 (66.0%)	
≥65	138 (51.1%)	51 (24.4%)		58 (38.7%)	51 (34.0%)	
Body mass index, n (%)			0.379			0.667
<Median	223 (82.6%)	166 (79.4%)		118 (78.7%)	121 (80.7%)	
≥Median	47 (17.4%)	43 (20.6%)		32 (21.3%)	29 (19.3%)	
ECOG status, n (%)			<0.001			0.627
0	151 (55.9%)	149 (71.3%)		96 (64.0%)	100 (66.7%)	
≥1	119 (44.1%)	60 (28.7%)		54 (36.0%)	50 (33.3%)	
Pathological types, n (%)			0.307			0.888
Squamous carcinoma	139 (51.5%)	93 (44.5%)		79 (52.7%)	78 (52.0%)	
Adenocarcinoma	115 (42.6%)	103 (49.3%)		63 (42.0%)	62 (41.3%)	
Other	16 (5.9%)	13 (6.2%)		8 (5.3%)	10 (6.7%)	
Stages, n (%)			0.924			0.535
Stage III	76 (28.1%)	58 (27.8%)		50 (33.3%)	45 (30.0%)	
Stage IV	194 (71.9%)	151 (72.2%)		100 (66.7%)	105 (70.0%)	
Type of ICIs, n (%)			0.652			0.531

(Continued)

TABLE 1 Continued

Characteristics	Before PSM			After PSM		
	BMD-lower	BMD-higher	*P* value	BMD-lower	BMD-higher	*P* value
PD-1	251 (93.0%)	192 (91.9%)		136 (90.7%)	139 (92.7%)	
PD-L1	19 (7.0%)	17 (8.1%)		14 (9.3%)	11 (7.3%)	
Smoking, n (%)			0.411			0.815
Yes	151 (55.9%)	109 (52.2%)		89 (59.3%)	87 (58.0%)	
No	119 (44.1%)	100 (47.8%)		61 (40.7%)	63 (42.0%)	
Diabetes, n (%)			0.606			0.395
Yes	27 (10.0%)	18 (8.6%)		10 (6.7%)	14 (9.3%)	
No	243 (90.0%)	191 (91.4%)		140 (93.3%)	136 (90.7%)	
Hypertension, n (%)			0.011			0.899
Yes	98 (36.3%)	53 (25.4%)		45 (30.0%)	44 (29.3%)	
No	172 (63.7%)	156 (74.6%)		105 (70.0%)	106 (70.7%)	
Hyperlipidemia, n (%)			0.549			1.000
Yes	87 (32.2%)	62 (29.7%)		49 (32.7%)	49 (32.7%)	
No	183 (67.8%)	147 (70.3%)		101 (67.3%)	101 (67.3%)	
COPD, n (%)			0.405			0.274
Yes	28 (10.4%)	17 (8.1%)		20 (13.3%)	14 (9.3%)	
No	242 (89.6%)	192 (91.9%)		130 (86.7%)	136 (90.7%)	
Alkaline phosphatase, mean (SD)	97.7 (48.0)	106.1 (66.4)	0.121	99.0 (48.0)	99.2 (51.6)	0.979
Lactic dehydrogenase, mean (SD)	228.0 (103.7)	245.6 (120.7)	0.087	227.9 (116.9)	233.7 (103.5)	0.645
Ca, mean (SD)	2.2 (0.1)	2.2 (0.2)	0.965	2.2 (0.1)	2.2 (0.1)	0.642
Blood urea nitrogen, mean (SD)	5.4 (1.8)	5.3 (1.7)	0.377	5.5 (1.9)	5.4 (1.8)	0.868
Hemoglobin, mean (SD)	123.0 (16.6)	124.9 (15.7)	0.209	124.1 (15.9)	124.9 (16.3)	0.660
A/G ratio, mean (SD)	1.5 (1.3)	1.4 (0.3)	0.400	1.4 (0.3)	1.4 (0.3)	0.603
NLR, n (%)			0.624			0.222
≤2	42 (15.6%)	36 (17.2%)		30 (20.0%)	22 (14.7%)	
>2	228 (84.4%)	173 (82.8%)		120 (80.0%)	128 (85.3%)	
PLR, n (%)			0.176			0.633
≤150	104 (38.5%)	68 (32.5%)		58 (38.7%)	54 (36.0%)	
>150	166 (61.5%)	141 (67.5%)		92 (61.3%)	96 (64.0%)	
Prior radiation therapy, n (%)			<0.001			1.000
Yes	30 (11.1%)	2 (1.0%)		3 (2.0%)	2 (1.3%)	
No	240 (88.9%)	207 (99.0%)		147 (98.0%)	148 (98.7%)	
Vertebral bone metastasis, n (%)			0.008			1.000
Yes	68 (25.2%)	32 (15.3%)		22 (14.7%)	22 (14.7%)	
No	202 (74.8%)	177 (84.7%)		128 (85.3%)	128 (85.3%)	
Corticosteroid application, n (%)			0.009			0.299
Yes	155 (57.4%)	95 (45.5%)		81 (54.0%)	72 (48.0%)	
No	115 (42.6%)	114 (54.5%)		69 (46.0%)	78 (52.0%)	

(Continued)

TABLE 1 Continued

Characteristics	Before PSM			After PSM		
	BMD-lower	BMD-higher	*P* value	BMD-lower	BMD-higher	*P* value
Osteopenia treatment, n (%)			<0.001			0.590
Yes	63 (25.2%)	24 (11.5%)		16 (10.7%)	19 (12.7%)	
No	207 (76.7%)	185 (88.5%)		134 (89.3%)	131 (87.3%)	

PSM, propensity score matching; BMD, bone mineral density; SD, standard deviation; ICIs, immune checkpoint inhibitors; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; COPD, chronic obstructive pulmonary disease; A/G ratio, albumin to globulin ratio; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

patients. Finally, the cutoff value was determined to be 138 HU, and 270 patients were classified into the BMD-lower group and 209 patients were classified into the BMD-higher group.

To demonstrate osteopenia, this study performed a correlation analysis between two BMD measurements taken from the first lumbar vertebra and the tenth thoracic vertebra in the same subject. Scatter plots showed significant correlations between the two BMD measurements before (R=0.947, *P*<0.001) and after PSM analysis (R=0.955, *P*<0.001) (Supplementary Figures 3A, B).

Tumor response

The tumor responses of the BMD-lower group and BMD-higher group before and after PSM analysis are shown in Supplementary Tables 2, 3. Overall, short-term therapeutic effects were similar between the two groups. Before PSM analysis, the ORR and DCR of the BMD-lower group were 43.3% and 91.1%, respectively, and were 43.5% and 94.3% in the BMD-higher group, with no statistical difference (ORR, *P* = 0.964; DCR, *P* = 0.195). Similarly, there were also no statistically significant differences in ORR (44.7% vs. 44.7%, *P* ;= 1.000) and DCR (93.3% vs. 96.7%, *P* = 0.190) between the two groups after PSM analysis. It is worth noting that before PSM analysis, compared with the BMD-higher group, the proportion of patients in the BMD-lower group reaching PD was higher (8.9% vs. 5.7%). After PSM analysis, the difference was still existed (6.6% vs. 3.3%).

SRE

The incidences of SRE in the BMD-lower group and BMD-higher group are shown in Supplementary Table 4. Compared with the BMD-higher group, the incidences of SRE in the BMD-lower group was higher (17.4% vs. 4.8%, *P*<0.001).

Survival analysis

The median follow-up time was 22.0 months (IQR, 17.0-29.0 months), and during follow-up, 88 of 270 (32.6%) patients died in the BMD-lower group and 40 of 209 (19.1%) patients died in the BMD-higher group. Kaplan-Meier survival curves of PFS and OS were conducted between patients with baseline BMD ≤ 138 HU and with baseline BMD > 138 HU. The log-rank tests indicated that the BMD-lower group had the shorter PFS (16.0 months vs. 18.0 months, *P* = 0.067) and OS (20.5 months vs. 23.0 months, *P*< 0.001) than the BMD-higher group before the PSM analysis (Figures 2A, B). Likewise, after the PSM analysis, the BMD-lower group still had a shorter PFS (17.0 months vs. 19.0 months, *P* = 0.095) than the BMD-higher group, although there is no statistical difference (Figure 2C). And the OS (20.0 months vs. 23.0 months, *P* = 0.008) of the BMD-lower group was significantly shorter than that of the BMD-higher group, reaching statistical significance (Figure 2D).

Patients were divided into 3 groups according to the tertiles of BMD. Kaplan–Meier curves showed patients in the highest tertile of BMD had better OS compared to those in the lowest tertile before PSM analysis (*P* = 0.030) (Supplementary Figure 4A). This trend was still significant after PSM analysis (*P* = 0.042) (Supplementary Figure 4B). Meanwhile, we performed a correlation analysis to determine if an association existed between BMD and OS. Scatter plots showed the correlation between BMD and OS before (R=0.325, *P*< 0.001) and after PSM analysis (R=0.337, *P*< 0.001) (Supplementary Figures 5A, B). Furthermore, the relationship of BMD, clinical features and survival of patients in different BMD groups before and after PSM analysis are shown in heat maps (Figures 3A, B).

To decrease the influence of age on our findings, we calculated each patient's age-adjusted standard BMD and divided patients into the osteopenia group (n=314) and the non-osteopenia group (n=165) based on the standard BMD. Notably, the OS of the non-osteopenia group was better than that of the osteopenia group (*P* = 0.019) and a similar trend was found in the PFS of the two groups (*P* =0.059) (Supplementary Figures 6A, B).

In addition, to explore the predictive value of BMD in non-immunotherapy patients, we randomly selected 100 patients with NSCLC who receive standard chemotherapy at the same time. Patients were divided into the low group (n=65) and the high group (n=35) based on calculated age-adjusted standard BMD values. We found that there were no significant differences in the PFS between the two groups (*P* =0.671) (Supplementary Figure 7A). The high group had a longer OS than the low group, although there is no statistical difference (*P* =0.063) (Supplementary Figure 7B).

It is worth noting that some patients in the BMD-lower group received osteopenia treatment. After analysis, there were no significant differences in the PFS between the two groups (*P* =0.429) (Supplementary Figure 8A). Patients who received osteopenia treatment had a longer OS than those who did not, although the difference did not reach statistical significance (*P* =0.097) (Supplementary Figure 8B).

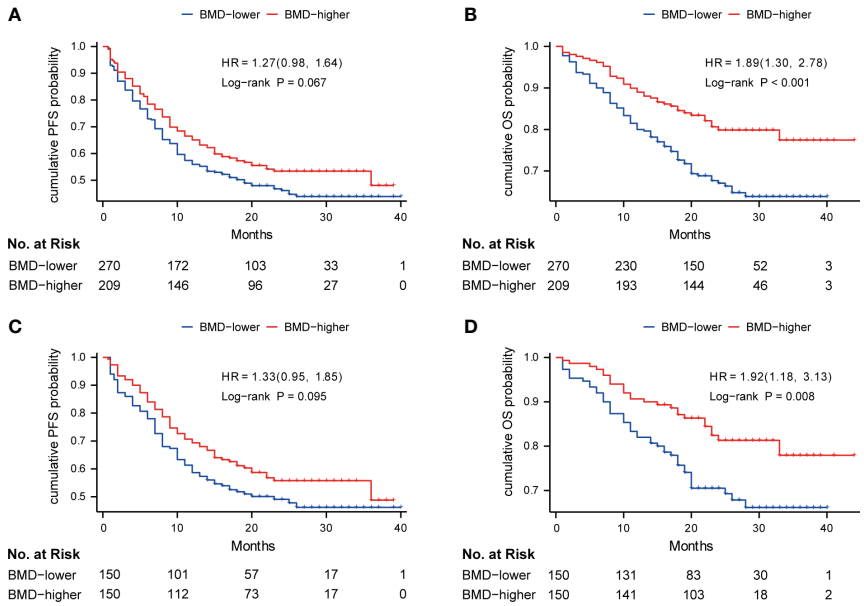

FIGURE 2
Kaplan-Meier curve of PFS **(A)** and OS **(B)** in the BMD-lower group (blue) and BMD-higher group (red) before PSM analysis; Kaplan-Meier curve of PFS **(C)** and OS **(D)** in two groups after PSM analysis. Analyses were conducted using LogRank tests. BMD, bone mineral density; PFS, progression-free survival; OS, overall survival; PSM, propensity score matching.

Cox regression analysis and subgroup analysis

Before PSM analysis, ECOG status, stages, alkaline phosphatase, blood urea nitrogen, albumin to globulin ratio, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, vertebral bone metastasis, corticosteroid application, skeletal-related events and group were identified as potential predictors for PFS, and age, ECOG status, stages, hypertension, alkaline phosphatase, albumin to globulin ratio, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, vertebral bone metastasis, corticosteroid application, and group were identified as potential predictors for OS in the univariable regression analysis. These covariates were further included in the multivariate regression analysis. In the multivariate analyses, stage IV (HR, 1.72[95%Cl, 1.21 to 2.46]; P =0.003), lower albumin to globulin ratio (HR, 1.75 [95%Cl, 1.12 to 2.70]; P = 0.013)and corticosteroid application (HR, 1.39 [95%Cl, 1.06 to 1.81]; P = 0.017) were significantly associated with shorter PFS (Supplementary Table 5), and aged over 65 years old (HR, 1.53 [95%Cl, 1.04 to 2.24]; P =0.029), ECOG status ≥1 (HR, 1.43 [95%Cl, 1.00 to 2.05]; P = 0.048), stage IV (HR, 1.64[95%Cl, 1.00 to 2.70]; P =0.049), higher alkaline phosphatase (HR, 1.00 [95%Cl, 1.00 to 1.01]; P = 0.006)and BMD-lower group (HR, 1.60[95%Cl,1.07 to 2.40]; P = 0.022) were significantly associated with shorter OS (Supplementary Table 6). After PSM analysis, higher albumin to globulin ratio (HR, 1.00 [95%Cl, 1.00 to 1.01]; P =0.049) and stage IV (HR, 2.13[95%Cl, 1.35 to 3.33]; P<0.001) were significant risk factors associated with a shorter PFS (Table 2), and aged over 65 years old (HR, 1.81[95%Cl, 1.10 to 2.97]; P = 0.020), stage IV (HR, 1.97 [95%Cl, 1.06 to 3.66]; P =0.032), higher alkaline phosphatase (HR, 1.00 [95%Cl, 1.00 to 1.01]; P = 0.025), lower albumin to globulin ratio (HR, 2.38 [95%Cl, 1.00 to 5.56]; P = 0.044), and BMD-lower group (HR, 1.90 [95%Cl, 1.16 to 3.12]; P = 0.011) were significant risk factors associated with a shorter OS (Table 3).

We performed a subgroup analysis of patients after PSM analysis based on baseline characteristics and observed relatively consistent results for PFS and OS, and hazard ratios for each subgroup were derived from the univariate Cox model. Among each subgroup of PFS (Figure 4), we found an interaction between age and ICI use. Except for the subgroup aged ≥ 65 years, the risk of PFS in the BMD-lower group was higher than that in the BMD-higher group. In the subgroup analysis of OS (Figure 5), the BMD-lower group showed a higher risk in all subgroups, including those aged ≥ 65 years, although some subgroups did not reach statistical differences.

Discussion

BMD, as an imaging marker, has been shown to provide potential predictive value for various cancer entities, such as breast cancer and hepatocellular carcinoma (17, 18). This is the first study to report the association between baseline BMD and the short-term efficacy and long-term prognosis in NSCLC patients treated with ICIs. We used X-tile software to scientifically determine the optimal cutoff value. At the same time, considering the impact of factors such as age and sex on BMD and prognosis, we balanced the baseline characteristics of the two groups through PSM, and further analyzed possible influencing factors through Cox regression and subgroup analysis. To reduce the effect of age on

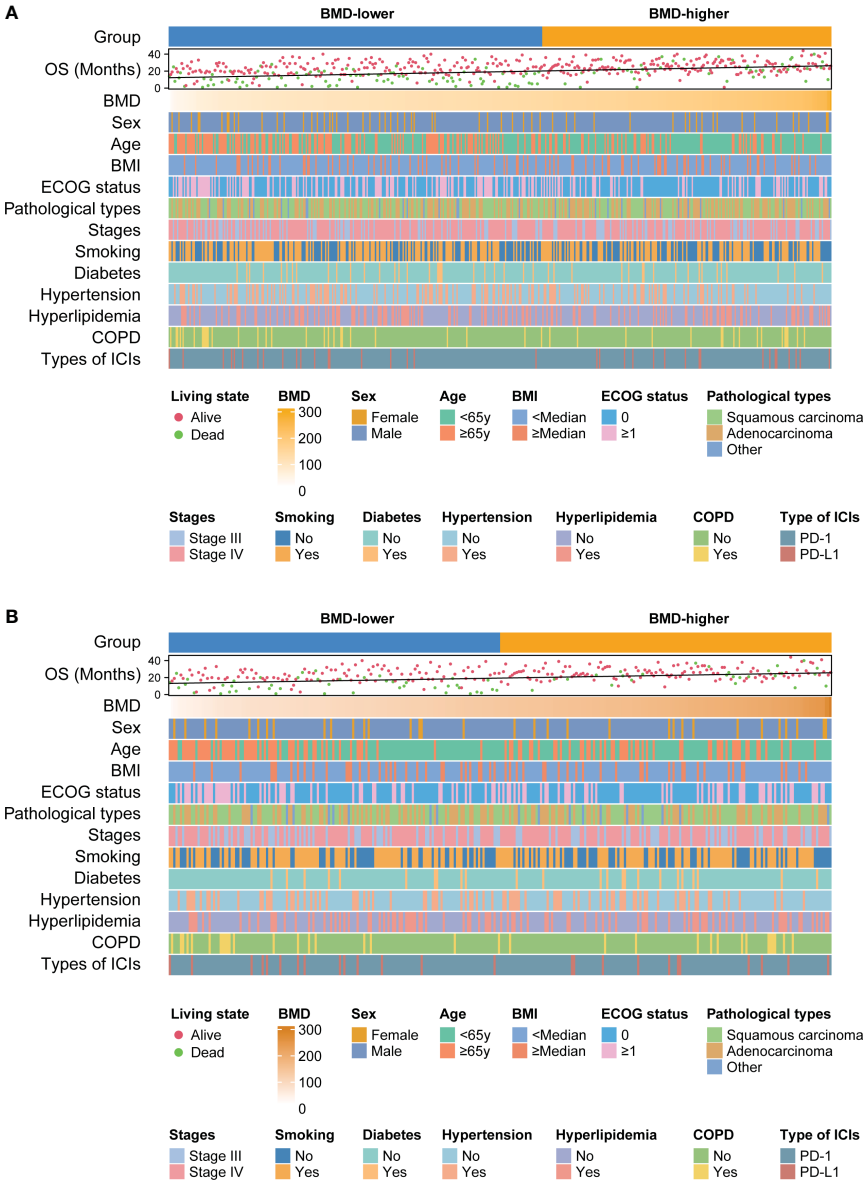

FIGURE 3
Heat maps shows the relationship between BMD, clinical features of patients and survival outcomes before **(A)** and after **(B)** PSM analysis. BMD, bone mineral density; OS, overall survival; COPD, chronic obstructive pulmonary disease; ICIs, immune checkpoint inhibitors; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PSM, propensity score matching.

study results, we grouped patients again based on the age-adjusted standard BMD. We further explored the predictive value of BMD in non-immunotherapy patients. In this study, CT-derived BMD was used to evaluate baseline BMD. Although DXA was the standard for assessing BMD, more and more studies indicated that CT scans were suitable for predicting vertebral fractures and consecutive measurements of bone loss, correlating well with BMD measured by DXA (19–21). Our results showed that lower baseline BMD was associated with shorter OS, both before and after PSM. Moreover, we found that patients with lower baseline BMD had a higher incidence of SRE. Ilic et al. (22) reported that low preoperative BMD was the independent predictor of patients with NSCLC-related brain metastasis (BM) post-surgical mortality. They measured the BMD value of the first lumbar vertebra in preoperative CT scans and divided it into pathological BMD (median, 99 HU; IQR, 75 to 195 HU) and physiological BMD (median, 140 HU; IQR, 113 to 159 HU) (22). The results showed that pathological BMD was associated with shorter OS (6.0 months vs. 15.0 months, P = 0.002) and higher 1-year mortality (OR, 0.5 [95%Cl, 0.2 to 1.0]; P = 0.03), which was similar to the results of our study. Similar findings have been reported in other tumors. Watanabe et al. (11) summarized and analyzed 11 studies (2330 patients) on the relationship between gastrointestinal cancer and BMD and found that osteopenia is independently associated with poor prognosis in these patients. The above studies have shown the unique role of BMD in predicting tumor progression. In addition, recent studies

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TABLE 2 Univariate and multivariate Cox proportional hazards analyses for PFS after PSM analysis.

Parameter	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Sex				
Male	Reference			
Female	1.12 (0.68, 1.87)	0.651		
Age				
<65	Reference			
≥65	1.08 (0.76, 1.52)	0.673		
ECOG status				
0	Reference		Reference	
≥1	1.64 (1.17, 2.28)	0.004	1.36 (0.97, 1.91)	0.077
Pathological types				
Squamous carcinoma	Reference			
Adenocarcinoma	1.20 (0.85, 1.69)	0.294		
Other	1.02 (0.51, 2.05)	0.947		
Stages				
Stage III	Reference		Reference	
Stage IV	2.17 (1.54, 3.57)	<0.001	2.13 (1.35, 3.33)	<0.001
Smoking				
No	Reference			
Yes	0.99 (0.71, 1.38)	0.936		
Diabetes				
No	Reference			
Yes	1.23 (0.71, 2.14)	0.464		
Hypertension				
No	Reference			
Yes	1.31 (0.93, 1.85)	0.126		
Hyperlipidemia				
No	Reference			
Yes	0.84 (0.59, 1.21)	0.353		
COPD				
No	Reference			
Yes	1.34 (0.83, 2.18)	0.234		
Alkaline phosphatase	1.00 (1.00, 1.01)	0.018	1.00 (0.99, 1.00)	0.596
Ca	0.50 (0.15, 1.66)	0.260		
Blood urea nitrogen	0.91 (0.83, 1.00)	0.059	0.91 (0.83, 1.00)	0.059
Albumin to globulin ratio	0.55 (0.32, 0.94)	0.028	0.56 (0.32, 1.00)	0.049
Neutrophil to lymphocyte ratio				
≤2	Reference		Reference	
>2	1.47 (0.91, 2.39)	0.116	1.14 (0.68, 1.90)	0.616
Platelet to lymphocyte ratio				
≤150	Reference		Reference	
>150	1.54 (1.08, 2.20)	0.018	1.23 (0.83, 1.82)	0.297
Vertebral bone metastasis				
No	Reference		Reference	
Yes	1.65 (1.09, 2.50)	0.018	1.01 (0.62, 1.65)	0.976
Corticosteroid application				
No	Reference		Reference	
Yes	1.47 (1.05, 2.04)	0.024	1.30 (0.91, 1.84)	0.149
Skeletal-related events				
No	Reference		Reference	
Yes	2.58 (1.51, 4.42)	<0.001	1.83 (1.00, 3.35)	0.051
Group				
BMD- higher	Reference		Reference	
BMD- lower	1.33 (0.95, 1.85)	0.094	1.37 (0.98, 1.92)	0.068

PFS, progression-free survival; PSM, propensity score matching; Cl, confidence interval; COPD, chronic obstructive pulmonary disease; BMD, bone mineral density.

and case reports have found that cancer patients have an increased risk of early fractures after starting ICIs, which may be related to reduced bone density and osteoporosis caused by T cell activation

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TABLE 3 Univariate and multivariate Cox proportional hazards analyses for OS after PSM analysis.

Parameter	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Sex				
Male	Reference			
Female	0.84 (0.39, 1.84)	0.663		
Age				
<65	Reference		Reference	
≥65	1.83 (1.15, 2.92)	0.011	1.81 (1.10, 2.97)	0.020
ECOG status				
0	Reference		Reference	
≥1	1.67 (1.04, 2.66)	0.033	1.33 (0.82, 2.14)	0.251
Pathological types				
Squamous carcinoma	Reference			
Adenocarcinoma	1.03 (0.64, 1.66)	0.897		
Other	0.42 (0.10, 1.74)	0.231		
Stages				
Stage III	Reference		Reference	
Stage IV	2.03 (1.13, 3.65)	0.018	1.97 (1.06, 3.66)	0.032
Smoking				
No	Reference			
Yes	0.92 (0.58, 1.48)	0.744		
Diabetes				
No	Reference			
Yes	1.48 (0.71, 3.10)	0.294		
Hypertension				
No	Reference			
Yes	1.30 (0.80, 2.12)	0.291		
Hyperlipidemia				
No	Reference			
Yes	0.85 (0.51, 1.42)	0.536		
COPD				
No	Reference			
Yes	1.28 (0.65, 2.50)	0.472		
Alkaline phosphatase	1.00 (1.00, 1.01)	0.011	1.00 (1.00, 1.01)	0.025
Ca	0.43 (0.08, 2.36)	0.332		
Blood urea nitrogen	0.95 (0.83, 1.08)	0.410		
Albumin to globulin ratio	0.36 (0.17, 0.78)	0.010	0.42 (0.18, 1.00)	0.044
Neutrophil to lymphocyte ratio				
≤2	Reference			
>2	1.33 (0.68, 2.59)	0.407		
Platelet to lymphocyte ratio				
≤150	Reference			
>150	1.39 (0.84, 2.31)	0.194		
Vertebral bone metastasis				
No	Reference			
Yes	1.51 (0.85, 2.67)	0.157		
Corticosteroid application				
No	Reference		Reference	
Yes	1.72 (1.06, 2.79)	0.027	1.41 (0.86, 2.31)	0.176
Skeletal-related events				
No	Reference		Reference	
Yes	2.49 (1.19, 5.20)	0.015	1.49 (0.69, 3.21)	0.308
Group				
BMD- higher	Reference		Reference	
BMD- lower	1.91 (1.18, 3.10)	0.009	1.90 (1.16, 3.12)	0.011

OS, overall survival; PSM, propensity score matching; CI, confidence interval; COPD, chronic obstructive pulmonary disease; BMD, bone mineral density.

(23–25). This suggests that ICIs also promote bone loss and thus affect clinical outcomes, although we did not follow up the changes in BMD after ICI treatment. Due to the double blow of

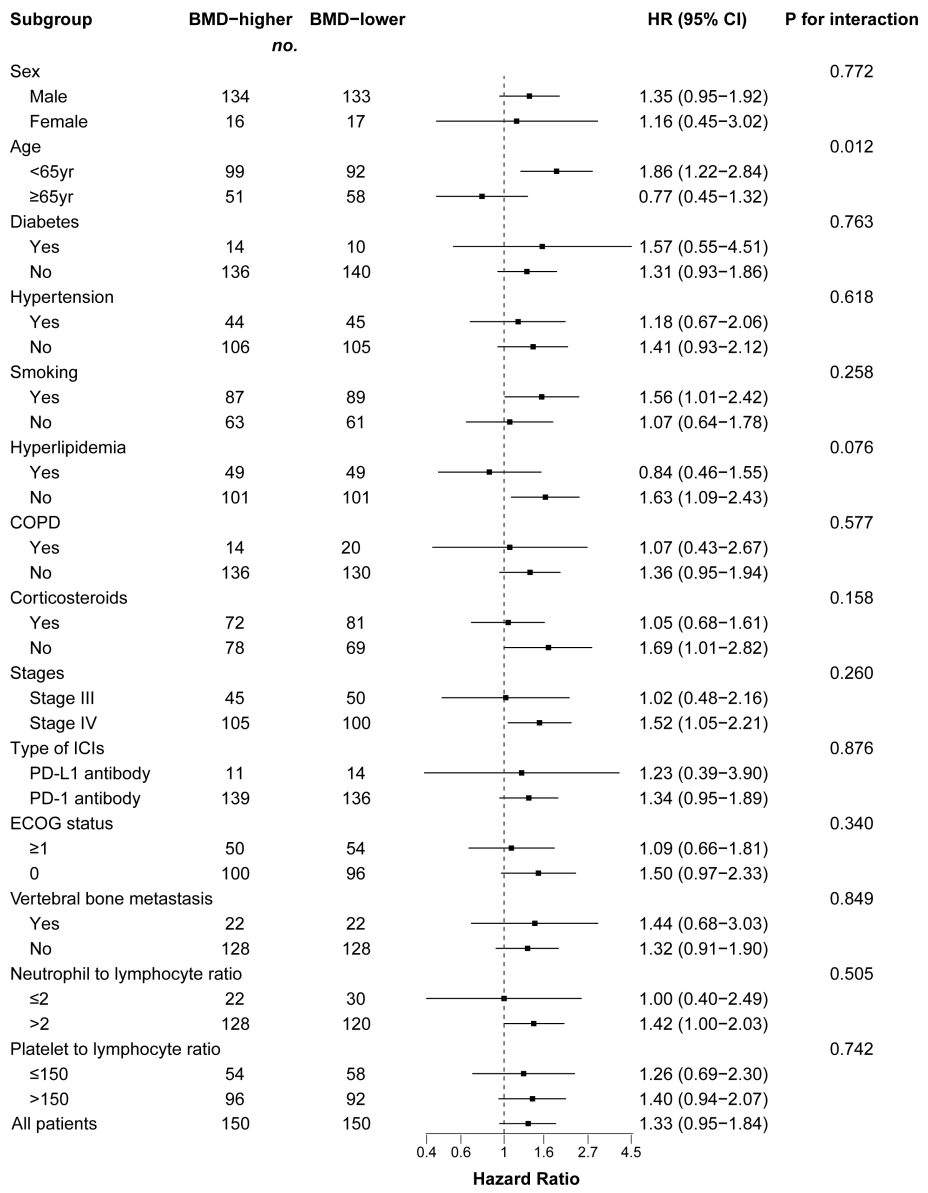

FIGURE 4
Forest plot of subgroup analysis in progression-free survival between the BMD-lower group and BMD-higher group. Dashed line indicates Hazard ratio of 1. BMD, bone mineral density; HR, hazard ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ICIs, immune checkpoint inhibitors; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1.

immunotherapy and tumors to bone loss, we should pay more attention to the bone condition of patients before treatment and intervene accordingly.

Our finding suggest that OS was better in patients initially presenting with higher BMD, however there is no difference concerning PFS, ORR or DCR between the two groups. PFS, ORR and DCR have been implemented as early clinical end points and have been extensively used in the evaluation of anti-tumor therapy. However, the relationship between these early end points and OS has not been formally established, which may be influenced by multiple factors (26). Notably, several immunotherapy trials demonstrated improvements in OS without improvements in PFS and/or ORR (27, 28). The ICIs may alter tumor growth kinetics rather than solely act via direct cytotoxicity, which may be the reason for the divorce between ORR, PFS and OS. Furthermore, our study found that corticosteroid application was associated with shorter PFS and was a potential risk factor for OS, although statistical differences were not reached after PSM. Corticosteroids, as immunosuppressive drugs, could exert several mechanisms to reduce immune activity. However, ICIs are designed to enhance the immune system's inherent antitumor activity (29). Based on our results, we speculate that there may be an antagonistic effect between corticosteroids and ICIs. However, more research is needed to further verify the interactions of corticosteroid use and ICI.

It is still unclear the association between the lower baseline BMD and immunotherapy efficacy and cancer progression, but

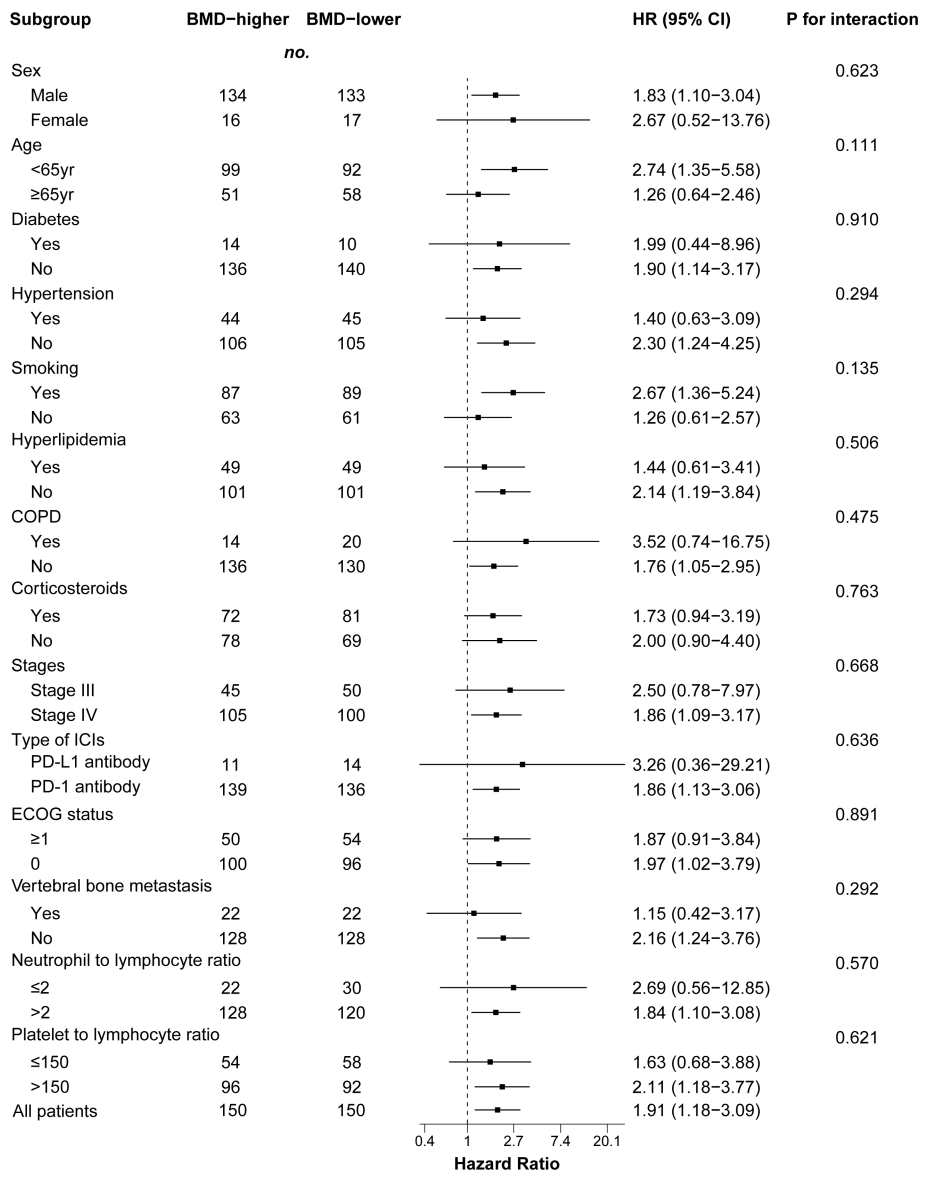

FIGURE 5
Forest plot of subgroup analysis in overall survival between the BMD-lower group and BMD-higher group. Dashed line indicates Hazard ratio of 1. BMD, bone mineral density; HR, hazard ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ICIs, immune checkpoint inhibitors; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1.

increasing evidence suggests that the immune systems are closely closed to skeletal systems. Cytokines (such as PTHrP, interleukin (IL)-1, IL-6, and IL-8) derived from cancer cells could activate osteoclasts and subsequently activate the RANKL/RANK pathway which had been proven to be correlated with poor prognosis in cancer patients (30, 31). Animal experiments have shown that reduced bone mineral density, trabecular thickness, and mineralization can be observed in NSCLC mice in the absence of tumor cell metastasis (32). From another point of view, BMD not only reflected the general condition and nutritional status of patients but was also associated with tumor progression to a degree. Bisphosphonates and Denosumab, the widely-used long-term treatment of osteolytic bone diseases and bone metastasis, were reported to exert direct and indirect anti-tumor effects, including inhibition of tumor cell proliferation and adhesion, reduction tumor cells secrete factors that increase RANKL expression, enhancement of immune surveillance, and prevention of angiogenesis (33–35). Clinical and preclinical experiments have shown that they can not only inhibit the progression of bone metastases and reduce SREs, but also prevent the growth of non-small cell lung cancer (36–38).

Our study had limitations. First, this study was retrospective, and data was collected previously. Prospective multicenter clinical trials are needed to validate our results in the future. Second, it should be considered whether the evaluation of CT-derived BMD was reliable, although there have been a number of previous studies using this method. Finally, bone loss was a continuous pathological process under the long-lasting negative effects of tumor, but we did

not evaluate changes in BMD during follow-up. Therefore, besides the baseline BMD, the extent of decline in BMD during ICI treatment and its association with prognosis should also be investigated with great care in the future studies. In spite of these limitations, we reported the relationship between baseline BMD and prognosis in NSCLC patients treated with ICIs for the first time.

Conclusions

In conclusion, our study found that for patients with NSCLC, baseline BMD before ICI treatment affects the long-term prognosis of them, although there is no difference in their short-term efficacy. Routine testing of BMD before receiving immunotherapy will help clinicians make better decisions.

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 humans were approved by The Research Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Institutional Review Board No. S054). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because clinical data were analyzed retrospectively and anonymously.

Author contributions

JL: Conceptualization, Data curation, Writing – original draft. BG: Data curation, Writing – original draft. YL: Data curation, Writing – original draft. YG: Conceptualization, Data curation, Resources, Writing – original draft. LL: Data curation, Writing – original draft. JW: Investigation, Project administration, Supervision, Writing – review & editing. WL: Data curation, Resources, Validation, Writing – original draft. ZY: Data curation, Resources, Writing – original draft. HZ: Investigation, Methodology, Writing – original draft. FP: Software, Validation, Writing – review & editing. BL: Formal Analysis, Project administration, Supervision, Writing – review & editing. LY: Project administration, Supervision, Visualization, Writing – review & editing. GZ: Conceptualization, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by grant from National Nature Science Foundation of China (No.82172034).

Acknowledgments

We would like to thank Aodong Xiao of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology for helpful technical advice on this work.

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/fimmu.2024.1332303/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 23 April 2024
DOI 10.3389/fonc.2024.1395654

OPEN ACCESS

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RECEIVED 04 March 2024
ACCEPTED 04 April 2024
PUBLISHED 23 April 2024

CITATION
Chen R, Jian Y, Liu Y and Xie J (2024) ALK-rearranged and EGFR wild-type lung adenocarcinoma transformed to small cell lung cancer: a case report.
Front. Oncol. 14:1395654.
doi: 10.3389/fonc.2024.1395654

ALK-rearranged and EGFR wild-type lung adenocarcinoma transformed to small cell lung cancer: a case report

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Background: Cases of ALK-rearranged EGFR wild-type lung adenocarcinoma (LUAD) transforming into small cell lung cancer (SCLC) are rarely reported, and diagnosis is often delayed. The emergence of this transformation phenomenon is often regarded as a consequence of acquired resistance mechanisms.

Case presentation: A 47-year-old male diagnosed with poorly differentiated adenocarcinoma of the right middle lung (pT2N2M0, stage IIIA) achieved a 46-month progression-free survival (PFS) following surgery and adjuvant chemotherapy. During routine follow-up, tumor recurrence and metastasis was detected. Genetic testing revealed ALK rearrangement and wild-type EGFR, prompting treatment with ALK-TKIs. In May 2023, abdominal CT scans showed significant progression of liver metastases and abnormal elevation of the tumor marker NSE. Immunohistochemical results from percutaneous liver biopsy indicated metastatic SCLC.

Results: After resistance to ALK-TKIs and transformation to SCLC, the patient received chemotherapy combined with immunotherapy for SCLC, but the patient's disease progressed rapidly. Currently, the patient is being treated with albumin-bound paclitaxel in combination with oral erlotinib and remains stable.

Conclusion: Histological transformation emerges as a compelling mechanism of resistance to ALK-TKIs, necessitating the utmost urgency for repeat biopsies in patients displaying disease progression after resistance. These biopsies are pivotal in enabling the tailor-made adaptation of treatment regimens to effectively counteract the assorted mechanisms of acquired resistance, thus optimizing patient outcomes in the battle against ALK-driven malignancies.

KEYWORDS

non-small cell lung cancer, small cell lung cancer, transformation, ALK-TKIs, liver metastasis

Introduction

Chromosomal rearrangements within the Anaplastic lymphoma kinase (ALK) gene play a pivotal role in determining the heightened sensitivity of a subset of non-small cell lung cancers (NSCLC) to small molecule ALK tyrosine kinase inhibitors (ALK-TKIs) (1). As per guideline recommendations, targeted therapy has now become the primary standard of care for patients with locally advanced and metastatic ALK-positive NSCLC (2). The advent of ALK-TKIs, including first-generation crizotinib, second-generation alectinib, brigatinib, and ensartinib, as well as third-generation Lorlatinib, has significantly revolutionized treatment choices and prognosis for individuals with ALK-positive NSCLC. Nevertheless, it is essential to acknowledge the inevitability of drug resistance alongside challenges like distant metastasis, the potential for severe adverse effects, and a diminished quality of life encountered during therapy (3). Common drug resistance mechanisms include secondary mutations in ALK, amplification of ALK fusion gene copies, and activation of bypass and downstream pathways (4). While ALK-TKIs are still being updated and iterated to address issues such as drug resistance, the silent phenomenon of SCLC transformation does not appear to have received much attention.

Indeed, this transformation from NSCLC to SCLC occurs mostly in patients resistant to EGFR-TKIs (5–7). This transformation has been described as a mechanism of acquired resistance occurring in approximately 5% of patients who develop resistance to EGFR-TKIs (8). Despite case reports, transformation from NSCLC to SCLC due to resistance to ALK-TKIs remains a rare phenomenon.

We present a case of a patient with NSCLC who developed recurrence and metastasis after surgery, progressed and transformed to SCLC after being resistant to treatment with ALK-TKIs. Subsequent application of therapy targeting SCLC led to transient control of the disease. The current treatment regimen is albumin-bound paclitaxel in combination with anlotinib, and the patient's condition is still stable.

Case report

A 47-year-old male patient with a smoking history was admitted for evaluation of mild discomfort in the right chest and back on 30th November 2016. Physical examination revealed that the patient had diminished breath sounds and audible rales in the right middle lung. Chest CT revealed a nodular shadow near the hilum of the right middle lobe, suggesting a high possibility of lung cancer with enlarged mediastinal lymph nodes. The patient had mild hypertension, no other diseases and no family history of tumors. After excluding contraindications for surgery, the patient underwent video-assisted thoracoscopic surgery for right middle lobe resection and mediastinal lymph node dissection on 23rd December 2016. Postoperative pathology revealed poorly differentiated adenocarcinoma of the right middle lung (pT2N2M0, stage IIIA), with positive immunohistochemical markers for CK, CK7, TTF-1, CgA, and Ki67 (40% positive staining) (Supplementary Figure 1). Starting from 17th January 2017, the patient received four cycles of post-operative adjuvant chemotherapy using the PP regimen (Pemetrexed 500mg/m^2 and cisplatin 75mg/m^2 once every three weeks) (specific treatment process illustrated in Figure 1), followed by regular outpatient follow-ups. The patient achieved a PFS of 46 months postoperatively.

In early September 2020, the patient experienced occasional hemoptysis. On October 19, 2020, a follow-up chest CT revealed slightly increased multiple nodules in the mediastinum compared to previous findings (Supplementary Figures 2A, B). An upper abdominal MRI indicated hepatic metastases and enhanced lesions in the lumbar vertebrae, suggesting bone metastasis (Supplementary Figure 2C). These findings suggested tumor recurrence and metastasis in the patient, prompting us to conduct genetic testing on the patient (Jinyu Medical Laboratory Center, Guangzhou, China). Due to the time required for waiting for genetic testing results, considering the progression of the patient's condition and the patient's desire for treatment, systemic chemotherapy was administered as the first step. Starting from November 1, 2020, the patient received two cycles of chemotherapy, including pemetrexed 500mg/m^2 and Nedaplatin 80-100mg/m^2 once every three weeks. After chemotherapy, patients exhibited poor tolerance, characterized by Grade II gastrointestinal adverse reactions and significant hematological adverse events. After aggressive symptomatic treatment, the patient's symptoms were partially relieved. However, this led to a slight delay in subsequent treatment. Concurrently, genetic testing revealed the patient's epidermal growth factor receptor (EGFR) mutation to be negative, while harboring an ALK rearrangement. At the beginning of 2021, the patient presented with lower back pain. Physical examination revealed tenderness in the lumbar region, without evidence of spinal deformities or signs of neural involvement. Considering the patient's symptoms and lumbar spine MRI findings (Supplementary Figure 2D), we considered that the lumbar pain is induced by bone metastasis. From March to April of the same year, palliative radiotherapy was administered to the L4 metastatic lesion in the lumbar spine at a dose of 39Gy in 13 fractions, whole-brain radiotherapy at a dose of 30Gy in 15 fractions, and a local boost of 12Gy in 6 fractions. The patient's

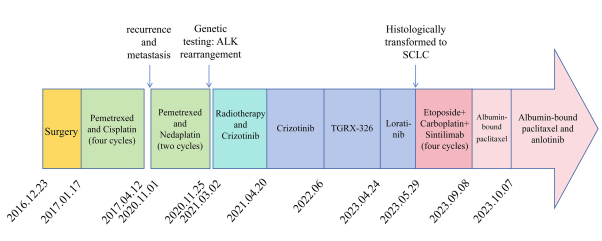

FIGURE 1
Patient's treatment history and medication details.

Abbreviations: NSCLC, non-small cell lung cancer; ALK-TKIs, ALK tyrosine kinase inhibitors; SCLC, small cell lung cancer; LUAD, lung adenocarcinoma; NSE, neural-specific enolase; SD, disease stability; PD, disease progression; EML4, echinoderm microtubule-associated protein-like 4; PFS, progression-free survival; CNVs, copy number variations; NGS, next-generation sequencing; WES, Whole-exome sequencing.

symptoms improved (additional brain metastasis images from the patient can be seen in Supplementary Figure 3). Since genetic testing revealed the presence of an ALK rearrangement, the patient received oral crizotinib at a dose of 250 mg twice daily after radiotherapy and achieved a PFS of 15 months. In June 2022, a follow-up upper abdominal MRI showed progression of hepatic lesions compared to previous findings. Patients opted into the clinical trial after giving full informed consent. The patient's treatment was switched to TGRX-326 (a third-generation ALK/ROS1 tyrosine kinase inhibitor) at a dose of 60 mg once daily to continued targeted therapy, with disease stability (SD) as the assessed treatment response, until April 24, 2023. The patient achieved a PFS of 11 months. Subsequently, progressive disease (PD) was observed (Figures 2A, B), leading to the patient's withdrawal from the clinical trial. Treatment was then switched to oral administration of the third-generation targeted therapy drug loratinib, at a dosage of 100mg once daily.

Patient revisited on 2023-05-22 and was found to have a high level of neural-specific enolase (NSE), a tumor marker, reaching 287.6 ng/mL (Figure 3). Percutaneous liver biopsy was performed, and pathological examination revealed metastatic small cell carcinoma, most likely from the lungs. Immunohistochemistry results showed: CD56 (+), CgA (+), broad-spectrum CK (+), P40 (-), TTF-1 (+), Ki-67 (+, 40%), CK7 (+), Napsin A(-), CK19 (+), CD10 (+), AFP (-), GPC-3 (+), HSP70 (+), GS (+), HepPar-1 (-), Syn (+) (Figure 4). Therefore, we transitioned the treatment protocol to target SCLC. Starting from 2023-05-29, as palliative first-line treatment following transformation of the pathological type, the patient received treatment with 100mg/m^2 of etoposide on days 1-3, carboplatin injection (AUC of 5 mg/ml/min) on day 1, and 200mg of sintilimab on day 1, once every three weeks, for a total of 4 cycles. After treatment, NSE was significantly reduced compared to before and the patient achieved a PFS of 3 months. On 2023-09-06, follow-up chest and abdominal CT scans indicated increased lymph node metastasis in the mediastinum and multiple liver lesions compared to previous scans (Figure 2C). In light of the deterioration in the patient's condition, we opted to switch to paclitaxel-based medication as palliative second-line treatment for SCLC. Starting on 8 September 2023, patients received a single course of systemic chemotherapy with intravenous albumin-bound paclitaxel. Nevertheless, the patient's tumor marker NSE continued to rise. Considering the patient's satisfactory physical condition (with a PS score of 2) and tolerance, a combined approach with anlotinib targeted therapy was initiated. On October 7, 2023, the patient was readmitted for treatment and received intravenous administration of albumin-bound paclitaxel at a dose of 260mg/m^2 every 3 weeks, along with concurrent oral administration of anlotinib at a daily dosage of 8 milligrams, administered for two weeks followed by a one-week break. After treatment, the patient's mental state and appetite were satisfactory and his condition was stable (see Supplementary Table 1 for detailed patient information).

Discussion

Lung cancer is the leading cause of cancer-related deaths globally, with NSCLC accounting for approximately 80-85% of lung cancer cases (9). Up to 8% of NSCLC patients have an ALK rearrangement, most commonly a fusion between ALK and the echinoderm microtubule-associated protein-like 4 (EML4), resulting in an EML4-ALK fusion that drives continuous cell proliferation and ultimately tumor formation (10). Crizotinib is currently the standard first-line therapy for ALK-positive NSCLC patients. However, due to various resistance mechanisms, most patients experience relapse within one year of treatment. The Phase III CROWN Study demonstrated that compared to the first-generation crizotinib, the third-generation ALK-TKI lorlatinib can improve progression-free survival (PFS) and reduce central nervous system progression in patients with advanced ALK-positive NSCLC (11). Nonetheless, drug resistance remains inevitable, with the most common mechanism being drug-resistant mutations in ALK. Case report have demonstrated that the NSCLC patient with ALK fusion mutations developed ALK fusion V1180L mutation and transformed into SCLC after acquiring resistance to alectinib (12). And, there was also a LUAD patient who developed ALK G1202R mutation and SCLC transformation after treatment resistance to second-generation ALK-TKIs (13). Although these are rare case reports, they carry significant implications. The poor response of patients to ALK-TKIs not only suggests the potential emergence of

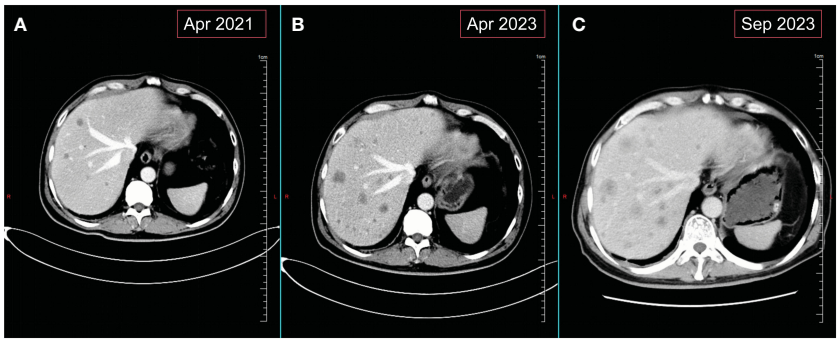

FIGURE 2
Changes in liver metastases on abdominal CT. **(A)** Images of liver metastases before monotherapy with crizotinib. **(B)** Images of the liver at the time of disease progression following treatment with TGRX-326. **(C)** Hepatic imaging of disease progression following use of 4 courses of chemotherapy combined with immunotherapy for SCLC.

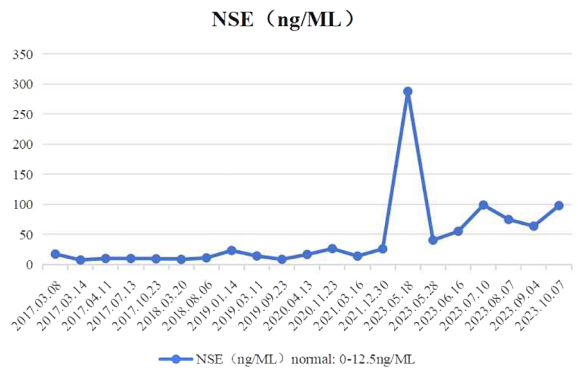

FIGURE 3
The profile of NSE in serum.

drug-resistant mutations but also necessitates consideration of the possibility of SCLC transformation.

In fact, cases of transformation from NSCLC to SCLC occurred mainly in patients with EGFR-mutated LUAD who were resistant to EGFR-TKIs (5–7, 14). To explore the molecular mutational mechanisms underlying this transformation, researchers have conducted next-generation sequencing (NGS) or whole-exome sequencing (WES) on samples from LUAD patients who transformed into SCLC after developing resistance to EGFR-TKIs. Multiple studies have consistently shown that this histological transformation is closely associated with inactivation mutations in the Retinoblastoma1 (Rb1) and TP53 genes, indicating that the inactivation of Rb1 and TP53 is an effective predictive factor for the transformation of LUAD into SCLC (7, 8, 15–18). Additionally, researchers reported a case of RET-rearranged LUAD transforming into SCLC, with acquired resistance to pralsetinib. Molecular analysis revealed the presence of the same RET fusion and TP53 mutation in the primary LUAD and recurrent SCLC (19). Previously, a patient with ALK-rearranged NSCLC experienced disease progression after treatment with ALK-TKIs, followed by SCLC transformation. Genomic profiling revealed the retention of ALK rearrangement, accompanied by inactivating Rb1 mutation (C706Y) and p53 exon deletion, which were not detected in the original tumor tissue at diagnosis (13). This also provides evidence supporting the significant roles of p53 and Rb1 loss in SCLC transformation. Additionally, other mutations possibly associated

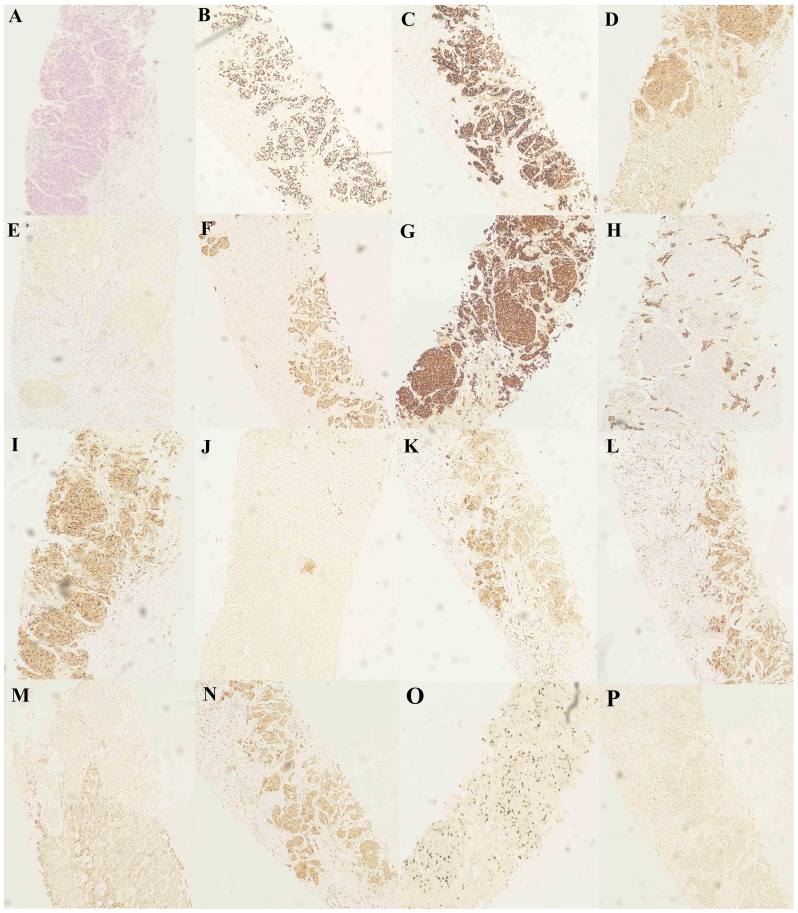

FIGURE 4
Hematoxylin and eosin (H&E) staining and immunohistochemical staining (IHC) staining of the liver biopsy specimen. **(A)** H&E staining of the liver biopsy specimen. **(B-D)** IHC staining confirmed positive for TTF-1, CgA and CD56. IHC staining confirms **(E)** AFP-negative and **(F-H)** CK, CK7 and CK19-positive. IHC staining confirmed **(I)** CD10 positivity, **(J)** Napsin A negativity, **(K)** GPC-3 positivity, and **(L)** GS positivity. IHC staining confirmed **(M)** HepPar-1 negativity, **(N)** HSP70 positivity, **(O)** Ki-67 positivity, and **(P)** P40 negativity.

with transformation include PIK3CA mutation, WNK1 mutation, etc. (20, 21). Furthermore, research suggests that the presence of neuroendocrine differentiation in NSCLC may be one of the factors leading to SCLC transformation (22). The origin of this transformation from NSCLC to SCLC is a controversial topic, as it is not entirely clear whether the original lung cancer tissue harbored mixed components (23).

Transformed SCLC typically manifests rapid disease progression and poses therapeutic challenges. Currently, there is no standardized treatment strategy for patients who develop SCLC transformation after resistance to ALK-TKIs. Chemotherapy combined with immunotherapy is the most common treatment option. In the entirety of this patient's therapeutic journey, the regimen devised was personalized, integrating the patient's actual condition while adhering to treatment norms. For instances, following the pathological transformation to SCLC, commonly used treatment options carboplatin, etoposide, and atezolizumab or durvalumab (24, 25), were not selected; rather, carboplatin, etoposide, and sintilimab were chosen. The choice of immunotherapeutic agents primarily stemmed from the patient's limited financial capacity, unable to afford imported PD-L1 inhibitors, thus opting for domestically produced sintilimab in combination with chemotherapy, yielding a 3-month PFS. Indeed, studies have demonstrated that sintilimab can serve as maintenance therapy post-chemotherapy for SCLC (26). The combination of sintilimab and anlotinib as second-line or beyond therapy for extensive disease (ED)-SCLC exhibits favorable antitumor activity with manageable toxicity (27). Additionally, prior reports have shown the efficacy of sintilimab in ED-SCLC refractory to multi-line treatments (28). Therefore, we opted for this regimen. This case underscores the necessity, in clinical practice, to tailor treatment approaches to individual patients by considering treatment guidelines alongside factors such as the patient's actual physical condition and economic status.

According to the literature, it has been pointed out that a rapid elevation of serum NSE and poor response to targeted drugs usually indicate a transformation from LUAD to SCLC (29). This trend was also observed in the present case, where a follow-up liver imaging examination after approximately 26 months of taking ALK-TKIs indicated disease progression and an elevated NSE level of 287.6ng/ML (normal range: 0-12.5ng/ML). This also suggests that during treatment, clinicians should monitor serum tumor markers or perform genomic sequencing, especially in patients with disease progression, as this may aid in the early detection of SCLC transformation. Repeat biopsies may be performed if necessary, and treatment plans can be adjusted promptly based on molecular pathological examination results to achieve personalized and comprehensive management of patients. Additionally, in this case, despite developing drug resistance and multiple metastases during the course of treatment, the patient's survival time since the initial diagnosis has over seven years. In a certain sense, regular follow-up visits, improved doctor-patient communication, and enhanced patient compliance hold great practical significance in improving patient prognosis.

Conclusion

This study reports a case of LUAD patient with postoperative recurrence and metastasis who developed acquired resistance and underwent SCLC transformation following treatment with ALK-TKIs. This finding has influenced clinical practice, highlighting the importance of dynamic assessment of tumor markers and repeat biopsies when necessary. The results suggest the necessity of early development of personalized treatment plans for patients experiencing SCLC transformation after ALK-TKIs resistance, with regular follow-up appointments and timely adjustment of treatment strategies advised. This subset of patients appears to exhibit faster disease progression compared to typical SCLC patients.

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

RC: Investigation, Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft. YJ: Supervision, Writing – review & editing. YL: Supervision, Writing – review & editing. JX: Supervision, Writing – review & editing, Investigation.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The present study was funded by the National Natural Science Foundation of China (grant nos. 81960425 and 81160294).

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.2024.1395654/full#supplementary-material

SUPPLEMENTARY FIGURE 1
H&E staining and IHC staining of surgical resection specimens. **(A)** H&E staining of surgically excised specimens. **(B-D)** IHC staining of the surgically resected specimen showed positivity for TTF-1, CK, and Ki67.

SUPPLEMENTARY FIGURE 2
CT images of the patient's chest and MRI images of the abdomen. **(A, B)** Mediastinal window of chest CT demonstrating the patient's mediastinal lymph nodes. **(C, D)** Abdominal MRI images showed tumor invasion of the patient's lumbar spine at L4.

SUPPLEMENTARY FIGURE 3
MRI image of the patient's cranium. **(A)**. Cranial MRI images of the patient before whole brain radiotherapy. **(B, C)** are cranial MRI images of the patient after whole brain radiotherapy.

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 08 May 2024
DOI 10.3389/fonc.2024.1402017

OPEN ACCESS

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RECEIVED 16 March 2024
ACCEPTED 17 April 2024
PUBLISHED 08 May 2024

CITATION
Ma S, Nie H, Wei C, Jin C and Wang L (2024) Association between immune-related adverse events and prognosis in patients with advanced non-small cell lung cancer: a systematic review and meta-analysis. *Front. Oncol.* 14:1402017. doi: 10.3389/fonc.2024.1402017

Association between immune-related adverse events and prognosis in patients with advanced non-small cell lung cancer: a systematic review and meta-analysis

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Background: The emergence of immune checkpoint inhibitors (ICIs) provides a variety of options for patients with advanced non-small-cell lung cancer (NSCLC). After the application of ICIs, the immune system of patients was highly activated, and immune-related adverse events (irAEs) could occur in some organ systems, and irAEs seemed to be associated with the survival prognosis of patients. Therefore, we evaluated the association between survival outcomes and irAEs in NSCLC patients and conducted a systematic review and meta-analysis.

Methods: We conducted systematic reviews of PubMed, Embase, Cochrane, and Web of Science databases until December 2021. The forest map was constructed by combining the hazard ratio (HR) and 95% confidence interval (CI). I^2 estimated the heterogeneity between studies. A meta-analysis was performed using R 4.2.1 software.

Results: Eighteen studies included 4808 patients with advanced NSCLC. In pooled analysis, the occurrence of irAEs was found to be a favorable factor for improved prognosis (PFS: HR: 0.48, 95% CI: 0.41-0.55, P <0.01; OS: HR: 0.46, 95% CI: 0.42-0.52, P <0.01). In subgroup analyses, cutaneous irAE, gastrointestinal irAE, endocrine irAE and grade ≥3 irAEs were associated with improvements in PFS and OS, but pulmonary and hepatic irAEs were not.

Conclusion: Existing evidence suggests that the occurrence of irAEs may be a prognostic biomarker for advanced NSCLC. However, further research is needed to explore the prospect of irAEs as a prognostic biomarker in patients undergoing immunotherapy.

Systematic review registration: https://www.crd.york.ac.uk/PROSPEROFILES/405333_STRATEGY_20240502.pdf, identifier CRD42023405333.

KEYWORDS

non-small-cell lung carcinoma, immune checkpoint inhibitors, immune-related adverse events, prognosis, meta - analysis

Introduction

Lung cancer represents 11.4% of all malignancies and 18% of all cancer-related fatalities, making it the primary cause of mortality from cancer, according to Global Cancer Statistics 2020 (1). Non-small-cell lung cancer (NSCLC) comprises approximately 80–85% of all lung cancer cases and exhibits a poor 5-year survival rate (2). Patients with early NSCLC typically undergo surgery followed by adjuvant therapy to reduce the risk of cancer recurrence and enhance patient survival (3). With the progress of clinical diagnosis and treatment technology, the early detection rate of lung cancer has increased significantly, and the 5-year survival rate of patients has improved (4). However, some patients are diagnosed with advanced lung cancer and cannot benefit from surgery. The emergence of targeted therapy and immunotherapy provides a variety of options for lung cancer patients. ICIs relieve the suppression of immune function caused by immune checkpoints by blocking the binding of immune checkpoints with their ligands so as to reactivate immune cells to play an anti-tumor role (5). Tumor mutational burden (TMB) and programmed cell death-ligand 1 (PD-L1) expression are often utilized biomarkers for assessing therapy response and prognosis in patients. However, they are not considered the optimal biomarkers due to considerations including high cost, lengthy processing time, and inadequate tumor samples (6–8). After the application of ICIs, the immune system of patients is highly activated, and immune-related adverse events (irAEs) can occur in some organ systems, the most common of which are the cutaneous, gastrointestinal tract, endocrine system, liver, and lung. Others include nervous system, blood system, heart, eye, and rheumatic system involvement (9, 10). Previous studies have shown that the development of irAEs is associated with improved melanoma prognosis (11). The emergence or development of irAEs may be used as an alternative indicator to judge the efficacy of ICIs and evaluate the survival and prognosis of patients. This connection makes it crucial to monitor the adverse reactions after treatment with ICIs. However, the results of existing studies are not the same. Therefore, in order to strengthen the relationship between irAEs and the survival outcome of NSCLC patients, this study conducted a systematic review of the studies of patients with advanced NSCLC receiving immunotherapy and developing irAEs to investigate the relationship between irAEs and the survival prognosis of NSCLC patients.

Materials and methods

Literature retrieval strategy

We utilized the PICOS framework to formulate study questions and conduct literature searches. The participants were individuals diagnosed with lung cancer, namely NSCLC. The intervention was immune checkpoint inhibitor therapy, and the result was irAEs. We searched PubMed, Embase, Cochrane, and Web of Science databases for studies reporting irAEs and prognosis after ICIs in NSCLC patients from database creation until December 2021. Key search terms included lung cancer, non-small cell lung cancer, irAEs, immune checkpoint inhibitors, programmed death- 1 (PD-1) or PD-L1 inhibitors, and cytotoxic t lymphocyte-associated antigen-4 (CTLA-4) inhibitors, as well as those identified by the Food and Drug Administration. The food and drug administration (FDA) approved immune checkpoint inhibitor drug already on the market.

Inclusion and exclusion criteria

The included studies met the following criteria: (1) prospective or retrospective studies to investigate the effect of irAEs on prognosis in patients with NSCLC; (2) have been clinically diagnosed with advanced non-small cell lung cancer and have been treated with at least one or more ICIs; (3) strictly in accordance with the definition of irAEs classification and clear grouping; (4) articles including hazard ratios (HR) and 95% confidence intervals (CI) for overall survival (OS) and progression-free survival (PFS). (5) Research published in English. The exclusion criteria were as follows: (1) The patient was known to have an autoimmune disease, and the adverse events reported in the study were not significantly associated with ICIs; (2) In order to avoid confusion about adverse reactions caused by other drugs, studies receiving immunotherapy in combination with other anti-tumor therapies, including combination chemotherapy, radiation therapy, targeted therapy, and antiangiogenic therapy, were excluded. (3) Studies without HR and 95% CI data. (4) Review articles, case reports, animal studies, and cost-benefit studies.

Data collection and quality assessment

Two researchers are responsible for the first phase of independent screening of titles and abstracts and the second stage of full text screening, a full text review of all potentially relevant citations to determine the final inclusion of the study. If there are any unresolved differences, discuss them with the third researcher and resolve them. The extracted data included author, publication year, sample size, population of irAEs occurrence, irAEs type and grade, and OS and PFS of patients with and without irAEs. HR, 95% CI, and P-value were extracted from the Cox regression analysis and survival curve. According to the occurrence of irAEs, they were divided into an irAEs group and a non-irAEs group. In addition, HR provided by irAEs of any grade or organ is selected when HR of irAEs of any grade or organ, graded irAEs, and single organs is presented simultaneously in the study. When both univariate and multivariate HR are provided for any grade or any type of irAEs in the study, the HR provided in multivariate analysis is selected.

Meta analysis

The primary goal of this study was to evaluate the association between OS, PFS, and irAEs in NSCLC receiving immunotherapy. The secondary objective was to evaluate the relationship between irAEs organ and irAEs grade with OS and PFS. Meta-analysis was performed using R4.2.1. The forest map was constructed by combining HR and 95% CI. Heterogeneity between studies was estimated by I^2. If I^2 >50% indicates significant heterogeneity, the meta-analysis uses a random

effects model (12). Instead, a fixed effects model is used (13). P-values below 0.05 were considered statistically significant.

Results

Literature search results

We searched PubMed, Embase, and Cochrane databases, respectively. There were 232 citations found in PubMed, 3,767 citations found in Embase, and 1,072 citations found in Cochrane, for a total of 5,071 citations. After sifting through the titles and abstracts, we collected from them 27 studies that might qualify. Finally, after a full review of the articles, we selected 18 studies. The reasons for exclusion are as follows: Two reports had survival data but fell under the category of case reports. Two studies only reported OS and PFS data but did not report the corresponding HR. Two studies reported survival data only for irAE patients involving a single organ system; one study had incomplete data and could not be included in the analysis; and in one study, survival data of patients with other types of tumors were pooled. Survival data for patients with NSCLC were indistinguishable. The study was a combination of ICIs and non-ICIs drugs and radiation therapy and could not clearly distinguish the source of adverse events. The detailed retrieval process is shown in Figure 1. The meta-analysis included 18 studies of 4808 patients with advanced NSCLC, with a sample size ranging from 23 to 1010 patients. Sixteen studies were retrospective, and two were prospective (14–31). Main characteristics of the included studies as shown in Table 1.

The correlation between irAEs occurrence, PFS and OS

In the meta-analysis, 18 studies all provided HRs for PFS (14–31) and 16 studies evaluated HRs for OS (14, 15, 17–26, 28–31), and the pooled analysis showed that the occurrence of irAEs was a favorable factor for improvement in PFS and OS (PFS: [HR: 0.48, 95% CI: 0.41-0.55, P <0.01]; OS: [HR: 0.46, 95% CI: 0.42-0.52, P <0.01]. As shown in Figure 2, Synthetic analysis showed moderate heterogeneity between irAEs and OS studies (I^2 = 46%, P = 0.02) and significant heterogeneity between PFS studies (I^2 = 56%, P <0.01). The heterogeneity may be related to the organ and grade of irAEs. Therefore, we conducted a subgroup analysis of the correlation between the occurrence and prognosis of irAEs.

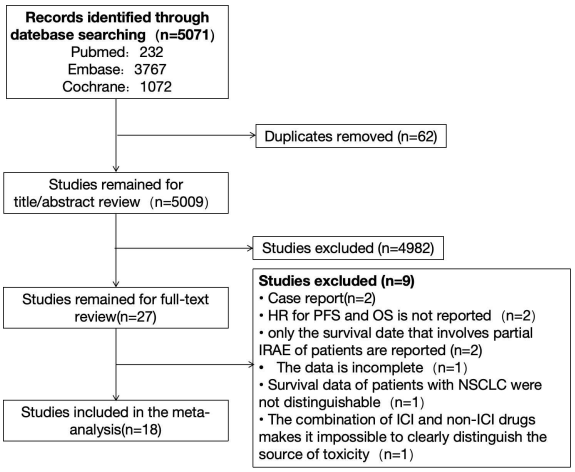

FIGURE 1
Flowchart and the detailed process of eligible studies.

Subgroup analysis based on irAEs types and grades showed that cutaneous irAE [PFS: (HR: 0.53, 95% CI: 0.45-0.63, P <0.01); OS: (HR: 0.47, 95% CI: 0.37-0.60, P <0.01)], gastrointestinal irAE [PFS: (HR=0.67, 95% CI=0.54-0.82, P <0.01); OS: (HR: 0.56, 95% CI: 0.43-0.73, P <0.01)], endocrine irAE [PFS: (HR: 0.58, 95% CI: 0.46-0.72, P <0.01); the OS: (HR: 0.50, 95% CI: 0.40-0.63, P <0.01)], and grade ≥3 irAEs [PFS: (HR: 0.90, 95% CI: 0.73-1.11, P = 0.33); OS: (HR: 0.72, 95% CI: 0.56-0.92, P <0.01)] is a favorable factor for the improvement of PFS and OS. However, pulmonary irAE [PFS: (HR: 0.95, 95% CI: 0.76-1.18, P = 0.63); OS: (HR: 1.01, 95% CI: 0.79-1.29, P = 0.95)] and hepatic irAE [PFS: (HR: 0.98, 95% CI: 0.76-1.26, P = 0.86); the OS: (HR: 0.96, 95% CI: 0.71-1.30, P = 0.80)] happened not improvement factor of PFS and OS (p > 0.05). As shown in Figures 3 and 4.

Sensitivity analysis and publication bias

In the sensitivity analysis, the results of OS and PFS remained significant regardless of which study was deleted, indicating that the significant associations between the occurrence of irAEs and the response to ICIs and survival outcomes in NSCLC patients remained stable (Additional File 1: Supplementary Figures S1, S2). In the meta-analysis, funnel plots and Begg tests were used to assess publication bias (32). As can be seen from the funnel plot, the symmetrical spread of the effect points of the independent studies and the Begg test showed no significant asymmetry for PFS (p = 0.058) (Attached File 1: Supplementary Figure S3). For OS, the funnel plot shows a symmetrical spread of the independent study effect points, and the Begg test also shows no significant asymmetry for OS (p = 1.000) (Supplementary 1: Supplementary Figure S4).

Discussion

Although the underlying pathophysiology has not been explicitly articulated to date, there is growing evidence that the occurrence of irAEs is an independent predictor of NSCLC patients receiving immunotherapy. This study provides a more comprehensive and extensive analysis of the relationship between irAEs and patient survival outcomes. In our analysis, we found that the presence of irAEs was a favorable factor for the survival prognosis of patients. Possible explanations are that irAEs are caused by overactivation of autoreactive T cells and that patients who respond to ICIs are at greater risk of developing irAEs. Stratified analysis based on irAEs type showed that cutaneous, gastrointestinal, and endocrine irAEs were favorable factors for the improvement of OS and PFS (P<0.05). However, no significant

TABLE 1 Main characteristics of the included studies.

Study	n	ICIs	irAEs(%)	PFS		OS	
				irAEs+	irAEs-	irAEs+	irAEs-
Ahn et al., 2019 (14)	155	Nivolumab	61.93%	11.63	3.27	24.05	7.39
		Pembrolizumab					
Akamatsu et al., 2020 (15)	23	Nivolumab	65.21%	19.10	5.60	27.80	16.10
		Pembrolizumab					
		Atezolizumab					
Chen et al., 2020 (16)	97	Pembrolizumab	46.39%	11.30	2.80	17.90	–
		Nivolumab					
Chen et al., 2021 (17)	191	PD-1/PD-L1	36.60%	8.80	3.90	21.00	14.80
Conde-Estévez et al., 2021 (18)	70	Nivolumab	44.30%	13.00	1.90	30.10	5.10
		Pembrolizumab					
		Atezolizumab					
Cortellini et al., 2019 (19)	559	Nivolumab	41.32%	10.10	4.10	20.50	8.50
		Pembrolizumab					
Cortellini et al., 2020 (20)	1010	Pembrolizumab	32.97%	19.90	7.80	–	16.10
Daniello et al., 2021 (21)	894	PD-1/PD-L1	22.10%	17.00	10.00	37.00	15.00
Grangeon et al., 2019 (22)	270	PD-1/PD-L1	45.92%	5.20	1.97	–	8.21
Haratani et al., 2018 (23)	134	Nivolumab	51.49%	9.20	4.80	not reached	11.10
Hosoya et al., 2020 (24)	76	Nivolumab	57.89%	4.00	1.90	not reached	13.00
K.Komiya et al., 2019 (25)	61	nivolumab	29.50%	9.30	1.90	not reached	8.70
		pembrolizumab					
Naqash et al., 2020 (26)	531	Nivolumab	32.58%	6.10	3.10	14.90	7.40
Noguchi et al., 2020 (27)	94	Pembrolizumab	67.02%	12.40	2.20	not reached	not reached
Ricciuti et al., 2019 (28)	195	Nivolumab	43.58%	5.70	2.00	17.80	4.00
Riudavets et al., 2020 (29)	267	PD-1/PD-L1	56.90%	12.40	4.10	28.20	12.50
Sonehara et al., 2022 (30)	80	Nivolumab	31.25%	6.80	1.90	37.80	8.10
		Pembrolizumab					
		Atezolizumab					
Y. Wu et al., 2022 (31)	101	PD-1/PD-L1	44.60%	7.00	4.00	17.00	9.00

ICIs, immune checkpoint inhibitor; irAEs, Immune-related adverse events; PFS, progression-free survival; OS, Overall survival; "-", indicates data not reported in the original publication.

association was found between hepatobiliary irAEs, pulmonary irAEs, and favorable outcomes. The possible reason is that adverse events in the liver, lung, and other important organs can lead to irreversible damage to their function, and they cannot tolerate other anti-tumor therapy, thus affecting the prognosis. In contrast, adverse events related to the cutaneous, gastrointestinal tract, and endocrine system are relatively easier to control, which also leads to a difference in the occurrence site and prognosis of irAEs.

Although in this study, the presence of grade 3 or above irAEs showed a good correlation with survival outcomes, which is inconsistent with previous studies. The authors suggest that there was no significant correlation between the occurrence of grade 3 or higher irAEs and good survival outcomes. First of all, we went back to the original text and found that in the Cortellini et al. (19, 20) study, the gastrointestinal tract was the most common type of grade 3 or above irAEs, followed by hepatopulmonary irAEs, cutaneous irAEs, and endocrine irAEs. However, if the above high-grade irAEs are included in the analysis simultaneously without subgroup analysis for different types of high-grade irAEs, the adverse effects of hepatopulmonary irAEs on survival outcomes are likely to be masked by cutaneous, gastrointestinal, and endocrine irAEs with

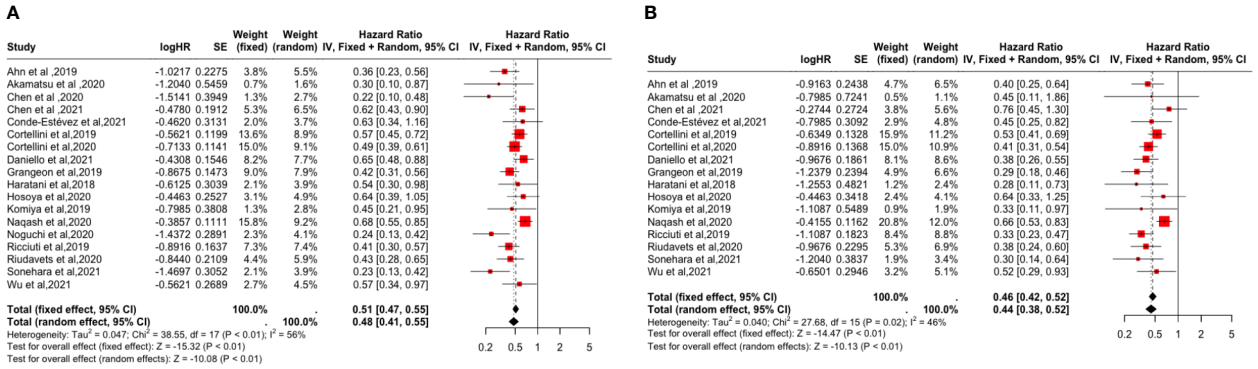

FIGURE 2
Forest plot of the association between the occurrence of irAEs and PFS and OS. **(A)** Progression-Free Survival (PFS); **(B)** Overall Survival (OS).

better prognosis, which may lead to bias in the final results. It even produces a better prognosis. Therefore, more studies are needed in the future to conduct subgroup analyses of high-level irAEs to confirm this problem. Second, according to the guidelines, the occurrence of grade 3 irAEs requires the suspension or permanent discontinuation of ICIs therapy, which will eventually lead to disease progression and affect survival outcomes. However, there are still differences between the organs of grade 3 or higher irAEs and the prognosis; for example, except for grade ≥3 bullous dermatitis, Stevens-Johnson syndrome, and toxic epidermal necrolysis requiring permanent disuse of ICIs, most of the other types of irAEs, such as rashes and pruritus, can be relieved or cured after local or systemic steroid treatment. Endocrine-related irAEs can also continue ICIs therapy after receiving alternative therapy or symptomatic therapy. The main manifestations of gastrointestinal irAEs are diarrhea or colitis, both of which can be well controlled by hormone therapy. However, checkpoint inhibitor pneumonitis (CIP), once detected, requires immediate suspension or discontinuation of ICIs and symptomatic treatment. In addition, the occurrence of CIP is closely related to PD-L1 and programmed death-ligand 2 (PD-L2), and studies have shown that PD-L1 and PD-L2 have important but opposite roles in regulating airway hyper reactivity (AHR) and invariant natural killer T (iNKT) cell-mediated activation and maintaining internal environment stability. Under normal circumstances, the interaction of the two can inhibit the inflammatory response of T helper 2 (Th2) cells, and when ICIs disrupt this balance, it can lead to CIP (33, 34). Direct inhibition of PD-1 also increases the likelihood of increased toxicity (35). The reason for the poor prognosis of CIP may be due to the fact that CIP can appear in grades ≥3 irAEs in the early stages of the

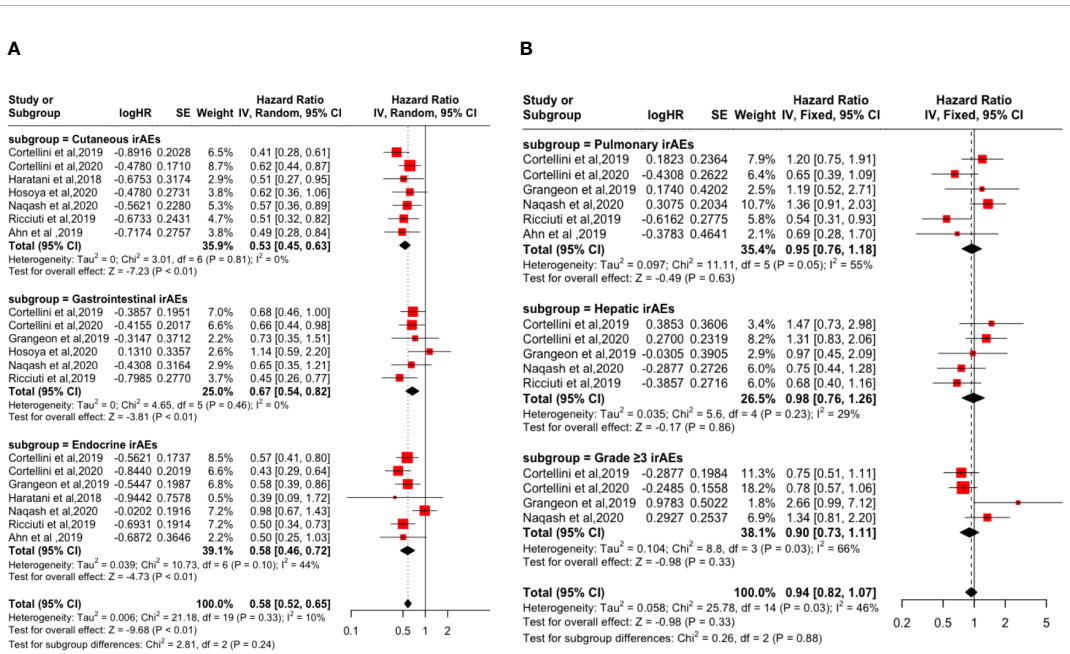

FIGURE 3
Forest plot of the association between the occurrences of different irAEs types and grades and PFS. **(A)** Cutaneous irAEs; Gastrointestinal irAEs; Endocrine irAEs; **(B)** Pulmonary irAEs; Hepatic irAEs; Grade ≥3 irAEs.

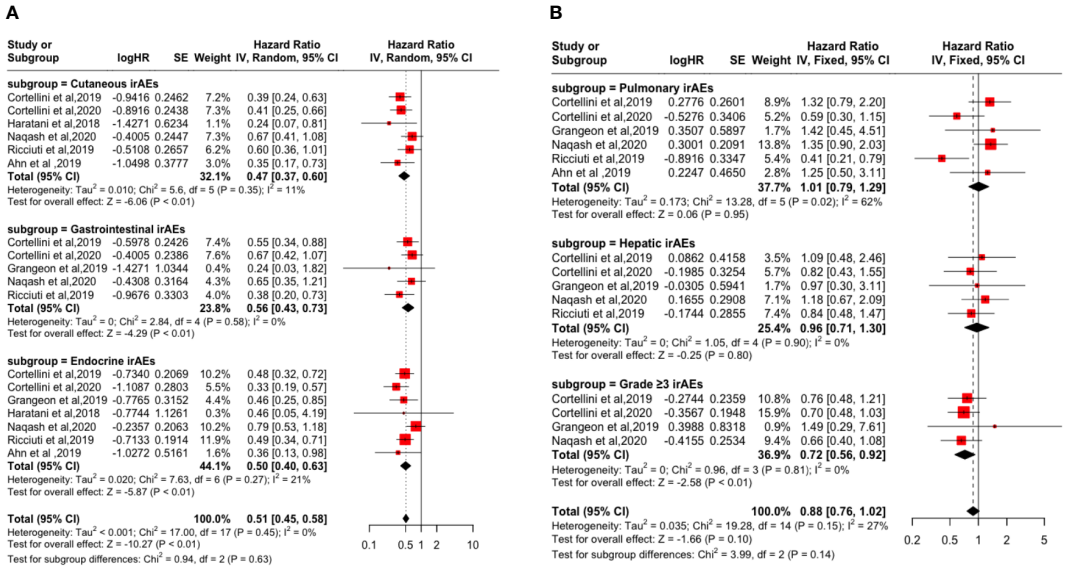

FIGURE 4
Forest plot of the association between the occurrences of different irAEs types and grades and OS. **(A)** Cutaneous irAEs; Gastrointestinal irAEs; Endocrine irAEs; **(B)** Pulmonary irAEs; Hepatic irAEs; Grade ≥3 irAEs.

disease, and the disease progresses more rapidly (36). Therefore, this relationship leads us to realize that the absence of adverse events after ICIs treatment may indicate a lack of efficacy. On the contrary, because different types of adverse events have different pathophysiological mechanisms, the response to ICIs and the degree of damage to the body are also different. Patients with pulmonary, hepatogenic, and high-grade adverse events often have a poor prognosis, possibly due to the need to discontinue ICIs after irAEs, combined with organ system damage that prevents further antitumor therapy in the short term and ultimately leads to disease progression. Therefore, not all irAEs can improve the prognosis of patients. Close attention should be paid to the occurrence of pulmonary, hepatogenic, and high-grade adverse events, and identification and active treatment should be carried out as early as possible to effectively control the progression of the disease.

This study was subject to several limitations inherent in the study design and the included studies. First, we included HR reported in the study rather than individual patient data. In addition, synthetic analyses of OS and PFS showed significant heterogeneity, which may be due to different types and grades of irAEs. Although subgroup analysis of irAEs was performed in this study to reduce the influence of heterogeneity, cutaneous, gastrointestinal, and endocrine-related adverse events were more common in irAEs, and the prognosis was good, while liver and lung irAEs showed poor prognosis. In the analysis of irAEs grade, if the type of irAEs includes liver and lung irAEs, the study results may be overshadowed by irAEs such as cutaneous with a better prognosis. Therefore, future research needs to further investigate this issue. However, despite these limitations, we provide a meta-analysis of irAEs versus survival outcomes in patients with NSCLC, and irAEs can serve as a promising prognostic biomarker in patients with NSCLC.

Conclusion

In conclusion, the available evidence suggests that irAEs may be a prognostic biomarker for patients with NSCLC. However, further research is needed to explore the prospect of irAEs as a prognostic biomarker for patients on immunocombination therapy.

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

SM: Conceptualization, Funding acquisition, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. HN: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. CW: Formal analysis, Investigation, Methodology, Writing – original draft. CJ: Investigation, Methodology, Writing – original draft. LW: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1402017/full#supplementary-material.

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 16 May 2024
DOI 10.3389/fonc.2024.1385761

OPEN ACCESS

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RECEIVED 13 February 2024
ACCEPTED 22 April 2024
PUBLISHED 16 May 2024

CITATION
Shen H and Li C (2024) Global research trends in immunotherapy for non-small cell lung cancer patients with KRAS mutations: a bibliometric analysis. *Front. Oncol.* 14:1385761. doi: 10.3389/fonc.2024.1385761

Global research trends in immunotherapy for non-small cell lung cancer patients with KRAS mutations: a bibliometric analysis

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Background: Immunotherapy, frequently combined with conventional chemotherapy, is crucial for treating NSCLC. Kirsten rat sarcoma virus (KRAS) is a poor prognostic factor in patients with NSCLC, particularly lung adenocarcinoma, where binding of conventional inhibitors to mutated KRAS proteins is challenging. Field profiles, research hotspots, and prospects for immunotherapy for patients with NSCLC-carrying KRAS mutations were uncovered in this study.

Methods: Microsoft Excel 2019, Bibliometrix, VOSviewer software, and Citespace were utilized to conduct a comprehensive scientometric analysis and understand a specific research field's knowledge base and frontiers aided by bibliometrics.

Results: Between 2014 and 2023, 398 eligible documents in the English language were acquired using the WoSCC database, of which 113 and 285 were reviews and articles, respectively. The growth rate per year was 34.25 %. The most cited articles were from the United States, and China published the highest number of articles. Cancers was the journal, with increased publications in recent years. The keywords with the strongest citation bursts were analyzed using Citespace. "Immune checkpoint inhibitors," "co-occurring genomic alterations," and "KRAS" are among the research hotspots in this field.

Conclusion: Using bibliometric and visual analyses, we examined immunotherapy for patients with KRAS-mutant NSCLC over the previous decade. The whole analysis showed a steady, quick increase in yearly publications in this area. Our findings will provide a roadmap for future research on the mechanisms of immunotherapy and immune checkpoint inhibitor action in treating KRAS-mutant NSCLC.

KEYWORDS

immunotherapy, immune checkpoint inhibitors, KRAS mutations, NSCLC, bibliometric

Introduction

Lung cancer ranked first in mortality since 2020 and second in incidence, according to the most recent cancer statistics (1). Its five-year survival rate remains among the lowest despite recent advancements in early detection, molecular characterization, and development of innovative therapeutic approaches. Approximately 85% of lung cancer cases are of non-small cell lung cancer (NSCLC) (2–4). KRAS mutation is typically linked to a poor prognosis in NSCLC, with an incidence rate of 20-40% (5, 6). Treatment and medication for patients with NSCLC carrying KRAS mutation remain challenging. KRAS has four main mutational subtypes: G12C, G12V, G12D, and G12A. Among all patients with NSCLC carrying KRAS mutations, the incidences of G12C, G12V, G12D, and G12A subtypes are approximately 40%, 21%, 17%, and 8% (7–9).

Some clinical trials have reported promising results for new small-molecule inhibitors of KRAS-G12C subtype (10, 11), sotorasib (AMG510) (12), and adagrasib (MRTX849) (13), indicating their potential for use in these patients. A retrospective study showed that MRTX1133, as a non-covalent and selective KRAS-G12D inhibitor, has shown potential for tumor regression in preclinical data across multiple solid tumor models (7). Recently, the pan KRAS inhibitor BI-2865, reported by the Memorial Sloan Kettering Cancer Center and Boehringer Ingelheim (BI), has been shown to effectively inhibit the growth of various tumor cells (14). However, except for the KRAS-G12C subtype, targeted therapy for other subtypes of KRAS-mutant NSCLC is lacking. Considered undruggable, KRAS modulates the immune response in pancreatic and colorectal cancers (15). For metastatic NSCLC, immune checkpoint inhibitors (ICIs) are used as a monotherapy or combination therapy in the frontline and subsequent lines of treatment. The higher the threshold for tumor positivity for programmed death ligand 1 (PD-L1) expression, the greater the benefit. In most clinical trials examining PD-L1's role in NSCLC, the response to ICIs has been predicted (16). Upon treatment with checkpoint therapy, clinically significant KRAS-mutated NSCLC shows a better overall survival rate than the KRAS wild-type NSCLC (17). In KRAS-mutant NSCLC, PD-L1 expression is more significant for predicting the effectiveness of ICIs compared to the other mutant types of NSCLC (18).

In 1969, Alan Pritchard introduced the concept of bibliometrics. It quantitatively examines indicators such as the volume, frequency of citations, and importance of scholarly literature. Bibliometrics gathers and processes data to thoroughly and accurately observe and characterize various patterns and phenomena. Bibliometrics aids the understanding of a specific research field's knowledge base and frontiers (19, 20). This multi-perspective, time-phased, and dynamic technique of visual analysis of literature can automatically identify the research frontiers of the discipline and present the evolution of knowledge disciplines by displaying author networks, scholarly communication, connections between scholars, and advancements in knowledge through citation nodes and co-citation clustering. It offers a significant and workable systematic method for determining the importance of published literature. Three publications on bibliometric analyses of immunotherapy for lung cancer exist (21–23); however, to date, no bibliometric analysis on immunotherapy for NSCLC linked to KRAS mutations has been published.

In addition to examining research trends, hotspots, and boundaries from 2014 to 2023, this study aimed to conduct a bibliometric analysis in the field of immunotherapy for patients with NSCLC carrying KRAS mutations. Collaborative relationships between countries, institutions/organizations, authors, journals, references, and keywords were analyzed using Bibliometrix, VOSviewer software, Citespace, and Microsoft Excel 2019 to identify research priorities and boundaries in this area.

Methods

Data collection and retrieval strategy

We searched the Web of Science Core Collection (WoSCC) database from January 1, 2014, to December 31, 2023, to obtain all publications on immunotherapy for NSCLC linked to KRAS mutations. The search strategy was as follows: TS= (non-small cell lung cancer OR non-small cell lung carcinoma) AND TS= (immunotherapy OR immunotherapeutic OR immune checkpoint inhibitor OR immune checkpoint blockade) AND TS= (KRAS OR Kirsten rat sarcoma virus). Only articles and reviews were accepted as document types, and only English was used as the language of publication. Figure 1 illustrates the comprehensive processes of data retrieval and inclusion.

Data analysis and visualization

Microsoft Excel 2019, Bibliometrix, VOSviewer software, and Citespace (6.2.7) were utilized to conduct a comprehensive scientometric analysis. The yearly number of publications on KRAS-mutant NSCLC immunotherapy was analyzed and plotted using Microsoft Excel 2019. We employed Bibliometrix, an R-tool available on R software (4.0.3), that generates visual representations of the results to facilitate the comprehensive scientometric analysis and statistical data. The VOSviewer 1.6.19 software was used to perform a thorough literature visualization and bibliometric analysis (24), focusing on quantifying the extent of research related to NSCLC immunotherapy across biological fields. VOSviewer was used to analyze countries, institutions, references, and keywords intuitively. CiteSpace was utilized to provide an intuitive understanding of the research bursts and evolutionary process (25, 26).

Results

Publication outputs

A total of 398 publications related to immunotherapy and KRAS mutation in NSCLC between 2014 and 2023 were obtained from the WOS core collection database. As shown in Figure 2A, with an average of 40 published papers annually, the lowest number of

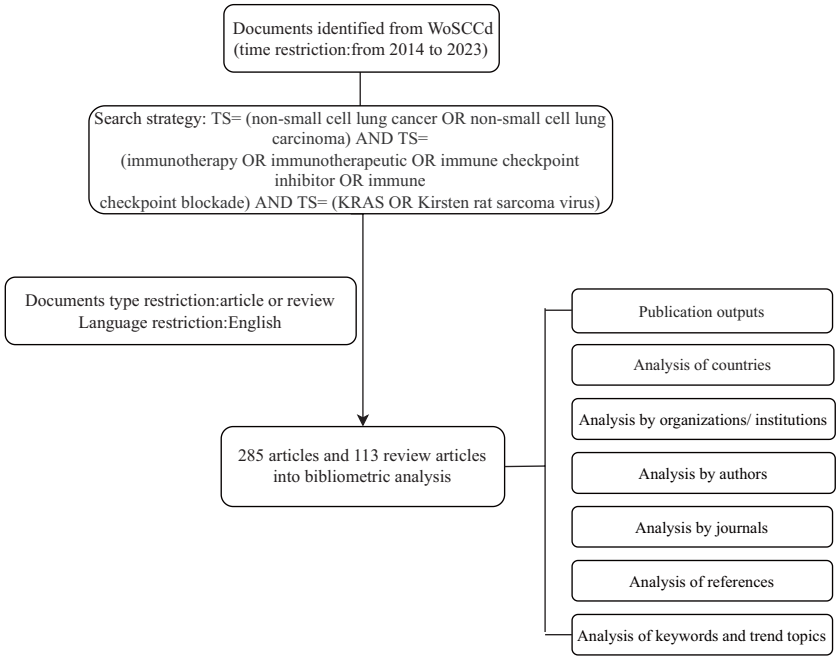


FIGURE 1
Retrieval workflow for publications related to immunotherapy for patients with KRAS mutations in NSCLC.

published papers in any given year was 2 in 2015, and the highest was 94 in 2022. A statistically significant relationship (R^2 = 0.9844) between the number of publications and the year was obtained by fitting a mathematical function to the annual number of publications curve. The fitting curve indicated an upward trend in published articles since 2013. The number of publications on this topic has rapidly increased over the past decade, and more research opportunities exist at present. The annual mean total citations in immunotherapy for patients with NSCLC carrying KRAS mutation is shown in Figure 2B.

Analysis by countries

Figure 3A shows the geographic distribution of research on immunotherapy for patients with NSCLC carrying KRAS mutations. The countries of the top five corresponding authors were the People's Republic of China (111, 27.9%), the United States (106, 26.6%), Italy (33, 8.3%), France (30,7.5%), and Germany (20, 5.0%). The United States has the highest number of multiple-country publications (MCP), while China has the most single-country publications (SCP). A collaborative network world map shows the collaboration between countries (Figure 3B). The most closely connected countries were China and the United States. A map overlay visualization of nations/regions working together on immunotherapy for patients with NSCLC carrying KRAS mutations (Figure 3C). Figure 3D displays the time trend visualization for the nation-wise co-authorship networks. The node's color, ranging from blue to red, indicates the country's academic activity time, while the node's size indicates the nation's output.

Analysis by organizations/institutions

In total, 1045 organizations and institutions released 398 documents. Visualization analysis included 57 organizations that met the inclusion criteria (publications > 4). The top three most productive organizations were Shanghai Jiao Tong University (14 documents), Dana-Farber Cancer Institute (17 documents), and Memorial Sloan-Kettering Cancer Center (21 documents). Six of the top ten publishing organizations were based in the United States, three in China, and one in the United Kingdom. Table 1 shows the total yearly publications of the top ten institutions. We analyzed the co-authorship between the organizations (Figure 4A). A co-authorship network map of all the countries was created using VOSviewer to examine the collaboration between organizations. Excluding three institutions without connection with other organizations, all 54 top publishing institutions could be divided into eight clusters. Research from organizations in China, such as Nanjing University, Zhejiang University of Traditional Chinese Medicine, and Capital Medical University, is relatively new according to the overlay visualization maps of organizations (Figure 4B).

Analysis by authors

In total, 3524 authors contributed to this field over the past decade. Among these, the top three authors are shown in Figure 5A. The top author with the most publications was Kwok-Kin Wong. Studies focus on STK11/LKB1 mutations with the immune microenvironment of NSCLC harboring KRAS mutations, and the impact of TSC1/TSC2 deficiency on immune checkpoint blockade in

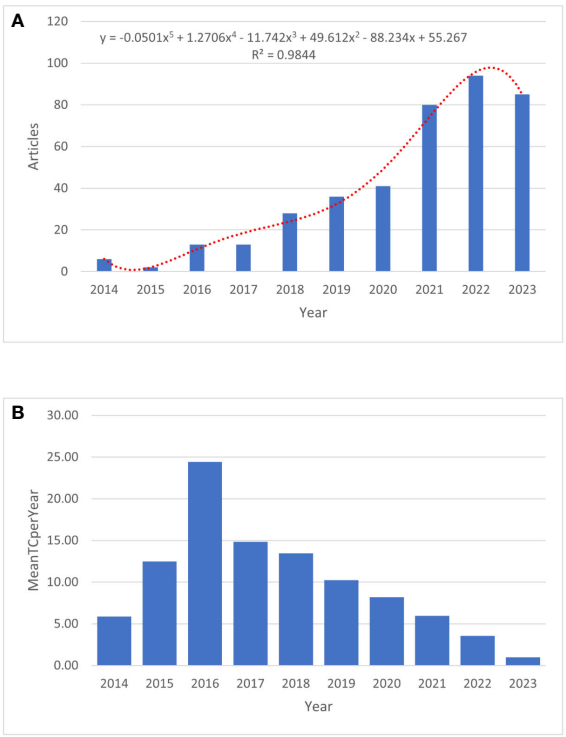


FIGURE 2
The publication trend of immunotherapy in NSCLC patients with KRAS mutation. **(A)** The bar graph displays the annual publication volume. **(B)** The annual mean total citations.

NSCLC (27–29). Figure 5B shows the authors' production over the past decade. The authors who have published more articles in the past two years are Mark M Awad (30) and Jing Wang (31).

Analysis by journals

Papers on immunotherapy for patients with NSCLC carrying KRAS mutations were published in 143 journals. As shown in Figure 6A, the five leading journals in publications were *Cancers* (N = 38), *Lung Cancer* (N = 28), *Frontiers in Oncology* (N = 25), *Translational Lung Cancer Research* (N = 16), *Clinical Cancer Research* (N = 11), *Journal of Thoracic Oncology* (N = 11), *International Journal of Molecular Sciences* (N = 10), *Cancer Medicine* (N = 8), *Clinical Lung Cancer* (N = 7), and Frontiers in Immunology (N = 7). More detailed journal information is listed in Table 2, according to the "2022 Incites Journal Citation Report," journal citation report (JCR) quartile and impact factor (IF) were defined. Figure 6B shows the sources' production over time. The publication volume of *Cancers* has increased significantly in recent years. The primary citation lines are indicated in orange in Figure 6C, which presents an overlay map of journals showing the citation trajectory of interdisciplinary collaboration. The studies published in molecular biology and immunology journals mainly cited reports published in journals on molecular biology and genetics. The journal's discipline in the figure is indicated by the label on the right, where the cited paper was published. As a journal publishes more papers, the vertical axis of the ellipse in the figure on the left extends, while the horizontal axis increases as the number of authors increases.

Analysis of references

Co-cited references and co-cited sources analyzed using VOSviewer are shown in Figures 7A, B. It is divided into two clusters, and most references cited in the documents were published in *Journal of Thoracic Oncology*, *The New England Journal of Medicine*, *Clinical Cancer Research*, *Journal of Clinical Oncology*, and *Annals of Oncology*. Citation bursts are a useful indicator for tracking the interest of academics in a field over time. Figure 7C shows the top 15 references with the strongest citation bursts from our study, as determined using CiteSpace. The article titled "Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer" published in 2015" (32), ranked first in terms of strength, with a value of 19.38. The citation bursts for articles authored by Hong DS, Liu CM, Hallin J, and Skoulidis F

TABLE 1 The top 10 most publishing institutions according to publications.

Rank	Organization	Documents	Citations	Country
1	Memorial Sloan-Kettering Cancer Center	21	2523	USA
2	Dana-Farber Cancer Institute	17	2128	USA
3	Shanghai Jiao Tong University	14	233	China
4	Harvard Medical School	13	464	USA
5	UT MD Anderson Cancer Center	12	1596	USA
6	Chinese Academy Medical Science & Peking Union Medical College	10	255	China
7	AstraZeneca	9	446	UK
8	Southern Medical University	9	683	China
9	Brigham and Women's Hospital	8	1667	USA
10	Weill Cornell Medical College	8	708	USA

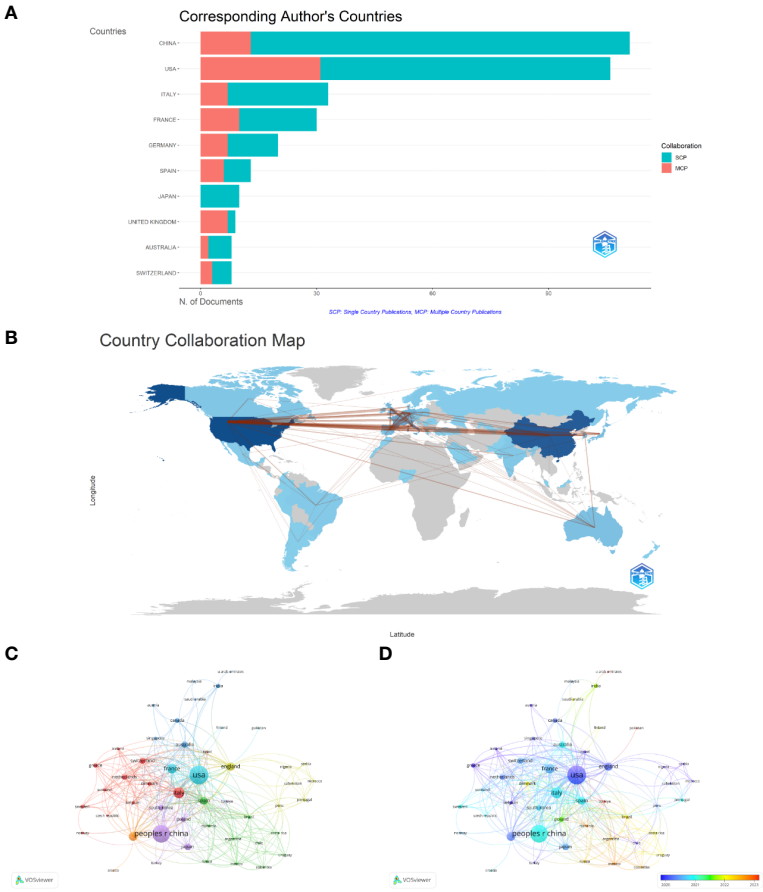


FIGURE 3
Contributions of countries. **(A)** Geographic distribution of research. **(B)** Country collaboration map. **(C)** Country/territory collaboration analysis by VOSviewer. **(D)** The time trend visualization for country co-authorship networks.

have been continuously cited from 2021 to 2023 (33–36), demonstrating ongoing consideration for the authors' research direction.

Analysis of keywords and trend topics

After merging synonyms and removing superfluous terms, a visualization map of keywords was generated using the VOSviewer program. Consequently, 812 keywords were identified, including 47 terms with five or more occurrences. Eight clusters were formed (Figure 8A). Overlay visualization maps showed around 2020, researchers focused more on immunotherapy for NSCLC patients with KRAS mutations, and in the past two years, they have focused on targeted drugs such as KRAS-G12C-related targeted therapy research. (Figure 8B). The timeline view of keywords intuitively showed the changing trend of research topics over time (Figure 8C). ICIs, tumor microenvironment, and PD-1 were early research subjects in this field. KRAS-G12C, NSCLC, and target therapy, located at the far right of this line, are new research trends in this field. Figure 8D shows the evolution of research hotspots in the past decade. As shown in the figure, "immune checkpoint inhibitors," "co-occurring genomic alterations," and "KRAS" were the keywords with the strongest citation bursts from 2021 to 2023.

Discussion

Overview of the results

This study identified documents on immunotherapy for patients with NSCLC carrying KRAS mutations from 2014 to 2023 by conducting a thorough literature review based on the WoSCC database. The present scientometric study included 398 English-language publications from 143 journals. The annual growth rate is 34.25%. The results indicate an increasing trend in the number of publications, suggesting growing interest in this topic, particularly due to recent advancements in the field of immunotherapy for patients carrying KRAS mutations. The annual global publication is an intuitive measure of the progress made in a particular field of study. Over the last decade, research on immunotherapy for patients with NSCLC who carry KRAS

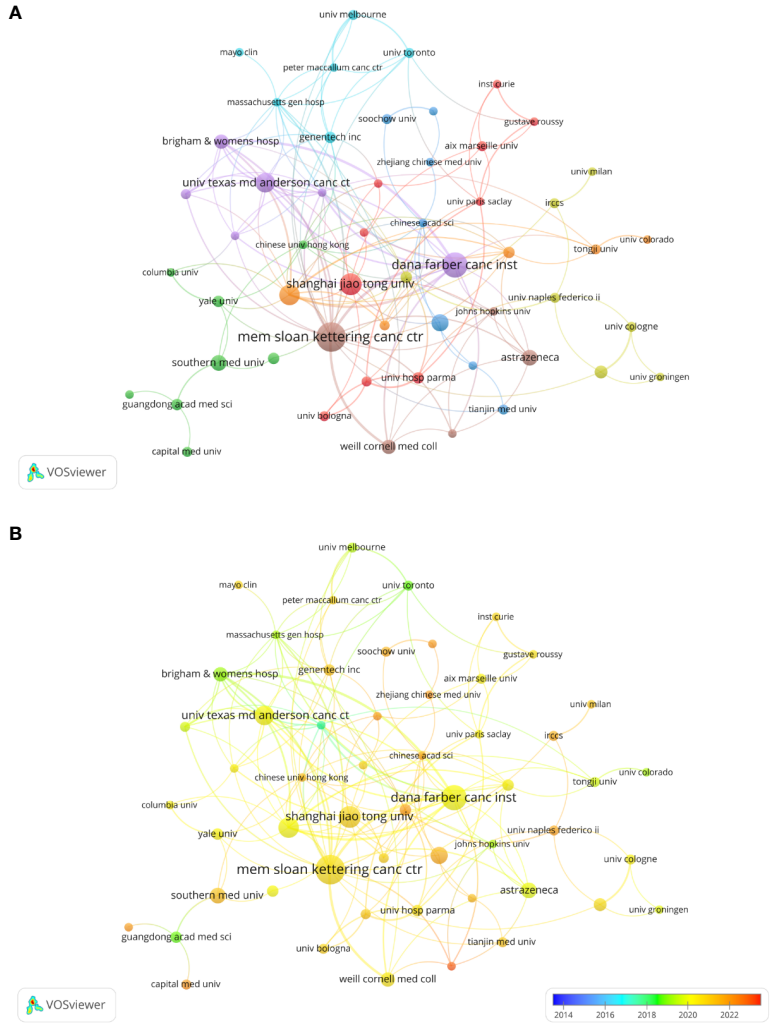


FIGURE 4
Analysis of organization/institutions. **(A)** Network visualization for institutional co-authorship analysis by VOSviewer. **(B)** Overlay map of institutional publications and average years.

mutations has increased. Between 2020 and 2021, there was a 48.75% growth rate in publications related to immunotherapy for patients with NSCLC who carry KRAS mutations. This implies that new clinical applications have emerged along with the uncovering of the underlying mechanisms of immunotherapies for these patients. More researchers are investing time and attention in this field, as evidenced by the sharp increase in publications and citations. In the upcoming years, the attention rate of immunotherapy for KRAS-mutant NSCLC will increase, particularly for treating patients with advanced NSCLC.

Although China has the highest number of publications, its citation count is not as high as that of the USA. More than half of the reports were from the United States, indicating its outstanding position in this field. The United States is home to some of the best cancer research facilities and medical facilities worldwide, such as the Memorial Sloan-Kettering Cancer Center, Dana-Farber Cancer Institute, and UT MD Anderson Cancer Center. These institutions conduct basic research to explore the root causes of cancer and strive to develop new diagnostic tools, treatment methods, and prevention strategies. Through keyword analysis, it was found that researchers have been paying more attention to KRAS-G12C, NSCLC, and target therapy in the past two years. This might be related to the higher prevalence of KRAS-G12C mutation in NSCLC patients among KRAS mutant cancer patients. A recent analysis of a series of patients with metastatic KRAS-mutant NSCLC showed that G12C has a higher tumor mutation burden (TMB) and PD-L1 expression, which may be sensitive to immunotherapy.

Status of research

Targeted therapy for patients with advanced KRAS mutations remains challenging. According to existing guidelines,

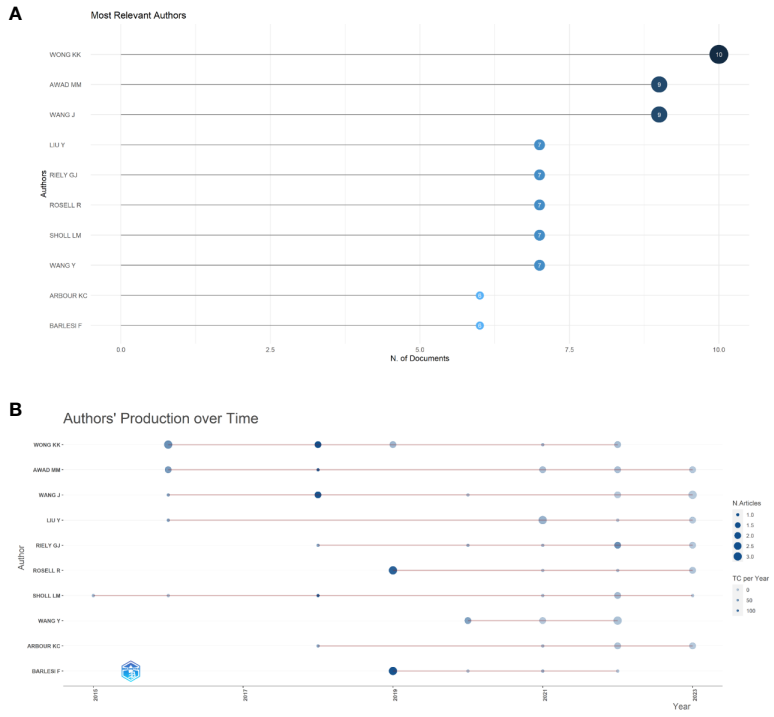


FIGURE 5
Analysis of authors' output. **(A)** Top 10 productive authors in immunotherapy for NSCLC patients with KRAS mutations. **(B)** Authors' production over past decade.

immunotherapy targeting PD1 and PD-L1 is the best treatment method (37, 38). KRAS mutation is associated with the efficacy of ICIs (39). A real-world retrospective study revealed that the effectiveness of first-line ICIs, either alone or in combination with chemotherapy, did not differ among patients with different isoforms of KRAS mutations. Patients with KRAS-G12D and KRAS-G12A mutations showed a shorter median progression-free survival (mPFS), which did not reach statistical significance (40). The intermediate mechanism of immunotherapy for various KRAS mutation subtypes merits investigation. Recent clinical studies have shown that patients carrying KRAS mutations respond well to ICI treatment after chemoradiotherapy (CRT) treatment (41). In late-stage NSCLC, monotherapy with $KRAS^{wt}$ resulted in poorer OS compared to KRAS-mutant patients (42). An increasing threshold for tumor positivity for PD-L1 expression was associated with a greater benefit. A trend was obtained toward a correlation between PD-L1 expression in tumor cells and the objective response rate (ORR) and progression-free survival (PFS). A recent study conducted by Wang found higher expression of immunotherapy indicators (43) (PD1, PD-L1, PD-L2, CYT, and GEP) in the KRAS-G12V and KRAS-G12D subtypes.

AMG510 and MRTX849 have been approved for treating patients with advanced NSCLC who carry G12C mutation (44). Patients with KRAS-G12C mutation in NSCLC received first-line treatment with the $KRAS^{G12Ci}$ MRTX849 in combination with pembrolizumab, resulting in a disease control rate (DCR) of 100% (45). $KRAS^{G12Ci}$ can reverse the immunosuppressive environment and make cancer cells sensitive to immunotherapies such as ICIs (46). However, $KRAS^{G12Ci}$ rapidly develops resistance, as evidenced in clinical trials (47), and less than 50% of patients benefit from $KRAS^{G12Ci}$. The combination of $KRAS^{G12Di}$ MRTX1133 and immune checkpoint inhibitors can activate the FAS pathway, continuously inhibit tumor growth, enhance the ability to clear cancer cells, and improve survival outcomes (48). This suggests that immunotherapy's effectiveness can be enhanced. Significant differences in TMB levels were observed among the four KRAS subtypes, with the KRAS-G12D subtype having the lowest TMB (49). In patients carrying KRAS mutations, the abundance of different immune cells varies across different subtypes. Th cells can spontaneously bind to PD-L1, blocking the anti-tumor immune response mediated by T cells. Outcomes of immunotherapy for patients with KRAS mutations are affected by several internal and external factors.

The co-mutation status is strongly suggested to affect the effectiveness of immunotherapy (50). KRAS mutations often co-exist with other mutations. A study by the National Network Genomic Medicine (NNGM) Lung Cancer Collaborator Group found that patients (PD-L1 ≥50%) carrying KRAS mutations, especially G12C and TP53 co-mutations, have better survival after receiving treatment with pembrolizumab (51–53). The co-existence of STK11 mutation and KRAS-G12C mutation can lead to poorer immune checkpoint inhibitor treatment efficacy in patients with LUAD (29). A recent study by UT MD Anderson Cancer Center found that when KEAP1, SMARCA4, and

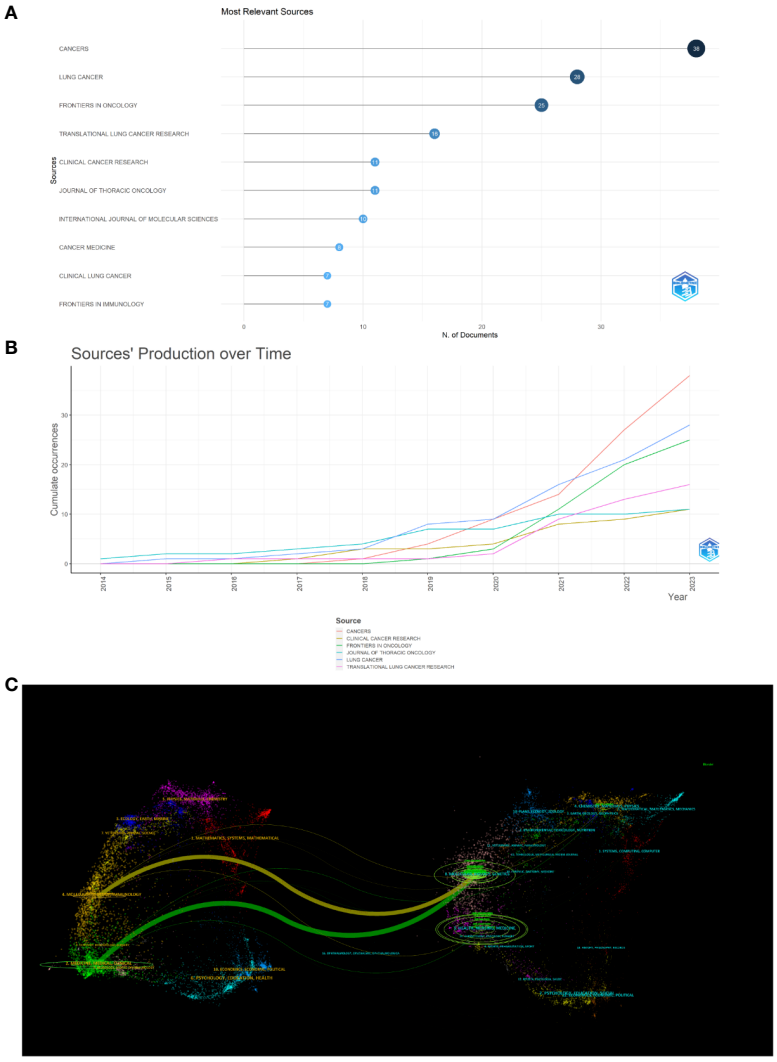
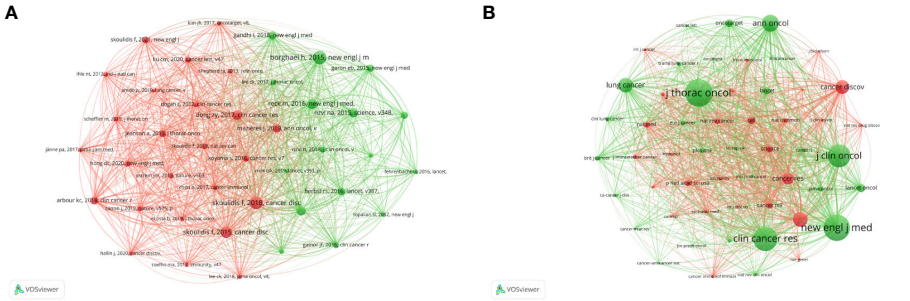


FIGURE 6
Analysis of journals. **(A)** Most relevant sources. **(B)** Sources' production from 2014 to 2023. **(C)** Overlay map of journals.

TABLE 2 The top 10 most productive journals according to publications.

Rank	Sources	Articles	IF (2022)	JCR
1	*Cancers*	38	5.2	Q1
2	*Lung Cancer*	28	5.3	Q2
3	*Frontiers in Oncology*	25	4.7	Q2
4	*Translational Lung Cancer Research*	16	4.0	Q2
5	*Clinical Cancer Research*	11	11.5	Q1
6	*Journal of Thoracic Oncology*	11	20.4	Q1
7	*International Journal of Molecular Sciences*	10	5.6	Q2
8	*Cancer Medicine*	8	4.0	Q2
9	*Clinical Lung Cancer*	7	3.6	Q2
10	*Frontiers in Immunology*	7	7.3	Q1



C

Top 15 References with the Strongest Citation Bursts

References	Year	Strength	Begin	End	2014 - 2023
Topalian SL, 2012, NEW ENGL J MED, V366, P2443, DOI 10.1056/NEJMoa1200690, DOI	2012	7.48	2014	2017	
Herbst RS, 2014, NATURE, V515, P563, DOI 10.1038/nature14011, DOI	2014	7.89	2015	2018	
Borghaei H, 2015, NEW ENGL J MED, V373, P1627, DOI 10.1056/NEJMoa1507643, DOI	2015	19.38	2016	2020	
Rizvi NA, 2015, SCIENCE, V348, P124, DOI 10.1126/science.aaa1348, DOI	2015	15.06	2016	2020	
Brahmer J, 2015, NEW ENGL J MED, V373, P123, DOI 10.1056/NEJMoa1504627, DOI	2015	11.91	2016	2020	
Garon EB, 2015, NEW ENGL J MED, V372, P2018, DOI 10.1056/NEJMoa1501824, DOI	2015	11.27	2016	2020	
Skoulidis F, 2015, CANCER DISCOV, V5, P860, DOI 10.1158/2159-8290.CD-14-1236, DOI	2015	10.24	2016	2020	
Herbst RS, 2016, LANCET, V387, P1540, DOI 10.1016/S0140-6736(15)01281-7, DOI	2016	8.97	2017	2021	
Fehrenbacher L, 2016, LANCET, V387, P1837, DOI 10.1016/S0140-6736(16)00587-0, DOI	2016	7.81	2017	2020	
Reck M, 2016, NEW ENGL J MED, V375, P1823, DOI 10.1056/NEJMoa1606774, DOI	2016	13.07	2018	2021	
Koyama S, 2016, CANCER RES, V76, P999, DOI 10.1158/0008-5472.CAN-15-1439, DOI	2016	7.94	2018	2021	
Hong DS, 2020, NEW ENGL J MED, V383, P1207, DOI 10.1056/NEJMoa1917239, DOI	2020	9.63	2021	2023	
Liu CM, 2020, CANCER LETT, V470, P95, DOI 10.1016/j.canlet.2019.10.027, DOI	2020	9.44	2021	2023	
Hallin J, 2020, CANCER DISCOV, V10, P54, DOI 10.1158/2159-8290.CD-19-1167, DOI	2020	8.63	2021	2023	
Skoulidis F, 2019, NAT REV CANCER, V19, P495, DOI 10.1038/s41568-019-0179-8, DOI	2019	7.55	2021	2023	

FIGURE 7
Analysis of references. **(A)** co-cited references. **(B)** co-cited sources. **(C)** Top 15 references with the strongest citation bursts.

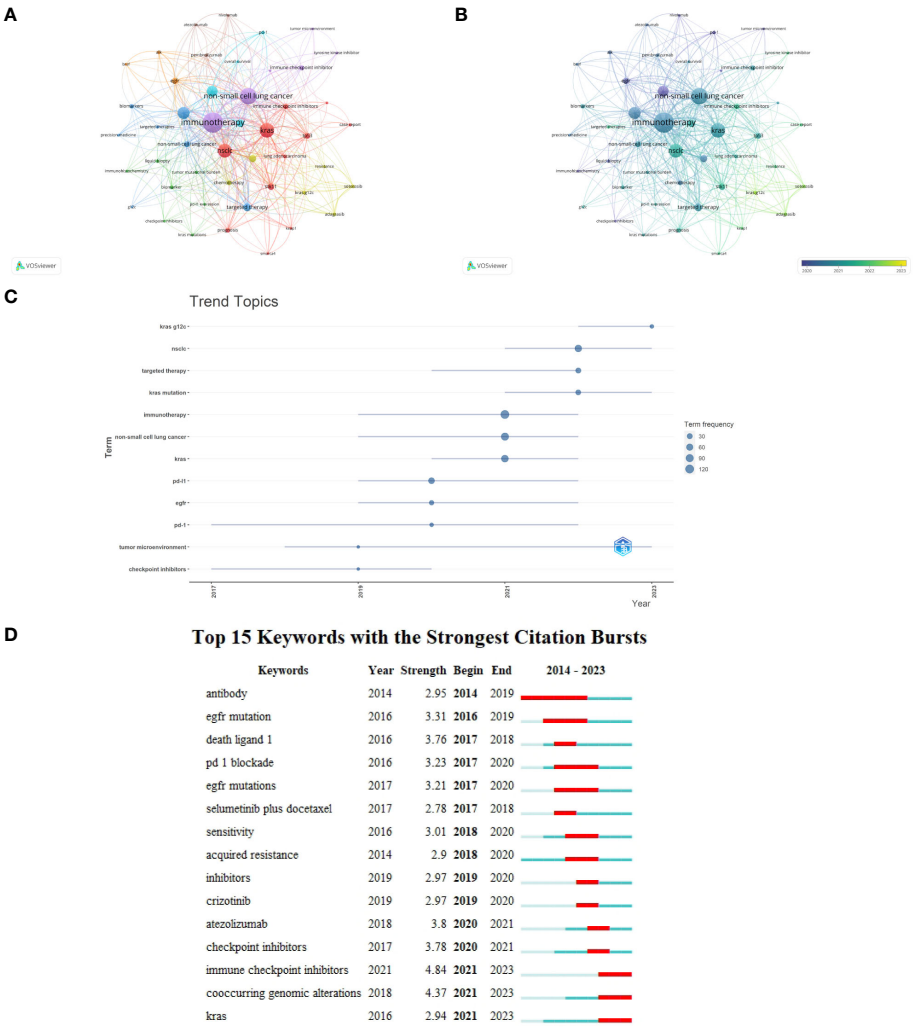
CDKN2A co-mutate with KRAS-G12C, KRAS^{G12Ci} monotherapy is ineffective in treating patients with advanced lung cancer (54). TP53 and STK11 are two common co-mutations of KRAS-G12D, and KRAS-G12D/STK11 co-mutations may be negatively correlated biomarkers for immunotherapy (55). The loss of function mutation of NKX2-1/CDKN2A can induce tumor development in patients with KRAS-G12D mutation in lung mucinous adenocarcinoma (7). For patient stratification and treatment option selection, other biological factors, such as TMB, co-mutation status, and KRAS mutation subtypes, need to be considered in addition to PD-L1 status.

Limitations

First, this research was restricted to relying on data from the WoSCC database and did not consider literature from other databases. The analysis was limited to documents in the English language, publication type of article, and reviews. While bibliometric analyses are valuable for identifying trends and hotspots in a field, they inherently focus on quantitative metrics, such as publication volume and citation counts. Software limitations may have prevented the modification of case formats and abbreviations; inevitably, this led to the partial inclusion of articles. Thus, this study may not have fully captured the quality of research, the clinical applicability of findings, or the nuances of scientific debate within the field. Second, due to the regular updation of the database, there was a certain lag in the data obtained, such as the number of articles and citations. Finally, the dearth of keywords or abstracts may increase the chances of being excluded due to poor discoverability.

Conclusion

Using bibliometric and visual analyses, we examined immunotherapy for patients with KRAS-mutant NSCLC over the previous decade. The whole analysis showed a steady, quick increase in yearly publications in this area. In terms of research, the United States is currently in the lead. Bibliometric analysis of keywords revealed researchers focus on the survival of certain patients carrying KRAS mutations, targeted therapy combined with immunotherapy is a highly effective therapy for patient survival, but it is also necessary to monitor whether patients have target co-mutations. However, the nature of this intermediate mechanism remains unclear. Future international cooperation



Keywords	Year	Strength	Begin	End	2014 - 2023
antibody	2014	2.95	2014	2019	
egfr mutation	2016	3.31	2016	2019	
death ligand 1	2016	3.76	2017	2018	
pd 1 blockade	2016	3.23	2017	2020	
egfr mutations	2017	3.21	2017	2020	
selumetinib plus docetaxel	2017	2.78	2017	2018	
sensitivity	2016	3.01	2018	2020	
acquired resistance	2014	2.9	2018	2020	
inhibitors	2019	2.97	2019	2020	
crizotinib	2019	2.97	2019	2020	
atezolizumab	2018	3.8	2020	2021	
checkpoint inhibitors	2017	3.78	2020	2021	
immune checkpoint inhibitors	2021	4.84	2021	2023	
cooccurring genomic alterations	2018	4.37	2021	2023	
kras	2016	2.94	2021	2023	

FIGURE 8
Analysis of keywords. **(A)** Clusters for keywords co-occurrence by VOSviewer. **(B)** Overlay visualization maps of keywords. **(C)** Trend Topics through Bibliometrix. **(D)** Top 15 keywords with the strongest citation bursts.

between nations, organizations, and writers is expected to hasten the development of immunotherapy targeting KRAS mutations in NSCLC in conjunction with additional treatment trials. This can aid early disease diagnosis and offer useful approaches to both treatment and prevention.

Data availability statement

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

Author contributions

HS: Conceptualization, Funding acquisition, Investigation, Methodology, Software, Validation, Writing – original draft. CL: Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Technology Plan of Wuxi Municipal Health Commission Funds (No. Q202355), Medical Education Collaborative Innovation Found of Jiangsu University (No. JDYY2023138).

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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frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 05 June 2024
DOI 10.3389/fimmu.2024.1384121

OPEN ACCESS

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RECEIVED 08 February 2024
ACCEPTED 15 May 2024
PUBLISHED 05 June 2024

CITATION
Berland L, Gabr Z, Chang M, Ilié M, Hofman V, Rignol G, Ghiringhelli F, Mograbi B, Rashidian M and Hofman P (2024) Further knowledge and developments in resistance mechanisms to immune checkpoint inhibitors. *Front. Immunol.* 15:1384121. doi: 10.3389/fimmu.2024.1384121

Further knowledge and developments in resistance mechanisms to immune checkpoint inhibitors

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The past decade has witnessed a revolution in cancer treatment, shifting from conventional drugs (chemotherapies) towards targeted molecular therapies and immune-based therapies, in particular immune-checkpoint inhibitors (ICIs). These immunotherapies release the host's immune system against the tumor and have shown unprecedented durable remission for patients with cancers that were thought incurable, such as metastatic melanoma, metastatic renal cell carcinoma (RCC), microsatellite instability (MSI) high colorectal cancer and late stages of non-small cell lung cancer (NSCLC). However, about 80% of the patients fail to respond to these immunotherapies and are therefore left with other less effective and potentially toxic treatments. Identifying and understanding the mechanisms that enable cancerous cells to adapt to and eventually overcome therapy can help circumvent resistance and improve treatment. In this review, we describe the recent discoveries on the onco-immunological processes which govern the tumor microenvironment and their impact on the resistance to PD-1/PD-L1 checkpoint blockade.

KEYWORDS

cancer, immunotherapy, checkpoint blockade, resistance, biomarkers

Introduction

The onco-immunology field has witnessed a remarkable boom in the past decade after years of controversial dogmas and inconsistent findings. The upgraded comprehension of the cancer-immune system interactions and the tremendous technological progress have revived the hope of curing cancer with immune-based therapies. The target of these treatments has shifted from the tumor to the host's immune system, mobilizing immune cells to recognize and eventually eliminate cancer cells. Hallmarks of immunotherapy are the long-lasting response, through immunological memory, and the specificity of a trained immune system to target cancer cells. However, its effectiveness is currently limited to a subset of patients.

ICIs have proven remarkable clinical effects in a wide range of metastatic tumor types. In particular, the PD-1/PD-L1 blocking antibodies act by reactivating pre-existing tumor-infiltrating lymphocytes (TILs) (1). Yost et al. demonstrated that the majority of tumor-specific TILs after anti-PD-1 treatment have TCR specificity not found in the tumor before the therapy, indicating their recruitment post-treatment (2, 3).

Furthermore, a recent scientific investigation has unveiled that innate T cell responses, triggered by ICIs therapies, effectively eliminate tumors by specifically targeting a restricted set of immunodominant neoantigens. The findings of this study also propose that neoTCRs present in polyclonal T cells play a crucial role in generating robust anti-tumor immunity (4).

Independently of their primary immune-related effects, PD-1 and PD-L1 were recently found to induce intrinsic pro-tumoral effects. The expression of PD-1 in melanoma cells has been found to promote tumor growth in immunocompetent as well as in immunocompromised mice (5). Additionally, PD-L1 expression was reported to promote cancer cell survival by conferring resistance to apoptosis induced by T cell cytolytic effectors, cytotoxic drug like staurosporine and interferons (6, 7).

We still lack a comprehensive understanding of the molecular signaling of PD-1 and PD-L1. However, the perspective of using ICIs to reinvigorate the cytotoxic immune responses and concomitantly induce the metabolic reprogramming of tumor cells has made anti-PD-1/PD-L1 immunotherapies even more attractive. Despite the unprecedented durable responses obtained with the anti-PD-1/PD-L1 agents, a large number of patients do not benefit from the treatment (primary resistance) (Tables 1A, 1B), and some responders relapse after a period of response (acquired resistance) (Table 2). Moreover, some cancer patients may experience an unexpected acceleration of tumor growth after starting immunotherapy and present with poor outcome in retrospective studies (hyper progressive disease) (Figure 1) (Table 3) (95, 96).

Thus, it is crucial to address the primary and acquired resistances to ICIs, which emerge as significant clinical challenges. However, it is important to remember that the immune response is constantly evolving and unique to each patient. By understanding the host's environmental and genomic factors that influence the immune response, we aim to develop more effective treatment interventions, ultimately improving patient outcomes (56).

Primary resistance to immune checkpoint blockade

Multiple studies demonstrated that a combination of both tumor-intrinsic and tumor-extrinsic factors may contribute to immunotherapy resistance (57). Tumor-intrinsic mechanisms include genetic and epigenetic modifications that prevent the processing and presentation of tumor neoantigen, as well as T cell infiltration or action within the tumor microenvironment (TME) (97, 98) (Table 1A). Tumor-extrinsic factors include inadequate T cell function, non-cancerous stromal or immune cells, and other systemic influences that can act with cancer cells to promote resistance to ICIs (57) (Table 1B). These mechanisms can either contribute to the primary resistance when detected at the time of the initial diagnosis or highlight the adaptive resistance when detected later during the evolution of cancer under treatment.

Tumor intrinsic factors

Genetic mutations

With each scientific advancement, our comprehension of the fundamental mechanisms governing cancer resistance advances. Attempts to understand the mechanisms of resistance have uncovered that specific genetic mutations can affect the oncogenic signaling, influencing the extent and type of immune infiltration within the TME. Most notably, alterations of *STK11/LKB1* in the presence of *KRAS* mutations have been linked to primary resistance to PD-1 inhibitors in lung adenocarcinoma patients undergoing chemoimmunotherapy (8). The loss of *STK11/LKB1* promotes the production of IL-6, which recruits neutrophils, inhibits recruitment of T cells, and is associated with high levels of T cell exhaustion markers such as PD-1 and TIM-3, and decreased expression of PD-L1 on tumor cells (9, 10). Notably, *KRAS* mutant adenocarcinoma tumors exhibiting LKB1 loss exhibit a significant prevalence of simultaneous *KEAP1* mutations. These mutations activate the KEAP1/NRF2 pathway, a pivotal route in cytoprotection against oxidative stress. This phenomenon contributes to the cancer cells' ability to resist cytotoxic agents and cytotoxic T cells, enhancing their defense mechanisms against external threats (11, 99). Similarly, the *KRAS-G12D* point mutation has been shown to contribute to an immune-suppressive TME and negatively correlated with $CD8^+$ TILs and PD-L1 levels. Specifically, in NSCLC, *KRAS* mutation triggers both the MEK-ERK pathway and the P70S6K/PI3K/AKT pathways, leading to low PD-L1 levels. This also leads to a reduced secretion of the CXCL10 and CXCL11 chemokines by downregulation of HMGA2 signaling, leading to a decrease in $CD8^+$ TILS. This results in an immunosuppressive TME, resistant to PD-1/PD-L1 ICIs (12).

SMARCA4 mutations are detected in 10% of NSCLC cases and are correlated with an immune desert TME, characterized by the absence of tertiary lymphoid structures (TLS) within the TME. Notably, NSCLC with *SMARCA4* mutations exhibits a low response to ICIs, with objective response rates consistently below 20%. A significant proportion of patients also demonstrates minimal

10.3389/fimmu.2024.1384121

TABLE 1A Primary resistance – tumor intrinsic mechanism.

Mechanism	Cause	Consequences	Citation
Genetic mutations	STK11/LKB1 or KEAP1 alterations	Recruits neutrophils, Inhibits recruitment of T cells, Associated with increased expression of PD-1 and TIM-3	(8–11)
		Inhibits CD8+ T cell infiltration	(12)
	KRAS-G12D point mutation	Increase PD-L1 level	
	Mutations B2M or CASP8 gene	Impaired cell surface expression of MHC class I Defective antigen presentation Lack of CD8 T cells recognition	(13–19)
	MATP loss of function	Impaired T cell infiltration and functionality	(20)
Epigenetic changes	IPRES signature	Upregulation of epithelial to mesenchymal transition, hypoxia, angiogenesis, and wound healing	(21)
	Tumor dedifferentiation	Expression of negative regulatory immune molecules	(22–25)
	Modifications in gene expression of immune-related genes	Impact antigen processing, presentation, and tumor immune evasion	(26–29)
Alteration in the IFNg signaling pathway	Mutations in IFRNGR1 and IFNGR2, JAK1 and JAK2, IRF-1 and STATs	Diminished IFNγ sensitivity, reduced expression of HLA, PD-L1, and anti-tumoral chemokines	(14, 30–33)
	Loss of function in PBAF complex	Facilitates the transcription of IFN-γ-inducible genes leading to the recruitment of T cells and NK cells into the TME	(34)
	Loss of function of ADAR1	Leads to tumor inflammation and growth inhibition	(35)
Modification of PD-L1 expression	Oncogenic addiction Inflammatory cytokines PI3K/AKT mutations PTEN deletions EGFR mutations ALK rearrangements MYC overexpression CDK4/CDK6 disruption Increase in PD-L1 transcript	Inhibition of anti-tumor T cell responses	(19, 36–44)
Expression of immuno-suppressive cytokines	TGF-b	Increase of cancer cells invasiveness and promote metastasis	(45–52)
	CCL5, CCL7, CXCL8, CXCL12 or CCL22 CCR1, CXCR2, or CXCR4	Promotion of an immunosuppressive TME through recruitment of MDSCs and Tregs	(53–55)

TABLE 1B Primary resistance – tumor extrinsic mechanism.

Mechanism	Cause	Consequences	Citation
Infiltration of immune suppressor cells	Macrophages	Support neoplastic cell survival, proliferation, angiogenesis, and immune suppression	(53, 56–61)
	Low-density circulating neutrophils	Suppress therapy-induced T cell expansion and effector function	(62, 63)
Induction of co-inhibitory molecules expression	Upregulation of CTLA-4, IDO, TIM-3, LAG-3, CD73, and VISTA	Inhibit the function of anti-tumor T cells and dendritic cells	(64–72)

infiltration of cytotoxic T cells, while showcasing higher infiltration of pro-tumoral macrophages (100). The current understanding of *SMARCA4* mutations in NSCLC is constrained by a paucity of comprehensive studies and a limited patient cohort available for in-depth analysis. Further complicating matters is the concurrent occurrence of *SMARCA4* mutations with *STK11* and *KEAP1* mutations. The complexity of this molecular interplay makes it challenging to draw conclusions about the impact of *SMARCA4* mutations on the dynamics of immune response and treatment outcomes in NSCLC.

Alternate oncogenic mutations can also hinder the generation of anti-tumor T cells and their exclusion from the TME. This phenomenon has been associated with changes in β-catenin/WNT signaling, a pathway intricately involved in the initiation and progression of various types of cancer. Those modifications lead to reduced CCL4 production and impaired infiltration of $CD103^{+}$

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TABLE 2 Secondary resistance mechanism.

Mechanism	Cause	Consequences	Citation
T cell dysfunction	Defects in the antigen presentation machinery of T cells	Failure of T cell activation	(30)
	Mutations in the IFNg receptor pathway (JAK1 and JAK2)	Tumor escape due to decreased antigen presentation	(33)
		Decreased in T cell infiltration due to lower expression of T cell chemoattractant	
	De novo DNA methylation	Irreversible T cell exhaustion	(73, 74)
Changes in the mutational landscape	Low TMB but high intratumor heterogeneity	Low neoantigens exposure, leading to decreased effector functions	(75–77)
	Decreased expression or mutations in tumor neoantigens	Immune escape	(78–80)
Induced expression of alternative immune checkpoints	LAG-3, TIGIT, TIM-3 and VISTA re-expression	Promotes immune escape and the suppressive function of MDSCs in the TME	(78–81)
Metabolic alterations	Increased expression of extracellular adenosine	Inhibits T-cell proliferation, cytotoxic activity and promotes metastasis	(45, 82–86)
	Induction of the LXR pathway	Diminish the clonal expansion of T lymphocytes	(87)
		Leads to a Th17 phenotype associated with an inhibition of the anti-tumoral immune response	
		Inhibits the maturation and migration of dendritic cells (DCs)	
	Warburg effect	Decrease in ROS production	
		Impair the anti-tumor activity of CD8+ T cells	
	Hypoxia	Boost the immunosuppressive cell populations such as MDSCs, TAM, Th2 CD4+ T cells and Tregs	
Alterations within the TME	Increased angiogenesis	Decreased number of antitumoral T cells and increased number of TAMs	(88, 89)

dendritic cells, hampering effective anti-tumor immune responses (13). $CD103^+$ dendritic cells secrete CXCL9 and CXCL10 chemokines, crucial components of the anti-tumoral immune response as they attract $CXCR3^+$ effector T cells and NK cells. Upon binding to the CXCR3 receptor, CXCL9/CXCL10 chemokines induce effector T cells and NK infiltration to the TME (101).

T cells exclusion from the TME is also associated with loss of function of *CDK2A* and *CDK2B*, two tumor suppressor genes, located at the 9p21 locus and contributing to resistance against immune checkpoint blockade. However, recent investigations by Gjuka et al. have presented an alternative perspective (20). Located at 100 kb from *CDK2A* and *CDK2B*, lies the *MTAP* gene. Gjuka et al.'s research has elucidated that the loss of *MTAP* function is the actual determinant of the deficiency in TILs. The functional impairment of *MTAP* results in the accumulation of methyladenosine (MTA), which detrimentally affects T cell function. MTA promotes the inhibition of protein arginine methyltransferase 5 (PRMT5) and induces activation of adenosine receptor which impedes T cells effector function. Gjuka et al. demonstrated that the administration of MTA-depleting enzymes effectively reinstated TILs infiltration, thereby reducing tumor growth. Furthermore, this intervention synergistically enhanced the efficacy of ICIs, providing empirical validation for the mechanistic association between *MTAP*, MTA accumulation, and T cell dysfunction in the context of immunotherapy resistance.

Moreover, several genomic alterations, such as mutations in beta-2-microglobulin (*β2M*), *JAK1/2* loss of function mutations or *CASP8* gene, which lead to an impaired cell surface expression of MHC class I, defective antigen presentation, and lack of CD8 T cells recognition, have been identified to partially explain the treatment unresponsiveness (14–19).

Finally, aneuploidy, also known as Somatic Copy Number Alteration (SCNAs) is considered one of the main factors driving cancer development, and suspected to be involved in cancer immune evasion. The mechanisms underlying this phenomenon were studied in a comprehensive analysis of 5255 samples from The Cancer Genome Atlas project. This investigation involved examining SCNAs levels and their correlation with the types and number of mutations. Intriguingly, SCNAs levels emerged as a more robust predictor of cytotoxic T cell infiltration than tumor mutational burden (TMB). Additionally, increased SCNAs levels were associated with poorer survival outcomes in patients treated with ICIs, suggesting its potential as a prognostic tool (17).

Epigenetic changes

Apart from genetic mutations, resistance to ICIs has also been associated with epigenetic changes, such as the transcriptional IPRES signature (Innate anti-PD-1 Resistance). The IPRES signature consists of the concurrent overexpression of genes involved in the regulation of mesenchymal to epithelial transition, cell adhesion, extracellular matrix remodeling, angiogenesis, and wound healing (21), and was found across various cancer types.

Recent evidence also suggests that tumor dedifferentiation or stemness may also play a role in the resistance to anti-PD-1/PD-L1 blockade. Tumor-initiating stem cells have been found to express

10.3389/fimmu.2024.1384121

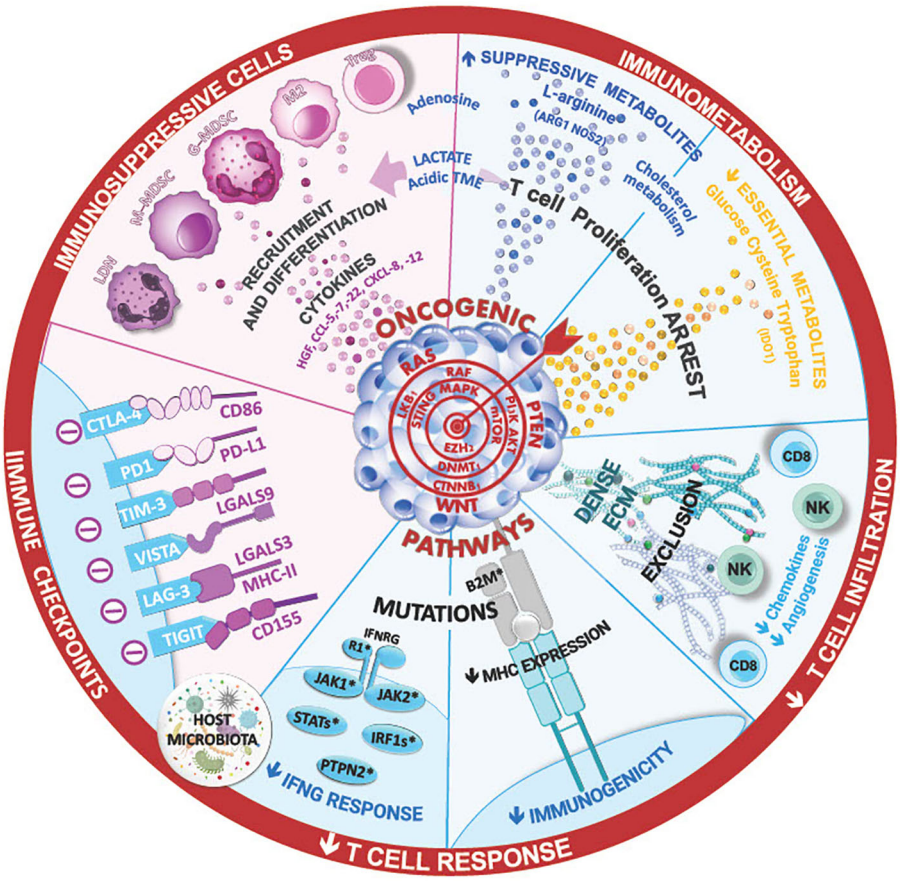

FIGURE 1
The ineffectiveness of immune checkpoint inhibitor therapies in lung cancer can stem from various mechanism, such as insufficient T-cell infiltration ang high levels of immunosuppressive cells in the tumor microenvironment. Cytokines and the composition of lung and tumor microbial can shape this TME and the immune anti-tumor responses, limiting the efficacy of ICI. Additionally, innate or acquired intrinsic resistance mechanism within cancer cells, such as low tumor mutational burden, mutations in IFN signaling, and antigen presentation pathways, may contribute to treatment resistance. Ongoing research aims to unravel these complexities foir improved therapeutic strategies.

TABLE 3 Hyperprogressive disease.

Mechanism	Cause	Consequences	Citation
Genetic alteration	EGFR alteration and MDM2 amplification DNMT3a alteration	Inhibition of p53	(90, 91)
Alteration in oncogenic pathways	Alteration of FGF2/β-catenin oncogene pathway	Escape through a T cell dependant mechanism	(92)
Modification of the immune infiltration	Imbalance between Teff and regulatory T cells (Treg) Increased infiltration of Type 2 macrophages	Escape through a T cell dependant mechanism Activated through the Fc portion of the ICIs	(93) (91, 92, 94)

negative regulatory immune molecules, such as CD80, PD-L1, and NKG2D (22–25). Interestingly, the β-catenin/WNT signaling, described above in immunotherapy resistance, is also involved in tumor stemness and dedifferentiation (26). Upstream, several epigenetic changes in cancer cells may lead to modifications in gene expression of immune-related genes, which can impact antigen processing, presentation, and tumor immune evasion (56). In pre-clinical studies, this is demonstrated by epigenetic modifying agents, including DNA-methyltransferase inhibitors and histone modifiers. Their mechanism of action involve rescuing the re-expression of components of antigen-processing and presentation machinery, tumor neoantigens, and cytokines, with a potential for therapeutic impact (27–29).

In summary, while checkpoint blockade resistance may stem from a spectrum of genetic and epigenetic modifications, it is essential to recognize that these alterations do not represent the exclusive mechanisms by which tumors evade immune destruction. The dysregulation of immune pathways, exemplified by the interferon-gamma (IFNγ) signaling pathway, emerges as another pivotal factor contributing to the facilitation of tumor immune escape.

Alteration in the IFNγ signaling pathway

Critical to the regulation of inflammation and cell-mediated immune responses, mutations within the IFNγ signaling pathway wield a double-edged sword effect in the context of immunotherapy.

Indeed, the sequencing of tumors from patients who did not respond to anti-PD-1 or anti-CTLA4 blockade revealed a high prevalence of loss-of-function mutations in the IFNγ receptor chains (*IFNRGR1, IFNGR2*), the pathway components (*JAK1, JAK2*), the interferon regulatory factor 1 (*IRF1*), the signal transducer and activators of transcription (*STATs*), and the tyrosine-protein phosphatase non-receptor 2 (*Ptpn2*) (14, 30–33). Consequently, upon IFNγ exposure, such mutations would lead to increase expression of PD-L1, leading to cancer cell immunoediting and immune escape (33, 45, 102).

Inversely, mutations in the IFNγ pathway can increase tumor cell sensitivity to ICIs by enhancing the secretion of chemokines that recruit effector T and NK cells to the tumor tissue. For instance, the loss of function of PBAF complex genes (*Pbrm1, Arid2, and Brd7*) can increase the transcription of IFN-γ-inducible genes, increasing the production of effector T cells and NK chemoattractant cytokines (CXCL9/CXCL10) (34). The upregulation of IFNγ expression also leads to an increased expression of the antigen presentation machinery, enhancing cancer cell recognition and facilitating more effective killing. Additionally, reactivation of endogenous retroviral elements and the loss of function of *ADAR1*, an RNA-editing enzyme, could make the cancer more vulnerable to immunotherapy by *viral mimicry* (35).

The dysregulation of the IFNγ pathway is a complex event that can increase sensitivity to immune checkpoint blockade by attracting immune cells and inducing HLA expression on tumor cells. However, it also further exacerbates the evasion tactics employed by cancer cells through PD-L1 overexpression, creating a barrier against the effectiveness of immune-based interventions.

PD-L1 expression

Within the TME, PD-L1 is constitutively expressed in response to oncogenic signaling or induced by inflammatory cytokines. Its primary function is to actively inhibit immune anti-tumor T-cell responses. A locus in chromosome 9p24.1 containing the genes for *PD-L1*, *PD-L2*, and *JAK2* is amplified in Hodgkin lymphoma and seems correlated to a high clinical response rate to anti-PD-1 therapy (19).

Co-amplification of *JAK2* and *PD-L1* were also detected in various solid tumors and may be associated with potential valuable metrics in predicting response to immunotherapy (103–107). Other mechanisms that may lead to constitutive PD-L1 expression in tumor cells include *PI3K/AKT* mutations, *PTEN* deletions, *EGFR* mutations, *ALK* rearrangements, *MYC* overexpression, *CDK4/CDK6* disruption, and an increase in PD-L1 transcripts stabilized by truncation of the 3' UTR of the gene (36–42). In the context of NSCLC, patients with oncogene addiction were frequently excluded from ICIs registration trials. As a result, we have limited clinical knowledge about the efficacy of ICIs in the subgroup of NSCLC patients with oncogene addiction (43). The available data mainly concerns patients with *EGFR* mutation or *ALK* rearrangement, while data for the other less common NSCLC subtypes is lacking. The Immunotarget registry recently demonstrated that ICIs may induce regression in some NSCLC tumors with actionable driver alterations, but clinical activity is significantly lower compared with the *KRAS* group, and the *ALK* group has a notable lack of response (44). Thus, patients with actionable tumor alterations should first receive targeted therapies and chemotherapy before considering immunotherapy as a single agent. Moreover, given the negative impact of the oncogene on the inflammatory TME, a combination of tyrosine kinase inhibitors with ICI may be clinically beneficial for long-term disease control, as recently suggested (43).

Tumor extrinsic mechanisms

Immunosuppressive cytokines

Tumor cells, regulatory T cells (Treg) and M2 macrophages secrete immunosuppressive cytokines to suppress anti-tumor immune responses. Transforming growth factor-β (TGF-β) plays a vital role in immunosuppression by inhibiting the infiltration of cytotoxic T cells through extracellular matrix remodeling (46) and by promoting the activation of Tregs (47–49). Combining anti-TGFβ with anti-CTLA-4 or radiation therapy demonstrated synergistic anti-tumor responses in pre-clinical models (50, 51). TGFβ is also known to induce the expression of transcription factors involved during epithelial-to-mesenchymal transition (EMT) in cancer, such as SNAIL. This process leads to transcription of the Zinc finger protein SNA1 that promotes repression of the E-cadherin cohesion molecule (52). This expression of SNAIL leads to increased production of immunosuppressive molecules such as IL-10 and TSP1 that increase cancer cell invasiveness and metastasis. The expression of these transcription factors also increases the transcription of immunosuppressive elements such as IL-10 and CSF1. Numerous results indicate that the use of TGFβ or TGFβ-related immunosuppressive molecules (IL-10, IL-6, IL-8, VEGF, and CSF1) can be beneficial. Additionally, inhibitors of cells such as TAMs/MDSCs and Tregs may help rescue an immune response to anti-PD-1/PD-L1 immunotherapy (45).

Similarly to TGF-β, certain chemokines (e.g., CCL5, CCL7, CXCL8, CXCL12, or CCL22), along with their corresponding chemokine receptors (e.g., CCR1, CXCR2, or CXCR4) play a significant role in creating an immunosuppressive TME. These are responsible for the attraction of myeloid-derived suppressor cells (MDSCs) and Tregs to the tumor (53, 54). For instance, CCL22 recruits immunosuppressive $CCR4^+$ Tregs or $CSF1R^+$ macrophages and MDSCs into tumors (55). Furthermore, this intricate interplay between TGF-β, chemokines, and their receptors not only shapes the immunosuppressive milieu within tumors but also bears significant implications for ICIs resistance through the recruitment of immunosuppressive cells.

Immune suppressor cells

The impact of the immune system, primarily mediated by T cells, is pivotal in determining the response to checkpoint blockade. However, it is essential to acknowledge that various other immune

cell populations also shape the outcomes of immunotherapeutic interventions. Tregs, MDSCs, M2-polarized tumor-associated macrophages, and Th2 $CD4^+$ T cells have been linked to ICIs resistance (56, 57). These cells promote an immune suppressive microenvironment that suppresses effector T cell responses through the secretion of cytokines and chemokines or by direct cell contact. In lung cancer, macrophages, pivotal regulators of tumor angiogenesis, secrete growth factors such as VEGF-A and angiopoietin-2. These factors support neoplastic cell survival, angiogenesis, and immune suppression at ectopic sites (58, 59). Many pre-clinical studies have demonstrated that the depletion of those immunosuppressive populations may restore a more robust immune response to cancer, overcoming resistance to ICIs (53, 60, 61). Recent studies have also established a clear link between resistance to checkpoint blockade and the presence of low-density circulating neutrophils (LDN) (62). Elevated blood neutrophil levels are correlated with increased serum hepatocyte growth factor (HGF) concentrations, likely linking these factors to immunotherapeutic resistance. HGF/c-MET signaling mobilizes neutrophils, which acquire immunosuppressive properties in T-cell inflamed tissues. Notably, c-MET+ neutrophils suppress therapy-induced T-cell expansion and effector functions, making the C-MET/neutrophil axis a primary oncogenic driver of ICI resistance (63). It is worth noting that while LDN are associated with immunotherapy resistance in single-therapy scenarios it is not observed when combined with chemotherapy, possibly due to observed neutrophil depletion in the latter case.

Immunosuppressive immune cells significantly contribute to patients' resistance to immunotherapy. This phenomenon underscores the complexity of immune regulation in the TME and emphasizes the necessity for in-depth scientific investigation to decipher the underlying mechanisms.

Induction of co-inhibitory molecule expression

The partial response to immunotherapy has frequently been correlated with the notable upregulation of other inhibitory checkpoints, such as CTLA-4, IDO, TIM-3, LAG-3, CD73, and VISTA, upon PD-(L)1 blockade (64, 65). Indeed, it has been observed that cancer patients who develop recurrent disease after anti-PD-1 treatment have increased TIM-3 expression on T cells (65). Pre-clinical models have demonstrated that the combination of checkpoint blockade using LAG-3+PD-1 and TIM-3+PD-1 led to improved responses (66, 67). Additionally, myeloid- and tumor-cell-derived indoleamine-2,3-dioxygenase (IDO) catabolizes tryptophan to the immune suppressive kynurenine, which can contribute to peripheral tolerance and negatively affects T cell function (68). Other immune suppressive enzymes, such as arginase 1, work in cooperation with the IDO pathway to inhibit the function of dendritic cells (108). Moreover, IFNγ induces the upregulation of IDO and another inhibitory molecule, the carcinoembryonic antigen cell adhesion molecule-1 (CEACAM1) (69, 70). Therapeutic antibodies blocking CEACAM1, and TIM-3 have demonstrated improved anti-tumor immune responses (67, 71, 72).

By identifying both tumor-intrinsic and tumor-extrinsic mechanisms of primary resistance, immuno-oncology has paved the way for multiple lines of attack against cancer. Currently, nearly a thousand clinical trials are testing a combination of anti-PD1 with other therapies. While the precise pathways underlying ICIs resistance have yet to be completely identified, the strong associations between specific axes, signaling pathways, and mutations bring us a step closer to further studies and, ultimately the development of precision immunotherapy.

In conclusion, the challenge of checkpoint blockade resistance is multifaceted. The mechanisms underlying *tumor intrinsic mechanisms of* resistance to checkpoint blockade include genetic and epigenetic alterations, disruption in IFNγ signaling, upregulation of PD-L1 expression, and the influence of immunosuppressive cytokines. Moreover, *tumor extrinsic mechanisms* resistance to ICIs involves adaptive changes within the TME, which poses significant challenges to sustained immunotherapeutic responses. Tumor-extrinsic mechanisms involve non-cancerous stromal or immune suppressive cells, expression of alternate co-inhibitory immune checkpoints, immune suppressive cytokines, or other systemic influences (e.g., host microbiota) that can act in concert with cancer cells to promote resistance to ICIs (97, 109). Altogether, these intricate processes underscore the complexity of tumor immune evasion strategies and emphasize the need for comprehensive research and innovative therapeutic approaches to overcome resistance and enhance the effectiveness of immunotherapy in cancer treatment.

Acquired resistance to immune checkpoint blockade

While antibodies targeting PD-1 or PD-L1 have shown remarkable and long-lasting clinical effectiveness in some individuals with NSCLC, a significant number of patients who initially respond will eventually experience relapse due to acquired resistance (110). Similarly, it is estimated that one-quarter to one-third of patients with metastatic melanoma who initially respond to anti-PD-1/PD-L1 will experience disease recurrence over time, even when they continue to receive therapy (111). This suggests that the anti-tumor immune response is dynamic, and the mechanisms initially blocked by the treatment tend to turn on inhibitory genes and pathways to tightly regulate immune escape.

Acquired resistance can manifest through various mechanisms, most of which are shared with primary resistance (Table 2).

T cell dysfunction

The primary process of acquired resistance is through T-cell dysfunction. The latter can occur through downregulation of tumor antigen presentation, epigenetic alterations, and acquisition of escape mutations, ultimately leading to T cell exhaustion (56). For instance, a mutation in *β2M* leading to the absence of surface expression of MHC class I was identified in tumor cells from a patient with late acquired resistance to anti-PD-1 treatment (30).

Similar defects in T cell effector functions can lead to acquired resistance to anti-PD-1. In patients with melanoma, anti-PD-1 treatment can induce mutations in the IFNγ receptor pathway, a

pathway also prone to disruption in primary resistance. By analyzing melanoma tumor biopsies that relapsed after PD-1 treatment, acquired homozygous loss-of-function mutations were identified in the kinases associated with the interferon-gamma receptor pathway: Janus kinase 1 (JAK1) and Janus kinase 2 (JAK2). Inactivation of *JAK1* and *JAK2* impairs the ability of IFNγ to exert its antitumor effects and renders the tumor unresponsive to anti-PD-L1 (33).

Another mechanism through which patients acquire PD-1 resistance occurs at the T-cell post-effector level. Working with preclinical models, Youngblood et al., have discovered how T cells become exhausted and unable to attack cancer cells as a result of PD-1 treatment. Whole-genome bisulfite sequencing of murine $CD8^+$ T cells identified progressive *de novo* methylation programs that restrict their effector function. This provides the rationale of combining ICI with the epigenetic drug decitabine to rescue T cell rejuvenation during PD-1 blockade treatment (73, 74).

Changes in the mutational landscape

For anti-PD1/PDL-1 therapy to remain effective, the tumor must maintain a sufficient level of immunogenicity. Melanoma is amongst the cancers that are most immunogenic and has one of the highest objective response rates to PD-1 checkpoint blockade (75). Data suggests that anti-tumor T cells activated by checkpoint blockade are specific to tumor antigens presented by the MHC. Those antigens, absent in normal tissues, are called neoantigens. The prevailing understanding in immunotherapy suggests that a higher TMB is a crucial biomarker for identifying cancer patients who are likely to benefit from ICIs. This hypothesis is based on the observed correlation between high TMB and enhanced neoantigen presentation, which amplifies tumor immunogenicity. However, a pre-clinical study conducted on mouse melanoma models found that a higher TMB does not correlate with a better immune checkpoint response. On the other hand, a low intra-tumor heterogeneity (ITH) has been associated with better overall response in immune checkpoint cohorts. This suggests that diminishing the diversity of tumor mutations might make reactive neoantigens more exposed to tumor-infiltrating T cells, leading to a better effector function (75). Therefore, it is not necessarily the increased number of mutations that will lead to a better response but rather the level of diversity of these tumors, with excessive mutational diversity leading to a poor prognosis in immune checkpoint blockade melanoma cohorts.

Additionally, tumors can develop acquired ICIs resistance through decreased expression or mutations in their tumor neoantigens. Over time, this will lead to the killing of immunogenic tumor clones and the growth of the clones harboring poorly immunogenic mutated tumor antigens, leading to immune escape. Consequently, variation of neoantigen level has been proposed as a key mechanism contributing to acquired resistance.

In summary, although tumors with a high clonal neoantigen burden may initially show a favorable response to ICIs and longer progression-free survival, patients may develop acquired resistance to ICIs due to the evolving mutational landscape of tumor neoantigens (76, 77).

Induced expression of alternative immune checkpoints

Other alternative immune checkpoint molecules may contribute to acquired resistance to PD-1/PD-L1 blockade. LAG-3, TIGIT, TIM-3, and VISTA, four inhibitory checkpoints, are often re-expressed in the TME after an initial response or at the time of relapsed disease (78). Interestingly, hypoxia-induced VISTA promotes the suppressive function of MDSCs in the TME, suggesting that targeting VISTA may mitigate the deleterious effects of hypoxia on anti-tumor immunity (81). Several clinical trials are currently underway to test antibodies against these inhibitory pathways, both as monotherapy and combination therapy strategies (79, 80).

Metabolic alterations

In addition to the intricate web of immunosuppressive mechanisms within the tumor microenvironment, cancer cells undergo significant metabolic alterations to support their aggressive growth and evade immune surveillance. A key player in promoting immunosuppression is extracellular adenosine. This molecule is produced by the hydrolysis of extracellular AMP, catalyzed by the ectonucleotidases CD39 and CD73. Extracellular adenosine can have diverse implications in anti-tumor immunity, by triggering specific signaling pathways. Specifically, adenosine binding to the A2A receptor inhibits T-cell proliferation and cytotoxic activity (82). Additionally, its engagement with the A2B receptor can promote metastasis, contributing to the development of acquired resistance in cancer (45).

Of interest, the upregulation of CD39 was also shown to suppress $CD8^+$ T-cell function and contribute to resistance to PD-1/PD-L1 blockade (83, 84). Thus, co-inhibition of CD39 and PD-L1 could improve anti-tumor immune response and could benefit a large percentage of ICI treated patients (83, 85). Similarly, high levels of soluble CD73 in peripheral blood were associated with a poor response to anti-PD-1 immunotherapy and A2A blockade given concurrently could rescue ICI efficacy (86). Accordingly, both CD39 and CD73 could be used as a potential biomarker of ICI resistance.

Another critical metabolic pathway in the context of tumor-acquired resistance is cholesterol metabolism, which plays a key role in the modulation of the immune response (87). Cholesterol oxidation produces epoxycholesterol and hydroxycholesterol that can bind to the liver X receptor (LXR), leading to its activation. The LXR pathway can diminish the clonal expansion of T lymphocytes, a mechanism that is essential for the activation of these immune cells. In mice, the inhibition of cholesterol esterification by administration of avasimibe, an esterase acetyl-CoA acetyltransferase (ACAT1) inhibitor, enhanced the inhibitory cytotoxic T cells activity [99]. The LXR pathway is essential for the activation but also the polarization of the adaptive

immune response. Its activation leads to a Th17 phenotype associated with an inhibition of the anti-tumoral immune response. This pathway also impacts the innate immune response by inhibiting the maturation and migration of DCs, crucial intermediaries bridging the innate and adaptive immunity.

Additionally, cancer cells undergo a metabolic shift known as the Warburg effect, favoring glycolysis and the pentose phosphate pathway over mitochondrial metabolism. This alteration aims to generate ATP and nucleic acids, facilitating rapid proliferation. The lack of mitochondrial activity leads to a decrease of reactive oxygen species (ROSs), protecting tumor cells from cellular damage and promoting their survival.

Cytotoxic T cells are also dependent on the glucose metabolism. Cancer cells impair the anti-tumor activity of $CD8^+$ T cells by outcompeting them for glucose consumption. In contrast, T regs rely on fatty acid oxidation (FAO) and remain unimpacted by this competition, enabling them to maintain their immune suppressive activity. Thus, the upregulation of glycolysis and decrease of the amount of ROS produced in cancer cells represents a mechanism of immunosuppression leading to acquired resistance (112).

Furthermore, metabolic abnormalities in the TME are reinforced by poor vascularization. The inadequate formation of blood vessels (vasculature) within the tumor and its surrounding tissue is a hallmark of cancer. This leads to poor supply of oxygen in the TME (hypoxia), making the cancer cells revert to anaerobic glycolysis. As a result, lactate levels are upregulated, which further exacerbates the acidic state of the TME. Low pH boosts the immunosuppressive cell populations such as MDSCs, TAM, Th2 $CD4^+$ T cells and Tregs which all have been shown to induce acquired resistance following ICI treatment (88, 89).

Alterations within the tumor microenvironment

Along this line, the reshaping of the TME following the administration of immunotherapy has been extensively studied. A therapy induced mechanism of resistance was observed in a combination therapy of anti-angiogenic agents and anti PD-1 agents in NSCLC. In cancer, pathological angiogenesis is mediated by the vascular endothelial growth factor A (VEGFA) and angiopoietin-2 (ANGPT2), which both constitute good targets of anti-angiogenic therapies. Their dual inhibition in murine KP and NSCLC mouse models was shown to mediate anti-tumoral effects, through the immune reprogramming of the TME characterized by an increased number of antitumoral T cells and a decrease in TAMs. However, adding PD-1 to that dual inhibition led to relapse (113).

The dual inhibition of angiogenesis was observed to result in the recruitment of $PD\text{-}1^+$ T regs at a higher proportion than anti-tumoral $CD8^+$ T cells. These $PD\text{-}1^+$ Treg cells were more effectively targeted and activated by anti-PD-1 antibodies. Additionally, intratumoral $PD\text{-}1^+$ Treg's were shown to be activated as a result of their interaction with $PD\text{-}L1^+$ TAMs in murine KP lung tumors. Therefore, within the tumor microenvironment, the infiltration of $PD\text{-}1^+$ T regs activated by PD-1 antibodies poses an additional obstacle to the efficacy of PD-1 blockade.

To summarize, acquired resistance can arise through a multitude of mechanisms. Those can be grouped into main categories: defects in T cell activation or function, reduced immunogenicity of the tumor, immunosuppression through the reshaping of metabolic pathways or of the tumor microenvironment. Deeper comprehension of fundamental biology holds the potential to enhance therapeutic approaches, allowing to find more precise ways of using and combining immunotherapies in order to circumvent and reverse ICIs acquired resistance.

Hyper progressive disease

There remains ongoing debate within the scientific community regarding the status of hyper progressive disease (HPD), with divergent opinions regarding whether it represents a distinct pathological entity or merely signifies patients with inherently poor prognostic factors from the onset (114). Cases of patients with advanced cancers, such as NSCLC (13.8%) or head and neck cancer (29%) (115), who experience rapid progression pose serious safety concerns. These cases, identified in 9% of individuals with advanced cancers compared to 2% undergoing targeted therapy, significantly undermine the prospects of success associated with immunotherapy (95, 116). Also observed with PD-1/PD-L1 blockers, hyper progressive disease (HPD) is characterized by accelerated tumor proliferation, high metastatic burden, and early death (mean overall survival of 3.4 months) within the first two months of treatment. HPD is defined as a tumor burden increase of more than 50%, a tumor growth rate exceeding 2-fold, and a time to treatment failure (TTF) of less than 2 months, as outlined in previous studies (95). Although critical, the predictive factors of HPD in patients with cancer treated with anti-PD-1/PD-L1 remain unknown (Table 3).

Enhancing our comprehension of HPD is essential for the early identification of susceptible individuals before the initiation of treatment. This understanding is essential in preventing these patients from undergoing potentially detrimental and costly treatment regimens. Identifying HPD early on can facilitate the redirection of these individuals towards alternative therapeutic modalities thereby optimizing the chances of therapeutic success and patient outcomes.

Genomic profiling emerges as a promising avenue for discerning HP disease, as evidenced by a case report study implicating *EGFR* alteration and *MDM2* amplification as potential indicators for HPD in NSCLC) (90, 91). Notably, *MDM2/MDM4* amplification was universally detected in all hyper progressive patients, all experiencing cessation of immunotherapy merely two months post-treatment initiation. Additionally, patients exhibiting *DNMT3a* alteration demonstrated hyper progressive disease in four out of five cases. The concurrent presence of *EGFR* mutation and *MDM2/MDM4* amplification correlated with a TTF of less than two months. *MDM2*, a known inhibitor of *p53*, underpins these observed associations. Further comprehensive

investigation is needed to elucidate the intricate molecular mechanisms underlying hyper progressive disease (92, 117).

An integrative study was necessary to gain deeper insights and elucidate the underlying complexities of the mechanisms involved in HPD. Li et al. examined the intricate interplay among immunogenic, metabolic, and oncogenic pathways of cancer patients undergoing immunotherapy (92). Surprisingly, patients who experienced either complete responses (CR) or HPD exhibited similar levels of immune factors, such as IFNγ and $CD8^+$ T cell infiltration, as well as comparable T cell clonal diversity. The expression of FoxP3, a T regulatory marker, was also comparable across patients with CR or HPD. While certain gene signatures like *KRAS*, *NOTCH*, and *EGF* demonstrated similarities, HPD patients displayed an increased activity in pathways associated with *FGF2*, Wnt β-catenin, and stemness invasiveness compared to other groups. These findings were reproducible in several mouse models, including the LLC1 lung adenocarcinoma model, where increased T cell infiltration was evident in HPD cases. Notably, depleting CD8+ T cells resulted in slower tumor growth, suggesting a T cell-dependent mechanism. Further investigation revealed that IFNγ selectively altered NAF^+/β-catenin signaling in HPD-prone tumor models, confirming the key role of T cells in this mechanism. The disruption of FGF2/β-catenin oncogene pathway was also validated in patients who did not respond to immunotherapy, confirming the study's clinical relevance.

The role of T effector cells was also confirmed in a study using Near-Infrared Photoimmunotherapy (NIR PIT) (93). NIR PIT is a technology that enables depletion of a specific target population while leaving neighboring cells unaffected. By specifically targeting CD8β, it became possible to deplete effector T cells, leading to an imbalance between Teff and Treg and thereby replicating the immune microenvironment of hyper-progressive tumors. When mice lacking Teff cells were subjected to checkpoint blockade therapy, a significantly accelerated tumor growth was observed compared to the control group lacking Teff cells untreated with checkpoint inhibitors, confirming the key role of $CD8^+$ T effector cells in the regulation of HPD.

But T eff cells are not the only component of the immune system playing a crucial role in the development of HPD. In particular, the blockade of PD-1/PD-L1 signaling pathways is known to induce immunosuppression by modulating interactions with innate immune cells. Analysis of pre-treatment tissue samples from patients revealed increased infiltration of Type 2 macrophages within tumors, a phenomenon more pronounced in patients who later exhibited hyper progressive diseases (118). Consistently, murine models also demonstrated enrichment of tumor-associated macrophages within the tumor microenvironment. Notably, in patients, the presence of Type II macrophages expressing the $CD163^+$ $CD33^+$ $PD\text{-}L1^+$ phenotype positively correlated with hyper progressive disease, while PD-L1 expression alone showed an inverse correlation. This observation suggests that PD-1 blockade might induce immunosuppression through the interaction of the Fc domain with the inhibitory FcγRIIb receptor, expressed on DCs and monocytes (119). Experimental evidence supporting this notion was derived from athymic nude mice treated with checkpoint blockade, where the removal of the Fc domain from the protein construct led to a decelerated tumor growth rate. Furthermore, administration of nivolumab lacking the Fc domain prevented hyper progressive disease in this model, corroborating the significance of this interaction in the context of immunosuppressive responses (120).

The primary challenge in studying HPD lies in the absence of pre-treatment, as well as during and post-treatment samples. To gain deeper insights, future investigations should focus on collecting tumor and blood samples from HPD patients both before and during treatment. This approach can provide valuable data to elucidate the molecular and cellular mechanisms accelerating disease progression and their direct connection to the treatment process.

Perspectives

Presently, there is an urgent need to overcome obstacles that hinder the clinical advancements in the field of onco-immunology. These challenges include developing accurate pre-clinical models that mimic human immunity, gaining a comprehensive understanding of the molecular and cellular determinants of primary and secondary resistance, and designing the most effective combinations of personalized immune-based therapies for individual patients (121). Meeting these challenges will require the combined efforts of researchers and clinicians, to accelerate our understanding of the complex interactions between cancer and the immune system, and ultimately develop improved treatment options for cancer patients.

Combination strategies

In the pipeline, combinatory therapeutic strategies have been explored to target diverse molecular and cellular pathways of resistance (Table 4). One established strategy is to combine chemotherapy and immunotherapy. Although counterintuitive at first due to chemotherapy-induced myelosuppression, the chemo-immunotherapy approach has shown significant promise in improving patient outcomes. Chemotherapy inhibits the generation of immunosuppressive immune cells such as T regs, MDSCs, TAMs, thereby promoting a more inflammatory immune infiltrate (122). Additionally, chemotherapy induces tumor cell death, leading to increased presentation of neoantigens (123). A retrospective analysis of NSCLC patients treated with a combination of chemotherapy and immunotherapy demonstrated enhanced overall survival and progression-free survival. Currently, multiple clinical trials (NCT02486718, NCT02657434, NCT02409342, NCT02367781, NCT02366143) are underway to validate the efficacy of Atezolizumab in combination with chemotherapy, aiming to stimulate a robust immune response in NSCLC patients.

Directing therapeutic efforts toward cancer cells via chemotherapy holds promise, yet the effectiveness of

10.3389/fimmu.2024.1384121

TABLE 4 Emerging therapies.

Combination	Registration number	Strategy	Phase
Chemotherapy + Immunotherapy	NCT02486718	Atezolizumab compared with best supportive care following adjuvant cisplatin-based chemotherapy	III
	NCT02657434	Atezolizumab in combination with carboplatin or cisplatin + pemetrexed	III
	NCT02409342	Atezolizumab compared with cisplatin or carboplatin in combination with either pemetrexed or gemcitabine	III
	NCT02367781	Atezolizumab in combination with carboplatin + nab-paclitaxel	III
	NCT02366143	Atezolizumab in combination with carboplatin + paclitaxel with or without bevacizumab compared with carboplatin + paclitaxel + bevacizumab	III
Anti VEGF + Immunotherapy	NCT00790010	Bevacizumab plus ipilimumab	I
	NCT05063552	Chemotherapy + cetuximab vs chemotherapy + bevacizumab vs atezolizumab + bevacizumab	II/III
ACT +/- Immunotherapy	NCT03168438	NY-ESO-1 specific (c259) T cells alone or in combination with pembrolizumab	I
	NCT02992743	NY-ESO-1c259 T cells	II
	NCT02588612	Autologous T cells expressing enhanced TCRs specific for NY-ESO-1	I
	NCT03709706	Autologous T-Cells expressing enhanced TCRs (T Cell receptors) specific for NY-ESO-1/LAGE-1a alone, or in combination with pembrolizumab	Ib/IIa
Emerging therapies- CAR-T cells	NCT05060796	CXCR5 Modified EGFR Targeted CAR-T Cells	EarIy phase I
	NCT04153799	CXCR5 Modified EGFR Targeted CAR-T Cells	I
	NCT03525782	Anti-MUC1 CAR T Cells and PD-1 Knockout Engineered T Cells	I and II
	NCT02414269	Anti-MSLN CAR T Cells	I and II
	NCT05693844	CD40 Ligand Expressing MSLN-CAR T Cell Treatment	I and II
Emerging therapies - Cytokine therapy + Immunotherapy	NCT02748564	IL-2 in combination with Pembrolizumab	II
	NCT04905316	Canakinumab (IL-1β inhibitor) With Chemoradiation and Durvalumab	I and II

immunotherapy can be increased by direct intervention in the tumor microenvironment. One of the most critical mechanisms in tumor progression is angiogenesis, which fuels nutrients and oxygen to tumor growth. As hoped, integrating anti-VEGF bevacizumab with immunotherapy helps stabilize the tumor vasculature, support the penetration of immune cells and drugs into the tumors and hence boost immunotherapy effectiveness in pre-clinical models (94, 124). Ongoing clinical trials support the potential of this approach in cancer treatment (NCT00790010, NCT05063552).

Checkpoint inhibitors also showed synergistic activity when combined with adoptive cell therapy (ACT). T cells with a transduced TCR can specifically recognize and target cancer cells with high specificity and low toxicity, making them a promising tool in the management of cancer patients. They are currently being investigated in several clinical trials in various cancer types in combination with checkpoint inhibitors such as pembrolizumab or nivolumab (NCT03168438, NCT02992743, NCT02588612, NCT03709706).

Another strategy receiving significant attention is the integration of immunotherapy with cancer vaccines. Therapeutic cancer vaccines are able to enhance the efficacy of ICIs. One approach involves vector-based vaccines like TG4010, which utilize modified viruses encoding specific proteins. In pre-clinical studies, TG4010 has shown significant potential, leading to ongoing phase II clinical trials in combination with ICIs. Another avenue explores dendritic cell-based vaccines like AdCCL21-DC, where genetically modified cells displayed enhanced immune responses. These vaccines have demonstrated encouraging results in animal models, paving the way for phase I clinical trials in patients with advanced cancers.

Exploring diverse combination therapies, including chemotherapy and immunotherapy, anti-angiogenic agents, adoptive cell therapy, and cancer vaccines, offers promising avenues to prevent resistance to ICIs. These innovative approaches, supported by clinical trials, demonstrate the potential to improve cancer immunotherapy, providing patients with more effective and personalized solutions.

Emerging immunotherapies

More than twenty years after the discovery of the first checkpoint blockade, immunology continues to be the focal point of cancer research, and recent advancements in the past years indicate a promising future. For example, CAR T cells represents

a groundbreaking advancement in treating liquid tumors, demonstrating significant efficacy with patients achieving complete remission and experiencing limited toxicities. However, the translation of CAR T cells to solid tumors remains a challenge due to the scarcity of suitable targets. Numerous potential targets for CAR T cell development, including EGFR, HER2, mesothelin (MSLN), prostate stem cell antigen (PSCA), mucin 1 (MUC1), and carcinoembryonic antigen (CEA), among others, have been explored. Nevertheless, only a few have progressed to clinical trials (125).

In a phase I clinical trial assessing the impact of CXCR5-modified CAR T cells targeting EGFR in advanced non-small cell lung carcinoma (NCT05060796), patients exhibited favorable tolerance to the treatment. Subsequent investigations in a second trial (NCT04153799) aimed at optimizing the dosage of EGFR CAR T cell therapy confirmed the low toxicity profile observed in the initial study. However, due to the early stage of these investigations, conclusive remarks regarding the efficacy of anti-EGFR CAR T cells are premature. The need for further exploration will necessitate the initiation of phase II and III clinical trials to comprehensively assess the therapeutic potential of this promising approach.

Exploring an alternative target, a pilot study (NCT03525782) investigates the combined use of MUC1 CAR T cells with PD-1 knock-out T cells, revealing efficacy in primary tumor reduction. However, the findings for metastases present a less encouraging picture.

Mesothelin has also been a focal point of interest as a target for developing CAR T cells designed for solid tumors. However, current clinical trials have not produced promising results, with patients enduring severe toxicities (NCT02414269). Ongoing trials continue to assess the potential toxicities associated with targeting MSLN using CAR T cells. Additionally, investigations are underway to explore the prospect of enhancing CAR T cells through co-expression with CD40L (NCT05693844).

In addition to their associated toxicities, CAR T cells exhibit inherent drawbacks (126). Challenges include the absence of adequate vascularization, downregulation of adhesion molecules, the immunosuppressive tumor microenvironment (TME), and the exhaustion and/or limited infiltration of CAR T cells into the TME, collectively contributing to the observed lack of efficacy. Furthermore, the presence of targeted markers in healthy tissues can lead to aberrant activation of CAR T cells, potentially responsible for their toxicities.

To address these limitations, ongoing research is focused on investigating new markers to enhance the targeting and specificity of CAR T cells for tumors. Promising candidates, such as ephrin-A receptor 2 (EphA2), tissue factor (TF), and protein tyrosine kinase 7 (PTK7), are being explored. These endeavors instill hope that the success achieved by CAR T cells in treating liquid cancers may be replicated in the challenging landscape of solid tumors.

Interleukin therapies have also recently gained prominence due to their promising ability to activate and enhance the cytotoxic capabilities of T cells, including CAR T cells. IL-2, in particular, was the pioneering immunotherapy to exhibit significant antitumor efficacy, with patients demonstrating complete and durable responses in melanoma and renal cell carcinoma. Notably, High-Dose IL-2 (Aldesleukin) stands as the sole interleukin therapy currently approved by the FDA.

Combining HD-IL2 with pembrolizumab holds the potential for a more potent eradication of tumor cells. This combination resulted in partial responses in 11% of treated patients, along with one complete response, underscoring the feasibility and safety of synergizing these two therapies (NCT02748564).

IL-1β is another emerging cytokine of interest. Remarkably, inhibiting its receptor in the context of atherosclerotic disease has shown a reduced incidence of lung cancer. To further explore the potential therapeutic implications, an ongoing phase II clinical trial (NCT04905316) is investigating whether the combination of canakinumab (an anti-IL-1β monoclonal antibody) with chemoradiation and durvalumab proves to be an effective and safe treatment for locally advanced NSCLC. These findings highlight the emerging role of interleukin therapies in enhancing the therapeutic landscape for cancer treatment.

The downside of these therapeutic options lies in the toxicities resulting from on-target or off-site effects. An ideal solution would involve directing the delivery of these drugs specifically to the tumor microenvironment, either through passive or active mechanisms (127).

The principle of passive targeting revolves around delivering drugs through nanocarriers via their passive diffusion or convection through the interstices of tumor capillary pores. Illustrative examples include liposomes, which deliver drugs to the tumor through fusion with the cell membrane, and polymeric nanoparticles (PEG) that enhance drug absorption and blood circulation (128).

Conversely, active targeting entails modifying specific ligands, antibodies, or other molecules on the surface of nanoparticles to identify and attach to particular cells or tissues at the targeted site, ensuring more precise drug delivery. This includes antibody-based targeting, peptide-based targeting, aptamer-based targeting, and small-molecule-based targeting (129, 130).

Emerging predictive biomarkers

Immunotherapy plays a central role in the treatment of lung cancer and identifying biomarkers that predict response to ICIs (and other immunotherapies) is key. While the predictive power of PD-L1 expression and TMB has long been studied and documented, their accuracy and robustness aren't consistently reliable. Numerous studies have shed light on emerging biomarkers that can further help with therapeutic response prediction.

The detection of pretreatment PD-L1 protein expression on tumor cells and immune cells by immunohistochemistry is currently the standard practice in the clinical setting. However, it is becoming increasingly clear that PD-L1 remains a controversial biomarker, primarily due to the intratumoral and intertumoral heterogeneity of its expression. Moreover, treatments such as radiotherapy or EGFR tyrosine kinase inhibitor are known to induce changes in its expression levels overtime.

A emerging solution appears with liquid biopsy, allowing the analysis of cancer-related signals in biological fluids. It presents the advantage of being less invasive while being of easier access than tumor biopsies and enabling the analysis of tumor biomolecular features. In a study using liquid biopsies on a cohort of patients, the interest of monitoring the levels of blood PD-L1 and its expression (including PD-L1 mRNA, circulating exosomal PD-L1 and soluble PD-L1) was demonstrated. Blood PD-L1 was shown to have a positive correlation with tumor PD-L1 expression in various malignancies and its upregulation has been correlated with good efficacy and survival for ICIs treatments (131).

Other novel biomarkers are emerging, hoping for better predictor of response than the PD-L1 gold standard. In a retrospective study on a cohort of advanced NSCLC patients, mutations in ARID1A and ARID1B have been proposed as biomarkers for the prognosis and sensitivity to ICI treatment. Deficiencies in those recently discovered oncogenic drivers have been shown to be tightly associated with cancer mutability, PD-L1 expression and are associated with good prognosis for ICIs treatment (132).

Similarly, a recent study identified ZFHX3 mutations as prognostic predictors of NSCLC immunotherapy. Associated with longer overall survival after immunotherapy and demonstrating a positive correlation with other predictive biomarkers such as TMB, ZFHX3 mutations can be used as a novel potential predictive marker to direct NSCLC ICI treatment (133).

Looking at the transcriptome expression profile rather than just the genomics of cancer has also proven to be a valuable tool. Notably, compared to the currently recognized expression of CD274 gene which encodes for PD-L1, the expression of CSF1R and HCST has been shown to have better efficacy in predicting the response to anti-PD-1 therapy in NSCLC. Those genes participates in antigen processing and presentation and T cell receptor signaling pathways, underscoring their significance in this context (134).

Another compelling biomarker is the neutrophil-to-lymphocyte ratio (NLR). It has been extensively studied in recent years as a potential predictive and prognostic tool in patients with NSCLC treated with PD-1/PD-L1 inhibitors. NLR can be used as an inflammation marker and thus has clinical potential in identifying patients that can durably respond to treatment, although prospective studies are needed to confirm its clinical value (135, 136).

Extracellular vesicles (EVs) (which include exosomes and microvesicles) derived from tumor tissues also hold promises as a potential non-invasive biomarker. They play a crucial role in cellular communication by transporting bioactive molecules such as microRNAs, presenting a valuable predictive value. For instance, EV-miR-625–5p has been described as a novel biomarker of response to ICIs in NSCLC patients with PD-L1 expression ≥50% that can thus help stratify them (137).

Microbiota profiling is also increasingly considered a useful tool in predicting response to ICI in NSCLC patients. While an imbalanced respiratory tract microbiome has been associated with tumor progression, a more diverse lung microbiome is correlated with higher levels of CXCL9, a chemokine associated with better immune response in the tumor. More specifically, using 16S RNA sequencing has identified specific microbial enrichments in NSCLC patients with differential ICI responses (138, 139).

Finally, several studies have demonstrated that certain characteristics of the TCR repertoire, such as diversity and density, can influence the effectiveness of immunotherapy in various cancer types. By studying the TCR repertoire before and during treatment, clinicians may be able to identify patients who are more likely to respond to immunotherapy, thereby guiding treatment decisions and improving patient outcomes.

Major developments in TCR sequencing and T-cell antigen specificity prediction have helped with predicting patient outcomes, making it a useful emerging biomarker in the context of cancer (140). As an example, in patients with melanoma, which tend to have a greater T-cell diversity and richness in their peripheral blood and in lymph node metastases, had longer progression-free and overall survival (140). In NSCLC, patients with T cell repertoires that are highly homologous between the tumor and non-involved tumor-adjacent lung showed a lower survival, suggesting that a higher T cell clonality in tumors is correlated with a better prognosis (141).

TCR sequencing characterizes both intratumor as well as intertumoral heterogeneity, which have important implications in explaining mechanisms of cancer immunity and predicting therapeutic responses to immunotherapy. Furthermore, TCR repertoire metrics can also inform about potential immunotherapy-related toxicities. Clonality was assessed in the context of immune-related adverse events (irAEs) after anti-CTLA-4 treatment of prostate cancer patients, showing that the expansion more than 55 CD8+ T-cell clones in the peripheral blood preceded the development of severe irAEs (140).

Thus, there is an ongoing exploration of additional biomarkers, attempting to elucidate why patient responses to immunotherapies differ. Efforts to translate these emergent biomarkers into clinical practice will help strengthen the personalized approach in cancer immunotherapy treatments.

In addition to refining existing therapeutic strategies, it is crucial to enhance patient selection for immunotherapy by excluding individuals who are unlikely to respond or may experience significant side effects. Obtaining tumor tissue before and after treatment initiation is essential for a systematic analysis, enabling a comprehensive understanding of the resistant mechanisms at play (121). Thus, the strategy in identifying the mechanisms of response and resistance to ICIs involves the assessment of serial tumor specimens throughout the course of treatment, together with the development of minimally invasive biomarkers (e.g., liquid biopsy, PBMCs) (56, 142). This approach is important because it encompasses traditional static time points research and aims to recognize superior diagnosis biomarkers by analyzing dynamic responses to ICIs.

Conclusion

While the revolution of cancer immunotherapy is hurtling down, there is little, if any, time to standardize the companion/complementary tests for routine clinical practice. Whatever the biomarkers and their promise, we are in the rush of their early phases of development; and we require time for global acceptance by large-scale collaborative efforts worldwide (143, 144).

To date, there is no clinically validated biomarker of resistance to ICIs. The onco-immunology research has never been as intriguing, prosper, and promising as nowadays. The revolution of cancer immunotherapies has shed light on a promising decade of success in cancer management, yet large-scale collaborative efforts are crucial to overcoming actual detection, stratification, and resistance obstacles.

Bringing therapeutic benefit to most of patients involves a thorough understanding of the mechanisms that would cause an effective anti-tumor response and the various cell-intrinsic and -extrinsic tumor factors that would give rise to primary, adaptive, and acquired immunotherapy resistance. Elucidating these pathways will provide important insights into the next approaches that need to be taken to effectively resolve immunotherapy resistance.

Author contributions

LB: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. ZG: Writing – original draft, Writing – review & editing. MC: Writing – original draft, Writing – review & editing. MI: Writing – original draft, Writing – review & editing. VH: Writing – original draft, Writing – review & editing. GR: Writing – original draft, Writing – review & editing. FG: Writing – original draft, Writing – review & editing. BM: Writing – original draft, Writing – review & editing. MR: Writing – original draft, Writing – review & editing. PH: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. LB received a fellowship from the University Côte d'Azur (Nice, France) for her PhD program. This work was supported by the "Region Provence Alpes Côte d'Azur", " Conseil départemental des Alpes-Maritimes", "Association pour la Recherche contre le Cancer" (ARC Grants n° SL220110603478, ARC CANC'AIR GENExposomics and ARC Sign'it 2019), Cancéropôle PACA, Plan Cancer INSERM, FHU Oncoage, IHU RespirERA, and French national research agency ("Investments for the Future" LABEX SIGNALIFE: program reference # ANR-11-LABX-0028–01 and "STEATOX" # ANR-13-CESA-0009–01).The funding organizations had no role in the design and conduct of the study.

Conflict of interest

MI has received honoraria for travel support and consulting/advisory roles for AstraZeneca, Bristol-Myers Squibb, Roche, Boehringer-Ingelheim and Merck & Co. outside the submitted work. PH has received honoraria for travel support and consulting/advisory roles for AstraZeneca, Roche, Bristol-Myers Squibb, Novartis, Pfizer, MSD, Qiagen, Thermo-Fisher Scientist, Janssen, Abbvie, Biocartis, Pierre Fabre, Sanofi, and Merck & Co. outside the submitted work.

The remaining author declares 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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frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 05 July 2024
DOI 10.3389/fonc.2024.1398357

OPEN ACCESS

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RECEIVED 11 March 2024
ACCEPTED 24 June 2024
PUBLISHED 05 July 2024

CITATION
Fan B, Sun X, Han W, Zou Y, Chen F, Lan F, Li W and Mao Y (2024) Immune checkpoint inhibitor increased mortality in lung cancer patients with *Pneumocystis jirovecii* pneumonia: a comparative retrospective cohort study. *Front. Oncol.* 14:1398357. doi: 10.3389/fonc.2024.1398357

Immune checkpoint inhibitor increased mortality in lung cancer patients with *Pneumocystis jirovecii* pneumonia: a comparative retrospective cohort study

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Introduction: *Pneumocystis jirovecii* pneumonia (PJP) is a life-threatening infection in immunocompromised individuals. Immune checkpoint inhibitor (ICI) has brought significant survival benefit in lung cancer patients. Although the few studies showed there was high mortality in PJP patients with ICI use, these studies had no comparative control groups.

Methods: A retrospective study was conducted to compare the mortality in PJP patients with lung cancer between those treated with ICI and a concurrent control group treated without ICI.

Results: A total number of 20 non-human immunodeficiency virus (HIV) patients with confirmed PJP and co-existing lung cancer were included in the current study, and classified into ICI group (n=9) and non-ICI group (n=11).There was a clear trend to a shorter onset of PJP in ICI group than non-ICI group (118.9 ± 60.9 vs 253.0 ± 185.1 days), although without statistical significance (p=0.053). Bronchoscopic alveolar lavage fluid were collected from all patients and used to identify *Pneumocystis jirovecii*. In both groups, metagenomics next-generation sequencing (mNGS) were the most used diagnostic techniques. Within 28 days after the onset of PJP, mortality was significantly higher in the ICI group than non-ICI group (33.3% vs 0, p=0.042)

Conclusion: Lung cancer patients with ICI use had a higher mortality rate after PJP infection than patients without ICI use. Prospective studies with larger sample size and a multi-center design are warranted to further verify the present results.

KEYWORDS

immune checkpoint inhibitor, lung carcinoma, Pneumocystis jirovecii pneumonia, mortality, metagenomics next-generation sequencing

Introduction

Pneumocystis jirovecii (PJ) is an opportunistic pathogen that is responsible for life-threatening manifestations of *Pneumocystis jirovecii* pneumonia (PJP) in immunocompromised individuals (1). PJP remains the most prevalent opportunistic infection in patients infected with the human immunodeficiency virus (HIV) (2). In recent years, with increasing use of corticosteroids and/or immunosuppressive agents, chemotherapy and radiotherapy for malignancies, and advancement of organ transplantation, PJP has been increasingly reported in non-HIV patients as well (3–5). The prognosis of non-HIV-infected PJP patients tends to be worse, and the reported mortality of PJP in immunocompromised non-HIV patients ranges from 48% to 67% (6). Recently, because wide application of molecular diagnostic techniques has made timely diagnosis and prompt treatment a reality, the mortality of PJP have been greatly reduced (7). But PJP is still a health threat to immunocompromised individuals.

Lung cancer is a malignancy with high prevalence and mortality worldwide. PJP could occur in lung cancer patients (8). A retrospective study in France showed that 3% of non-HIV patients with PJP had lung cancer (9). Another study in Japan showed that in non-HIV solid tumor patients with PJP, lung cancer was the most common underlying tumor, which accounted for 30% of PJP cases (10). Like other non-HIV-infected PJP patients, lung cancer patients with PJP had poor prognosis. A retrospective analysis by Lee et al. revealed that lung cancer patients with PJP had an all-cause mortality rate of 61.6% during 3-month PJP treatment (11). So the high mortality of PJP in lung cancer patients warrant attention from physicians.

In recent years, immune checkpoint inhibitor (ICI) has revolutionized the treatment of lung cancer and brought significant survival benefit (12). Their use has been widely recommended in lung cancer patients by major guidelines. Since the introduction of ICI into clinical practice, concerns have emerged regarding their potential to cause infection. Now increasing evidence show that ICI use might not increase in risk of infection, but it might increase risk of infection in patients developing immune-related adverse events (irAE) and treated with additional immunosuppressive such as corticosteroids (13–15). In melanoma patients with ICI treatment, bacteria were the most common pathogen of serious infection, followed by fungus, virus and parasite (13).The study by Malek et al. showed that in lung cancer patients treated with ICI, pneumonia was the most common infection encountered, and bacteria were the dominant type of pathogens, followed by virus and fungus (14).

A meta-analysis, which included a total of 21,451 cancer patients from 36 studies, showed that ICI were associated with a similar risk of infections versus non-ICI treatments (16). So these findings have greatly relieved the concern about ICI's detrimental effect on infection. But the concerns persist in patients with use of corticosteroids, who had increased risk of infection.

So far PJP has been reported in patients with ICI use, but the clinical features and prognosis of PJP with ICI use remains mostly unknown. There were only over a dozen PJP cases associated with ICI reported in literature. In an analysis base on the Food and Drug AdministrationAdverse Event Reporting System (FAERS) database of Food and Drug Administration (FDA), researchers identified 677 reports of PJP associated with ICI, in which 300 (44.3%) PJP cases with fatal outcome (8). They also found that male gender and age >65 years were predominant in PJP cases associated with across all ICI. Although the few studies showed there was high mortality in PJP patients with ICI use, these studies had no comparative control groups. To better evaluate the mortality risk of ICI in patients with lung cancer, we compared the mortality in PJP patients with lung cancer between those treated with ICI and a concurrent control group treated without ICI.

Methods

Ethical approval

This was a retrospective study of patients conducted in an academic teaching tertiary hospital (The Second Hospital of Zhejiang University School of Medicine, China). The ethical approval was sought and granted by Ethics Committee of Second Affiliated Hospital of Zhejiang University School of Medicine (Approval Number: 2023–0847). As the non-interventional retrospective study was determined to be no greater than minimal risk, the Ethics Committee of 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.

Patient selection

All patients admitted to the study hospital with a discharge diagnosis of PJP between January 2017 and February 2022 were retrieved from the Electrical Medical Records System (EMRS). Patients with prior HIV infection were excluded from the study. Records were further reviewed by two pulmonologists (FL and YMZ) to confirm the diagnosis of PJP. When the opinions differed, a third pulmonologist (WL) was involved in decision. The diagnosis of PJP were made according to clinical manifestations, imaging

Abbreviations: PJP: *Pneumocystis jiroveci* pneumonia; PJP: *Pneumocystis jiroveci*; HIV: human immunodeficiency virus; ICI: immune checkpoint inhibitor; irAE: immune-related adverse events; FAERS: FDA Adverse Event Reporting System; FDA: Food and Drug Administration; EMRS: Electrical Medical Records System; PCR: polymerase chain reaction; mNGS: metagenomic next-generation sequencing; PD-1: immune blockade programmed death receptor 1; PD-L1: programmed death ligand 1; IBM: International Business Machines Corporation; SD: standard deviation; IQR: interquartile range; BMI: body mass index; COPD: chronic obstructive pulmonary disease; BALF: bronchoscopic alveolar lavage fluid; TMP/SMZ: trimethoprim/sulfamethoxazole; CIP: checkpoint inhibitor associated pneumonia; NSCLC: non-small cell lung cancer; CT: Computer Tomography; DNA: Deoxyribonucleic Acid.

examinations, and microbiological test results as described before (17). The criteria were as follows: (1) compatible clinical symptoms including fever, cough, sputum, and dyspnea; (2) radiological findings compatible with PJP such as uni- or bilateral ground-glass opacity or patchy consolidation; and (3) microbiologic finding including conventional or immunofluorescence staining, and molecular diagnosis by polymerase chain reaction (PCR) or metagenomics next-generation sequencing (mNGS) via respiratory specimens (sputum specimens or bronchoalveolar lavage fluid) and blood samples.

Data collection

Demographic data, lab test results on admission, disease comorbidities, and pharmacotherapy were collected from EMRS. ICI included programmed cell death protein 1 (PD-1) agents and programmed cell death receptor ligand-1 (PD-L1) agents. The survival status of patients was assessed by medical record review and phone interview in late August 2023.

Data analysis

The results were analyzed using International Business Machines Corporation (IBM) SPSS Statistics 20. Continuous data was 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 the 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. Statistical significance was set at $p< 0.05$.

Results

A total of 92 patients discharged with diagnosis of PJP between June 2017 and February 2022 were extracted from the EMRS. After screening, a total number of 20 non-HIV patients with confirmed PJP and co-existing lung cancer were included for further analysis (Figure 1). Of these 20 patients, there were 9 patients who had a history of ICI use (ICI group) and 11 patients who had no history of ICI use (non-ICI group).

Baseline characteristics

Baseline demographics, comorbidities, and lung function test results were similar between two groups, except for body mass index (BMI) (Table 1). The ICI group had an average age of 69.11 ± 4.99 which was similar to non-ICI group (average age of 66.27 ± 6.20). The majority of patients in both groups were males (non-ICI group vs ICI group: 81.8% vs 100%). BMI were within the normal adult range in both groups, although ICI group had significantly higher BMI than non-ICI group (23.53 ± 2.45 vs 20.75 ± 2.17, p=0.015). The most common comorbidities in both groups were chronic obstructive pulmonary disease (COPD) (non-ICI group vs ICI group: 36.4% vs 44.4%) and hypertension (non-ICI group vs ICI group: 36.4% vs 44.4%). There were 2 patients (22.2%) with renal insufficiency in the ICI group, and none in the non-ICI group.

History of lung cancer and ICI use

The cancer subtypes and stage were similar between both groups. The percentage of patients receiving chemotherapy and surgery in both groups were similar as well (Table 2). The ICI group were less likely to receive chest radiotherapy than non-ICI group with borderline significance (44.4% vs 90.1%, p=0.05). Five patients (45.4%) in non-ICI group used corticosteroids prior to onset of PJP due to radiotherapy

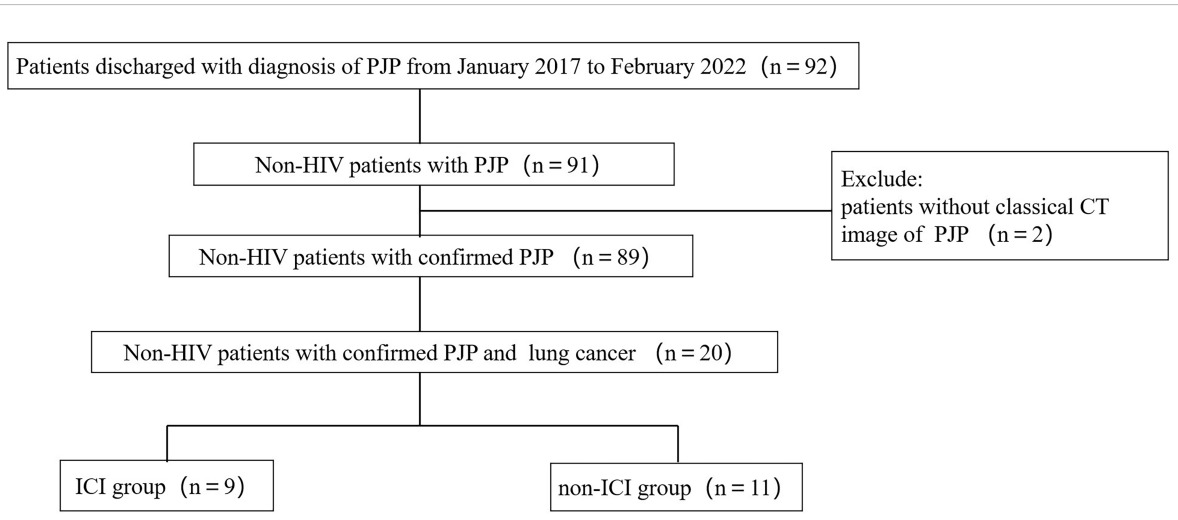

FIGURE 1
Flow chart of study population. PJP, *Pneumocystis jiroveci* pneumonia; HIV, human immunodeficiency virus; ICI, immune checkpoint inhibitor.

associated adverse events. Three patients (33.3%) in ICI group used prior corticosteroids. Of those 3 patients, two patients used corticosteroids due to interstitial pneumonia, and one patient used corticosteroids due to acute exacerbation of COPD. The ICI used in ICI group were as follows: tislelizumab (33.3%), pembrolizumab (22.2%), camrelizumab (22.2%) and sintilimab (22.2%).

PJP characteristics and treatment

There was a clear trend to a shorter onset of PJP in ICI group, although without statistical significance (ICI group vs non-ICI group: 118.9 ± 60.9 vs 253.0 ± 185.1 days, p=0.053) (Table 3). The CURB65 score was not different between two groups, which indicated that the severity of PJP between two groups was similar. Bronchoscopic alveolar lavage fluid (BALF) were collected from all patients and were the specimens from which PJ were identified. In both groups, mNGS were the most used diagnostic techniques (ICI group vs non-ICI group: 72.7% vs 66.6%). The cellular immunity profile was similar between two groups. Corticosteroids treatment after diagnosis of PJP were similar. Patients in both groups were treated with trimethoprim/sulfamethoxazole (TMP-SMZ) except for one patient in ICI group due to rapid death after admission. Most patients in both groups received corticosteroids use after diagnosis of PJP (ICI group vs non-ICI group: 90.1% vs 66.7%).

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

Variables	Non-ICI group (n=11)	ICI group (n=9)	p
Age	66.27 (6.20)	69.11 (4.99)	0.282
Male	9 (81.8%)	9 (100%)	0.167
BMI	20.75 (2.17)	23.53 (2.45)	**0.015**
Smoking history			0.638
Ever	4 (36.4%)	2 (22.2%)	
Current	5 (45.4%)	6 (66.6%)	
Never	2 (18.2%)	1 (11.1%)	
Pack-years	40 (30.00, 40.00)	40 (30.00, 47.50)	0.648
Comorbidities			
COPD	4 (36.4%)	4 (44.4%)	0.888
Asthma	0	0	—
ILD	0	0	—
Hypertension	4 (36.4%)	4 (44.4%)	0.731
Diabetes mellitus	0	0	—
Renal insufficiency	0	2 (22.2%)	0.169
Lung function test#			
FEV1	2.09 (0.46)	1.94 (0.76)	—
FEV1% predicted	85.05 (16.08)	70.08 (22.79)	—
FVC	2.75 (0.52)	2.91 (0.80)	—
FVC % predicted	90.42 (17.85)	81.35 (17.99)	—
DLCO % predicted	5.17 (0.44)	4.56 (2.03)	—
No spirometry performed	4 (36.4%)	6 (66.6%)	—

All data are presented as No. (%), median (interquartile range), or mean (standard deviation). BMI, body mass; COPD, chronic obstructive pulmonary disease; ILD, interstital 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 very small sample size.
"—", means that no statistical analysis can be performed between two groups due to very small sample size or no comparison.

TABLE 2 History of lung cancer and ICI use.

Variables	Non-ICI group (n=11)	ICI group (n=9)	p
Histology			0.465
Adenocarcinoma	5 (45.4%)	2 (22.2%)	
Squamous cell carcinoma	4 (36.4%)	5 (55.5%)	
Small cell carcinoma	2 (18.2%)	1 (11.1%)	
Others	0	1 (11.1%)	
Cancer Stage			0.463
II	2 (18.2%)	0 (0%)	
III	6 (54.5%)	4 (44.4%)	
IV	3 (27.3%)	5 (55.5%)	
Prior cancer treatment			
Thoracic surgery	4 (36.4%)	3 (33.3%)	1.00
Thoracic radiotherapy	10 (90.1%)	4 (44.4%)	0.050
Chemotherapy	10 (90.1%)	9 (100%)	1.00
Corticosteroids use prior to onset of PJP			
Use of corticosteroids	5 (45.4%)	3 (33.3%)	0.67
Daily dose of corticosteroids	28.80 (20.26)	17.67 (19.50)	0.475
Cause of corticosteroids use			
Radiotherapy associated adverse events	5 (45.4%)	—	
Interstitial pneumonia	—	2 (22.2%)	
AECOPD	—	1 (11.1%)	
ICI			
Pembrolizumab	—	2 (22.2%)	—
Camrelizumab	—	2 (22.2%)	—
Tislelizumab	—	3 (33.3%)	—
Sintilimab	—	2 (22.2%)	—

All data are presented as No. (%), median (interquartile range), or mean (standard deviation). ICI, immune checkpoint inhibitors; PJP, P. jirovecii pneumonia; AECOPD, acute exacerbation of chronic obstructive pulmonary disease.
"—", means that no statistical analysis can be performed between two groups due to very small sample size or no comparison.

TABLE 3 PJP characteristics and treatment.

Variables	Non-ICI group (n=11)	ICI group (n=9)	p
Onset time of PJP	253.0 (185.1)	118.9 (60.9)	0.053
Baseline Performance Status	0.00 (0.0,1.0)	1.00 (0.0,1.75)	0.254
CURB65	1.00 (0,2.0)	1.0 (0.5,2.0)	0.754
Bronchoscopic alveolar lavage	11 (100%)	9 (100%)	1.00
Diagnostic tools			
Hexamine silver staining of BALF	4 (36.4%)	4 (44.4%)	1.00
PCR of BALF	4 (36.4%)	4 (44.4%)	1.00
mNGS of BALF	8 (72.7%)	6 (66.6%)	1.00
Blood test results			
CRP	43.6 (12.1, 102)	32.7 (19.28,64.35)	0.414
D-dimer	880 (540,1420)	1455 (655,2427.5)	0.305
Albumin	34.69 (4.60)	31 (5.00)	0.103
White blood cell count	5.77 (2.86)	6.05 (1.82)	0.801
Neutrophil count	4.74 (2.71)	4.79 (1.89)	0.962
Lymphocyte count	0.58 (0.48)	0.66 (0.21)	0.651
Eosinophil count	0.03 (0.01, 0.1)	0.04 (0.025, 0.075)	0.541
Hemoglobin	110.18 (21.87)	109.78 (24.94)	0.97
Platlets count	127.18 (63.74)	165.78 (38.91)	0.13
Cellular immunity			
Percentage of total T-cells	75.67% (10.46%)	71.87% (11.46%)	0.500
Total T-cells count	308.49 (110.32,842.80)	445.28 (280.80,608.31)	0.115
Helper T-cells (CD3+CD4+)count	138.75 (61.43,419.87)	278.08 (124.60,325.62)	0.203
Percentage of Helper T-cells (CD3+CD4+)	36.2% (8.48%)	41.53% (16.53%)	0.456
Cytotoxic T-cells (CD3+CD8+)count	157.68 (48.06,387.93)	175.20 (138.60,272.49)	0.643
Percentage of Cytotoxic T-cells (CD3+CD8+)	39.52% (6.28%)	28.78% (12.04%)	0.054
CD4/CD8 ratio	1.02 (0.64,1.29)	1.83 (0.90,1.88)	0.064
Fungal G test			
Bronchoalveolar lavage fluid	263.21 (167.98)	360.28 (240.78)	0.357
Blood	42 (10, 178)	38 (38, 38)	0.862
Corticosteroids use after diagnosis of PJP			
Use of corticosteroids	10 (90.1%)	6 (66.7%)	0.285
Cumulative dose of corticosteroids	3500 (1820, 4970)	960 (666, 3860)	0.115
Daily dose of corticosteroids	219 (193, 268)	179 (135, 238)	0.608
Duration of corticosteroids use	14 (10.25, 21)	9 (4, 16)	0.158
Other treatment			
TMP-SMZ	11 (100%)	8 (88.9%)	0.450
IVIG	1 (9.1%)	2 (22.2%)	0.566
Non-invasive ventilation	1 (9.1%)	1 (11.1%)	1.00
Invasive ventilation	1 (9.1%)	2 (22.2%)	0.566

All data are presented as No. (%), median (interquartile range), or mean (SD).
PJP, pneumocystis jiroveci pneumonia; CURB65, confusion, urea, respiratory rate, blood pressure and age; BALF, bronchoscopic alveolar lavage fluid; PCR, polymerase chain reaction; mNGS, metagenomic next-generation sequencing; TMP-SMZ, trimethoprim/sulfamethoxazole; CRP, C-reactive protein; IVIG, intravenous immunoglobins.

Kaplan-Meier analysis

Kaplan-Meier analysis revealed a significant difference in all-cause mortality after PJP onset between the two groups. Within 28 days after the onset of PJP, mortality was significantly higher in the ICI group than non-ICI group (33.3% vs 0, p=0.042) (Figure 2).

Discussion

Our study reported that lung cancer patients with ICI use had a higher mortality rate after PJP infection than patients without ICI use. Our study also revealed that there was a trend towards shorter onset of PJP in patients receiving ICI. To the best of our knowledge, this study was the first retrospective study of the impact of ICI on mortality of PJP in lung cancer patients with including a comparator group. Although the overall incidence of PJP was low in patients with ICI use, it might bring severe consequence. So when there were patients presented with ground-glass opacity, physicians should be alert to the occurrence of PJP. In the future, prospective studies with larger sample size and a multi-center design are warranted to further verify the present results.

The full picture of PJP with ICI use remained mostly unknown. Most reported studies in this area were case reports/series (8). So far the most comprehensive study about PJP infection associated with ICI was an analysis base on the FDA FAERS database. The indications of ICI use in the study were lung cancer, melanoma, renal cell carcinoma and Hodgkin's disease. In the study, 677 reports of PJP associated with ICI were identified, in which 300 (44.3%) PJP cases with fatal outcome (8). The ICI showed a lower

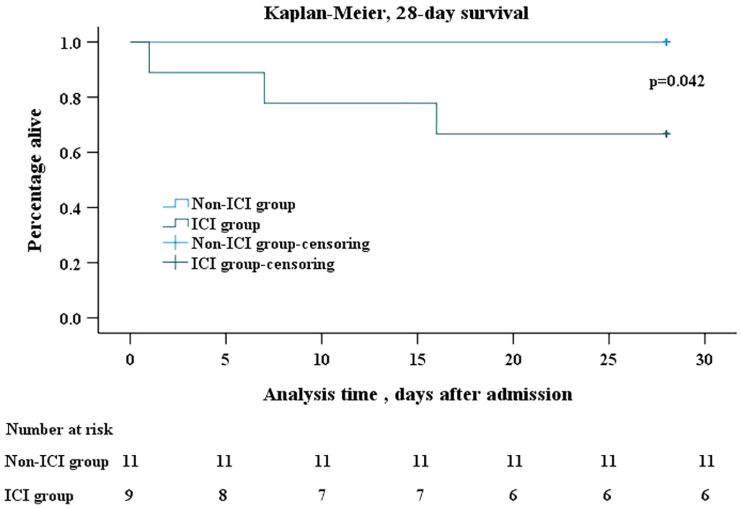

FIGURE 2
Kaplan-Meier survival analysis. Kaplan–Meier analysis of survival in 28 days after onset of PJP showed that mortality was significantly higher in ICI group than in non-ICI group (log rank, p=0.042). In the Kaplan–Meier analysis, censoring mean the total survival time for that subject cannot be accurately determined. The days after admission refer to the days after the patents' admission to hospital due to PJP. The number at risk refer to patients infected with PJP who were still alive but at risk of death. PJP, *Pneumocystis jiroveci* pneumonia; ICI, immune checkpoint inhibitor.

signal of PJP than traditional chemotherapy. Male gender and age >65 years were predominant in PJP cases associated with across all ICI. With expanding use of ICI worldwide and continuing release of new ICI agents, the absolute number of PJP cases were expected to rise. More studies on the area were warranted, and the current study aimed to evaluate the mortality risk of ICI in PJP patients with lung cancer.

In current study, lung cancer patients with ICI use had a higher risk of death after PJP infection than patients without ICI use. As far as we knew, there was no similar report before. In the study conducted by Malek et al, researchers reported a similar infection-related mortality between patients treated with ICI combined with chemotherapy and those treated with chemotherapy alone. But those infectious episodes were most caused by bacteria, and none was PJP. So far it was generally believed that ICI use didn't increase the risk of infection including PJP in cancer patients, but it remained unknown if ICI use increased the risk of death after PJP infection. Our study provided preliminary evidence to show ICI use might increase the risk of death after PJP infection. But our finding should be interpreted with caution, because of small sample size. So future multi-center studies with large sample size were needed to further verify our findings.

A possible reason why ICI use brought higher death risk was potential confounding checkpoint inhibitor-associated pneumonia (CIP) (18). As potentially fatal irAE caused by ICI, CIP was characterized by the presence of new infiltrative shadows on chest imaging and respiratory signs/symptoms related to a new emerging infiltration viewed on a chest imaging but excluding new infections or alternative etiologies (19). The incidence of CIP ranged from 2% to 38% in non-small cell lung carcinoma (NSCLC) in clinical trials and 4.8% to 39.3% in real-world studies (20).Although there was no consensus on the diagnostic evaluation of CIP, exclusion of new infection was a prerequisite for diagnosis (21). PJP and CIP may present with similar clinical manifestations. On chest Computer Tomography (CT), PJP presented as bilateral interstitial infiltrates and bilateral ground-glass exudate (22). But pulmonary ground-glass exudate, the classic radiographic pattern of PJP, was also a common radiographic pattern in CIP (23). By the current consensus definition, PJP and CIP couldn't co-exist. But it was possible that patients had PJP and CIP at the same time, and the diagnosis of PJP based on detection of PJP from respiratory specimens excluded CIP. Consequently, the underdiagnosis of CIP may lead to improper management, resulting in increased mortality in patients. This may be a possible reason for the higher mortality rate in patients with ICI use. But with current definition of CIP, this possibility couldn't be verified.

The mortality of PJP reported in current study was lower than previous reports. The current study reported a 28-day mortality of 33.3% in the ICI group and of 0 in non-ICI group. In published studies, the mortality of PJP in non-HIV-infected patients varied from 35% to 55% (5, 7, 24). A retrospective study conducted in Germany reported a mortality of 40% in patients with solid malignancies (5). This discrepancy might be explained by timely and accurate diagnosis of PJP via wide use of BALF sample and mNGS. Early diagnosis of PJP was critical for improving clinical outcomes, and early initiation of TMP/SMZ was significantly associated with reduced mortality (4, 17). But *in vitro* culture of *Pneumocystis jiroveci* was extremely difficult, and establishing a microbiological diagnosis of PJP remained a challenge. So the selection of the proper samples and detection methods was crucial in diagnosis of PJP. On one hand, the current gold standard sample for diagnosis of PJP was BALF, which was considered to be the highest quality respiratory sample (4). The main superiority of BALF was its proximity to the site of pulmonary infection, which was a good indication of the local lung environment (24). In the current study, BALF were collected from all patients and used for detection of PJ,

which provided excellent sensitivity and specificity. On other hand, there were various detection methods of PJ, with different sensitivity and specificity. In the past, PJP was usually diagnosed based on direct-view techniques with different staining tests or immunofluorescence method, which had proven to be insensitive (25). Molecular tests such as PCR showed good sensitivity and specificity in diagnosis of PJP (25, 26). But suspicion of PJP was an essential prerequisite for physician to order PCR test, which were not necessarily the case in clinical practice. In recent years, mNGS had been developed to provide information on the Deoxyribonucleic Acid (DNA) sequence of microbial genomes (27). The mNGS allowed sequence-based identification of all potential pathogens, and it helped to identify specific pathogens for most unexpected cases, which might be lifesaving in critical scenarios. Previous reports showed that the mNGS was highly efficient in the diagnosing PJP (28–30). According to a meta-analysis, which included 418 cases diagnosed with PJP and 925 controls, the pooled sensitivity and specificity of BALF mNGS for diagnosis of PJP was 0.957 and 0.939 respectively (24). So the combination of BALF sample and mNGS might improve diagnosis efficiency of PJP, and timely and accurate diagnosis of PJP subsequently promoted targeted therapy against PJP and reduced mortality.

The profile of patients included in the study was in agreement with that of previous study. The ICI group had an average age of 69.11 ± 4.99 and 100% of male. In the FAERS database analysis of PJP, it was reported that male gender and age >65 years were predominant in PJP cases associated with ICI (8). This was also consistent with published case reports. By Xia's account, on published case reports, 53.3% PJP cases associated with ICI were male and age more than 65 (8). This similarity lent more credibility to our findings.

The current study also revealed that there was a trend towards shorter onset of PJP in patients receiving ICI, although without statistical significance. As far as we knew, there was no similar report before. In the study by Malek, the results showed that duration between therapy initiation and infection onset was similar between patients treated with ICI combined with chemotherapy and those treated with chemotherapy alone (14). This finding suggested that ICI use might accelerate the onset of PJP in lung cancer patients, but it needed further validation.

The current study has a potentially important clinical implication for physicians. According to our findings, although ICI might not increase the incidence of PJP, it might cause higher mortality in PJP patients. It is well known that TMP-SMZ are very effective for both prevention and treatment of PJP (1, 31). So on one hand, the physicians should be in alert to determine those patients who are at greatest risk for developing PJP. Although so far no general strategy exists for identifying such populations, at least patients with long-term use of corticosteroids should be considered to be potential candidates for TMP-SMZ prophylaxis (2). On the other hand, when there is new onset of respiratory symptoms and ground-glass opacity on CT, physicians need to be vigilant regarding the possible development of PJP. In that case, mNGS for BALF samples should be preferred and used on time. The proper prophylaxis and timely treatment of PJP would bring significant survival benefit to the patients.

The major strength of our study was that it was the first to compare mortality in PJP patients with lung cancer between those treated with ICI and a concurrent control group treated without ICI. However, our study was subject to some limitations. First, the single-center retrospective design made it impossible to determine the causal relationship between ICI use and mortality. The retrospective design was also prone to missing data and bias due to reliance on documents available for review. Second, due to small size of PJP patients with lung cancer, no propensity score matching could not be applied to minimize bias. Third, despite the combined use of clinical symptoms, radiographic findings, and pathogen detection for PJP diagnosis, the possibility of including patients with PJ colonization cannot be fully eliminated.

Conclusion

To the best of our knowledge, the present study provided preliminary evidence to show that lung cancer patients with ICI use had a higher mortality rate after PJP infection than patients without ICI use for the first time. Although the overall incidence of PJP was low in patients with ICI use, it might bring severe consequence. So when there were patients presented with ground-glass opacity, physicians should be alert to the occurrence of PJP. In the future, prospective studies with larger sample size and a multi-center design are warranted to further verify the present results.

Data availability statement

Due to the potential compromise of patient privacy, the data sets generated and/or analyzed in the current study are not publicly available, but are available from the corresponding authors upon reasonable request. Requests to access these datasets should be directed to YM (email: 2314023@zju.edu.cn).

Ethics statement

The studies involving humans were approved by Ethics Committee of Second Affiliated Hospital of Zhejiang University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because the non-interventional retrospective study was determined to be no greater than minimal risk.

Author contributions

BF: Conceptualization, Software, Writing – original draft, Data curation, Formal analysis, Investigation, Visualization. XS: Conceptualization, Data curation, Writing – original draft, Investigation. WH: Conceptualization, Data curation,

Writing – original draft, Formal analysis, Investigation. YZ: Conceptualization, Writing – original draft, Data curation. FC: Conceptualization, Data curation, Writing – original draft, Investigation. FL: Funding acquisition, Writing – review & editing, Methodology, Project administration. WL: Funding acquisition, Writing – review & editing, Methodology, Project administration. YM: Methodology, Writing – review & editing, Conceptualization, Project administration, Resources, Supervision, Funding acquisition, Validation.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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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frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 09 August 2024
DOI 10.3389/fonc.2024.1403392

OPEN ACCESS

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RECEIVED 19 March 2024
ACCEPTED 23 July 2024
PUBLISHED 09 August 2024

CITATION
Sun T, Liu J, Yuan H, Li X and Yan H (2024) Construction of a risk prediction model for lung infection after chemotherapy in lung cancer patients based on the machine learning algorithm.
Front. Oncol. 14:1403392.
doi: 10.3389/fonc.2024.1403392

Construction of a risk prediction model for lung infection after chemotherapy in lung cancer patients based on the machine learning algorithm

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Purpose: The objective of this study was to create and validate a machine learning (ML)-based model for predicting the likelihood of lung infections following chemotherapy in patients with lung cancer.

Methods: A retrospective study was conducted on a cohort of 502 lung cancer patients undergoing chemotherapy. Data on age, Body Mass Index (BMI), underlying disease, chemotherapy cycle, number of hospitalizations, and various blood test results were collected from medical records. We used the Synthetic Minority Oversampling Technique (SMOTE) to handle unbalanced data. Feature screening was performed using the Boruta algorithm and The Least Absolute Shrinkage and Selection Operator (LASSO). Subsequently, six ML algorithms, namely Logistic Regression (LR), Random Forest (RF), Gaussian Naive Bayes (GNB), Multi-layer Perceptron (MLP), Support Vector Machine (SVM), and K-Nearest Neighbors (KNN) were employed to train and develop an ML model using a 10-fold cross-validation methodology. The model's performance was evaluated through various metrics, including the area under the receiver operating characteristic curve (ROC), accuracy, sensitivity, specificity, F1 score, calibration curve, decision curves, clinical impact curve, and confusion matrix. In addition, model interpretation was performed by the Shapley Additive Explanations (SHAP) analysis to clarify the importance of each feature of the model and its decision basis. Finally, we constructed nomograms to make the predictive model results more readable.

Results: The integration of Boruta and LASSO methodologies identified Gender, Smoke, Drink, Chemotherapy cycles, pleural effusion (PE), Neutrophil-lymphocyte count ratio (NLR), Neutrophil-monocyte count ratio (NMR), Lymphocytes (LYM) and Neutrophil (NEUT) as significant predictors. The LR model demonstrated superior performance compared to alternative ML algorithms, achieving an accuracy of 81.80%, a sensitivity of 81.1%, a specificity of 82.5%, an F1 score of 81.6%, and an AUC of 0.888(95%CI(0.863-0.911)). Furthermore, the SHAP method identified Chemotherapy cycles and Smoke as the primary decision factors influencing the ML model's predictions. Finally, this study successfully constructed interactive nomograms and dynamic nomograms.

Conclusion: The ML algorithm, combining demographic and clinical factors, accurately predicted post-chemotherapy lung infections in cancer patients. The LR model performed well, potentially improving early detection and treatment in clinical practice.

KEYWORDS

lung infection, chemotherapy, machine learning, logistic regression, predictive model, nomogram

1 Introduction

Lung cancer, being one of the most prevalent malignant neoplasms globally, presents a substantial risk to both the survival and well-being of affected individuals (1). The World Health Organization's data indicates that lung cancer exhibits the highest incidence and mortality rates among all cancer types (2). Despite notable advancements in lung cancer therapy, the effective management of post-chemotherapy complications remains a significant hurdle (3–5). Of particular concern is the high prevalence of lung infections following chemotherapy in lung cancer patients, which seriously affects the therapeutic effect and survival quality of patients (6). The presence of lung infections in lung cancer patients not only exacerbates their health status but also has the potential to impede or halt chemotherapy, thereby impacting the overall efficacy of treatment. Furthermore, lung infections contribute to escalated medical expenses, extended hospital stays, and heightened mortality rates (7). Consequently, the timely and precise identification of the likelihood of lung infections following chemotherapy is crucial for informing clinical interventions and enhancing patient outcomes.

The utilization of ML technology in the healthcare sector has experienced significant growth in recent years, showcasing robust data processing and pattern recognition capabilities. ML algorithms have exhibited promise and efficacy in lung cancer diagnosis, treatment selection, and prognosis assessment (8, 9). Notably, the analysis of extensive clinical data through ML algorithms can aid healthcare professionals in identifying potential disease development patterns, facilitating personalized treatment strategies, and enhancing treatment outcomes (10–12). Conventional approaches to evaluating the risk of lung infection rely heavily on the subjective judgment and clinical expertise of healthcare professionals, necessitating a greater degree of objectivity and precision. In light of this prevailing situation, the utilization of ML technology presents novel opportunities for addressing this issue by leveraging ML algorithms to analyze extensive patient data, potential correlations and patterns can be identified, enabling healthcare providers to make more precise predictions regarding the likelihood of lung infection following chemotherapy in individuals with lung cancer.

In recent studies, researchers have utilized various ML algorithms to create predictive models aimed at aiding physicians in evaluating the likelihood of complications in lung cancer patients following chemotherapy or surgical procedures. While previous research has explored the application of ML in forecasting complications in lung cancer patients, there is a notable scarcity of studies focusing on predicting the likelihood of lung infection following chemotherapy. Consequently, the current study seeks to address this gap by introducing and refining a prediction model utilizing ML algorithms to identify lung cancer patients at risk of post-chemotherapy lung infection. This study posits that an interpretable ML-based algorithm will achieve the most accurate predictions if significant predictors are identified through an effective feature selection method. Therefore, the objective of this study was to create and evaluate a proficient and interpretable ML system for forecasting the likelihood of lung infection following chemotherapy in Chinese lung cancer patients. Our research findings offer a novel approach for early identification of infection risk in lung cancer patients while also contributing to the advancement of ML in oncology clinical investigations. Moving forward, we intend to enhance the precision and reliability of the model, facilitate its integration into clinical settings, and offer enhanced scientific and precise assistance for the care and oversight of lung cancer patients.

2 Materials and methods

2.1 Study design

This study was conducted to develop a machine learning-based model for predicting the risk of lung infections following chemotherapy in lung cancer patients. The retrospective study included a cohort of 502 lung cancer patients who had undergone chemotherapy, aged 18 years and above, and had completed at least one cycle of treatment. Data encompassing demographic details, medical history, chemotherapy specifics, and blood test results were extracted from the hospital's electronic medical record system. The SMOTE algorithm is used to solve the category imbalance problem. The Boruta algorithm and LASSO regression performed feature screening to identify the features most associated with the risk of lung infection. Subsequently, a range of ML models, including LR, RF, GNB, MLP, SVM, and KNN, were developed and refined by

applying a 10-fold cross-validation methodology. The performance of these models was assessed using various metrics, including accuracy, sensitivity, specificity, positive predictive value, negative predictive value, F1 score, Kappa score, AUC, calibration curve, calibration curves, Clinical Impact Curve and confusion matrix. To enhance the transparency and interpretability of the model, the SHAP method was employed to interpret the predicted results and elucidate the impact of each feature on the predictions, thereby offering a practical reference for clinicians. Figure 1 explains the overall workflow of the proposed system more clearly.

2.2 Study data

This retrospective study examined data from lung cancer patients at The Central Hospital of Shaoyang between January 2020 and December 2023. The study included adult patients aged 18 years and older who had not experienced lung infections within a week before receiving chemotherapy. Patient records with missing or abnormal data were excluded to maintain data quality. The study's rigorous inclusion and exclusion criteria aimed to ensure the completeness and reliability of the information on included cases, thus providing a high-quality database for evaluating the risk of lung infections in lung cancer patients after chemotherapy. Inclusion criteria: (i) adult patients aged ≥18 years, (ii) patients diagnosed with lung cancer and treated with chemotherapy, (iii) patients who did not have any lung infection before chemotherapy, and (iv) patients with complete clinical information; Exclusion criteria: (i) patients with mental illness or intellectual disability, (ii) patients with missing or abnormal data, and (iii) exclusion of patients with a combination of other tumors.

2.3 Research variables

The study encompassed 36 predictors related to demographic factors (gender, age), lifestyle habits (history of alcohol consumption, history of smoking), medical history (history of diabetes, history of hypertension, history of coronary heart disease), physical characteristics (BMI), disease severity (stage at diagnosis, histologic features, presence or absence of pleural effusion), treatment information (cycles of chemotherapy,

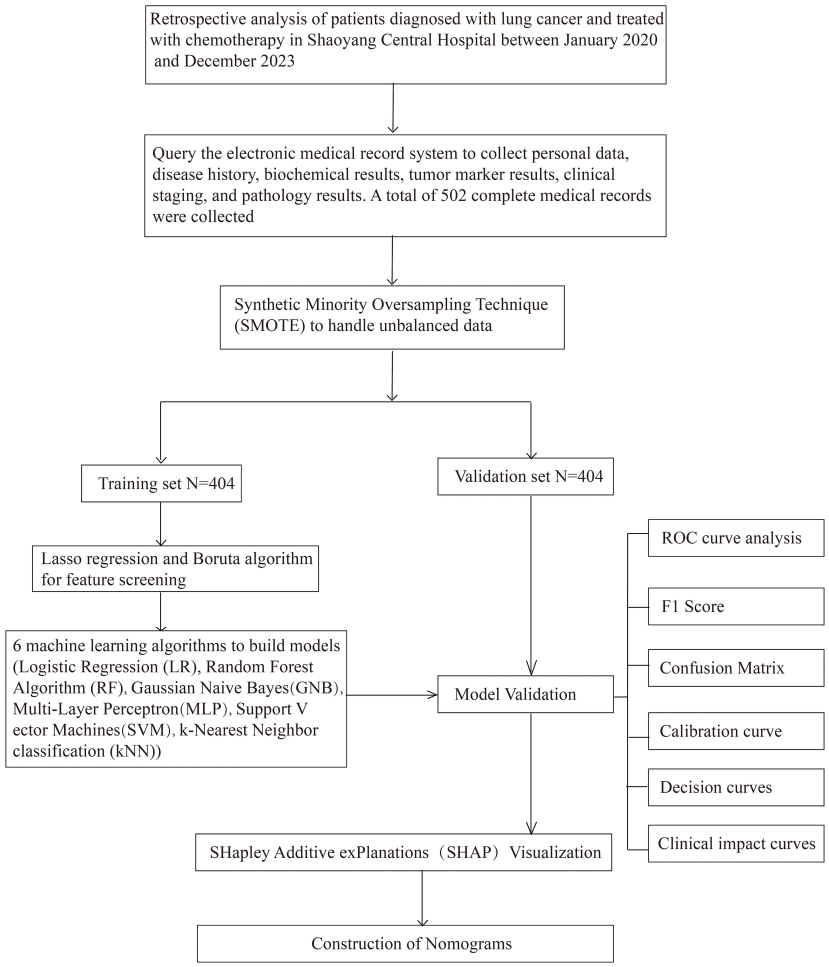

FIGURE 1
Research flowchart.

number of hospitalizations), and laboratory values (leukocytes, erythrocytes, hemoglobin, platelets, percentage of neutrophils, percentage of lymphocytes, percentage of monocytes, NLR, NMR, neutrophil-platelet count ratio (NPR), indirect bilirubin, alanine aminotransferase, glutamine aminotransferase, total bilirubin, direct bilirubin, total protein, albumin, globulin, white globule ratio, urea, creatinine, uric acid, and CEA). Of these, gender, age, history of alcohol consumption, history of smoking, history of diabetes mellitus, history of hypertension, history of coronary artery disease, BMI, tumor typing, cycles of chemotherapy, number of hospitalizations, and the presence or absence of pleural effusions were the data before the last chemotherapy session. The other laboratory data were obtained after the last chemotherapy. A brief description of the study variables is given in Table 1.

2.4 Diagnostic criteria of pulmonary infection after chemotherapy

The diagnostic criteria for pulmonary infection in patients with lung cancer following chemotherapy encompass a body temperature exceeding 38°C, the presence of clinical symptoms indicative of pulmonary infection (e.g., cough and expectoration),

TABLE 1 Description of the study variables.

SN	Predictors	Description	Types	Values
1	Gender	Sex of the patient	Categorical	1 male 2 female
2	Age	Age of the patient (years)	Continuous	35-83
3	Drink	History of alcohol consumption	Categorical	0 No history of alcohol consumption 1 History of alcohol consumption
4	Smoke	History of smoking	Categorical	0 No history of smoking 1 History of smoking
5	Diabetes	History of diabetes	Categorical	0 No history of diabetes 1 History of diabetes
6	Hypertension	History of Hypertension	Categorical	0 No history of hypertension 1 History of hypertension
7	CHD	History of coronary heart disease	Categorical	0 No history of coronary heart disease 1 History of coronary heart disease
8	BMI	Body mass index (kg/m2)	Continuous	11.43-31.83
9	Stage	Stage at diagnosis, Count (%)	Categorical	Stage 1 24(4.78%) Stage 2 59(11.75%) Stage 3 204(40.64%) Stage 4 215(42.83%)
10	Histology	Histologic features, Count (%). 1, Adenocarcinoma; 2, Squamous; 3, SCLC; 4, Other lung cancers	Categorical	Grade1 222(44.22%) Grade2 189(37.65%) Grade3 80(15.94%) Grade4 11(2.19%)
11	Chemotherapy cycles	The Number of chemotherapy cycles	Continuous	1-32
12	Hospitalizations	Total number of hospitalizations	Continuous	1-45
13	PE	The presence of pleural effusion	Categorical	0 No pleural effusion 1 With pleural effusion
14	WBC	White blood cell	Continuous	1.31-60.80
15	RBC	Red blood cell	Continuous	1.44-6.20
16	HGB	Hemoglobin	Continuous	53.00-9792.00
17	PLT	Platelet	Continuous	22.00-631.00
18	NEUT	Percentage of Neutrophil	Continuous	0.44-98.21
19	LYM	Percentage of Lymphocytes	Continuous	1.42-65.50
20	NLR	Neutrophil-Lymphocyte count ratio	Continuous	0.02-69.16

(Continued)

TABLE 1 Continued

SN	Predictors	Description	Types	Values
21	NMR	Neutrophil-Monocyte count ratio	Continuous	0.01-311.37
22	NPR	Neutrophil-Platelet count ratio	Continuous	0.01-3.67
23	MONO	Percentage of Monocytes	Continuous	0.30-63.20
24	IBIL	Indirect bilirubin	Continuous	2.20-90.40
25	ALT	Glutamic pyruvic transaminase	Continuous	2.70-888.60
26	AST	Aspartate aminotransferase	Continuous	3.80-591.20
27	TBIL	Total bilirubin	Continuous	1.90-297.60
28	DBIL	Direct bilirubin	Continuous	0.13-207.20
29	TP	Total protein	Continuous	22.50-85.80
30	ALB	Albumin	Continuous	10.70-51.04
31	GLB	Globulin	Continuous	11.96-51.90
32	A/G	White ball ratio	Continuous	0.48-3.99
33	Urea	Urea	Continuous	1.25-32.97
34	CREA	Creatinine	Continuous	34.70-367.90
35	UA	Uric acid	Continuous	78.30-1201.40
36	CEA	CEA	Continuous	0.20-1500.00

the identification of moist rales in the lungs, and the visualization of a distinct infectious focus on CT imaging. Should a lung cancer patient meet at least three of these criteria within 14 days post-operation, a diagnosis of post-chemotherapy lung infection is warranted.

2.5 Feature screening

2.5.1 Least absolute shrinkage and selection operator

The LASSO regression enhances model refinement by implementing a penalty function that compresses certain regression coefficients, thereby enforcing a constraint on the sum of their absolute values to be below a predetermined threshold (13, 14). We utilize the glmnet package in R for LASSO regression, setting family="binomial" to apply to our binary outcome data. The key parameter alpha is set to 1, and the LASSO method is used entirely. Through cross-validation with the cv.glmnet function, we chose two lambda values: lambda.min and lambda.1se. The former minimizes the cross-validation error, while the latter provides a cleaner model, which together help us to balance the complexity of the model with the prediction accuracy. Ultimately, we filter out variables that are significant to the predictions based on non-zero coefficients, simplifying the model and improving its interpretability.

2.5.2 Boruta

The Boruta algorithm is a Random Forest-based feature selection and packaging algorithm that evaluates the importance of features by generating "shadow variables" corresponding to each original variable in the dataset (15). In particular, Boruta (Version: 8.0.0) is executed to perform feature selection, where the algorithm iteratively compares the importance of each original variable with its shadow variable, and determines the importance of each variable over 500 iterations or until all variables are stable. Importance results are extracted with the attStats function and formatted with a customized adjustdata function (16).

2.6 Machine learning algorithms

2.6.1 Logistic regression algorithm

In this study, we used a logistic regression (LR) model to predict the probability of infection in patients receiving chemotherapy, defined as a binary classification problem that predicts the risk of infection based only on clinical features (17). The logistic regression model used L2 regularization with the regularization factor (C) set to 1.0, a maximum number of iterations of 100, and a convergence tolerance (tol) of 0.0001.These parameters help prevent model overfitting while ensuring convergence and computational efficiency of the algorithm.

2.6.2 Random forest algorithm

The RF algorithm is an ML technique that enhances predictive accuracy by generating multiple decision trees. RFs excel in analyzing extensive datasets with high-dimensional features, effectively managing intricate relationships among data variables (18). In this research, RFs are employed to identify non-linear associations and enhance the model's ability to generalize. In the Random Forest model, the Gini Index is used as the splitting

criterion, the number of trees is set to 20, the maximum depth of the tree is not restricted, and the minimum impurity reduction is set to 0.0. This parameter configuration is designed to allow the model to fully learn the complex structure in the data, and to improve the accuracy and generalization of the prediction.

2.6.3 Gaussian Naive Bayes algorithm

The GNB classifier is a straightforward probabilistic model grounded in Bayes' theorem, predicated on the feature independence assumption. While this assumption may not hold true in all practical scenarios, GNB remains highly effective in numerous instances owing to its simplicity and computational efficiency (19). The Gaussian Naive Bayes model does not set a specific prior probability, and the variable smoothing parameter is set to 1e-09. this setting allows the model to be more accurate when performing probability calculations, especially when dealing with datasets with continuous characteristics.

2.6.4 Multi-layer perceptron algorithm

The MLP is a feed-forward artificial neural network model capable of processing data through multiple layers to learn non-linear features (20). It is well-suited for complex pattern recognition tasks. In this research, we employ MLP to develop a sophisticated predictive model for assessing the risk of lung infection following chemotherapy, the multilayer perceptron model uses ReLU as the activation function, and the structure of the hidden layer is set to two layers containing 20 and 10 neurons, respectively, with a maximum number of iterations of 20.

2.6.5 Support vector machine algorithm

Support Vector Machine (SVM) is robust classifiers utilized to discern between classes by identifying optimal decision boundaries within data points. SVMs are especially adept at processing high-dimensional data and excel in scenarios where data boundaries are ambiguous (21, 22). In this study, the SVM model selects Radial Basis Function (RBF) as the kernel function, with the regularization parameter C set to 1.0 and the tolerance to 0.001. This setting helps the model to effectively identify complex decision boundaries while controlling overfitting when dealing with high-dimensional data.

2.6.6 K-Nearest neighbor algorithm

The KNN is utilized to predict the category of a given sample point by examining the categories of its K-nearest neighbors. This method, known for its simplicity and intuitive nature, does not necessitate explicit model training (23). In this study, The number of neighbors of the KNN model is set to 5 and a uniform weighting method is used. This setting simplifies the computational process of the model and allows the model to predict the classification of new samples based directly on the nearest few samples for effective classification.

2.7 SHAP interpretability analysis

The SHAP is a technique utilized to interpret predictions generated by ML models, particularly those that are intricate and incorporate numerous features (24). The fundamental principle underlying this method involves the computation of the incremental impact of individual features on the model's output, enabling interpretation of the model's behavior at both a global and local scale. This is achieved through the development of an additive explanatory model that considers all features as contributors, thereby facilitating the calculation of the average incremental impact of each feature across all feasible feature combinations to derive a SHAP value for each feature, which provides both global and local interpretations, helping to understand which features are the main influences on model predictions, as well as the predictions of individual samples—factors, as well as the prediction results for a single sample (25).

2.8 Statistical analysis

All data analyses in this study were performed using SPSS (17.0), R language (version 4.3.2), Matlab (version R2021a), and Python (version 3.7). The initial analysis of the data set involved the application of descriptive statistics. Data points adhering to a normal distribution were represented as mean ± standard deviation, while those deviating from normal distribution were represented as median (quartiles). Subsequently, the independent samples t-test was employed to compare two groups with normally distributed data. In contrast, the Mann-Whitney U test was utilized to compare two groups with non-normally distributed data. For count data, frequencies and percentages were used to characterize group variances, while the chi-square test or Fisher's exact probability method was employed to assess inter-group discrepancies. We solved the problem of sample imbalance by oversampling a small number of classes and thereby solving the sample imbalance problem through the SMOTE algorithm based on Matlab software. To construct the predictive model, the dataset was partitioned randomly into a training subset comprising 70% of the total data and a test subset comprising 30%. Subsequently, six ML algorithms were employed to train the model using the training subset data. During the model training process, a 10-fold cross-validation method is used to optimize the model parameters and prevent the occurrence of overfitting phenomenon. LASSO regression analysis was conducted utilizing the glmnet package [4.1.7] in R to analyze cleaned data and derive coefficient values of variables, logarithmic values of lambda, and regularized values of L1, followed by data visualization. The Boruta algorithm was implemented using Boruta 8.0.0 [4.1.7] in R. Interpretability analysis was carried out using the Python libraries shap=0.43.0. Statistical significance levels were established at $P<0.05$.

3 Results

3.1 Patient characteristics

This study assembled a cohort of 502 lung cancer patients who did not have lung infections before undergoing chemotherapy. The median age of the patients was 65 years (range: 58-71 years), with 404 (80.48%) being male and 98 (19.52%) being female. We used

the SMOTE algorithm for data imbalance. The original data of 502 cases contained 404 non-infected cases, 98 infected cases, and 19.52% of infected cases, and the processed data of 808 cases contained 404 non-infected cases, 404 infected cases, and 50.00% of infected cases. A comparison of baseline characteristics between the two groups revealed statistically significant differences in chemotherapy cycles, hospitalizations, WBC, pulmonary embolism, Gender, CREA, Histology, alcohol consumption, smoke, CHD, NEUT, LYM, NMR, NPR, IBIL, TBIL, and NLR ($P < 0.05$), as shown in Table 2.

3.2 Predictor screening

A total of 808 patients undergoing chemotherapy for lung cancer after data imbalance were divided into a training group consisting of 565 patients and a test group consisting of 243 patients, following a ratio of 7:3. Statistical analysis revealed no significant differences between the two groups (Table 3). Utilizing the Boruta algorithm, an extension of the RF algorithm, enabled the identification of the actual feature set by accurately estimating the importance of each feature. The Boruta algorithm identified 35 key factors, including Drink, Smoke, Chemotherapy cycles, Hospitalizations, PE, NEUT, LYM, MONO, NLR, and NMR, etc (Figure 2A). In contrast, LASSO regression serves as a compression estimation method that accomplishes variable selection and complexity adjustment through the formulation of an optimization objective function incorporating penalty terms. In this study, LASSO regression was utilized to identify characteristic factors such as Gender, Drink, Smoke, Chemotherapy cycles, PE, NEUT, NLR, NMR, and AST (Figures 2B, C). Through a comparative analysis of the outcomes obtained from LASSO regression and Boruta algorithm screening, we identified a common subset of feature variables selected by both methods. These selected features were ultimately utilized in the construction of the model and consisted of Gender, Drink, Smoke, Chemotherapy cycles, PE, NEUT, AST, NLR, and NMR (Figure 2D).

TABLE 2 Baseline characterization and comparison.

Variables	Total (n = 808)	Pulmonary infection after chemotherapy for lung cancer		*P*
		No (n = 404)	Yes (n = 404)	
Age	65.00 [59.00, 70.00]	65.00 [58.00, 71.00]	65.00 [59.00, 69.00]	0.985
BMI	21.50 [19.70, 23.70]	21.80 [19.50, 24.10]	21.20 [19.80, 23.40]	0.129
Chemotherapy cycles	5.00 [2.00, 8.00]	3.00 [1.00, 5.00]	7.00 [5.00, 11.00]	**<0.001**
Hospitalizations	7.00 [4.00, 12.00]	4.50 [2.00, 7.00]	10.00 [6.00, 15.30]	**<0.001**
WBC	6.90 [5.49, 9.17]	6.56 [5.19, 8.78]	7.07 [5.93, 9.55]	**<0.001**
RBC	3.77 [3.30, 4.15]	3.76 [3.28, 4.17]	3.78 [3.33, 4.15]	0.943
HGB	112.00 [99.90, 125.00]	112.00 [99.00, 125.00]	112.00 [101.00, 124.00]	0.603
PLT	209.00 [160.00, 258.00]	208.00 [161.00,268.00]	212.00 [160.00,241.00]	0.357
NEUT	72.10 [64.30, 79.20]	70.60 [63.10, 78.30]	74.10 [66.00, 79.60]	**<0.001**
LYM	17.30 [12.00, 23.30]	18.80 [12.80, 25.10]	16.10 [11.50, 21.60]	**<0.001**
MONO	7.30 [5.40, 9.48]	7.40 [5.50, 9.73]	7.11 [5.20, 9.10]	0.147
NLR	4.38 [2.83, 7.09]	3.74 [2.53, 6.10]	4.90 [3.33, 7.67]	**<0.001**
NMR	10.10 [7.11, 14.40]	9.34 [6.74, 13.20]	10.90 [7.57, 15.40]	**<0.001**
NPR	0.35 [0.27, 0.43]	0.33 [0.26, 0.42]	0.36 [0.29, 0.44]	**0.001**
IBIL	7.30 [5.70, 9.39]	7.00 [5.31, 9.42]	7.45 [6.10, 9.31]	**0.007**
ALT	18.00 [12.70, 26.30]	17.90 [13.00, 28.30]	18.20 [12.30, 24.90]	0.218
AST	23.40 [19.40, 29.40]	23.80 [18.90, 29.80]	23.20 [19.80, 28.70]	0.858
TBIL	9.89 [7.63, 12.60]	9.46 [7.22, 12.70]	10.10 [8.08, 12.30]	**0.013**
DBIL	2.40 [1.59, 3.40]	2.30 [1.50, 3.43]	2.50 [1.63, 3.38]	0.199
TP	66.80 [62.30, 69.90]	66.30 [61.70, 71.10]	66.90 [62.50, 69.20]	0.636
ALB	40.00 [36.50, 42.50]	39.90 [36.50, 42.70]	40.20 [36.80, 42.30]	0.764
GLB	26.30 [23.50, 29.40]	26.30 [22.30, 30.60]	26.30 [24.30, 28.60]	0.897
A/G	1.54 [1.31, 1.75]	1.52 [1.26, 1.81]	1.55 [1.34, 1.71]	0.943

(Continued)

TABLE 2 Continued

Variables	Total (n = 808)	Pulmonary infection after chemotherapy for lung cancer		*P*
		No (n = 404)	Yes (n = 404)	
Urea	5.89 [4.74, 7.56]	5.73 [4.58, 7.20]	6.05 [4.99, 7.64]	0.059
CREA	78.80 [66.00, 92.30]	76.60 [63.70, 91.90]	82.00 [68.60, 93.10]	**0.004**
UA	330.00 [278.00,394.00]	326.00 [265.00,398.00]	332.00[288.00,393.00]	0.180
CEA	3.69 [2.11, 9.74]	3.70 [2.05, 9.43]	3.68 [2.25, 9.81]	0.556
PE				**<0.001**
No	608 (75.20%)	353 (87.40%)	255 (63.10%)	
Yes	200 (24.80%)	51 (12.60%)	149 (36.90%)	
Gender				**<0.001**
Male	683 (84.50%)	315 (78.00%)	368 (91.10%)	
Female	125 (15.50%)	89 (22.00%)	36 (8.90%)	
Drink				**<0.001**
No	683 (84.50%)	377 (93.30%)	306 (75.70%)	
Yes	125 (15.50%)	27 (6.70%)	98 (24.30%)	
Smoke				**<0.001**
No	518 (64.10%)	322 (79.70%)	196 (48.50%)	
Yes	290 (35.90%)	82 (20.30%)	208 (51.5%)	
Diabetes				0.999
No	731 (90.50%)	366 (90.60%)	365 (90.30%)	
Yes	77 (9.50%)	38 (9.40%)	39 (9.70%)	
Hypertension				0.667
No	636 (78.70%)	321 (79.50%)	315 (78.00%)	
Yes	172 (21.30%)	83 (20.50%)	89 (22.00%)	
CHD				**0.008**
No	759 (93.90%)	370 (91.60%)	389 (96.30%)	
Yes	49 (6.10%)	34 (8.40%)	15 (3.70%)	
Stage				0.053
Stage I	29 (3.60%)	21 (5.20%)	8 (2.00%)	
Stage II	96 (11.90%)	46 (11.40%)	50 (12.40%)	
Stage III	330 (40.80%)	171 (42.30%)	159 (39.40%)	
Stage IV	353 (43.70%)	166 (41.10%)	187 (46.30%)	
Histology				**0.005**
Adenocarcinoma	327 (40.50%)	183 (45.30%)	144 (35.60%)	
Squamous	333 (41.20%)	151 (37.40%)	182 (45.00%)	
SCLC	135 (16.70%)	60 (14.90%)	75 (18.60%)	
Other lung cancers	13 (1.60%)	10 (2.50%)	1 (0.70%)	

Statistically significant differences are marked with bold font.

TABLE 3 Training set and Test set variability analysis.

Variable	Total (N = 808)	Train set (N = 565)	Test set (N = 243)	*P*
Age	65.00 [59.00, 70.00]	65.00 [59.00, 70.00]	65.00 [58.00, 70.00]	0.727
BMI	21.50 [19.7, 23.70]	21.50 [19.60, 23.70]	21.60 [20.00, 23.60]	0.497
Chemotherapy cycles	5.00 [2.00, 8.00]	5.00 [2.00, 8.00]	5.00 [2.00, 9.00]	0.737
Hospitalizations	7.00[4.00, 12.00]	7.00 [4.00, 12.00]	7.00 [3.00, 12.00]	0.927
WBC	6.90 [5.49, 9.17]	6.82 [5.49, 8.94]	7.37 [5.47, 9.89]	0.075
RBC	3.77 [3.30, 4.15]	3.77 [3.33, 4.14]	3.76 [3.29, 4.19]	0.644
HGB	112.00 [99.90, 125.00]	112.00 [99.30, 124.00]	112.00 [100.00, 125.00]	0.810
PLT	209.00 [160.00, 258.00]	210.00 [159.00, 262.00]	209.00 [163.00, 243.00]	0.411
NEUT	72.10 [64.30, 79.20]	71.60 [63.60, 78.90]	73.30 [66.10, 80.10]	0.073
LYM	17.30 [12.00, 23.30]	17.50 [12.20, 23.70]	16.80 [11.40, 23.00]	0.249
MONO	7.30 [5.40, 9.48]	7.23 [5.50, 9.50]	7.40 [5.05, 9.30]	0.456
NLR	4.38 [2.83, 7.09]	4.27 [2.73, 6.89]	4.46 [2.97, 7.60]	0.137
NMR	10.10 [6.79, 14.03]	10.20 [7.00, 14.40]	10.00 [7.46, 14.50]	0.375
NPR	0.35 [0.27, 0.43]	0.34 [0.27, 0.43]	0.36 [0.28, 0.44]	0.108
IBIL	7.30 [5.70, 9.39]	7.24 [5.79, 9.30]	7.40 [5.60, 9.72]	0.700
ALT	18.00 [12.70, 26.30]	18.20 [12.40, 27.00]	17.40 [13.30, 24.30]	0.853
AST	23.40 [19.40, 29.40]	23.40 [19.30, 29.00]	23.50 [19.70, 29.70]	0.805
TBIL	9.89 [7.63, 12.60]	9.80 [7.70, 12.40]	9.90 [7.50, 12.80]	0.955
DBIL	2.40 [1.59, 3.40]	2.44 [1.60, 3.40]	2.30 [1.48, 3.39]	0.467
TP	66.80 [62.30, 69.90]	66.70 [62.20, 70.00]	67.20 [62.40, 69.80]	0.939
ALB	40.00 [36.50, 42.50]	39.90 [36.70, 42.40]	40.20 [36.30, 42.70]	0.931
GLB	26.30 [23.50, 29.40]	26.30 [23.70, 29.10]	26.40 [22.80, 30.00]	0.967
A/G	1.54 [1.31, 1.75]	1.54 [1.32, 1.75]	1.55 [1.26, 1.77]	0.975
Urea	5.89 [4.74, 7.56]	5.92 [4.69, 7.63]	5.84 [4.89, 7.32]	0.540
CREA	78.80 [66.00, 92.30]	78.50 [66.60, 92.20]	79.80 [64.00, 92.90]	0.889
UA	330.00 [278.00, 394.00]	330.00 [279.00, 394.00]	330.00 [277.00, 395.00]	0.951
CEA	3.69 [2.11, 9.74]	3.69 [2.14, 9.87]	3.66 [2.03, 8.29]	0.447
Gender, n (%)				0.298
Male	683 (84.50%)	483 (85.50%)	200 (82.30%)	
Female	125 (15.50%)	82 (14.50%)	43 (17.70%)	
Drink, n (%)				0.385
No	683 (84.50%)	473 (83.70%)	210 (86.40%)	
Yes	125 (15.50%)	92 (16.30%)	33 (13.60%)	
Smoke, n (%)				0.087
No	518 (64.10%)	351 (62.10%)	167 (68.70%)	
Yes	290 (35.90%)	214 (37.90%)	76 (31.30%)	
Diabetes, n (%)				0.864
No	731 (90.50%)	510 (90.30%)	221 (90.90%)	
Yes	77 (9.50%)	55 (9.70%)	22 (9.10%)	

(Continued)

TABLE 3 Continued

Variable	Total (N = 808)	Train set (N = 565)	Test set (N = 243)	*P*
Hypertension, n (%)				0.371
No	636 (78.70%)	450 (79.60%)	186 (76.50%)	
Yes	172 (21.30%)	115 (20.40%)	57 (23.50%)	
CHD, n (%)				0.226
No	759 (93.90%)	535 (94.70%)	224 (92.20%)	
Yes	49 (6.10%)	30 (5.30%)	19 (7.80%)	
Stage, n (%)				0.779
Stage I	29 (3.60%)	20 (3.50%)	9 (3.70%)	
Stage II	96 (11.90%)	71 (12.60%)	25 (10.30%)	
Stage III	330 (40.80%)	232 (41.10%)	98 (40.30%)	
Stage IV	353 (43.70%)	242 (42.80%)	111 (45.70%)	
Histology, n (%)				0.537
Adenocarcinoma	327 (40.50%)	221 (39.10%)	106 (43.60%)	
Squamous	333 (41.20%)	241 (42.70%)	92 (37.90%)	
SCLC	135 (16.70%)	93 (16.50%)	42 (17.30%)	
Other lung cancers	13 (1.60%)	10 (1.80%)	3 (1.20%)	
PE				0.237
No	608 (75.20%)	418 (74.00%)	190 (78.20%)	
Yes	200 (24.80%)	147 (26.00%)	53 (21.80%)	

3.3 Model performance

In the training dataset, the RF model exhibited superior predictive performance with an AUC of 1.00, indicating a high level of accuracy in prediction. In contrast, the AUC values for the remaining five models were as follows: 0.888, 95%CI(0.863-0.911) for LR, 0.822, 95%CI(0.791-0.852) for GNB, 0.792, 95%CI(0.760-0.825) for MLP, 0.719, 95%CI(0.681-0.758) for SVM, and 1.000, 95%CI (NaN- NaN) for KNN (Figure 3A). The F1 scores for these models were as follows: LR 0.816, RF 0.998, GNB 0.756, MLP 0.736, SVM 0.679, and KNN nan. In the test set, the AUC values for LR, RF, GNB, MLP, SVM, and KNN were 0.876(95%CI(0.806-0.953)), 0.923(95% CI(0.866-0.979)), 0.817(95%CI(0.726-0.909)), 0.777(95%CI(0.674-0.880)), 0.709(95%CI(0.590-0.828)), and 0.837(95%CI(0.750-0.923)), respectively (Figure 3B). The corresponding F1 scores were 0.791, 0.837, 0.747, 0.716, 0.658, and nan for LR, RF, GNB, MLP, SVM, and KNN, respectively. The forest plot comparing the AUC scores of the six ML models is presented in Figure 3C. In this study, the accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and kappa value of each model were computed and compared (Figures 3D, E). While the RF model exhibited exceptional performance on the training set, the Logistic Regression model was ultimately selected as the optimal model due to concerns regarding potential overfitting.

3.4 The logistic regression model

The results of the univariate logistic analysis are summarized in Supplementary Table 1. 12 variables were statistically significant: Gender, Drink, Smoke, CHD, Chemotherapy cycles, Hospitalizations, PE, NEUT, LYM, NLR, NMR, and CEA. Table 4 presents the coefficients and odds ratios (OR) for the nine predictor variables included in the model. The logistic equation was as follows:

y = - 2.954 - 0.424×Gender - 0.049×Drink + 1.754×Smoke + 0.395×Chemotherapy cycles + 1.417×PE + 0.083×NLR + 0.017×NMR + 0.009×AST - 0.008×NEUT. In this study, we evaluated the prediction accuracy and calibration of the model by calibration curve analysis of the training and test sets. The calibration curve results showed that the model in the training set had high prediction accuracy with a Somers' D coefficient of 0.777 and an area under the ROC curve of 0.888, indicating that the model had excellent discriminative ability (Figure 4A). In addition, the logistic regression calibration slope of the training set model was close to the ideal value of 1.000, with an intercept of 0.000, showing excellent calibration. The Brier score of 0.134 reflected the high reliability of the model predictions. In contrast, the model in the test set maintained a high discriminative power with an area under the ROC curve of 0.876, although there was a slight decrease in prediction accuracy (Somers' D coefficient of 0.751) (Figure 4B).

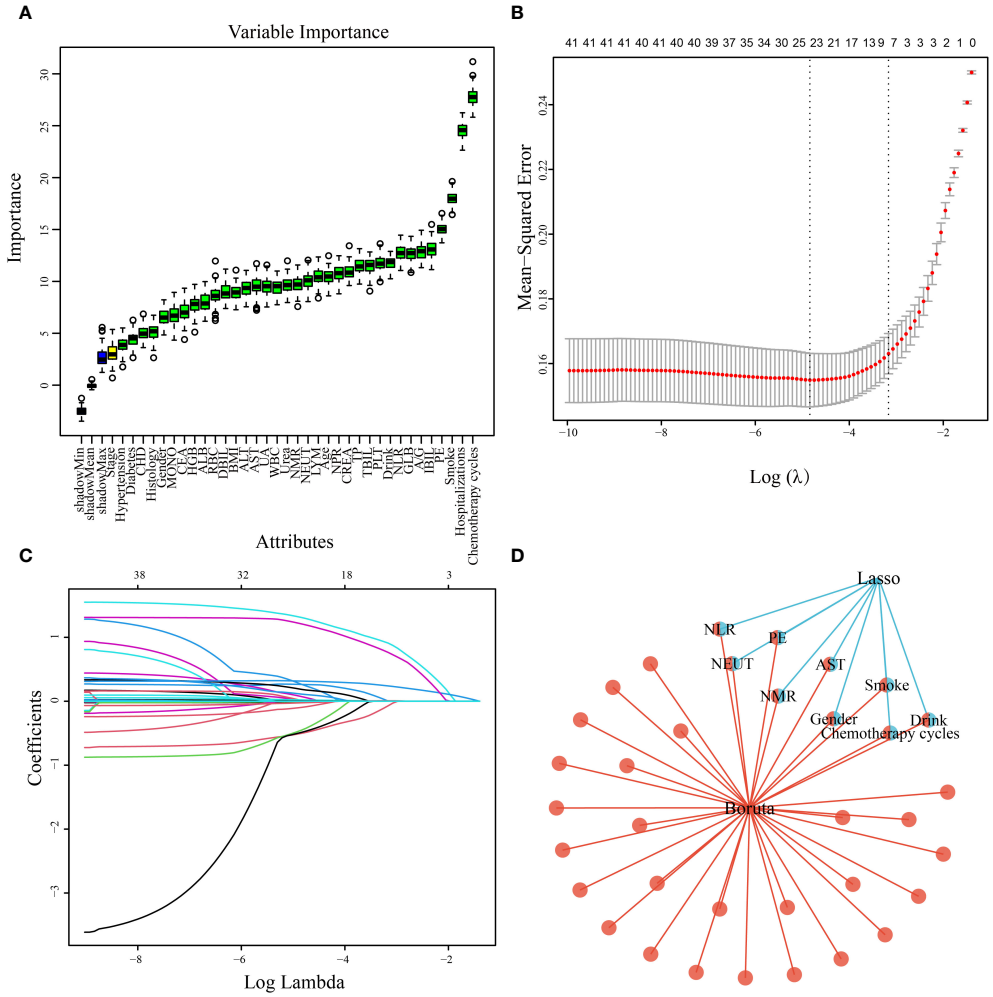

FIGURE 2
Predictor screening results. **(A)** Boruta; **(B)** Factor screening based on the LASSO regression model, with the left dashed line indicating the best lambda value for the evaluation metrics (lambda. min) and the right dashed line indicating the lambda value for the model where the evaluation metrics are in the range of the best value by one standard error (lambda.1se); **(C)** LASSO regression model screening variable trajectories; **(D)** common predictors between Boruta and LASSO.

The decision curve for the training set (Figure 4C) shows that the model provides significantly higher net gains than the baseline strategy when the threshold probabilities are between 0.1 and 0.9. On the test set (Figure 4D), the model similarly demonstrates good net returns, especially in the range of threshold probabilities from 0.1 to 0.85, where it maintains a high level of net returns. The confusion matrix results show the difference in the model's performance on different datasets. In the training set (Figure 4E), the model correctly identified 320 true negatives and 283 true positives, and misidentified 42 false positives and 82 false negatives, with a true positive rate (sensitivity) of 77.5% and a true negative rate (specificity) of 88.4%. In the test set (Figure 4F), the model correctly identified 32 true negatives and 27 true positives and misidentified 10 false positives and 12 false negatives, for a true positive rate of 69.2% and a true negative rate of 76.2%. Finally, we plotted clinical impact curves (CICs) to assess the net gain in clinical utility and applicability of the model with the highest diagnostic value. The clinical impact curves (Figures 4G, H) provide information on the ability of the models to predict high-risk patients at different cost-benefit ratio thresholds. The curves for both the training and test sets show that when the threshold probability is greater than the 65% predictive score probability value, the predictive model's determination of those at high risk of developing an infection in the lungs after chemotherapy is highly matched to those who actually develop an infection, confirming that the predictive model is clinically highly effective.

3.5 SHAP-based model interpretability analysis

This study assessed the relative significance of various factors influencing the susceptibility to lung infections following chemotherapy in patients with lung cancer. Figure 5A visually represents this ranking, with each point denoting a sample and the color gradient from blue to red indicating the magnitude of the sample eigenvalues. The vertical axis displays the importance ranking of features, along with the correlation and distribution of each

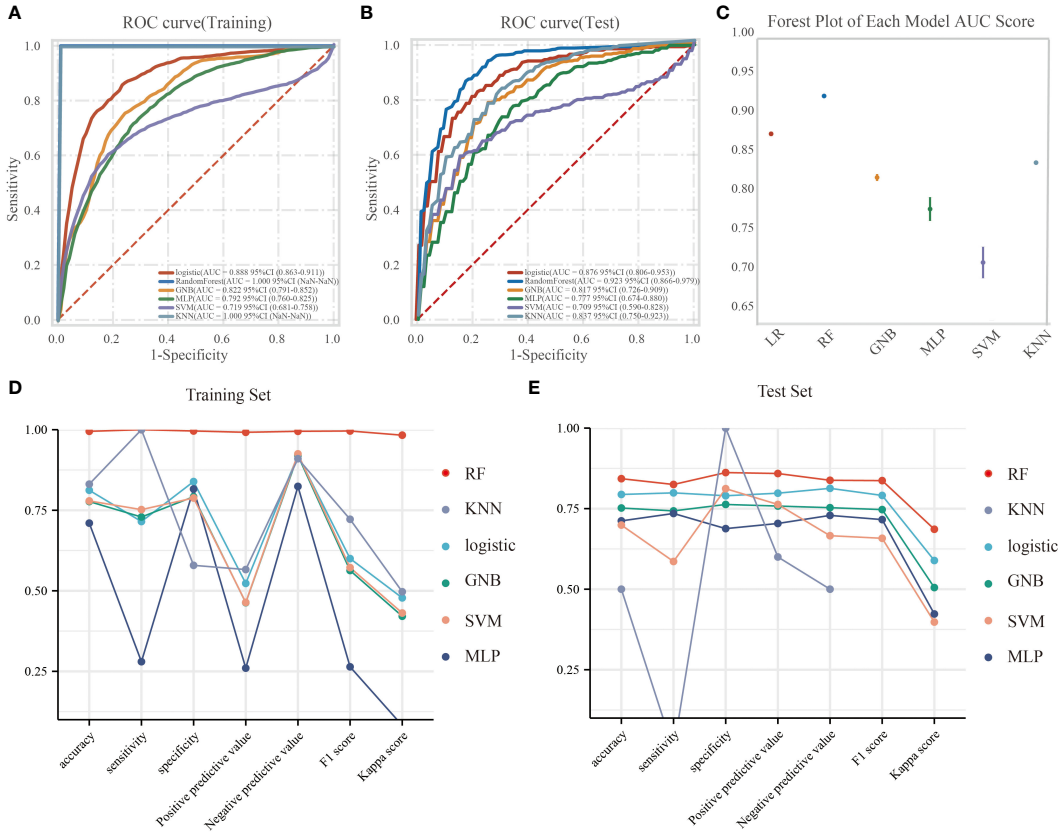

FIGURE 3
The performance and comparison of six different predictive models. **(A)** The training set ROC curve; **(B)** The test set ROC curve; **(C)** Forest plot of AUC values; **(D)** Evaluation metrics for the training set; **(E)** Evaluation metrics for the test set.

eigenvalue with the SHAP value. The impact of the top nine features in the importance ranking on prediction outcomes is illustrated in Figure 5B. Specifically, Chemotherapy cycles, Smoke, and PE exhibit positive contributions to the predictive results, while NEUT demonstrate negative influences on the model's output. Figure 5B illustrates the hierarchical significance of features in the logistic regression model. The vertical axis displays individual features in descending order of importance, while the horizontal axis represents average SHAP values. The analysis reveals that Chemotherapy cycles, Smoke, PE, NMR, and NLR are the top five features ranked by importance, indicating their critical influence on the presence of a lung infection. To enhance comprehension of the model's decision-making process at the individual level, we conducted a detailed interpretability analysis on two representative samples, as illustrated in Figures 5C, D. By visualizing the SHAP values of these samples, we could discern the impact of each feature on the model's predictions for these specific instances.

TABLE 4 Risk factors and their parameters of the logistic model.

Variables	Coefficients	OR(95%CI)	p
Intercept	-2.954	0.052(0.006-0.395)	**0.006**
Gender	-0.424	0.655(0.321-1.297)	0.233
Drink	-0.049	0.952(0.464-1.963)	0.893
Smoke	1.754	5.776 (3.292-10.375)	**<0.001**
Chemotherapy cycles	0.395	1.484(1.380-1.606)	**<0.001**
PE	1.417	4.123(2.389-7.274)	**<0.001**
NLR	0.083	1.087(1.007-1.174)	**0.034**
NMR	0.017	1.018(0.998-1.038)	0.077
AST	0.009	1.009(1.003-1.019)	**0.020**
NEUT	-0.008	0.992(0.962-1.026)	0.639

OR, odds ratio; CI, confidence interval.
Statistically significant differences are marked with bold font.

3.6 Construction of nomograms

In this study, two nomograms were constructed, integrating nine important predictor variables such as alcohol consumption, smoking, and chemotherapy cycle to visually assess the risk of lung infection after chemotherapy. Figure 6A shows an interactive nomogram with a score of 3.51 for the example patient, corresponding to a 94.5% probability of infection, providing a quick and easy-to-interpret risk assessment. Figure 6B illustrates a dynamic nomogram with different risk profiles derived from 10 combinations of variables.

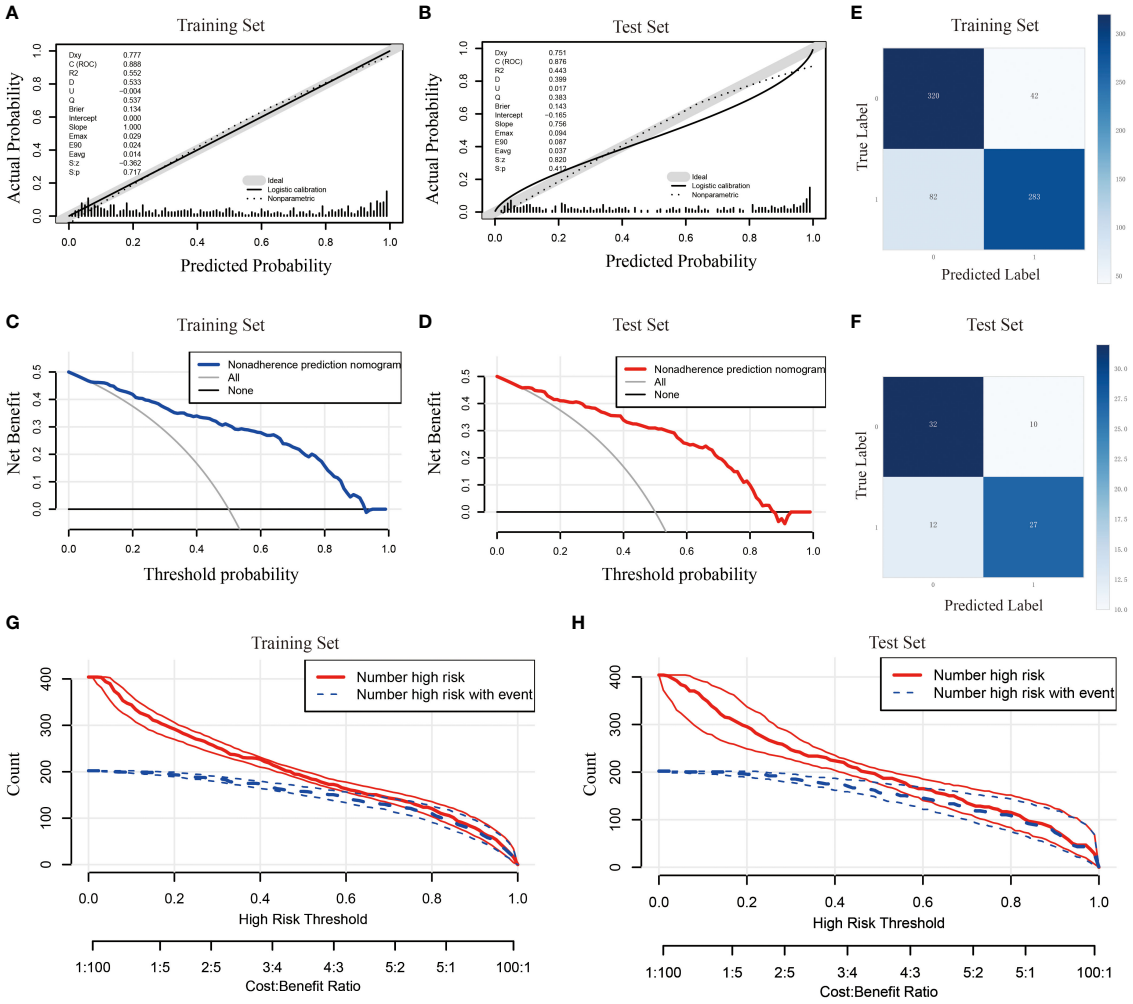

FIGURE 4
Comprehensive evaluation of the logistic regression model. **(A)** Calibration curve for the training set; **(B)** Calibration curve for the test set; **(C)** Decision curve analysis for the training set; **(D)** Decision curve analysis for the test set; **(E)** Confounding matrix for the training set; **(F)** Confounding matrix for the test set; **(G)** Clinical impact curve for the training set; **(H)** Clinical impact curve for the test set.

4 Discussion

This research investigated the predictive factors associated with post-chemotherapy lung infection in patients with lung cancer and developed a logistic regression-based predictive model that effectively estimates the likelihood of lung infection following chemotherapy. By employing meticulous feature selection and conducting multi-model comparative validation, this study highlights the significance of various key predictors and offers a valuable tool to aid in clinical decision-making.

Zhou D et al. conducted a retrospective analysis of 244 non-small cell lung cancer (NSCLC) patients who underwent surgical interventions from June 2015 to January 2017. Through applying LASSO regression and logistic regression analyses, the researchers identified independent risk factors for postoperative pulmonary infection (PPI) in NSCLC patients and subsequently developed a predictive model based on these findings (26). Jong-Ho Kim and colleagues pioneered the application of ML techniques for the prognostication of postoperative pulmonary complications (PPCs), employing a suite of five algorithms, namely LR, random forests (RFs), light-gradient boosting machines (LightGBM), extreme-gradient boosting machines (XGBoost) and MLP for the construction and assessment of predictive models (27). Xue et al. established a predictive model utilizing preoperative and intraoperative data to detect the likelihood of postoperative pneumonia. Their research delved into the application of machine learning in predicting a range of postoperative complications, including pneumonia, within the context of PPCs. Nevertheless, the authors failed to emphasize unique characteristics and risk factors beyond pneumonia linked to PPCs, potentially diverting attention away from PPCs (28). While predictive models have been created for complications in lung cancer patients, there is a scarcity of predictive models utilizing ML algorithms for assessing the risk of lung infection following chemotherapy for lung cancer.

The dysregulation of the autoimmune system, exacerbated by chemotherapy-induced immune cell depletion, tumor cell infiltration, impaired antibody-complement generation, and dysregulation of the inflammatory system, disrupts immune homeostasis and heightens

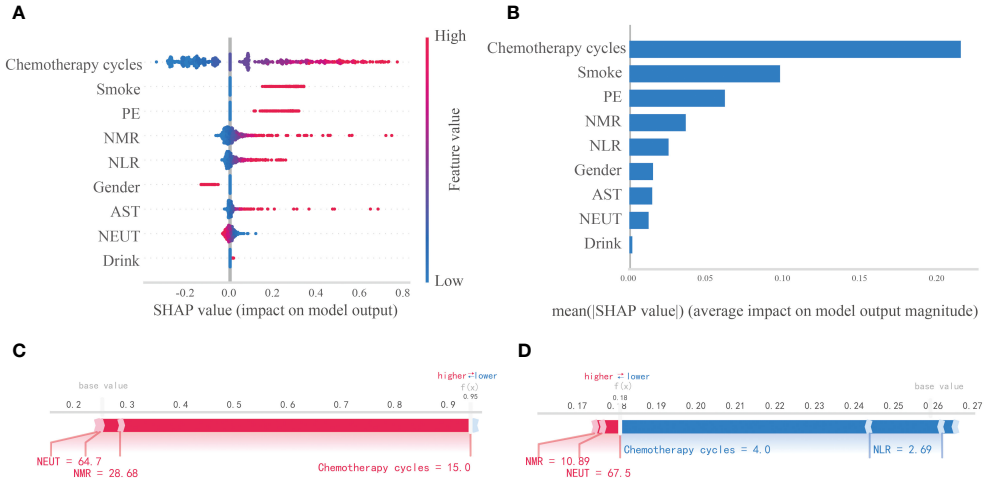

FIGURE 5
Interpretability analysis of logistic regression models. **(A)** SHAP dendrogram of features of the logistic regression model. **(B)** Importance ranking plot of features of the logistic regression model. **(C, D)** Interpretability analysis of 2 independent samples.

susceptibility to concurrent lung infections (29–31). This risk is further compounded in individuals with comorbidities such as chronic bronchitis, chronic obstructive pulmonary disease, interstitial lung disease, pulmonary atelectasis, and other organic diseases (32, 33). The occurrence of lung infection during chemotherapy is a prevalent and challenging complication that hinders the efficacy of treatment and exacerbates the health status of patients, ultimately impacting their prognosis and increasing the financial burden of medical care. As such, our research holds significant clinical importance in examining the determinants of lung infection during chemotherapy and implementing timely and efficient interventions for patients with lung cancer.

This study employed a dual methodology of Boruta's algorithm and LASSO regression to identify predictors for accurate feature selection and model stability. The selected features encompassed variables such as alcohol consumption status, smoking habits, chemotherapy cycles, hospitalization frequency, presence of lung pleural fluid, neutrophil count, AST, NLR, and NMR, all of which have demonstrated significant correlations with the prognosis of lung cancer patients in prior research. Wei Guo et al. colleagues created a predictive model utilizing artificial neural network (ANN) technology to forecast infection rates in lung cancer patients undergoing chemotherapy (34). The researchers employed a logistic regression (LR) model to analyze the data and identify statistically significant variables. Their results indicated a positive correlation between length of hospital stay and infection risk, which aligns with our research findings. However, the researchers discovered that a prior diagnosis of diabetes was linked to an increased likelihood of lung infection, a finding that did not align with our results. This discrepancy may be attributed to the limited sample size of the previous study, which only included 80 cases. Zhouzhou Ding et al. explored the risk factors for PPI in patients with non-small cell lung cancer (NSCLC), developed a risk model, and conducted predictive modeling for PPI. Their research revealed that the chemotherapy cycle, identified as an independent risk factor, had a notable impact on the occurrence of PPI (26). This is in general agreement with our findings. Our findings emphasize the importance of monitoring and managing these factors during chemotherapy management.

After comparing these models, it is observed that while the RF model exhibits superior performance in the training set, its

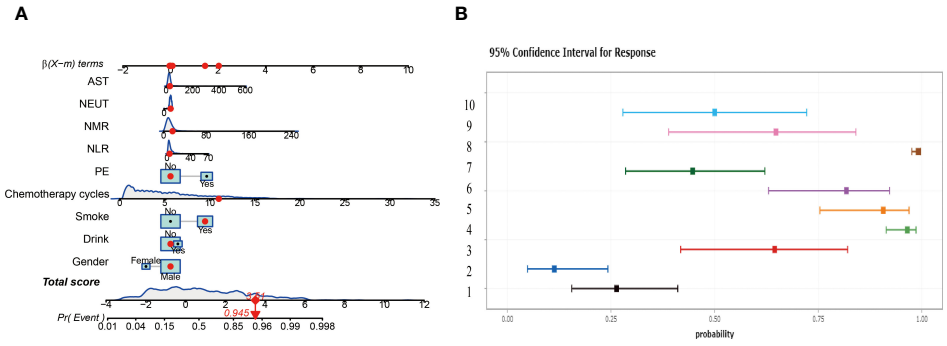

FIGURE 6
Construct two different nomograms. **(A)** Interactive Nomogram. **(B)** Dynamic Nomogram showing risk profiles for ten scenarios.

propensity for overfitting necessitates the selection of the logistic regression model as the optimal choice due to its strong generalization capabilities in the external test set. Logistic regression models are favored for their predictive accuracy and interpretability, which are essential qualities for practical clinical implementation. The importance of constructing disease prediction models lies in identifying high-risk patients and mitigating the risk for individuals who may fall into the high-risk category, thereby benefiting patients overall. Consequently, the clinical interpretability of ML models holds significant value in medical practice. In this research, we utilized the SHAP method to provide both global and local interpretations of the ML model, enhancing its visual representation and transparency. Kaidi Gong et al. have observed that the SHAP method exhibits superior consistency and performance compared to conventional weight-based interpretation methods, and the SHAP algorithm demonstrates greater stability across various models. In contrast to the Local Interpretable Model-agnostic Explanations (LIME) method, SHAP demonstrates strong performance in both global and individual interpretation tasks, while LIME shows less consistency in individual analysis (35). Yasunobu Nohara and colleagues further substantiated that SHAP values exhibit superior interpretability compared to the coefficients of generalized linear regression models, as evidenced through a comparative analysis of interpretation outcomes with other established methodologies. Additionally, they found that SHAP summary plots offer more effective visualization of results than feature importance plots (36). The utilization of SHAP value analysis in this research offers a novel lens through which to comprehend the model's decision-making process. Through this method, we were able to elucidate the specific contributions of individual predictors to the model's decision-making, ultimately improving the transparency and interpretability of the model. Notably, factors such as chemotherapy cycle, smoking, PE, and NMR were underscored for their significance, consistent with prior research findings and reaffirmed their pivotal role in predicting post-chemotherapy lung infections.

Despite the results of this study, there are some limitations. Firstly, being a retrospective study, there is a potential for omitted data and selection bias to impact the results. Secondly, the small sample size of this study and the fact that the sample was collected from a single center may limit the generalizability of the findings. The potential incorporation of prospective design and multicenter data in future studies, coupled with integrating additional patient data and utilizing advanced machine learning techniques, is anticipated to enhance model performance. This improvement aims to validate the robustness and generalizability of the model, ultimately leading to the development of more personalized and precise treatment management strategies for patients with lung cancer.

5 Conclusion

This study has effectively developed a predictive tool utilizing logistic regression modeling to forecast lung infections following chemotherapy in lung cancer patients. The tool demonstrates high predictive accuracy and holds substantial clinical relevance. By identifying and assessing crucial predictors, this research establishes a valuable scientific foundation for the prevention and treatment of post-chemotherapy complications in lung cancer patients, ultimately enhancing patient survival quality and prognostic outcomes. Future work will focus on further validating the model's validity and exploring integrating these predictive tools into clinical practice to improve the prediction of treatment consequences in lung cancer patients.

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 humans were approved by The Medical Ethics Committee of the Central Hospital of Shaoyang (No: 20231207). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

TS: Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JL: Formal analysis, Software, Writing – original draft. HQY: Methodology, Software, Validation, Writing – review & editing. XL: Conceptualization, Investigation, Writing – review & editing. HY: Resources, Software, Validation, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank the Extreme Analytics platform for statistical support (https://www.xsmartanalysis.com); we would also like to thank Home for Researchers for proofreading the paper (https://www.home-for-researchers.com).

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

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1403392/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Systematic Review
PUBLISHED 18 October 2024
DOI 10.3389/fonc.2024.1334957

OPEN ACCESS

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RECEIVED 12 November 2023
ACCEPTED 02 October 2024
PUBLISHED 18 October 2024

CITATION
Kang W, Cheng J, Pan L, Zhan P, Liu H, Lv T, Han H and Song Y (2024) Heterogeneity between subgroups of first-line chemoimmunotherapy for extensive-stage small cell lung cancer patients: a meta-analysis and systematic review.
Front. Oncol. 14:1334957.
doi: 10.3389/fonc.2024.1334957

Heterogeneity between subgroups of first-line chemoimmunotherapy for extensive-stage small cell lung cancer patients: a meta-analysis and systematic review

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Objectives: Differences in clinicopathological characteristics of extensive-stage small cell lung cancer (ES-SCLC) patients may influence the immune response. This study aims to evaluate the heterogeneity of response to first-line chemoimmunotherapy between subgroups in ES-SCLC to screen out suitable populations.

Materials and methods: We searched the PubMed, EMBASE, and Cochrane Library databases from inception to December 3, 2022 for randomized controlled trials (RCTs) of ES-SCLC chemoimmunotherapy. We also reviewed main conferences from January 1, 2021 to October 1, 2023. A trial-specific hazard ratio (HR) ratio for each subgroup was calculated, and these ratios were then pooled using the deft approach.

Results: A total of 9 RCTs with 4099 patients were finally included. The pooled ratios were 0.92 (95% CI = 0.77 to 1.09) for OS-HRs and 0.79 (95% CI = 0.55 to 1.13) for PFS-HRs in women versus men. The pooled ratios of OS-HRs and PFS-HRs in patients with positive versus negative PD-L1 expression were 1.26 (95% CI = 0.91 to 1.73) and 1.08 (95% CI = 0.77 to 1.52), respectively. The pooled ratios of OS-HRs and PFS-HRs in patients without versus with brain metastasis were 0.77 (95% CI = 0.59 to 1.01) and 0.71 (95% CI = 0.44 to 1.12). No statistically significant differences were also found in terms of subgroups for age, liver metastasis, smoking status, ECOG PS, LDH level, type of platinum salt and race.

Conclusion: Women or patients with negative PD-L1 expression or with LDH ≤ ULN or without brain metastasis tend to benefit more from first-line chemoimmunotherapy in ES-SCLC. More trials are needed to prospectively validate the therapeutic heterogeneity among clinicopathological characteristics.

Systematic review registration: https://inplasy.com/inplasy-2023-3-0064/ identifier, INPLASY202330064.

KEYWORDS

ES-SCLC, therapeutic heterogeneity, subgroup analysis, first-line chemoimmunotherapy, deft method

1 Introduction

Lung cancer is among the most common malignant tumors, with small cell lung cancer (SCLC) accounting for approximately 15% of all cases (1). SCLC is an aggressive neuroendocrine tumor originating from bronchial epithelial cells, and about 60%-70% of patients already have distant metastasis at diagnosis (2). Over the past 30 years, chemotherapy and radiotherapy were the primary clinical treatments for extensive-stage small cell lung cancer (ES-SCLC) patients, whereas effective time of them is short, and local recurrence or distant metastasis will occur soon. Overall, the 5-year survival rate of ES-SCLC patients is less than 2% (3, 4). Thus, we urgently need new treatment options for this recalcitrant cancer.

Immune checkpoint inhibitors (ICIs) can interrupt the immune escape system of tumors, enhance anti-tumor immunity and ultimately improve patient survival (5). However, the application of ICIs alone as a first-line treatment for SCLC patients is unsatisfactory, likely due to the rapid progression of SCLC, potential immune escape mechanisms and high potential risk of not undergoing chemotherapy (6, 7). Fortunately, immunotherapy can reverse the resistance of tumor cells to chemotherapy and reduce the toxicity of chemotherapy, while chemotherapy can enhance the anti-tumor activity in coordination with immunotherapy by enhancing tumor cell immunogenicity, removing immunosuppression and regulating the immune response (8, 9). Currently, chemoimmunotherapy seems to be the better first-line treatment option for ES-SCLC patients with a growing accumulation of phase II and III clinical researches data. The CAPSTONE-1 trial demonstrated that adebrelimab plus chemotherapy significantly improved survival in ES-SCLC patients, further validating the results of programmed death-ligand 1 (PD-L1) inhibitors plus chemotherapy in IMpower133 trial and CASPIAN trial (10–12). The ASTRUM-005 trial was the first to show that programmed death-1 (PD-1) inhibitors plus chemotherapy can also significantly prolong the survival of ES-SCLC patients (13). Moreover, serplulimab has been granted Orphan-Drug Designation (ODD) by the United States Food and Drug Administration (FDA) for the treatment of SCLC. The results of the RATIONALE-312 trial, presented at the 2023 World Conference on Lung Cancer (WCLC), further confirmed that ES-SCLC patients can achieve better survival outcomes (14).

It is well established that responses to chemoimmunotherapy vary among individuals, and it remains unclear which patients are most suited for this treatment. For example, NSCLC patients with positive PD-L1 expression may derive greater benefit from immunotherapy compared to those with negative PD-L1 expression. In view of the differences in clinical characteristics that may affect the efficacy of chemoimmunotherapy, we conducted this meta-analysis to directly explore potential therapeutic heterogeneity between subgroups and select the dominant groups more suitable for first-line chemoimmunotherapy in ES-SCLC, so as to maximize the therapeutic efficacy.

2 Materials and methods

2.1 Literature search

Two researchers (Kang and Han) independently searched the PubMed, EMBASE, and Cochrane Library databases from inception to December 3, 2022. The search terms included "extensive-small cell lung cancer", "chemoimmunotherapy", "PD-1 Inhibitors", "Pembrolizumab", "Nivolumab", "Serplulimab", "Cemiplimab", "PD-L1 Inhibitors", "Atezolizumab", "Durvalumab", "Adebrelimab", "Avelumab", "CTLA-4 Inhibitors", "Ipilimumab", "Tremelimumab", "randomized controlled trial" (Supplementary Table 1). We also reviewed main conferences from January 1, 2021 to October 1, 2023.

This meta-analysis was conducted under the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) (15) and registered on the INPLASY website (registration number: INPLASY202330064, https://inplasy.com/inplasy-2023-3-0064/).

2.2 Inclusion and exclusion criteria

Trials meeting the following criteria were included (1): phase II or III RCTs in patients with histological diagnosis of unresectable or advanced ES-SCLC (2); compared chemoimmunotherapy with chemotherapy as the first-line treatment (3); reported detailed outcomes including overall survival (OS), progression-free survival (PFS), objective response rate (ORR), disease control rate (DCR), treatment related adverse events (TRAEs) of grade 3 or higher and discontinuation rate (DR) (4); published in English. These trials with the latest and most comprehensive data were included.

2.3 Study selection and data extraction

Data collected included: trial name, first author, year of publication, treatment regimen, number of participants, and outcomes of included trials. To evaluate the therapeutic heterogeneity between subgroups, we also extracted HR and 95% confidence interval (CI) of OS and PFS in the following predefined subgroups: gender, age, PD-L1 expression level, brain metastasis, liver metastasis, smoking status, Eastern Cooperative Oncology Group performance status (ECOG PS), lactate dehydrogenase (LDH) level, the type of platinum salt and race. PD-L1 expression ≥ 1% tumor cell (TC) or tumor-infiltrating immune cell (IC) and PD-L1 tumor cell proportion score (TPS) ≥ 1% were considered to be positive PD-L1 expression (16). Finally, 9 RCTs were included (10–14, 17–23). Two authors (Kang and Han) independently extracted data and resolved the discrepancies by consensus.

2.4 Quality assessment and bias assessment

Using the Cochrane bias risk assessment tool (24), two authors (Kang and Han) independently assessed the risk of bias in each trial (Supplementary Figure 9). Studies were rated as low (low risk in all fields), high (high risk in one or more fields), and unclear risk of bias (more than 3 fields indicated unclear risk). Funnel plots were used to examine the presence of publication bias in our meta-analysis (Supplementary Figure 10).

2.5 Statistical analysis

All analyses were performed using a random-effects model. The primary endpoint was therapeutic heterogeneity between subgroups, measured by specific ratio of HRs (e.g. ratio of HR in women to HR in men). To avoid the risk of ecological bias for RCTs, the specific ratio of HR was calculated for each RCT and then combined using the deft method (25). We further performed subgroup analysis to explore therapeutic heterogeneity among patients receiving different types of chemoimmunotherapy.

The Q test was used to evaluate the heterogeneity between studies, and the I^2 statistics were also calculated to represent the percentage of the total observed variability due to heterogeneity (26, 27). Sensitivity analysis was performed using a "one study deletion" approach. All tests were two-sided, and the results were considered statistically significant when the P value was less than 0.05. All analyses were performed using R software (version 4.2.2).

3 Results

3.1 Literature search and study selection

8070 studies were identified on the initial literature search. A total of 9 RCTs with 4099 patients were finally included (Figure 1). The baseline characteristics of 9 RCTs were shown in Table 1, and patient characteristics across subgroups of trials were shown in Table 2. OS-HR data of subgroups were reported in 7 trials (Supplementary Table 2), and PFS-HR data of subgroups were reported in 4 trials (Supplementary Table 3).

3.2 Comparison of the efficacy of chemoimmunotherapy versus chemotherapy as the first-line treatment of ES-SCLC patients

Compared with chemotherapy alone, no obvious advantages of chemoimmunotherapy were observed in ORR (RR = 1.07, 95% CI = 1.00 to 1.14; Figure 2A) and DCR (RR = 1.00, 95% CI = 0.97 to 1.03; Figure 2B). Notably, PFS (HR = 0.71, 95% CI = 0.63 to 0.81; Figure 2C) and OS (HR = 0.77, 95% CI = 0.70 to 0.84; Figure 2D) were significantly prolonged in ES-SCLC patients receiving chemoimmunotherapy. As for the safety of chemoimmunotherapy, it resulted in an increase in DR (RR = 2.03, 95% CI = 1.13 to 3.66; Figure 2E), but no statistically significant increase in TRAEs (RR = 1.03, 95% CI = 0.98 to 1.08; Figure 2F).

3.3 Heterogeneity between subgroups of chemoimmunotherapy as the first-line treatment of ES-SCLC patients

Women and men benefited more from chemoimmunotherapy than chemotherapy in ES-SCLC. (women: pooled OS-HR = 0.82, 95% CI = 0.65 to 1.05, pooled PFS-HR = 0.70, 95% CI = 0.54 to 0.90, men: pooled OS-HR = 0.64, 95% CI = 0.55 to 0.74, pooled PFS-HR = 0.66, 95% CI = 0.56 to 0.79; Figures 3A, B). The pooled ratio of OS-HRs in women versus men reported in each trial was 0.92 (95% CI = 0.77 to 1.09; Figure 3C), and the pooled ratio of PFS-HRs was 0.79 (95% CI = 0.55 to 1.13; Figure 3D). It suggested that women tend to benefit more from first-line chemoimmunotherapy in ES-SCLC.

Compared to chemotherapy, both patients with positive or negative PD-L1 expression benefit more from OS (PD-L1+: pooled OS-HR = 0.82, 95% CI = 0.65 to 1.05, PD-L1-: pooled OS-HR = 0.64, 95% CI = 0.55 to 0.74; Figure 4A) and PFS (PD-L1+: pooled PFS-HR = 0.70, 95% CI = 0.54 to 0.90, PD-L1-: pooled PFS-HR = 0.66, 95% CI = 0.56 to 0.79; Figure 4B) in chemoimmunotherapy. Respectively, the pooled ratios of OS-HRs and PFS-HRs reported in patients with positive versus negative PD-L1 expression were 1.26 (95% CI = 0.91 to 1.73; Figure 4C) and 1.08 (95% CI = 0.77 to 1.52; Figure 4D), and this heterogeneity indicated that ES-SCLC patients with negative PD-L1 expression may be more suitable candidates for chemoimmunotherapy.

In patients with or without brain metastasis, chemoimmunotherapy demonstrated superior efficacy than chemotherapy. Considering the heterogeneity between two groups, the pooled ratios of OS-HRs and PFS-HRs in patients without or with brain metastasis were calculated (pooled ratio of OS-HRs = 0.77, 95% CI = 0.59 to 1.01; pooled ratio of PFS-HRs = 0.71, 95% CI = 0.44 to 1.12; Figure 5). This heterogeneity indicated that ES-SCLC patients without brain metastases may achieve better survival outcomes from chemoimmunotherapy.

We performed several similar analyses to assess therapeutic heterogeneity on other clinicopathological characteristics (Supplementary Figures 1-7). Eventually, we concluded that non-smokers, Asians, patients older than 65 years, patients without liver metastasis, patients with LDH below upper limit of normal (ULN) or using etoposide-cisplatin may have longer OS from chemoimmunotherapy with no statistically significant differences.

3.4 Heterogeneity between subgroups of different types of chemoimmunotherapy as the first-line treatment of ES-SCLC patients

Therapeutic heterogeneity between subgroups was analyzed for different treatment regimens, including cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors plus chemotherapy, PD-

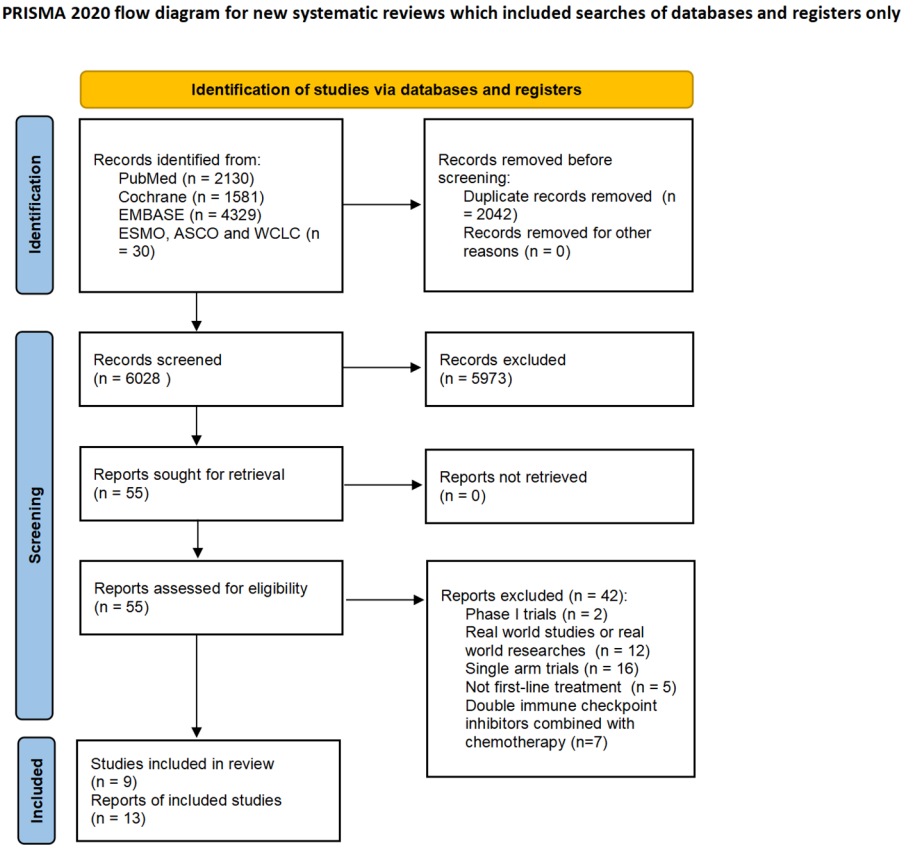

FIGURE 1
Flowchart of study selection and design.

L1 inhibitors plus chemotherapy, and PD-1 inhibitors plus chemotherapy, to identify the dominant population for each regimen (Table 3). Statistically significant pooled ratios of PFS-HRs (0.58, 95% CI = 0.34 to 0.99; Table 3) indicated that smokers were more suitable for PD-1 inhibitors plus chemotherapy than non-smokers. No statistically significant differences were observed in other subgroups.

3.5 Sensitivity analysis and publication bias

We performed sensitivity analyses on subgroups by gender, PD-L1 expression level, brain metastasis, LDH level, the type of platinum salt and race (Supplementary Figure 8). Statistically significant pooled ratios of OS-HRs (0.74, 95%CI = 0.55 to 0.98) in brain metastases subgroup indicated that ES-SCLC patients without brain metastases were the dominant population for first-line chemoimmunotherapy. The pooled ratios of PFS-HRs in gender subgroup (0.66, 95% CI = 0.49-0.89) was statistically significant, indicating that women were more suitable for first-line chemoimmunotherapy. The funnel plot for publication bias was shown in the Supplementary Figure 10, and no significant publication bias was observed.

4 Discussion

This meta-analysis, which included 9 RCTs, demonstrated that first-line chemoimmunotherapy was more effective than chemotherapy in ES-SCLC patients. Not coincidentally, this result was consistent with the conclusion of a meta-analysis of 6 RCTs published in 2021 (28). Gristina et al. found that specific patient clinical characteristics (such as ECOG PS of 1, the use of cisplatin and the absence of brain metastases) seemed to be associated with a survival gain using chemoimmunotherapy in ES-SCLC patients, and patients both with and without liver metastases receiving chemoimmunotherapy may have better survival outcomes (28). Based on this study, we used the deft approach to directly compare the potential therapeutic heterogeneity between subgroups, which could further assist ES-SCLC patients to choose personalized treatment. By analyzing the therapeutic heterogeneity between subgroups, we concluded that women or non-smokers or Asians or patients over 65 years old or with negative PD-L1 expression or with LDH ≤ ULN or without brain metastasis or without liver metastasis or using etoposide-cisplatin may achieve longer OS from first-line chemoimmunotherapy. Among them, specific patient clinical characteristics tended to obtain longer OS and PFS,

TABLE 1 Main baseline characteristics of each included trial considered in this meta-analysis.

Trial	NCT number	Design	Experimental arm 1 (n)	Experimental arm 2 (n)	Control arm (n)
CA184-041	NCT00527735	A randomized, multicenter, double -blind, parallel, phase 2 trial	(n = 42) phase regman: Ipilimumab + CP	(n = 43) concurrent regman: Ipilimumab + CP	(n = 45) CP
CA184-156	NCT01450761	A randomized, multicenter, double-blind, parallel, phase 3 trial	(n = 478) Ipilimumab + EP		(n = 476) Placebo + EP
IMpower133	NCT02763579	A randomized, multicenter, double-blind, parallel, phase 3 trial	(n = 201) Atezolizumab + EP		(n = 202) Placebo + EP
EA5161	NCT03382561	A randomized, open-label, parallel, phase 2 trial	(n = 80) Nivolumab + EP		(n = 80) EP
KEYNOTE-604	NCT03066778	A randomized, multicenter, double-blind, parallel, phase 3 trial	(n = 228) Pembrolizumab + EP		(n = 225) Placebo + EP
CASPIAN	NCT03043872	A randomized, multicenter, open-label, parallel, phase 3 trial	(n = 268) Durvalumab + EP	(n = 268) Durvalumab + Tremelimumab + EP	(n = 269) EP
CAPSTONE-1	NCT03711305	A randomized, multicenter, double-blind, parallel, phase 3 trial	(n = 230) Adebrelimab + EP		(n = 232) Placebo + EP
ASTRUM-005	NCT04063163	A randomized, multicenter, double-blind, parallel, phase 3 trial	(n = 389) Serplulimab + EP		(n = 196) Placebo + EP
RATIONALE-312	NCT04005716	A randomized, multicenter, double-blind, parallel, phase 3 trial	(n = 227) Tislelizumab + EP		(n = 230) Placebo + EP

including women or patients with negative PD-L1 expression or with LDH ≤ ULN or without brain metastasis. Notably, the OS prolongation trends of patients with negative PD-L1 expression or with LDH ≤ ULN in all RCTs were completely consistent, and the PFS prolongation trends of patients without brain or liver metastasis were completely consistent.

Our analysis of the difference in the efficacy of chemoimmunotherapy between women and men was similar to a previous meta-analysis which showed that women with advanced lung cancer achieved more statistically significant survival improvement from PD-1/PD-L1 inhibitors plus chemotherapy than men (29). Except for KEYNOTE-604 trial, other trials were observed a consistent trend that women receiving chemoimmunotherapy may have better survival outcomes than men. After sensitivity analysis, the PFS improvement of women undergoing chemoimmunotherapy was observed to be statistically significant. Given the complexity of sex-dimorphism of immune system function and responses, women may benefit more than men from different immunotherapy strategies (30). The possible mechanisms underlying this gender heterogeneity include: First, the X chromosome contained immune-related genes that can escape X chromosome inactivation. Second, sex hormone-induced signaling pathways could be regulated by sex chromosome-linked genes. Moreover, PD-1 expression and function could be regulated by estrogen, and PD-L1 was expressed in an estrogen-dependent and sex-dependent manner (31–34). A study using a cRaf transgenic disease model assessed commonalities in sex-specific NSCLC gene regulations between mice and humans, and confirmed the role of estrogen receptor α in affecting immune cells in the tumor microenvironment and regulating tumor growth genes (35). Up to now, several studies have shown that gender heterogeneity should be considered in chemoimmunotherapy for lung cancer patients.

We analyzed the therapeutic heterogeneity of PD-L1 expression level subgroup, and concluded that patients with negative PD-L1 expression may have better survival outcomes. Detection of PD-L1 expression level can guide the use of PD-1/PD-L1 inhibitors and assist to screen potential candidates of immunotherapy. High PD-L1 expression will reduce the immunity of patients, especially in solid tumors, which may seriously affect the survival benefit of patients (36). A phase III RCT has demonstrated that advanced NSCLC patients with high PD-L1 expression and high immune infiltration were the dominant population for chemoimmunotherapy (37). However, a recent study suggested that the predictive value of PD-L1 expression level was not significantly heterogeneity between squamous cell carcinoma and adenocarcinoma patients receiving ICIs plus chemotherapy (38). The aforementioned results were not consistent with our study, which focused on ES-SCLC. Except for KEYNOTE-604 trial, results were consistently observed that patients with negative PD-L1 expression may have longer OS and PFS from chemoimmunotherapy. As a biomarker for ICIs treatment, PD-L1 expression level has been used as an auxiliary diagnosis in selecting immunotherapy options for NSCLC patients (11), but it is not suitable to predict the efficacy of immunotherapy in SCLC. Possible reasons for this difference were as follows: First, the heterogeneity of tumor immune

TABLE 2 Patient characteristics of each included trial considered in this meta-analysis.

Patient characteristics		CA184-041		CA184-156		IMpower133		CASPIAN		CAPSTONE-1		EA5161		KEYNOTE-604		ASTRUM-005		RATIONALE-312	
		CT+IO n=43 (%)	CT n=45 (%)	CT+IO n=478 (%)	CT+PLA n=476 (%)	CT+IO n=201 (%)	CT+PLA n=202 (%)	CT+IO n=268 (%)	CT n=269 (%)	CT+IO n=230 (%)	CT+PLA n=232 (%)	CT+IO n=80 (%)	CT n=80 (%)	CT+IO n=228 (%)	CT+PLA n=225 (%)	CT+IO n=389 (%)	CT+PLA n=196 (%)	CT+IO n=227 (%)	CT+PLA n=230 (%)
Sex	Male	33 (76.7)	33 (73.3)	317 (66.3)	326 (68.5)	129 (64.2)	132 (65.3)	190 (70.9)	184 (68.4)	184 (80.0)	188 (81.0)	35 (43.7)	36 (45.0)	152 (66.7)	142 (63.1)	317 (81.5)	164 (83.7)	186 (81.9)	186 (80.9)
	Female	10 (23.3)	12 (26.7)	161 (33.7)	150 (31.5)	72 (35.8)	70 (34.7)	78 (29.1)	85 (31.6)	46 (20.0)	44 (19.0)	45 (56.3)	44 (55.0)	76 (33.3)	83 (36.9)	72 (18.5)	32 (16.3)	41 (18.1)	44 (19.1)
Age	<65	35 (81.4)	36 (80.0)	299 (62.6)	277 (58.2)	111 (55.2)	106 (52.5)	167 (62.3)	157 (58.4)	155 (67.4)	147 (63.4)	NA	NA	115 (50.4)	101 (44.9)	235 (60.4)	119 (60.7)	138 (60.8)	149 (64.8)
	≥65	8 (18.6)	9 (20.0)	179 (37.4)	199 (41.8)	90 (44.8)	96 (47.5)	101 (37.7)	112 (41.6)	75 (32.6)	85 (36.6)	NA	NA	113 (49.6)	124 (55.1)	154 (39.6)	77 (39.3)	89 (39.2)	81 (35.2)
Race	Asian	NA	NA	108 (22.6)	107 (22.5)	33 (16.4)	36 (17.8)	36 (13.4)	42 (15.6)	230 (100)	232 (100)	NA	NA	52 (22.8)	32 (14.2)	262 (67.4)	139 (70.9)	NA	NA
	Non-Asian	NA	NA	370 (77.4)	369 (77.5)	168 (83.6)	166 (82.2)	232 (86.6)	227 (84.4)	0 (0)	0 (0)	NA	NA	176 (77.2)	193 (85.8)	127 (32.6)	57 (29.1)	NA	NA
ECOG PS	0	8 (18.6)	12 (26.7)	137 (28.7)	147 (30.9)	73 (36.3)	67 (33.2)	99 (36.9)	90 (33.5)	33 (14.3)	30 (12.9)	23 (28.7)	24 (30.0)	60 (26.3)	56 (24.9)	71 (18.3)	32 (16.3)	35 (15.4)	34 (14.8)
	1	34 (79.1)	33 (73.3)	340 (71.1)	328 (68.9)	128 (63.7)	135 (66.8)	169 (63.1)	179 (66.5)	197 (85.7)	202 (87.1)	57 (71.3)	56 (70.0)	168 (73.7)	169 (75.1)	318 (81.7)	164 (83.7)	192 (84.6)	196 (85.2)
Platinum salt	Carboplatin	43 (100)	45 (100)	314 (65.7)	317 (66.6)	201 (100)	202 (100)	201 (75.0)	201 (74.7)	230 (100)	232 (100)	NA	NA	161 (70.6)	156 (69.3)	389 (100)	196 (100)	180 (79.3)	181 (78.7)
	Cisplatin	0 (0)	0 (0)	164 (34.3)	159 (33.4)	0 (0)	0 (0)	67 (25.0)	68 (25.3)	0 (0)	0 (0)	NA	NA	67 (29.4)	69 (30.7)	0 (0)	0 (0)	47 (20.7)	49 (21.3)
Brain mts	Yes	0 (0)	0 (0)	55 (11.5)	45 (9.5)	17 (8.5)	18 (8.9)	28 (10.4)	27 (10.0)	5 (2.2)	5 (2.2)	NA	NA	33 (14.5)	22 (9.8)	50 (12.9)	28 (14.3)	1 (0.4)	4 (1.7)
	No	43 (100)	45 (100)	423 (88.5)	431 (90.5)	184 (91.5)	184 (91.1)	240 (89.6)	242 (90.0)	225 (97.8)	227 (97.8)	NA	NA	195 (85.5)	203 (90.2)	339 (87.1)	168 (85.7)	226 (99.6)	226 (98.3)
Liver mts	Yes	NA	NA	NA	NA	77 (38.3)	72 (35.6)	108 (40.3)	104 (38.7)	73 (31.7)	74 (31.9)	NA	NA	95 (41.7)	92 (40.9)	99 (25.4)	51 (26.0)	64 (28.2)	59 (25.7)
	No	NA	NA	NA	NA	124 (61.7)	130 (63.4)	160 (59.7)	165 (61.3)	157 (68.3)	158 (68.1)	NA	NA	133 (58.3)	133 (59.1)	290 (74.6)	145 (74.0)	163 (71.8)	171 (74.3)
Smoking status	Smoker	38 (88.4)	41 (91.1)	268 (56.1)	271 (56.9)	192 (95.5)	199 (98.5)	246 (91.8)	254 (94.4)	180 (78.3)	179 (77.2)	NA	NA	220 (96.5)	217 (96.4)	308 (79.2)	161 (82.1)	174 (76.7)	171 (74.3)
	Non-Smoker	5 (11.6)	4 (8.9)	172 (36.0)	167 (35.1)	9 (4.5)	3 (1.5)	22 (8.2)	15 (5.6)	50 (21.7)	53 (22.8)	NA	NA	8 (3.5)	8 (3.6)	81 (20.8)	35 (17.9)	53 (23.3)	59 (25.7)
LDH level	≤ULN	NA	NA	242 (50.6)	246 (51.7)	NA	NA	NA	NA	116 (50.4)	115 (49.6)	NA	NA	100 (43.9)	95 (42.2)	NA	NA	114 (50.2)	109 (47.4)
	>ULN	25 (58.1)	19 (42.2)	231 (48.3)	228 (47.9)	NA	NA	NA	NA	114 (49.6)	117 (50.4)	NA	NA	127 (55.7)	129 (57.3)	NA	NA	113 (49.8)	121 (52.6)
PD-L1 expression level	<1%	NA	NA	NA	NA	36/ 64 (56.3)	36/ 73 (49.3)	NA	NA	196 (85.2)	200 (86.2)	NA	NA	97 (42.5)	78 (34.7)	317 (81.5)	152 (77.6)	NA	NA
	≥1%	NA	NA	NA	NA	28/ 64 (43.8)	37/ 73 (50.7)	NA	NA	24 (10.4)	20 (8.6)	NA	NA	88 (38.6)	97 (43.1)	62 (15.9)	34 (17.3)	NA	NA
	Unknow	NA	NA	NA	NA	NA	NA	NA	NA	10 (4.3)	12 (5.2)	NA	NA	43 (18.9)	50 (22.2)	10 (2.6)	10 (5.1)	NA	NA

CT, platinum-based chemotherapy; IO, immune-oncology; PLA, placebo; PD-L1, programmed death-ligand 1; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase; ULN, upper limit of normal; mts, metastases; NA, not available.

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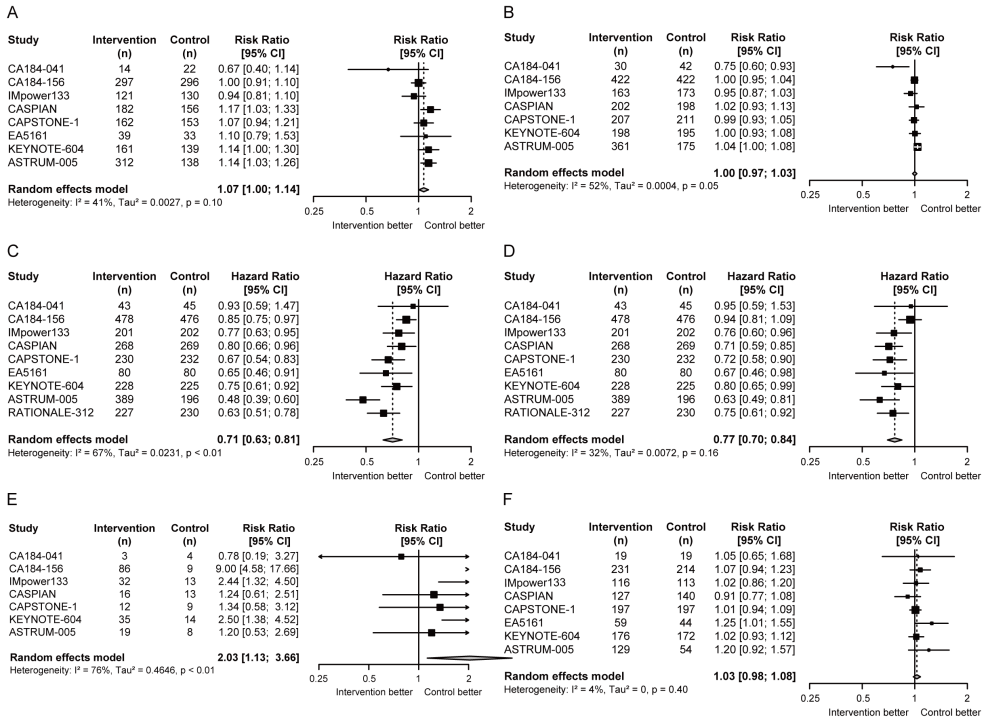

FIGURE 2
Forest plots of efficacy and safety endpoints in ES-SCLC patients receiving first-line chemoimmunotherapy versus chemotherapy. **(A)** RRs of ORR; **(B)** RRs of DCR; **(C)** HR of PFS; **(D)** HR of OS; **(E)** RRs of DR; **(F)** RRs of TRAEs. ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; OS, overall survival; DR, discontinuation rate; TRAEs, treatment-related adverse events; RR, risk ratio; HR, hazard ratio; CI, confidence interval.

microenvironment in SCLC and NSCLC affects the clinical efficacy of chemoimmunotherapy (39). Second, the expression level of PD-L1 in SCLC was generally lower than that in NSCLC. In Checkmate-032 trial (40), PD-L1 expression was observed to be greater than 1% in only 17% of ES-SCLC patients, and greater than 5% in only 5% of ES-SCLC patients. The sample size of patients with positive PD-L1 expression accounted for only 26% in our meta-analysis, potentially limiting the assessment of therapeutic heterogeneity regarding PD-L1 expression level. Moreover, there were various evaluation approaches and detection methods for PD-L1 expression. For example, PD-L1 expression of tissues in SCLC could not be reflected by fine needle aspiration specimens (41). More studies are needed to explore the feasibility of using PD-L1 expression level as a biomarker in ES-SCLC patients receiving chemoimmunotherapy.

In the heterogeneity analysis of brain metastasis subgroup, patients without brain metastasis may respond better to first-line chemoimmunotherapy than those with brain metastasis. A study of PD-1 inhibitors plus chemotherapy as first-line treatment for advanced nonsquamous NSCLC with brain metastases showed favorable intracranial anti-tumor activity and tolerability of this regimen. For patients with negative PD-L1 expression, this regimen also demonstrated efficacy which provided strong evidence to support the application of chemoimmunotherapy in patients with brain metastasis (42). The study of Rudin et al. similarly found that ES-SCLC patients without brain metastases were the dominant group of first-line chemoimmunotherapy (1). Notably, except for ASTRUM-005 trial, there was a trend that patients without brain metastases receiving chemoimmunotherapy have more significant survival benefits. Varied criteria for brain metastases in the included studies may account for this discrepancy. For example, patients with asymptomatic and stable brain metastases were included in ASTRUM-005 trial (13), while patients with lesions confined to the supratentorial region and cerebellum and without central nervous system progression after stereotactic treatment or whole brain radiotherapy were also included in CAPSTONE-1 trial. In our meta-analysis, patients with brain metastases accounted for only 9.14% of all included patients, which may limit the generalizability even statistical significance of the results. Additionally, due to complex tumor microenvironment of brain metastases and different ability of ICIs to penetrate the blood-brain barrier (43), whether chemoimmunotherapy can be used as the first-line treatment for ES-SCLC patients with brain metastases remains to be studied.

Non-smokers, Asians, patients without liver metastasis or with LDH ≤ UNL or using etoposide-cisplatin were observed to tend to achieve longer OS from chemoimmunotherapy, although these findings did not reach statistical significance. However, the OS and PFS benefits of patients in the age and ECOG PS subgroups were inconsistent after receiving chemoimmunotherapy. Different from our results, a meta-analysis showed that smokers receiving chemoimmunotherapy had a better therapeutic effect than non-smokers in metastatic NSCLC (44). Recently, a study revealed the distinct immune microenvironment of lung adenocarcinoma in non-smokers and smokers, further explaining the poor response of

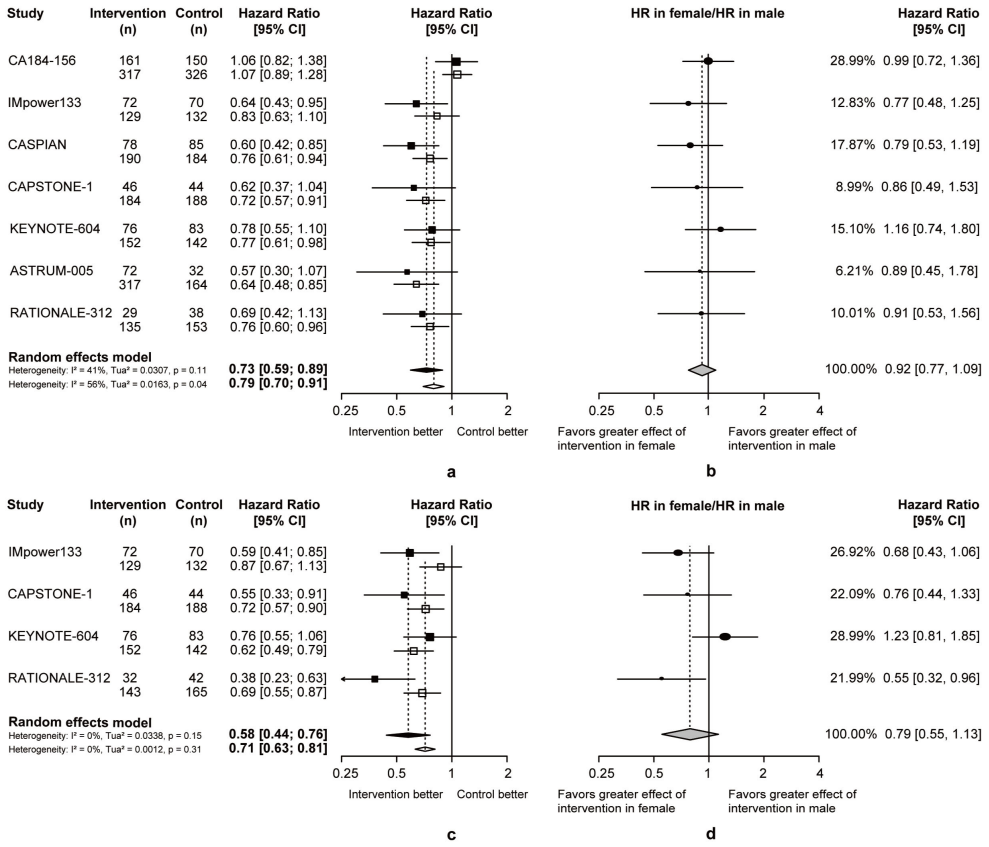

FIGURE 3
Heterogeneity of efficacy between gender subgroup. **(A)** The OS-HRs of the intervention and control groups are compared in gender subgroup. **(B)** The PFS-HRs of the intervention and control groups are compared in gender subgroup. Squares indicate study-specific hazard ratios. Values less than 1 indicate intervention is better than control. Size of the square is proportional to the precision of the estimate. Horizontal lines indicate the 95% CI. Diamonds indicate the meta-analytic pooled HRs, calculated separately in women and men, with their corresponding 95% CIs. The dashed line represents the specific combined risk ratio of gender subgroup, and the solid line represents a risk ratio of 1, which is the null hypothesis value. **(C)** The pooled ratio of OS-HRs reported in gender subgroup. **(D)** The pooled ratio of PFS-HRs reported in gender subgroup. Each filled circle indicates the study-specific ratio of HRs. Values less than 1 indicate that the effect of the intervention compared with control is greater for women than men. Size of the circle is proportional to the precision of the estimate. Horizontal lines indicate the 95% CI. The diamond indicates the meta-analytic pooled ratio of HRs, with its corresponding 95% CI. The solid line represents a risk ratio of 1, which is the null hypothesis value.

non-smokers to PD-1/PD-L1 inhibitors (45). Similarly, the distinct immune microenvironment of SCLC and NSCLC may lead to different efficacy of immunotherapy for smokers. The reliable efficacy and safety of first-line chemoimmunotherapy in ES-SCLC patients have been confirmed by several real-world researches (RWR) of different regions (46–50), but some studies on the therapeutic heterogeneity of different ethnicities lacked sufficient evidence. A meta-analysis in NSCLC and SCLC patients with liver metastasis indicated that the presence of liver metastases did not significantly affect the OS benefit of ICIs in NSCLC patients, while a small amount of data showed that liver metastasis inhibited OS benefit in SCLC patients (51). By developing a two-site tumor system model, a study found that liver metastasis can inhibit systemic anti-tumor immune response, and proposed that CTLA-4 inhibitors or enhancer of zeste homolog 2 (EZH2) inhibitors combined with PD-1 inhibitors can restore systemic anti-tumor immune activity (52). Therefore, PD-1 inhibitors combined with targeted drugs may be required for patients with liver metastasis. The study of R. Zeng et al. found that OS benefit of PD-L1/PD-1 inhibitors plus chemotherapy in ES-SCLC patients with LDH ≤ ULN was superior to those with LDH > ULN (53). High level of LDH expression has been reported to promote epithelial to mesenchymal transition (54), angiogenesis, cellular invasion and migration, which is associated with a poor prognosis in patients with various solid tumors (55). According to the guidelines, etoposide-carboplatin is selected in more cases (56). Furthermore, a study has shown that the survival advantage associated with cisplatin was not superior to that of carboplatin in single chemotherapy regimen of ES-SCLC, and less toxic carboplatin-etoposide plus chemotherapy regimen may be better (57). In future clinical studies, more patients using etoposide-cisplatin should be considered to provide better treatment options for ES-SCLC patients. A study of chemoimmunotherapy for advanced NSCLC showed that age was negatively correlated with survival in patients receiving ICIs combined with or without chemotherapy, indicating that the differential use of chemoimmunotherapy across age groups was unlikely to account for age-related survival differences (58). A previous meta-analysis also analyzed the impact of age on the

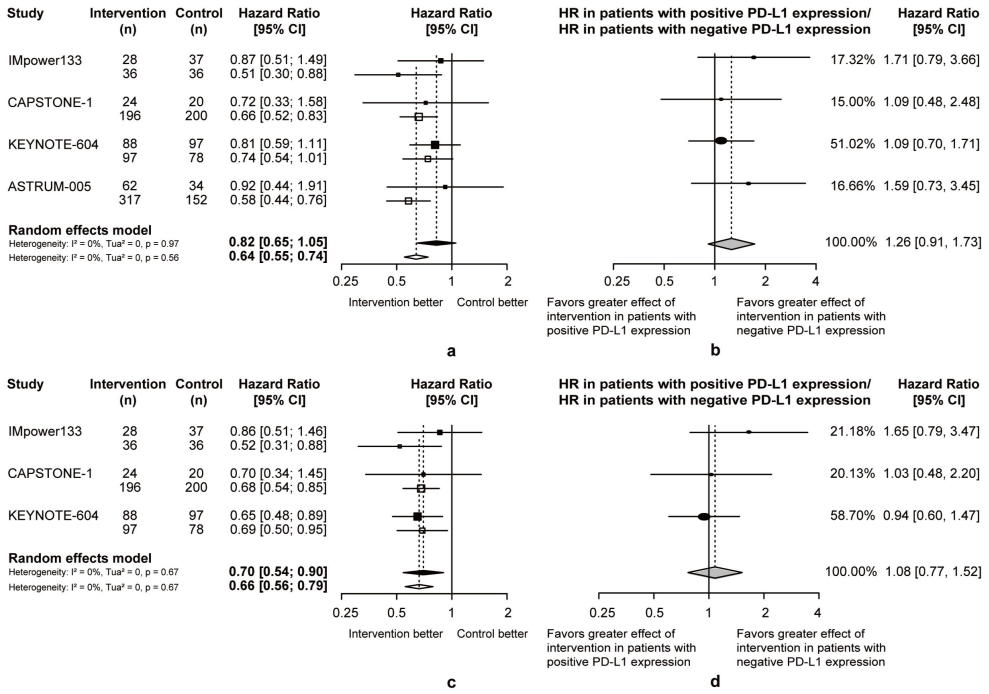

FIGURE 4
Heterogeneity of efficacy between PD-L1 expression level subgroup. **(A)** The OS-HRs of the intervention and control groups are compared in PD-L1 expression level subgroup. **(B)** The PFS-HRs of the intervention and control groups are compared in PD-L1 expression level subgroup. Squares indicate study-specific hazard ratios. Values less than 1 indicate intervention is better than control. Size of the square is proportional to the precision of the estimate. Horizontal lines indicate the 95% CI. Diamonds indicate the meta-analytic pooled HRs, calculated separately in patients with positive PD-L1 expression and patients with negative PD-L1 expression, with their corresponding 95% CIs. The dashed line represents the specific combined risk ratio of PD-L1 expression level subgroup, and the solid line represents a risk ratio of 1, which is the null hypothesis value. **(C)** The pooled ratio of OS-HRs reported in PD-L1 expression level subgroup. **(D)** The pooled ratio of PFS-HRs reported in PD-L1 expression level subgroup. Each filled circle indicates the study-specific ratio of HRs. Values greater than 1 indicate that the effect of the intervention compared with control is greater for patients with negative PD-L1 expression than patients with positive PD-L1 expression. Size of the circle is proportional to the precision of the estimate. Horizontal lines indicate the 95% CI. The diamond indicates the meta-analytic pooled ratio of HRs, with its corresponding 95% CI. The solid line represents a risk ratio of 1, which is the null hypothesis value.

efficacy of chemoimmunotherapy in lung cancer patients, and no statistically significant effect was observed (29). For most RCTs, the majority of patients included were ECOG PS 0 or 1, and patients with ECOG PS ≥ 2 were usually excluded, leading to unclear efficacy of chemoimmunotherapy in these patients. A meta-analysis of 19 studies containing 3600 NSCLC patients showed that the efficacy of chemoimmunotherapy in patients with ECOG PS ≥ 2 was comparable to that of chemotherapy (59). Regardless of ECOG PS, ICIs have been approved and routinely administered, so whether ECOG PS is a predictor of chemoimmunotherapy efficacy remains to be confirmed.

One advantage of the analysis is that all the data were derived from large RCTs with similar trial designs and enrolled populations. Additionally, our meta-analysis is the latest and most detailed assessment of heterogeneity between subgroups of first-line chemoimmunotherapy for ES-SCLC patients, including therapeutic heterogeneity among clinicopathological characteristics and subgroup analyses of different chemoimmunotherapy regimens. Notably, patients with negative PD-L1 expression may have better survival outcomes than those with positive PD-L1 expression. Although this result did not reach a statistically significant level, it provided guidance for the treatment of ES-SCLC patients and underscores the need for further clinical and basic research to explore the significance of PD-L1 expression in ES-SCLC.

Nevertheless, there are several limitations in our meta-analysis. First, this meta-analysis is based on published clinical trial data and lacks individual patient-level data, which hinders more in-depth analysis and may lead to potential publication bias. Second, although all included trials are RCTs, the imbalance of baseline characteristics (selected patient population, sample size, low incidence, different treatment of brain metastases, etc.) of included trials should be considered. Third, these results should always be interpreted with caution since the included trials are subject to updates and several ongoing trials are not included.

In conclusion, we suggested that chemoimmunotherapy can significantly prolong OS and PFS in ES-SCLC patients compared with chemotherapy. By analyzing the therapeutic heterogeneity between subgroups, we concluded that women or patients with negative PD-L1 expression or with LDH ≤ ULN or without brain metastasis tend to benefit more from first-line chemoimmunotherapy in ES-SCLC. Additionally, patients with negative PD-L1 expression or LDH ≤ ULN have consistent trend toward prolonged OS, and patients without brain metastasis or liver metastasis have consistent trend toward prolonged PFS. In aggregate, the findings of this meta-

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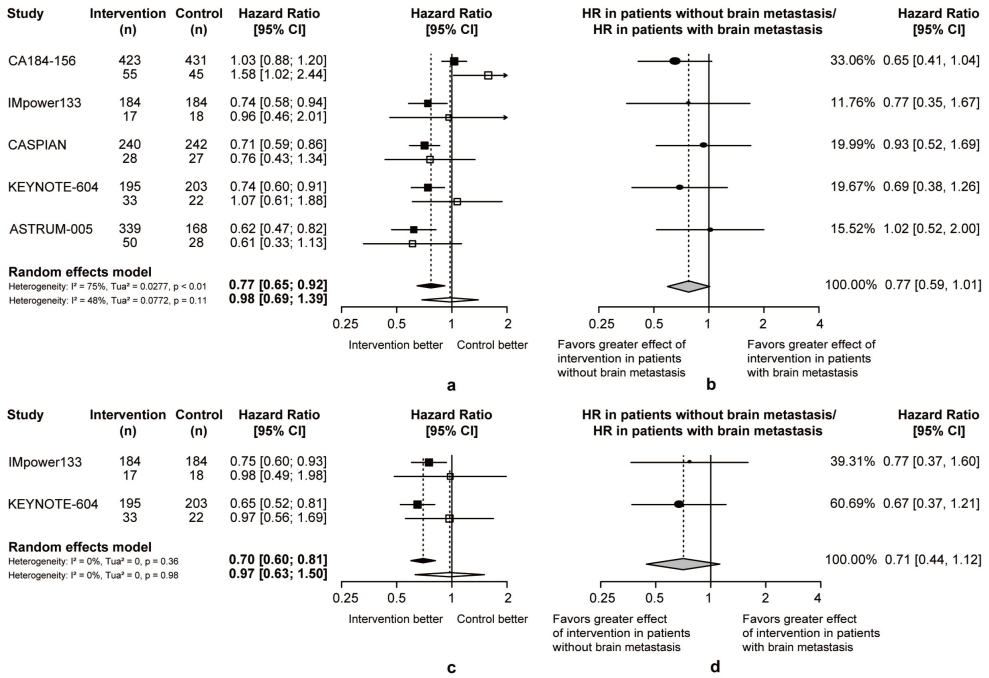

FIGURE 5
Heterogeneity of efficacy between brain metastases subgroup. **(A)** The OS-HRs of the intervention and control groups are compared in brain metastases subgroup. **(B)** The PFS-HRs of the intervention and control groups are compared in brain metastases subgroup. Squares indicate study-specific hazard ratios. Values less than 1 indicate intervention is better than control. Size of the square is proportional to the precision of the estimate. Horizontal lines indicate the 95% CI. Diamonds indicate the meta-analytic pooled HRs, calculated separately in patients without brain metastasis and patients with brain metastasis, with their corresponding 95% CIs. The dashed line represents the specific combined risk ratio of brain metastases subgroup, and the solid line represents a risk ratio of 1, which is the null hypothesis value. **(C)** The pooled ratio of OS-HRs reported in brain metastases subgroup. **(D)** The pooled ratio of PFS-HRs reported in brain metastases subgroup. Each filled circle indicates the study-specific ratio of HRs. Values less than 1 indicate that the effect of the intervention compared with control is greater for patients without brain metastasis than patients with brain metastasis. Size of the circle is proportional to the precision of the estimate. Horizontal lines indicate the 95% CI. The diamond indicates the meta-analytic pooled ratio of HRs, with its corresponding 95% CI. The solid line represents a risk ratio of 1, which is the null hypothesis value.

TABLE 3 Subgroup analysis of overall survival and progression-free survival among different types of chemoimmunotherapy in this meta-analysis.

	HR (95% CI)									
	Sex	Age	PD-L1 expression level	Brain mts	Liver mts	ECOG PS	LDH level	Race	Smoking status	Platinum salt
OS										
CTLA-4 Inhibitors	0.99 (0.72-1.36)	1.06 (0.76-1.47)	NA	0.65 (0.41-1.04)	NA	0.77 (0.56-1.07)	1.02 (0.76-1.37)	NA	0.94 (0.68-1.28)	0.82 (0.59-1.12)
PD-L1 Inhibitors	0.80 (0.61-1.05)	0.91 (0.64-1.29)	1.39 (0.79-2.42)	0.87 (0.54-1.40)	0.80 (0.63-1.02)	1.01 (0.77-1.33)	1.41 (0.90-2.19)	0.88 (0.53-1.46)	0.90 (0.58-1.38)	0.88 (0.58-1.34)
PD-1 Inhibitors	1.02 (0.75-1.38)	0.89 (0.69-1.15)	1.20 (0.82-1.76)	0.82 (0.52-1.28)	1.05 (0.78-1.41)	1.13 (0.82-1.56)	1.10 (0.82-1.47)	1.28 (0.88-1.88)	0.93 (0.69-1.26)	0.97 (0.70-1.35)
PFS										
PD-L1 Inhibitors	0.71 (0.50-1.01)	0.94 (0.69-1.29)	1.31 (0.77-2.23)	0.77 (0.37-1.60)	0.88 (0.65-1.21)	0.94 (0.65-1.34)	0.91 (0.60-1.38)	NA	0.58 (0.34-0.99)	NA
PD-1 Inhibitors	0.84 (0.38-1.84)	1.11 (0.83-1.48)	0.94 (0.60-1.47)	0.67 (0.37-1.21)	0.78 (0.58-1.05)	1.06 (0.75-1.52)	1.10 (0.82-1.46)	NA	0.91 (0.65-1.28)	NA

OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; PD-L1, programmed death-ligand 1; PD-1, programmed death-1; ECOG PS, Eastern Cooperative Oncology Group Performance Sstatus; LDH, lactate dehydrogenase; mts, metastases; NA, not available.

analysis could assist in achievement of personalized treatment by screening out more suitable candidates for chemoimmunotherapy, as well as the design and interpretation of future trials on therapeutic heterogeneity in ES-SCLC patients.

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 authors.

Author contributions

WK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. JC: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing. LP: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. HL: Conceptualization, Data curation, Formal analysis, Resources, Supervision, Visualization, Writing – review & editing. PZ: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Visualization, Writing – review & editing. TL: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Visualization, Writing – review & editing. HH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. YS: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Key Medical Discipline of Jiangsu Province (No. JSDW202208), the Natural Science Foundation of Jiangsu Province (No. BK20210146) and the Clinical Research Project of Jinling Hospital (No. 22LCYY-QH7).

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

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1334957/full#supplementary-material

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frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 03 February 2025
DOI 10.3389/fonc.2025.1385794

OPEN ACCESS

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RECEIVED 14 February 2024
ACCEPTED 13 January 2025
PUBLISHED 03 February 2025

CITATION
Jiang C and Guo S (2025) Tacrolimus and mycophenolate mofetil in corticosteroid-resistant hepatitis secondary to tislelizumab: a case report.
Front. Oncol. 15:1385794.
doi: 10.3389/fonc.2025.1385794

Tacrolimus and mycophenolate mofetil in corticosteroid-resistant hepatitis secondary to tislelizumab: a case report

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Tislelizumab is a monoclonal antibody with high binding affinity for programmed death-1 (PD-1) receptors. In patients with extensive-stage small-cell lung cancer (ES-SCLC), the first-line use of tislelizumab combined with chemotherapy has shown significant efficacy. However, with the widespread use of PD-1 inhibitors, there are increasing reports of immune-related adverse events (irAEs) in clinical practice, with immune-related hepatitis (IRH) being particularly common. This article reports a case of an ES-SCLC patient (cT3N3M0 cStage IIIB) who developed corticosteroid-resistant hepatitis and recovered through dual immunosuppressant therapy. The patient was a 67-year-old male, diagnosed with ES-SCLC, who received a combination therapy of etoposide, cisplatin, and tislelizumab. Three weeks after the fourth treatment cycle, the patient experienced symptoms, such as decreased appetite, itching, yellow urine, and jaundice, and was diagnosed with IRH, manifested as "Grade 3 total bilirubin increase," "Grade 3 alanine transaminase increase," and "Grade 3 aspartate transaminase increase." Despite intravenous injection of methylprednisolone (MP) 100 mg/day (2 mg/kg) and oral administration of mycophenolate mofetil (MMF) 1 g twice daily, liver function continued to be impaired. In this context, tacrolimus (TAC) (5 mg, twice daily) was added to the therapy, and the IRH level was reduced from Grade 3 to normal. Subsequently, TAC and MMF were gradually reduced and eventually discontinued. Unfortunately, after discontinuing immunosuppressants, IRH recurred. Although the patient still responded to TAC combined with MMF, liver function recovery took a longer time. Due to persistent liver dysfunction, the patient failed to receive second-line chemotherapy and ultimately passed away due to disease progression. Through this case, we hope to emphasize the importance of reasonably extending the use of immunosuppressants to avoid the recurrence of IRH and reduce the premature discontinuation of immunosuppressants. Besides, when tumor progression and IRH recurrence occur simultaneously, providing effective immunosuppressive therapy and reasonably arranging systemic anti-tumor therapy may bring clinical benefits to patients.

KEYWORDS

immune-related hepatitis, tacrolimus, mycophenolate mofetil, corticosteroid, tislelizumab

Introduction

The emergence of immune checkpoint inhibitors (ICIs) has revolutionized the treatment landscape for patients with advanced solid malignancies demonstrating significant clinical benefits (1). However, the activation of T cells by ICIs can also lead to attacks on non-tumor normal tissues resulting in organ toxicity and immune-related adverse effects (irAEs), including immune-related hepatitis (IRH) (2). Most cases of IRH are mild to moderate, and interrupting ICI treatment and using corticosteroids can effectively control them (3). However, in a small number of patients with Grades 3 and 4 liver injury, corticosteroid alone may not be sufficient, and additional immunosuppressants, such as mycophenolate mofetil (MMF) or tacrolimus (TAC), are required (4, 5).

Tislelizumab is a human IgG4 monoclonal antibody developed by BeiGene Ltd. that binds to and blocks the programmed cell death-1 (PD-1) receptor expressed on activated immune cells, including T lymphocytes (6). Tislelizumab can enhance anti-cancer immune activity by blocking the binding of PD-1 to its ligand. Two early studies have shown that tislelizumab monotherapy has anti-tumor activity in patients with advanced refractory solid tumors (7, 8). The combination of tislelizumab and platinum-based chemotherapy as first-line treatment for advanced small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) exhibited robust responses in a phase 2 study (9). In the RATIONALE-312 study, the incidence of IRH was 1.3%, and only one patient in the tislelizumab group (n = 227) reported ≥Grade 3 IRH (10). Here, we report a case of a Grade 3 IRH patient who was resistant to corticosteroid treatment and gradually recovered liver function after receiving MMF and TAC. Unfortunately, after discontinuing immunosuppressants, IRH recurred. Although the patient still responded to MMF and TAC, it took a longer time to improve liver function. During the treatment of hepatotoxicity, the patient was unable to receive systemic anti-tumor therapy and ultimately passed away due to disease progression.

Case presentation

A 67-year-old man with extensive-stage small-cell lung cancer (ES-SCLC) (cT3N3M0 cStage IIIB) received etoposide plus cisplatin in combination with tislelizumab (200 mg). Three weeks after completing the fourth treatment cycle, the patient started experiencing symptoms such as decreased appetite, itching, yellow urine, and jaundice. Liver function tests were conducted revealing the following results: total bilirubin (TB): 136 μmol/L (normal range: 0–26 μmol/L), alanine transaminase (ALT): 526 U/L (normal range: 0–50 U/L), aspartate transaminase (AST): 350 U/L (normal range: 0–40 U/L), alkaline phosphatase (ALP): 362 U/L (normal range: 45–125 U/L), and γ-glutamyl transpeptidase (GTP): 264 U/L (normal range: 10–60 U/L) (Figure 1D). According to the Common Terminology Criteria for Adverse Events Version 5.0, the patient was diagnosed with "Grade 3 total bilirubin increase," "Grade 3 alanine transaminase increase," and "Grade 3 aspartate transaminase increase." Coagulation function and hemogram were normal. Chest CT showed near-complete resolution of lung lesions. Abdominal CT and ultrasound did not indicate liver metastasis or abnormalities in the hepatobiliary system. Anti-nuclear antibodies and anti-smooth muscle actin antibodies were negative. Tests for hepatitis B, hepatitis C virus, and HIV were also negative. The necessity of liver biopsy remains controversial in cases of suspected immune-related liver injury (11), and the patient declined this procedure, so no liver biopsy was conducted. Given the patient's treatment history and clinical presentation, it was strongly suspected that liver dysfunction was related to immunotherapy. Therefore, the patient was diagnosed with IRH, and the PD-1 inhibitor tislelizumab was interrupted. The patient continued to receive ursodeoxycholic acid (UDCA) and was administered intravenous pulse methylprednisolone (MP) at a dose of 100 mg (2 mg/kg) for 3 days. Subsequently, the patient's TB level and liver enzyme value decreased. Continuing the use of MP for 3 days resulted in a decrease in liver enzymes, but TB level increased. Although liver enzymes continued to decrease over the next 3 days, the persistent increase in TB led us to decide to add MMF at 1 g twice daily. However, we did not observe any improvement in TB level 3 days later. After starting oral MMF, TAC (5 mg) was added twice daily 4 days later. TB level gradually decreased by the 14th day of admission. Meanwhile, due to the patient's resistance to corticosteroid, the MP dose was gradually tapered and eventually discontinued on the 50th day of admission. ALT and AST levels returned to normal by the 28th day of admission. The patient was discharged 39 days after hospitalization. Liver function was regularly followed up post-discharge. TB level returned to normal on the 52nd day since the initial detection of liver dysfunction. Then, the dose of TAC was decreased to 3 mg twice daily and ultimately discontinued on the 62nd day due to normalization of TB, ALT, and AST levels. MMF was then discontinued 10 days after stopping TAC (on the 72nd day).

The patient was readmitted on the 78th day due to dizziness, headache, and left supraclavicular lymph node enlargement. Pulmonary imaging examination revealed stable disease, and magnetic resonance imaging (MRI) detected brain metastasis (Figure 1A). An ultrasound demonstrated enlargement of the left supraclavicular lymph nodes. Liver function tests showed that TB, ALT, AST, and ALP were all within normal ranges (on the 79th day). The patient underwent a lymph node biopsy, and pathological results confirmed SCLC. A plan was devised to administer radiotherapy combined with second-line chemotherapy to the brain and left supraclavicular lymph nodes. However, before treatment commenced, the patient experienced nausea, yellow urine, jaundice, and liver dysfunction. Liver function analysis indicated abnormal TB at 50 μmol/L, ALT at 209 U/L, AST at 220 U/L, ALP at 479 U/L, and GTP at 1,409 U/L (on the 89th day). After the initial detection of liver dysfunction, the patient did not take any other medications except for UDCA, MP, and

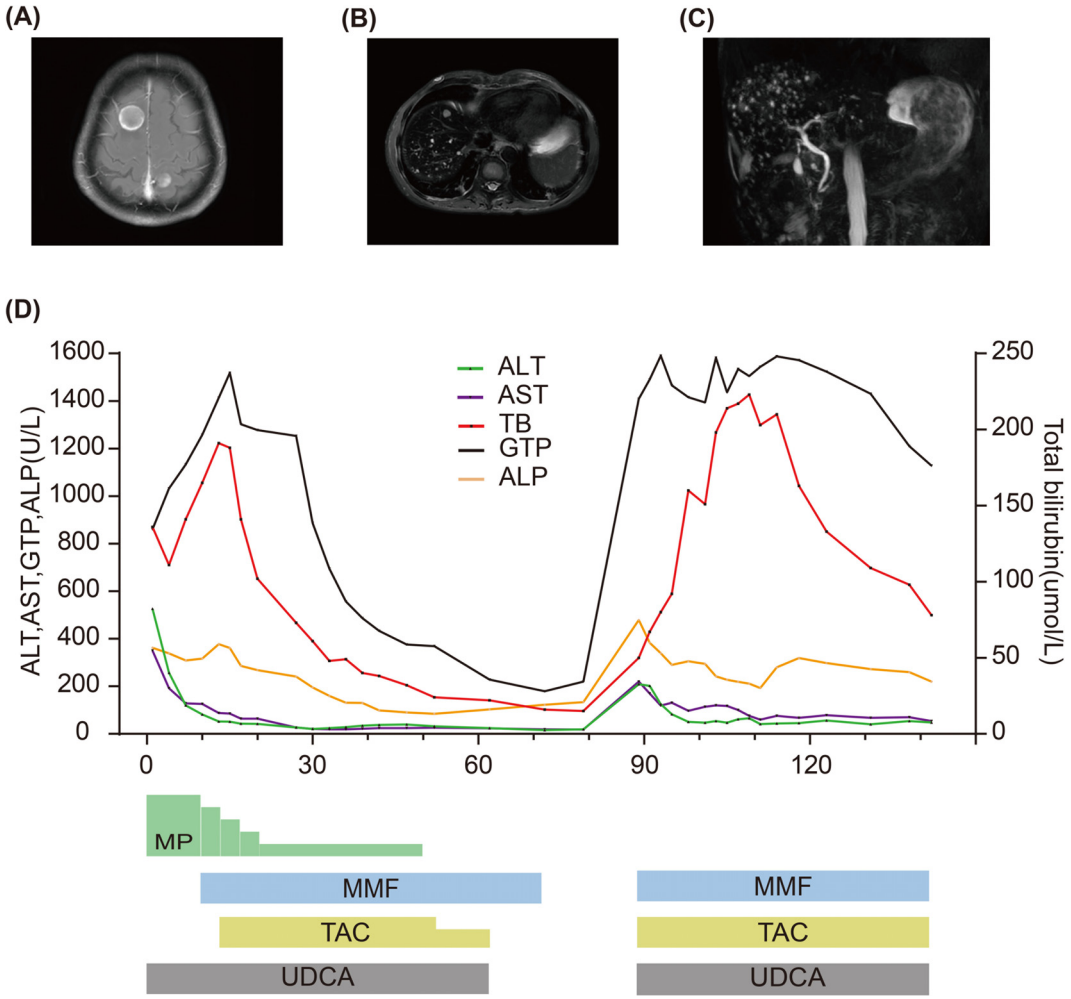

FIGURE 1
(A) MRI of the brain shows brain metastases. **(B)** MRI of the abdomen shows liver metastatic lesions. **(C)** MRCP performed when TB level was re-increased showing normal biliary tract. **(D)** Summary of clinical course and biochemical examinations.

immunosuppressants. Based on the previous treatment history, the patient was diagnosed with IRH relapse. The treatment was restarted with 1 g of MMF twice daily, 5 mg of TAC twice daily, and UDCA for liver dysfunction. The patient underwent radiotherapy for the brain and left supraclavicular lymph nodes but could not receive chemotherapy owing to abnormal liver function. Following immunosuppressive therapy, the patient's liver enzymes gradually decreased, but TB significantly increased. An abdominal MRI revealed multiple small metastatic liver lesions, with the largest lesion diameter being approximately 1 centimeter. Magnetic resonance cholangiopancreatography (MRCP) did not show any signs of biliary tract obstruction on the 93rd day (Figures 1B, C). After 28 days of oral administration of MMF and TAC, a gradual improvement in TB was observed. However, due to liver dysfunction and an Eastern Cooperative Oncology Group (ECOG) performance status of 3, the patient failed to receive second-line systematic chemotherapy. The patient required discharge on the 39th day of hospitalization (on the 107th day). Follow-up liver function tests showed a gradual recovery of TB. Due to the inability to receive systemic anti-tumor treatment, the patient eventually succumbed to disease progression.

Discussion

ICIs have revolutionized the treatment of various solid tumors demonstrating remarkable clinical benefits (12). However, with the widespread application of these innovative therapies, the incidence of irAEs in clinical practice has gradually increased, with IRH being the most common type. The incidence of IRH caused by PD-1 inhibitor alone was approximately 1%–4%, while the incidence rate can rise to as high as 33% in patients receiving PD-1 inhibitors combined with CTLA-4 inhibitors (10, 13, 14). IRH is typically defined as an increase of at least three times in liver enzyme levels (15, 16). Its typical manifestations include elevated ALT and AST levels, which may or may not be accompanied by an increase in bilirubin (17). The onset of IRH usually occurs within 1–3 months, but it can also arise at any time. Notably, the management of IRH

lacks strong randomized evidence, and existing treatment recommendations are primarily based on expert consensus from the European Society for Medical Oncology, the American Society of Clinical Oncology, the Society for Immunotherapy of Cancer, and the National Comprehensive Cancer Network (15, 16, 18). Generally, IRH associated with liver enzyme abnormalities may spontaneously resolve after discontinuing ICI treatment. Some patients require corticosteroid therapy to control IRH, while a small number of IRH patients who are resistant to corticosteroids necessitate the addition of immunosuppressants such as MMF, TAC, and cyclosporine. To date, the incidence rate of corticosteroid-resistant IRH is not well defined, and the evidence for its diagnosis and treatment is relatively limited. According to previous retrospective studies, approximately 23%–48% of IRH patients require additional use of immunosuppressants (19, 20). There is debate in clinical practice regarding the necessity of liver biopsy as an auxiliary tool for diagnosing corticosteroid-resistant IRH, but most guidelines recommend considering the need for liver biopsy based on specific clinical circumstances (15, 16, 18).

Herein, we report a case of corticosteroid-resistant Grade 3 IRH induced by tislelizumab. The patient developed IRH on the 15th week after starting tislelizumab treatment. The main clinical symptoms included decreased appetite, yellow urine, and jaundice. In addition to clinical manifestations, laboratory abnormalities associated with ICI included "Grade 3 total bilirubin increase," "Grade 3 alanine aminotransferase increase," and "Grade 3 aspartate aminotransferase increase." According to a recent systematic review, approximately half of Grades 3–4 IRH patients achieved remission without receiving corticosteroid treatment (11), but this situation did not apply to our case. Despite pulse therapy with intravenous MP at 2 mg/kg, there was a decreasing trend in liver enzyme levels, but TB continued to rise.

For patients who did not respond to first-line MP treatment, MMF was recommended as a second-line treatment option and has been successfully applied to some corticosteroid-resistant IRH patients (5). Although MMF has achieved partial success in some cases of IRH, some patients have not seen significant therapeutic effects after treatment with steroids and MMF. Given the presence of CD8+ T-lymphocyte infiltration in histopathology, immunosuppressive agents that specifically target T cells, such as cyclosporine, TAC, and anti-thymoglobulin, may be the preferred third-line treatment. These therapies have been successfully applied in several cases (21–23). In addition, tocilizumab (IL-6 receptor antagonist) (24) and plasma exchange (25) have also been successfully used in some cases of steroid-refractory IRH. There are reports that the anti-TNF inhibitor infliximab normalizes liver function in steroid-refractory IRH patients (26), but not all guidelines recommend the use of this type of drug in IRH due to its potential hepatotoxicity (15, 16, 18).

In this case, despite the addition of the immunosuppressive agent MMF, an increase in TB was still observed. Immune-related cholestatic hepatitis is typically characterized by elevated levels of bilirubin, ALP, and GTP, indicating resistance to corticosteroids and a poor prognosis (27). Abdominal ultrasound or MRCP plays a key role in excluding factors of biliary obstruction. The abdominal ultrasound examination of this patient did not show any signs of biliary tract obstruction. Due to the patient's refusal, a liver biopsy could not be performed. In this context, administering the calcineurin inhibitor TAC resulted in a gradual return of TB to normal level. Later, we adjusted the dosage of TAC and ultimately discontinued MMF and TAC. Unfortunately, after stopping MMF and TAC, the patient's TB and liver enzymes showed abnormalities again. Although abdominal MRI showed metastatic liver lesions, MRCP showed no signs of biliary tract obstruction, and the patient did not take any hepatotoxic substances, the possibility of IRH relapse remained the top priority. MMF and TAC were orally administered again, and an improvement in liver enzymes was observed, but TB began to decrease until 28 days of therapy. Chemotherapy was not administered during the treatment of liver dysfunction, and the patient died due to tumor progression.

The process of IRH treatment suggests that IRH exhibits significant clinical heterogeneity, and its management remains challenging due to poorly understood pathogenesis, difficult diagnosis, and serious clinical consequences. Currently, there is insufficient evidence to support a specific duration for the use of immunosuppressants. Ziogas reported that resolving corticosteroid-resistant IRH with effective immunosuppressants may take up to 3 months, and the patient did not experience a recurrence of IRH (28). Hence, 3 months may be a reasonable duration for the use of immunosuppressants in these cases. In our subsequent clinical practice, immunosuppressants were administered for 3 months in corticosteroid-resistant IRH patients, and no patients experienced a recurrence of IRH.

In conclusion, our IRH patient exhibited resistance to corticosteroids but responded well to dual immunosuppressive therapy with MMF and TAC. Given the recurrence of IRH after discontinuing immunosuppressants, prolonging the treatment time of immunosuppressants to stabilize liver function may help to obtain opportunities for anti-tumor treatment. In addition, when tumor progression and IRH recurrence occur simultaneously, timely anti-tumor treatment may bring clinical benefits to patients, in addition to using initially effective immunosuppressive therapy.

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

CJ: Conceptualization, Writing – original draft. SG: Software, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors would like to express their gratitude to the medical, nursing, and pharmacy staff who were involved in the patient's hospitalization.

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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