ImmunoTargets and Therapy Dovepress

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Patients with Extensive-Stage Small Cell Lung Cancer Harboring Less Than 4 Metastatic Sites May Benefit from Immune Checkpoint Inhibitor Rechallenge by Reshaping Tumor Microenvironment

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Background: Immune checkpoint inhibitors (ICIs) has prolonged survival in patients with extensive-stage small cell lung cancer (ES-SCLC) as first-line treatment. However, whether ICI rechallenge could bring survival benefit to patients with ES-SCLC following its failure as first-line treatment remains unknown. Therefore, we aim to address the issue and identify the cohort of patients that may derive such benefit.

Methods: Patients with ES-SCLC from both the IMpower133 study and Shandong Cancer Hospital and Institute (shanzhong cohort) who failed first-line ICI were included. Kaplan Meier analysis was performed to compare overall survival (OS). Both univariate and multivariate Cox regression analyses were conducted to identify factors affecting survival. Tumor immune cell infiltration was evaluated by the CIBERSORT algorithm and detected by multiplex immunofluorescence (mIF).

Results: A total of 125 ES-SCLC patients undergoing atezolizumab and 161 patients undergoing ICI as first-line treatment were recruited from IMpower133 and shanzhong cohort. Those receiving ICI rechallenge had a longer OS than those without in IMpower133 ($P = 0.08$) and shanzhong cohort ($P = 0.013$). In IMpower133 cohort, subgroup analyses found that patients with <4 metastatic sites derived more survival benefit from atezolizumab ($P = 0.008$). For patients with ES-SCLC harboring <4 metastatic sites, there was significant OS difference between atezolizumab versus non-atezolizumab as retreatment ($P = 0.036$). Moreover, for ES-SCLC patients with <4 metastatic sites, atezolizumab improved survival compared with non-atezolizumab (hazard ratio [HR]: 0.457; 95% CI: 0.256–0.817; $P = 0.008$). These findings were confirmed in shanzhong cohort. Those harboring <4 metastatic sites had fewer M2 macrophage and more CD4 naïve T cells infiltration, which was further confirmed by mIF of ES-SCLC samples from shanzhong cohort.

Conclusion: Our study provides rationale for ICI rechallenge among ES-SCLC patients with <4 metastatic sites, suggesting beneficial outcome by reshaping TME.

Keywords: extensive stage small cell lung cancer, ICI rechallenge, survival, metastatic sites, tumor microenvironment

Received: 17 June 2024
Accepted: 22 October 2024
Published: 26 October 2024

Introduction

Patients with extensive stage-small cell lung cancer (ES-SCLC) quickly progress on platinum-based treatment.[1,2] There have been few effective treatment options available after progression with first-line therapy. Considering the aggressiveness and fast progression of ES-SCLC, the combination of immune checkpoint inhibitors (ICIs) with platinum-based chemotherapies has represented a novel therapeutic as first-line treatment.[3,4] It has been reported that induction with atezolizumab plus carboplatin and etoposide and maintenance with atezolizumab resulted in improved survival in SCLC, as demonstrated in IMpower133. Survival benefits of immune checkpoint inhibitors ascended to 6 to 7 months in both IMpower 133 and CASPIAN study.[5,6] And long-term follow-up of large randomized clinical trials has shown that durable responses can be maintained after ICIs.[7] This raises the issue of what is the most optimal option for treatment following ICI treatment in first-line. The previous dogma assumes that a different drug should be adopted because disease progression would occur due to drug resistance. However, this perception has been challenged recently by the mechanism of action of ICI, where the immune memory would be reset and reactivated.[8,9] Retreatment with ICI as subsequent therapies, known as ICI rechallenge, has been documented in several cancer types but seldomly reported in SCLC.[10–12] In a case study, ICI rechallenge using the combination of penpulimab and anlotinib demonstrated improved survival in a patient with ES-SCLC.[13] Currently, there have been no studies that address the critical clinical issues such as the utility of ICI retreatment for patients with ES-SCLC following first-line ICI.

Therefore, in the present study, we aim to investigate whether ICI rechallenge could improve survival following its first-line among patients with ES-SCLC. Additionally, we explored potential biomarkers for ICI rechallenge advantageous populations following first-line among patients with ES-SCLC.

Tumor microenvironment (TME), an indispensable component of cancer, has been reported to play a vital role in the modulation of ICI responses.[14,15] The TME is profoundly immunosuppressive, which provides the rationale of ICI against cancer. It is well recognized that the clinical efficacy of ICI is associated with the immune cell infiltration.[16,17] We therefore also dug into the mechanisms underlying the beneficial role of ICI rechallenge among such populations by analyzing their TME landscapes.

Methods

Study Design and Data Collection

The data of our study was obtained from IMpower133 study (IMpower133 cohort) and ES-SCLC patients from Shandong Cancer Hospital and Institute (shanzhong cohort). The IMpower133 study is a randomized, double-blind, Phase I/III study, demonstrated that adding atezolizumab to carboplatin plus etoposide for first-line treatment of ES-SCLC resulted in significant improvement in overall survival (OS) and progression-free survival (PFS) versus placebo plus carboplatin and etoposide.[5] The study and data have been published; thus, informed consent and ethical committee approval were not warranted. All patients in this study provided written informed consent and this study was approved by the Ethics Committee of Shandong Cancer Hospital and Institute (approval ID number: 2024006145). It also conforms to the provisions of the Declaration of Helsinki. The recruited patients from both IMpower133 and shanzhong cohort were subject to further analyses.

Estimation of Immune Cell Type Fractions and Gene Expressions

The ES-SCLC bulk RNAseq data from IMpower133 was downloaded from the European Genome-Phenome Archive (https://ega-archive.org/studies). To quantify the abundance of 22 immune cells in ES-SCLC specimens, we applied CIBERSORT to provide an estimation of the proportions of cell types in a mixed cell population using normalized data.[18] The 22 types of infiltrating immune cells inferred by CIBERSORT include B cells, plasma cells, T cells, NK cells, monocytes, macrophages, dendritic cells, mast cells, eosinophils, neutrophils. Processed gene expression data were downloaded from public databases and normalized using the limma package in R software (version 4.2.3).

Tissue Multiplex Immunofluorescent (mIF) Staining

ES-SCLC samples were obtained by biopsies. 5 um thick formalin-fixed paraffin-embedded (FFPE) tissue sections from shanzhong cohort were evaluated with mIF technology for the following markers: CD68 (KP1; Abcam, cat#ab955), CD206 (E6T5J; CST, cat#24595), CD4 (EPR6855; Abcam, cat#ab133616), CD45RA (D9M8I; CST, cat#13917), CCR7

(E75; Abcam, cat#ab32075), S100A16 (Abcam, ab130419). All IF slides were counterstained with DAPI. The results from each experiment were reviewed by the study pathologist. All scans were performed using the Vectra Polaris at 20X. The patient inclusion process and the study design were shown in Figure 1.

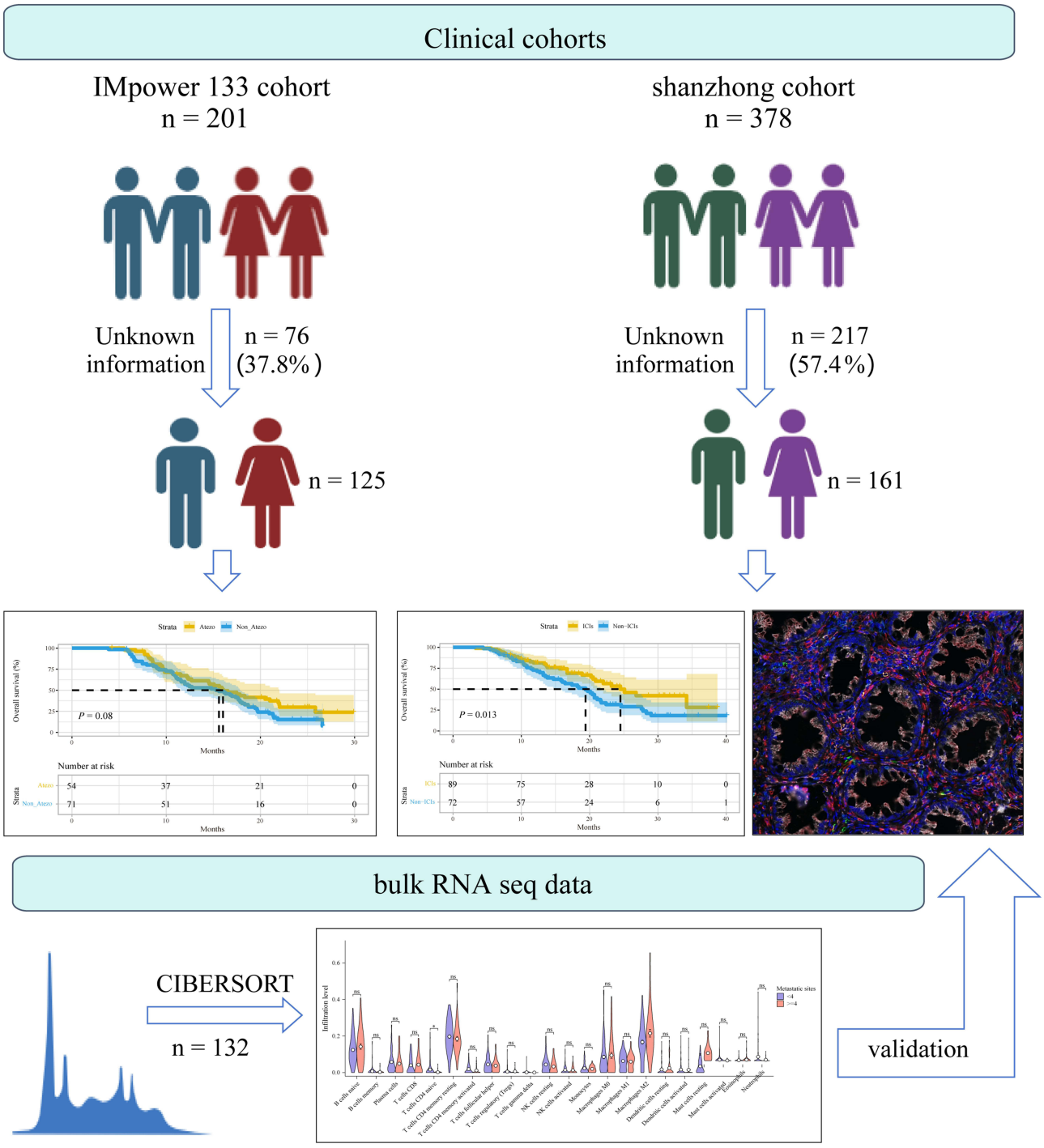

Figure 1 Flow chart of included patients. A total of 201 SCLC patients from IMPower133 were recruited in the current study. Patients with unknown information and untreated patients following progression were excluded, with 125 patients remaining. These patients were divided into two groups, with a total of 54 patients in the atezolizumab-treated group and 71 patients in non-atezolizumab treated group. Moreover, a total of 378 SCLC patients undergoing ICI as first-line treatment were recruited from Shandong Cancer Hospital and Institute (shanzhong cohort). After exclusion of 217 patients without known second-line treatment strategies, a total of 161 patients were ultimately recruited. Among these 161 patients, a total of 89 patients received ICI retreatment and the 72 remaining received non-ICI treatment. *: $P < 0.05$, ns: not significant.

Statistical Analysis

The χ^2 test (categorical variables) and Wilcoxon rank-sum test (continuous variable) were performed to analyze the difference in clinical variables based on the adoption of atezolizumab. Survival curves were plotted using Kaplan Meier analyses and compared by Log rank test. Univariate and multivariate Cox regression analysis were conducted to analyze the hazard ratio (HR) of OS in patients with SCLC according to different clinical variables. The correlation between M2 macrophage infiltration and S100A16 expression was analyzed with Pearson's $\chi 2$ test.[19] The subgroup analysis results are presented in corresponding forest plots. All statistical analyses were performed using R (version 4.2.3), and a p-value <0.05 was considered statistically significant.

Results

Clinicopathological Characteristics of ES-SCLC

A total of 201 SCLC patients from IMpower133 were recruited in the current study. Next, both patients with unknown information and untreated patients following progression were excluded, with 125 patients remaining. Among these patients, 54 patients were treated with atezolizumab on or after the first progression disease (PD). Another 71 patients received other anti-cancer therapy excluding atezolizumab on or after the first PD. Data collected included age, sex, Eastern Cooperative Oncology Group (ECOG), tobacco history (TOBHX), objective response, number of metastatic sites, body mass index (BMI) and neutrophil to lymphocyte ratio (NLR). Moreover, a total of 378 ES-SCLC patients undergoing ICI as first-line treatment were recruited from shanzhong cohort. After exclusion of 217 patients without known second-line treatment strategies, a total of 161 patients were ultimately recruited. Among these 161 patients, a total of 89 patients received ICI retreatment on or after the first PD and the 72 remaining received non-ICI treatment on or after the first PD. Among all these patients, 54 (43.20%) underwent atezolizumab treatment on or after first PD (Atezo) and 71 (56.80%) underwent other anti-cancer therapy excluding atezolizumab on or after first PD (Non-Atezo). Baseline demographical characteristics included the following categorical variables, such as age, sex, ECOG, TOBHX, objective response, number of metastatic sites. Results have shown that there was no significant difference in age, sex, ECOG, TOBHX, objective response, number of metastatic sites between those treated with atezolizumab and those without atezolizumab ($p > 0.05$ for all) (Table 1). Since the effects of both BMI and NLR on survival have been

Table 1 Clinicopathological Characteristics of ES-SCLC

Variables	Atezo (n = 54)	Non-Atezo (n = 71)	*P*
Age (%)			0.424
<65	25 (20%)	38 (30.4%)	
≥65	29 (23.2%)	33 (26.4%)	
Sex (%)			0.086
Female	24 (19.2%)	21 (16.8%)	
Male	30 (24%)	50 (40%)	
ECOG (%)			0.606
0	23 (18.4%)	27 (21.6%)	
1	31 (24.8%)	44 (35.2%)	
TOBHX (%)			0.681
PREVIOUS	36 (28.8%)	42 (33.6%)	
CURRENT	17 (13.6%)	27 (21.6%)	
NEVER	1 (0.8%)	2 (1.6%)	
Objective response (%)			0.635
Yes	43 (34.4%)	54 (43.2%)	
No	11 (8.8%)	17 (13.6%)	
Number of metastatic sites (%)			0.433
<4	40 (32%)	48 (38.4%)	
≥4	14 (11.2%)	23 (18.4%)	

Abbreviations: ES-SCLC, extensive-stage small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; TOBHX, tobacco history.

Table 2 The Comparison of Body Mass Index (BMI) and Neutrophil to Lymphocyte Ratio (NLR) Between the Atezolizumab and Non-Atezolizumab Groups in Patients with ES-SCLC

Variables	Atezo N=54	Non-Atezo N=71	*P*
BMI (median [IQR])	27.95 [23.50, 30.30]	24.97 [21.85, 27.72]	0.016
NLR (median [IQR])	2.97 [2.05, 5.46]	3.55 [2.53, 5.78]	0.080

Abbreviations: BMI, body mass index; NLR, neutrophil to lymphocyte; ES-SCLC, extensive-stage small cell lung cancer.

reported in patients with SCLC, these two continuous variables were also compared between the atezolizumab and non-atezolizumab groups. Median BMI was 27.95 (range: 23.50–30.30) in patients treated with atezolizumab and 24.97 (range: 21.85–27.72) in patients without atezolizumab treatment, respectively, which was of statistical significance (p=0.016). With regard to NLR, SCLC patients in cross-line treatment undergoing atezolizumab had numerically lower NLR [2.97 (range: 2.05–5.46)] than that [3.55 (range: 2.53–5.78)] of those without atezolizumab treatment, as shown in Table 2.

Survival Difference Between Atezolizumab and Non-Atezolizumab Treatment for ES-SCLC Patients Following Progression from ICI as First-Line Treatment

The survival difference in atezolizumab versus non-atezolizumab in subsequent treatments was compared using Kaplan Meier analysis in ES-SCLC patients who progressed after first-line ICI therapy. For these ES-SCLC patients, those undergoing atezolizumab had numerically longer survival than those without atezolizumab treatment, despite being statistically insignificant ($P = 0.08$). Besides, the ratio of risk at death is relatively higher in those with non-atezolizumab than those with atezolizumab treatment following progression (Figure 2A). Additionally, using ES-SCLC patients undergoing ICI as first-line treatment from shanzhong cohort, we demonstrated that ICI-treated patients have significantly longer OS than non-ICI-treated after progression of ICI as first-line treatment ($P = 0.013$). Similarly, the ratio of risk at death is relatively higher in those with non-ICI than those with ICI following progression (Figure 2B).

Beneficial Population from ICI Rechallenge

Since a trend towards a numerical longer OS has been observed in patients with ES-SCLC treated with atezolizumab than those without atezolizumab as cross-line therapy, we next attempted to ascertain vital factors that may impact the survival of ES-SCLC patients who failed atezolizumab as first-line treatment. Therefore, survival benefit was also compared

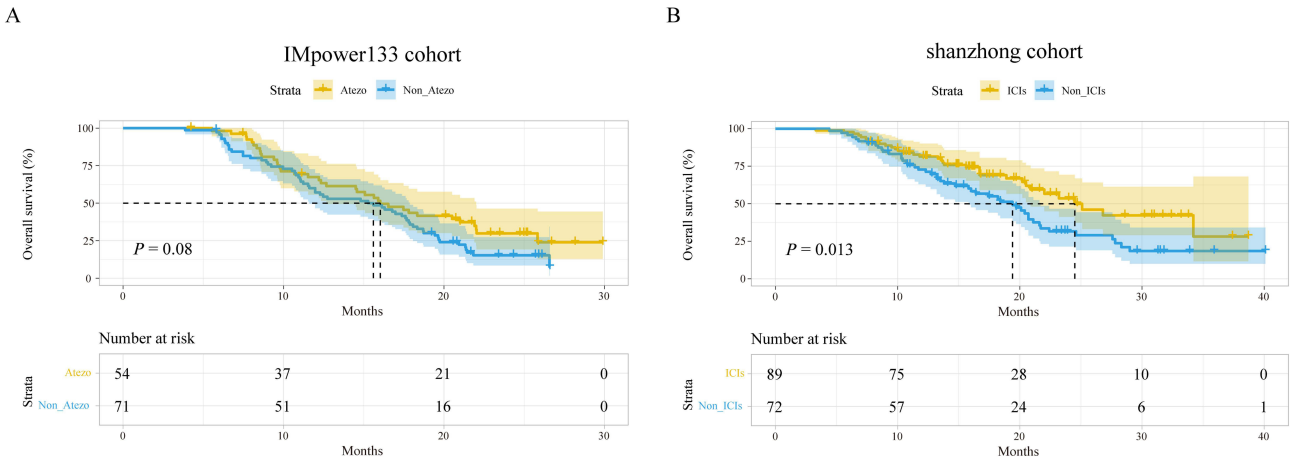

Figure 2 The effect of ICI rechallenge on survival among ES-SCLC patients following progression of ICI as first-line treatment. **(A)** Comparison of survival for ES-SCLC patients treated with atezolizumab or without atezolizumab using Kaplan Meier analysis using IMpower133 data. **(B)** Comparison of survival for ES-SCLC patients treated with ICI or without ICI using Kaplan Meier analysis using data from shanzhong cohort.

between atezolizumab- and non-atezolizumab-treated ES-SCLC patients according to the clinical variables including age, sex, ECOG, TOBHX, objective response and number of metastatic sites. Subgroup analyses found that for ES-SCLC patients with less than four metastatic sites, survival benefit was observed in the treatment with atezolizumab than those without atezolizumab (HR, 0.457; 95% CI, 0.256–0.817; $P = 0.008$) (Figure 3A). To further validate that SCLC with <4 metastatic sites may benefit from ICI rechallenge, using our patient cohort, we analyzed the role of less than 4 metastatic sites in survival of SCLC patients with and without ICI rechallenge. It showed that for patients with SCLC receiving ICI rechallenge, OS is significantly longer in those with <4 metastatic sites than those with ≥4 metastatic sites ($P = 0.025$) (Figure 3B). For SCLC patients receiving non-ICI treatment, OS is numerically longer in those with <4 metastatic sites than those with ≥4 metastatic sites ($P = 0.14$) (Figure 3C). Taken together, our results showed that for patients with ES-SCLC harboring less than 4 metastatic sites, they would benefit from ICI rechallenge.

ES-SCLC Patients with Metastatic Sites Less Than 4 May Derive Survival Benefit from ICI Rechallenge

Survival curves for OS from IMpower133 cohort were also plotted among those patients with ES-SCLC harboring less than 4 metastatic sites, and the results showed that for patients with less than 4 metastatic sites, those treatment with atezolizumab had significantly longer OS compared with those without atezolizumab treatment ($P = 0.036$), as shown in Figure 4A. Using data from shanzhong cohort, we also found that for ES-SCLC harboring less than 4 metastatic sites,

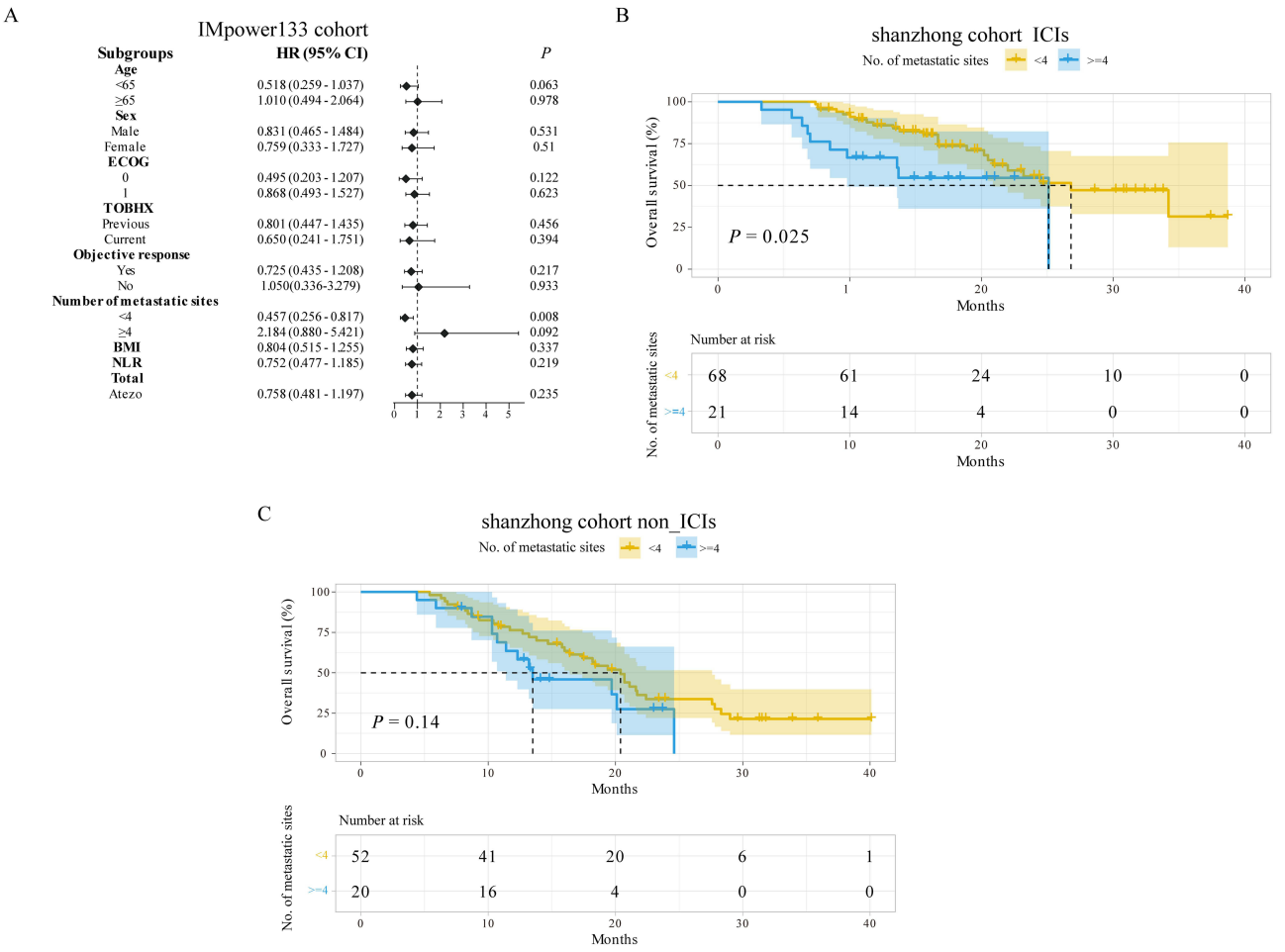

Figure 3 Effect of metastatic sites in affecting survival in ES-SCLC patients with and without ICI rechallenge. **(A)** Forest plot of subgroup analysis of survival according to clinical variables in ES-SCLC patients with atezolizumab rechallenge. Clinical variables include age, sex, ECOG, TOBHX, objective response, number of metastatic sites. HR, hazard ratio; CI, confidence interval. (**B**) The effect of metastatic sites on survival of ES-SCLC patients with ICI rechallenge. (**C**) The effect of metastatic sites on survival of ES-SCLC patients without ICI rechallenge.

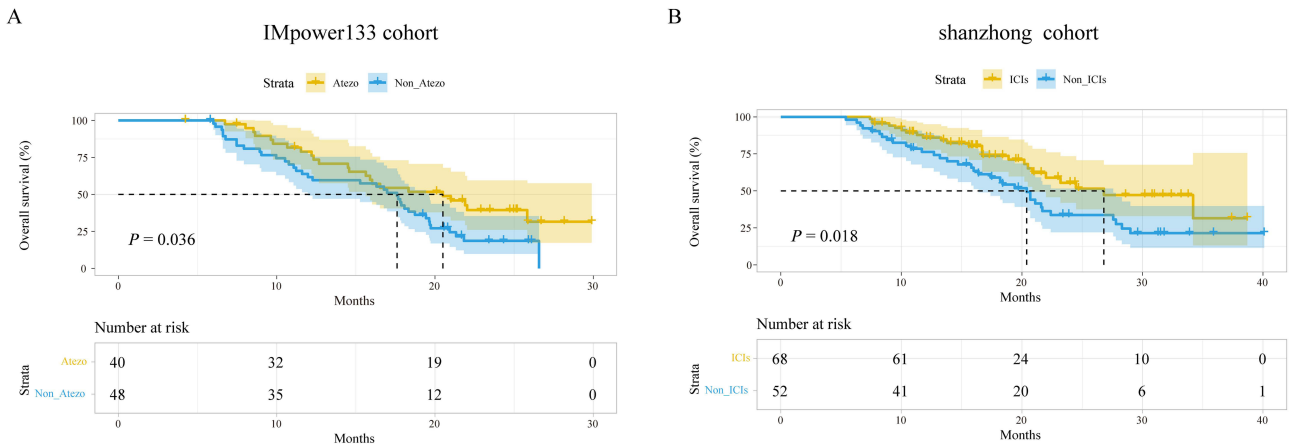

Figure 4 For ES-SCLC patients with < 4 metastatic sites, the effect of ICI re-challenge on survival. **(A)** For ES-SCLC patients with < 4 metastatic sites, the effect of atezolizumab re-challenge on survival using IMpower133 data. **(B)** For ES-SCLC patients with < 4 metastatic sites, the effect of ICI rechallenge on survival using shanzhong cohort data.

those with ICI treatment had significantly longer OS than those with non-ICI treatment following failure of ICI as first-line (P = 0.018) (Figure 4B). To further validate our hypothesis that patients with ES-SCLC harboring less than 4 metastatic sites may benefit from ICI rechallenge, we next evaluated prognostic factors for OS among patients with ES-SCLC harboring less than 4 metastatic sites using univariate and multivariate Cox proportional hazards regression analyses. The multivariate cox model analysis showed that treatment with atezolizumab after first PD of first-line treatment (HR, 0.457; 95% CI, 0.256–0.817; P = 0.008), age ≥65 (HR, 0.409; 95% CI, 0.227–0.738; P = 0.003) were protective factors for favorable OS whereas ECOG (HR, 3.787; 95% CI, 2.045–7.011; P < 0.001) was found to be a risk factor for worse OS (Table 3). Altogether, our results revealed that ES-SCLC patients with <4 metastatic sites may derive survival benefit from ICI rechallenge.

Table 3 The Prognostic Factors for OS Among Patients with ES-SCLC Harboring Less Than 4 Metastatic Sites Using Univariate and Multivariate Cox Proportional Hazards Regression Analyses

Variables	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P	HR (95% CI)	P
Atezolizumab treatment				
Non-Atezolizumab	Reference		Reference	
Atezolizumab	0.574 (0.340–0.970)	**0.038**	0.457 (0.256–0.817)	**0.008**
Age				
<65	Reference		Reference	
≥65	0.727 (0.438–1.208)	0.218	0.409 (0.227–0.738)	**0.003**
Sex				
Female	Reference		Reference	
Male	1.225 (0.717–2.093)	0.458	0.863 (0.485–1.536)	0.616
ECOG				
0	Reference		Reference	
1	2.589 (1.510–4.438)	**< 0.001**	3.787 (2.045–7.011)	**< 0.001**
TOBHX				
PREVIOUS	Reference		Reference	
NEVER	0.634 (0.087–4.630)	0.653	0.339 (0.041–2.827)	0.317
CURRENT	0.800 (0.472–1.357)	0.409	0.601 (0.339–1.066)	0.081

(*Continued*)

Table 3 (Continued).

Variables	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P	HR (95% CI)	P
Objective response				
Yes	Reference		Reference	
No	1.230 (0.666–2.272)	0.509	1.285 (0.647–2.552)	0.474
BMI	0.992 (0.944–1.043)	0.762	0.981 (0.932–1.033)	0.473
NLR	1.057 (0.954–1.171)	0.285	0.966 (0.863–1.081)	0.542

Notes: The bold numbers are P values of statistical significance in Table 3.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; TOBHX, tobacco history; BMI, body mass index; NLR, neutrophil lymphocyte ratio.

Patients with ES-SCLC Harboring Less Than 4 Metastatic Sites May Benefit from ICI Rechallenge by Reshaping Tumor Microenvironment

To further explore the possible mechanisms of longer survival in ES-SCLC patients with <4 metastatic sites from ICI rechallenge. Using data from IMpower133 study, we compared the immune cells in between ES-SCLC patients with <4 metastatic sites and those with ≥4 metastatic sites. Results have found that elevated CD4 naïve T cells and decreased M2 macrophage infiltration in SCLC patients with <4 metastatic sites compared with those with ≥4 metastatic sites ($P < 0.05$) (Figure 5A). Markers of M2 macrophages ($CD68^+CD206^+$) and CD4 naïve T cells ($CD4^+CD45RA^+CCR7^+$) were stained from SCLC samples in shanzhong cohort, shown in the mIF in Figure 5B, which corroborated the above findings.

In the cohort with <4 metastatic sites, SCLC patients with low infiltration of M2 macrophage had significantly longer survival than those with high M2 macrophage infiltration ($P = 0.023$, Figure 5C); those with high immersion of CD4 naïve T cells had numerically longer OS than those with low CD4 naïve T cell infiltration ($P = 0.89$) (Supplementary Figure 1). Additionally, the types of metastases among the included patients from IMpower 133 and shanzhong cohort were demonstrated in Supplementary Figure 2. For those with ≥4 metastatic sites, survival was numerically prolonged in those with low M2 macrophage infiltration compared with that in high M2 macrophage cohort ($P = 0.31$; Figure 5D). In summary, these revealed that patients with ES-SCLC harboring <4 metastatic sites may benefit from ICI rechallenge by reshaping TME.

S100A16 May Regulate M2 Macrophage Infiltration in SCLC Patients with Less Than 4 Metastatic Sites

Using IMpower133 data, we analyzed the significantly altered genes between SCLC patients with <4 metastatic sites and those with ≥4 metastatic sites. Results have found that S100A16 and S100A14 were significantly higher in SCLC patients with ≥4 metastatic sites than those with <4 metastatic sites (Supplementary Figure 3A). SCLC patients from shanzhong cohort validated higher S100A16 levels in those with ≥4 metastatic sites than those with <4 metastatic sites, as shown in immunofluorescence (Supplementary Figure 3B). Correlation analysis found the negative correlation of S100A16 with CD4 naïve T cells ($R = -0.483$, $P < 0.001$), whereas the positive correlation with M2 macrophage ($R = 0.200$, $P = 0.022$) (Supplementary Figure 3C and 3D). We further explored the impact of S100A16 on OS. It showed that for SCLC patients with <4 metastatic sites, lower S100A16 level was associated with significantly better OS, indicating the prognostic effect of S100A16 in the prediction of poor survival among SCLC patients with <4 metastatic sites (P=0.014) (Supplementary Figure 3E).

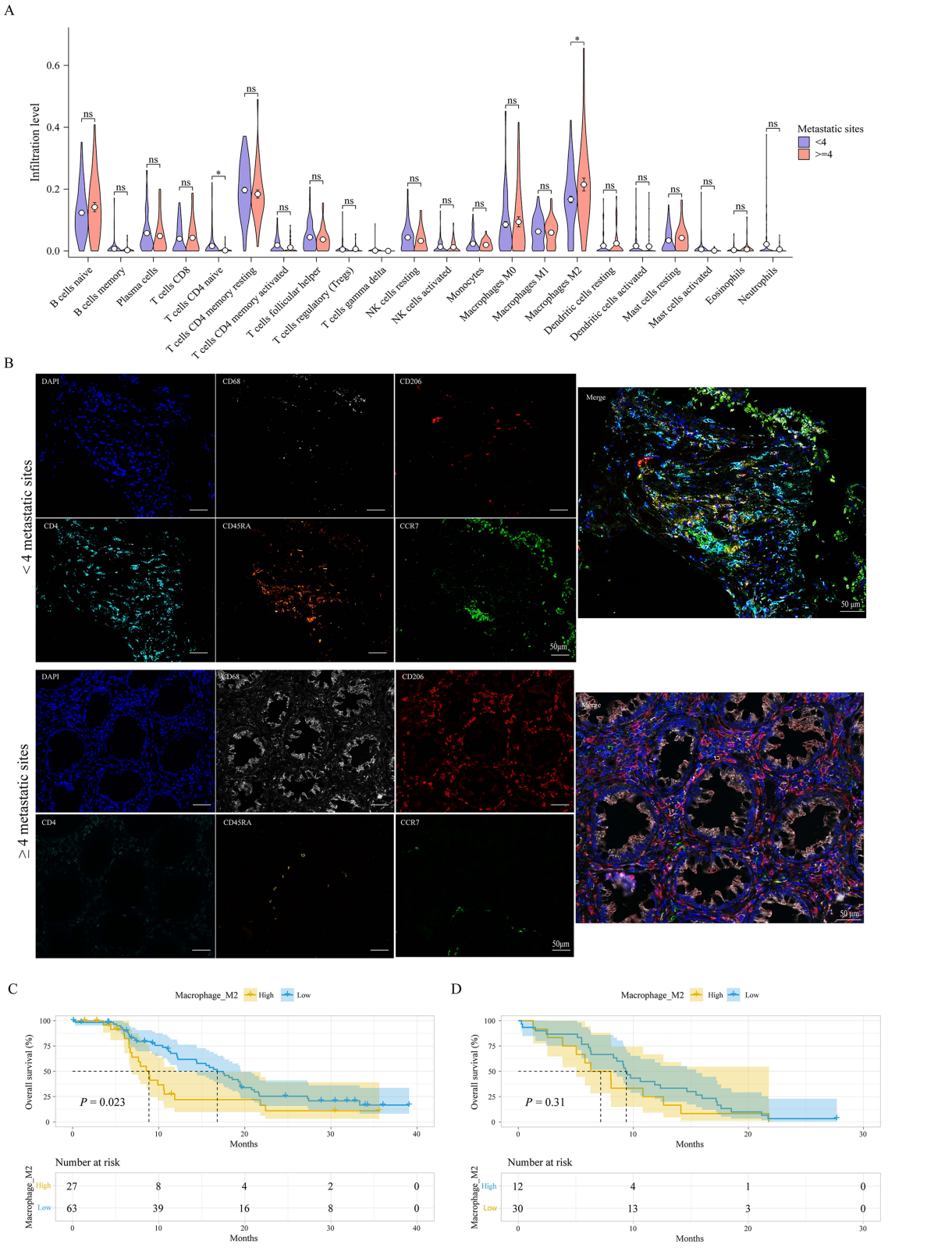

Figure 5 The landscape of tumor microenvironment in ES-SCLC patients with ICI treatment. **(A)** Comparison of tumor immune cells in ES-SCLC patients harboring < 4 metastatic sites and those with ≥ 4 metastatic sites using IMpower133 data. **(B)** mIF of M2 macrophage and CD4 naïve T cells in SCLC patients with ICI treatment. Representative images of M2 macrophages and CD4+ naïve T cells by staining of CD206 (red), CD68 (white) and CD4 (light-blue), CD45RA (Orange), CCR7 (green) and nuclei (dark-blue) in SCLC tissues from patients treated with ICIs. Scale bars representing 50μm and 20μm are shown in images. **(C)** Subgroup survival analysis between M2-macrophage high and M2-macrophage low in ES-SCLC patients harboring < 4 metastatic sites. **(D)** Subgroup survival analysis between M2-macrophage high and M2-macrophage low in ES-SCLC patients harboring ≥ 4 metastatic sites *: $P < 0.05$, ns: not significant.

Discussion

Despite the achievements made in SCLC by utilizing ICI in the first-line treatment,[20,21] it still warrants much more attention on the role of ICI as retreatment after failure as first-line therapy. Our study first compared survival in patients with ES-SCLC undergoing either atezolizumab or non-atezolizumab after progression from it as first-line treatment. We next showed their responses with and without atezolizumab in subgroup patients. It was revealed that the patients with ES-SCLC harboring less than 4 metastatic sites derived survival benefit from atezolizumab than non-atezolizumab, after progression as first-line therapy.

The primary goal of this study was to 1) explore the value of atezolizumab as ICI rechallenge following progression as first-line treatment among patients with ES-SCLC, 2) to ascertain the subgroup of ES-SCLC patients that would benefit from atezolizumab after progression as first-line therapy. A pivotal point found here is that after progression of atezolizumab combined with chemotherapy, patients with less than 4 metastatic sites derived longer survival from continuing to use atezolizumab compared with non-atezolizumab. It should be noted that our study is the first study to investigate the cohort of ES-SCLC patients appropriate for atezolizumab after progression as first-line treatment. Most previous studies have only focused on the role of atezolizumab as first-line treatment in patients with ES-SCLC.

Most studies have revolved around the role of ICI as first-line therapy among patients with ES-SCLC. For instance, the efficacy of atezolizumab in combination with carboplatin and etoposide was evaluated in the randomized, double-blind, placebo-controlled, multinational, Phase III IMpower133 trial, which has improved survival in patients with ES-SCLC.[5] A study conducted by Reck demonstrated that induction with atezolizumab plus chemotherapy and maintenance treatment with atezolizumab would contribute to the survival advantage in ES-SCLC.[22] Additionally, the CASPIAN trial, a randomized, controlled, open-label, Phase 3 trial, has revealed that first-line treatment of durvalumab with platinum-etoposide significantly improved OS in ES-SCLC patients as compared to control group who merely received chemotherapy.[6]

However, the role of ICI rechallenge in ES-SCLC has been far from satisfactory, as tested in series of clinical trials. CheckMate 331 has tested the effect of nivolumab as second-line in relapsed SCLC. Results have found that nivolumab did not improve survival versus chemotherapy in relapsed SCLC, whereas in exploratory analyses, select baseline characteristics were linked with better OS for nivolumab.[23] IFCT-1603 Trial, a randomized non-comparative Phase II study of atezolizumab or chemotherapy as second-line therapy in patients with SCLC, failed to show significant efficacy.[24] Moreover, results from both the KEYNOTE-028 and KEYNOTE-158 studies showed that pembrolizumab presented durable antitumor activity in a subset of patients with recurrent or metastatic SCLC who had subject to two or more previous lines of therapy, regardless of PD-L1 expression.[25] Based on these clinical trials, the effect of subsequent adoption of ICI in ES-SCLC remains unsolid and further data are needed. Therefore, in the present study, we sought to explore the value of atezolizumab rechallenge in ES-SCLC.

We found that there was a numerical survival benefit for patients with SCLC undergoing atezolizumab treatment compared with those without atezolizumab after failure of atezolizumab as first-line treatment. However, the superiority over survival brought by atezolizumab treatment was limited without patient selection in the following treatment setting. Heterogeneous subgroups may respond distinctively to the treatment of atezolizumab, highlighting a necessity for the role of atezolizumab versus non-atezolizumab in the subgroup patients of ES-SCLC. We therefore sought out for atezolizumab-beneficial subgroups among these patients.

Previous studies have demonstrated that age is one of the important factors affecting the immune system. Therefore, it has been assumed that the elderly would have a weakened response to immunotherapy, which might be linked with the telomere length attrition.[26] As age increases, the innate immune system would forge a pro-inflammatory landscape and the adaptive immune response would result in T cell dysfunction, which might be associated with debilitated immunotherapeutic effects.[27] Further subgroup analyses found the superiority over survival in atezolizumab-treated ES-SCLC in subgroup patients with less than four metastatic sites. Our findings may have clinical implications regarding the adoption of atezolizumab in patients with ES-SCLC harboring less than 4 metastatic sites.

The underlying explanations for the phenomenon that the subgroups of patients with less than four metastatic sites benefit more from treatment with atezolizumab as later treatment needs to be explored. The TME, a dynamic niche in SCLC, is widely involved in SCLC metastasis.[28–30] Recently, the impact of TME on immune cells in SCLC has been

gradually revealed, which is intimately associated with the effects of the immunotherapy. And the efficacy of immunotherapy is largely dependent on the immune contexture within the TME. It is found that patients with SCLC could be divided into "hot" (highly infiltrated) and "cold" (non-infiltrated) categories. Those with "cold" property might have debilitated responses to immunotherapies.[31,32]

Additionally, it should be noted that the immunity of both metastasis numbers and sites of patients with ES-SCLC should not be ignored in the responses to immunotherapies, which might be associated with the efficacy of immunotherapies. TME is dependent on metastatic sites. The specific TME modulates tumor growth and development, as well as affects treatment responses. It has been reported that metastasis sites stemmed from urothelial carcinoma to lymph nodes were sensitive to ICI, whereas metastasis sites to the liver demonstrated resistance to ICI.[33] Such phenomenon could be explained by the fact that the existence of numerous immune cells in particular organs such as lymph nodes, lung and skin where antitumor immunity could be achieved. However, it has been reported that liver metastasis dampens immunotherapy efficacy via $CD8^+$ T cell deletion.[34] We therefore speculate patients with SCLC harboring different metastasis sites might be possessed with quite different TME and immune cells, which might be linked with various immunotherapeutic responses. We therefore analyzed the association between immune landscape and metastasis number and sites in the present study.

The TME is consisted of a cluster of immune cells that may affect the responses of ICI and the survival of patients. As cancer is a complex niche consisting of inflammatory cells, macrophage, as a key player in inflammation, is intimately associated with tumor progression.[35,36] Macrophages in the TME are predominantly M2 polarized, due to the "re-education" of tumor cells. It has been generally accepted that a high M2 macrophage immersion is correlated with poor prognosis in a variety of cancers. An amounting number of studies have demonstrated the contributory role of M2 macrophage to immunosuppression in the TME, while the precise mechanisms remain unclarified.[37,38] In a study led by Iriki T, it was suggested that the involvement of macrophage M2 in SCLC progression via STAT3 activation.[39] The most extensively used human macrophage marker is CD68, which serves as a pan macrophage marker. However, the existence of CD68 could be found in both stromal cells and tumor cells. Thus, CD206 was also employed to identify M2 macrophage.[40] Besides, the indispensable role of $CD4^+$ T cell in the recognition of the MHC class II binding neoantigens, thus initiating the antitumor immunity, has been reported. It allows for a substantial supply of cytotoxic T cells, which helps cancer cell killing activity of $CD8^+$ T cells.[41] In this study, we demonstrated debilitated M2 macrophage and enhanced CD4 naïve T cell infiltration in ES-SCLC patients with <4 metastatic sites treated with ICI, which may partially account for the effect of ICI rechallenge.

In the present study, we also showed a positive association between S100A16 and M2 macrophage whereas its negative association with CD4 naïve T cells, which suggest a possibility of its role in the modulation of TME. The interactions between S100A16 and M2 macrophage as well as CD4 naïve T cells may be complex and warrant our further study.

Undoubtedly, there are limitations to this study. More detailed subgroup analyses according to the various atezolizumab treatment regimens, such as atezolizumab monotherapy, atezolizumab + chemotherapy, dual ICI therapy (atezolizumab plus another ICI). Second, some clinical variables that may affect immunotherapy were not included in our study, which may result in deviation of the results. Third, patients with ES-SCLC harboring metastases could be classified into different metastases types. Unfortunately, we did not analyze survival between atezolizumab versus non-atezolizumab according to the concrete metastatic site. Fourth, the present study was based on the IMpower133 trial, we only validated using a small number of patients in our cohort, which warrants larger-scale studies in other patient cohorts and clinical settings to verify our conclusions.

Although shortfalls may exist, our study can still be considered meaningful and hold clinical significance. We tried to ensure the validity of patients' clinical characteristics. We also interpreted the results with caution. Both the multivariate analysis which adjusted for possible confounding factors and the subgroup analyses were conducted, which suggested that the number of metastatic sites may be an important factor affecting the efficacy of atezolizumab retreatment. Additionally, patients from our cohort were adopted to validate our previous finding, thus render our conclusion more convincing.

To the best of our knowledge, we provided first evidence that patients harboring <4 metastases could benefit from ICI rechallenge following its first-line in ES-SCLC possibly by reshaping the TME. However, the relevant mechanisms warrant further validations by experiments and cohort studies in a real-world clinical setting.

Data Sharing Statement

The data supporting the findings of the present study are available upon request from the corresponding authors.

Acknowledgments

This publication is based on research using data from data contributors *Roche* that has been made available through Vivli, Inc. Vivli has not contributed to or approved, and is not in any way responsible for, the contents of this publication.

Funding

This study was supported jointly by the Special Funds for Taishan Scholars Project (Grant No. tsqn201812149) and Academic promotion program of Shandong First Medical University (2019RC004) and the National Natural Science Foundation of China (No. 82003996).

Disclosure

The authors declare that they have no competing interests in this work.

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