

Original Research article

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Oral etoposide plus Anlotinib and subcutaneous Envafolelimab as a platinum-free, non-intravenous first-line regimen for elderly extensive-stage small cell lung cancer: a phase II trial with biomarker discovery

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How to cite this article: Zhang M, Sun H, Xing S, Li Y, Zhao G, Wang H, Zuo R, Li S, Qi J, Wang B, Yan Z, Xu J, Pan Z, Jiang Z. Oral etoposide plus Anlotinib and subcutaneous Envafolelimab as a platinum-free, non-intravenous first-line regimen for elderly extensive-stage small cell lung cancer: a phase II trial with biomarker discovery. *Int Open Med J*. 2026;1:227-43. <https://dx.doi.org/10.65364/iomj.2026.12>

Received: 27 May 2026 **Accepted:** 10 Aug 2026 **Published:** 21 Sep 2026

Abstract

Objective: To evaluate the efficacy and safety of a non-IV, platinum-free regimen (oral etoposide + Anlotinib + Envafolelimab) in treatment-naïve elderly patients with extensive-stage small cell lung cancer (ES-SCLC).



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Methods: Twenty-five elderly patients with ES-SCLC were enrolled. The regimen included etoposide 100 mg PO daily (days 1-7), Anlotinib 12 mg PO daily (days 1-14), and Envafolimab 300 mg SC per dose, repeated every 21 days for 4-6 cycles until progression or intolerance.

Results: Mean age was 73.7 ± 3.2 years. ORR was 68%, DCR 96%. Median progression-free survival (PFS) was 5.23 months (95% CI: 4.5-NA) and median OS 7.53 months (95% CI: 6.28-NA). Grade ≥ 3 AEs included neutropenia (16.0%), ALT/AST elevation (8.0%), immune-related pneumonitis (8.0%), and cardiac dysfunction (4.0%). No platinum-related severe toxicities occurred; 56.0% required no antiemetics. Responders showed upregulation of FAM230A and NAT16, downregulation of Bone Morphogenetic Protein 4 (BMP4), and increased eosinophil infiltration. Non-responders featured naive/memory B-cell infiltration. Longer PFS correlated with high RET/WDR65 expression and cell cycle/immune activation pathways, while shorter PFS was associated with plasma cell infiltration and aggressive tumor features.

Conclusion: This oral, platinum-free, non-IV regimen demonstrated efficacy comparable to standard first-line therapy in elderly ES-SCLC, with significantly lower toxicity, better convenience, and superior tolerability, supporting its use as a preferred first-line option. The identified biomarkers may aid individualized efficacy prediction.

Keywords: Elderly, extensive-stage small cell lung cancer, first-line treatment, platinum-free regimen, non-intravenous therapy

INTRODUCTION

Small cell lung cancer (SCLC) is a poorly differentiated neuroendocrine tumor characterized by rapid proliferation, high invasiveness, and early metastasis. Approximately 60%-70% of patients are diagnosed with extensive-stage disease. Although SCLC is sensitive to radiotherapy and chemotherapy, most patients experience relapse within one year^[1]. Approximately half of patients with SCLC are aged over 65 years, with about 30% aged over 70 years^[2]. Elderly patients often have reduced organ function and comorbidities, leading to reduced tolerance of traditional platinum-based intravenous chemotherapy. This population exhibits a significantly increased incidence of myelosuppression, gastrointestinal reactions, and nephrotoxicity, frequently resulting in dose reduction, treatment delays, or discontinuation, which may ultimately compromise survival outcomes.

Since the IMpower133 study^[3] and CASPIAN^[4] established the foundation for immunotherapy-based combination regimens as first-line standard treatment for extensive-stage small cell lung cancer (ES-SCLC), multiple immunotherapeutic agents have demonstrated positive results in ES-SCLC treatment in recent years, offering renewed therapeutic prospects for patients with SCLC. Although elderly patients are underrepresented in clinical trials, subgroup analyses and real-world data from multiple studies indicate that immunotherapy combined with chemotherapy still demonstrates a favorable trend in clinical outcomes in elderly SCLC patients^[3-8]. The current standard of care for ES-SCLC primarily comprises three-drug immunochemotherapy combinations, with median progression-free survival (PFS) ranging from 4.5 to 5.8 months and median overall survival (OS) from 12.3 to 15.8 months. The ETER701 study^[9], which added anti-angiogenic therapy to the three-drug immunochemotherapy combination, achieved a median PFS of 6.9 months and a median OS of 19.3 months, significantly prolonging survival in ES-SCLC patients. However, due to the unique physiological characteristics and clinical complexity of elderly patients, these standard regimens may not be universally applicable to all elderly ES-SCLC patients, and the optimal treatment paradigm warrants further refinement.

Anlotinib is an oral multi-target tyrosine kinase inhibitor (TKI) that primarily targets vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and c-Kit, thereby exerting broad-spectrum inhibitory effects on tumor angiogenesis and growth^[10,11]. The ALTER1202 study^[12], a phase II clinical trial of anlotinib versus placebo in the third-line or later setting for SCLC, demonstrated that the anlotinib group yielded a significant improvement in OS compared to placebo while reducing the risk of death. Furthermore, as a small molecule anti-angiogenic TKI, anlotinib can reshape the tumor microenvironment, reverse the immunosuppressive state of the microenvironment, and enhance sensitivity to immunotherapy^[13].

Etoposide, as a topoisomerase II inhibitor, is a cell cycle-specific drug. Its clinical efficacy exhibits significant time- and dose-dependency, and existing research has confirmed that oral and intravenous administration routes show comparable bioavailability^[14]. A previous study indicated that single-agent oral etoposide capsules significantly prolonged the duration of maintenance therapy in patients with small cell lung cancer^[15].

Envafolelimab, as the world's first approved subcutaneous administered PD-L1 antibody, is a monospecific antibody composed of a single-domain antibody (sdAb) and an Fc region, with a molecular weight only half that of conventional antibodies. This reduced molecular weight enables rapid and uniform distribution throughout the body with excellent tissue penetration capability. The CN006 study^[16] demonstrated that among patients with dMMR/MSI-H tumors, its anti-tumor efficacy was non-inferior to other PD-1/PD-L1 antibodies [objective response rate (ORR) 42.7% (95% CI: 33.0-52.8), DCR 66.0% (95% CI: 56.0-75.1), mPFS 11.1 months (95% CI: 5.5-NA); 12-month OS rate 74.6% (95% CI: 64.7-82.1)].

Based on the anti-tumor efficacy and ease of administration of etoposide capsules, Anlotinib, and Envafolelimab in clinical practice, we propose a non-intravenous, platinum-free treatment regimen combining oral etoposide with Anlotinib and Envafolelimab as first-line therapy for elderly ES-SCLC patients, to evaluate the efficacy and safety of oral chemotherapy combined with anti-angiogenic therapy and subcutaneous immunotherapy in this population, and to provide a new treatment paradigm for these patients in the immunotherapy era.

MATERIALS AND METHODS

This phase II clinical trial was conducted at the Tianjin Cancer Hospital Airport Hospital to primarily evaluate the efficacy and safety of oral etoposide capsules in combination with anlotinib and envafolelimab among elderly patients with ES-SCLC. This trial was registered on ClinicalTrials.gov under identifier NCT05685550 (<https://register.clinicaltrials.gov/prs/beta/records>). The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. The study protocol received approval from the Institutional Review Board and Ethics Committee of the Tianjin Cancer Hospital Airport Hospital (Approval No. JS-2022-0048). Written informed consent was obtained from all patients prior to their participation in the study.

Patients and study design

Inclusion criteria:

- (1) Histologically or cytologically confirmed extensive-stage small cell lung cancer;
- (2) No prior systemic anti-tumor treatment for small cell lung cancer. If subjects received adjuvant therapy after completing curative treatment for limited-stage small cell lung cancer and experienced disease relapse, the interval between completion of adjuvant therapy and administration of the first dose in this study must

exceed 6 months, and toxicities from adjuvant therapy must have resolved ($\leq$ Grade 1 per CTCAE v5.0, except for alopecia);

(3) Age $\geq$ 65 years and provision of signed informed consent;

(4) Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 3 with expected survival $>$ 6 months;

(5) At least one measurable lesion as target lesion (per RECIST 1.1 criteria);

(6) Adequate organ function;

(7) Ability to communicate well with investigators and comply with study-specified visits, treatment, laboratory tests, and other relevant regulations.

Exclusion criteria:

(1) Prior treatment with anti-Programmed Death-1 (anti-PD-1), anti-Programmed Death Ligand-1 (anti-PD-L1), anti-Programmed Death Ligand-2 (anti-PD-L2), anti-T cell Immunoreceptor with Ig and ITIM domains (anti-TIGIT), or any other antibodies or drugs specifically targeting T cell co-stimulation or checkpoint pathways;

(2) Active leptomeningeal disease or untreated or uncontrolled brain metastases;

(3) Active autoimmune disease or history of autoimmune disease with potential for recurrence;

(4) Any condition requiring systemic corticosteroid therapy (dose $>$ 10 mg/day prednisone or equivalent) or other immunosuppressive therapy within 14 days before the first study drug administration; washout periods are specified based on the nature of prior treatments.

Intervention

Following enrollment, patients were administered oral etoposide (100 mg once daily for 7 days) and subcutaneous Envafolelimab (300 mg per dose) in combination with oral Anlotinib (12 mg once daily for 14 days), constituting a 21-day treatment cycle. Guided by safety data and investigator assessment, patients underwent the aforementioned combination therapy for 4 to 6 cycles. Patients who did not experience disease progression continued with maintenance therapy with Envafolelimab and Anlotinib until disease progression, unacceptable toxicity, or death.

Endpoints and assessments

The primary endpoints were PFS. Secondary endpoints included OS, ORR, disease control rate (DCR), and safety. PFS was defined as the time from randomization or treatment initiation to disease progression or death from any cause. OS was defined as the time from randomization or treatment initiation to death from any cause. ORR was defined as the proportion of patients achieving complete remission (CR) and partial remission (PR). Treatment efficacy was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The efficacy outcomes assessed included complete remission (CR), PR, SD, progressive disease (PD), ORR, and disease control rate (DCR). Adverse events were graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

RNA sequencing

Total RNA was isolated from formalin-fixed paraffin-embedded (FFPE) samples using the miRNeasy FFPE Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Libraries were prepared from extracted RNA using the KAPA Stranded mRNA-Seq Kit (KAPA Biosystems, Wilmington, MA). The pooled libraries were paired-end sequenced. The sequencing data underwent initial demultiplexing and FASTQ file quality control using Trimmomatic^[17]. Next, BWA (version 0.7.17) was employed to remove reads corresponding to transfer RNA (tRNA) and/or ribosomal RNA (rRNA). Additionally, adaptor and primer sequences, as well as reads shorter than 20 bp were excluded. Qualified data were mapped to the human reference genome hg19 using STAR (version 2.7.3a) with default parameters^[18], followed by isoform and gene level quantification using RSEM (version 1.3.0)^[19].

Statistical analysis

This study is a single-arm, exploratory phase II clinical trial, primarily investigating the efficacy and safety of a non-intravenous platinum-free regimen consisting of oral etoposide capsules combined with Anlotinib plus Envafoleimab as first-line treatment for elderly patients with ES-SCLC, and aims to provide key design parameters (such as median progression-free survival, event rate, and safety signals) for subsequent confirmatory clinical studies. Given the exploratory/pilot nature of this study, the sample size is not based on formal superiority or non-inferiority hypothesis testing power analysis.

Given the actual patient flow of elderly ES-SCLC patients at this center, the strictness of inclusion and exclusion criteria, the planned study duration, and the enrollment scales observed in similar single-center exploratory studies, it is estimated that approximately 25-30 patients will be screened and enrolled within the study period. Assuming patient survival time follows an exponential distribution, with a planned enrollment of 25 evaluable patients, the expected event rate during the follow-up period is 70%-80% (approximately 18-20 progression-free survival events). This sample size is anticipated to provide a clinically meaningful precision range for median progression-free survival, thereby supporting the hypothesis-generating nature of this exploratory research.

Statistical analysis was performed using R (version 4.3.3) and IBM SPSS 27.0. Continuous variables with normal distribution were expressed as mean $\pm$ standard deviation (SD), and between-group comparisons were performed using independent samples *t*-tests; continuous variables with non-normal distribution were expressed as median (Q1, Q3), and between-group comparisons were performed using the Mann-Whitney *U* test. Categorical variables were expressed as *n* (percentages), and between-group comparisons were performed using Chi-square test or Fisher's exact test. Survival analysis was performed using the Kaplan-Meier method, with between-group comparisons using the Log-rank test. A *P*-value < 0.05 was considered statistically significant. Differential expression analysis was carried out for different subgroups using the R package 'DESeq2'. The thresholds for identifying differentially expressed genes (DEGs) were $\log_2FC > 1$ or $\log_2FC < -1$, and a false discovery rate (FDR)-adjusted *P*-value < 0.05 , unless otherwise specified. Gene set enrichment analysis (GSEA)^[20] was performed to identify significantly enriched pathways between groups, with $|\text{normalized enrichment score (NES)}| > 1$, *P*-value < 0.05 , and FDR < 0.05 as thresholds for statistical significance. To estimate the proportions of immune cell subsets present in samples using RNA-seq data, the analytical tool xCell was used. The input data was Transcripts Per Million normalization of the RNA-seq data, done by normalizing each gene length with the total read counts. A *P*-value less than 0.05 was statistically significant. The receiver operating characteristic (ROC) curve was generated and the corresponding area under the curve (AUC) was measured to assess the sensitivity and specificity of relevant features using the R package 'pROC'.

Table 1. Baseline clinical characteristics of elderly patients with extensive-stage small cell lung cancer

Characteristics	Overall
Age, mean $\pm$ SD	73.7 $\pm$ 3.2
Sex, <i>n</i> (%)	
Female	5 (20.0)
Male	20 (80.0)
Smoke, <i>n</i> (%)	
Y	23 (92.0)
N	2 (8.0)
ECOG Score, <i>n</i> (%)	
0	0 (0.0)
1	17 (68.0)
2	5 (20.0)
3	3 (12.0)
Radiotherapy, <i>n</i> (%)	
N	21 (84.0)
Y	4 (16.0)
Cycles, median (IQR)	7 (4, 10)
Liver M, <i>n</i> (%)	
N	18 (72.0)
Y	7 (28.0)
Bone M, <i>n</i> (%)	
N	19 (76.0)
Y	6 (24.0)
Brain M, <i>n</i> (%)	
N	20 (80.0)
Y	5 (20.0)
Pleura M, <i>n</i> (%)	
Y	12 (48.0)
N	13 (52.0)

SD: Standard deviation; ECOG: Eastern Cooperative Oncology Group; IQR: interquartile range.

RESULTS

Patient enrollment and baseline characteristics

Between December 2022 and September 2025, 27 elderly patients with extensive-stage small cell lung cancer were screened, of whom 2 withdrew informed consent. Ultimately, 25 patients were deemed eligible and received treatment. Baseline characteristics of the 25 patients are shown in Table 1. The median age was 73 years (range, 67-80); 20 patients (80%) were male, 5 (20%) were female, 23 (92%) were smokers, and 2 (8%) were non-smokers. Seventeen patients (68%) had an ECOG performance status of 1, 5 (20%) had a status of 2, and 3 (12%) had a status of 3. The most common metastatic sites involved the pleura (12 patients, 48%), bone (6, 24%), liver (7, 28%), and brain (5, 20%).

Efficacy analysis

With a data cutoff date of November 28, 2025, the median follow-up was 6.83 months (range: 2.83-19.61 months). Of the 25 patients, 17 (68.0%) achieved partial response (PR), 7 (28.0%) had stable disease (SD),

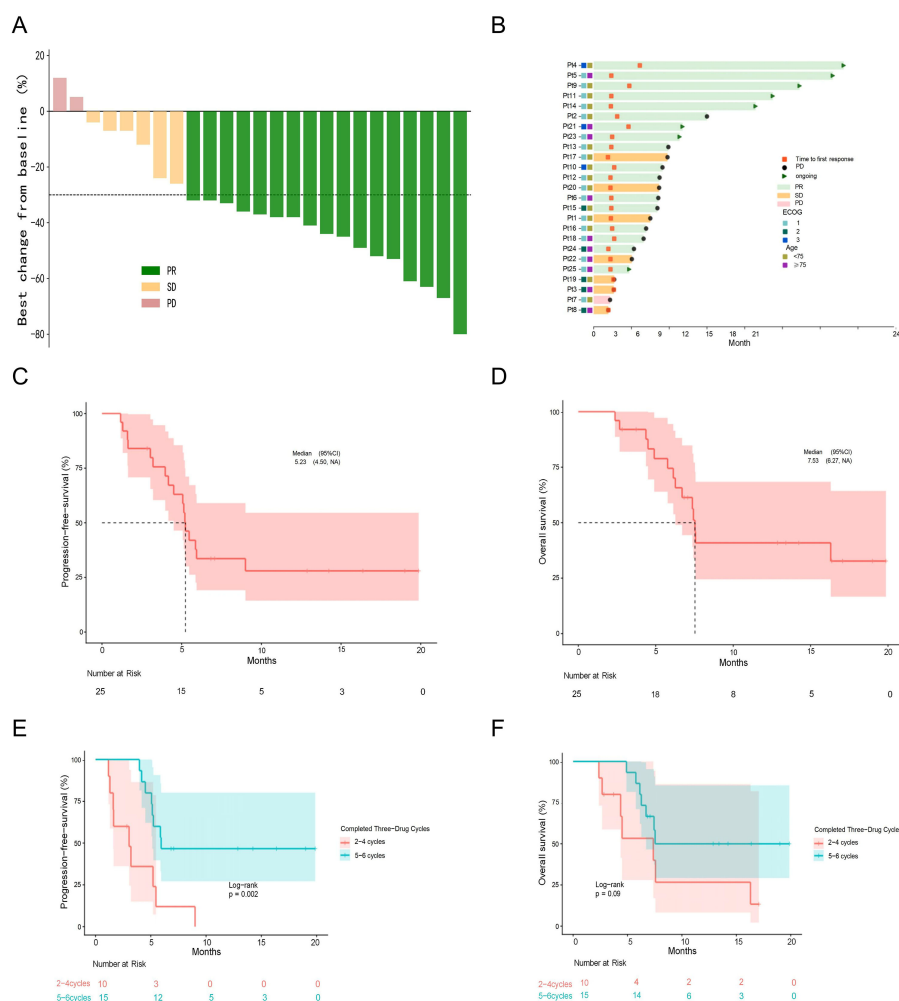


Figure 1. Anti-tumor activity and survival outcomes. (A) Best percentage changes in target lesion size from baseline in the efficacy analysis population; (B) Treatment response and duration of response; (C) Kaplan-Meier estimate of PFS of patients; (D) Kaplan-Meier estimate of PFS of different cycles of completed Three-Drug of patients. (E) Kaplan-Meier estimate of OS of patients. (F) Kaplan-Meier estimate of OS of different cycles of completed Three-Drug of patients. PR: Partial remission; ECOG: Eastern Cooperative Oncology Group; PFS: progression-free survival; OS: overall survival.

and 1 (4.0%) had progressive disease (PD). The ORR was 68.0% (95% CI: 46.5%-85.1%), and the DCR was 96.0% (95% CI: 79.6%-99.9%) [Figure 1A]. Among the 25 patients, 14 patients (56.0%) died, 8 patients (32.0%) were still receiving treatment, and 3 patients (12.0%) withdrew from the study [Figure 1B]. The median PFS was 5.23 months (95% CI: 4.5-NA) [Figure 1C], with a longest observed PFS of 19.61 months [Figure 1B]. Six patients (25%) achieved a best response depth exceeding 50% [Figure 1A]. The median OS was 7.53 months (95% CI: 6.27-NA) [Figure 1D]. Subgroup analysis showed that patients who completed 5-6 cycles of the triple-drug combination regimen had superior PFS and OS compared to those who completed 2-4 cycles [Figure 1E and F].

Safety analysis (AE-related dose modifications)

The most common all-grade treatment-related adverse events were neutropenia (80.0%), nausea (68.0%), elevated TSH (52.0%), hypertension (44.0%), proteinuria (40.0%), weight loss (36.0%), constipation (32.0%), elevated transaminases (24.0%), and oral mucositis (20.0%). Grade ≥ 3 treatment-related adverse events included neutropenia (16.0%), elevated ALT/AST (8.0%), immune-related pneumonitis (8.0%), and cardiac dysfunction (4.0%) [Table 2]. Neutropenia, elevated transaminases, and immune-related pneumonitis resolved after symptomatic treatment. One patient died secondary to cardiac dysfunction.

Table 2. Preferred terms of the treatment-related adverse events (TRAEs)

TRAE	Total (n,%)	Grade ≥ 3 (n,%)
Neutropenia	20 (80.0%)	4 (16.0%)
Nausea	17 (68.0%)	0 (0.0%)
Elevated TSH	13 (52.0%)	1 (4.0%)
Hypertension	11 (44.0%)	0 (0.0%)
Proteinuria	10 (40.0%)	0 (0.0%)
Weight loss	9 (36.0%)	0 (0.0%)
Constipation	8 (32.0%)	0 (0.0%)
Elevated ALT/AST	6 (24.0%)	2 (8.0%)
Oral mucositis	5 (20.0%)	1 (4.0%)
Thrombocytopenia	3 (12.0%)	0 (0.0%)
Anemia	3 (12.0%)	0 (0.0%)
Diarrhea	2 (8.0%)	1 (4.0%)
Pneumonia	2 (8.0%)	2 (8.0%)
Hemoptysis	2 (8.0%)	0 (0.0%)
Rash	1 (4.0%)	1 (4.0%)
Colitis	1 (4.0%)	1 (4.0%)
Elevated creatinine	1 (4.0%)	0 (0.0%)
Decreased cardiac function	1 (4.0%)	1 (4.0%)

TSH: Thyroid-stimulating hormone; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Among all treated patients, 9 patients (36.0%) received oral etoposide capsules for 6 cycles, 6 patients (24.0%) for 5 cycles, 6 patients (24.0%) for 4 cycles, and 4 patients (16.0%) for 2 cycles. [Figure 2A]. Among patients who developed neutropenia during treatment, 52.0% required granulocyte colony-stimulating factor (G-CSF) in cycle 1. As treatment cycles progressed, patient tolerability improved and the requirement for G-CSF support gradually decreased [Figure 2A and B]. Adverse events of anemia and thrombocytopenia did not exceed Grade 3; Grade 1 occurred in 2 patients (8.0%) for each condition, and Grade 2 in 1 patient (4.0%) for each, with interventions required only for Grade 2 cases. In terms of antiemetic use, 14 patients (56.0%) required no intervention, while 8 (32.0%) and 3 (12.0%) required intervention for 1-2 cycles and > 2 cycles, respectively [Figure 2C].

RNA-seq reveals transcriptomic and immune microenvironment phenotypic characteristics associated with efficacy and prognosis

To explore the potential molecular characteristics underlying the heterogeneity of response to this regimen, RNA-seq analysis was performed on 15 patients with complete tissue samples (Molecular subtyping is shown in Supplementary Figures 1 and 2). Patients were stratified by clinical objective response status (PR vs. non-PR) and PFS. Subsequently, molecular and immune microenvironment characteristics associated with efficacy and survival outcomes in this cohort were characterized.

Stratification by objective response status revealed that tumor tissues in the PR group exhibited significantly higher expression of FAM230A and NAT16 genes, while Bone Morphogenetic Protein 4 (BMP4) gene expression was significantly downregulated [Figure 3A]. Gene set enrichment analysis (GSEA) results showed that pathways significantly enriched in the PR group included complement and coagulation cascades, cytochrome P450-mediated drug metabolism, xenobiotic metabolism via cytochrome P450, and cell adhesion molecule pathways [Figure 3B, left], whereas those enriched in the non-PR group included

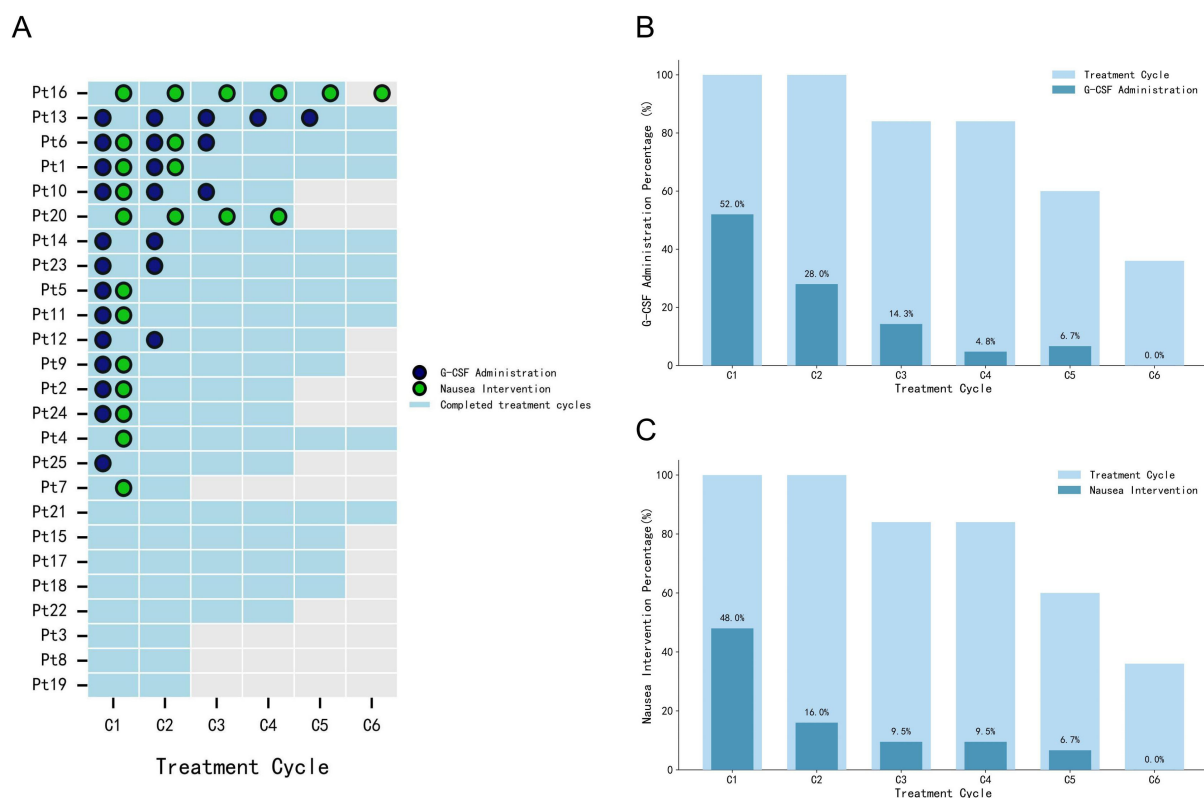


Figure 2. Interventions for AEs (A) Patient completion status of Triple-Drug combination and nausea intervention and G-CSF administration across different treatment cycles; (B) Percentage of patients receiving G-CSF administration across cycles; (C) Percentage of patients receiving nausea intervention across cycles. AE: Adverse event; G-CSF: granulocyte colony-stimulating factor.

ribosome, purine metabolism, TGF- β signaling pathway, and pyrimidine metabolism pathways [Figure 3B, right]. These pathway enrichment differences suggest that the state of immune regulatory networks within the microenvironment and macromolecular metabolic remodeling may be closely associated with the initial response to this targeted combination immunotherapy in elderly SCLC patients.

Furthermore, analysis of tumor immune infiltration characteristics revealed that compared with the non-PR group, patients in the PR group exhibited significantly higher levels of eosinophil infiltration, whereas memory B cells and naïve B cells demonstrated higher infiltration levels in the non-PR group; however, no significant difference was observed in common myeloid progenitor cell infiltration between the two groups [Figure 3C and D]. These findings suggest that the differential infiltration patterns of B cell subsets and eosinophils may serve as potential immune markers indicative of the efficacy of this targeted combination immunotherapy regimen.

To further identify transcript-level changes associated with long-term survival benefit, based on median PFS stratification into long PFS and short PFS groups, comparison of differentially expressed genes between the two groups showed that the long PFS group had 50 upregulated genes and 38 downregulated genes compared with the short PFS group. Representative DEGs included *RET*, *WDR65*, *NBFP15*, *APOA1*, and *FTCD* [Figure 4A]. GSEA results showed that the long PFS group was significantly enriched in cell cycle, proteasome, DNA replication, P53 signaling pathway, pyrimidine metabolism, and cytokine-cytokine receptor interaction pathways [Figure 4B, left], while the short PFS group was significantly enriched in ECM-receptor interaction, focal adhesion, and TGF- β signaling pathway [Figure 4B, right]. Violin plots showed that compared with the long PFS group [Figure 4C], patients in the short PFS group had higher degrees of plasma cell infiltration [Figure 4D], suggesting that abnormal plasma cell infiltration may be a key microenvironmental characteristic of early disease progression in elderly small cell lung cancer patients.

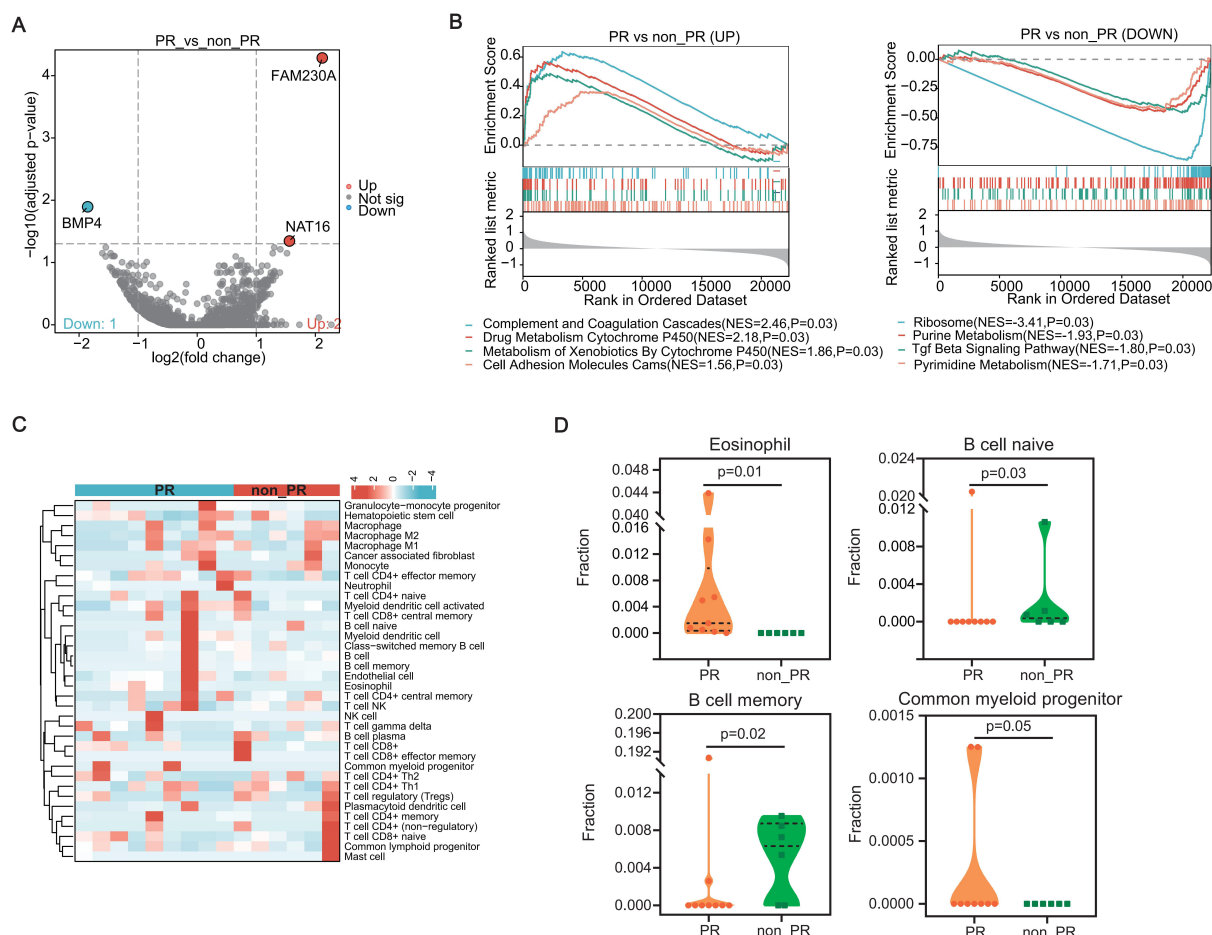


Figure 3. Distinct transcriptomic profiles, pathway enrichment patterns, and immune microenvironment landscapes between PR and non-PR subgroups; (A) Volcano plot illustrating DEGs in the PR vs. non-PR comparison. Red, blue, and gray dots denote upregulated, downregulated, and non-significant genes, respectively. Representative DEGs including FAM230A, BMP4, and NAT16 are highlighted; (B) Gene set enrichment analysis (GSEA) identifying significantly perturbed canonical pathways. Left: Enrichment plots of upregulated pathways (Complement and Coagulation Cascades, Drug Metabolism Cytochrome P450, Metabolism of Xenobiotics, Cell Adhesion Molecules Cams); Right: Enrichment plots of downregulated pathways (Ribosome, Purine Metabolism, TGF- β Signaling Pathway, Pyrimidine Metabolism). Normalized enrichment score (NES) and adjusted P-values are annotated for each pathway; (C) Unsupervised hierarchical clustering heatmap of immune cell subset abundances in PR and non-PR samples. Color gradient represents the relative infiltration level of each immune cell type; (D) Violin plots comparing the fractions of eosinophils, naive B cells, memory B cells, and common myeloid progenitor between PR and non-PR groups. P -values were derived from non-parametric tests, indicating statistically significant differences in immune cell composition. PR: Partial remission; DEG: differentially expressed gene.

DISCUSSION

This study is the first to propose a novel non-intravenous treatment regimen incorporating anti-angiogenic therapy, immunotherapy, and chemotherapy as first-line therapy for elderly ES-SCLC patients. The results showed that this regimen achieved an ORR of 68% (95% CI: 46.5%-85.1%), the DCR of 96% (95% CI: 79.6%-99.9%), and the median PFS of 5.23 months (95% CI: 4.5-NA), with a maximum PFS of 19.61 months, which is generally consistent with previously reported studies^[3-5]. Furthermore, through optimization of the chemotherapy regimen, this study found that the incidence of chemotherapy-related adverse events was significantly reduced, with 56.0% of patients not requiring antiemetic intervention throughout the treatment course. Compared to previous platinum-containing regimens, the cumulative severity of myelosuppression was significantly reduced, with 48.0% of patients requiring G-CSF intervention in cycle 1, 12.0% in cycle 3, and 0.0% in cycle 6. Moreover, no grade ≥ 3 thrombocytopenia or anemia occurred in this study. Finally, this study also screened genes correlated with efficacy and survival through pre-treatment RNA-seq testing in patients, providing an important basis for guiding prognosis.

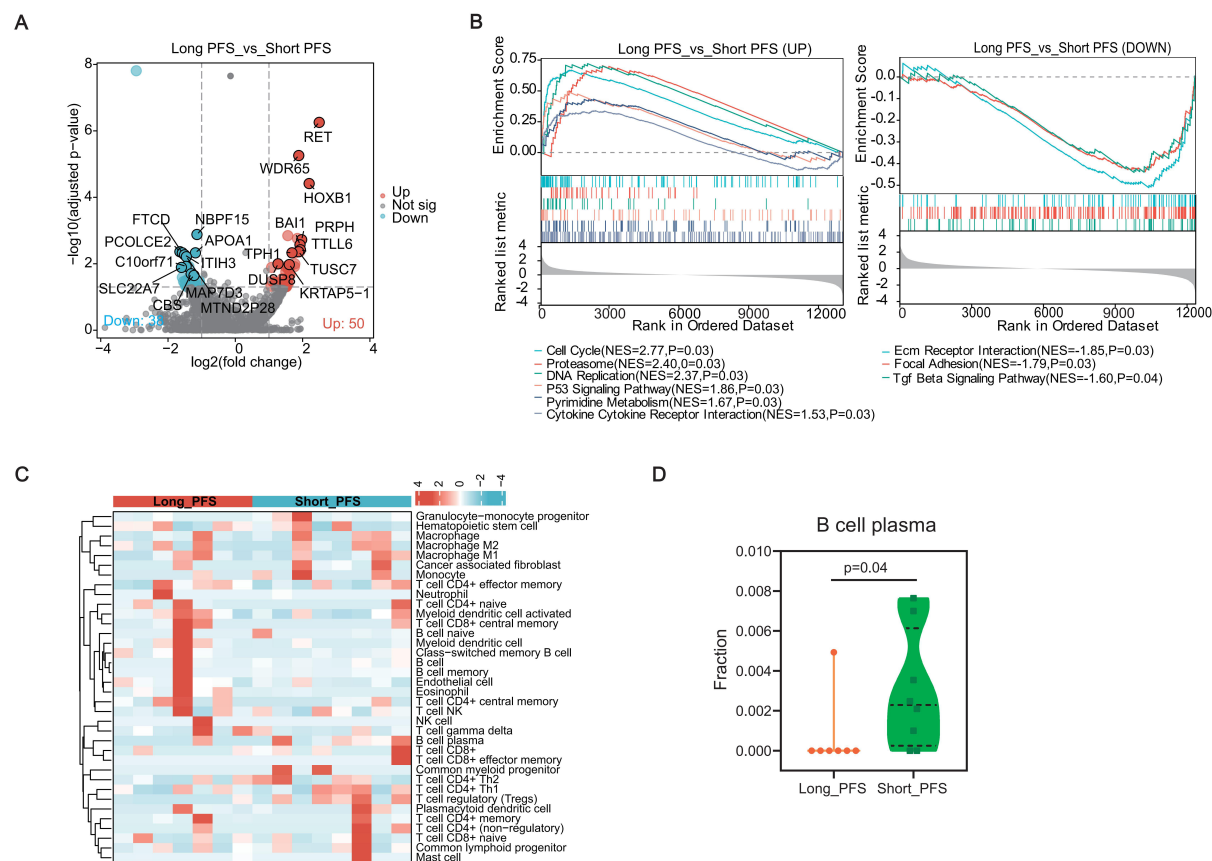


Figure 4. Distinct transcriptomic profiles, pathway enrichment patterns, and immune microenvironment landscapes between long PFS and short PFS subgroups. (A) Volcano plot illustrating DEGs in the Long PFS vs. Short PFS comparison. Red, blue, and gray dots denote upregulated, downregulated, and non-significant genes, respectively. Representative DEGs including *RET*, *WDR65*, *NBPF15*, *APOA1*, and *FTCD* are highlighted; (B) GSEA identifying significantly perturbed canonical pathways. Left: Enrichment plots of upregulated pathways (Cell Cycle, Proteasome, DNA Replication, P53 Signaling Pathway, Pyrimidine Metabolism, Cytokine Cytokine Receptor Interaction); Right: Enrichment plots of downregulated pathways (ECM Receptor Interaction, Focal Adhesion, TGF-Beta Signaling Pathway). NES and P-values are annotated for each pathway; (C) Unsupervised hierarchical clustering heatmap of immune cell subset abundances in Long PFS and Short PFS samples. Color gradient represents the relative infiltration level of each immune cell type; (D) Violin plots comparing the fractions of B cell plasma between Long PFS and Short PFS groups. P-values were derived from non-parametric tests, indicating a statistically significant difference in B cell plasma composition between the two groups. DEG: Differentially expressed gene; PFS: progression-free survival; DEG: differentially expressed gene; GSEA: gene set enrichment analysis; NES: normalized enrichment score; TGF:

Currently, prospective clinical data regarding elderly patients with ES-SCLC are extremely scarce, with most evidence derived from subgroup analyses of large clinical trials and retrospective studies, which poses significant challenges for clinical decision-making. Across both three-drug combination studies, such as IMpower133^[3], CASPIAN^[4], and ASTRUM-005^[6], and the four-drug combination ETER701 study^[9], subgroup analyses showed that patients aged ≥ 65 years receiving immunotherapy-based treatment demonstrated trends toward a survival benefit, suggesting that age is not a contraindication for immunotherapy in SCLC patients. A meta-analysis including IMpower133^[3], CASPIAN^[4], and KEYNOTE-604^[21] showed that in the population aged ≥ 65 years, immunotherapy combined with chemotherapy provided similar OS benefit compared to those aged < 65 years, with HRs of 0.84 and 0.86, respectively^[22]. Current treatment for ES-SCLC patients primarily involves three-drug immunochemotherapy combinations^[3-8], with ORR ranging from 60.2%-87.0%, median PFS from 4.5-5.8 months, and median OS from 12.3-15.8 months. The results of this study showed an ORR of 68% and PFS of 5.23 months, which are generally consistent with the IMpower133 study^[3] (ORR 60.2%, PFS 5.2 months) and CASPIAN^[4] study (ORR 68%, PFS 5.1 months). However, it is noteworthy that this study regimen did

not use carboplatin or cisplatin, yet the ORR and PFS results were not affected, suggesting that in the immunotherapy era, whether platinum-based combination chemotherapy regimens remain as important warrants further discussion.

Furthermore, the median age of patients enrolled in this study was 73 years. As of the follow-up date, 8 patients (32%) were still in the study, among whom 5 patients (20%) in the maintenance treatment phase had PFS ranging from 12.71-19.61 months, with no intolerable toxicities. Additionally, although 8 patients (32%) enrolled in this study had an ECOG performance status of 2-3, this factor did not adversely affect overall efficacy or PFS. The NEJ045A study^[23] was a single-arm, multicenter, phase II clinical trial conducted in Japan for treatment-naïve ES-SCLC patients with poor performance status (ECOG 2-3). This study used dose-reduced regimens for PS 2-3 patients (PS 2: carboplatin AUC 4, etoposide 80 mg/m², durvalumab 1500 mg; PS 3: carboplatin AUC 3, etoposide 60 mg/m², durvalumab 1,500 mg). The results showed that treatment tolerability and completion were key factors affecting prognosis. Patients capable of completing 4 cycles of induction treatment ($n = 34$) demonstrated a significantly longer median OS compared to those unable to do so ($n = 19$) (15.0 months vs. 3.8 months; $P < 0.0001$). These results suggest that for patients with poor performance status, the active management of supportive care and comorbidities to facilitate treatment tolerance and completion may be crucial for achieving improved survival outcomes.

High tolerability is also a key finding of this study. In terms of safety, the most common grade ≥ 3 treatment-related adverse events in the NEJ045A study^[23] were hematologic toxicities, such as neutropenia (64%), leukopenia (43%), and lymphopenia (30%), while the incidence of immune-related adverse events was low and predominantly consisted of low-grade events. In this study, the most common grade ≥ 3 treatment-related adverse events were neutropenia (16.0%), ALT/AST elevation (8.0%), and immune-related pneumonitis (8.0%). Furthermore, regarding treatment-related neutropenia and nausea in this study, the proportion of patients requiring symptomatic interventions (e.g., granulocyte colony-stimulating factor or antiemetic therapy) was low, indicating good tolerability.

Small cell lung cancer is characterized by highly aggressive biology and significant heterogeneity. Compounded by the clinical challenge of obtaining high-quality tumor specimens, universally applicable and clinically relevant biomarkers for predicting immunotherapy efficacy have not yet been established, representing a critical bottleneck that hinders the advancement of individualized treatment for elderly SCLC. Previous studies^[24] indicate that the infiltration of different immune cell subsets in the local tumor microenvironment is a key factor determining treatment response and long-term prognosis; specifically, CD4+/Cluster of Differentiation 8-positive (CD8+) CXCR5+ follicular helper T cells, CD8+CD103+ tissue-resident memory T cells, and B cells are linked to improved treatment responses and prognoses. However, within the context of the triple-combination regimen comprising anti-angiogenic agents, PD-L1 inhibitors, and chemotherapy, the microenvironmental characteristics associated with therapeutic efficacy remain incompletely elucidated. Based on exploratory RNA-seq analysis of this elderly SCLC cohort, this study provides an initial characterization of the transcriptional and immune infiltration profiles associated with clinical response and survival benefits from this triple-drug regimen, offering initial insights into its potential resistance mechanisms.

At the transcriptome level, we observed that high response and long-term benefit from this targeted combination immunotherapy regimen were associated with specific metabolic pathways and cell cycle characteristics. This study found that upregulation of FAM230A and NAT16 genes and downregulation of BMP4 gene were associated with better efficacy, while upregulation of RET and WDR65 genes and downregulation of NBFP15 and APOA1 genes were associated with longer PFS. Although FAM230A is a long non-coding RNA, its role in tumorigenesis and development remains unclear due to limited reporting.

This study is the first to reveal its correlation with SCLC immunotherapy combination response, providing a new direction for subsequent mechanistic exploration. The NAT16 gene^[25], which belongs to the N-acetyltransferase family, participates in protein post-translational modifications and the regulation of multiple key cellular processes. As a member of the bone morphogenetic protein family, the BMP4 gene^[26] has been shown in previous studies to exert tumor-suppressive effects by inducing apoptosis and cell cycle arrest; furthermore, it significantly reduces CD8+ T cell infiltration in tumors, increases the proportion of exhausted CD8+ T cells and regulatory T cells (Tregs), and alters the ratio of CD8+ T cells to Tregs. Furthermore, BMP4 promotes tumor immune escape by upregulating immunosuppressive cytokines [such as Interleukin-6 (IL-6), Interleukin-10 (IL-10), Transforming Growth Factor-beta (TGF- β), Monocyte Chemoattractant Protein-1 (MCP-1), and G-CSF] and inhibiting the anti-tumor immune stimulatory factor Interferon-gamma (IFN- γ), shaping an immunosuppressive microenvironment^[27]. The correlation between low BMP4 expression and treatment resistance in this study is consistent with the above mechanisms of BMP4-mediated tumor immune escape.

As a proto-oncogene, RET exhibits abnormal activation associated with the development of various tumors. Dabir's study^[28] found that overexpression of RET in SCLC cell lines activated the ERK signaling pathway, upregulated MYC expression, and promoted cell proliferation, with mutant RET showing more significant carcinogenic effects. Research by Huang *et al.*^[29] indicated that RET variant tumors were accompanied by increased infiltration of various immune cells (such as Tc cells, Tex cells, CD4+ T cells, and CD8+ T cells). As the primary protein component of high-density lipoprotein (HDL), APOA1 participates in lipid metabolism, inflammation regulation, and the modulation of immune responses^[30]. Zeng *et al.* reported^[31] that ApoA1 is an important predictive indicator of treatment response and prognosis in patients with locally advanced esophageal squamous cell carcinoma. This study found that downregulation of APOA1 was associated with poor prognosis in SCLC, further substantiating the prognostic value of APOA1 in malignant tumors. It is noteworthy that in the context of immunotherapy combined with chemotherapy, the interaction between tumors and the immune system may be more complex, and the specific regulatory mechanisms of the above genes still require functional experimental validation.

Analysis of the tumor immune microenvironment in this study revealed that patients in the PR group exhibited higher levels of eosinophil infiltration, whereas memory B cells and naïve B cells showed higher infiltration in non-PR patients. Compared with the long PFS group, the short PFS group exhibited greater plasma cell infiltration. Previous studies^[32] have reported that B cells enhance the efficacy of PD-L1 inhibitors and prolong patient survival by forming tumor-associated lymphoid structures (TLS) and facilitating interactions with T cells. Moreover, in NSCLC, B cell subsets show dynamic functional differentiation, with naïve B cells inhibiting tumor growth in early-stage tumors, whereas their decreased numbers in advanced stages are associated with a poor prognosis. In contrast, plasma cells exert anti-tumor activity in early-stage disease but may promote tumor progression in advanced disease through the secretion of specific immunoglobulins (such as IgG subclasses) or interaction with the microenvironment^[32,33]. This study preliminarily confirmed that the platinum-free, non-intravenous treatment regimen of Envafoimab combined with oral etoposide and Anlotinib showed good clinical benefit in elderly ES-SCLC patients. By avoiding the cytotoxicity of traditional platinum agents, this regimen significantly reduced chemotherapy-related hematologic and gastrointestinal side effects, presenting a lower incidence of toxicities and a controllable safety profile. The non-intravenous administration mode greatly improved treatment convenience, effectively reducing hospitalization frequency and healthcare resource utilization, which is particularly beneficial for elderly populations with mobility limitations or difficulty accessing medical care.

Although the ORR and PFS in this study were generally consistent with data from previous large-scale clinical studies, the OS data in this study were lower than those reported in other studies, possibly attributable to the combined effects of multiple factors. First, the median age of enrolled patients was higher, and this population exhibited reduced tolerance to subsequent treatments, contributing to a less favorable baseline prognosis. Second, during the study period, some patients were unable to receive effective second-line and subsequent treatments due to disease progression and compromised physiological state, which shortened post-progression survival. Additionally, the relatively limited sample size restricted the power of subgroup analysis, potentially exacerbating the influence of the above confounding factors on overall survival data.

This study has several limitations. First, this was a single-center study with a relatively small sample size, which may introduce selection bias. Some subgroup analyses were underpowered, and the external generalizability of the study conclusions warrants validation in multicenter, large-sample cohorts. Second, constrained by the significant challenges associated with obtaining tumor tissue from elderly advanced SCLC, this exploratory analysis only included baseline samples from 15 patients. Additionally, due to sample collection limitations, this study precluded the dynamic monitoring of paired samples before and after treatment, restricting in-depth analysis of dynamic changes in various indicators within the microenvironment. As the core findings rely primarily on bioinformatics analyses and clinical associations, they lack validation through *in vitro* or *in vivo* functional experiments; consequently, the inferred causal relationships require further experimental confirmation.

In conclusion, for elderly ES-SCLC patients, a comprehensive assessment should be performed to further optimize first-line treatment selection of immunotherapy combined with chemotherapy $\pm$ anti-angiogenic therapy. Based on the current data from this study, the platinum-free, non-intravenous regimen of oral etoposide combined with anlotinib and envafolimab may serve as a new first-line treatment option for elderly ES-SCLC patients with poor ECOG performance status who are intolerant to platinum-based therapy. However, further exploration through clinical studies with expanded sample sizes remains necessary.

DECLARATIONS

Authors' contributions

Conceived this study: Jiang Z

Drafted the research protocol: Zhang M, Jiang Z, Pan Z

Were responsible for patient treatment and management: Zhang M, Sun H, Li S, Zuo R, Wang H, Qi J, Wang B

Performed the pathological diagnosis: Zhao G

Handled patient recruitment: Yan Z, Xu J, Pan Z, Jiang Z

Coordinated the trial procedures: Sun H, Xing S, Li Y

Were responsible for the collection of data and samples: Sun H, Xing S, Li Y

Served as clinical investigators: Pan Z, Jiang Z

Analyzed and interpreted the clinical data: Zhang M, Sun H, Xing S, Li Y, Zuo R, Qi J

Completed the bioinformatics analyses and generated the figures: Wang H, Qi J, Wang B, Li S

Wrote the initial draft of the manuscript: Zhang M, Jiang Z

All authors have read and approved the final version of this paper.

Availability of data and materials

Data are available upon request by contacting the corresponding author, Jiang Z (zhjiang@tmu.edu.cn). Approval from the ethics committees is required for access. Clinical data are not publicly accessible due to patient privacy concerns but can be obtained from the corresponding author for a period of three years. Individual de-identified patient data will be provided for clinical study analyses. All other data are included in the Manuscript and [Supplementary Materials](#).

Financial support and sponsorship

This study was supported by the National Natural Science Foundation of China (No. 82274607 and 82503763), the Tianjin Municipal Health Commission (No. 2024013), the Tianjin Key Medical Discipline (Specialty) Construction Project (No. TJYXZDXK-009A), Beijing Xisike Clinical Oncology Research Foundation (No. Y-HS202102-0120 and Y-HS202202-0005), Health Science and Technology Development Promotion Project - Lung Cancer Diagnosis and Treatment Research Project (PMY202410110097-041), the Beijing Bethune Charitable Foundation (No. STLKY0040), and the Science and Technology Program of Tianjin Binhai New Area Health Commission (No. 2022BWKQ026) . The funders had no role in study design, data collection and analysis, or manuscript writing.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Written informed consent was obtained from all patients and/or their legal guardians prior to study enrollment. The study protocol was approved by the Ethics Committee of Tianjin Cancer Hospital Airport Hospital (Approval No. JS-2022-0048). All procedures were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Ethical issues were carefully considered throughout the entire study process.

Consent for publication

Not applicable.

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