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Pre-treatment Circulating Tumor Cell Associated White Blood Cell Clusters Independently Predict Poor Survival in Patients with Extensive-disease Small Cell Lung Cancer

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Abstract

Background: Patients with extensive disease (ED)-small cell lung cancer (SCLC) commonly suffer a more inferior prognosis than those with limited disease (LD)-SCLC.

Objectives: This study aims to investigate the heterogeneity and prognostic significance of various aneuploid circulating tumor cells (CTCs) subtypes and CTC-associated white blood cell (CTC-WBC) clusters in patients with LD-and ED-SCLC respectively.

Design: This prospective, non-interventional, single-center study included 48 patients with LD-SCLC and 47 patients with ED-SCLC.

Methods: A total of 95 SCLC patients were prospectively enrolled and serial blood samples were obtained before chemotherapy administration (t_0) and after 2 cycles of chemotherapy (t_1). Comprehensive in situ co-detection of CTCs and CTC-WBC clusters were performed in all enrolled patients.

Results: The analysis revealed no significant difference in CTCs quantity between LD-SCLC and ED-SCLC patients ($P = .610$). However, significant morphologic heterogeneity in CTCs, including cell size and chromosome 8 (Chr8) ploidy in CTCs was observed between the 2 groups ($P < .001$ and $P < .001$). Patients with post-therapeutic small cell CTCs $\geqslant$ 2/6 ml or triploid CTCs $\geqslant$ 2/6 ml exhibited reduced overall survival (OS) compared to those with small cell CTCs $<$ 2/6 ml or triploid CTCs $<$ 2/6 ml in the ED-SCLC ($P = .011$ and $P = .018$). Additionally, the positive detection of post-therapeutic tetraploid CTCs was associated with inferior survival in both LD-and ED-SCLC ($P = .041$ and $P = .049$). The presence of CTC-WBC clusters at baseline and after treatment significantly correlated with inferior OS in ED-SCLC ($P = .016$ and $P = .028$) but not in LD-SCLC ($P = .355$ and $P = .621$). Multivariate analysis identified brain metastasis and pre-treatment CTC-WBC clusters as independent prognostic factors for OS in ED-SCLC patients ($P = .004$ and $P = .013$).

Conclusion: Ideal biomarkers should be more specific for survival prediction in patients with different disease stages. Pre-treatment CTC-WBC clusters can independently predict inferior OS in ED-SCLC but not LD-SCLC.

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Plain language summary: Different from the diploid of normal cells, aneuploid is a common feature of human tumor cells. Circulating tumor cells and circulating tumor cell associated white blood cell clusters are widely present in the occurrence and development of tumors after invasion and metastasis of tumor cells into lymph and blood systems. Patients with extensive stage small cell lung cancer generally have shorter survival than patients with limited-stage small cell lung cancer. Therefore, we compared and analyzed the difference between different aneuploid circulating tumor cells and circulating tumor cell associated white blood cell clusters in extensive-stage small cell lung cancer and limited-stage small cell lung cancer, and what the clinical implications are.

Keywords
CTCs, CTC-WBC clusters, heterogeneity, survival, LD-SCLC, ED-SCLC

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Introduction

Small cell lung cancer (SCLC), which accounts for approximately 13%-15% of all lung cancer cases, is the most lethal subtype of lung cancer both in China and worldwide.[1] Despite decades of research, the prognosis for SCLC patients remains remarkably poor, with an estimated 2-year survival rate of 41% for limited disease (LD) and just 9% for extensive disease (ED) SCLC.[2] In clinical practice, curative-intent surgery is recommended for only 5% of patients with T1-2N0M0 stages. Treatment strategies and prognosis predictions for most patients are primarily based on the Veterans Administration Lung Cancer Study Group (VALSG) staging system, which categorizes SCLC into limited and extensive stages.[3] However, this imaging-based classification reflects only the bulk of the tumor mass and fails to account for micro metastatic burden and intra-tumor heterogeneity in patients with different disease stages.[4]

Liquid biopsy is a valid noninvasive alternative for representing tumor cell heterogeneity and dynamically tracking tumor progression.[5,6] Circulating tumor cells (CTCs) that detach from the primary tumor mark the initial key event in cancer cell dissemination, existing in the blood circulation either as single CTC or as clusters with white blood cell (CTC-WBC clusters).[7] Since disease progression is often accompanied by tumor cell evolution, distinct tumor cell populations may contribute differently at different disease stages in the process of disease progression. While evidence has proved the detection and alteration of CTCs are correlated with treatment resistance and dismal prognosis,[8,9] understanding the cellular drivers at each step of SCLC development remains a formidable undertaking. To date, few studies have specifically investigated the distribution of heterogeneous CTC subpopulations and CTC-WBC clusters in patients with LD- and ED-SCLC separately. Furthermore, the prognostic significance of CTCs and CTC-WBC clusters in patients with different stages has yet to be fully clarified.

In the current prospective study, we utilized a novel platform that integrates subtraction enrichment and immunostaining-fluorescence in situ hybridization (SE-iFISH) to detect and characterize different cell size aneuploid CTCs as well as CTC-WBC clusters at different time points in SCLC patients undergoing first-line treatment. The aim of our study is to investigate the heterogeneity of CTCs and CTC-WBC clusters in patients with LD-and ED-SCLC. Furthermore, we specifically evaluated the role of different subtypes of aneuploid CTCs and CTC-WBC clusters in predicting prognosis for SCLC patients with different disease stages.

Methods

Study Design

This prospective, non-interventional, single-center study was conducted at Beijing Chest Hospital, Capital Medical University, to systematically investigate the heterogeneity and prognostic significance of diverse subtypes of CTCs and CTC-WBC clusters in patients with LD-and ED-SCLC. The study design and workflow diagram are shown in Figure 1. Eligible participants included patients aged ⩾18 years with newly diagnosed inoperable SCLC and a performance status (PS) of 0 to 2. Patients with a history of other malignant tumors were excluded. All enrolled patients received standard first-line treatment according to National Comprehensive Cancer Network (NCCN) clinical guidelines and were ultimately eligible for analysis. Peripheral blood (PB) samples were collected to detect CTCs and CTC-WBC clusters prior to chemotherapy administration (t_0) and after 2 cycles of chemotherapy (t_1). The study was conducted in accordance with the Declaration of Helsinki Principles and all participants signed written informed consent. Blood sample collection was approved by the Ethics Committees of Beijing Chest Hospital, Capital Medical University (No. KY-2018-002).

SE-iFISH

With a few modifications, the SE-iFISH was conducted according to the kit's instructions and the previously published technique (Cytelligen, San Diego, USA).[10] Briefly, 6 ml of blood was drawn into a tube that contained acid-citrate dextrose (ACD) anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at room temperature for 15 minutes (1500 r/min) to separate the plasma. The supernatant above the brown-red precipitate was discarded. The blood cell pellets were then diluted with 6 ml of CRC buffer (Cytelligen, San Diego, CA, USA) and gently added into 3 ml of sample density separation liquid in a 50 ml centrifuge tube, followed by centrifugation at 3000 r/min for

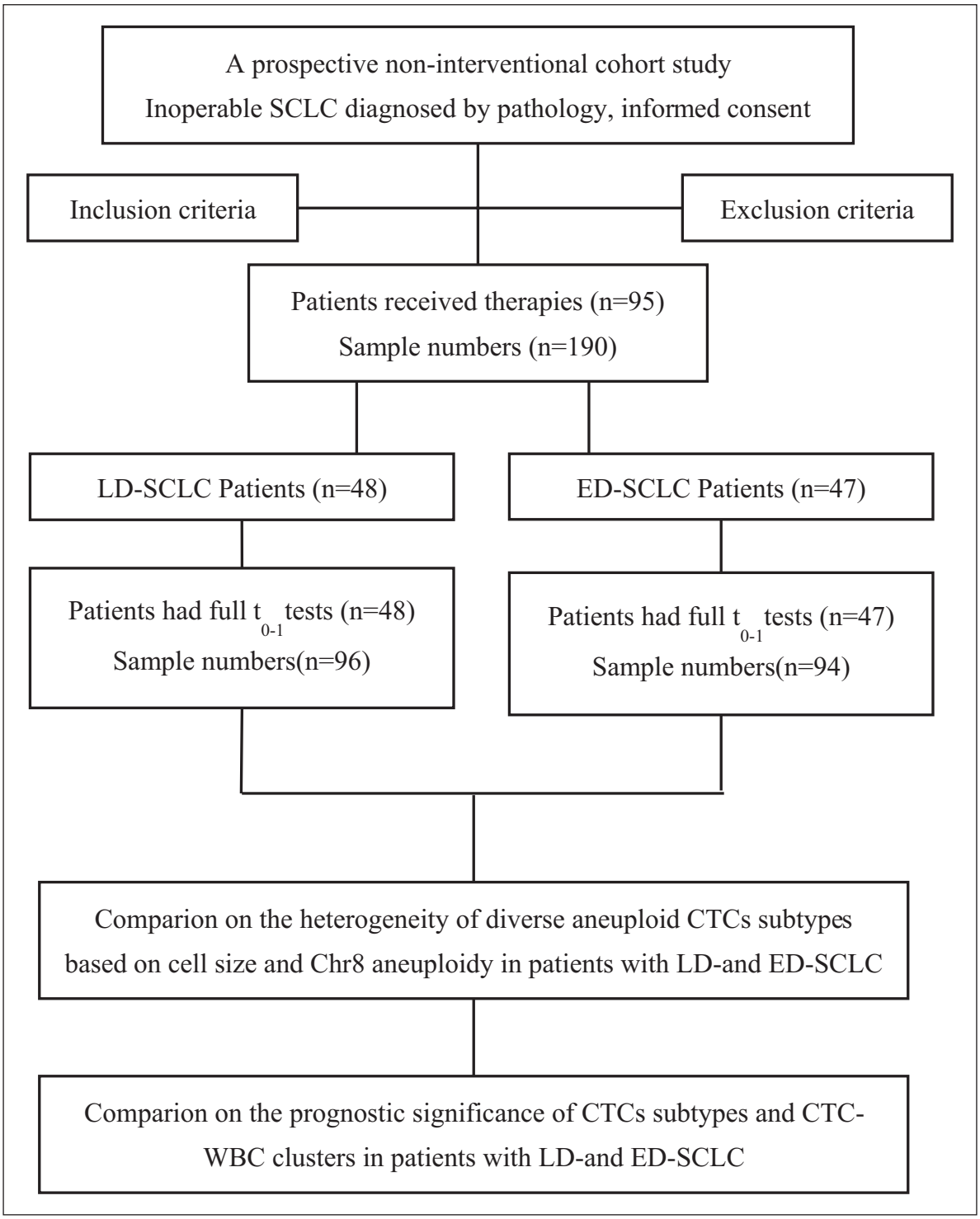


Figure 1. Study design and workflow diagram.
CTCs, circulating tumor cells; ED, extensive disease; LD, limited disease; SCLC, small cell lung cancer; WBC, white blood cell, t_0, prior to chemotherapy administration; t_1, after 2 cycles of chemotherapy.

7 minutes. A solution containing white blood cells (WBCs) and tumor cells above red blood cells (RBCs) was collected into a 50 ml tube. Next, the non-hematologic cell separation matrix was poured over the top of the sedimented blood cells, which had been resuspended in 3 ml of hCTCs solution. After centrifugation, the solution above the RBCs was collected. The WBC-containing solution was treated for 20 minutes at room temperature with immuno-magnetic beads conjugated to a cocktail of anti-leukocyte monoclonal antibodies. Using a magnetic separator appropriate for 50 ml tubes, the WBC-bound immuno-beads were removed (Cytelligen). The remaining non-hematologic cells were combined with a cell fixative, spread onto prepared CTC slides, and allowed to dry in preparation for subsequent iFISH investigations. Six-channel iFISH was conducted as previously described.[11] Using a ThermoBrite FISH Slides

Table 1. Clinical characteristics of enrolled SCLC patients.

Characteristics	Number of patients (%)	Characteristics	Number of patients (%)
Age (year)		Liver metastasis	
<60	41(43.16%)	Yes	16(16.84%)
≧60	54(56.84%)	No	79(83.16%)
Sex		Bone metastasis	
Male	72(75.79%)	Yes	17(17.89%)
Female	23(24.21%)	No	78(82.11%)
Smoking history		Brain metastasis	
Yes	76(80.00%)	Yes	7(7.37%)
No	19(20.00%)	No	88(92.63%)
PS score		Treatment efficacy	
0	17(17.89%)	PR	66(69.48%)
1	78(82.11%)	SD	20(21.05%)
VALSG stage		PD	9(9.47%)
Limited	48(50.53%)	chemotherapy regimens	
Extensive	47(49.47%)	Carboplatin + etoposide	51(53.68%)
		Cisplatin + etoposide	41(43.16%)
		ICI + carboplatin + etoposide	3(3.16%)

ICI, immune checkpoint inhibitor.

Processing System (Leica Biosystems, Buffalo Grove, IL, USA), the coated slides containing dried monolayer cells were rinsed with PBS, dehydrated, and then FISH hybridized for 3 hours with the centromere probe for human chromosome 8 (CEP8 Spectrum Orange, Vysis, Abbott Laboratories, Chicago, IL, USA). The samples were then treated in the dark at room temperature for 20 minutes with specific monoclonal antibodies, including Cy5-anti-CD31 (Clone WM59), Alexa Fluor (AF)594-anti-CD45 (Clone 9.4), and Cy7-anti-Vimentin (Clone 1D3). After washing, the samples were mounted on a medium containing DAPI (Vector Laboratories, Burlingame, CA, USA) and subsequently processed by Cytelligen for automated CTC image scanning and analysis.

Automated CTC Scanning and Image Analysis by Metafer-i•FISH

The Metafer-i•FISH imaging technology, developed in collaboration with Carl Zeiss (Oberkochen, Germany), MetaSystems (Altlussheim, Germany), and Cytelligen, was utilized to scan and analyze CTC slides. Automated characterization and categorization of aneuploid CTCs were performed upon cell size, immunostaining intensity of tumor marker expression and the chromosome 8 (Chr8) ploidy. The criteria for identifying CTCs include: $DAPI^+/CD45^-/CD31^-/Vimentin^+$ or aneuploid cells, as well as $Vimentin^+$ diploid cells. Small cell CTCs were defined as those with a maximum diameter smaller than 5 μm, while large cell had a maximum diameter larger than 5 μm. CTC-WBC clusters were defined as CTCs adhered to WBCs.

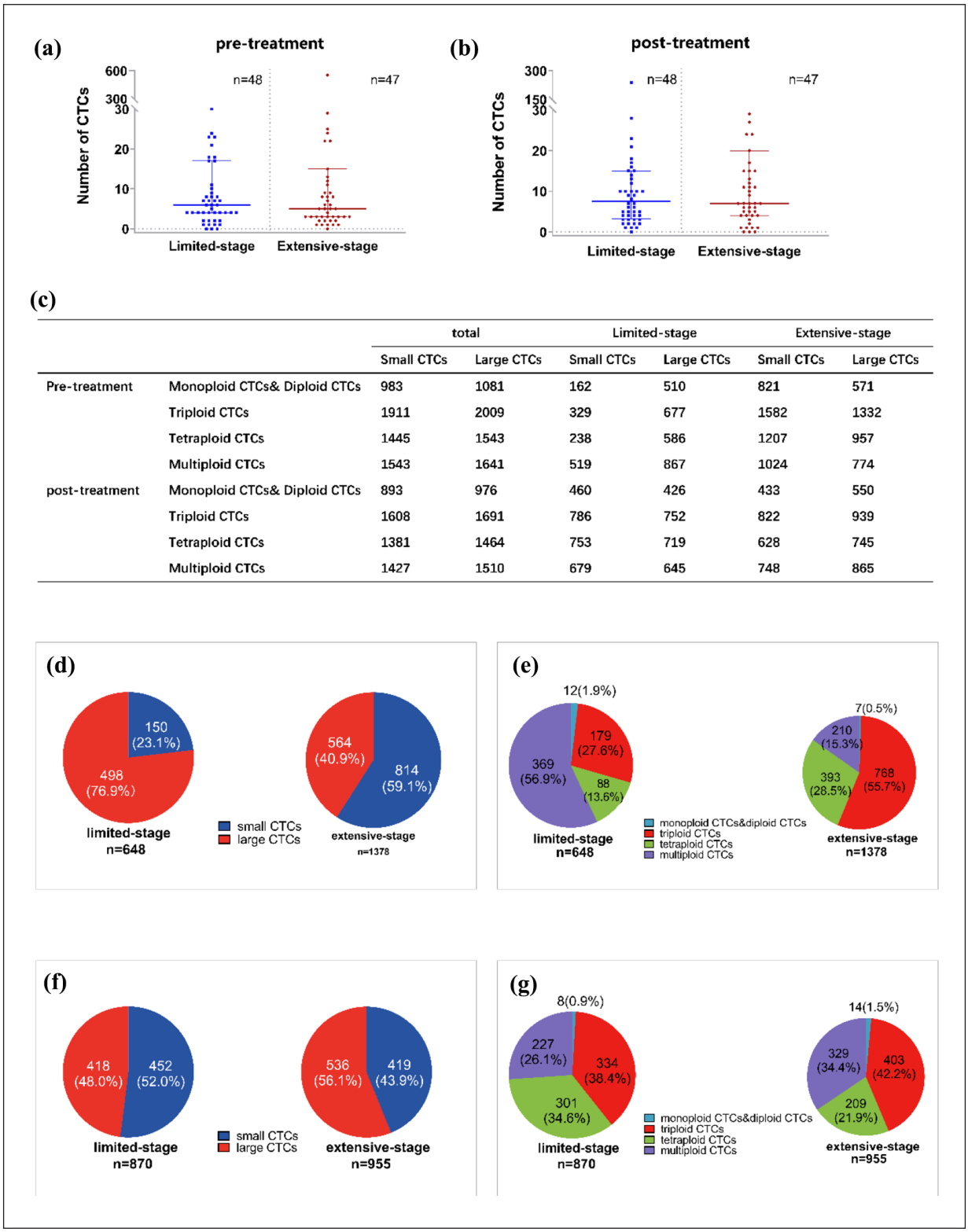
Statistical Analysis

We estimated that approximately 90 patients would be enrolled in the study. SPSS 25.0 (Chicago, IL, USA) and GraphPad 7.0 (GraphPad, San Diego, CA, USA) software were applied for statistical analyses. The chi-square test and Fisher's exact test were used to compare categorical variables. Continuous variables were expressed as the mean ± standard deviation or the median (interquartile range) and were compared using the Mann-Whitney test. Depicted in Supplemental Table 1, the median numbers of diverse CTC subtypes were used as cut-off values, for CTC-WBC clusters which the median number was 0, 1was applied as the cut-off point. Kaplan-Meier plots were created for OS based on diverse CTC subtypes or CTC-WBC clusters, with log-rank tests used to compare the survival curves. OS independent prognostic indicators were identified using univariate and multivariate Cox proportional hazards regression models, reporting hazard ratios and 95% confidence intervals. All P values were 2-sided and $P < .05$ was considered statistically significant.

Results

Co-Detection and Categorical Analysis of Diverse Subtypes of CTCs in SCLC Patients

As summarized in Table 1, a total of 95 eligible SCLC patients were prospectively enrolled from January 2018 to January 2020, including 48 limited-stage and 47 extensive-stage subjects. As of the data cut-off date of November 30, 2022, 70 out of 95 patients had passed away, resulting in a median OS was 16.53 months (range, 1.80-55.90 months). As shown in Figure 1, all participants provided serial blood samples, with a total of 190 samples collected, covering baseline (t_0) and post-treatment blood draws (t_1). Six-channel iFISH was utilized to perform in situ morphologic and karyotypic characterization of different subtypes of aneuploid CTCs enriched from SCLC patients. Representative images of CTCs subtypes and CTC-WBC clusters are shown in Supplemental Figure 1. Quantification of CTCs revealed their presence in 91 out of 95 patients (45 out of 48



(c)

		total		Limited-stage		Extensive-stage	
		Small CTCs	Large CTCs	Small CTCs	Large CTCs	Small CTCs	Large CTCs
Pre-treatment	Monoploid CTCs& Diploid CTCs	983	1081	162	510	821	571
	Triploid CTCs	1911	2009	329	677	1582	1332
	Tetraploid CTCs	1445	1543	238	586	1207	957
	Multiploid CTCs	1543	1641	519	867	1024	774
post-treatment	Monoploid CTCs& Diploid CTCs	893	976	460	426	433	550
	Triploid CTCs	1608	1691	786	752	822	939
	Tetraploid CTCs	1381	1464	753	719	628	745
	Multiploid CTCs	1427	1510	679	645	748	865

Figure 2. Quantitative, morphologic and karyotypic analysis of CTCs longitudinally detected in SCLC patients: (a) distribution of CTCs in pre-treatment LD-and ED-SCLC patients, (b) distribution of CTCs in overall post-treatment LD-and ED-SCLC patients, (c) quantitative analysis of characterized CTCs in different cell sizes with diverse Chr8 ploidy in LD-and ED-SCLC patients, (d) morphological analysis of CTCs in pre-treatment SCLC patients with different disease stages, (e) karyotype analysis of CTCs in pre-treatment LD-and ED-SCLC patients, (f) morphological analysis of CTCs in post-treatment SCLC patients with different disease stages, and (g) karyotype analysis of CTCs in post-treatment LD-and ED-SCLC patients.

for LD-SCLC and 46 out of 47 for ED-SCLC patients), with CTC-WBC clusters detected in 31 out of 95 patients. As illustrated in Figure 2a and b, the median values of CTCs before treatment were 6 (blue, IQR 4-17) in LD-SCLC and 5 (red, IQR 3-15) in ED-SCLC patients. After 2 cycles of chemotherapy, the median values of CTCs were 7 for both

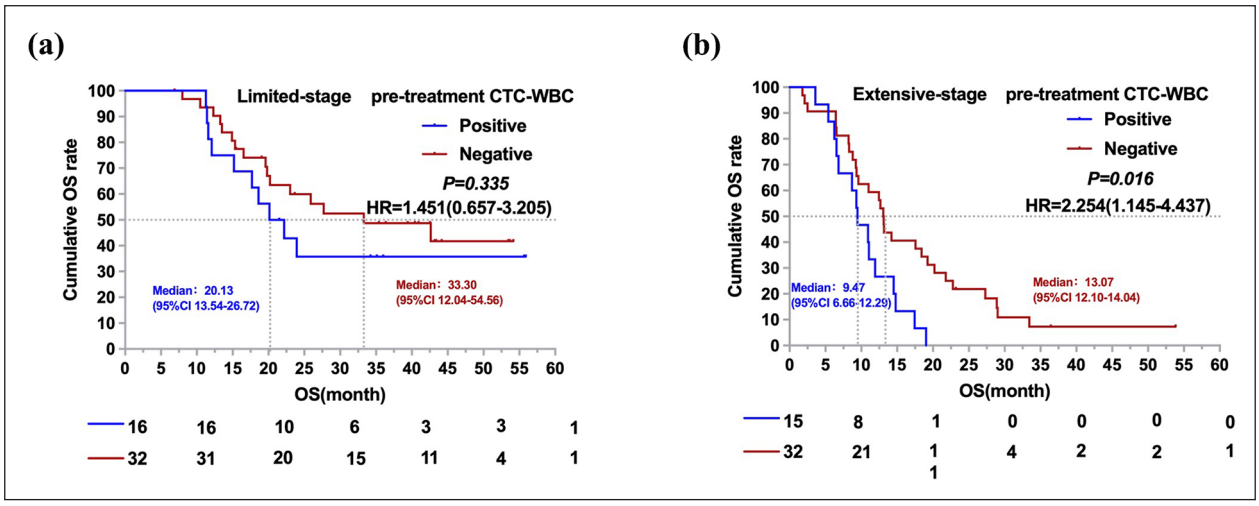


Figure 3. Analysis of pre-treatment CTC-WBC clusters with LD-and ED-SCLC patients' OS: (a) the OS curves of LD-SCLC patients with negative and positive pre-treatment CTC-WBC clusters, (b) the OS curves of ED-SCLC patients with negative and positive pre-treatment CTC-WBC clusters.

LD-SCLC (blue, IQR 3-15) and ED-SCLC (red, IQR 4-20). Comparison of CTCs quantities between patients with different disease stages showed no statistical differences ($P = .610$ at t_0 and $P = .444$ at t_1). We further conducted morphologic and karyotypic analysis of CTCs in LD-SCLC and ED-SCLC patients respectively. Figure 2c presents a quantitative analysis of characterized CTCs based on cell sizes and diverse Chr8 ploidy in both patient groups. As shown in Figure 2d, before treatment, small CTCs account for 23.1% of the total in LD patients while large CTCs comprised 76.9%. In ED patients, small CTCs represented 59.1%, and large CTCs 40.9% of the total. Depicted in Figure 2e, karyotype analysis of Chr8 revealed that multiploid cells accounts for over half (56.9%) of the total CTCs in pre-treatment LD-SCLC patients, with triploid CTCs constituting 27.6%, followed by tetraploid (13.6%) and mono/diploid (1.9%) CTCs. Significant morphologic heterogeneity in CTCs, including cell size and Chr8 ploidy was observed between pre-treatment patients with different disease stages ($P < .001$ and $P < .001$ at t_0). Figure 2f and g present similar morphologic and karyotypic analysis of CTCs in post-treatment patients classified by disease stages, with results consistent with those observed in pre-treatment patients ($P < .001$ and $P < .001$ at t_1).

Association of Pre-treatment CTCs Subtypes and CTC-WBC Clusters With OS

The prognostic significance of various subtypes of CTCs, subcategorized according to cell size and Chr8 aneuploidy, was evaluated in LD-and ED-SCLC patients separately. As shown in Supplemental Table 2, no positive relationship was established between the pre-treatment categorical CTCs numbers and patients' survival. The prognostic significance of CTC-WBC clusters was specifically evaluated. Shown in Figure 3a, although the presence of pre-therapeutic CTC-WBC clusters (t_0) in LD-SCLC patients demonstrated a shorter median OS of 20.13 months (95% CI:13.54-26.72 months) compared to a median OS of 33.30 months (95% CI:12.04-54.56 months) for those without CTC-WBC clusters (t_0), no statistical significance was achieved in OS between the 2 groups in our study ($P = .335$, log-rank test, Figure 3a). Further analysis of pre-therapeutic CTC-WBC clusters in ED-SCLC patients revealed that those with detectable CTC-WBC clusters had a median OS of 9.47 months (95% CI:6.66-12.29 months), which was shorter than 13.07 months (95% CI:12.10-14.04 months) observed in patients without detectable CTC-WBC clusters. This difference in OS was statistically significant ($P = .016$, log-rank test, Figure 3b), indicating that the presence of pre-treatment CTC-WBC clusters can predict poor survival in ED-SCLC, but not in LD-SCLC patients.

Prognostic Values of Post-Treatment Categorical CTCs Subtypes and CTC-WBC Clusters in LD-and ED-SCLC Patients

All enrolled patients are available for the second blood sample and the prognostic significance of post-treatment categorical CTCs subtypes and CTC-WBC clusters were systemically evaluated, as depicted in Supplemental Table 3. Univariate analysis indicated no significant difference in OS between LD-SCLC patients with small CTCs $\geq 3/6$ ml and those with small CTCs $< 3/6$ ml ($P = .880$, log-rank test, Figure 4a). However, ED-SCLC patients with small CTCs $\geq 3/6$ ml exhibited reduced OS compared to patients with small CTCs $< 3/6$ ml ($P = .011$, log-rank test, Figure 4b). Further analysis of the correlation between Chr8 aneuploidy-based CTC subtypes and OS revealed a statistically significant relationship between triploid CTCs and OS in ED-SCLC patients ($P = .018$, log-rank test, Figure 4d) but not LD-SCLC patients ($P = .442$, log-rank test, Figure 4c). Additionally, patients with post-treatment tetraploid CTCs $\geq 1/6$ ml had significantly shorter OS than those possessing tetraploid CTCs $< 1/6$ ml in both LD-and ED-SCLC ($P = .041$ and $P = .049$, log-rank test, Figure 4e and f).

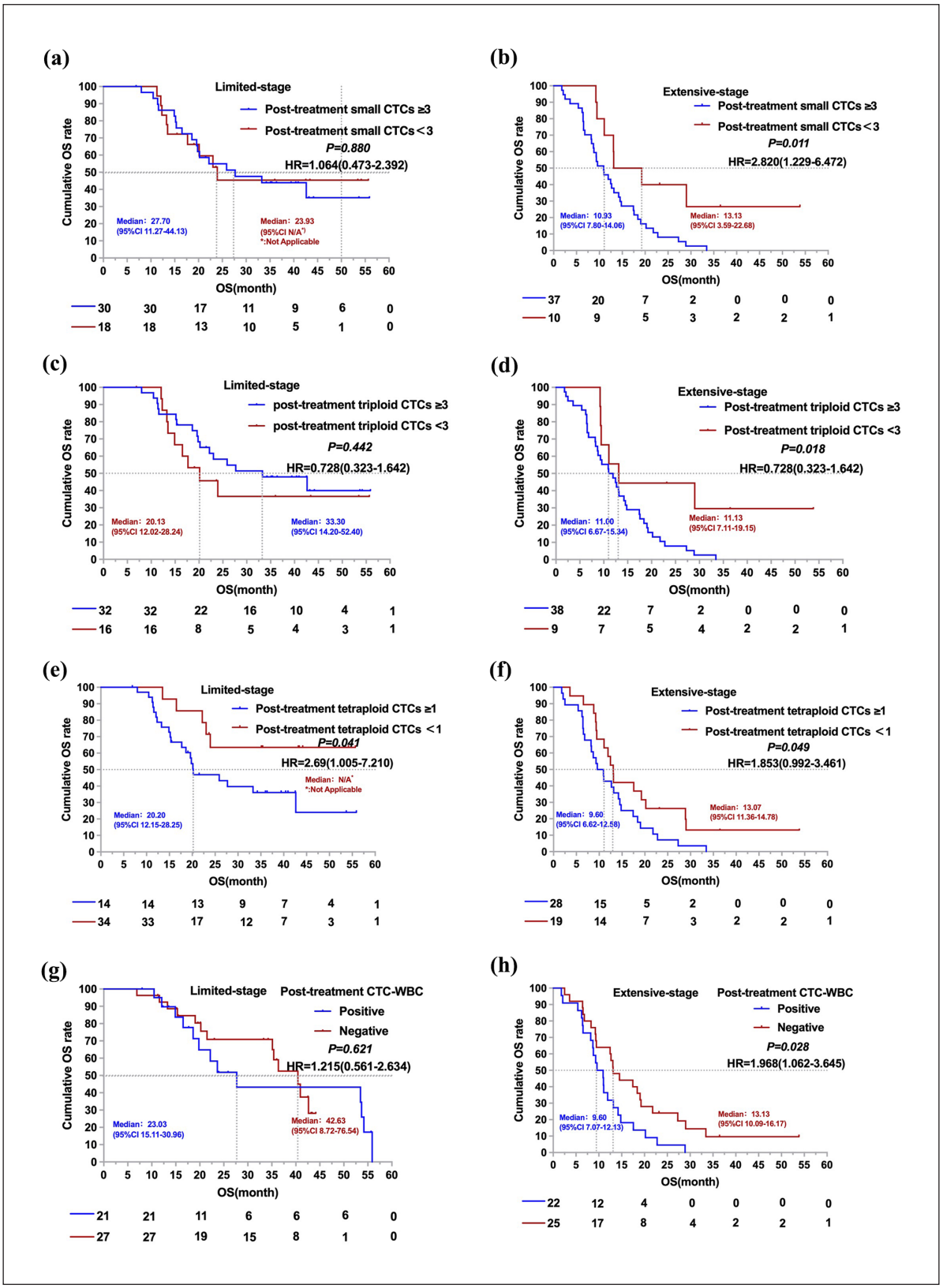


Figure 4. Analysis of diverse aneuploid post-treatment CTCs count and CTC-WBC clusters with LD-and ED-SCLC patients' OS: (a) the OS curves of LD-SCLC patients with post-treatment small CTCs ⩾ 3/ml and post-treatment small CTCs < 3/ml, (b) the OS curves of ED-SCLC patients with post-treatment small CTCs ⩾ 3/ml and post-treatment small CTCs < 3/ml, (c) the OS curves of LD-SCLC patients with post-treatment triploid CTCs ⩾ 3 ml and post-treatment triploid CTCs < 3/ml, (d) the OS curves of ED-SCLC patients with post-treatment triploid CTCs ⩾ 3/ml and post-treatment triploid CTCs < 3/ml, (e) the OS curves of LD-SCLC patients with post-treatment tetraploid CTCs ⩾ 1/ml and post-treatment tetraploid CTCs < 1/ml, (f) the OS curves of ED-SCLC patients with post-treatment tetraploid CTCs ⩾ 1/ml and post-treatment tetraploid CTCs < 1/ml, (g) the OS curves of LD-SCLC patients with negative and positive baseline CTC-WBC clusters, and (h) The OS curves of ED-SCLC patients with negative and positive baseline CTC-WBC clusters.

Table 2. Univariate and multivariate analysis for OS predictors in ED-SCLC patients.

	Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)	*P*	HR (95% CI)	*P*
Age <60 versus ≧60	1.058(0.577-1.941)	.856		
Sex Male versus Female	1.309(0.625-2.740)	.473		
PS score 0 versus 1	1.982(0.877-4.479)	.093		
Smoking history Yes versus No	1.786(0.752-4.241)	.182		
Liver metastasis Yes versus No	1.606(0.856-3.012)	.136		
Bone metastasis Yes versus No	1.419(0.763-2.641)	.266		
Brain metastasis Yes versus No	2.506(1.092-5.751)	.025	3.771(1.543-9.216)	.004
Post-treatment triploid CTCs ≥4 versus <4	2.926(1.201-7.130)	.014	1.178(0.309-4.494)	.811
Post-treatment Tetraploid CTCs ≥1 versus <1	1.863(0.998-3.480)	.047	0.898(0.377-2.139)	.808
Post-treatment small CTCs ≥3 versus <3	2.913(1.271-6.675)	.008	2.477(0.655-9.365)	.181
Baseline CTC-WBC clusters Pos versus Neg	2.575(1.304-5.084)	.005	2.572(1.224-5.406)	.013
Post-treatment CTC-WBC clusters Pos versus Neg	1.901(1.027-3.518)	.038	1.454(0.732-2.889)	.285

Regarding CTC-WBC clusters, our results demonstrated that patients with detectable post-therapeutic CTC-WBC clusters exhibited inferior survival compared to those without detectable CTC-WBC clusters in ED-SCLC, but not LD-SCLC patients ($P=.028$ and $P=.621$, log-rank test, Figure 4g and h).

Positive Detection of Pre-treatment CTC-WBC Clusters and Brain Metastases Independently Predict Poor OS in ED-SCLC Patients

As of the last follow-up, 44 out of 47 ED-SCLC patients died, with a median OS of 11 months. Univariate analysis of CTC subpopulations identified several factors significantly associated with a shorter OS: pre-treatment CTC-WBC clusters(t_0) ≥ 1/6 ml ($P=.005$), post-treatment small CTCs(t_1) ≥ 3/6 ml ($P=.008$), triploid CTCs (t_1) ≥ 4/6 ml ($P=.014$), tetraploid CTCs(t_1) ≥ 1/6 ml ($P=.047$) and post-treatment CTC-WBC clusters (t_1) positive($P=.038$). The correlation of standard clinical factors with OS was also evaluated, revealing that brain metastases were statistically significant in univariate analysis ($P=.025$). To further investigate prognostic factors affecting OS in ED-SCLC patients, clinical factors along with CTCs variables that showed statistical significance in univariate analysis were included in a multivariate Cox proportional hazard model, as demonstrated in Table 2. The analysis confirmed that pretreatment CTC-WBC clusters (t_0) and brain metastases were independent factors associated with OS in ED-SCLC patients [HR:2.572, 95% CI:(1.224-5.406), $P=.013$; HR:3.771, 95% CI:(1.543-9.216), $P=.004$, respectively, Table 2].

Discussion

This study is the first prospective study to systematically investigate the heterogeneity and prognostic significance of various aneuploid CTCs subtypes and CTC-WBC clusters in patients with LD-and ED-SCLC. It reveals significant morphologic and karyotypic CTCs heterogeneity in patients with different disease stages and validated that pre-treatment CTC-WBC clusters can independently predict inferior OS in ED-SCLC but not LD-SCLC.

Increasing evidence suggests that cancer development is a complex process, with disease progression intricately linked to tumor cell clonal evolution.[12] However, little is known about the intratumor heterogeneity and tumor cell plasticity during the disease progression in SCLC due to the limited number of operable patients. The rarity of tissue samples in clinical practice poses a significant challenge in SCLC research. Liquid biopsy has emerged as a valuable tool, reflecting the clonal dynamics of primary and metastatic tumor cells, and serves as an important adjunct to tissue analyses.[13] Recent advancements in CTCs detection technology have greatly enhanced our understanding of tumor biology in various cancers, including breast, gastric, pancreatic, and lung cancer.[5,14-16] Previous studies have demonstrated that CTCs are heterogenous and can predict treatment response, as well as longitudinal monitoring

disease progression in SCLC.[10] However, whether CTCs in different disease stages are of significant difference remains unclear. In the current study, CTCs were detected in 93.75% LD-SCLC and 97.87% ED-SCLC, suggesting the presence of micro metastasis at the early disease stage. Although many studies have shown a correlation between levels of CTCs in PB samples and tumor disease stage,[17,18] our quantitative analysis revealed no significant difference in CTCs between LD-SCLC and ED-SCLC. Both groups exhibited a similar "invisible" micro metastatic tumor burden. This inconsistency may be attributed to biological differences in various cancer types. Given that a deeper understanding of the tumor evolution in metastatic disease could elucidate differences in therapeutic vulnerabilities between primary and metastatic tumors,[13] we conducted extensive investigation of morphologic and karyotypic characteristics in CTCs from LD-SCLC and ED-SCLC patients. Our results revealed significant heterogeneity in CTCs between the 2 disease stages, indicating the onset of morphologic and chromosomal instability may contribute to the evolution of metastatic disease in SCLC patients.

The clinical utility of CTCs in both SCLC and NSCLC has been well documented in numerous studies.[19-21] Given that biological behavior of SCLC differs significantly from that of NSCLC, CTCs are detectable in peripheral blood at early stages of the disease, with substantially higher numbers found in SCLC patients compared to those with NSCLC.[22,23] However, to our knowledge, few studies have systemically investigated how different CTCs subtypes based on cell size and Chr8 aneuploidy correlate with clinical outcomes in SCLC with different disease stages. In the current study, we evaluated the prognostic significance of different cell sizes and diverse aneuploid CTCs subpopulations at baseline and after treatment in both LD-SCLC and ED-SCLC patients. Based on cell size CTCs were subdivided into small and large cell CTCs, on the other hand, CTCs were subcategorized according to karyotyping of Chr8 ploidy as monoploid, diploid, triploid, tetraploid, and multiploidy CTCs in SCLC patients. Discordance with previous reports, our findings showed no positive relationship between baseline CTCs subtypes and survival time in LD-or ED-SCLC patients. Possible explanations for this discrepancy included the small number of patients and confounding factors related to different CTCs detection platforms or cut-off values used. Further longitudinal investigation revealed that patients with post-therapeutic small cell CTCs $\geqslant$ 2/6 ml or triploid CTCs $\geqslant$ 2/6 ml displayed reduced OS than those with small cell CTCs $<$ 2/6 ml or triploid CTCs $<$2/6 ml in ED-SCLC but not LD-SCLC patients, which was in line with our previous work that small cell CTCs and triploid CTCs are of prognostic significance in lung cancer.[24] Moreover, patients with post-therapeutic tetraploid CTCs exhibited inferior survival compared to those without tetraploid CTCs in both LD-and ED-SCLC patients. Hence, detailed morphologic and karyotypic characterization is crucial for understanding the heterogeneity of tumor cells, highlighting the imperative need for specific biomarkers to better guide clinical practice in the treatment of SCLC with different disease stages.

In addition to CTCs' intrinsic features that contribute to disease progression, the interactions between CTCs subtypes and nonmalignant cells in bloodstream also play a significant role in the metastasis process.[25,26] It has been demonstrated that WBC may promote CTCs development and metastasis by binding to CTCs directly through intercellular adhesion molecule-1 to shield them from natural killer cells or macrophage attacks, and indirectly affecting CTCs by changing the tumor microenvironment.[27] Existing data suggests that CTCs and immune cells can play different roles at different stages in the process of tumor progression, thus patients are likely to respond differently to therapies as their tumor evolves.[13] Gain a better understanding of how and when cell-cell interactions influence disease progression of SCLC may be translated into new therapeutic options to limit metastatic spread and treat metastatic SCLC. In recent years, CTC-WBC clusters have garnered increased interest due to their contribution to cancer-promoting effects.[7,28] Given that WBCs may facilitate the metastasis process through direct interaction with CTCs and CTC-WBC clusters were proven to predict inferior prognosis in many carcinomas including lung cancers. Despite our previous small sample study in SCLC has demonstrated the CTC-WBC clusters' prognostic utility,[7] its role at different disease stages remains unclear. The present study suggested that the baseline and posttreatment presence of CTC-WBC clusters were significantly correlated with inferior overall survival (OS) in ED-SCLC but not LD-SCLC patients. This prognostic role of CTC-WBC clusters was further validated in multivariate analysis. After adjusting for clinically significant factors, both brain metastasis and pre-treatment CTC-WBC clusters were established as independent prognosis factors for OS in ED-SCLC patients. SCLC is known to be a highly aggressive subtype of lung cancer and its current management is challenging. LD-SCLC is potentially curable, with long-term survival rates of 15% to 20% when treated with concurrent chemoradiotherapy.[29] In contrast, ED-SCLC patients may benefit from the combination of immune checkpoint inhibitors and chemotherapy, which can prolong survival and improve quality of life, but long-term survival remains rare.[30] Since the treatment goals are different for LD-and ED-SCLC patients, that is, cure versus palliation, separate derivation markers are required to discriminate patients who are more likely to achieve favorable outcomes thus conducting risk stratification into patients' management. The present study systematically evaluated the prognostic value of diverse subpopulations of CTCs in different disease stages, establishing that brain metastasis and pre-treatment CTC-WBC clusters independently predict poor survival in ED-SCLC but not LD-SCLC patients. Therefore, CTCs and immune cells may play distinct roles in promoting disease progression at different disease stages of SCLC. However, our study has some limitations that should be acknowledged. First, this single-center study enrolled a relatively small number of patients, which may limit the statistical power of the analysis. Additionally, the underlying mechanisms by which CTC-WBC clusters contribute to disease progression in ED-SCLC remains to be investigated.

Conclusion

In conclusion, our findings demonstrate that co-detection and molecular characterization of aneuploid CTCs and CTC-WBC clusters can significantly enhance personalized clinical management and risk stratification in SCLC patients. Our observations revealed important morphological and karyotypical discrepancy in CTCs between patients with LD- and ED-SCLC. Notably, pre-treatment CTC-WBC clusters can independently predict inferior OS in ED-SCLC but not LD-SCLC. Ideal biomarkers should be more specific for survival prediction across different disease stages. However, further investigations are necessary to fully understand the underlying mechanisms of cancer cell-immune cell communication mechanisms.

Abbreviations

ACD: Acid-citrate dextrose
Chr 8: chromosome 8
CTCs: Circulating tumor cells
ED: Extensive disease
LD: Limited disease
NCCN: National Comprehensive Cancer Network
PB: Peripheral blood
PS: Performance status
RBCs: Red blood cells
RECIST: Response Evaluation Criteria in Solid Tumors
SCLC: Small cell lung cancer
SE-iFISH: Subtraction enrichment and immunostaining-fluorescence in situ hybridization
VALSG: Veterans Administration Lung Cancer Study Group
WBC: White blood cell

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Ethical Considerations

The study was approved by the Ethics Committees of Beijing Chest Hospital, Capital Medical University (No. KY-2018-002).

Consent to Participate

All patients signed written informed consent.

Author Contributions

Ying Wang: Formal analysis; Writing - original draft; Writing - review & editing.
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Cen Chen: Formal analysis; Writing - original draft; Writing - review & editing.
Yanxia Liu: Formal analysis; Writing - review & editing.
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Zhanliang Ren: Conceptualization; Formal analysis; Writing - review & editing.
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Declaration of conflicting interests

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Data Availability Statement

All datasets generated during this study are available from the corresponding author for reasonable request.

Supplemental Material

Supplemental material for this article is available online.

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