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PD-L1 status on circulating tumor cells: a promising predictor in advanced lung cancer with PD-1/PD-L1 immunotherapies

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Abstract

Background Tumor heterogeneity and invasive sampling limit traditional tissue-based PD-L1 testing in advanced lung cancer. We explored a non-invasive alternative by assessing PD-L1 expression on circulating tumor cells (CTC PD-L1) via liquid biopsy to predict immunotherapy outcomes.

Methods Fifty-two advanced lung cancer patients were recruited, and 38 individuals received a combination of chemotherapy and PD-1/PD-L1 inhibitor therapy, with serial blood samples (baseline, during treatment, and at progression) analyzed using the LiquidBiopsy™ platform and CTC PD-L1 assay. Primary endpoints included objective response rate (ORR), progression-free survival (PFS), and overall survival (OS).

Results CTC PD-L1 was identified on special phenotype with a ratio of CK/CD45 > 1.7 and PD-L1/CD45 > 3.2. At baseline, 71.2% (37/52) of patients had detectable CTCs, with 50.0% (26/52) being CTC PD-L1$^+$. CTC PD-L1$^+$ patients exhibited significantly higher ORR (84.2% vs. 36.8%), longer median PFS (16 months vs 4 months; HR = 0.28, 95% CI 0.096–0.78, $p = 0.0041$), and superior OS (median survival, undefined; HR = 0.19, 95% CI 0.05–0.74, $p = 0.017$). Multivariate analysis confirmed CTC PD-L1$^+$ as an independent predictor of response ($p = 0.003$) and potential as a complementary biomarker to tPD-L1.

Conclusions We developed a novel procedure to detect and characterize PD-L1 expression on CTCs, which was a feasible, non-invasive biomarker for potentially predicting the combination of chemotherapy and immunotherapy efficacy in advanced lung cancer, addressing tissue sampling limitations and enhancing patient stratification and monitoring.

Trial registration: The study was approved by the Medical Ethics Committee of Fourth Affiliated Hospital of Soochow University, with ethics approval number 220154 on 23th January, 2022 and retrospectively registered under clinical trial number ChiCTR2500096312 on 21th January, 2025.

Keywords Circulating tumor cells, CTC PD-L1, Objective response rate (ORR), Progression-free survival (PFS), Overall survival (OS)

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Introduction

Immunotherapy with PD-1/PD-L1 blockade is a standard treatment for advanced non-small cell lung cancer (NSCLC) and is increasingly used in small cell lung cancer (SCLC) [1–4]. However, durable clinical benefit is observed in only a subset of patients, with only 20–30% showing lasting benefits from PD-1/PD-L1 blockade immune-therapies, underscoring the need for reliable predictive biomarkers [5]. Furthermore, immune-related adverse events, including skin reactions and liver abnormalities, and potentially fatal conditions like pneumonitis, hepatitis, and myocarditis, underscore the critical importance of early identification of patients likely to benefit from PD-1/PD-L1 blockade therapy early in the treatment process [6].

Currently, PD-L1 expression on tumor tissue (tPD-L1) is a key predictive biomarker, particularly in NSCLC [7]. However, its clinical utility is limited by several factors: the invasiveness and risk of serial biopsies, intra-tumoral heterogeneity, limited tumor tissue size obtained from biopsies [8, 9], and temporal changes in expression due to prior therapies [10, 11]. Moreover, significant variability exists across different PD-L1 immunohistochemistry assays, as highlighted in lung cancer-specific comparative studies [12]. This variability likely stems from the dynamic regulation of PD-L1 expression, which can be modulated by treatments such as targeted therapy, chemotherapy [13], or radiation therapy [14]. In SCLC, the predictive role of tPD-L1 is less established and its assessment is not routinely standardized [15, 16]. These limitations highlight the need for minimally invasive, dynamic biomarkers.

Circulating tumor cells (CTCs) represent a real-time, liquid biopsy approach to capture tumor biology [17–20]. The expression of PD-L1 on CTCs has emerged as a potential biomarker of interest [21, 22]. However, existing data on its predictive value for immunotherapy outcomes are conflicting. Some studies associate PD-L1-positive CTCs with worse prognosis, while others suggest a correlation with better response to PD-1/PD-L1 blockade [23, 24]. Notably, the predictive role of CTC PD-L1 specifically in the context of first-line chemo-immunotherapy—the current standard for many advanced lung cancer patients—remains unclear, and studies often pool NSCLC and SCLC despite their distinct biologies.

To address this, we developed a novel assay to detect PD-L1 expression on CTCs using the LiquidBiopsy™ platform. In this study, we longitudinally assessed CTC PD-L1 in patients with both advanced NSCLC and SCLC undergoing first-line chemotherapy combined with immunotherapy. Our aim was to evaluate the correlation between baseline and dynamic changes in CTC PD-L1 expression and treatment outcomes, including objective response rate (ORR) and survival.

Materials and method

Study design

This study was reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines. It was a non-interventional, double-blind, prospective, real-world investigation conducted at the Fourth Affiliated Hospital of Soochow University (Ethics review board approval number 220003), enrolling advanced lung cancer patients without autoimmune diseases. Participants received at least two cycles of a combination of chemotherapy and PD-1/PD-L1 blockade immunotherapy starting from April 2022, with informed consent and under a registered clinical trial number (ChiCTR2500096312). To maintain the blind, peripheral blood samples (10 mL) were collected at baseline (T0), after two cycles (T1), after four cycles (T2), and at disease progression (PD) for CTC detection and PD-L1 expression analysis at Zhuhai Sanmedbio (Fig. 1 and Supplementary materials Figure S1), the effects of response to the combination of chemotherapy and immune checkpoint inhibitor (ICI) therapy and follow-up care are being studied at the Fourth Affiliated Hospital of Soochow University; all the data were sent to the third party for storage and analysis immediately. The primary objective was to assess the predictive value of baseline CTC PD-L1 for objective response rate (ORR), progression-free survival (PFS), and overall survival (OS). Secondary objectives included monitoring changes in CTC counts and their relationship to therapeutic response. All the patients were followed up with for more than 3 months for ORR evaluation.

CTC isolation and detection by using LiquidBiopsy™

CTCs were isolated and counted using the LiquidBiopsy™ system (Zhuhai Sanmed Biotech LTD, Zhuhai, China), as detailed elsewhere [25]. PBMCs were processed using Ficoll from 10 mL blood, fixed with PFA, and incubated with capture cocktail kits and streptavidin beads for CTC capture. Anti-pan CK antibody, anti-PD-L1 antibody, and anti-CD45 antibody, along with DAPI, were used for detection. CTCs were identified based on specific phenotypes with a threshold of CK mean of fluorescence intensity (MFI) and ratio of CK/CD45 [26]. CTC PD-L1 was analyzed on HRP-labeled anti-PD-L1 antibody with anti-HRP IgG Alexa Fluor594 secondary antibody (Supplementary materials Figure S2) and classified with a threshold of PD-L1 MFI and ratio of PD-L1/CD45. Patients with one or more PD-L1 positive CTCs were classified as CTC PD-L1^{+}, while those with one or more CK-positive

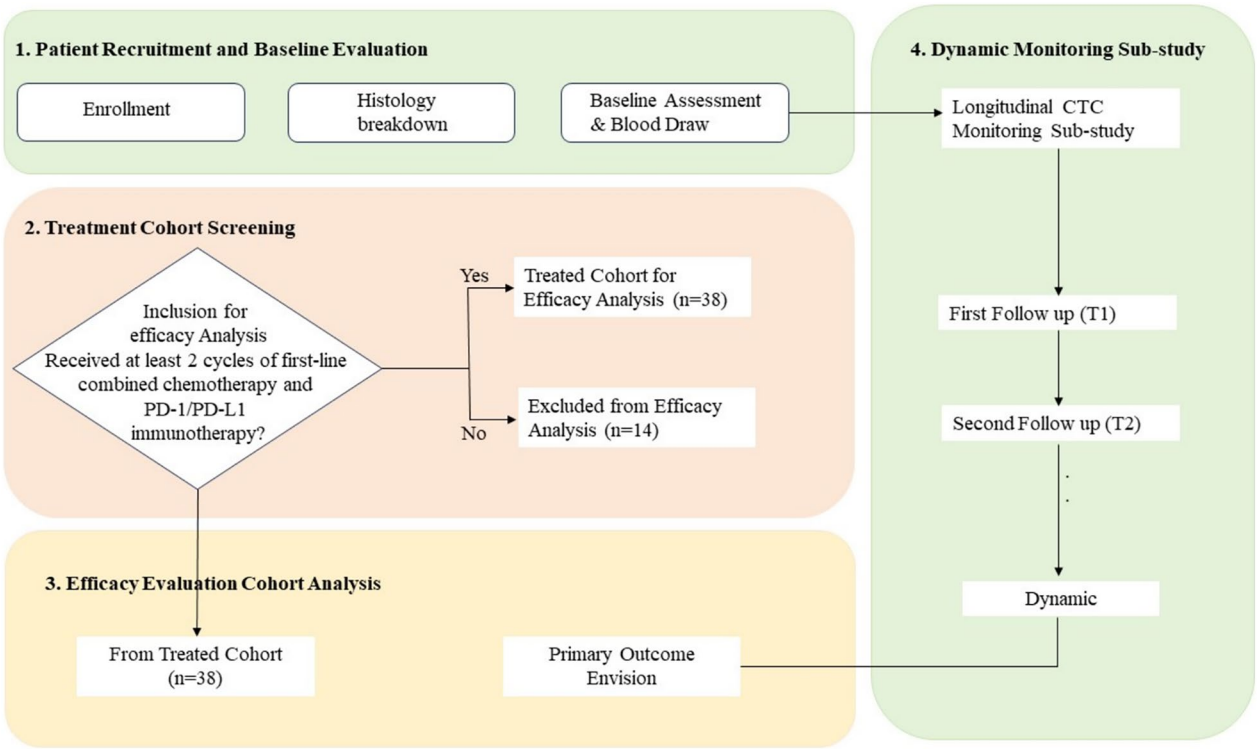

Fig. 1 Study flowchart and patient cohort. A total of 52 advanced lung cancer patients were enrolled in this study, among whom 38 received at least 2 cycles of combination chemotherapy and PD-1/PD-L1 immunotherapy. The relationship between baseline CTC PD-L1 expression levels and ORR, PFS, and OS was evaluated

CTCs were classified as CTC positive. 22C3 pharmDx (Dako) was used for evaluation of PD-L1 expression in tumor tissues (13 surgical resection specimens and 25 diagnostic biopsy specimens (12 from primary tissue and 13 from metastatic site)) according to a previous study [27]. The clinical standard threshold of TPS ≥ 1% was used to define tissue PD-L1 positivity.

Treatment response and disease progression assessment

Tumor responses were radiologically assessed after two treatment cycles using the RECIST 1.1 criteria. ORR was defined as the ratio of patients with complete response (CR) or partial response (PR) to all patients. Disease control rate (DCR) included patients with CR, PR, or stable disease (SD). PFS was measured from the start of the combination of chemotherapy and PD-1/PD-L1 immunotherapy to the first documented disease progression or death from any cause (death from any cause (including death without documented radiological progression) was counted as an event for PFS), and OS was measured from the start of PD-1/PD-L1 immunotherapy in combination with chemotherapy to death after recruitment. PR patients were defined as responders at the combination of chemotherapy and PD-1/PD-L1 immunotherapy.

Statistical analysis

The study analyzed the total number of CTCs and CTC PD-L1 to characterize patient disease features. Patients were categorized into two groups: those with at least one PD-L1-positive CTC (CTC PD-L1$^+$) or none (CTC PD-L1$^-$). A receiver operating characteristic (ROC) curve determined the optimal cutoff value to distinguish between treatment responders and non-responders. A univariate logistic regression model was then used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) at this cutoff.

Differences in quantities of CTC PD-L1$^+$ between the partial response (PR) group and the stable disease/progressive disease (SD/PD) group were assessed using a two-tailed, unpaired t-test for variables with normal distribution. Fisher's exact tests evaluated the association between CTC PD-L1 status and treatment response, as well as the link between changes in total CTC counts and treatment outcomes. These tests also assessed variations in the objective response rate (ORR) based on categorical variables like age, gender, cancer subtype, tissue PD-L1 (tPD-L1) status, type of immunotherapy, CEA, CYFRA21-1, NSE, SCC, and other clinical factors.

Survival analysis was conducted using the Kaplan–Meier method to plot curves and the Mantel–Cox test to

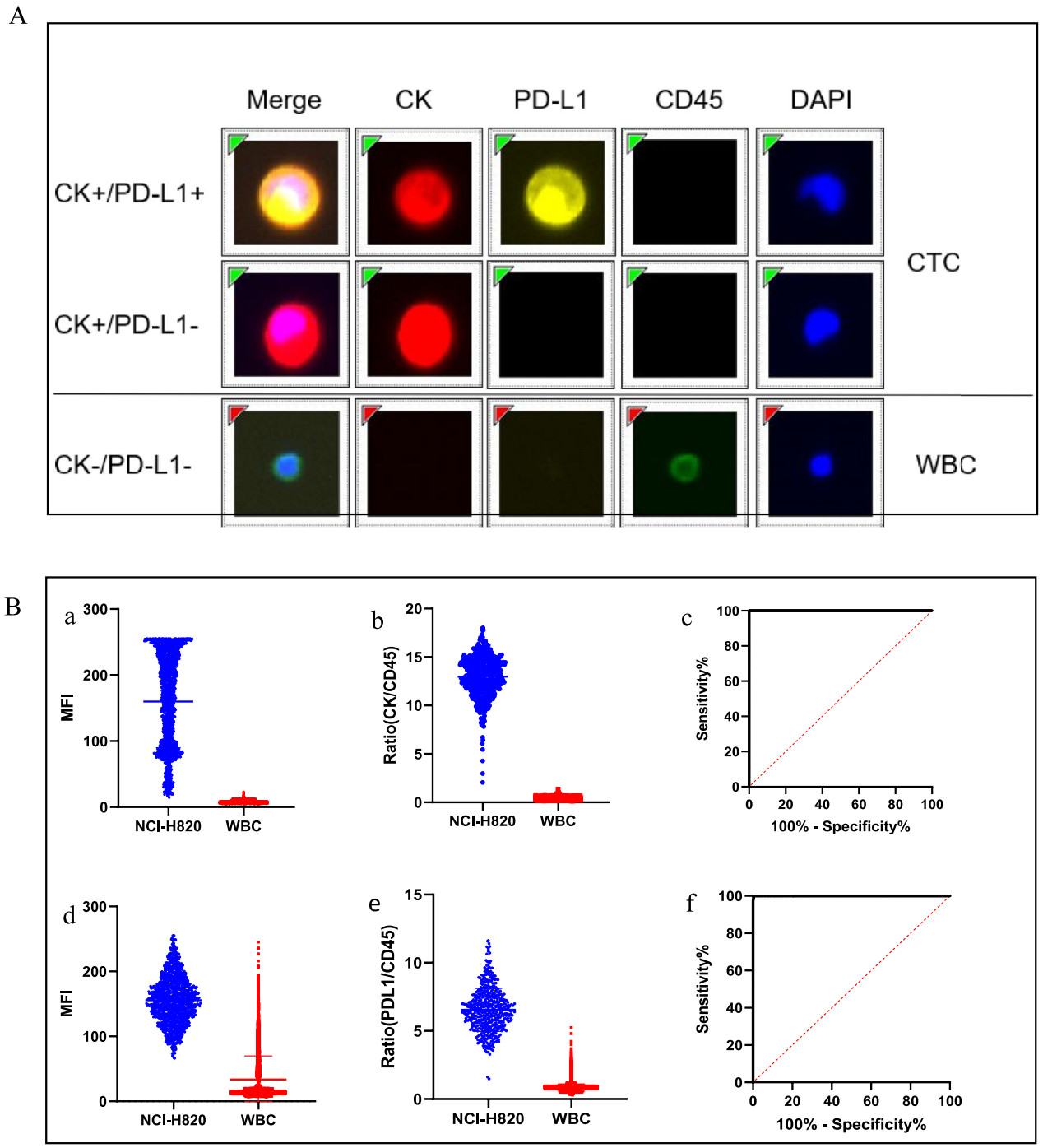

Fig. 2 Characterization of CTC in immunofluorescence staining. CTCs were identified as CK+/PD-L1 +/DAPI+/CD45–, or CK+/PD-L1–/DAPI+/CD45–; WBCs were detected by using CD45 antibody with CK–/PD-L1–/DAPI+/CD45+. To establish an interpretive method for CK and PD-L1, we compared the CK staining fluorescence intensity between the NCI-H820 tumor cell line and leukocytes WBC, as well as the PD-L1 staining fluorescence intensity between NCI-H820 and leukocytes. Due to the fact that some leukocytes also express PD-L1, there is partial overlap in the PD-L1 fluorescence signals between tumor cells and leukocytes. Therefore, we further calculated the ratio of PD-L1 to CD45 staining signal intensity and performed an analysis using the ROC curve. **A** Identification of CTCs and their PD-L1 expression. **Ba**, **Bb** CK fluorescence intensity and CK/CD45 ratio to distinguish tumor cells from leukocytes. **Bc** ROC analysis evaluating the CK/CD45 ratio for tumor cell discrimination [Sensitivity and specificity was 100% (95% CI 99.52–100.0%) and 100% (95% CI 99.27–100.0%), respectively at a threshold of 1.7 with AUC 1.0 (95% CI 1.0–1.0, $p < 0.0001$)]. **Bd**, **Be** PD-L1 fluorescence intensity and PD-L1/CD45 ratio to distinguish tumor-specific PD-L1 expression. **Bf** ROC analysis evaluating the PD-L1/CD45 ratio for specific PD-L1 expression assessment [Sensitivity and specificity was 99.90% (95% CI 99.81–99.95%) and 99.62% (95% CI 98.61–99.93%), respectively, at a threshold of 3.2 with AUC 0.9999 (95% CI 1.0–1.0, $p < 0.0001$)]

Table 1 Characteristics of recruited patients and CTC detection

Characteristic	N	CTC positive (%)	CTC PD-L1 (%)
Total	52	37 (71.2%)	26 (50.0%)
Gender			
Female	8	6 (75.0%)	4 (50.0%)
Male	44	31 (70.5%)	22 (50.0%)
Cancer type			
Adenocarcinoma	15	8 (53.3%)	6 (40.0%)
Squamous cell carcinoma	23	19 (82.6%)	13 (56.5%)
Small cell lung cancer	14	10 (71.4%)	7 (50.0%)
Therapy type			
Chemo + PD-1	30	21 (70.0%)	15 (50.0%)
Chemo + PD-L1	8	6 (75.0%)	4 (50.0%)
Chemo	14	11 (78.5%)	7 (50.0%)

calculate hazard ratios. Both univariate and multivariate Cox proportional hazards regression models assessed the correlation between CTC PD-L1 status and progression-free survival (PFS) and overall survival (OS), considering factors like age, gender, cancer subtype, treatment type, CEA, CYFRA21-1, NSE, SCC, smoking status, hypertension, diabetes, and ECOG score. Statistical analyses were performed using GraphPad Prism 8.2.0 and IBM SPSS Statistics version 25, with p-values of less than 0.05 considered statistically significant.

Results

Baseline characteristics and CTC detection

From April 2022 to February 2024, we prospectively enrolled 52 patients with advanced lung cancer (stages III–IV) in the Department of Respiratory and Critical Care Medicine at the Fourth Affiliated Hospital of Soochow University (Fig. 1). The cohort comprised 44 males (84.6%) and 8 females (15.4%), with a median age of 67.8 years (range: 45–82 years). Histological subtypes included adenocarcinoma ($n=15$, 28.8%), squamous cell carcinoma ($n=23$, 44.2%), and small cell lung cancer ($n=14$, 27.0%). All patients were driver gene-negative and received PD-1/PD-L1 inhibitor therapy in combination with chemotherapy.

At baseline assessment, CTCs, which were identified based on specific phenotypes, with CK+/DAPI+/CD45−, or CK+/PD-L1+/DAPI+/CD45− (Fig. 2A) at a ratio of CK/CD45 > 1.7 [sensitivity and specificity was 100% (95% CI 99.52–100.0%) and 100% (95% CI 99.27–100.0%), respectively, with AUC 1.0 (95% CI 1.0–1.0, $p<0.0001$)] (Fig. 2Ba–Bc), while CTC PD-L1 was classified as a ratio of PD-L1/CD45 > 3.2 [sensitivity and specificity was 99.90% (95% CI 99.81–99.95%) and 99.62% (95% CI 98.61–99.93%), respectively, with AUC 0.9999 (95% CI 1.0–1.0, $p<0.0001$)] with CK+/PD-L1+/DAPI+/CD45− (Fig. 2Bd–Bf). CTCs were successfully detected in 37 patients (71.2%) out of the 52 patients recruited, with varying detection rates across histological subtypes: adenocarcinoma (8/15, 53.3%), squamous cell carcinoma (19/23, 82.6%), and small cell lung cancer (10/14, 71.4%). CTC PD-L1$^+$ were identified in 26 patients (50.0%), with subtype-specific positivity rates of 40.0% (6/15) in adenocarcinoma, 56.5% (13/23) in squamous cell carcinoma, and 50.0% (7/14) in small cell lung cancer (Table 1).

Association between CTC PD-L1 status and clinical response

Of the 52 patients enrolled, 38 who completed at least two cycles of combination of chemotherapy and immunotherapy were assessed for treatment response per RECIST 1.1 (follow-up through 24 February 2024; Fig. 1); the other 14 were excluded because they received chemotherapy only. CTCs were detected in 27 patients (71.1%), with 19 (50.0%) individuals exhibiting CTC PD-L1$^+$. The accordance of CTC PD-L1 and tPD-L1 was 52.6% (20/38). In the CTC PD-L1$^+$ group ($n=19$), 16 patients achieved partial response (PR), 2 maintained stable disease (SD), and 1 developed progressive disease (PD), resulting in an objective response rate (ORR) of 84.2% (Fig. 3A) and disease control rate (DCR) of 94.7% (Fig. 3C). In contrast, the CTC PD-L1$^-$ group ($n=19$) demonstrated significantly lower efficacy measures, with an ORR of 36.8%

(See figure on next page.)

Fig. 3 Correlation between disease status and the counts of CTC PD-L1. PD-L1 expression on CTC was evaluated before combining chemotherapy and PD-1/PD-L1 immunotherapy (at baseline) from 38 patients. ORR and DCR were recorded after 2 cycles of therapy. Association of CTC PD-L1 to ORR, DCR had been analyzed. Difference of CTC PD-L1$^+$ counts was analyzed on PR and SD/PD by using student's t-test. Sensitivity and specificity of CTC PD-L1 were analyzed by using ROC curve. **A** Objective response rate of patients with or without CTC PD-L1 at baseline (84.2% vs 36.8%). **B** Objective response rate of patients with or without tPD-L1 at baseline (76.0% vs 41.2%). **C** Disease control rate of patients with or without CTC PD-L1 at baseline (94.7% vs 68.4%). **D** Disease control rate of patients with or without tPD-L1 at baseline (95.2% vs 64.7%). **E** Count distribution of PD-L1 positive CTCs in the PR, SD, and PD groups at baseline ($p=0.046$). **F** Percentage of tPD-L1 positive in the PR, SD, and PD groups at baseline ($p=0.9124$). **G** Analyzed the cutoff of CTC PD-L1 to ORR by ROC curve (AUC = 0.7900, 95% CI 0.6373–0.9427, $p=0.0037$; cutoff = 1, sensitivity = 69.5%, specificity = 80%). **H** Analyzed the cutoff of tPD-L1 to ORR by ROC curve (AUC = 0.6391, 95% CI 0.4450–0.8333, $p=0.1517$; cutoff = 1%, sensitivity = 69.5%, specificity = 80%)

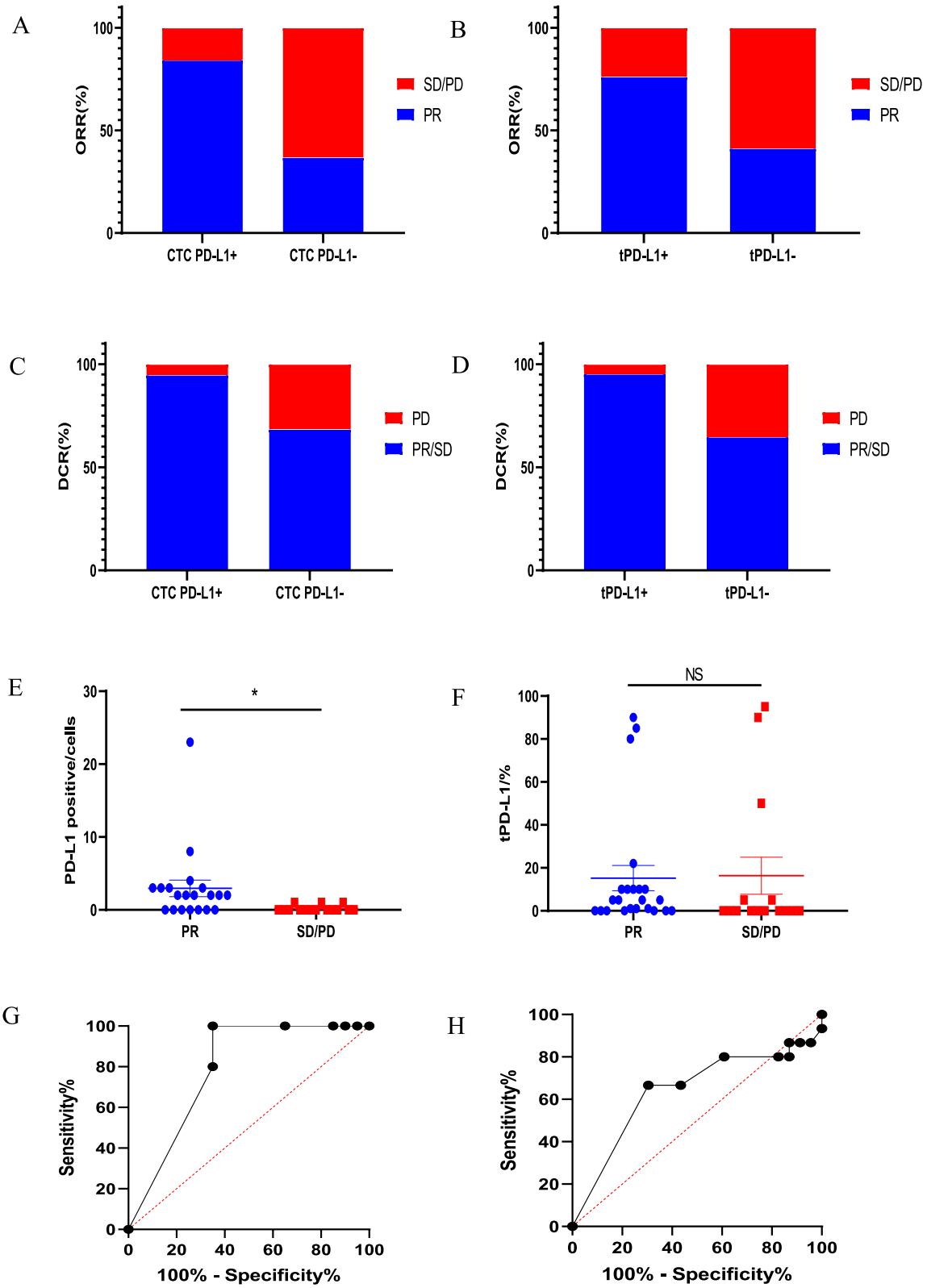

Fig. 3 (See legend on previous page.)

Table 2 Comparison of clinical and biological factors between patients with PD/SD and PR responses

	PD/SD	PR	*p*
Age	66.5 ± 13.4	69.3 ± 8.9	0.439
Gender			0.28
Male	12	22	
Female	3	1	
Cancer subtype			0.44
NSCLC	13	17	
SCLC	2	6	
PD-1 or PD-L1 monotherapy			0.66
PD-1	14	21	
PD-L1	1	2	
CTC PD-L1			0.003*
Positive	3	16	
Negative	12	7	
tPD-L1 positive cells			0.028*
≥ 1%	5	16	
< 1%	10	7	
CEA	10.9 (4.86, 65.7)	3.63 (1.93, 7.65)	0.091
CYRFRA19	6.07 (3.12, 11.4)	4.41 (3.29, 10.3)	0.153
NSE	15.7 (12.7, 23.0)	16.2 (12.5, 19.8)	0.153
SCC	1.97 (0.82, 4.34)	1.81 (1.26, 3.8)	0.062

* Significant relevance at $p < 0.05$

and DCR of 68.4%, which was similar to the results of ORR (Fig. 3C; 76.0% vs 41.2%) and DCR (Fig. 3D; 95.2% vs 64.7%) to tPD-L1. The positive and negative predictive values (PPV and NPV) of CTC PD-L1 for ORR were 84.2% and 63.2%, respectively.

Quantitative analysis revealed significantly higher quantities of CTC PD-L1$^+$ in responders (mean ± SD: 2.9 ± 1.14) compared to non-responders (mean ± SD: 0.2 ± 0.11, $p = 0.046$) (Fig. 3E), but not in tPD-L1 (Fig. 3F). ROC curve analysis established an optimal CTC PD-L1 cutoff value of 1 (AUC = 0.7900, 95% CI 0.6373–0.9427, $p = 0.0037$; cutoff = 1, sensitivity = 69.5%, specificity = 80%), yielding a sensitivity of 69.5% and specificity of 80.0% (Fig. 3G), which was similar to tPD-L1 with a cutoff value of 1% (AUC = 0.6391, 95% CI 0.4450–0.8333, $p = 0.1517$; cutoff = 1%, sensitivity = 69.5%, specificity = 80%) (Fig. 3H). Furthermore, the sensitivity and the specificity would be improved to 86.9% and 78%, respectively, when combined CTC PD-L1 and tPD-L1, which meant that CTC PD-L1 was potential act as a complementary biomarker for tPD-L1 in the prediction of benefit from the combination of chemotherapy and immunotherapy.

Additionally, a multivariate logistic regression analysis evaluated the prognostic value of various factors including age, gender, cancer subtype, type of ICI drugs (PD-1 or PD-L1 antibody), CEA, CYFRA21-1, NSE, and SCC. Only CTC PD-L1 and tPD-L1 showed significant differences between the PR and SD/PD groups ($p = 0.003$ and $p = 0.028$) (Table 2), confirming their role as independent predictors of ORR in ICI therapy and potential to act as complementary biomarkers for tPD-L1.

Survival analysis based on CTC PD-L1 status

Kaplan–Meier survival analysis demonstrated significant differences between CTC PD-L1$^+$ and CTC PD-L1$^-$ groups. The median progression-free survival (PFS) was markedly longer in CTC PD-L1$^+$ patients (16 months vs 4 months; HR = 0.28, 95% CI 0.096–0.78, $p = 0.0041$) (Fig. 4A) but not in tPD-L1 (median survival, 12 months vs undefined; HR = 0.83, 95% CI 0.28–2.5, $p = 0.74$) (Fig. 4C). Similarly, overall survival (OS) showed significant improvement in the CTC PD-L1$^+$ group, with median OS not reached compared to the CTC PD-L1$^-$ group (HR = 0.19, 95% CI 0.05–0.74, $p = 0.017$) (Fig. 4B), also not in tPD-L1 (HR = 0.88, 95% CI 0.23–3.4, $p = 0.85$) (Fig. 4D).

Multivariate analysis incorporating clinical variables (age, gender, histology, treatment modality) and laboratory parameters (CEA, CYFRA21-1, NSE, SCC) identified CTC PD-L1 as the sole independent predictor of both PFS ($p = 0.039$) and OS ($p = 0.033$) (Table 3).

Dynamic CTC monitoring during treatment

Serial blood sampling was performed in 21 patients at day 42 (T1) to evaluate CTC dynamics. The overall CTC positivity rate decreased from 71.2% at baseline to 33.3% at T1. In the PR patients (16/21), 14 patients (87.5%) exhibited CTC decline or stable, correlating with clinical response.

Extended monitoring at day 84 (T2) was obtained in 8 patients, of which 7 patients were PR and 1 patient was SD. CTC analysis revealed consistent patterns: 7 PR patients showed either decreased or stable CTC counts, while the SD single patient presented increased CTC counts. Notably, NSE levels showed better correlation with treatment response compared to other conventional markers (CEA, CYFRA21-1, SCC), suggesting its potential complementary role in monitoring treatment efficacy.

These comprehensive findings demonstrate the robust predictive and prognostic value of CTC PD-L1 assessment in advanced lung cancer patients receiving a combination of chemotherapy and immunotherapy, offering a promising liquid biopsy approach for treatment stratification and monitoring (Fig. 5).

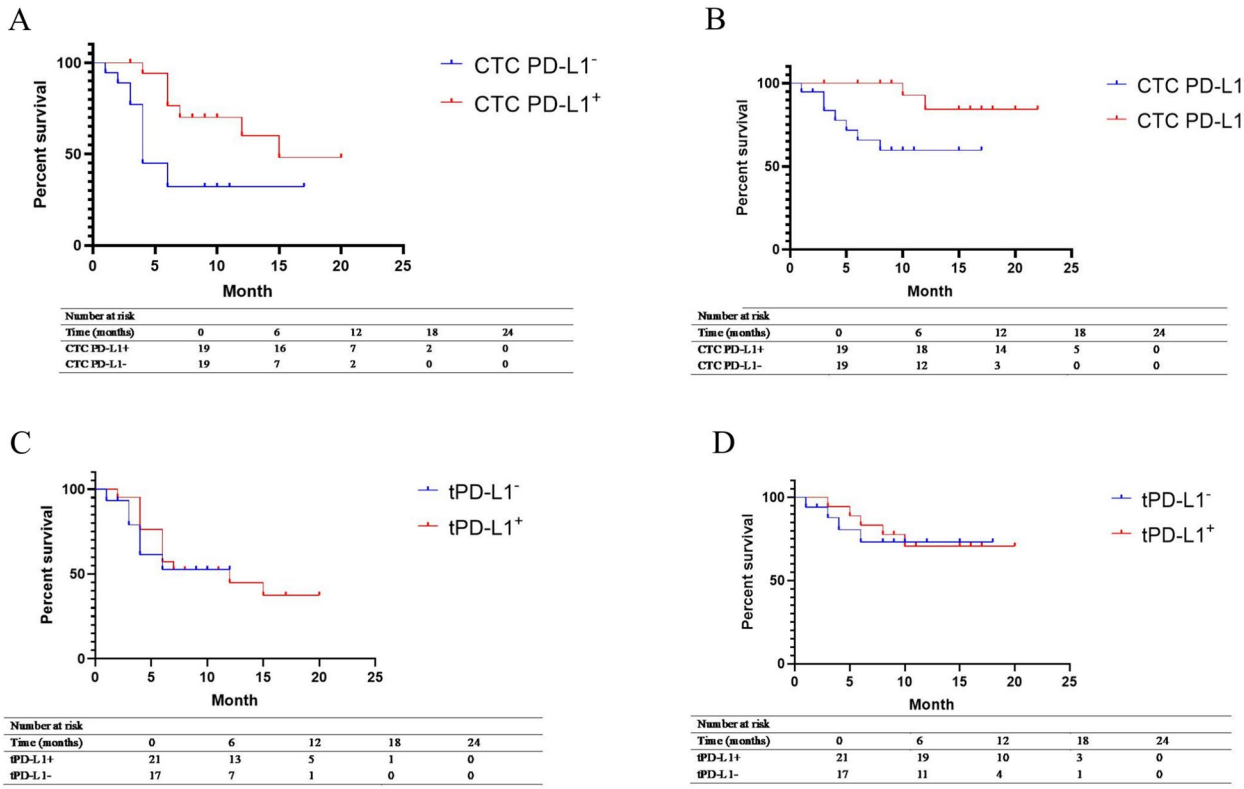

Fig. 4 Association between clinical outcome and CTC PD-L1 in the study. A total of 38 patients who received combined chemotherapy and PD-1/PD-L1 immunotherapy were followed up for more than 3 months. PFS and OS were evaluated. Kaplan–Meier survival curves were used to analyze the differences in PFS and OS between patients with baseline CTC PD-L1$^+$ and baseline CTC PD-L1$^-$ status, and baseline tPD-L1$^+$ and baseline tPD-L1$^-$ status. **A** Kaplan–Meier evaluates PFS for patients in the subgroups of CTC PD-L1$^+$ and CTC PD-L1$^-$ at baseline (median survival, 16 months vs 4 months; HR = 0.28, 95% CI 0.096–0.78, $p = 0.0041$). **B** Kaplan–Meier evaluates OS for patients in the subgroups of CTC PD-L1$^+$ and CTC PD-L1$^-$ at baseline (median survival, undefined; HR = 0.19, 95% CI 0.05–0.74, $p = 0.017$). **C** Kaplan–Meier evaluates PFS for patients in the subgroups of tPD-L1$^+$ and tPD-L1$^-$ at baseline (median survival, 12 months vs undefined; HR = 0.83, 95% CI 0.28–2.5, $p = 0.74$). **D** Kaplan–Meier evaluates OS for patients in the subgroups of tPD-L1$^+$ and tPD-L1$^-$ at baseline (median survival, undefined; HR = 0.88, 95% CI 0.23–3.4, $p = 0.85$)

Table 3 Univariate analysis of potential factors affecting progression-free survival (PFS) and overall survival (OS)

Factors	PFS	OS
Age	0.746	0.554
Gender	0.063	0.137
Cancer subtype	0.502	0.934
PD-1 or PD-L1 monotherapy	0.684	0.847
Smoking or not	0.144	0.074
Hypertension	0.059	0.106
Diabetes	0.765	0.748
ECOG score	0.072	0.203
CTC PD-L1	0.039*	0.033*
tPD-L1	0.405	0.844
CEA	0.051	0.658
CYRFRA19	0.361	0.688
NSE	0.567	0.122
SCC	0.777	0.556

* Significant relevance at $p < 0.05$

Discussion

This prospective study in 52 patients with advanced lung cancer developed a new assay to detect PD-L1 expression on CTCs and evaluated its association with outcomes of chemo-immunotherapy. The core finding was that positivity for CTC PD-L1 at baseline was significantly associated with a higher ORR and emerged as an independent prognostic factor for improved PFS and OS. These associations showed consistent trends in both NSCLC and SCLC subgroups (Supplementary materials Figure S3 and S4). Notably, despite this strong prognostic signal, the concordance between CTC PD-L1 status and tPD-L1 expression was only 52.6% (Supplementary materials Table S2), suggesting that CTCs and tissue biopsies may capture distinct, complementary biological information. Furthermore, dynamic monitoring indicated that an early decrease in CTC counts was associated with treatment response.

Compared to traditional tissue biopsies, CTC testing in patients with advanced lung cancer was previously shown to provide higher feasibility in real-time assessment of tumor changes during PD-1/PD-L1 antibody immunotherapy [28]. CTC dynamic monitoring suggested that changes in CTC counts might act as a prognostic indicator for therapy efficacy [29]. Moreover, CTC PD-L1 was associated with poor PFS and OS, indicating its potential prognostic value in prediction for lung cancer [30]. Although prior investigations have provided preliminary insights into the potential utility of CTC PD-L1 assessment during immunotherapy, comprehensive evaluations delineating the quantitative and qualitative concordance between CTC PD-L1 and tPD-L1 expression remain conspicuously absent. Moreover, the clinical relevance of CTC PD-L1 as a predictive biomarker for combining chemotherapy and PD-1/PD-L1 immunotherapy remains contentious, with conflicting data regarding its association with therapeutic benefit [31, 32]. In the current study, we investigated not only the correlation between CTC PD-L1 and tPD-L1, but also the clinical benefit of short-term treatment outcomes (ORR and DCR) and long-term survival (PFS and OS), providing valuable insights for clinical practice. Moreover, we also explored dynamic changes of CTC before and after therapy, and the role of CTC testing in combination with tumor markers in predicting the efficacy of immunotherapy (Supplementary materials Table S3).

In summary, the study employed a prospective cohort design, enrolling 52 patients with advanced lung cancer who were intended to be treated with immunotherapy and followed for up to 2 years, ensuring data integrity and reliability. Multivariate analysis adjusted for multiple clinically relevant covariates (e.g., age, gender, histology, treatment modality, CEA, CYFRA21-1, NSE, SCC), allowing for a more accurate and comprehensive assessment of the independent predictive value of CTC PD-L1. Additionally, the study evaluated PD-L1 status in CTCs not only at baseline, but also dynamically during treatment, providing a comprehensive perspective on treatment response. Furthermore, monitoring the changes in CTC counts in combination with tumor markers would be better for tracking the disease progression of patients. This integrated study design and data analysis strategy offer new insights into the role of CTC monitoring in immunotherapy for advanced lung cancer, which may help identify patients with poor treatment response early, thereby optimizing therapeutic strategies.

The study has several limitations that should be considered when interpreting the results. First, the small sample size (n=38 in treated cohort), particularly within the SCLC subgroup, limits the statistical power of our analyses and increases the risk of overfitting in the multivariate models. Second, the exclusion of 27% of initially enrolled patients who proceeded to chemotherapy only introduces a potential selection bias, although we have provided baseline characteristics for both the screened and treated cohorts to improve transparency (Table 1 and supplementary materials table S1). Third, despite performing separate analyses for NSCLC and SCLC, the pooling of these biologically distinct entities in the primary analysis may still obscure histology-specific associations. Fourth, the heterogeneity in specimen type (surgical vs. biopsy), origin (primary vs. metastatic), and the different PD-L1 antibodies in the evaluation of tissue and CTC may act as potential factors contributing to the observed discordance with CTC PD-L1 status. Finally, as a single-center study with a specific CTC detection platform, the generalizability of our findings requires validation in larger, multi-center cohorts using standardized methodologies.

In conclusion, the LiquidBiopsy™ detection system for evaluating CTC PD-L1 proved feasible, effective, and non-invasive. The baseline CTC PD-L1 was significantly

(See figure on next page.)

Fig. 5 Comparing circulating biomarkers with CTCs for monitoring tumor dynamics. CTC and tumor biomarkers in blood samples were evaluated at multiple time points for 8 patients: pre-treatment (baseline, T0), during treatment (after the second cycle and before the third cycle, T1), and after the fourth cycle and before the fifth cycle (T2). The association between the dynamic changes of CTC and tumor biomarkers and ORR was analyzed. Each case represents data from a different patient, and the red boxes indicate the corresponding tumor tissue. Case 1: a patient with lung squamous cell carcinoma who received combination therapy of gemcitabine, carboplatin, and tislelizumab had PR after 2 cycles and PR after 4 cycles therapy (**A**); Case 2: a patient with lung adenocarcinoma who received combination therapy of pemetrexed, pembrolizumab had SD after 2 cycles and PR after 4 cycles therapy (**B**); Case 3: a patient with SCLC who received combination therapy of etoposide, carboplatin, serplulimab had PR after 2 cycles and PR after 4 cycles therapy (**C**); Case 4: A patient with SCLC who received combination therapy of Etoposide, Carboplatin, Serplulimab had PR after 2 cycles and PR after 4 cycles therapy (**D**); Case 5: a patient with lung squamous cell carcinoma who received combination therapy of paclitaxel, cisplatin, tislelizumab had SD after 2 cycles and PR after 4 cycles therapy (**E**); Case 6: a patient with lung squamous cell carcinoma who received combination therapy of albumin paclitaxel, sintilimab had PR after 2 cycles and PR after 4 cycles therapy (**F**); Case 7: a patient with lung squamous cell carcinoma who received combination therapy of paclitaxel, cisplatin, sintilimab had SD after 2 cycles and SD after 4 cycles therapy (**G**); Case 8: a patient with lung adenocarcinoma who received combination therapy of carboplatin, pemetrexed, pembrolizumab had PR after 2 cycles and PR after 4 cycles therapy (**H**)

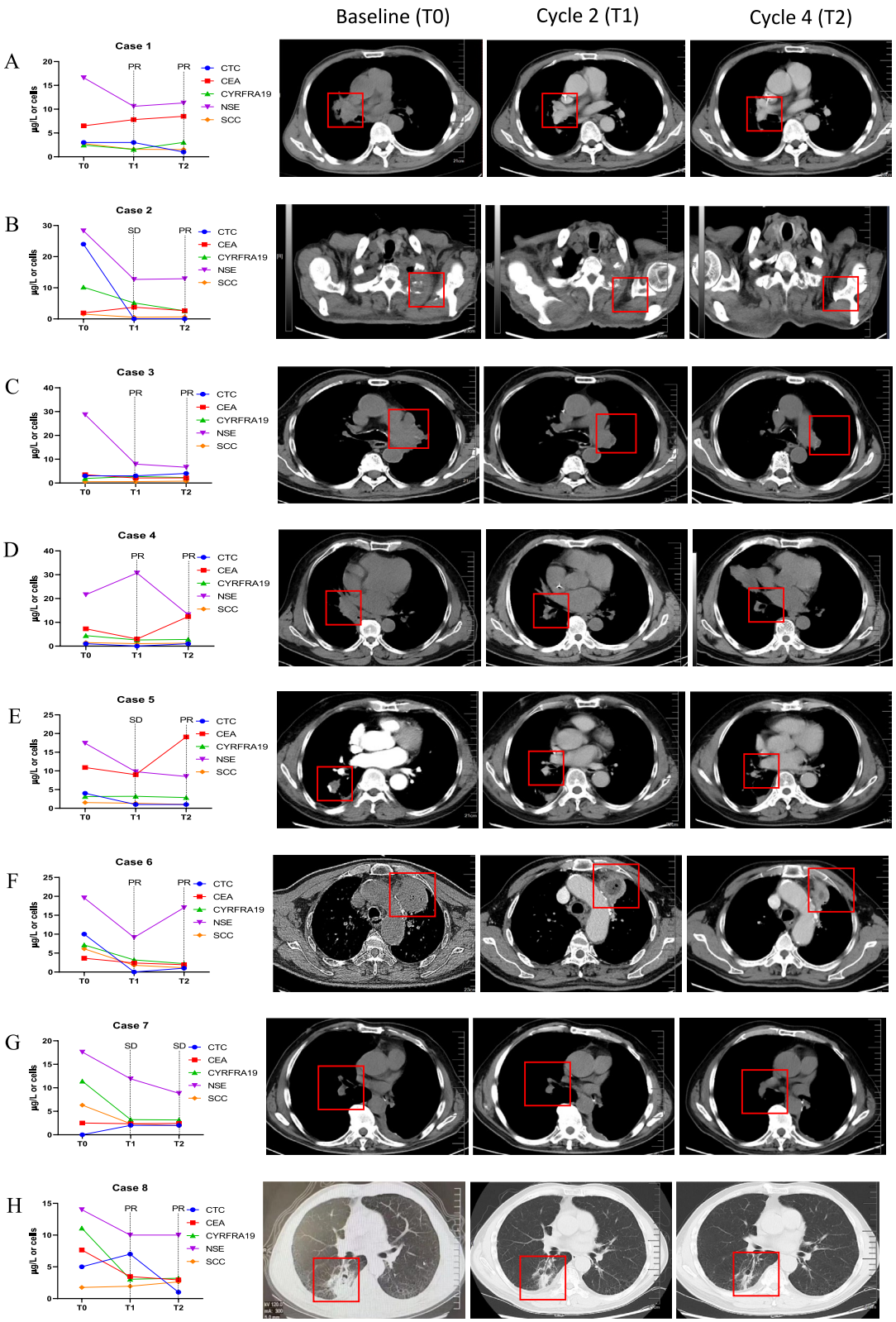

Fig. 5 (See legend on previous page.)

correlated with clinical outcomes in combination of chemotherapy and PD-1/PD-L1 blockade therapy, including objective response rate (ORR), progression-free survival (PFS), and overall survival (OS). The study suggests that CTC PD-L1 may be a potential predictive biomarker in integrating into therapeutic decision-making algorithms for combination of chemotherapy and PD-1/PD-L1 inhibitor therapy with larger, histology-specific cohorts for validation.

Abbreviations

AUC	Area under curve
CD45	Leukocyte common antigen 45
CEA	Carcinoembryonic antigen
CI	Confidence interval
CK	Cytokeratin
CTCs	Circulating tumor cells
CYRFRA19	Cytokeratin fragment antigen 19
DAPI	4′,6-Diamidino-2-phenylindole
DCR	Disease control rate
HR	Hazard ratio
HRP	Horseradish peroxidase
ORR	Objective response rate
NSE	Neuron-specific enolase
OS	Overall survival
PD	Progression disease
PD-1	Programmed death 1
PD-L1	Programmed death ligand 1
PFS	Progression-free survival
PR	Partial response
ROC	Receiver operating characteristic curve
SCC	Squamous cell carcinoma antigen
SD	Stable disease
WBC	White blood cell

Supplementary Information

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Supplementary material 1.

Author contributions

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Data availability

All data relevant to the study are included in the article.

Declarations

Ethics approval and consent to participate

This study was approved by the Fourth Affiliated Hospital of Soochow University (Approval number 220154) and under a registered clinical trial number (ChiCTR2500096312: Prediction value of PD-L1 statue on circulating tumor cells to PD-1/PD-L1 immunotherapy of NSCLC). Written informed consent was obtained from all participants.

Consent for publication

Written informed consent for publication was obtained from the patient or their legal guardian.

Competing interests

The authors declare no competing interests.

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