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Circulating tumor cells PD-L1 expression detection and correlation of therapeutic efficacy of immune checkpoint inhibition in advanced non-small-cell lung cancer

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Abstract

Introduction: This study investigated whether programmed death-ligand 1 (PD-L1) expression of circulating tumor cells (CTCs) in peripheral blood can serve as a predictive biomarker for immunotherapy efficacy in patients with advanced non-small-cell lung cancer (NSCLC).

Methods: We employed a negative enrichment method to isolate CTCs. We identified PD-L1 + CTCs as PD-L1+/4′,6-diamidino-2-phenylindole (DAPI)+/CD45-circulating tumor cells through an immunofluorescence method. Tumor tissue PD-L1 expression was determined by immunohistochemical staining. The correlation between CTC PD-L1 expression and patients' prognostic features was estimated through the Kaplan–Meier method.

Results: CTCs released a higher detection rate of PD-L1 expression than tumor tissues (53.0% vs. 42.1%). No correlation was observed between them. Forty-nine NSCLC patients received anti-PD-1/PD-L1 immunotherapy (three with combined anti-PD-1/PD-L1 and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), two with four cycles of combined immune checkpoint inhibitors [ICIs] plus chemotherapy and ICI monotherapy for maintenance). Patients with PD-L1 expression on tissue or CTCs had a median progression-free survival (mPFS) of 5.6 months ($n = 36$, 95% confidence interval [CI] 3.6–7.5 months), significantly longer than those without PD-L1 detection ($n = 9$, mPFS of 1.4 months, 95% CI 1.3–1.5 months, log-rank $p = 0.032$). The multivariable Cox proportional-hazard model suggested that the tissue or CTC PD-L1 expression was associated with a lower risk of progression (hazard ratio 0.45, 95% CI 0.21–0.98, $p = 0.043$).

Conclusions: CTCs and tumor tissues reveal heterogeneous expression of PD-L1 in NSCLC patients. Patients with baseline PD-L1 expression on CTCs or tissue showed prolonged mPFS and may help to identify the subsets of patients who potentially benefit from immunotherapy.

KEYWORDS
circulating tumor cells, immunotherapy, non-small-cell lung cancer, programmed death-ligand 1

INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths worldwide and in China. Recently, the clinical success of immune checkpoint inhibitors (ICIs) has revolutionized the treatment of patients with advanced lung cancer. Stratification of patients for ICI risks and benefits is crucial for individualized treatment. Various clinical trials have confirmed that the high expression status of programmed death-ligand 1 (PD-L1) predicted better efficacy of anti-PD-1/PD-L1

antibodies, especially in the mono ICI strategy.[1–5] However, approximately 5–10% of patients without PD-L1 expression can still benefit from immunotherapy.[6–8] Single tumor biopsy dose not adequately demonstrate the heterogeneity of PD-L1 expression in patients with metastatic tumor lesions. Furthermore, one single biopsy will miss the dynamic changes of PD-L1 under various antitumor therapies.[9,10]

To better reflect tumor cell genotype and behavior features, many researchers have focused on circulating tumor cells (CTCs) as a "liquid biopsy" method.[11] CTCs are cancer cells shed from tumor lesions into the peripheral blood and are closely related to tumor recurrence and metastasis in several malignancies, including lung cancer.[12,13] Researchers have conducted numerous studies to isolate and identify CTCs and explore whether the features of CTCs (such as number, types, or biomarkers like PD-L1) can serve as a promising predictive for ICI efficacy. However, the results remain controversial.[14–17]

In this study, we enrolled patients with advanced or metastatic non-small-cell lung cancer (NSCLC) and used a previously reported negative enrichment method to identify CTCs in peripheral blood samples.[18–20] We analyzed the baseline PD-L1 expression of CTCs as well as its correlation with the efficacy of immunotherapy.

METHODS

Patients and blood samples

The Ethics Committee of the Chinese Academy of Medical Sciences and the Peking Union Medical College Hospital approved this prospective observational study (ID ZS-1961). The inclusion criteria were age ≥18 years, histology or cytology confirmed NSCLC, stage IIIb or IV according to IASLC 8th version, no tumor history, and no previous immunotherapy. One hundred thirty-nine patients admitted to the Lung Cancer Center of PUMCH from July 2017 to October 2018 met the inclusion criteria and were included in this study after signing informed consent. The treatment strategies of each participant were determined by experienced lung cancer specialists based on clinical practice guidelines. Efficacy evaluation based on the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) was performed every two cycles and when disease progression was clinically suspected during the treatment.

On written consent, 3.2 ml of peripheral blood was drawn within 1 week before the first course of antitumor treatment and stored in a BD Vacutainer tube (Becton, Dickinson and Company). We collected baseline blood samples from all 149 participants for CTC isolation and CTC PD-L1 detection. We also performed PD-L1 immunohistochemical staining on matched tumor tissues of these patients to explore the correlation of PD-L1 expression on tissues and CTCs. All blood samples were processed within 24 h.

Forty-nine patients received immunotherapy after evaluation. The clinical and prognostic features of these patients were collected for the analysis of CTC PD-L1 expression and ICI efficacy.

Isolation, enumeration, and identification of CTCs

Enrichment of CTCs

We followed the Cyttel method (Cyttel Biosciences Inc.) to isolate CTCs. We added CS1 (Cyttel Biosciences Inc.) buffer to 3.2 ml of peripheral blood and discarded the supernatant to deplete plasma. CS2 (Cyttel Biosciences Inc.) was then added to lyse red blood cells. CD45, also known as the leucocyte common antigen, expressed on almost all haematopoietic cells except for mature erythrocytes and was recruited as the molecular marker to distinguish blood-derived cells and CTCs in our research. Anti-CD45 antibody-conjugated immuno-magnetic beads (Cyttel Biosciences Inc.) were used, and samples were passed through a magnetic frame to remove blood-derived cells. We chose the absence of CD45 expression as the criterion for detecting malignant cells. The resulting solution containing non-blood-derived cells was smeared on one slide, fixed and dried for subsequent analysis. The human lung adenocarcinoma cell line NCI-H1975 was used as a positive control, and normal human peripheral blood was used as a negative control.

Identification of PD-L1+/DAPI +/CD45-circulating tumor cells

PD-L1 expression detection was conducted through the immunofluorescence (IF) method. After enrichment, CTC slides were washed, permeabilized, blocked and prepared to stain. Diluted PD-L1 antibody and CD45 fluorescent antibody were added. After incubation for 2 h at room temperature, the slides were washed and fluorochrome-conjugated secondary antibody was added. Finally, we added 4′,6-diamidino-2-phenylindole (DAPI) and placed the slides under a fluorescence microscope or read the slides with a fluorescence scanner. Unstained circulating tumor cells were regarded as negative expression, and "+" and "+++" were regarded as positive expression.

Figure 1 shows one example of a positive cell. PD-L1 is labeled with green fluorescence, CD45 with red, and DAPI with blue. Cells with PD-L1 signal located in a circle or part of a circle outside the nucleus, indicating PD-L1 expressed in the cell membrane or cytoplasm, were identified as PD-L1+. The cells with PD-L1 +, CD45 negative and DAPI positive were defined as positive cells. These cells were PD-L1+ non-blood-derived circulating tumor cells associated with malignancy refer to previous research.[15] We defined these positive cells as PD-L1+ CTCs.

PD-L1 expression analysis in tumor tissue

PD-L1 immunohistochemical staining in tumor tissue was processed in tissue sections (3-μm thick) using the 22C3

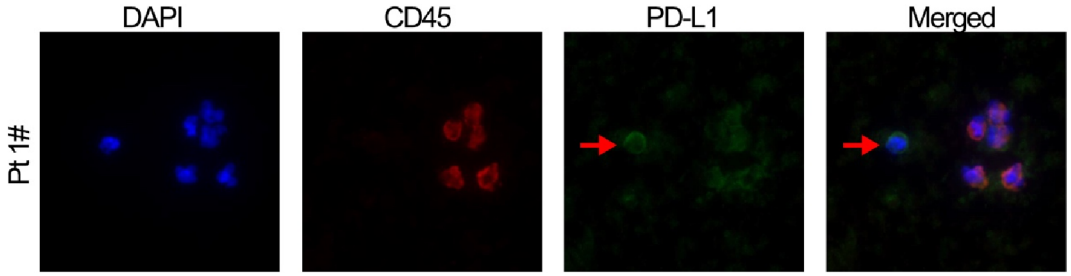

FIGURE 1 Analysis of PD-L1 in circulating tumor cells.

clone (22C3 PharmDx Kit, Dako) and analyzed by the practicing anatomic pathologist blinded to CTCs analysis. The PD-L1 protein tumor proportion score (TPS) was assessed based on the proportion of partial or complete staining (≥1%) cells relative to all viable tumor cells.

Statistical analysis

The data were analyzed by median and range for continuous variables and by frequency and percentage for qualitative variables. Survival was estimated by the Kaplan–Meier method and the differences were evaluated with a stratified log-rank test. Multivariable analysis with the Cox proportional-hazard model was used to explore possible prognostic factors on survival. We included age, gender, stage status, and Eastern Cooperative Oncology Group - Performance Status (ECOG-PS) as covariates in the Cox proportional-hazard model. Chi-squared test and Fisher exact test were used for the comparisons for qualitative variables and independent sample *t*-test for continuous variables. The two-sided probability of type I error was 0.05 in the analysis and p values<0.05 were considered significant. Statistical analysis was conducted by SPSS version 22.

RESULTS

Patient cohorts

This study consecutively enrolled 139 patients with unresectable advanced NSCLC from July 2017 to October 2018 (Figure 2). One hundred and seventeen patients had baseline CTC PD-L1 detection. The other 22 samples failed in enrichment or IF staining due to specimen hemolysis, coagulation, and severe structural damage affecting staining interpretation. Ninety-five of 139 patients had available and enough tumor tissue for PD-L1 testing. The resource of tumor tissues included small biopsies obtained from bronchoscopy or computed tomography-guided biopsies and surgical resection specimens. The correlation of PD-L1 expression on CTCs and tumor tissues was evaluated in cohort 1.

Out of 139 enrolled patients, a total of 49 patients underwent immunotherapy and were referred to as the ICI cohort (cohort 2, $N = 49$). All patients had baseline CTC PD-L1 detection and 43 of them had matched tumor tissues PD-L1 staining.

The flowchart of the patient cohort is shown in Figure 2.

PD-L1 expression in blood samples and matched tumor tissues

CTCs and tumor tissues revealed heterogeneous expression of PD-L1 in our research. We ran tissue PD-L1 staining in 95 patients and 40 patients (42.1%) were PD-L1 + on tissues (PD-L1 tumor proportion score (TPS) ≥1%). Among 117 patients with a baseline peripheral CTC PD-L1 test, 62 (53.0%) were PD-L1 + on circulating tumor cells (DAPI +/PD-L1+/CD45- cells ≥1 cell/3.2 ml).

Seventy-eight patients had pre-treatment PD-L1 detection on both CTCs and tissues. The results are presented in Table 1. No correlation was observed ($\kappa = -0.154$, $p = 0.17$). The concordance rate was 42.3% ($n = 33/78$). Of the 45 discordant cases, 26 were negative on tissue but positive on CTCs, and 19 detected positive on tissue but not on CTCs.

Correlation of PD-L1 expression and immunotherapy efficacy

Out of 139 patients in our cohort, a total of 49 patients had ICI treatment. The majority ($n = 44$, 89.8%) of patients underwent anti-PD-1/PD-L1 monotherapy, two patients had four cycles of combined anti-PD-1/PD-L1 plus chemotherapy and ICI monotherapy for maintenance, and three patients had combined anti-PD-1/PD-L1 and CTLA-4 therapy. In addition, 12.2% patients received first-line immunotherapy, while 63.3% received second-line. In terms of efficacy evaluation, 12 patients showed partial response (PR), 17 had stable disease (SD), 14 showed progressive disease (PD), two had severe adverse events and quit ICIs, and four patients were lost to follow-up. PD-L1 expression was identified in 19 of 43 (44.2%) tumor tissue samples, while DAPI+/PD-L1+/CD45 CTCs were detected in 25/49 (51.0%) cases (range 1–55 cells/3.2 ml). The demographic characteristics of the 49 patients are shown in Table 2.

Tissue PD-L1 expression was associated with a better response to ICI therapy

The median progression-free survival (mPFS) of all 49 patients in ICIs cohort was 4.8 months (range 0.3–32.0 months). Survival was analyzed by the Kaplan–Meier method, and the differences were evaluated with a stratified log-rank test.

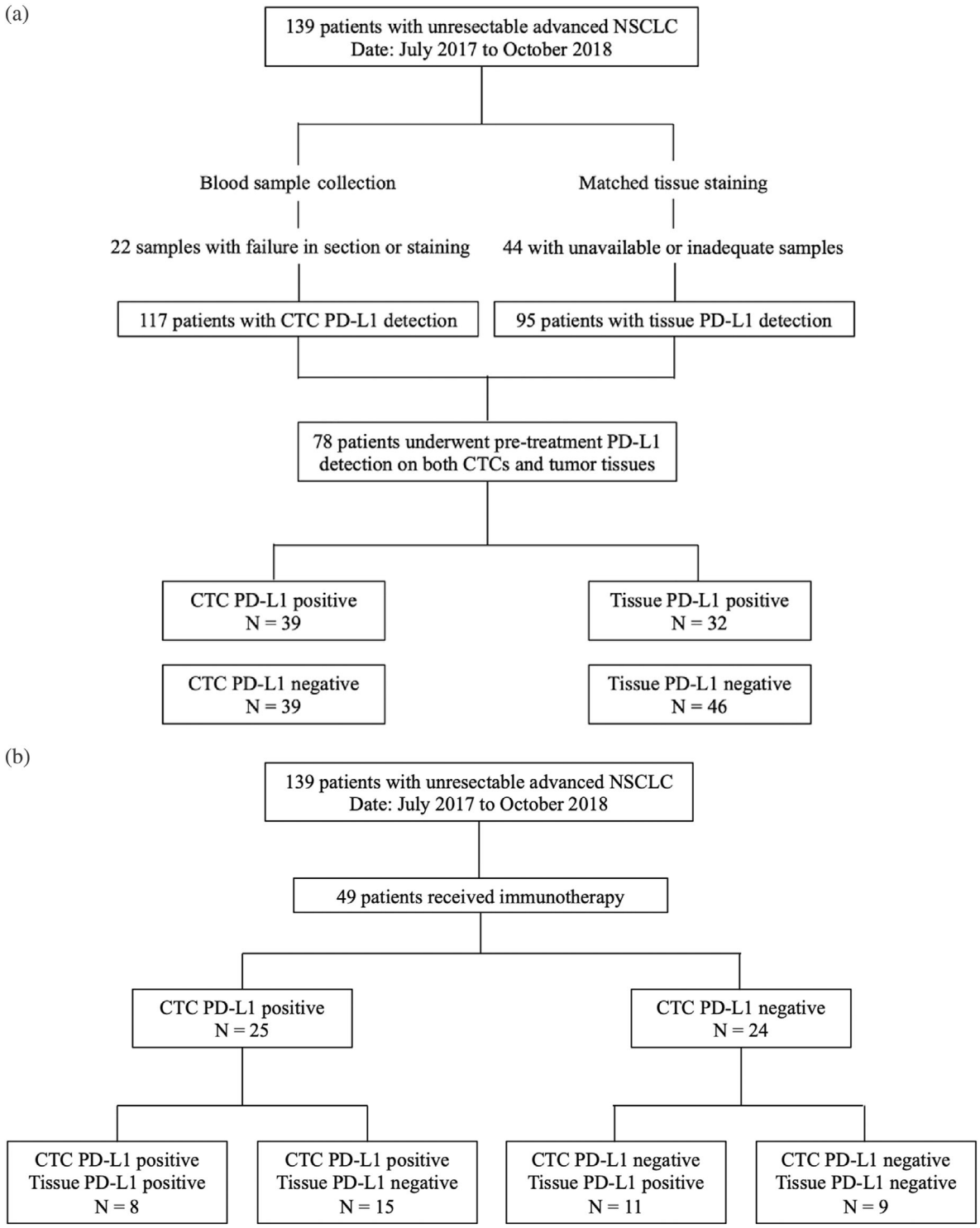

FIGURE 2 Flowchart of the patient cohort. (a) Cohort 1 included a total of 78 patients with matched pretreatment CTCs and tumor tissues. The correlation of PD-L1 expression was analyzed in cohort 1. (b) Cohort 2 included 49 patients receiving ICI treatment. All patients underwent CTC PD-L1 detection and 43 patients had tumor tissue PD-L1 detection. CTC, circulating tumor cells; NSCLC, non-small-cell lung cancer; PD-L1, programmed death-ligand 1

We included age, gender, stage status, and ECOG-PS as covariates in the Cox proportional-hazard model to explore possible prognostic factors on survival. As shown in Figure 3a, patients with positive PD-L1 on tumor tissue demonstrated a trend of longer mPFS of 4.8 months (n = 18, 95% confidence interval [CI] 0.0–10.5 months) than the 3.6 months (n = 23, 95% CI 0.0–9.8 months) for those with negative tissue PD-L1, but the difference was not

TABLE 1 Correlation between tissue and CTC PD-L1 expression

	PD-L1detection on CTCs	
PD-L1 detection on tissue	**Negative ($N_a = 39$)**	**Positive ($N_b = 39$)**
Negative ($N_1 = 46$)	20 (43.5%)	26 (56.5%)
Positive ($N_2 = 32$)	19 (59.4%)	13 (40.6%)

Abbreviations: CTC, circulating tumor cells; N_1, patients with tissue PD-L1-; N_2, patients with tissue PD-L1+; N_a, patients with CTCs PD-L1-; N_b, patients with CTCs PD-L1+; PD-L1, programmed death-ligand 1.

statistically significant (log-rank $p = 0.151$). The overall response rate (ORR) to ICIs was 47.1% (8/17) in the tissue PD-L1 + subset versus 9.1% (2/22) in the tissue PD-L1 − subset ($p = 0.011$). The disease control rates (DCRs) were 70.6% (12/17) and 59.1% (13/22) ($p = 0.458$), respectively. Patients who harbored PD-L1 expression on tissue had prolonged mPFS and significantly enhanced ORR.

Table 3 shows that among 24 patients with tissue PD-L1 −, 10 cases could still obtain stable disease control (PR or SD) under ICIs. A quarter (6/24) of cases even released PFS for more than 12 months. Only relying on tissue PD-L1 identification to screen the suitable population for immunotherapy was insufficient.

PD-L1 on CTCs can serve as a notable supplementary predictor to the efficacy of immunotherapy

We wanted to determine whether the PD-L1+ CTCs can be a predictors for the survival of patients with PD-1/PD-L1 inhibition drugs. Figure 3b indicates that cases with PD-L1 + CTCs had prolonged mPFS ($n = 24$, mPFS 5.6 months, 95% CI 2.9–8.2 months) compared with those without PD-L1 expression ($n = 21$, mPFS 3.3 months, 95% CI 0.3–6.3 months), although this was not statistically significant (log-rank p value = 0.519). The ORR was 36.4% (8/22) in cases with PD-L1 + CTCs and 19.0% (4/21) in the CTC PD-L1 − group ($p = 0.206$). The DCR was significantly enhanced among CTC PD-L1 + patients (81.8% [18/22] vs. 47.6% [10/21], $p = 0.019$).

Table 3 shows that patients with PD-L1 + on both tumor tissues and CTC had the highest PR rate and the best mPFS of 8.4 months (95% CI 0–24.7 months). However, patients with an absence of PD-L1 expression on tissue and CTC showed resistance to ICIs and a markedly reduced mPFS of 1.4 months (95% CI 1.3–1.5 months). As patients with PD-L1- on tissue and CTC suggested worst prognosis, we separated patients based on PD-L1 expression statuses. Patients with PD-L1 + either on tissue or CTCs had significantly longer mPFS of 5.6 months ($n = 36$, 95% CI 3.6 to 7.5 months) than those without PD-L1 detection on tissue and CTCs (mPFS 1.4 months, $n = 9$, 95% CI 1.3 to 1.5 months) (log-rank p-value = 0.032). The Kaplan–Meier survival curve is shown in Figure 3c. The ORR and DCR were 32.4% and 73.5% for patients positive for PD-L1 on tissue or CTCs, while for PD-L1 − cases ORR and DCR were 11.1% ($p = 0.398$) and 44.4% ($p = 0.209$). Patients who harbored PD-L1 expression on tissue or CTC had significantly improved PFS compared to the PD-L1 − patients in our cohort.

TABLE 2 Clinical characteristics of the 49 patients in the ICI cohort

Characteristic	**$N = 49$**
Age (years)	62 (37–77)
Gender, number (%)	
Male	36 (73.5%)
Female	13 (26.5%)
Smoking status, number (%)	
Smoker	33 (67.3%)
Nonsmoker	16 (32.7%)
Pathology, number (%)	
Nonsquamous cell carcinoma	31 (63.3%)
Squamous cell carcinoma	18 (36.7%)
Stage status, number (%)	
Stage III	9 (18.4%)
Stage IVa	21 (42.9%)
Stage IVb	19 (38.8%)
EGFR mutation, number (%)	
Positive	5 (10.2%)
Negative	31 (63.3%)
N/a	13 (26.5%)
ALK mutation, number (%)	
Positive	0
Negative	40 (81.6%)
N/a	9 (18.4%)
ECOG-PS	
2	2 (4.1%)
0–1	47 (95.9%)
Immunotherapy	
First line	6 (2.0%)
Second line	31 (63.3%)
Third line and above	12 (24.5%)
ICI modalities	
BGB-A317	1 (2.0%)
IBI308	2 (4.1%)
Nivolumab	37 (75.5%)
Pembrolizumab	6 (12.2%)
Nivolumab + ipilimumab	3 (6.1%)
Tissue PD-L1 expression	
Positive	19 (38.8%)
Negative	24 (49.0%)
N/a	6 (12.2%)
CTC PD-L1 expression	
Positive	25 (51.0%)
Negative	24 (49.0%)

Abbreviations: ALK, anaplastic lymphoma kinase; BGB-A317, tislelizumab; CTC, circulating tumor cells; ECOG-PS, Eastern Cooperative Oncology Group - Performance Status; EGFR, epidermal growth factor receptor; IBI308, sintilimab; ICI, immune checkpoint inhibitors; N/a, not available; PD-L1, programmed death-ligand 1.

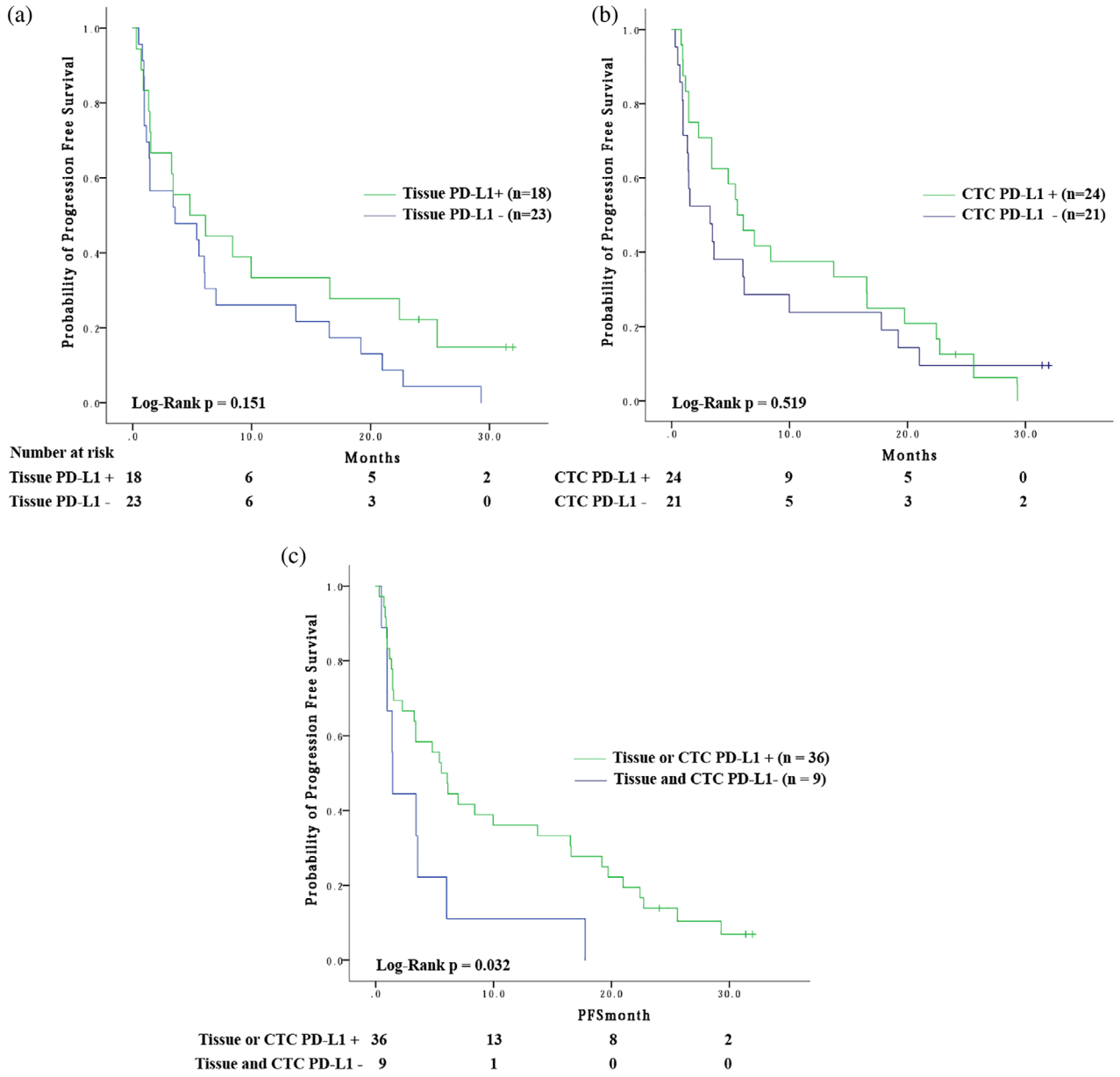

FIGURE 3 Kaplan–Meier analysis of progression-free survival (PFS) of non-small-cell lung cancer patients with immunotherapy. (a) PFS curve according to tissue PD-L1 expression, the median PFS (mPFS) was 4.8 months ($n = 18$, 95% CI 0.0–10.5 months) in tissue PD-L1+ patients versus 3.6 months ($n = 23$, 95% CI 0.0–9.8 months) in tissue PD-L1− patients. (b) PFS curve according to PD-L1 expression on CTCs, the mPFS was 5.6 months ($n = 24$, 95% CI 2.9–8.2 months) in patients with PD-L1+ CTCs versus 3.3 months ($n = 21$, 95% CI 0.3–6.3 months) in those without PD-L1 expression on CTCs. (c) PFS curve according to comprehensive PD-L1 detection, the mPFS was 5.6 months ($n = 36$, 95% CI 3.6–7.5 months) for patients with PD-L1 detected on tissue and/or CTCs versus 1.4 months ($n = 9$, 95% CI 1.3–1.5 months) for patients without PD-L1 expression either on tissue or CTCs. CTC, circulating tumor cells; PD-L1, programmed death-ligand 1

In the univariate and multivariate Cox regression analyses, only PD-L1 expression status was significantly associated with PFS. The multivariate Cox proportional hazards model revealed that patients with tissue or CTC PD-L1 expression had a lower risk of progression than those without PD-L1 expression (hazard ratio [HR] 0.45, 95% CI 0.21–0.98, $p = 0.043$) (Table 4). The PD-L1 detection on CTCs may serve as a promising predictor for better PFS and DCR of PD-1/PD-L1 inhibition.

DISCUSSION

CTCs can be detected in the peripheral blood and are considered to be the promotors of metastasis. However, the lack of a unified definition and amenable enrichment methods limited the clinical applications of CTCs in personalized cancer care. Existing methods for CTC identification utilize a wide variety of strategies to distinguish malignant cell, including physical properties (e.g., size, density, pH,

TABLE 3 PD-L1 expression status and the efficacy of immunotherapy in ICIs cohort

	PD-L1 expression status				
	Tissue PD-L1 + CTC PD-L1+ N = 8	Tissue PD-L1 + CTC PD-L1-N = 11	Tissue PD-L1-CTC PD-L1 + N = 15	Tissue PD-L1-CTC PD-L1-N = 9	Total $N = 43$[a]
Response to ICIs					
PR-No. (%)	5 (11.6%)	3 (7.0%)	2 (4.6%)	0 (0%)	10 (23.3%)
SD-No. (%)	2 (4.6%)	2 (4.6%)	8 (18.6%)	3 (7.0%)	15 (34.9%)
PD-No. (%)	0 (0%)	5 (11.6%)	3 (7.0%)	6 (14.0%)	14 (32.5%)
N/a-No. (%)	1 (2.3%)	1 (2.3%)	2 (4.6%)	0 (0%)	4 (9.3%)
mPFS (95% CI)-(month)	8.4 (0–24.7)	3.3 (0.4–6.2)	5.4 (1.4–9.4)	1.4 (1.3–1.5)	4.8 (2.4–7.2)

Abbreviations: CTC, circulating tumor cells; ICI, immune checkpoint inhibitors; mPFS, median progression -free survival; N/a, not available; PD, progressive disease; PD-L1, programmed death-ligand 1; PR, partial response; SD, stable disease.
[a]Two patients in the ICI cohort ($N = 49$) quit ICIs due to severe adverse events and four patients were lost to follow-up before efficacy evaluation.

TABLE 4 Multivariate Cox proportional hazards regression analysis for prediction of PFS

	At risk group		PFS risk		
Parameter	**Positive**	**Negative**	***p***	**HR**	**95% CI**
Age	≥65 years	<65 years	0.167	1.44	0.70–2.98
Gender	Female	Male	0.564	1.31	0.64–2.71
Stage	IV	IIIb	0.999	1.09	0.49–2.39
ECOG-PS	2	0–1	0.546	0.78	0.18–3.48
PD-L1 + on CTC or tissue	1	0	**0.043**	**0.45**	**0.21–0.98**

Abbreviations: CI, confidence interval; CTC, circulating tumor cells; ECOG-PS, Eastern Cooperative Oncology Group - Performance Status; HR, hazard ratio; PD-L1, programmed death-ligand 1; PFS, progression-free survival.
Note: p value < 0.05 (in bold).

deformability, and electrical charge) and specific biological characteristics such as peculiar surface protein, specific gene expression, aneuploids of chromosomes, and cellular function. CellSearch, employing the epithelial cell adhesion molecule (EpCAM) as the cell surface marker of CTCs, was the first FDA-approved CTC detection technology. However, this system failed to detect CTCs experiencing epithelial-to-mesenchymal transmission (EMT) process as the EpCAM becomes highly downregulated amid EMT.[21,22] The relatively low sensitivity and purity limited the application of te CellSearch system.[23,24] Different isolation platforms reported significantly diverse detection rates of CTCs in previous studies.[25,26]

The generally certified definition of CTC is nucleated cells with expression of cytokeratin (CK) and absence of leukocyte common antigen CD45, but not all relevant CTCs meet these criteria.[15,27] Boffa et al.[15] identified CK negative cells that share morphometric features consistent with CTCs and are not observed in healthy donors, and defined them as circulating cells associated with malignancy (CCAMs). In this research, we chose the negative enrichment method (lack of CD45 expression) to evade the selection bias of a specific CTC subpopulation based on selection markers.

We found that the concordance in PD-L1 expression between tissue and CTCs is low at 42.3%, which is similar to previous research. Guibert et al.[17] reported 83% (74/89) of patients were PD-L1 + on CTCs while 41% (28/69) were PD-L1 + on tumor tissues. Janning et al.[28] also described the percentage of PD-L1+ CTCs did not correlate with tissue ICH ($p = 0.179$). Some other studies showed controversial results with a 57–93% concordance in NSCLC patients.[16,29,30] The controversial conclusions may partially result from different CTC isolation techniques and anti-PD-L1 antibodies for tissue ICH. Our study confirmed that patients that were PD-L1 − on tissue but PD-L1 + on CTCs could still benefit from mono ICI therapy. Analysis of PD-L1 expression on CTCs may serve as an effective supplement to screen potential candidates for ICIs.

The predictive value of tissue PD-L1 expression for immunotherapeutic efficacy was previously confirmed in several studies. Patients with tissue PD-L1 + at baseline achieved a prolonged mPFS (4.8 vs. 3.6 months) in our research. However, the log-rank p value was not statistically significant, probably due to limited sample size.

The association of CTC PD-L1 expression and efficacy of immunotherapy remains controversial. A former pilot study associated high PD-L1 expression >50% with an improved overall response in a rather limited sample size (three of four patients),[31] whereas in other previous work, generally, PD-L1+ CTCs were associated with poor

outcome. Guibert et al.[17] isolated CTCs through size-based technology from 96 NSCLC patients before nivolumab treatment and at the time of progression. They found that patients with PD-L1+ CTCs at baseline were more frequently nonresponders (PFS <6 months) ($p = 0.04$). Cheng et al.[30] found that in 41 initially treated patients, those with high CTC PD-L1 expression experienced shorter PFS (5.12 vs. 6.07 months), but there was no significant difference ($p = 0.210$). The detailed treatment was not mentioned in Cheng's study. Kulasinghe et al.[32] found no association with PFS and CTC PD-L1 expression among 17 patients with advanced NSCLC (HR 1.646, 95% CI 0.5128–5.283, $p = 0.40$). In this research, CTCs were collected based on different platforms and may contain completely different proportions of CTC subpopulations.

In our research, we collected the CTCs based on the negative enrichment method to remove the blood-derived cells. The PD-L1 expression on CTCs was suggested to be a supplementary predictor for efficacy of immunotherapy. Patients with PD-L1 detected on CTCs or tissue achieved significantly prolonged mPFS compared to PD-L1 − patients.

CTCs detection as liquid biopsy allows real-time sampling and PD-L1 evaluation of patients through the course of the disease. Nicolazzo et al.[14] and Guibert et al.[17] reported that PD-L1+ CTCs were present in all patients at disease progression. The hypothesis lay in that the increasing PD-L1+ CTCs might mediate the immune escape.[33] Recent studies have focused on how a specific CTC subpopulation developed during treatments. Zhang et al.[34] identified that the number of multiploid (≥tetrasomy 8) PD-L1+ circulating tumor endothelial cells (CTECs) increased during progressive disease. Patients possessing multiploid PD-L1+ CTECs had a significantly shorter PFS (5 months vs. 8 months, $p = 0.012$). We need to conduct further research focusing on the dynamic changes of PD-L1+ CTC subtypes during ICI therapy.

The conventional needle biopsy employed for PD-L1 detection on tumor tissue may bring appreciable false negativity. Our study showed that the co-detection of PD-L1+ CTCs and PD-L1+ tissues may help to select patients suitable for immunotherapy. One limitation of our research was that CTC PD-L1 expression was analyzed qualitatively and failed to describe the proportion of PD-L1+ cells. Although without detailed descriptions of subtypes of CTCs, our research proposed a feasible method to detect PD-L1+ CTCs, and help to sceen patients who may benefit from immunotherapy. Future research should focus on establishing a unified and efficient standard operating procedure for CTC enrichment and collection, the additional biomarkers for CTC subpopulation identification, and the optimal portfolio of biomarkers (including PD-1/PD-L1expression) for monitoring prognostic features for immune checkpoint blockade therapy. Extensive trials of how CTCs, CCAMs, and their subgroups behave under immunotherapy will help to better understanding the interaction of tumor cells and hosts during therapy resistance.

CONCLUSIONS

PD-L1 expression was detectable on CTCs in patients with advanced NSCLC. No correlation was observed between PD-L1 expression on tumor tissues and CTCs. NSCLC patients with PD-L1 − on tissues but PD-L1 + on CTCs could still benefit from ICI therapy. The "liquid biopsy" method of CTC detection and co-identification of PD-L1+ CTCs or PD-L1+ tissues may help to identify patients who would benefit from immunotherapy.

AUTHOR CONTRIBUTIONS

M.Z.W. and Y.X. conceived the presented idea. Q.Z., B.T., J.L., and X.N.L. carried out the experiments. Q.Z. wrote the manuscript. X.Y.L., X.X.G., and Y.Q.S. performed the data collection. J.L. interpreted the immunohistochemical results. M.J.C., Y.X., J.Z., and W.Z. provided critical feedback and helped to shape the analysis and manuscript. All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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