

RESEARCH ARTICLE

Prognostic significance of programmed cell death-ligand 1 expression on circulating tumor cells in various cancers: A systematic review and meta-analysis

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Abstract

Background: The prognostic significance of programmed cell death-ligand 1 (PD-L1) expression on circulating tumor cells (CTCs) has been explored but is still in controversy. We performed, for the first time, a meta-analysis to systematically evaluate its prognostic value in human cancers.

Methods: Literature databases were searched for eligible studies prior to June 30, 2021. The pooled hazard ratios (HRs) and 95% confidence intervals (95% CIs) were calculated for the associations of pre-treatment and post-treatment PD-L1⁺ CTCs with progression-free survival (PFS) and overall survival (OS). Subgroup analyses with regards to cancer type, treatment, CTC enrichment method, PD-L1 detection method, cut-off, and specifically the comparison model were performed.

Results: We included 30 eligible studies (32 cohorts, 1419 cancer patients) in our analysis. Pre-treatment PD-L1⁺ CTCs detected by immunofluorescence (IF) tended to predict better PFS (HR = 0.55, 95% CI 0.28–1.08, $p = 0.084$) and OS (HR = 0.61, 95% CI 0.36–1.04, $p = 0.067$) for immune checkpoint inhibitor (ICI) treatment, but were significantly associated with unfavorable survival for non-ICI therapies (PFS: HR = 1.85, 95% CI 1.21–2.85, $p = 0.005$; OS: HR = 2.44, 95% CI 1.69–3.51, $p < 0.001$). Post-treatment PD-L1⁺ CTCs predicted markedly worse PFS and OS. The prognostic value was obviously modulated by comparison models. Among patients with detectable CTCs, PD-L1⁺ individuals had comparable survival to PD-L1⁻ individuals, except ICI treatment for which PD-L1⁺ may predict better PFS (HR = 0.42, 95% CI 0.17–1.06, $p = 0.067$). Patients with PD-L1⁺ CTCs had worse survival prognosis compared to those without PD-L1⁺ CTCs in overall analysis (PFS: HR = 2.10, 95% CI 1.59–2.77, $p < 0.001$; OS: HR = 2.55, 95% CI 1.70–3.81, $p < 0.001$) and in most subgroups.

Conclusions: Our analysis demonstrated that PD-L1 positive expression on CTCs predicted better survival prognosis for ICI treatment but worse survival for other therapies, which thus can be potentially used as a prognostic marker of

malignant tumor treatment. However, the prognostic value of PD-L1⁺ CTCs for ICI treatment needs validation by more large-scale studies in the future.

KEYWORDS

circulating tumor cells, immune checkpoint inhibitors, overall survival, programmed cell death-ligand 1, progression-free survival

1 | INTRODUCTION

Programmed cell death-ligand 1 (PD-L1) overexpression on tumor tissues has been explored as a promising biomarker that predicts response to immune checkpoint inhibitors (ICIs) therapy.¹ Patients with PD-L1 overexpression may benefit more from anti-PD-1/PD-L1 antibodies.² Immunohistochemistry (IHC)-based tests for PD-L1 expression on tumor tissues can help select patients suitable for these drugs.³ However, the predictive role of tumor PD-L1 expression is still in controversy and some limitations need to be overcome. About 10% of patients negative for PD-L1 tumor expression can also benefit from ICIs therapy,⁴ and the underlying mechanism needs further investigation. There is obvious spatial and temporal heterogeneity of PD-L1 expression on tumor tissues. The expression may significantly vary from tumor boundary to core, differ between primary and metastatic sites,^{5,6} and dynamically change along with disease progression.⁷ Therefore, the biopsy at a single tumor site or a certain time point may not be sufficiently representative of the overall PD-L1 status of tumor tissue. Since tumor tissue biopsy is invasive and may increase the risk of tumor metastasis, multi-site or longitudinal biopsies of tumor tissue, however, are considered not applicable.⁸

In view of the shortages of PD-L1 expression detected by conventional tissue biopsy and IHC, researchers have recently focused on circulating PD-L1 expressions in serum, plasma, circulating tumor cells (CTCs), and exosomes. These alternative methods allow a minimally invasive and real-time detection for a more accurate representation of the heterogeneous expression of PD-L1, and are feasible for dynamic monitoring of PD-L1 status during anti-cancer treatment.⁹ A recent meta-analysis involving 21 studies demonstrated that higher soluble PD-L1 (sPD-L1) was significantly correlated with worse survivals in various cancers.¹⁰ Significantly higher levels of serum/plasma-derived exosomal PD-L1 were found in melanoma, non-small cell lung cancer (NSCLC), head, and neck squamous cell carcinoma (HNSCC) than in healthy controls.¹¹⁻¹³ Patients with elevated exosomal PD-L1 level were less likely to respond to immunotherapy¹² and had shorter survivals^{14,15} than those with low levels. Thus, sPD-L1 and exosomal PD-L1 may be potential biomarkers for cancer therapies.¹⁶

PD-L1 expression on CTCs was first demonstrated in breast cancer,¹⁷ and then reported in colorectal cancer, bladder cancer, NSCLC, HNSCC, and melanoma.¹⁸⁻²² Subsequently, the clinical significance of PD-L1⁺ CTCs was explored. Wang Y et al found that PD-L1⁺ CTCs were associated with significantly shorter progression-free survival (PFS) in NSCLC patients undergoing radiochemotherapy.²³ Liu MY et al showed that gastric cancer patients with higher number of PD-L1⁺ CTCs had decreased PFS and worse overall survival (OS) than those with lower number of PD-L1⁺ CTCs.²⁴ Winograd P et al demonstrated that PD-L1⁺ CTCs predicted inferior OS in hepatocellular carcinoma patients undergoing ICIs therapy.²⁵ However, some researches have yielded inconsistent and even opposite results. Tada H et al observed prolonged survival in HNSCC patients with PD-L1⁺ CTCs.²⁶ In colorectal cancer patients receiving regorafenib, a receptor tyrosine kinases inhibitor, PD-L1⁺ CTCs predicted favorable survivals.²⁷ These results indicated a controversial prognostic value of PD-L1 expression on CTCs in human cancers.

The inconsistent results may be caused by many factors, such as the difference in cancer type, anti-cancer treatment, CTC enrichment method, or PD-L1 detection method. Here, we performed the first meta-analysis to systematically assess the prognostic role of PD-L1 expression on CTCs in various cancers.

2 | MATERIALS AND METHODS

2.1 | Studies selection

This study is performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA).²⁸ We comprehensively searched PubMed, Web of Science, and EMBASE prior to June 30, 2021, using the following search items: (CTCs OR neoplastic circulating cells OR CTCs) AND (programmed death ligand 1 OR PD-L1 OR CD274). Studies investigating the association between PD-L1⁺ CTCs and survival in cancer patients were a candidate for the present meta-analysis. The references of relevant articles were manually reviewed for additional candidate studies.

Candidate articles meeting the following criteria were included in the meta-analysis: (1) enriched and isolated

CTCs in blood samples of cancer patients and detected PD-L1 expression on CTCs; (2) reported hazard ratio (HR) and 95% confidence interval (95% CI) of PD-L1⁺ CTCs in association with PFS and/or OS, or provided sufficient data to calculate HR and 95% CI. Reviews, meta-analyses, case reports, and duplicated studies were excluded. Since this is a meta-analysis, ethical approval is not required.

cancer type, anti-cancer therapy, enrichment method and platform of CTCs, detection method and antibody of PD-L1, metastatic status, prognostic cut-off of PD-L1⁺ CTCs, cell-surface vimentin (CSV) expression on CTCs, time point of blood draw, HR, and 95% CI of survival outcomes. Discrepancies, if occurred, were resolved by discussion.

2.2 | Data extraction

Two independent researchers extracted the following information of eligible studies: first author, publication year,

2.3 | Quality assessment

Newcastle-Ottawa Scale was used to assess the quality of eligible studies in three categories: selection, comparability, and outcome. A total of nine stars were distributed

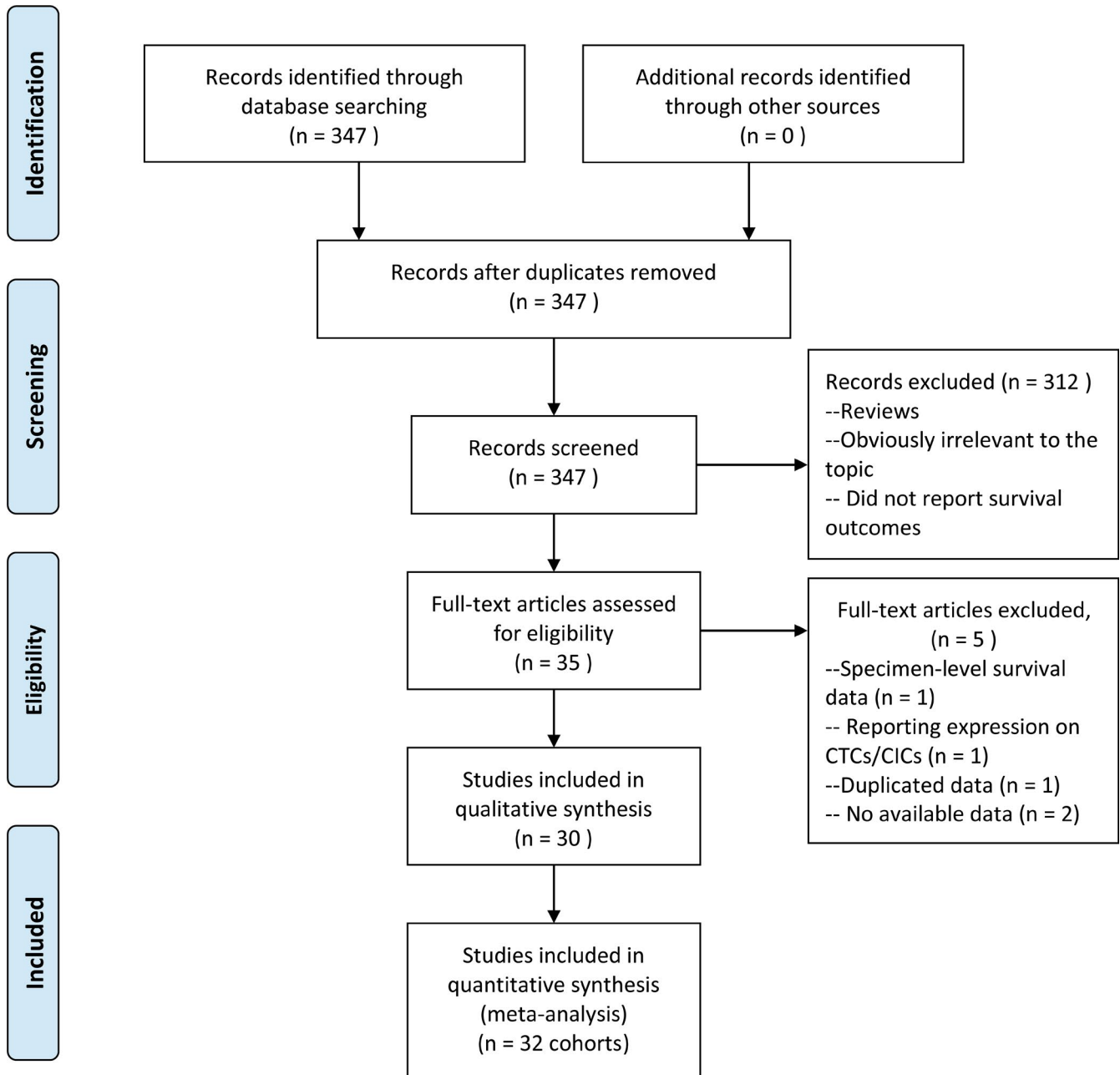


FIGURE 1 Flow chart for the literature search and study selection. CIC, circulating immune cell; CTC, circulating tumor cell

TABLE 1 Baseline characteristics of all studies included in meta-analysis

Study	Cancer	Therapy	CTC enrichment
Satelli A (2016)	Metastatic colon cancer, prostate cancer	Chemotherapy	Enrichment-free
Anantharaman A (2016)	Metastatic bladder cancer	Chemotherapy, ICI and others	Enrichment-free
Boffa DJ (2017)	Stage I–IV NSCLC	NR	Enrichment-free
Adams DL (2017)	Stage I–IV NSCLC	Radiotherapy	Size-based (CellSieve)
Strati A (2017)	Locally advanced HNSCC	Chemoradiotherapy	EpCAM-based (CellSearch)
Kallergi G (2018)	Metastatic NSCLC	Chemotherapy	Size-based (ISET)
Dhar M (2018)	Metastatic NSCLC	Pembrolizumab, nivolumab, avelumab	Size-based (Vortex HT chip)
Guibert N (2018)	Metastatic NSCLC	Nivolumab	Size-based (ISET)
Yue CY (2018)	Advanced gastrointestinal tumors	Sintilimab	EpCAM-based (Pep@MNPs)
Kulasinghe A (2018)	Stage I–IV HNSCC, metastatic NSCLC	Chemotherapy, ICI, TKIs	Size-based (ClearCell)
Wang Y (2019)	Non-metastatic NSCLC	Radiotherapy, chemoradiotherapy	EpCAM-based (GO chip)
Manjunath Y (2019)	Stage I–IIIA NSCLC	Surgery	Size-based (CellSieve)
Kotsakis A (2019)	Metastatic NSCLC	Chemotherapy	Size-based (ISET)
Dong JS (2019)	Stage I–III NSCLC	Surgery	Size-based (CanPatrol)
Liu MY (2020)	Advanced gastric cancer	Chemotherapy	EpCAM-based (Miltenyi Biotec)
Papadaki MA (2020)	Metastatic breast cancer	Chemotherapy, hormone therapy	Enrichment-free
Tada H (2020)	Stage I–IV HNSCC	NR	Size-based (CellSieve)
Pinato DJ (2020)	Neuroendocrine tumor	Surgery	EpCAM-based (CellSearch)
Khattak MA (2020)	Metastatic melanoma	Pembrolizumab	Enrichment-free
Cheng YX (2020)	Stage II–IV NSCLC	Initial treated	Size-based (ISET)
Bergmann S (2020)	Advanced urothelial carcinoma	NR	EpCAM-based (CellSearch)
Papadaki MA (2020)	Metastatic NSCLC	ICI	Size-based (Parsortix)
Jacot W (2020)	Metastatic breast cancer	NR	EpCAM-based (CellSearch)
Raimondi L (2020)	Metastatic colorectal cancer	Regorafenib	EpCAM-based (CellSearch)
Winograd P (2020)	Hepatocellular carcinoma	NR	EpCAM-based (NanoVelcro chip)
Chalfin HJ (2020)	Metastatic genitourinary cancer	Cabozantinib, nivolumab, ipilimumab	Enrichment-free
Tada H (2020)	Recurrent/metastatic HNSCC	Nivolumab	Enrichment-free
Polioudaki H (2020)	Metastatic breast cancer	Eribulin	Enrichment-free
Zavridou M (2021)	mCRPC	Chemotherapy, new hormonal agents	EpCAM-based (Dynabeads Epithelial Enrich)
Dall'Olio FG (2021)	Advanced NSCLC	nivolumab, pembrolizumab, atezolizumab	EpCAM-based (CellSearch)

Abbreviations: API: average pixel intensity of immunofluorescence staining; CTC: circulating tumor cell; HNSCC: head and neck squamous cell carcinoma; ICI: immune checkpoint inhibitor; IF: immunofluorescence; mCRPC: metastatic castration-resistant prostate cancer; NOS: Newcastle-Ottawa Scale; NR: not reported; NSCLC: non-small cell lung cancer; OS: overall survival; PD-L1: programmed cell death ligand 1; PFS: progression-free survival; RNA-ISH, RNA in situ hybridization; RT-qPCR: real-time quantitative polymerase chain reaction.

^aPercentage of patients with PD-L1⁺ CTCs in all patients.

^bPercentage of patients with PD-L1⁺ CTCs in CTC positive patients.

PD-L1 detection (antibody)	PD-L1 ⁺ CTC patient		Time point of blood draw, outcome	NOS
	Cut-off	Number		
IF (AHP-1703, AbD Serotec)	≥50% PD-L1 ⁺ CTCs	41/61 (67.2%) ^a , 23/30 (76.7%) ^a	Pre-treatment, PFS and OS	6
IF (E1L3N, Cell Signaling)	>1 PD-L1 ⁺ CTCs/ml	4/19 (21.0%) ^b	Pre-treatment, OS	6
IF (E1L3N, Cell Signaling)	>1.1 PD-L1 ⁺ CTCs/ml	14/112 (12.5%) ^a	Pre-treatment, OS	7
IF (130021, R&D system)	≥2 API	15/34 (44.2%) ^b	Pre- and post-treatment, PFS	8
RT-qPCR	Relative fold change	24/94 (25.5%) ^a	Pre- and post-treatment, PFS and OS	7
IF (B7-H1, Novus Biologicals)	>3 PD-L1 ⁺ CTCs/ml	2/30 (6.7%) ^a	Pre-treatment, PFS	7
IF (4059, ProSci Inc)	≥2 PD-L1 ⁺ CTCs	7/17 (41.2%) ^a	Pre-treatment, PFS	6
IF (D8T4X, Cell Signaling)	≥1% PD-L1 ⁺ CTCs	74/89 (83.1%) ^b	Pre-treatment, PFS and OS	8
IF (KN802, Kohnoor)	≥20% PD-L1 ⁺ CTCs	14/35 (40.0%) ^b	Pre- and post-treatment, PFS	8
IF (28--2, Abcam)	≥1 PD-L1 ⁺ CTCs	6/11 (54.5%) ^b , 11/17 (64.7%) ^b	Pre-treatment, PFS	7
IF (329802, BioLegend)	≥5% PD-L1 ⁺ CTCs	6/13 (46.2%) ^b	Pre-treatment, PFS	7
IF (D8T4X, Cell Signaling)	≥3 PD-L1 ⁺ CTCs	18/30 (60.0%) ^a	Pre-treatment, OS	6
IF (BioLegend)	≥1 PD-L1 ⁺ CTCs	7/34 (20.6%) ^a	Pre-treatment, PFS	8
RNA-ISH	≥1 PD-L1 ⁺ CTCs	56/110 (50.1%) ^b	Pre-treatment, OS	6
IF (Cell Signaling)	≥8 PD-L1 ⁺ CTCs/ml	18/32 (56.2%) ^a	Pre-treatment, PFS and OS	7
IF (E1L3N, Cell Signaling)	≥1 P PD-L1 ⁺ CTCs	5/98 (5.1%) ^a	Pre-treatment, PFS and OS	7
RT-qPCR	$2^{-\Delta\Delta Ct} > 1$	11/28 (39.3%) ^b	Pre-treatment, PFS	8
IF (FAB1561P, R&D System)	≥1 PD-L1 ⁺ CTCs	9/12 (75.0%) ^a	Pre-treatment, OS	8
IF	≥1 PD-L1 ⁺ CTCs	16/25 (60.0%) ^b	Pre-treatment, PFS and OS	7
IF (28-8, Abcam)	≥1% PD-L1 ⁺ CTCs	22/41 (53.6%) ^b	Pre-treatment, PFS	8
IF (E1L3N, Cell Signaling)	≥1 PD-L1 ⁺ CTCs	4/16 (25.0%) ^b	Pre-treatment, OS	7
IF (E1L3N, Cell Signaling)	≥1 PD-L1 ⁺ CTCs	3/15 (20.0%) ^a	Pre-treatment, PFS and OS	6
IF (FAB1561P, R&D System)	≥1 PD-L1 ⁺ CTCs	26/72 (36.1%) ^a	Pre-treatment, PFS and OS	6
IF (D8T4X, Cell Signaling)	≥1 PD-L1 ⁺ CTCs	24/38 (63.2%) ^b	Pre-treatment, PFS	7
IF (R&D System)	≥1 PD-L1 ⁺ CTCs	31/87 (35.6%) ^a	Pre-treatment, OS	8
IF (E1L3N, Cell Signaling)	≥1 PD-L1 ⁺ CTCs	7/67 (10.4%) ^a	Pre-treatment, PFS Post-treatment, OS	8
RT-qPCR	$40^{-\Delta Ct} > 24.98$	16/28 (57.1%) ^b	Pre-treatment, OS	6
IF (E1L3N, Cell Signaling)	≥1 PD-L1 ⁺ CTCs	5/38 (13.2%) ^a	Pre- and post-treatment, PFS and OS	7
RT-qPCR	Relative fold change	34/62 (54.8%) ^b	Pre-treatment, OS	7
IF (MIH3, BioLegend)	≥1 PD-L1 ⁺ CTCs	13/24 (54.2%) ^b	Pre-treatment, PFS and OS	7

to the assessment items and six or more stars indicated a high quality.

2.4 | PD-L1 expression status on CTCs and comparison model

As some cancer patients had undetectable CTCs, the PD-L1 expression status can be divided into three categories: CTCs negative (status 1), CTC positive plus PD-L1 negative expression on CTCs (status 2: PD-L1⁻ CTCs), CTC positive plus PD-L1 positive expression on CTCs (status 3: PD-L1⁺ CTCs). Therefore, the prognostic value of PD-L1⁺ CTCs, that is, status 3, can be analyzed under two comparison models. The first comparison model was performed among CTCs positive patients, that is, those with status 3 and those with status 2 (model 1: CTC PD-L1⁺ vs. CTC PD-L1⁻). The second comparison model was performed in all patients, that is, those with status 3 and those with status 1+2 (model 2: presence of PD-L1⁺ CTCs vs. absence of PD-L1⁺ CTCs).

2.5 | Statistical analysis

We assessed the heterogeneity by I^2 and Q test. $I^2 < 50%$ with p value of Q test > 0.10 indicated no obvious heterogeneity, and then a fixed-effect model was applied to combine HR and 95% CI of survival outcomes. Otherwise, a random-effect model was used. Subgroups analyses regarding prognostic cut-off of PD-L1⁺ CTCs, cancer type, CTC enrichment method, metastatic status, treatment, comparison model, CSV expression status, PD-L1 detection method were performed. Moreover, we analyzed the interactions between comparison models and the other variables. Sensitivity analysis was performed, and funnel plot and Egger's test were used to assess publication bias. The present meta-analysis was performed by STATA 12.0 (StataCorp).

3 | RESULTS

3.1 | Baseline features of eligible studies

Three hundred and fourteen articles were identified through literature search, and 35 studies were remained after discarding studies not relevant to the research topic. Furtherly, we excluded five candidate studies for the following reasons: one only provided specimen-level survival data,²⁹ one reported the correlation of PD-L1⁺ CTCs/circulating immune cells with survival,³⁰ one was duplicated with another study,^{18,31} and two did not report survival outcomes.^{32,33} Finally, we identified 30 studies

eligible for the present meta-analysis^{18-27,34-53} as shown in Figure 1. A total of 1419 patients with malignant tumors, including 208 breast cancer, 253 gastrointestinal cancer, 194 genitourinary cancer, 161 head and neck cancer, 25 melanoma, 12 metastatic neuroendocrine tumor, and 566 NSCLC, were analyzed. CTCs were enriched by epithelial cell adhesion molecule (EpCAM)-based or size-based methods or were enrichment-free in 11, 11, and 8 studies, respectively. PD-L1 was detected for protein expression on CTCs by immunofluorescence (IF) in 25 studies, and for mRNA expression in 5 studies. All studies detected pre-treatment PD-L1⁺ CTCs, while five studies also detected post-treatment PD-L1⁺ CTCs. The cut-off of ≥ 1 PD-L1⁺ CTCs was the most commonly used by 14 studies to define PD-L1 positive patients. As to the comparison model, 15 studies used model 1 while 15 studies used model 2. Specifically, two researches^{22,46} both had two cohorts of patients with different cancers, then each cohort was included as an individual study into quantitative analysis. The characteristics of all eligible studies are summarized in Table 1.

3.2 | Correlation between pre-treatment PD-L1⁺ CTCs and survival of cancer patients

Twenty-three studies comprising 992 patients evaluated the association of pre-treatment PD-L1⁺ CTCs with PFS (Table 2). There was obvious heterogeneity ($I^2 = 70.3%$, $p < 0.001$) and a random-effect model was applied. Pre-treatment PD-L1⁺ CTCs were not associated with PFS (HR = 1.33, 95% CI 0.88–2.01, $p = 0.170$). When stratified for treatment, we found that PD-L1⁺ CTCs detected by IF were associated with a better PFS (HR = 0.55, 95% CI 0.28–1.08, $p = 0.084$, Figure 2) for ICI treatment in a borderline significance, but a worse PFS for other therapies (HR = 1.85, 95% CI 1.21–2.85, $p < 0.001$, Figure 2).

The association between pre-treatment PD-L1⁺ CTCs and OS was evaluated in 20 studies comprising 1096 patients (Table 3). Pooled analysis using a random-effect model demonstrated that patients with PD-L1⁺ CTCs had significantly worse OS (HR 1.82, 95% CI 1.24–2.68, $p = 0.002$). When stratified for treatment, PD-L1⁺ CTCs seemed to predict a better OS (HR = 0.72, 95% CI 0.38–1.38, $p = 0.325$, Figure 3) for ICI treatment, but were significantly associated with worse OS for other therapies (HR = 2.44, 95% CI 1.69–3.51, $p < 0.001$, Figure 3). If we excluded only one study³⁵ detecting PD-L1 mRNA and remained the other studies detecting PD-L1 by IF, we found that PD-L1⁺ CTCs had a borderline association with prolonged OS (HR = 0.61, 95% CI 0.36–1.04, $p = 0.067$) for ICI treatment.

TABLE 2 Association between pre-treatment PD-L1⁺ CTCs and progression-free survival in cancers

Pre-treatment, PFS	No. of studies	No. of patients	Combined HR (95% CI)	p	Heterogeneity		Model
					I ² (%)	p	
Overall	23	992	1.33 (0.88–2.01)	0.170	70.3	<0.001	RE
Treatment							
ICIs	6	210	0.55 (0.28–1.08)	0.084	61.1	0.025	RE
Other therapies	17	782	1.85 (1.21–2.85)	0.005	60.6	<0.001	RE
Cancer type							
NSCLC	10	319	1.30 (0.76–2.21)	0.341	58.0	0.011	RE
Breast cancer	3	208	1.90 (1.24–2.91)	0.003	0	0.635	FE
Gastrointestinal cancer	4	210	0.74 (0.17–3.14)	0.684	84.6	<0.001	RE
Genitourinary cancer	2	97	4.81 (2.02–11.45)	<0.001	46.2	0.173	FE
HNSCC	3	133	1.18 (0.28–2.09)	0.826	79.2	0.008	RE
Enrichment method							
EpCAM-based	7	346	0.92 (0.41–2.08)	0.847	80.9	<0.001	RE
Size-based	10	321	1.30 (0.77–2.20)	0.326	56.1	0.015	RE
Enrichment-free	6	325	2.25 (0.92–5.52)	0.077	64.1	0.016	RE
Metastatic disease							
Yes	14	642	1.70 (1.09–2.64)	0.019	54.5	0.008	RE
Mixed	9	350	1.00 (0.49–2.06)	0.991	77.1	<0.001	RE
Comparison							
CTC PD-L1 ⁺ versus CTC PD-L1 ⁻	11	355	0.71 (0.37–1.37)	0.307	72.4	<0.001	RE
Presence versus absence of PD-L1 ⁺ CTCs	12	637	2.10 (1.59–2.77)	<0.001	7.0	0.377	FE
Vimentin expression							
Yes	4	205	2.47 (1.41–4.33)	0.002	9.1	0.347	FE
Not specified	19	787	1.15 (0.73–1.82)	0.542	72.3	<0.001	RE
Prognostic cut-off							
≥1 PD-L1 ⁺ CTCs	12	480	1.43 (0.83–2.46)	0.202	67.5	<0.001	RE
Other cut-offs	11	512	1.27 (0.68–2.38)	0.458	72.7	<0.001	RE
PD-L1 detection							
IF	21	870	1.44 (0.93–2.22)	0.101	69.9	<0.001	RE
mRNA expression	2	122	0.67 (0.14–3.20)	0.616	81.1	0.021	RE

Statistically significant values are indicated in bold.

Abbreviations: CSV, cell-surface vimentin; FE, fixed-effect model; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; ICIs, immune checkpoint inhibitors; IF, Immunofluorescence; NSCLC, non-small cell lung cancer; PFS, progression-free survival; RE, random-effect model.

3.3 | Subgroup analyses of pre-treatment PD-L1⁺ CTCs in association with survival

We performed subgroup analysis according to the cancer type (NSCLC, breast cancer, gastrointestinal cancer, genitourinary cancer, HNSCC), CTC enrichment method

(EpCAM-based, size-based, enrichment-free), metastatic status (yes, mixed), comparison model (model 1 and model 2), CSV expression (yes, no specified), prognostic cut-off (≥1 PD-L1⁺ CTCs, other cut-offs), and PD-L1 detection method (IF, mRNA expression). The results of subgroup analyses for PFS and OS are shown in Tables 2 and 3, respectively.

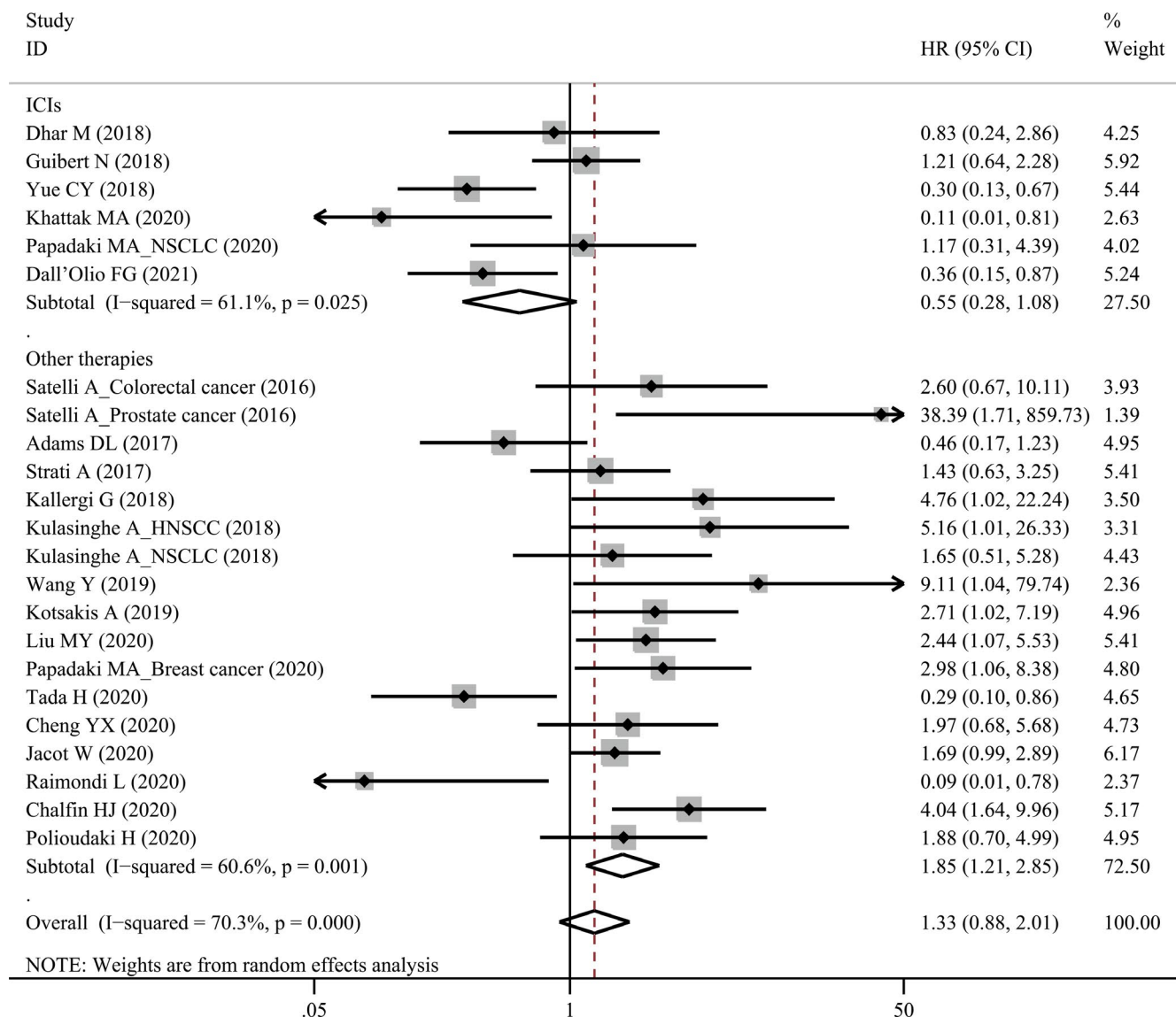


FIGURE 2 Forest plot of pre-treatment PD-L1⁺ circulating tumor cells with progression-free survival. ICI, immune checkpoint inhibitor; PD-L1, programmed cell death-ligand 1

3.3.1 | Cancer type

In NSCLC, PD-L1⁺ CTCs were neither associated with PFS (HR = 1.30, 95% CI 0.76–2.21, $p = 0.341$, Figure 4A) nor OS (HR = 1.43, 95% CI 0.59–3.46, $p = 0.424$, Figure 4B). We further stratified the analysis for treatment and found that ICIs-treated patients with PD-L1⁺ CTCs seemed to have prolonged survival (PFS: HR = 0.84, 95% CI 0.54–1.31, $p = 0.442$; OS: HR = 0.68, 95% CI 0.38–1.20, $p = 0.184$) although it did not reach statistical significance. In contrast, in NSCLC patients treated by other therapies, PD-L1⁺ CTCs predicted worse survival (PFS: HR = 1.96, 95% CI 0.91–4.22, $p = 0.086$; OS: HR = 3.34, 95% CI 1.68–6.64, $p = 0.001$).

Pre-treatment PD-L1⁺ CTCs were associated with inferior PFS in breast cancer (HR = 1.90, 95% CI 1.24–2.91) and genitourinary cancer (HR = 4.81, 95% CI 2.02–11.45), predicted significantly worse OS in breast (HR = 2.62, 95% CI 1.50–4.59), and gastrointestinal cancer (HR = 3.29, 95% CI 2.06–5.26), respectively. No association was found between PD-L1⁺ CTCs and survival in HNSCC.

3.3.2 | CTC enrichment method

PD-L1⁺ CTCs were not associated with PFS in any subgroup of the enrichment method. However, PD-L1⁺ CTCs predicted worse OS in enrichment-free studies (HR = 2.37, 95% CI 1.61–3.50).

TABLE 3 Association between pre-treatment PD-L1⁺ CTCs and overall survival in cancers

Pre-treatment, OS	No. of studies	No. of patients	Combined HR (95% CI)	p	Heterogeneity		Model
					I ² (%)	p	
Overall	20	1096	1.82 (1.24–2.68)	0.002	60.5	<0.001	RE
Treatment							
ICIs	5	181	0.72 (0.38–1.38)	0.325	43.0	0.135	RE
Other therapies	15	915	2.44 (1.69–3.51)	<0.001	42.2	0.043	RE
Cancer type							
NSCLC	6	380	1.43 (0.59–3.46)	0.424	71.3	0.004	RE
Breast cancer	3	208	2.62 (1.50–4.59)	0.001	0	0.467	FE
Gastrointestinal cancer	3	224	3.29 (2.06–5.26)	<0.001	0	0.645	FE
Genitourinary cancer	4	125	1.69 (0.97–2.93)	0.063	40.5	0.169	FE
HNSCC	2	122	0.87 (0.33–2.28)	0.773	52.1	0.148	RE
Enrichment method							
EpCAM-based	8	435	1.64 (0.82–3.28)	0.166	76.6	<0.001	RE
Size-based	4	244	1.38 (0.74–2.56)	0.312	0	0.421	FE
Enrichment-free	8	417	2.37 (1.61–3.50)	<0.001	40.3	0.110	FE
Metastatic disease							
Yes	12	555	1.65 (1.22–2.22)	0.001	21.3	0.234	FE
Mixed	8	541	2.12 (0.93–4.81)	0.074	79.3	<0.001	RE
Comparison							
CTC PD-L1 ⁺ versus CTC PD-L1 ⁻	8	371	1.06 (0.60–1.89)	0.840	53.2	0.037	RE
Presence versus absence of PD-L1 ⁺ CTCs	12	725	2.55 (1.70–3.81)	<0.001	41.4	0.065	RE
CSV expression							
Yes	5	235	3.46 (2.13–5.61)	<0.001	0	0.816	FE
Not specified	15	861	1.45 (0.93–2.27)	0.099	62.0	0.001	RE
Prognostic cut-off							
≥1 PD-L1 ⁺ CTCs	11	584	1.65 (0.91–3.00)	0.101	66.4	0.001	RE
Other cut-offs	9	512	2.02 (1.21–3.37)	0.007	55.5	0.022	RE
PD-L1 detection							
IF	16	802	2.13 (1.36–3.35)	0.001	60.3	0.001	RE
mRNA expression	4	294	1.04 (0.67–1.62)	0.852	4.2	0.372	FE

Statistically significant values are indicated in bold.

Abbreviations: CSV, cell-surface vimentin; FE, fixed-effect model; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; ICI, immune checkpoint inhibitor; IF, Immunofluorescence; NSCLC, non-small cell lung cancer; PFS, progression-free survival; RE, random-effect model.

3.3.3 | Metastatic disease, CSV expression, and treatment

Meta-analysis of studies enrolling patients with metastatic tumors revealed significant associations of PD-L1⁺ CTCs with worse PFS (HR = 1.70, 95% CI 1.09–2.64) and OS (HR = 1.65, 95% CI 1.22–2.22) as shown in Figure 5. Previous studies

found that CTCs undergoing epithelial-to-mesenchymal transition (EMT) were associated with invasion and metastasis and had increased expression of mesenchymal markers such as CSV.⁵⁴ Subgroup analysis involving five cohorts of patients^{22,24,34,45} showed that patients with CSV⁺ PD-L1⁺ CTCs had markedly worse PFS (HR = 2.47, 95% CI 1.41–4.33) and OS (HR = 3.46, 95% CI 2.13–5.61).

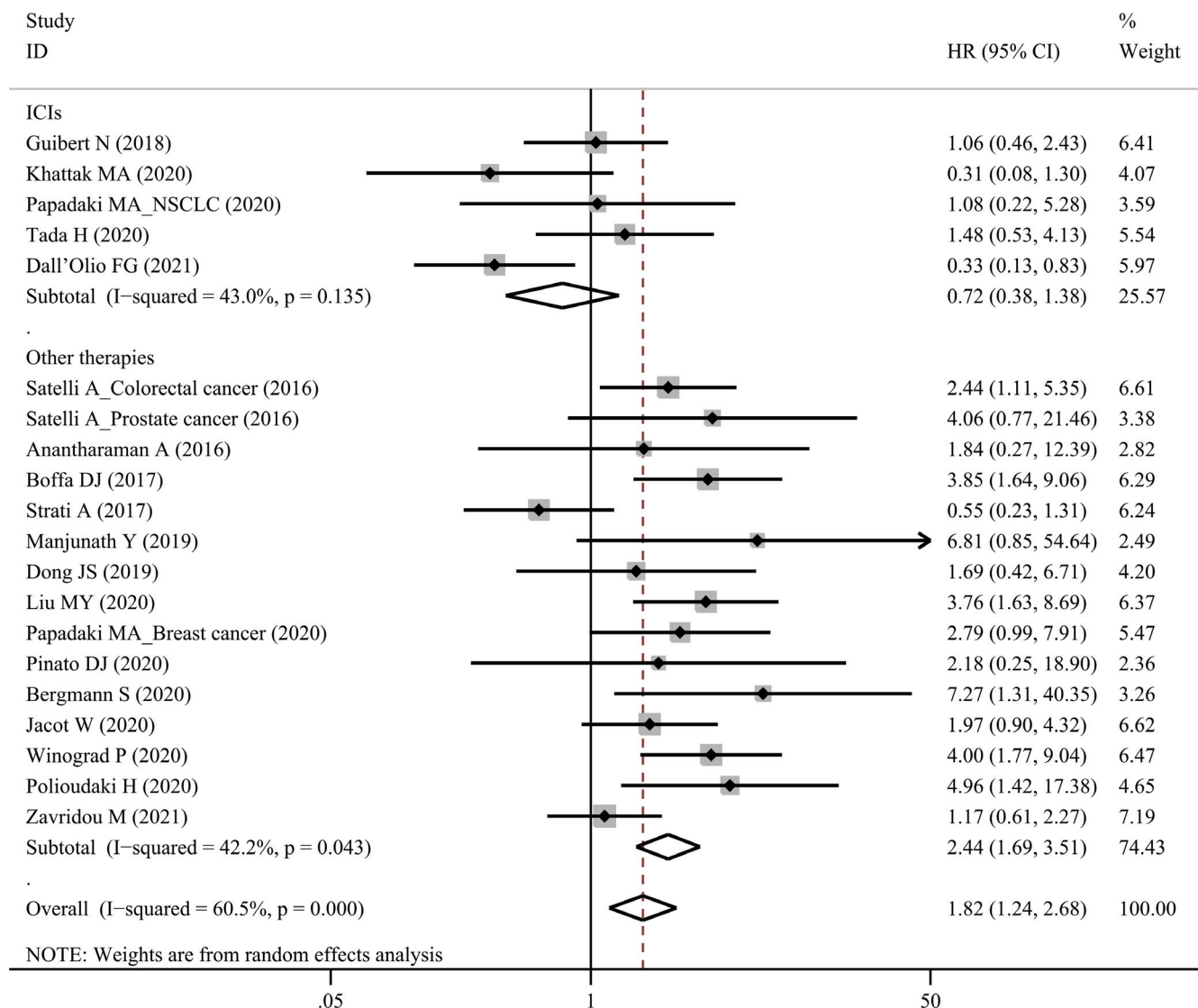


FIGURE 3 Forest plot of pre-treatment PD-L1⁺ circulating tumor cells with overall survival. ICI, immune checkpoint inhibitor; PD-L1, programmed cell death-ligand 1

3.3.4 | Prognostic cut-off and detection method of PD-L1

Using ≥ 1 PD-L1⁺ CTCs as cutoff, PD-L1⁺ CTCs were not associated with PFS (HR = 1.43, 95% CI 0.83–2.46) or OS (HR = 1.65, 95% CI 0.91–3.00) by random-effect model. Using the other cutoffs, PD-L1⁺ CTCs only predicted an unfavorable OS (HR = 2.02, 95% CI 1.21–3.37). Most of the included studies detected PD-L1 by IF, which showed worse OS (HR = 2.13, 95% CI 1.36–3.35) in patients with PD-L1⁺ CTCs by meta-analysis.

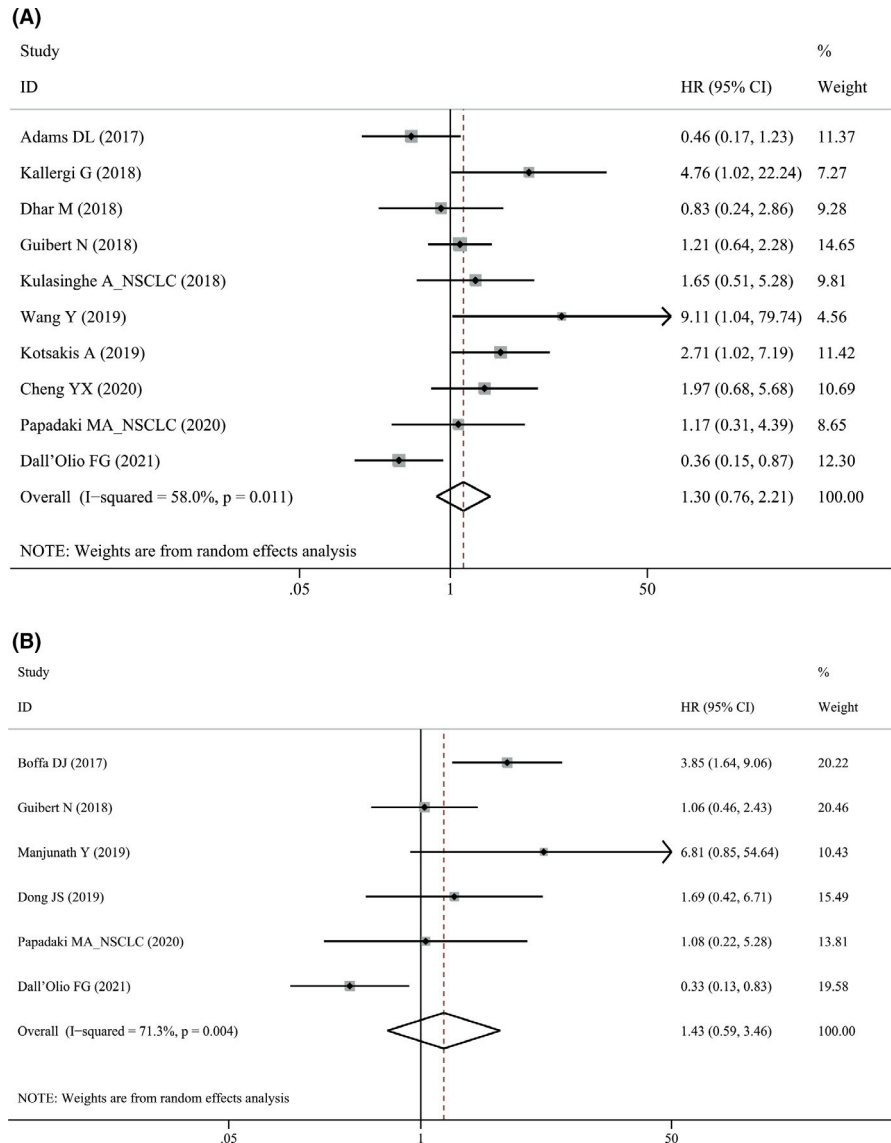
3.3.5 | Comparison model

Under model 1, there was no survival difference between CTC PD-L1⁺ and CTC PD-L1⁻ patients (Figure 6A,C).

Under comparison model 2, patients with PD-L1⁺ CTCs had unfavorable PFS (HR = 2.10, 95% CI 1.59–2.77, $p < 0.001$, Figure 6B) and OS (HR = 2.55, 95% CI 1.70–3.81, $p < 0.001$, Figure 6D) than those without PD-L1⁺ CTCs. Moreover, after excluding only one study detecting PD-L1 mRNA expression,¹⁹ there was no between-study heterogeneity, and the association of PD-L1⁺ CTCs with OS was still statistically significant (HR = 3.05, 95% CI 2.23–4.16, $p < 0.001$, $I^2 = 0$).

The results may indicate diverse predictive roles of PD-L1⁺ CTCs under different comparison models and a potential source of heterogeneity from the models. Therefore, we performed further subgroup analyses under each model to investigate the interactions between the models and other variables (Table 4). Under model 1, there was huge heterogeneity in most of the subgroups, and the correlations between CTC PD-L1⁺ and survival were not significant. Interestingly, among patients with detectable CTCs and

FIGURE 4 Forest plots of pre-treatment PD-L1⁺ circulating tumor cells with (A) progression-free survival and (B) overall survival in patients with non-small cell lung cancer. PD-L1, programmed cell death-ligand 1



who received ICIs, PD-L1 positive expression had borderline association with prolonged PFS compared to negative expression (HR = 0.42, 95% CI 0.17–1.06, $p = 0.067$). In contrast, there was very low between-study heterogeneity and PD-L1⁺ CTCs were associated with significantly inferior survival in most of the subgroups under model 2.

Previous studies have demonstrated that CTCs were independent prognostic factors for cancer treatment.^{55,56} Thus, we asked whether the prognostic role of PD-L1⁺ CTCs under comparison model 2 was largely dependent on the predictive role of CTCs. If so, there should be a correlation between the effect size, that is, HR, of both markers. We included seven studies that reported the associations of both markers with PFS^{22,24,34,38,42,49} and OS.^{22,24,34,37,38,42} Meta-regression analysis showed that HRs for CTCs did not modify the effect sizes of PD-L1⁺ CTCs with PFS and OS ($p = 0.870$ and 0.410 , respectively). Furthermore, we compared the pooled effect sizes of both markers with survival outcomes. Meta-analysis of PFS yielded HR of

1.74 (95% CI 1.23–2.47, $p = 0.002$, $I^2 = 19.3%$) for CTCs and 2.06 (95% CI 1.34–3.18, $p = 0.001$, $I^2 = 14.0%$) for PD-L1⁺ CTCs. Meta-analysis of OS yielded HR of 1.82 (95% CI 1.31–2.51, $p < 0.001$, $I^2 = 0$) for CTCs and 2.70 (95% CI 1.85–3.94, $p < 0.001$, $I^2 = 0$) for PD-L1⁺ CTCs. The effect sizes for PD-L1⁺ CTCs were slightly larger than those for CTCs. These results indicated an independent prognostic role of PD-L1⁺ CTCs under comparison model 2.

3.4 | Correlation between post-treatment PD-L1⁺ CTCs and survival

The associations of post-treatment PD-L1⁺ CTCs with PFS and OS were analyzed in 4 studies with 201 cases and 3 studies with 199 cases (Table S1), respectively. As shown in Figure 7, post-treatment PD-L1⁺ CTCs were significantly associated with PFS (HR = 2.34, 95% CI 1.45–3.77, $p < 0.001$) and OS (HR = 6.16, 95% CI 3.20–11.86, $p < 0.001$).

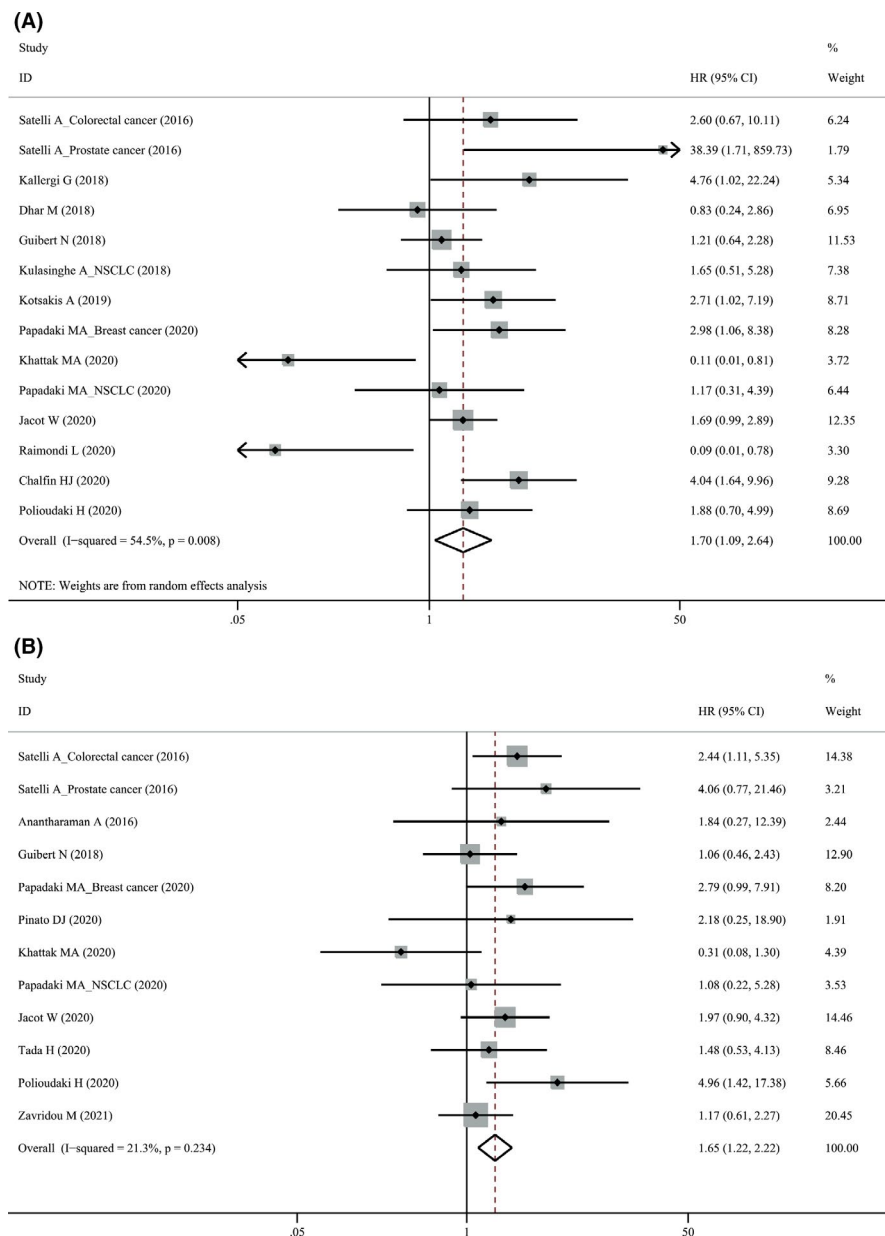


FIGURE 5 Forest plots of pre-treatment PD-L1⁺ circulating tumor cells with (A) progression-free survival and (B) overall survival in patients with metastatic tumors. PD-L1, programmed cell death-ligand 1

3.5 | Publication bias

Sensitivity analysis demonstrated that the results of our meta-analysis were robust and not significantly influenced by any single study. The symmetric funnel plots (Figure 8) and Egger's tests ($p > 0.05$) indicated that there was no obvious publication bias.

4 | DISCUSSION

We conducted the first meta-analysis to evaluate the clinical application of PD-L1 expression on CTCs in predicting the survivals of cancer patients, and to identify factors modulating the prognostic value. Overall, pre-treatment PD-L1⁺ CTCs may predict better survival for patients

receiving ICI treatment but worse survival for patients receiving other therapies. In addition, post-treatment PD-L1⁺ CTCs were correlated with worse survivals in cancers.

The PD-1/PD-L1 axis plays a crucial role in suppressing the activation, proliferation, and promoting the apoptosis of T cells, and consequently, its upregulation on tumor tissues leads to the immune escape of tumor cells.^{57,58} The PD-1/PD-L1 axis inhibitors disrupt the interaction between PD-1 and PD-L1, subsequently restore immune response toward tumor cells, and finally improve the survival outcomes of cancer patients.^{4,59} Patient selection for these drugs is vital, and PD-L1 tumor expression as a potential marker has been extensively investigated whereas there remain many unsolved issues.³ Some researchers have paid attention to PD-L1 expression on CTCs. In advanced gastrointestinal tumor patients who were treated

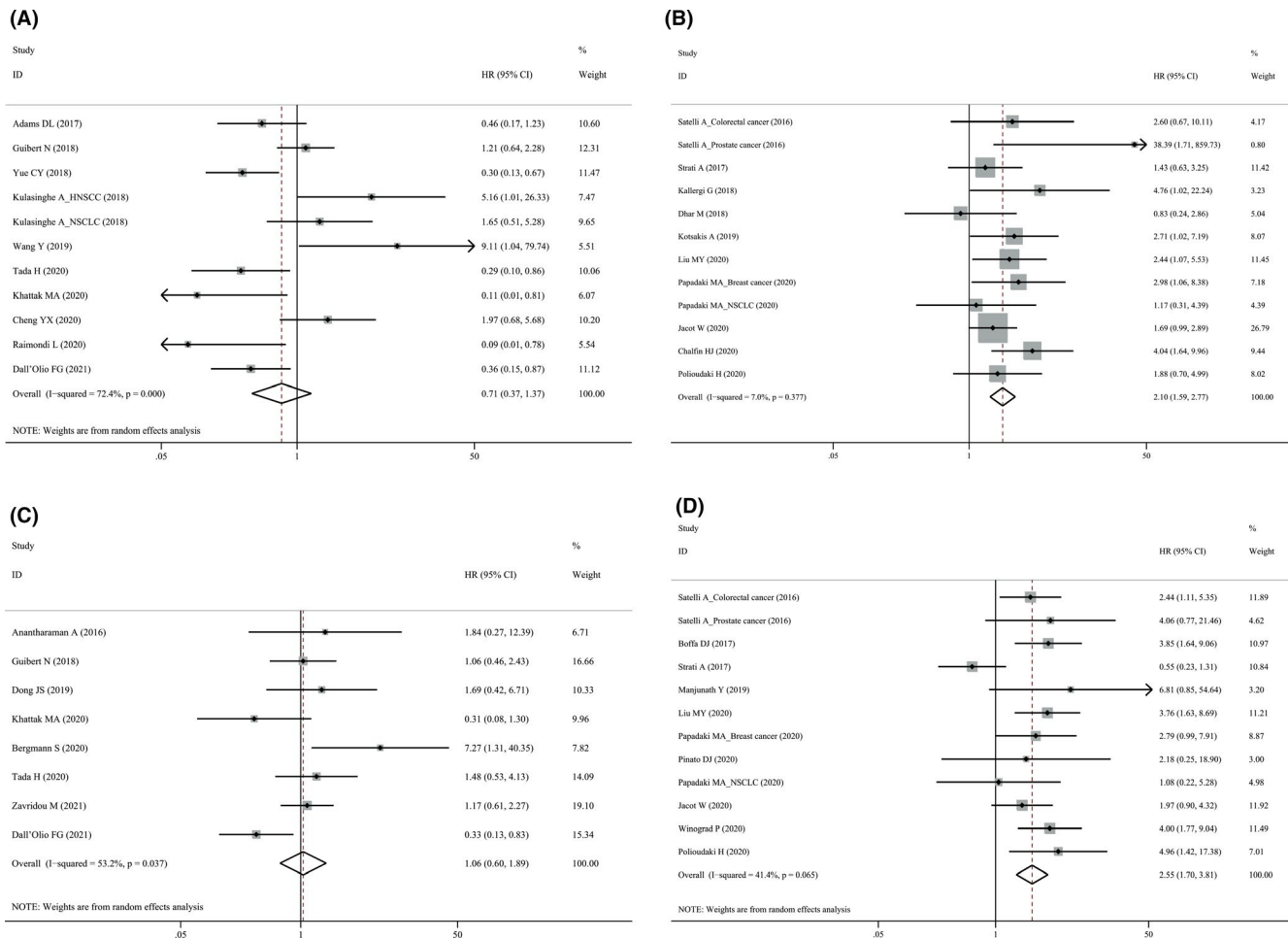


FIGURE 6 Forest plots of pre-treatment PD-L1⁺ CTCs with (A) progression-free survival (PFS) under comparison model 1, (B) PFS under comparison model 2, (C) overall survival (OS) under comparison model 1 and (D) OS under comparison model 2. Comparison model 1: PD-L1⁺ versus PD-L1⁻ among patients with detectable CTCs. Comparison model 2: Presence versus absence of PD-L1⁺ CTCs. CTC, circulating tumor cell; PD-L1, programmed cell death-ligand 1

with PD-1 inhibitors, Yue CY et al found that those with high PD-L1 expression on CTCs had prolonged PFS and higher disease control rate compared with those with low expression.⁴⁷ Khattak MA et al found similar results in advanced melanoma patients treated with pembrolizumab that PD-L1⁺ CTCs predicted prolonged PFS and were more likely to be responders.¹⁸ In NSCLC patients receiving ICI treatment, PD-L1⁺ CTCs were associated with better PFS and OS.⁵³ However, some studies did not find a significant association between PD-L1⁺ CTCs and survival for ICI treatment.^{38,48,49} Subgroup meta-analysis by pooling these studies together showed that patients having PD-L1⁺ CTCs and treated with PD-1/PD-L1 inhibitors may have prolonged PFS (HR = 0.55, 95% CI 0.28–1.08) and OS (HR = 0.61, 95% CI 0.36–1.04). Although the associations did not reach a significant level due to the small sample size (*n* = 210 for PFS and 153 for OS), PD-L1⁺ CTCs tend to predict favorable survival prognosis for ICI treatment as more evidence are accumulating. A recent study revealed that the number of CTC detected was correlated

with tumor size.⁵³ Moreover, tumor size calculated in total metabolic tumor volume was significantly associated with survival and response to ICI treatment.^{60,61} Thus, whether tumor size is associated with the positivity rate of PD-L1⁺ CTCs and whether it modulates the association between PD-L1⁺ CTCs and survival for ICI treatment need further investigation. Nonetheless, pre-treatment PD-L1 expression on CTCs is a potential prognostic marker for ICI treatment, which needs to be validated by more large-scale studies in the future. In contrast to ICI treatment, our meta-analysis showed additional evidence of a significant association between PD-L1⁺ CTCs and survival in patients receiving non-ICIs therapy that PD-L1⁺ CTCs predicted significantly shorter PFS and OS.

Apart from the baseline expression, the dynamic expression of PD-L1 on CTCs showed potentials in predicting response to anti-tumor therapies. Several researches have found decreased number or proportion of PD-L1⁺ CTCs upon treatment in responders but increased or unchanged expression in non-responders.^{18,32,47} These

TABLE 4 Interactions between comparison models and the other variables

Comparison model	Other variables	No. of studies and patients	I ²	HR (95% CI)	p
CTC PD-L1 ⁺ versus CTC PD-L1 ⁻	Cutoff: ≥1 PD-L1 ⁺ CTCs				
	PFS	6 (156)	75.2	0.72 (0.24–2.20)	0.568
	OS	5 (262)	69.5	0.95 (0.36–2.48)	0.912
	Cutoff: other cutoffs				
	PFS	5 (199)	74.2	0.66 (0.28–1.57)	0.349
	OS	3 (109)	0	1.29 (0.76–2.20)	0.345
	Metastatic disease: yes				
	PFS	4 (169)	71.4	0.53 (0.15–1.82)	0.311
	OS	5 (223)	0	1.08 (0.70–1.65)	0.727
	Metastatic disease: mixed				
	PFS	7 (186)	74.7	0.82 (0.35–1.89)	0.635
	OS	3 (148)	81.8	1.42 (0.24–8.45)	0.697
	PD-L1 detection: IF				
	PFS	10 (327)	73.1	0.79 (0.39–1.58)	0.501
	OS	5 (171)	68.5	0.94 (0.34–2.55)	0.897
	Treatment: ICIs				
	PFS	4 (173)	74.0	0.42 (0.17–1.06)	0.067
	OS	4 (166)	55.6	0.67 (0.31–1.45)	0.314
	Treatment: other therapies				
	PFS	7 (182)	73.0	1.05 (0.40–2.79)	0.922
	OS	4 (205)	22.3	1.54 (0.90–2.64)	0.117
	NSCLC				
	PFS	6 (218)	64.7	1.07 (0.53–2.16)	0.850
OS	3 (233)	60.0	0.78 (0.31–1.98)	0.604	
CTC enrichment: EpCAM-based					
PFS	4 (110)	64.0	0.46 (0.14–1.54)	0.209	
OS	3 (100)	81.5	1.19 (0.30–4.64)	0.805	
CTC enrichment: size-based					
PFS	6 (220)	64.0	1.08 (0.53–2.17)	0.839	
OS	2 (199)	0	1.20 (0.59–2.44)	0.617	
CTC enrichment-free					
OS	3 (72)	43.1	0.98 (0.46–2.10)	0.960	
Presence versus absence of PD-L1 ⁺ CTCs	Cutoff: ≥1 PD-L1 ⁺ CTCs				
	PFS	6 (324)	0	2.15 (1.52–3.04)	<0.001
	OS	6 (322)	0	2.74 (1.78–4.24)	<0.001
	Cutoff: other cutoffs				
	PFS	6 (313)	34.6	2.01 (1.27–3.19)	0.003
	OS	6 (403)	66.0	2.51 (1.21–5.20)	0.013
	Metastatic disease: yes				
	PFS	10 (473)	16.7	2.17 (1.58–2.98)	<0.001
	OS	7 (332)	0	2.47 (1.63–3.74)	<0.001
	Metastatic disease: mixed				
PFS	2 (164)	0	1.87 (1.05–3.33)	0.035	

(Continues)

TABLE 4 (Continued)

Comparison model	Other variables	No. of studies and patients	I^2	HR (95% CI)	<i>p</i>
	OS	4 (393)	74.7	2.67 (1.13–6.33)	0.026
	PD-L1 detection: IF				
	PFS	11 (543)	8.1	2.21 (1.64–2.96)	<0.001
	OS	11 (631)	0	3.05 (2.23–4.16)	<0.001
	Treatment: ICIs				
	PFS	2 (37)	0	0.97 (0.39–2.40)	0.954
	OS	1 (15)	-	1.08 (0.22–5.25)	0.924
	Treatment: other therapies				
	PFS	10 (600)	0	2.27 (1.70–3.04)	<0.001
	OS	11 (710)	43.4	2.66 (1.76–4.04)	<0.001
	NSCLC				
	PFS	4 (101)	27.0	1.86 (1.01–3.42)	0.046
	OS	3 (157)	19.5	3.19 (1.57–6.48)	0.001
	CTC enrichment: EpCAM-based				
	PFS	3 (236)	0	1.77 (1.19–2.62)	0.004
	OS	5 (335)	70.3	2.04 (0.93–4.47)	0.075
	CTC enrichment: size-based				
	PFS	4 (101)	27.0	1.86 (1.01–3.42)	0.046
	OS	2 (45)	47.4	2.12 (0.60–7.50)	0.242
	CTC enrichment-free				
	PFS	5 (300)	0	3.04 (1.83–5.06)	<0.001
	OS	5 (345)	0	3.23 (2.06–5.08)	<0.001

Statistically significant values are indicated in bold.

Abbreviations: CTC, circulating tumor cell; HR, hazard ratio; ICIs, immune checkpoint inhibitors; IF, immunofluorescence; NSCLC, non-small cell lung cancer; OS, overall survival; PD-L1, programmed cell death ligand 1; PFS, progression-free survival.

results were consistent with the findings that post-treatment PD-L1⁺ CTCs were associated with inferior PFS and OS. Therefore, the monitoring of PD-L1 expression on longitudinal CTC samples may help distinguish responders from non-responders and adjust treatment strategies.

Epithelial-mesenchymal transition is considered a pivotal process enabling tumor cells to metastasize, and vimentin is a mesenchymal marker upregulated during EMT.⁵⁴ CTCs may also undergo EMT, and CTCs over-expressing cell surface vimentin (CSV⁺ CTCs) indicates more progressive disease.⁶² Meta-analysis demonstrated that PD-L1⁺ CSV⁺ CTCs were markedly associated with survival outcomes and yielded larger HRs than PD-L1⁺ CTCs with unspecified CSV expression. The combination of these two markers may be potentially used to predict the prognosis of cancer patients.

It should be noted that the prognostic value of PD-L1⁺ CTCs is largely modulated by the comparison models, which has not to be reported by the studies included in our meta-analysis and should raise attention. PD-L1 expression was not associated with survival outcomes

among patients with detectable CTCs, whereas patients with PD-L1⁺ CTCs, in comparison with those without PD-L1⁺ CTC, had prolonged PFS and OS in overall and subgroup analyses, implying that CTCs but not PD-L1 may underlie the association of PD-L1⁺ CTCs with survival. However, further analyses showed no significant correlation between the effect sizes of both markers and even slightly larger effect sizes of PD-L1⁺ CTCs than those of CTCs, indicating an independent prognostic role of PD-L1⁺ CTCs. Nevertheless, the exact role of PD-L1⁺ CTCs modulating response to anti-tumor treatment and survival needs more investigation.

Despite research progress on the clinical relevance of PD-L1-expressing CTCs, some issues are needing to be solved. There is no consensus on CTC enrichment and PD-L1 detection, yet. CellSearch is the only Food and Drug Administration (FDA) approved platform of CTC enrichment and enriches CTCs by epithelial-related markers. But some studies enriched CTCs by size-based platforms or detected CTCs without enrichment. Two studies have detected PD-L1 expression on CTCs enriched by a size-based ISET platform and on matched

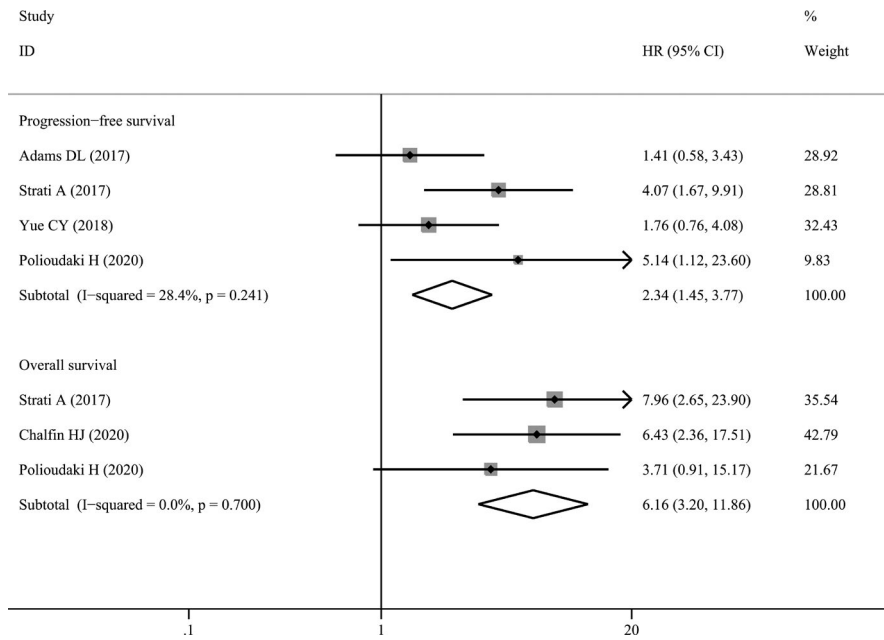


FIGURE 7 Forest plots of post-treatment PD-L1⁺ circulating tumor cells with survival outcomes. PD-L1, programmed cell death-ligand 1

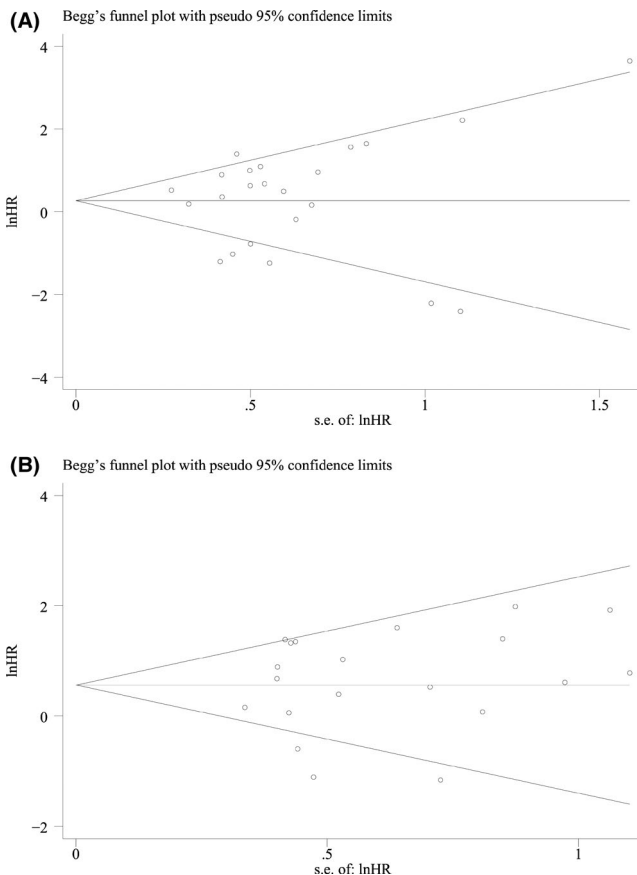


FIGURE 8 Funnel plots of pre-treatment PD-L1⁺ circulating tumor cells with (A) progression-free survival and (B) overall survival. PD-L1, programmed cell death-ligand 1

tumor tissues, and found high concordant PD-L1 classification.^{30,40} The CTC enumeration by Epic platform which detected CTCs without enrichment process was highly

consistent with that by the FDA-approved CellSearch system.⁶³ These results indicate that these non-marker-based platforms are also comparable and feasible for CTC enrichment. Most of the studies detected PD-L1 by IF with different antibodies, and only a few detected mRNA expression but the cut-offs for positive expression differed obviously.^{19,26,35,43,52} Thus, the establishment of standard procedures of CTCs enrichment and PD-L1 detection is in urgent need.

There are some limitations to our study. First, most eligible studies have very small sample sizes. Second, there is obvious heterogeneity in the overall analysis, which may be caused by cancer types, treatments, CTC enrichment and PD-L1 detection methods, cut-offs, and specifically the comparison models. Third, only a few studies were performed in patients undergoing PD-1/PD-L1 blockade therapy. More large-scale studies with patients of various cancers and receiving anti-PD-1/PD-L1 therapy are needed in the future to validate the findings of our meta-analysis.

5 | CONCLUSIONS

In summary, PD-L1⁺ CTCs are associated with better survival prognosis for ICI treatment but poor survival for non-ICI treatment. Thus, PD-L1 expression on CTCs may be potentially used to guide the clinical utility of ICIs in cancer patients, which needs validation in large-scale studies in the future.

CONFLICT OF INTEREST

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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REFERENCE

- Ma W, Gilligan BM, Yuan J, Li T. Current status and perspectives in translational biomarker research for PD-1/PD-L1 immune checkpoint blockade therapy. *J Hematol Oncol*. 2016;9(1):47.
- Meng X, Huang Z, Teng F, Xing L, Yu J. Predictive biomarkers in PD-1/PD-L1 checkpoint blockade immunotherapy. *Cancer Treat Rev*. 2015;41(10):868-876.
- Califano R, Lal R, Lewanski C, et al. Patient selection for anti-PD-1/PD-L1 therapy in advanced non-small-cell lung cancer: implications for clinical practice. *Future Oncol*. 2018;14(23):2415-2431.
- Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373(2):123-135.
- Buttner R, Gosney JR, Skov BG, et al. Programmed death-ligand 1 immunohistochemistry testing: a review of analytical assays and clinical implementation in non-small-cell lung cancer. *J Clin Oncol*. 2017;35(34):3867-3876.
- Kim S, Koh J, Kwon D, et al. Comparative analysis of PD-L1 expression between primary and metastatic pulmonary adenocarcinomas. *Eur J Cancer*. 2017;75:141-149.
- Vilain RE, Menzies AM, Wilmott JS, et al. Dynamic changes in PD-L1 expression and immune infiltrates early during treatment predict response to PD-1 blockade in melanoma. *Clin Cancer Res*. 2017;23(17):5024-5033.
- Wang YD, Yang XY, Jia ZQ, et al. Clinical utility of PD-L1 expression in circulating tumor cells in non-small cell lung cancer patients treated with immunotherapy. *Chin J Clin Thorac Cardiovasc Surgery*. 2021;28:110-115.
- Rossi G, Russo A, Tagliamento M, et al. Precision medicine for NSCLC in the era of immunotherapy: new biomarkers to select the most suitable treatment or the most suitable patient. *Cancers*. 2020;12(5):1125.
- Li X, Zheng Y, Yue F. Prognostic value of soluble programmed cell death ligand-1 (sPD-L1) in various cancers: a meta-analysis. *Target Oncol*. 2021;16(1):13-26.
- Li C, Li C, Zhi C, et al. Clinical significance of PD-L1 expression in serum-derived exosomes in NSCLC patients. *J Transl Med*. 2019;17(1):355.
- Chen G, Huang AC, Zhang W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature*. 2018;560(7718):382-386.
- Theodoraki MN, Yerneni SS, Hoffmann TK, Gooding WE, Whiteside TL. Clinical significance of PD-L1(+) exosomes in plasma of head and neck cancer patients. *Clin Cancer Res*. 2018;24(4):896-905.
- Zhang C, Fan Y, Che X, et al. Anti-PD-1 therapy response predicted by the combination of exosomal PD-L1 and CD28. *Front Oncol*. 2020;10:760.
- Lux A, Kahlert C, Grutzmann R, Pilarsky C. c-Met and PD-L1 on circulating exosomes as diagnostic and prognostic markers for pancreatic cancer. *Int J Mol Sci*. 2019;20(13):3305.
- Wang J, Zeng H, Zhang H, Han Y. The role of exosomal PD-L1 in tumor immunotherapy. *Transl Oncol*. 2021;14(5):101047.
- Mazel M, Jacot W, Pantel K, et al. Frequent expression of PD-L1 on circulating breast cancer cells. *Mol Oncol*. 2015;9(9):1773-1782.
- Khattak MA, Reid A, Freeman J, et al. PD-L1 expression on circulating tumor cells may be predictive of response to pembrolizumab in advanced melanoma: results from a pilot study. *Oncologist*. 2020;25(3):e520-e527.
- Strati A, Koutsodontis G, Papaxoinis G, et al. Prognostic significance of PD-L1 expression on circulating tumor cells in patients with head and neck squamous cell carcinoma. *Ann Oncol*. 2017;28(8):1923-1933.
- Boffa DJ, Graf RP, Salazar MC, et al. Cellular expression of PD-L1 in the peripheral blood of lung cancer patients is associated with worse survival. *Cancer Epidemiol Biomarkers Prev*. 2017;26(7):1139-1145.
- Anantharaman A, Friedlander T, Lu D, et al. Programmed death-ligand 1 (PD-L1) characterization of circulating tumor cells (CTCs) in muscle invasive and metastatic bladder cancer patients. *BMC Cancer*. 2016;16(1):744.
- Satelli A, Batth IS, Brownlee Z, et al. Potential role of nuclear PD-L1 expression in cell-surface vimentin positive circulating tumor cells as a prognostic marker in cancer patients. *Sci Rep*. 2016;6:28910.
- Wang Y, Kim TH, Fouladdel S, et al. PD-L1 expression in circulating tumor cells increases during radio(chemo)therapy and indicates poor prognosis in non-small cell lung cancer. *Sci Rep*. 2019;9(1):566.
- Liu M, Wang R, Sun X, et al. Prognostic significance of PD-L1 expression on cell-surface vimentin-positive circulating tumor cells in gastric cancer patients. *Mol Oncol*. 2020;14(4):865-881.
- Winograd P, Hou S, Court CM, et al. Hepatocellular carcinoma-circulating tumor cells expressing PD-L1 are prognostic and potentially associated with response to checkpoint inhibitors. *Hepatol Commun*. 2020;4(10):1527-1540.
- Tada H, Takahashi H, Kuwabara-Yokobori Y, Shino M, Chikamatsu K. Molecular profiling of circulating tumor cells predicts clinical outcome in head and neck squamous cell carcinoma. *Oral Oncol*. 2020;102:104558.
- Raimondi L, Raimondi FM, Di Benedetto L, Cimino G, Spinelli GP. PD-L1 expression on circulating tumour cells may be predictive of response to regorafenib in patients diagnosed with chemorefractory metastatic colorectal cancer. *Int J Mol Sci*. 2020;21(18):6907.
- Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol*. 2009;62(10):e1-e34.
- Zhang L, Zhang X, Liu Y, et al. PD-L1(+) aneuploid circulating tumor endothelial cells (CTECs) exhibit resistance to the checkpoint blockade immunotherapy in advanced NSCLC patients. *Cancer Lett*. 2020;469:355-366.
- Ilie M, Szafer-Glusman E, Hofman V, et al. Detection of PD-L1 in circulating tumor cells and white blood cells from patients with advanced non-small-cell lung cancer. *Ann Oncol*. 2018;29(1):193-199.
- Khattak MA, Reid A, Freeman J, et al. PD-L1 expression on circulating tumor cells may be predictive of response to

- pembrolizumab in advanced melanoma: results from a pilot study. *Oncologist*. 2020;25:e520.
32. Janning M, Kobus F, Babayan A, et al. Determination of PD-L1 expression in circulating tumor cells of NSCLC patients and correlation with response to PD-1/PD-11 inhibitors. *Cancers*. 2019;11(6):835.
 33. Bao H, Bai T, Takata K, et al. High expression of carcinoembryonic antigen and telomerase reverse transcriptase in circulating tumor cells is associated with poor clinical response to the immune checkpoint inhibitor nivolumab. *Oncol Lett*. 2018;15(3):3061-3067.
 34. Polioudaki H, Mala A, Gkimprixi E, et al. Epithelial/mesenchymal characteristics and PD-L1 co-expression in CTCs of metastatic breast cancer patients treated with eribulin: correlation with clinical outcome. *Cancers*. 2020;12(12):3735.
 35. Tada H, Takahashi H, Kawabata-Iwakawa R, et al. Molecular phenotypes of circulating tumor cells and efficacy of nivolumab treatment in patients with head and neck squamous cell carcinoma. *Sci Rep*. 2020;10(1):21573.
 36. Chalfin HJ, Pramparo T, Mortazavi A, et al. Circulating tumor cell subtypes and T-cell populations as prognostic biomarkers to combination immunotherapy in patients with metastatic genitourinary cancer. *Clin Cancer Res*. 2021;27(5):1391-1398.
 37. Jacot W, Mazel M, Mollevi C, et al. Clinical correlations of programmed cell death ligand 1 status in liquid and standard biopsies in breast cancer. *Clin Chem*. 2020;66(8):1093-1101.
 38. Papadaki MA, Sotiriou AI, Vasilopoulou C, et al. Optimization of the enrichment of circulating tumor cells for downstream phenotypic analysis in patients with non-small cell lung cancer treated with anti-PD-1 immunotherapy. *Cancers*. 2020;12(6):1556.
 39. Bergmann S, Coym A, Ott L, et al. Evaluation of PD-L1 expression on circulating tumor cells (CTCs) in patients with advanced urothelial carcinoma (UC). *Oncoimmunology*. 2020;9(1):1738798.
 40. Cheng Y, Wang T, Lv X, et al. Detection of PD-L1 expression and its clinical significance in circulating tumor cells from patients with non-small-cell lung cancer. *Cancer Manag Res*. 2020;12:2069-2078.
 41. Pinato DJ, Vallipuram A, Evans JS, et al. Programmed cell death ligands expression drives immune tolerogenesis across the diverse subtypes of neuroendocrine tumours. *Neuroendocrinology*. 2020;111:464-474.
 42. Papadaki MA, Koutsopoulos AV, Tsoulfas PG, et al. Clinical relevance of immune checkpoints on circulating tumor cells in breast cancer. *Cancers*. 2020;12(2):376.
 43. Dong J, Zhu D, Tang X, et al. Detection of circulating tumor cell molecular subtype in pulmonary vein predicting prognosis of stage I-III non-small cell lung cancer patients. *Front Oncol*. 2019;9:1139.
 44. Kotsakis A, Kallergi G, Aggouraki D, et al. CD8(+) PD-1(+) T-cells and PD-L1(+) circulating tumor cells in chemotherapy-naive non-small cell lung cancer: towards their clinical relevance? *Ther Adv Med Oncol*. 2019;11:1758835919853193.
 45. Manjunath Y, Upparahalli SV, Avella DM, et al. PD-L1 expression with epithelial mesenchymal transition of circulating tumor cells is associated with poor survival in curatively resected non-small cell lung cancer. *Cancers*. 2019;11(6):806.
 46. Kulasinghe A, Kapeleris J, Kimberley R, et al. The prognostic significance of circulating tumor cells in head and neck and non-small-cell lung cancer. *Cancer Med*. 2018;7(12):5910-5919.
 47. Yue C, Jiang Y, Li P, et al. Dynamic change of PD-L1 expression on circulating tumor cells in advanced solid tumor patients undergoing PD-1 blockade therapy. *Oncoimmunology*. 2018;7(7):e1438111.
 48. Guibert N, Delaunay M, Lusque A, et al. PD-L1 expression in circulating tumor cells of advanced non-small cell lung cancer patients treated with nivolumab. *Lung Cancer*. 2018;120:108-112.
 49. Dhar M, Wong J, Che J, et al. Evaluation of PD-L1 expression on vortex-isolated circulating tumor cells in metastatic lung cancer. *Sci Rep*. 2018;8(1):2592.
 50. Kallergi G, Vetsika EK, Aggouraki D, et al. Evaluation of PD-L1/PD-1 on circulating tumor cells in patients with advanced non-small cell lung cancer. *Ther Adv Med Oncol*. 2018;10:1758834017750121.
 51. Adams DL, Adams DK, He J, et al. Sequential tracking of PD-L1 expression and RAD50 induction in circulating tumor and stromal cells of lung cancer patients undergoing radiotherapy. *Clin Cancer Res*. 2017;23(19):5948-5958.
 52. Zavridou M, Strati A, Bournakis E, Smilkou S, Tserpeli V, Lianidou E. Prognostic significance of gene expression and DNA methylation markers in circulating tumor cells and paired plasma derived exosomes in metastatic castration resistant prostate cancer. *Cancers*. 2021;13(4):780.
 53. Dall'Olio FG, Gelsomino F, Conci N, et al. PD-L1 expression in circulating tumor cells as a promising prognostic biomarker in advanced non-small-cell lung cancer treated with immune checkpoint inhibitors. *Clin Lung Cancer*. 2021:S1525-7304(21)00057-7. doi:https://doi.org/10.1016/j.clcc.2021.03.005.
 54. Mimeault M, Batra SK. Molecular biomarkers of cancer stem/progenitor cells associated with progression, metastases, and treatment resistance of aggressive cancers. *Cancer Epidemiol Biomarkers Prev*. 2014;23(2):234-254.
 55. Alama A, Coco S, Genova C, et al. Prognostic relevance of circulating tumor cells and circulating cell-free DNA association in metastatic non-small cell lung cancer treated with nivolumab. *J Clin Med*. 2019;8(7):1011.
 56. Krebs MG, Sloane R, Priest L, et al. Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. *J Clin Oncol*. 2011;29(12):1556-1563.
 57. Frydenlund N, Mahalingam M. PD-L1 and immune escape: insights from melanoma and other lineage-unrelated malignancies. *Hum Pathol*. 2017;66:13-33.
 58. Qin A, Coffey DG, Warren EH, Ramnath N. Mechanisms of immune evasion and current status of checkpoint inhibitors in non-small cell lung cancer. *Cancer Med*. 2016;5(9):2567-2578.
 59. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378(22):2078-2092.
 60. Dall'Olio FG, Calabrò D, Conci N, et al. Baseline total metabolic tumour volume on 2-deoxy-2-[18F]fluoro-d-glucose positron emission tomography-computed tomography as a promising biomarker in patients with advanced non-small cell lung cancer treated with first-line pembrolizumab. *Eur J Cancer*. 2021;150:99-107.
 61. Seban RD, Mezquita L, Berenbaum A, et al. Baseline metabolic tumor burden on FDG PET/CT scans predicts outcome in advanced NSCLC patients treated with immune checkpoint inhibitors. *Eur J Nucl Med Mol Imaging*. 2020;47(5):1147-1157.
 62. Satelli A, Mitra A, Brownlee Z, et al. Epithelial-mesenchymal transitioned circulating tumor cells capture for detecting tumor progression. *Clin Cancer Res*. 2015;21(4):899-906.

63. Punnoose EA, Ferraldeschi R, Szafer-Glusman E, et al. PTEN loss in circulating tumour cells correlates with PTEN loss in fresh tumour tissue from castration-resistant prostate cancer patients. *Br J Cancer*. 2015;113(8):1225-1233.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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