

# PD-L1 on circulating tumor cells indicates poor prognosis in breast cancer

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## Research article

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# Abstract

## Background

In breast cancer, circulating tumor cells (CTCs) are associated with a poor prognosis, but research concerning programmed cell death protein 1 ligand (PD-L1) on CTCs in breast cancer is limited. This study investigated an association between PD-L1 on CTCs, tumor cells (TCs), or tumor infiltrating immune cells (TILs), respectively, and prognosis and clinicopathological features in breast cancer.

## Methods

Twenty patients with breast cancer were recruited; one was excluded for confirmed lymphoma. The PD-L1 on TCs and TILs was determined via immunohistochemistry (IHC); PD-L1 mRNA expression on CTCs was analyzed. The chi-squared test was used to determine associations between clinicopathological features and PD-L1 on TCs, TILs, and CTCs. Kaplan-Meier and Cox proportional hazards model analyses were applied to compare the survival of patients and related factors.

## Results

The follow-up time was 48 months. Of the 19 patients, 14 had > 1 CTC/10 mL peripheral blood. Among these, each had  $\geq 1$  CTC showing PD-L1. There was no association between the presence of PD-L1 on TCs, TILs, and CTCs and pathology, nor between PD-L1 on TCs and TILs and survival. Patients with high PD-L1 levels on CTCs or higher T status had poor overall survival ( $P = 0.034$  and  $0.003$ , respectively); and these were prognostic factors ( $P = 0.029$ ,  $0.010$ ).

## Conclusion

High levels of PD-L1 on CTCs may be a prognostic factor for shorter survival in breast cancer.

## Background

Breast cancer ranks first in prevalence of malignant tumors in women, worldwide; and in the year 2018, 2.28 million were newly diagnosed [1, 2]. Mortality in patients with breast cancer is mostly due to distant metastasis of circulating tumor cells (CTCs). Some scholars have proposed that this may be related to CTC immunity [3]. The major difference between CTC and tumor cell (TC) immunity is immune escape, i.e., CTCs that leave the primary tumor microenvironment (mainly formed with TCs and tumor infiltrating immune cells TILs) are exposed to the immune microenvironment of nontumor tissue. The number of peripheral immune cells (ICs) far exceeds the number of CTCs, so the circulatory system can be considered an adverse environment for breast cancer cells [4]. While primary tumors release daily a large number of cells into the bloodstream, only CTCs that escape immunity will develop into distant metastases [5–8].

PD-1/PD-L1 (programmed cell death protein 1/PD ligand 1) is considered the key factor in immune escape. PD-1/PD-L1 is widely present in many immune, epithelial, and tumor cells. Drugs relevant to PD-L1 (e.g., pembrolizumab and nivolumab) have shown a significant antitumor effect in a variety of malignant tumors, and are indicated as single-drug treatment in malignant melanoma, non-small cell lung cancer (NSCLC), and others. For the treatment of breast cancer, all of these drugs are in clinical trials [9], but have not significantly improved prognosis, and occasionally have serious side effects. Thus, the best to use when establishing PD-L1 status of patients and prognosis deserves more discussion.

Most studies have focused on PD-L1 on the surface of TCs. However, in clinical practice, immunotherapy after primary tumor resection is the major interest. While the study of PD-L1 on TCs is perforce indirect, it is easier to determine if there is an association between PD-L1 on CTCs and tumor immunity. It is already known that CTCs affect the prognosis of breast cancer, but research on the presence of PD-L1 on CTCs has been rare.

To improve the efficacy of PD-L1 inhibitors and further clarify the mechanism of immune escape mediated by PD-L1, more should be known about PD-L1 on TCs, TILCs, and CTCs in breast cancer. This study investigated an association between PD-L1 on TCs, TILCs, and CTCs and patients' clinicopathological features and prognosis.

## Methods

The patients provided written informed consent prior to enrollment in this observational study. Twenty patients with breast cancer were enrolled at Peking Union Medical College and Hospital in January 2016. Ten of the patients had primary breast cancer and 10 had metastatic breast cancer. For inclusion, the patients were aged 18 to 80 years; and were pre- or post-mastectomy.

Peripheral 10-mL blood samples were collected in EDTA (ethylenediamine tetraacetic acid) tubes by venipuncture and stored at 4 °C until cell isolation, which was all performed within 4 hours. The study cohort included 57 formalin-fixed paraffin-embedded (FFPE) tissue samples from 19 patients. PD-L1 staining was performed on each sample.

The complete clinicopathological data of all the patients were collected, and all the patients were followed up. Data included pathological type, tumor differentiation, T and N stages, estrogen receptor (ER) and progesterone receptor (PR) status, M1, M2, and HER2 (human epidermal growth factor receptor 2).

The end date of follow-up was 18 January 2020. The main endpoint of follow-up was death, up to 48 months. The clinicopathological data of the patients were analyzed, including date of surgery, tumor size, pathological type, lymph node metastasis, immunohistochemistry, clinical stage, and patient follow-up. The assessment of distant metastasis was determined in accordance with the tumor-node-metastasis staging system of the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology [10].

### Isolation of CTCs and expression of PD-L1

Canpatrol CTC assay (SurExam, Guangzhou, China; <http://www.surexam.com/>) was used to isolate and classify CTCs [11]. The protocol of the Canpatrol CTC assay has been described previously [12]. CTCs were epithelial, biophenotypic epithelial/mesenchymal, or mesenchymal. Erythrocyte lysis buffer, provided in the

assay kit, was added to the peripheral blood samples within 4 hours of venipuncture, which were then incubated for 30 min at room temperature. The blood samples were filtered using an 8- $\mu$ m diameter pore-calibrated membrane (EMD Millipore, Billerica, MA, USA) to collect CTCs. The concentrated CTCs were subjected to RNA *in situ* hybridization with a combination of epithelial (EpCAM and CK8/18/19), mesenchymal (vimentin and Twist1), and PD-L1 markers (provided by Canpatrol). CTCs were stained with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at 100 ng/mL for 5 min at room temperature and analyzed with an automated imaging fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany). All CTC subtypes were tested for PD-L1 and counted with a fluorescence microscope.

The amount of PD-L1 on each CTC was judged as none, low, medium, or high, based on the number of indications (as determined by Canpatrol), or scored nil, 1, 2, and 3 points, respectively. The total PD-L1 score for each patient was the sum of the scores on all CTCs. A total PD-L1 score  $\leq 5$  points was considered low, and a total score  $>5$  points was high.

### **PD-L1 expression on tumor tissue**

Four-micron-thick sequential histologic tumor sections were obtained from an archival FFPE representative tumor block and used for analysis via immunohistochemistry (IHC). After deparaffinization and rehydration by transfer through a series of graded concentrations of ethanol to distilled water, slides were incubated for 15 min with 3% H<sub>2</sub>O<sub>2</sub> (Dako, Glostrup, Denmark) for blocking endogenous peroxidase activity. Primary antibody OIT3 (Abxexa, Abx103361), secondary antibody horseradish peroxidase (HRP)-labeled goat anti-mouse/rabbit antibody (Bioworld, BS13278), and (HRP)-labeled streptavidin (Dako, Glostrup, Denmark) were applied; then, 3,3'-diaminobenzidine (DAB, Dako, Glostrup, Denmark) for 10 min as substrate enhancer for 3 min. Counterstaining was performed using hematoxylin (Dako) and the tissue sections were washed (tap water, 5 min). After dehydration, the sections were mounted on slides. A slide of normal tonsil was used as positive control in each IHC run. IHC was performed using antibodies against PD-L1 (clone EPR19759, dilution 1:250; ab213524, Abcam, Cambridge, UK). IHC in tissue samples were evaluated by light microscopy (Nikon DS-U3, Nikon Ci-S, Japan).

Sections were analyzed by two trained observers, who were blinded to all clinical data. For each sample, the tumor compartment was assessed, without considering the staining of stromal cells. For PD-L1, special emphasis was given so that only membrane staining was considered. The patient was classified as positive if at least one sample was positive. The results were interpreted based on published criteria for the combined positive score (CPS), with the tumor proportion score (TPS) and immune proportion score (IPS) included [13-14]. The scores were quantified by evaluating the ratio of PD-L1 positive TCs or TILCs to the number of all viable TCs. Based on the PD-L1 positive cell proportion, 4 categories were distinguished based on staining percentage: 0, none or  $< 1\%$ ; 1, weak or  $1\%$ ; 2, moderate or  $1-49\%$ ; or 3, strong or  $\geq 49\%$ . A percentage of PD-L1 positivity  $\geq 1\%$  was recorded as positive. The PD-L1 scores for TCs and TILCs were determined separately.

### **Statistical analysis**

The chi-squared and Fisher's exact tests were used to analyze the association between the amount of PD-L1 on TCs, TILCs, and CTCs and clinicopathological features. The Kaplan-Meier method and Cox proportional risk model were applied to analyze the factors influencing prognosis. The statistical analyses were 2-sided, and  $P <$

0.05 was statistically significant. Statistical analyses were conducted using SPSS software, version 24.0 (IBM, Armonk, NY, USA).

## Results

### CTC and PD-L1 expression on CTC

Among the 20 patients (all women), one was lost due to a diagnosis of lymphoma, leaving a final study population of 19 patients. Ten patients had received a diagnosis of metastatic breast cancer subsequent to surgery; 9 patients had newly diagnosed breast cancer and were prepared for surgery. The median age at diagnosis was 49 years (range, 24-80 y).

Of the 19 patients, 14 patients had more than one CTC in 10 mL peripheral blood. In each of the 14 patients, PD-L1 was detected on at least one CTC surface (Table 1). The median PD-L1 total score of the 14 patients was 6 (range, 1-44). Given that a total PD-L1 score  $\leq 5$  points was defined as low (including score = nil), and a total score  $> 5$  points as high, in all the 19 patients, 8 (42.11%) patients were high and 11 (57.89%) were low (Figure 1).

### PD-L1 expression on TCs and TILCs

The 19 patients had 57 FFPE tissue samples, and PD-L1 staining was performed on each sample. None of the 19 patients had PD-L1 expression on TCs ( $\geq 1\%$ ) and 15 patients had at least one sample with a positive PD-L1 presence on TILCs (78.95%). No statistically significant correlation was found between PD-L1 rates in TILCs and the clinical pathological characteristics.

### Patients' clinical and pathological characteristics

Among the 19 patients, there were 17 cases of invasive ductal carcinoma, and 1 case each of invasive tubular carcinoma and invasive lobular carcinoma. As of January 2016 (the time of recruitment and baseline), the percentages of patients with stage I, II, III, or IV were 31.58, 5.26, 10.53, and 52.6%, respectively. In January 2016, the 10 patients with metastatic breast cancer were with distant metastasis (timepoint M1). In January 2020, during follow-up, overall 11 patients were with distant metastasis (timepoint M2). The follow up was 48 months. Prognosis duration began with the date of CTC detection (January 2016; Table 2).

There were no associations between PD-L1 expression on TCs, TILCs, or CTCs and clinical pathological characteristics or metastatic status. These lack of associations for PD-L1 on TILCs, or CTCs are reflected by the following P-values, respectively: pathological type ( $P = 0.298, 0.485$ ); tumor differentiation ( $P = 0.740, 1.000$ ); T stage ( $P = 0.383, 0.387$ ); N stage ( $P = 0.111, 1.000$ ); ER status ( $P = 0.422, 1.000$ ); PR status ( $P = 1.000, 1.000$ ); M1 ( $P = 0.211, 1.000$ ); M2 ( $P = 0.228, 0.658$ ); and HER2 ( $P = 1.000, 1.000$ ).

### Survival analysis

The univariate Cox proportional hazards assessment showed that the following were associated with the prognosis of patients: T stage ( $P = 0.003$ , hazard ratio [HR] = 4.017, 95% confidence interval [CI]: 1.600-10.084), and PD-L1 on CTCs ( $P = 0.034$ , HR = 10.284, 95% CI: 1.189-88.918). However, the following showed no

association with prognosis: age ( $P = 0.533$ ); pathological type ( $P = 0.733$ ); tumor differentiation ( $P = 0.168$ ); N status ( $P = 0.251$ ); PD-L1 on TIICs ( $P = 0.924$ ); ER status ( $P = 0.469$ ); PR status ( $P = 0.722$ ); M1 ( $P = 0.139$ ); HER2 ( $P = 0.722$ ); and Ki67 ( $P = 0.774$ ).

Results of the multivariate Cox regression analysis indicated that patients with high levels of PD-L1 on CTCs had a lower overall survival rate ( $P = 0.029$ , HR = 94.618, 95% CI: 1.613-5551.594) compared with patients with low levels (Figure 2). In addition, T stage was a prognostic factor ( $P = 0.01$ , HR = 14.906, 95% CI: 1.905-116.612; Figure 3).

## Discussion

In clinical trials, PD-L1 inhibitors have benefited selected patients. However, targeted molecular therapy requires verified biomarkers to determine indication. Previous research on these issues has led to inconsistent results. For example, Baptista et al. [15] reported that PD-L1 expression emerged as a positive prognostic biomarker in breast cancer, while others disagreed [16]. Furthermore, the best cell type for determining the prognostic and predictive values of PD-L1 in breast cancer has not been established definitively. Herein, PD-L1 on TCs, TIICs, and CTCs were considered for this purpose. The present study found that patients with high levels of PD-L1 on CTCs had a lower overall survival rate. This can serve as a reference for selection of a PD-L1 inhibitor and monitoring during the entire treatment course.

We did not find PD-L1 expression on TCs, which is in accord with Ali et al.'s report (17), but this differed from the percentages found by the IMpassion130 trial (18). This may be due to the small sample size, and including patients with other than triple-negative breast cancer. Some previous studies reported that PD-L1 on TCs in breast cancer indicates a bad prognosis, as it does for NSCLC and melanoma (16,19), although Baptista et al. determined a good prognosis (15). We found no significant association between PD-L1 tissue expression and progression free survival or overall survival.

What is more, a recent study by Guiu et al. reported a 32.1% positive for TC PD-L1 rate and 36.1% positive for CTC PD-L1 rate and showed that the PD-L1 rate on CTCs has been considered a poor independent prognostic indicator in metastatic breast cancer, meanwhile professor Maria reported the same result (20–21), relative to that of TCs. Our results are consistent with these studies.

CTC evaluation appears of interest in this setting, as it probably faithfully reflects the PD-L1 status of the whole metastatic burden, and could represent an easy biomarker throughout the disease evolution. We were able to detect CTCs (whatever their PD-L1 status) in 73.68% of our patients, 31.58% of the patients presenting with 5 or more CTCs, a percentage in line with published MBC series and the published pooled analysis (22). We found an association between baseline CTCs and survival, as classically described in MBC (22–24).

Interestingly, in accord with the present study, Ali et al. (17) showed that PD-L1 protein in breast cancer is rare, but enriched in basal-like tumors and associated with infiltrating lymphocytes. Schott et al. (25) reported a very high number (94.5%) of breast cancer patients who were positive for PD-L1 on CTCs. This suggests that breast cancer cells may have little PD-L1 in the primary tumor but high PD-L1 levels on CTCs. The discrepancy could be due to the mechanism of tumor development, or differences in detection methods, antibodies used, or scoring systems. To date, there is no guideline for antibody selection, not a standard scoring system in breast

cancer. Therefore, the heterogeneity of data must be considered cautiously, when obtained via different means such as the CellSearchVR or Parsortix cell separation systems, or others.

Most of the detection systems have been applied in lung, head, and neck cancers; very few studies have focused on PD-L1 on CTCs of breast cancer. The quantification of PD-L1 on CTCs involves both CTC and PD-L1 detection, respectively. To detect CTCs, available systems include the CellSearch CTC Test (based on positive selection), Parsortix cell separation (physical properties), and isolation by size of epithelial TCs (ISET) (26–27). Combined systems are also widely used (28). It has been shown that Parsortix can improve the total detection rate of CTCs in NSCLC patients, compared to other systems (described above) (29). Some scholars think that this is because of the heterogeneity of some CTCs and the low expression of epithelial cell adhesion molecules (EpCAMs). A cell search based on EpCAMs may not be able to detect some subsets, such as EpCAM-negative CTCs (30, 31).

Considering the advantages of the above detection methods, we chose the Canpatrol CTC typing detection system (Yishan Biotechnology) which combines a variety of technologies to detect the presence of different CTC subtypes, and used nanotechnology to filter after lysis of red blood cells. The markers of epithelial cells (epithelial adhesion molecules and cytokeratins) and interstitial cells (vimentin and twist) were identified by multiple nucleic acid *in situ* hybridization. This not only guaranteed accuracy of the quantity of CTCs detected by the system, similar to that of Parsortix cell separation, but also the specificity by using the characteristics of CTC (32). To detect PD-L1, techniques such as western blot, flow cytometry, immunohistochemistry, and miRNA gene hybridization have been reported. In the present study, *in situ* hybridization of multiple nucleic acids was employed to detect PD-L1 mRNA expression on CTCs, which were counted under a fluorescence microscope (33). Currently there is no standard for CTC detection, but many methods are in the exploration stage.

The present study is the first to evaluate the intensity of PD-L1 on CTCs both qualitatively and quantitatively, as there is no standard method. The only other study concerning the detection of PD-L1 on the surface of breast cancer CTCs employs a PD-L1 immune score. Researchers have scored PD-L1 in SKBR3 TCs and SKBR3 TC clusters quantitatively as nil, 1, or 2, indicating nil, low, and high, respectively (34). In several other studies, more than three CTCs with PD-L1 were recorded as CTC PD-L1-positive. In another study, more than one PD-L1<sup>+</sup> CTC was considered as CTC PD-L1 positivity (28).

The present study clinically evaluated the PD-L1 intensity on CTCs both qualitatively and quantitatively. PD-L1 levels on CTCs was categorized as nil, low, medium, or high based on the number of indications, scored respectively as nil, 1, 2, or 3. For each patient, the total score was the sum of the scores on all CTCs. Thus, a total score of  $\leq 5$  points was considered low PD-L1 levels, while  $> 5$  points was high. So far, only a few studies have analyzed the levels of PD-L1 on CTCs for cancers of the breast (34), head and neck (35–37), prostate (38), and lung (39–40), and other solid tumors. The study that to analyze the levels of PD-L1 on CTCs in patients with breast cancer, and the association between PD-L1 on CTCs in such patients and prognosis is rare. Our research will deeply affect PD-L1<sup>+</sup> CTC research in the future and will be an indicator for monitoring during immune therapy. In most studies of PD-L1 on CTCs, different CTC enrichment and detection techniques, PD-L1 detection methods, and patient cohorts were used. Therefore, the comparison of clinical significance between these studies must be carefully explained, while our findings are consistent with the data of most studies.

Previous relevant studies have varied with regard to the timepoints of detection of PD-L1 on CTCs—for example, before and after surgery, before and after chemotherapy, during treatment, and disease progression. Most of the findings are in accord with ours. For example, after 6 months of treatment, all patients with NSCLC with progressed disease, and those who had died, had PD-L1<sup>+</sup> CTCs, while none of the responding patients showed this positivity (41). This is the evidence that PD-L1<sup>+</sup> CTCs are associated with a bad prognosis and poor response to treatment. In addition, in a study of 35 patients with different gastrointestinal tumors, 95% of those with advanced stage had high PD-L1<sup>+</sup> CTCs (i.e., 18/19 with progression) (42). In the past, NSCLC and head and neck cancers were the most relevant data concerning the significance of PD-L1<sup>+</sup> CTCs. Several studies indicated that PD-1<sup>+</sup> and PD-L1<sup>+</sup> CTCs could be detected in metastatic NSCLC patients before and after first-line chemotherapy. All of the studies show a high number of CTCs positive for PD-1 before treatment and an association with patients' poor prognosis (20, 21).

The current study is limited, in that all the patients received only conventional treatment, and no one received immunotherapy. This is because immune suppressants such as PD-L1 inhibitors had not been approved for breast cancer treatment, and most of them were in clinical trial at the time. In addition, PD-L1 on CTCs should be detected at various treatment timepoints and locations (tumor, lymph node, and site of metastasis), to clarify whether the treatment itself affects the status of PD-L1 on CTCs at different site, and thus prognosis.

Most importantly, this study determined that high levels of PD-L1 on CTCs may be a prognostic factor that can predict a poor prognosis, especially compared with that of TCs and TIICs. Our data warrants a larger cohort in a randomized clinical trial to investigate the value of PD-L1<sup>+</sup> CTCs for predicting response to immunotherapy and its association with prognosis. To continue the analysis of PD-L1 on CTCs as a potential biomarker for breast cancer, large-scale validation research must address the optimal method of collecting CTCs; the detection and evaluation criteria of PD-L1 on CTCs; and the optimal sites and timepoints for monitoring.

## Conclusions

Our data show that it is feasible to detect the PD-L1 status on CTCs at any stage of breast cancer treatment, and this has prognostic significance. High levels of PD-L1 on CTCs can be a prognostic factor. The dynamic monitoring of PD-L1 on CTCs may be a good choice. Future studies may also consider the effect of different levels of PD-L1 on CTCs. This will provide more information about the effect of PD-L1 on TCs in the course of anti-PD-1/PD-L1 treatment.

## Abbreviations

CTCs, circulating tumor cells; TCs, tumor cells; TIICs, tumor infiltrating immune cells; ER, estrogen receptor; PR progesterone receptor; PD-1/PD-L1, programmed cell death protein 1/PD ligand 1; PUMCH, Peking Union Medical College and Hospital; IDC, invasive ductal carcinoma; ITC, invasive tubular carcinoma; ILC, invasive lobular carcinoma.

## Declarations

*Ethics approval and consent to participate*



The protocol was approved by the Ethics Committee of Peking Union Medical College & Hospital (Beijing, China). Informed consent was attained for every patient included in the study.

### ***Consent for publication***

Not applicable

### ***Availability of data and materials***

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

### ***Competing interests***

The authors declare that they have no competing interests.

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### ***Authors' contributions***

WXF, ZGC, and SQ designed/performed most of the investigation, data analysis, and wrote the manuscript. WSF provided pathological assistance. LJX contributed to interpretation of the data and analyses. All of the authors have read and approved the manuscript.

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## Tables

**Table 1.** CTC and PD-L1 basic information in 19 patients with breast cancer \*

	Met **	CTCs, n	PD-L1 levels on CTCs				PD-L1 expression on FFPE		
			Nil	Low	Medium	High	Score	TPS score	IPS score
1	Yes	14	2	3	9	0	21	0	2
2	Yes	25	1	7	14	3	44	0	2
3	Yes	0	0	0	0	0	0	0	0
4	Yes	2	0	2	0	0	2	0	2
5	Yes	0	0	0	0	0	0	0	0
6	Yes	3	0	0	1	2	8	0	2
7	Yes	0	0	0	0	0	0	0	0
8	Yes	0	0	0	0	0	0	1	2
9	Yes	1	0	1	0	0	1	0	0
10	Yes	15	1	6	8	0	22	1	2
11	No	9	1	1	7	0	15	0	2
12	No	8	5	1	2	0	5	0	2
13	No	3	0	0	3	0	6	0	2
14	No	0	0	0	0	0	0	1	3
15	No	8	2	2	4	0	10	0	1
16	No	1	0	1	0	0	1	1	2
17	No	4	0	2	2	0	6	0	2
18	No	1	0	1	0	0	1	0	2
19	No	4	2	1	1	0	3	1	2

\* Data is reported as number (n), unless indicated otherwise

\*\* Met, metastasis on January 2016 (timepoint M1)

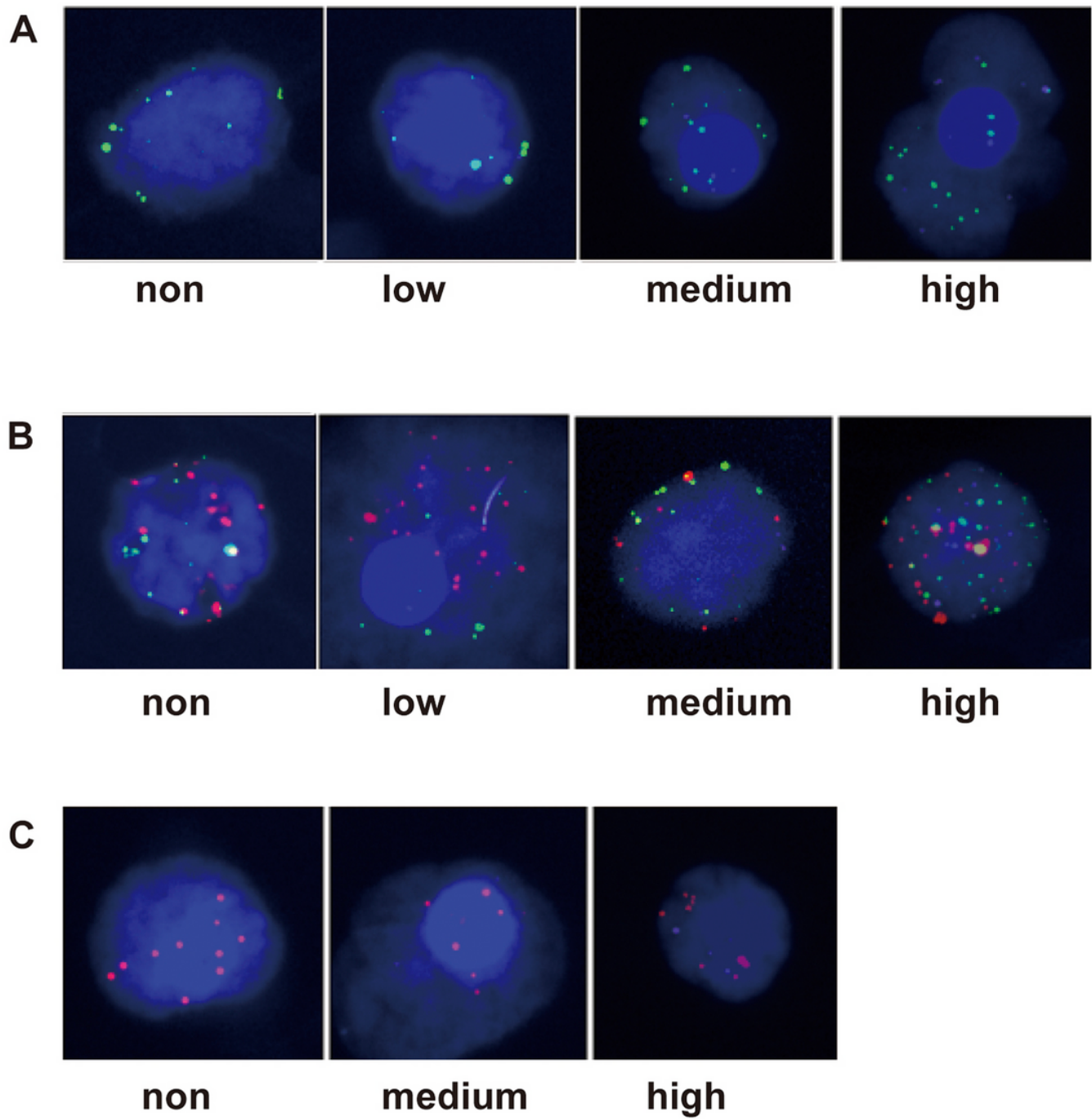
**Table 2.** Clinical and pathological characteristics of the 19 patients.

	Pathology	Differentiation	T	N	M1	M2	ER	PR	HER2	Ki67, %	Death	Months *
1	IDC	Moderately	1	1	1	1	+	+	-	30	Yes	47
2	IDC	Moderately	2	2	1	1	+	-	+	20	Yes	6
3	ITC	Well	1	0	1	1	+	+	-	2	No	48
4	IDC	Moderately	1	0	1	1	+	+	-	40	No	48
5	ILC	Well	3	3	1	1	+	+	-	10	Yes	47
6	IDC	Moderately	2	1	1	1	+	+	-	20	Yes	29
7	IDC	Moderately	1	2	1	1	+	+	-	20	No	48
8	IDC	Moderately	1	1	1	1	+	-	+	10	No	48
9	IDC	Poorly	1	1	1	1	-	-	+	20	No	48
10	IDC	Well	2	1	1	1	+	+	-	1	Yes	11
11	IDC	Poorly	1	0	0	0	+	+	-	90	No	48
12	IDC	Poorly	1	0	0	0	+	+	-	8	No	48
13	IDC	Poorly	1	0	0	0	-	-	-	40	No	48
14	IDC	Moderately	1	0	0	0	+	+	+	5	No	48
15	IDC	Moderately	1	0	0	0	+	+	-	50	No	48
16	IDC	Poorly	1	2	0	0	+	+	-	10	No	48
17	IDC	Poorly	3	2	0	0	+	-	+	75	Yes	10
18	IDC	Poorly	2	1	0	0	-	-	+	80	No	48
19	IDC	Moderately	1	1	0	1	+	+	-	40	No	48

\* Months of follow-up, or if death occurred.

IDC, invasive ductal carcinoma; ITC, invasive tubular carcinoma; ILC, invasive lobular carcinoma.

## Figures



**Figure 1**

PD-L1 intensity (nil, low, or medium) on 3 types of CTCs. (A) Mesenchymal; (B) epithelial-mesenchymal; (C) epithelial.

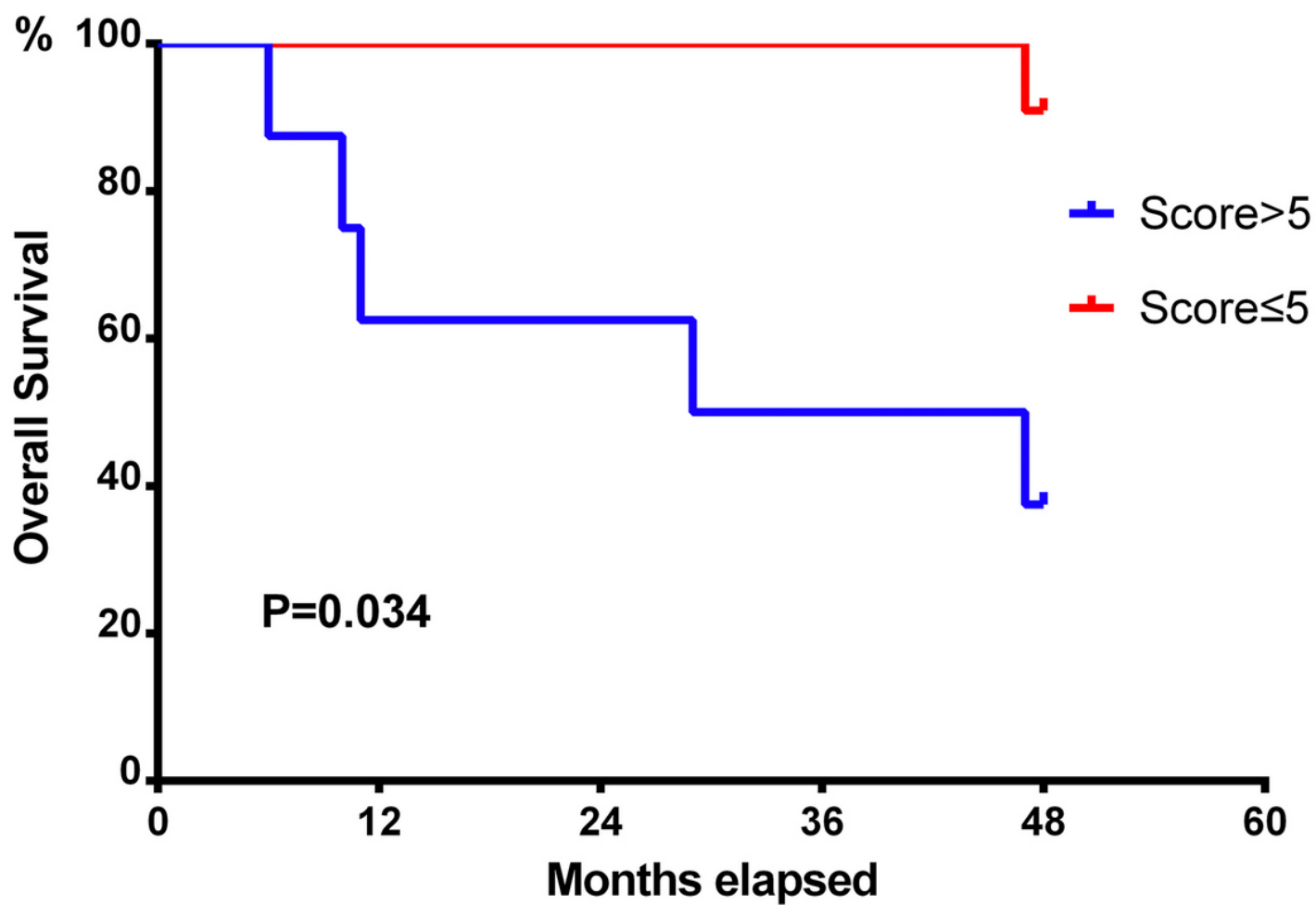


Figure 2

Overall survival analysis of PD-L1 levels on CTC (P = 0.034)

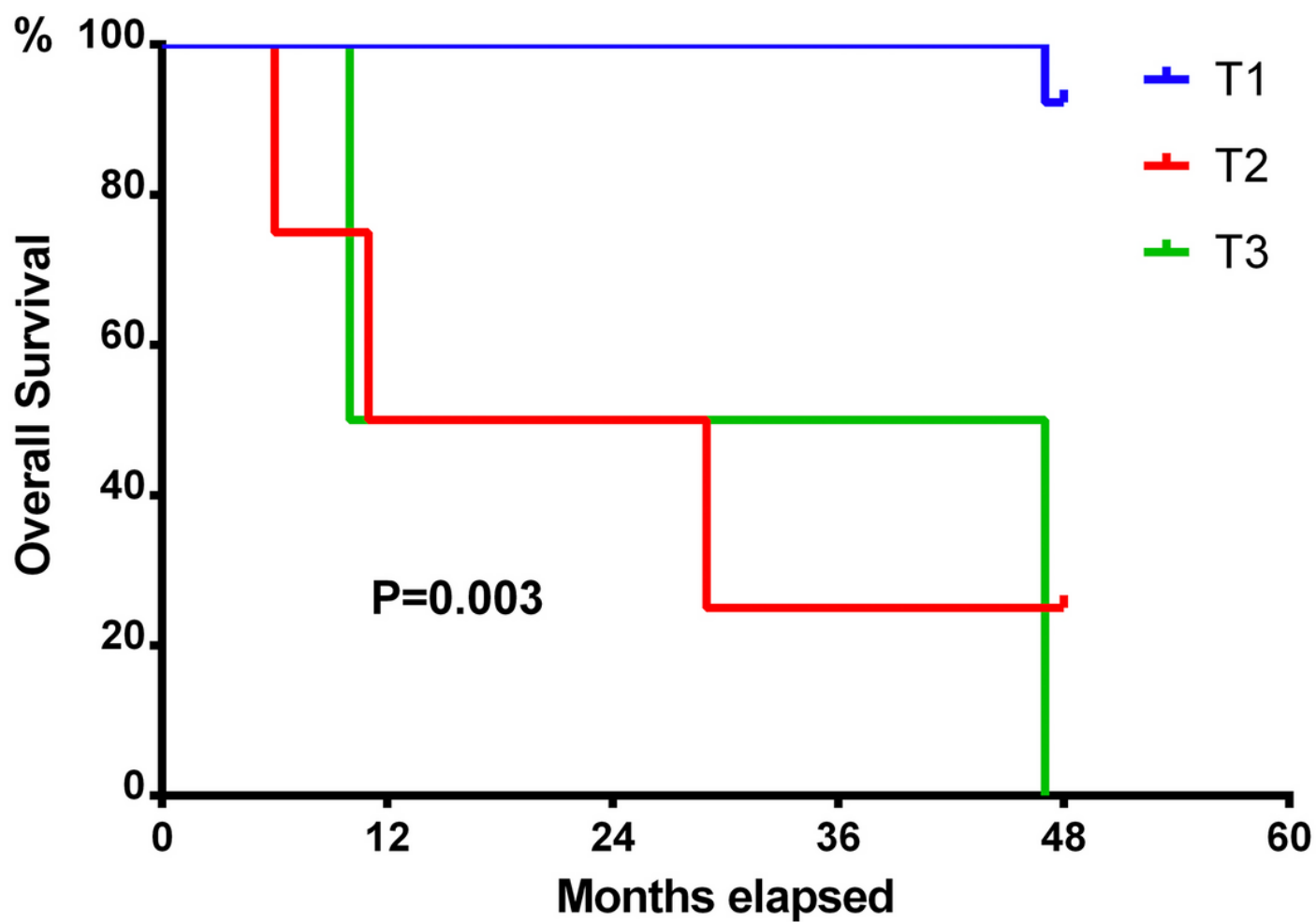


Figure 3

Overall survival analysis of T status (P = 0.003)