

Dynamic Changes in Blood PD-L1 Expression Predict the Efficacy and Overall Survival of Immune Checkpoint Inhibitor Treatment in Non-Small-Cell Lung Cancer Patients

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Research

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Abstract

Background Immune checkpoint inhibitors (ICIs) have become a high-profile treatment regimen for malignancy in recent years. However, only a small piece of patients obtain benefit. How to use reasonable biomarkers to optimally select suitable patients is currently a research hot topic.

Methods Paired tissue samples and blood samples from 51 patients with various malignancies were collected for correlation analysis. Dynamic changes in bPD-L1 expression, including PD-L1 mRNA, soluble PD-L1 (sPD-L1) and exosomal PD-L1 (exoPD-L1) protein, were detected in non-small-cell lung cancer (NSCLC) patients treated with ICIs. The best cutoff values for progression-free survival (PFS) and OS of all three biomarkers were calculated with R software.

Results In 51 malignancies patients, those with positive tPD-L1 expression had significantly higher PD-L1 mRNA expression than those with negative tPD-L1 expression. For sPD-L1 expression, only the TC1~2/IC1~2 group had higher expression than the TC0/IC0 group. In 40 NSCLC patients, those with a fold change (2 months compared to baseline) of PD-L1 mRNA over 2.03 had a better PFS, a better OS and the best objective response (bOR) rate. In addition, a fold change of exoPD-L1 over 1.85 was also found to be associated with better efficacy and OS in a small cohort. Furthermore, the combination of the biomarkers could better screen patients who could benefit from ICI treatment.

Conclusion Blood PD-L1 expression was positively correlated with tPD-L1 expression. Increased expression of exoPD-L1, PD-L1 mRNA, or both during early treatment could serve as biomarkers for predicting efficacy and OS in NSCLC patients treated with ICIs.

Background

Immune checkpoint inhibitor (ICI) treatment has become an increasingly high-profile treatment method for malignancies since it was listed as a top scientific innovation by Science Journal in 2013. Patients with malignancies obtain remarkable survival benefits from ICI treatment, for example, when antibodies against programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) are compared to traditional chemotherapy in non-small-cell lung cancer (NSCLC)^{1,2}. As effective as ICI treatment can be, only 10–40% of patients obtain dramatic responses³, and the five-year overall survival (OS) rate of ICI treatment ranges from 15.5–41% in advanced malignancies^{4–6}. Using single or multiple biomarkers to select patients who could benefit from ICIs or to exclude patients who may not respond to ICIs is the research focus of this study.

To date, various biomarkers, including tumor tissue PD-L1 (tPD-L1) protein expression, tumor mutation burden (TMB), tumor neoantigen burden, microsatellite instability, mismatch repair deficiency, tumor-infiltrating lymphocytes, T-cell receptor clonality, effector T-cell gene signature, DNA damage response and repair genes, intestinal microbiota, driven gene status, and so on, have been demonstrated to be associated with a better response rate and prolonged survival^{7–10}. Moreover, many blood biomarkers,

such as blood TMB (bTMB)¹¹, derived neutrophil/(leukocyte minus neutrophil) ratio¹², and circulating exosomal PD-L1 (exoPD-L1) protein expression¹³, have also been shown to predict efficacy and survival.

In the tumor microenvironment (TME), the PD-L1 protein is expressed on the surface of tumor cells (TCs) or immune cells (ICs). Its binding to PD-1 leads to the impairment of the antitumor function of T cells, similar to a blockade that impairs the flow of a pipeline. Anti-PD-1/anti-PD-L1 therapy could move the blockade away and restore the flow¹⁴. Hence, the detection of pretreatment PD-L1 protein expression on TCs or ICs by immunohistochemistry (IHC) is the most frequently used predictive biomarker in clinical practice. A phase I, multicohort study¹⁵ reported a much higher objective response rate (ORR) in patients with 50% or greater tPD-L1 expression than in patients with other tPD-L1 expression levels. In the KEYNOTE-001 study¹⁶, the clinical benefits, including progression-free survival (PFS) and OS, were enhanced in higher tPD-L1 expression subgroups. In addition, the dynamic changes in tPD-L1 expression help distinguish responders from nonresponders^{17,18}. Multiple PD-L1 IHC tests have been approved by the U.S. Food and Drug Administration for NSCLC, melanoma, bladder cancer, gastric cancer and cervical cancer patients with concomitant or complementary diagnosis, which helps screen suitable patients for ICI treatment. However, in the CHECKMATE-026 study¹⁹, the nivolumab subgroup did not have a PFS benefit compared with the platinum-based chemotherapy subgroup in patients with 5% or higher tPD-L1 expression. Hence, tPD-L1 expression is a controversial predictive biomarker in the clinic. Several factors may explain the reason. First, the complex regulation of PD-L1 protein expression in the TME includes constitutive expression and inducible expression. Constitutive expression may result from the activation of some oncogenic pathways or chromosomal abnormalities^{20,21}. Inducible expression may be induced by the activation of NF- κ B or IFN- γ , which is secreted by infiltrating lymphocytes^{22,23}. Second, there is heterogeneity of PD-L1 protein expression in the TME. Previous treatment had an effect on tPD-L1 expression. A study²⁴ demonstrated that radiotherapy led to an increase in tPD-L1 expression. However, EGFR-TKIs downregulated tPD-L1 expression²⁵. Third, because of the differences and quantitative subjectivity of the PD-L1 detection methods, it is difficult to form a consistent standard to measure the expression of tPD-L1 in the TME. In conclusion, tPD-L1 expression may not be a robust predictive biomarker.

Liquid biopsy is an emerging assay to obtain tumor-related molecular information. The sample sources of liquid biopsy included cerebrospinal fluid, saliva, pleural effusion, blood, ascites, urine, etc. Compared to tissue biopsy, liquid biopsy is noninvasive and convenient, which could help obtain multiple biopsies to monitor the molecular changes during ICI treatment. In addition, liquid biopsy could help to reduce the effect of tumor heterogeneity. Currently, blood-based liquid biopsy is mainly focused on cell-free circulating tumor DNA, circulating tumor cells (CTCs) and exosomes, and limited studies pay attention to blood PD-L1 (bPD-L1) expression. To explore the clinical role of bPD-L1 as a biomarker of ICI treatment, we sought to evaluate the correlation of tPD-L1 expression and bPD-L1 expression and investigated the predictive power of dynamic changes in multimodal bPD-L1 expression in patients who received ICIs.

Materials And Methods

Study design and patients

Paired tumor tissue samples and blood samples as well as clinicopathologic features were obtained from 51 various malignant tumor patients (ClinicalTrials.gov, NCT02890849). Repeated blood samples from forty other locally advanced/metastatic NSCLC patients treated with anti-PD-1/anti-PD-L1 antibody therapy were collected at baseline and at two months after the first intravenous transfusion (ClinicalTrials.gov, NCT03073902). In addition, blood samples from ten healthy donors (HDs) were collected. All patients and HDs provided informed consent. All tissue samples underwent overnight fixation in 10% phosphate-buffered formalin and then were processed and embedded in paraffin blocks for further analysis. All blood samples were centrifuged for 10 minutes at 2000 × g to obtain plasma and then stored at -80°C for further analysis. This study was approved by the ethics committee of the Xinqiao Hospital of Army Medical University (2016-No.054-01, 2017-No.011-01). The best objective response (bOR) to anti-PD-1/anti-PD-L1 antibody treatment was determined by iRECIST²⁶ and included complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). PFS was defined as the time from the first dose of ICI treatment to PD. OS was defined as the time from the first dose of ICI treatment to death for any reason.

PD-L1 IHC staining and scoring

PD-L1 IHC staining was conducted on 3 μm thick sections of formalin-fixed paraffin embedded (FFPE) tumor blocks according to the VENTANA SP142 PD-L1 immunohistochemistry assay (Ventana, AZ, USA). The score of tPD-L1 expression on both TCs and tumor-infiltrating ICs was evaluated by digital image analysis software (Aperio membrane v9 and Aperio Genie Classifier, LEICA CAMERA AG, Wetzlar, Germany). The scoring criteria used were from a previous study²⁷ (TC3, ≥50%; TC2, 5 to < 50%; TC1, ≥1 to < 5%; TC0, <1%; IC3, ≥10%; IC2, ≥5 to < 10%; IC1, ≥1 to < 5%; and IC0, < 1%). Additionally, all patients were divided into three groups according to tPD-L1 expression (TC0/IC0, TC1~2/IC1~2 and TC3/IC3).

Measurement of plasma PD-L1 mRNA

Total RNA was extracted using TRIzol Reagent (Invitrogen, Invitrogen, CA, USA), according to the manufacturer's instructions. After the concentration and purity of the total RNA were determined, reverse transcription was performed using a PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). PLACON (Supplementary Fig. 1), a self-designed novel plasma external reference, was used for amplification and comparison to detect plasma PD-L1 mRNA. The relative expression level of plasma PD-L1 mRNA in tumor patients was calculated by referring to the average expression level of plasma PD-L1 mRNA in 10 HDs samples. The formula is $y = 2^{-(\Delta CT_x - \Delta CT_0)}$. The following primer was used: PD-L1 (Forward: 5'-GCTATGGTGGTGCCGACTAC-3', Reverse: 5'-TTGGTGGTGGTGGTCTTACC-3').

Measurement of soluble PD-L1

Soluble PD-L1 (sPD-L1) expression in plasma was determined using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions. The

expression level of each sample was calculated according to standard curves. All samples were assayed in duplicate, and the mean value of each sample was reported.

Isolation of exosomes from plasma

Stored plasma samples were thawed in a water bath at 25°C. Exosomes were isolated from 200 µL of patient plasma using a Exosome Isolation Kit (Wayen Biotechnologies, Shanghai, China), according to the manufacturer's instructions. Then, isolated exosome samples were immediately stored at -80°C until further analysis.

Verification of isolated exosomes

We randomly selected one isolated exosome sample for verification. First, the size distribution of the isolated exosomes was determined through Nanosight Tracking Analysis (NTA) by utilizing ZetaView (Particle Metrix, Germany). Second, exosome morphology was analyzed by using transmission electron microscopy (TEM) (Tecnai G2 spirit BioTwin, FEI, USA). Third, exosomal proteins were subjected to SDS-PAGE followed by Western blotting (WB). The nitrocellulose membranes were blocked with 5% nonfat milk for 60 minutes at room temperature and incubated overnight at 4°C with the corresponding primary antibodies at dilutions recommended by the suppliers, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 1 hour. The blots were developed with enhanced chemiluminescence (ECL) Pierce™ detection reagents (Thermo Scientific). CD63, CD9, and calnexin were used as exosome markers. Finally, immunoreactive proteins were visualized using a chemiluminescence detection system (FluorChem HD2, USA).

Measurement of exoPD-L1

Exosomal PD-L1 protein was measured with a Simoa™ NF-light Reagent Kit (Quanterix Corp, Lexington, MA). In short, all isolated exosome samples were loaded at a mass of 280 µg and then diluted with sample diluent to 130 µL for single-well detection. Standard samples were added to a 96-well plate. After the completion of the sample preparation, beads, detector, and SBG were loaded into the reagent holder, and RGP was loaded into the tube holder. Then, the sample was transferred to the Simoa Disc, using oil to seal the sample so that the signal was only in the well. Finally, pictures were taken, and the concentration was analyzed on a Simoa HD-1 platform (Quanterix Corp).

Statistical analysis

The difference in PD-L1 mRNA and sPD-L1 expression in different subgroups was calculated by using independent-samples t-test. The difference in tPD-L1 expression and bOR in different subgroups was calculated by using Pearson's chi-square test or Fisher's exact test. SPSS version 23.0 (IBM, Armonk, NY, USA) was used for performing these statistical analyses. The 'survival' and 'survminer' packages from R software (version 3.5.2) were used for calculating the best cutoff point of each biomarker, conducting statistical calculations, and drawing Kaplan–Meier curves. A two-sided P value < 0.05 was considered statistically significant.

Results

Clinicopathologic features, tPD-L1 expression and bPD-L1 expression in 51 patients with various malignancies

Fifty-one patients with various malignancies were enrolled, including 33 NSCLC patients. Of these patients, 26 were less than 60 years old, 31 were male, 21 had a smoking history, and 33 had metastasis at biopsy (Table 1, see details in Supplementary Table 1). Furthermore, the differences in tPD-L1 and bPD-L1 expression between each subgroup were analyzed. In the overall population, no significant differences were found between subgroups (Supplementary Fig. 2). In 33 NSCLC patients, male patients had a higher PD-L1 mRNA expression than female patients. Patients with a smoking history had higher PD-L1 mRNA expression than those without a smoking history (Supplementary Fig. 3a). No differences were found between patients younger than 60 and older than 60 or between patients with metastasis and without metastasis. In addition, the expression levels of tPD-L1 and sPD-L1 showed no significant differences in each subgroup (Supplementary Fig. 3b, 3c).

Table 1
Baseline clinicopathological features of 51 patients with diverse malignancies.

Clinicopathologic feature	Number of patients (%)
Age	
< 60	26 (51%)
≥ 60	25 (49%)
Gender	
Male	31 (61%)
Female	20 (39%)
Smoking history	
Yes	21 (41%)
No	30 (59%)
Tumor type	
NSCLC	33 (65%)
Others	18 (35%)
Distant metastasis	
Yes	33 (65%)
No	18 (35%)
NSCLC, non-small cell lung cancer.	

In the overall population, the PD-L1 mRNA expression was higher in both the TC3/IC3 group ($p = 0.036$, Fig. 1a) and the TC1 ~ 2/IC1 ~ 2 group ($p = 0.026$, Fig. 1a) than in the TC0/IC0 group. There was also a trend that the TC3/IC3 group had a higher PD-L1 mRNA expression than the TC1 ~ 2/IC1 ~ 2 group ($p = 0.083$, Fig. 1a). For sPD-L1, only the TC1 ~ 2/IC1 ~ 2 group had significantly higher expression than the TC0/IC0 group ($p = 0.023$, Fig. 1b). No differences were found between the other groups. In the 33 NSCLC patients, there was a trend that patients with positive tPD-L1 expression had higher PD-L1 mRNA expression (Fig. 1c). The expression of sPD-L1 did not correlate with the PD-L1 mRNA expression (Fig. 1d).

Dynamic changes in bPD-L1 in 21 NSCLC patients treated with ICIs

Multimodal bPD-L1 expression detection, including PD-L1 mRNA, sPD-L1 and exoPD-L1, were performed in 21 locally advanced or metastatic NSCLC patients treated with ICIs. Only three out of twenty-one

patients had decreased exoPD-L1 expression levels at 2 months compared to baseline expression levels (Fig. 2a), and the fold change ranged from 0.40 to 113.76 times. Fifteen patients had increased PD-L1 mRNA expression, while the other six patients had decreased PD-L1 mRNA expression (Fig. 2b); the fold change ranged from 0.11 to 55.72 times. For sPD-L1 expression, nine patients had increased sPD-L1 expression, while the other twelve patients had decreased sPD-L1 expression (Fig. 2c); the fold change ranged from 0.54 to 4.72 times. An overview of the fold changes of all three kinds of bPD-L1 expression is shown in Fig. 2d.

Dynamic changes in PD-L1 mRNA expression served as a marker for predicting efficacy and OS

To explore the role of dynamic changes in PD-L1 mRNA expression in predicting efficacy and OS, we expanded the sample size into 40 NSCLC patients. According to iRECIST, 8 patients had PD; 11 had PR; 21 had SD; and no patients had CR. bPD-L1 mRNA expression levels at baseline and at 2 months were detected. The best cutoff value for fold change of PD-L1 mRNA expression was 2.03. The median PFS was 4.2 months (95% confidence interval [CI] 0.2–8.2) in patients with a fold change \leq 2.03. It was 10.0 months (95% CI 3.6–10.4) in patients with a fold change $>$ 2.03. The hazard ratio (HR) was 0.373 (fold change $>$ 2.03 vs. fold change \leq 2.03, 95% CI 0.174–0.797, $p = 0.011$) (Fig. 3a). The median OS was 7.0 months (95% CI 3.6–10.4) in patients with a fold change \leq 2.03 and 19.0 months (95% CI 9.1–28.9) in patients with a fold change $>$ 2.03 (HR 0.281, 95% CI 0.119–0.666, $p = 0.004$) (Fig. 3b). The bOR rate was 10.5% in patients with a fold change \leq 2.03 compared with 42.9% in patients with a fold change $>$ 2.03 ($p = 0.022$) (Fig. 3c).

Dynamic changes in exoPD-L1 and sPD-L1 served as markers for predicting efficacy and OS

To verify the isolated exosomes, TEM, NTA and WB were conducted. As shown in Supplementary Fig. 4a, the obtained exosomes had a distinctive cup shape. Then, positive marker proteins of exosomes, CD3 and CD69, were found in WB (Supplementary Fig. 4b). A negative marker protein, calnexin, was not found (Supplementary Fig. 4b). The size of exosomes ranged from 20 nm to 200 nm, and the average size was 117.5 nm (Supplementary Fig. 4c).

We conducted efficacy and OS analyses according to fold changes of exoPD-L1 and sPD-L1 expression. For exoPD-L1, patients with a fold change over 1.85 at 2 months compared to baseline had better PFS (9.9 vs. 4.3 months, $p = 0.001$; HR 0.165, 95% CI 0.052–0.525, $p = 0.002$) and OS (13.7 vs. 6.3 months, $p = 0.004$; HR 0.237, 95% CI 0.082–0.684, $p = 0.008$) as well as a higher bOR rate (33.3% vs. 11.1%, $p = 0.338$) (Fig. 4a-4c). For sPD-L1, no best cutoff point was found. The PFS, OS and bOR rates showed no differences (Fig. 4d-4f).

The combination of multimodal bPD-L1 expression served as a marker for predicting efficacy and OS

Since the fold changes of PD-L1 mRNA and exoPD-L1 were significant biomarkers, we conducted an analysis by combining the two biomarkers. Better PFS and OS were found in the combined high group compared with the single high group or the combined low group (PFS 11.2 vs. 7.0 vs. 3.2 months, $p <$

0.001; OS 22.0 vs. 13.0 vs. 4.0 months, $p < 0.001$) (Fig. 5a, 5b). The bOR rate in the combined high group and single high group was higher than that in the combined low group (33.3% vs. 33.3% vs. 0%, $p = 0.269$) (Fig. 5c).

Discussion

In this study, we identified the correlation among tPD-L1, bPD-L1 and clinicopathologic features in 51 patients with various malignancies. Then, we explored the predictive power of multimodal bPD-L1 expression, including exoPD-L1, PD-L1 mRNA and sPD-L1, in NSCLC patients treated with ICIs.

Our results demonstrated that patients with positive tPD-L1 expression had higher PD-L1 mRNA and sPD-L1 expression in plasma, which implied that bPD-L1 expression had a positive correlation with tPD-L1 expression at the same time points. Obviously, the acquisition of bPD-L1 expression is much more convenient, less expensive and less invasive and therefore could help dynamically monitor PD-L1 changes during ICI treatment.

Tumor-derived exosomes are extracellular vesicles with bilayer lipid membranes that carry many bioactive molecules. Tumor-derived exosomes are considered to be a key messenger in tumor progression and metastasis²⁸. Not surprisingly, the PD-L1 protein was found on the surface of tumor-derived exosomes²⁹. In vivo and in vitro³⁰, exoPD-L1 suppressed the function of T cells by binding to PD-1. Furthermore, PD-L1-positive exosomes could spread directly from the TME to the whole body to induce systemic immunosuppression. Exosomal PD-L1 exhibits the potential to serve as a biomarker in the clinic. In a cohort of 44 melanoma patients treated with pembrolizumab¹³, pretreatment exoPD-L1 expression was lower in responders than in nonresponders. In addition, pretreatment exoPD-L1 expression was positively correlated with circulating IFN- γ expression and overall tumor burden. In contrast, patients with an elevated exoPD-L1 expression fold change over 2.43 during early treatment compared to pretreatment exoPD-L1 expression had a much higher ORR. In our work, we also found an increased fold change (> 1.85) of exoPD-L1 in early treatment indicated better efficacy and OS in NSCLC patients.

The source and regulation of sPD-L1 remain unclear. A paper³¹ reported that sPD-L1 might be derived from TCs and retained the PD-1-binding domain. Plasma sPD-L1 could systemically impair host immunity and promote tumor progression. Zhou et al³² reported that malignant melanoma patients with higher pretreatment sPD-L1 expression were more likely to progress when treated with ICIs. Although early change in sPD-L1 showed no correlation with response, increased sPD-L1 expression over a 1.5-fold change at five months showed a positive correlation with PR. Okuma et al³³ reported that a higher baseline sPD-L1 expression was negatively associated with OS and ORR in NSCLC patients receiving nivolumab. In this study, the fold change of sPD-L1 in early ICI treatment showed no correlation with efficacy and OS.

Our study first demonstrated that plasma PD-L1 mRNA could predict the efficacy and survival in NSCLC patients treated with ICIs. The preliminary results were first published in the abstracts of the 2019 World Conference on Lung Cancer³⁴, which showed that in 21 NSCLC patients treated with ICIs, those with a fold change of PD-L1 mRNA over 2.03 at 2 months achieved better efficacy and OS. Then, we expanded the sample size to 40 patients and extended the follow-up time, and we still found the same conclusion. Inconsistent with our work, a study reported that decreased exosomal PD-L1 mRNA expression at 2 months was correlated with response to anti-PD-1 treatment, while increased expression was correlated with progression³⁵.

Additionally, tPD-L1 expression in the TME increased at early treatment in patients who responded to ICIs^{17, 18}. In addition, more ICs, especially CD8⁺ T cells, were found in responders. All these data suggested that in the early stage of ICI treatment, both tPD-L1 and bPD-L1 expression could increase. The underlying mechanism may be that higher levels of PD-L1 were expressed on TCs to cause a feedback and T-cell reinvigoration when patients responded to ICI treatment; however, this system is useless because ICIs block the interaction of PD-1 and PD-L1.

Furthermore, our work demonstrated that the combination of exoPD-L1 and PD-L1 mRNA in plasma could better determine NSCLC subgroups who may benefit from ICI treatment. Patients with both a fold change of exoPD-L1 over 1.85 and a fold change of PD-L1 mRNA over 2.03 had the best efficacy and OS.

To the best of our knowledge, this is the first report of changes in exoPD-L1 and PD-L1 mRNA to predict the efficacy of ICI treatment. Dynamic liquid biopsy of multimodal PD-L1 is a good way to occasionally monitor patients during ICI treatment. Our findings have crucial clinical significance. First, we may know which kind of patients would benefit more from ICI treatment and which patient subgroups may not have a long OS. Second, in clinical work, we may consider more frequent imaging examinations for those patients who have lower fold changes in exoPD-L1 and PD-L1 mRNA during early treatment. Third, these two biomarkers could predict potential progress approximately two months earlier than computed tomography imaging. Some interventions, such as the addition of chemotherapy, local radiotherapy, antivascular drugs, and multitarget inhibitors, could be used earlier before imaging progress.

In addition to the bPD-L1 indexes demonstrated in our study, bPD-L1 was also found on the surface of CTCs in peripheral blood^{36, 37}. Nicolazzo et al³⁸ continuously monitored PD-L1 expression on CTCs from baseline to 6 months in 24 advanced NSCLC patients treated with nivolumab. The results showed that those with continuous PD-L1 expression obtained PD, while those with negative PD-L1 expression at 6 months obtained clinical benefit. Another work³⁹ also supported that continuous PD-L1-positive CTCs were associated with poor prognosis. Yue et al³⁹ demonstrated that patients with a higher ratio of baseline PD-L1^{high} CTCs (abundance over 20%) had an obviously higher disease control rate and longer PFS than other patients. Meanwhile, reduced PD-L1^{high} CTCs at 9 weeks were observed in most patients with disease control.

There are some limitations in our work. The sample size is small in this trial. In the future, we plan to design a prospective clinical trial to confirm this conclusion. We did not include early-stage NSCLC patients. Thus, we do not know if bPD-L1 is an efficacy biomarker for presurgery neoadjuvant ICI treatment, postsurgery adjuvant ICI treatment or consolidation ICI treatment following concurrent chemoradiotherapy in locally advanced, unresectable stage NSCLC patients. All these questions could be explored and solved in future studies.

Conclusion

In summary, bPD-L1 expression has a positive correlation with tPD-L1 expression in malignant tumors. Increased fold change of exoPD-L1 expression or PD-L1 mRNA expression in plasma during early treatment could predict efficacy and survival. In particular, the combination of exoPD-L1 and PD-L1 mRNA could better screen patients who could benefit from ICI treatment. Our work offers a point of view that the dynamic changes in plasma exoPD-L1 and PD-L1 mRNA during early treatment could serve as biomarkers for predicting survival and monitoring efficacy in NSCLC patients treated with ICIs.

List Of Abbreviations

ICI – immune checkpoint inhibitor;

PD-L1 – programmed cell death ligand 1;

OS – overall survival;

bTMB – blood TMB;

TME – tumor microenvironment;

IC – immune cell;

ORR – objective response rate;

CTC – circulating tumor cell;

HD – healthy donor;

CR – complete response;

SD – stable disease;

sPD-L1 – soluble PD-L1;

TEM – transmission electron microscopy;

CI – confidence interval;

PD-1 – programmed cell death 1;
NSCLC – non-small-cell lung cancer;
tPD-L1 – tissue PD-L1;
exoPD-L1 – exosomal PD-L1;
TC – tumor cell;
IHC – immunohistochemistry;
PFS – progression-free survival;
bPD-L1 – blood PD-L1;
bOR – best objective response;
PR – partial response;
PD – progressive disease;
NTA – nanosight tracking analysis;
WB – western blotting;
HR – hazard ratio.

Declarations

Ethics approval and consent to participate:

This study was approved by the ethics committee of the Xinqiao Hospital of Army Medical University (2016-No.054-01, 2017-No.011-01). All patients and healthy donors provided informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current 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

Contributions to the conception: Luping Zhang, Jianguo Sun;

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The creation of new software used in the work: Feng Li, Lingxin Meng;

Draft the work and substantively revised it: Qiao Yang, Mingjing Chen, Jiaoyang Gu, Luping Zhang, Jianguo Sun.

All authors have approved to submit this study and any substantially modified version that involves the author's contribution to the study. And all authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Figures

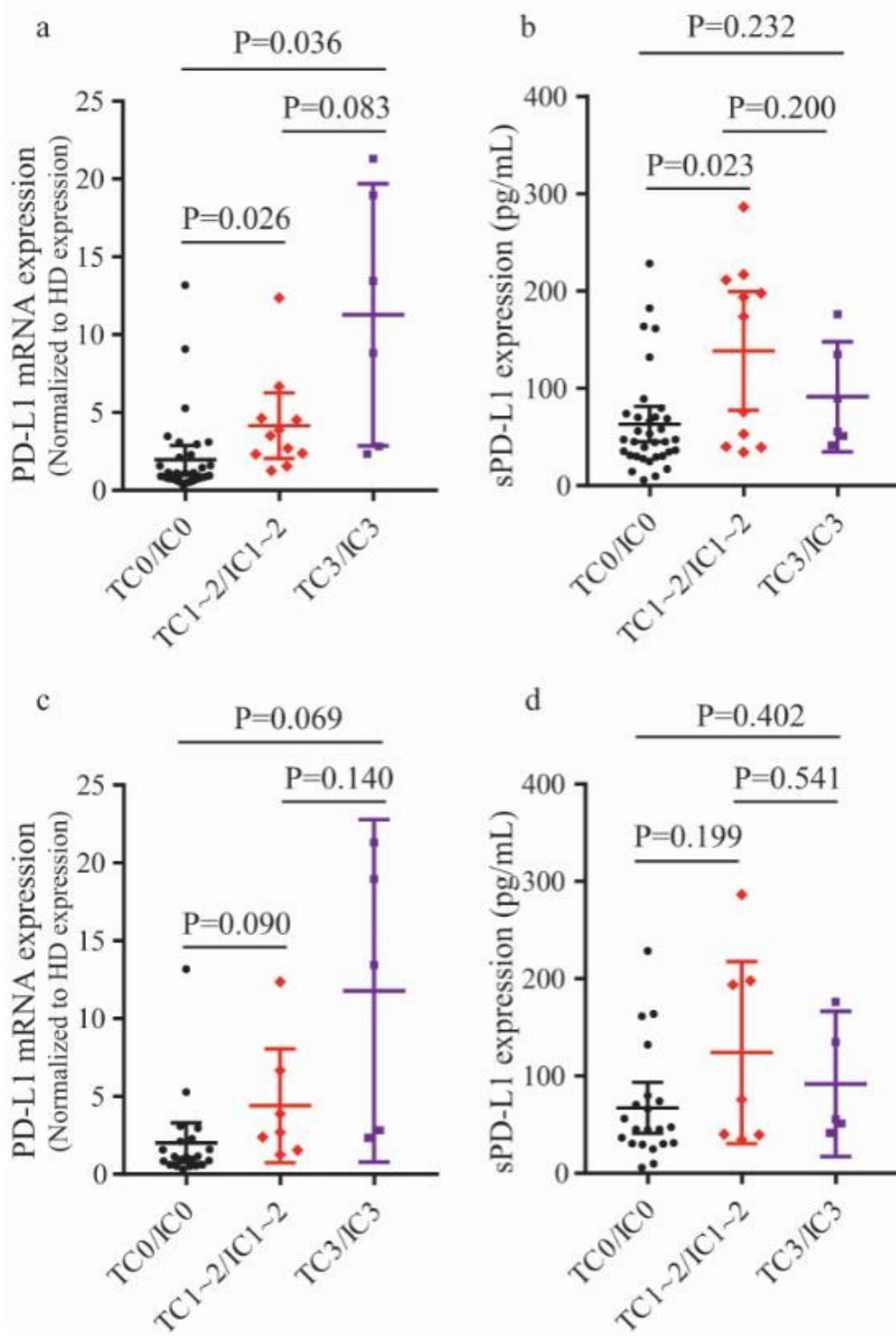


Figure 1

The correlation of tPD-L1 and bPD-L1. a, The correlation of PD-L1 mRNA and tPD-L1 in the overall population. b, The correlation of sPD-L1 and tPD-L1 in the overall population. c, The correlation of PD-L1 mRNA and tPD-L1 in 33 NSCLC patients. d, The correlation of sPD-L1 and tPD-L1 in 33 NSCLC patients. tPD-L1, tissue PD-L1; bPD-L1, blood PD-L1; sPD-L1, soluble PD-L1; NSCLC, non-small-cell lung cancer.

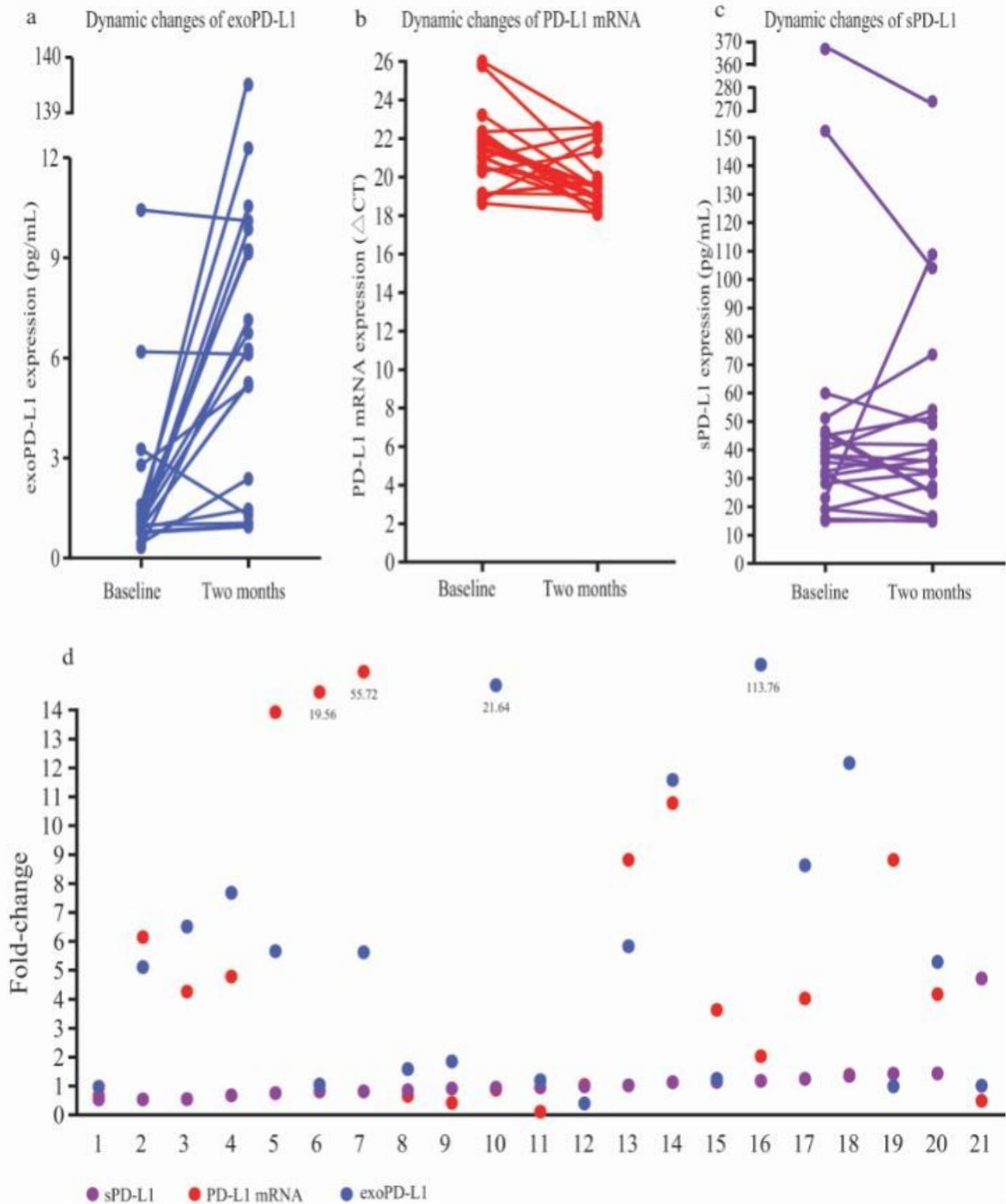


Figure 2

Dynamic changes in multimodal bPD-L1 expression during early treatment. a, Dynamic changes in exoPD-L1. b, Dynamic changes in PD-L1 mRNA (CT values). c, Dynamic changes in sPD-L1. d, An overview of fold changes of the three biomarkers. bPD-L1, blood PD-L1; exo-PD-L1, exosomal PD-L1; CT, cycle threshold; sPD-L1 soluble PD-L1.

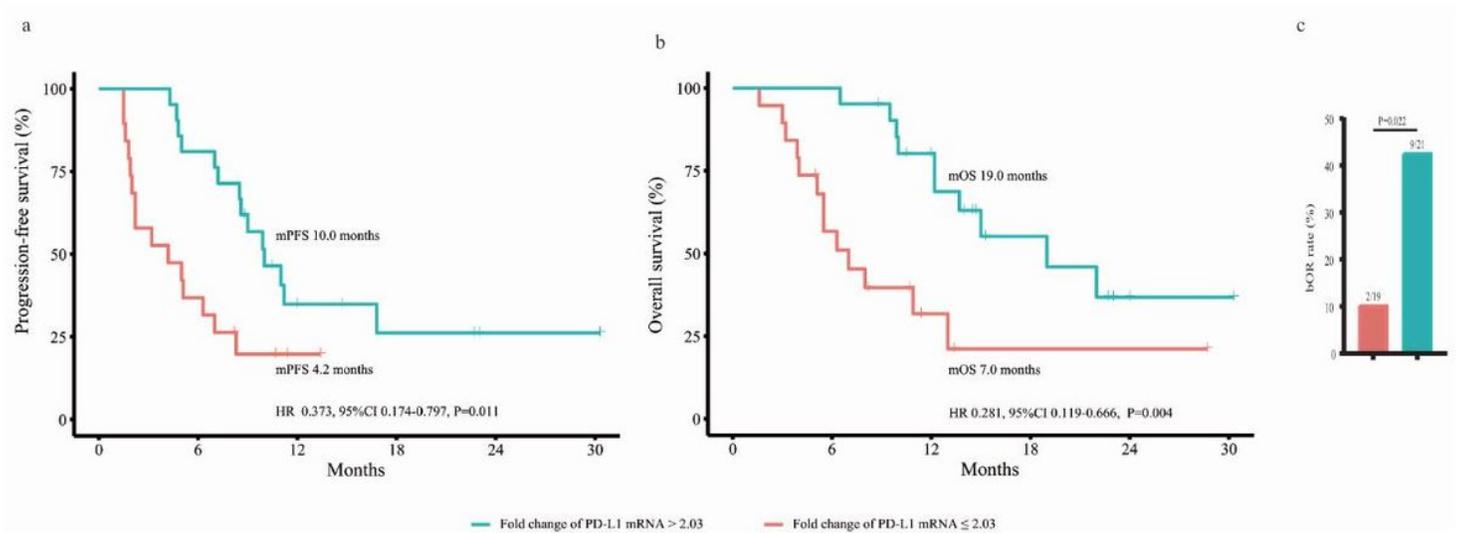


Figure 3

Dynamic change in PD-L1 mRNA expression for predicting efficacy and OS. a, PFS analysis based on PD-L1 mRNA expression. b, OS analysis based on PD-L1 mRNA expression. f, bOR rate differences based on PD-L1 mRNA expression. OS, overall survival; PFS, progression-free survival; bOR, best objective response.

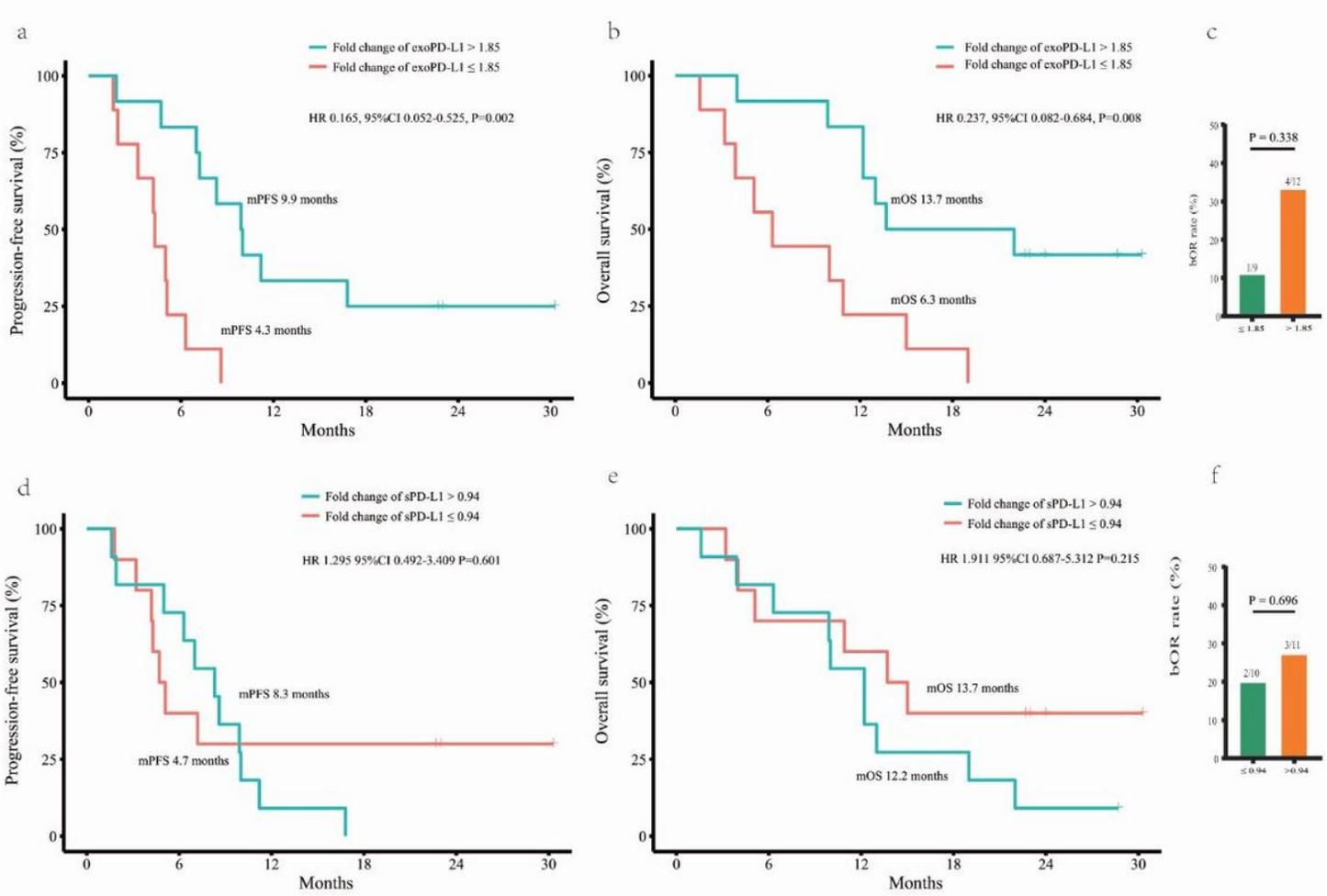


Figure 4

Efficacy and OS analyses based on fold change of exoPD-L1 and sPD-L1 expression. a, PFS analysis based on fold change of exoPD-L1 expression. b, OS analysis based on fold change of exoPD-L1 expression. c, bOR rate difference based on fold change of exoPD-L1 expression. d, PFS analysis based on fold change of sPD-L1 expression. e, OS analysis based on fold change of sPD-L1 expression. f, bOR rate difference based on fold change of sPD-L1 expression. OS, overall survival; exoPD-L1, exosomal PD-L1; sPD-L1, soluble PD-L1; PFS, progression-free survival; bOR, best objective response.

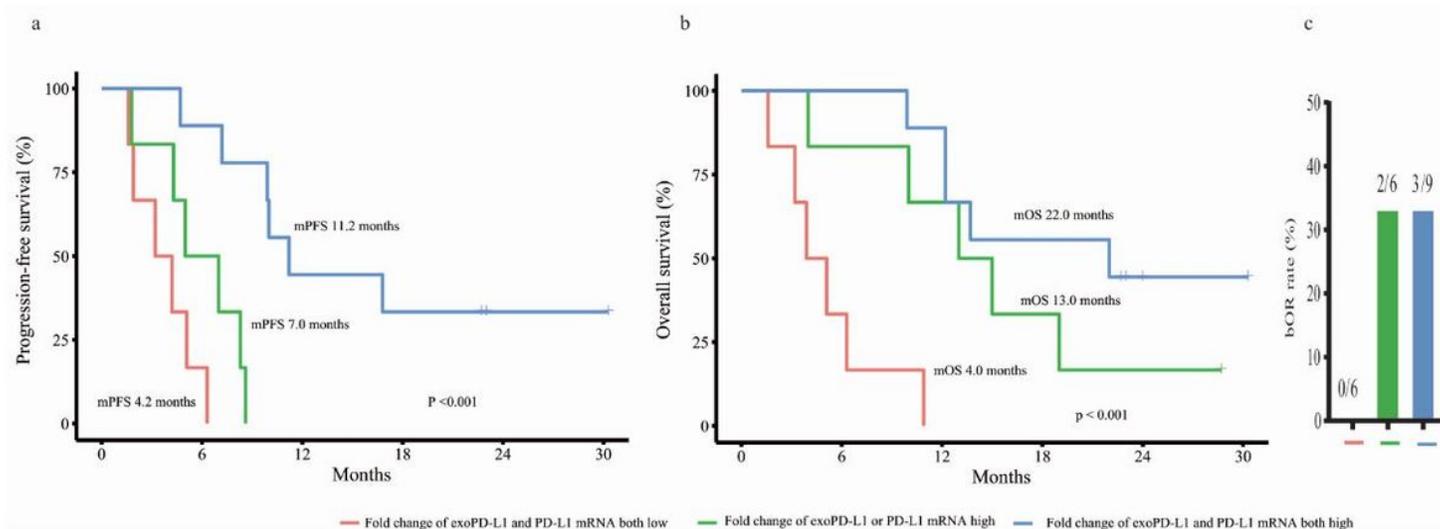


Figure 5

Efficacy and OS analyses based on the combination of PD-L1 mRNA and exoPD-L1 expression. a, PFS analysis based on the combination of two biomarkers. b, OS analysis based on the combination of two biomarkers. c, bOR rate difference based on the combination of two biomarkers. OS, overall survival; exoPD-L1, exosomal PD-L1; PFS, progression-free survival; bOR, best objective response.

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