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# Serum Cell Division Cycle 42 before and after Programmed Death-1 Inhibitor Therapy in Advanced Melanoma Patients: Correlation with Tumor Features, Clinical Response, and Survival

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Cell division cycle 42 (CDC42) mediates immune escape in cancers. This study aimed to investigate linkages of CDC42 with tumor features, treatment response, and survival in advanced melanoma patients receiving programmed death-1 (PD-1) inhibitors. Pre-treatment and post-treatment (after 2 cycles) serum CDC42 of 35 advanced melanoma patients receiving PD-1 inhibitor was assessed by enzyme-linked immunosorbent assay. Patients with tumor-node-metastasis (TNM) stage IV (vs. III) ($P$ = 0.050) and abnormal (vs. normal) lactate dehydrogenase (LDH) ($P$ = 0.022) had higher pre-treatment CDC42. After 2-cycle therapy, CDC42 was declined ($P$ < 0.001). Objective response and disease control rates were 34.3% and 62.9%, respectively. Additionally, pre-treatment and post-treatment CDC42 was reduced in patients with objective response and disease control than those without (all $P$ < 0.050). Concerning survival, pre-treatment with CDC42 > 700 pg/mL was associated with shorter progression-free survival (PFS) ($P$ = 0.013), but not overall survival (OS) ($P$ = 0.060). Specifically, the 12-month PFS rate was 26.7% and 66.2%, and the 12-month OS rate was 61.1% and 82.5% in patients with pre-treatment with CDC42 > 700 pg/mL and ≤ 700 pg/mL, respectively. Post-treatment with CDC42 > 700 pg/mL was correlated with shortened PFS ($P$ = 0.010) and OS ($P$ = 0.006). The 12-month PFS rate was 12.5% and 62.0%, and the 12-month OS rate was 42.3% and 88.0% in patients with post-treatment with CDC42 > 700 pg/mL and ≤ 700 pg/mL, accordingly. Furthermore, post-treatment with CDC42 > 700 pg/mL was independently related to PFS [hazard ratio (HR): 2.704, $P$ = 0.029 and OS (HR: 7.749, $P$ = 0.005)]. Elevated CDC42 correlates with advanced TNM, abnormal LDH, worse clinical response, and dismal survival in advanced melanoma patients receiving PD-1 inhibitors.

**Keywords:** advanced melanoma; cell division cycle 42; clinical response and survival; programmed death-1 inhibitor; tumor feature

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## Introduction

Melanoma attacks 300,000 people and causes nearly 60,000 deaths worldwide annually (Sung et al. 2021). In China, though the incidence of melanoma is relatively low, it is growing during the past decade and poses a threat to public health as well as a heavy economic burden (Cao et al. 2021; Zhang et al. 2021; Ke et al. 2022). Surgical excision with adequate margins remains the curative treatment for melanoma (Guo et al. 2015; Garbe et al. 2016; Joyce and Skitzki 2020), whereas advanced patients benefit less from surgery and tend to experience an unpleasing survival (Gershenwald et al. 2017). Besides, targeted therapy on some mutated driver genes has gained encouraging treatment response and revolutionized the standard of advanced melanoma management, whereas the inevitable occurrence of treatment resistance remains a concern (Guo et al. 2021). With the development of immunotherapy, programmed death-1 (PD-1) inhibitor has been increasingly used in the treatment of advanced melanoma (Guo et al. 2021; Jenkins

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and Fisher 2021; Li et al. 2023). However, a proportion of advanced melanoma patients may respond insufficiently to PD-1 inhibitors, leading to unfavorable treatment outcomes (Jenkins and Fisher 2021; Tawbi et al. 2022; Arance et al. 2023). Hence, exploring novel markers capable of estimating clinical outcomes of PD-1 inhibitor may facilitate individualized treatment for advanced melanoma patients.

Cell division cycle 42 (CDC42), ubiquitously expressed in bone marrow cells, is a small guanosine triphosphatase of Ras homologous family regulated by guanine nucleotide exchange factor; meanwhile, it controls diverse cellular functions, such as cytoskeletal assembly and organization (Lawson and Ridley 2018; Svensmark and Brakebusch 2019). CDC42 is found to correlate with tumor aggressive behaviors and prognosis in melanoma (Tucci et al. 2007; Tan et al. 2018; Wang et al. 2019). For instance, one study shows that CDC42 regulates melanoma cell proliferation by modulating mammalian target of rapamycin complex-1 (mTORC1) pathway (Tan et al. 2018). Another study elucidates that CDC42 activates Ras-related C3 botulinum toxin substrate 1 (Rac1) and further facilitates cell growth of melanoma (Wang et al. 2019). Furthermore, CDC42 is increased in melanoma patients with fetal outcomes (Tucci et al. 2007).

Inspiringly, evidence suggests that CDC42 regulates $CD8^+$ T cell activation and facilitates immune escape, which might serve as a regulator of cancer susceptibility (Jaksits et al. 2004; Marques et al. 2008). Clinically, previous studies disclose that CDC42 is linked with treatment outcomes of PD-1 inhibitor in patients with malignancy (Jiang et al. 2023; Xu et al. 2023). For instance, a study quantifies serum CDC42 in 30 advanced hepatocellular carcinoma patients before initiation of PD-1 inhibitor-based therapy, identifying that serum CDC42 is negatively related to disease control rate (Xu et al. 2023). Another study notices that CDC42 in mononuclear cells of peripheral blood is declined during the treatment of PD-1 inhibitor, and its elevation estimates unfavorable clinical response and worse survival of metastatic colorectal cancer patients (Jiang et al. 2023). However, the correlation of CDC42 with treatment outcomes of PD-1 inhibitors in advanced melanoma patients is unclear.

Therefore, this study determined serum CDC42 before and after treatment, aiming to investigate its association with tumor features, clinical response and survival in advanced melanoma patients receiving PD-1 inhibitor monotherapy.

## Methods

*Patients and treatments*

This study consecutively enrolled thirty-five advanced melanoma patients who were treated with PD-1 inhibitor monotherapy between July 2019 and February 2023. The enrollment criteria involved: i) histologically confirmed as melanoma; ii) older than 18 years old; iii) tumor-node-metastasis (TNM) stage III or IV; iv) unsuitable for surgical resection; v) Eastern Cooperative Oncology Group Performance Status (ECOG PS) score ≤ 1; vi) had ≥ 1 measurable lesion via Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 on imaging (Eisenhauer et al. 2009). The exclusion criteria involved: i) had a prior systemic therapy for advanced melanoma; ii) had an active central nervous system metastasis; iii) had inadequate organ functions (the disturbance of organ function, including brain, liver, adipose, pancreas islet, kidney, heart, small intestine, etc.). The study had the approval from the Ethics Committee of the Affiliated Hospital of Hebei Engineering University (approval No. 2023[K]084-01). Each patient signed informed consent.

Patients received PD-1 inhibitor monotherapy, including nivolumab (3 mg/kg, 2-weekly), camrelizumab (200 mg, 2-weekly), and pembrolizumab (200 mg, 2-weekly) until disease progression, patient death, or the occurrence of intolerable toxicity. The dose of PD-1 inhibitor was consistent with the package insert, and the dose adjustments were allowed. The choice of a PD-1 inhibitor was based on a combination of patient wishes, disease conditions, and physician suggestions.

*Assessments*

Progression was assessed on imaging every 2 cycles (monthly) for the first four months, and then every two months for follow-up assessments until June 2023. The median duration of follow-up was 10.2 months (ranging 2.1-24.0 months). The tumor response rate was calculated using the third-month assessment according to RECIST v.1.1 (Response Evaluation Criteria in Solid Tumors). Progression-free survival (PFS) and overall survival (OS) were assessed using the follow-up assessments. Besides, serum CDC42 level was assessed before treatment (pre-treatment) as well as after 2 treatment cycles/1 month (post-treatment) by enzyme-linked immunosorbent assay (ELISA) using the kits (Cat. No. JM-1116H2) from JINGMEI Biotechnology Company (Shanghai, China).

*Statistical analyses*

Analyses were completed by SPSS v.22.0 (IBM, New York, NY, USA). Normality was determined by the Shapiro-Wilk test. Comparison analyses were detected by the Wilcoxon rank sum or Wilcoxon signed-rank test. Accumulating PFS and OS rates were demonstrated by Kaplan Meier curves, which were analyzed using a log-rank test, among which the CDC42 level was dichotomized by 700.0 pg/mL due to the median value of the pre-treatment CDC42 level being approximately 700.0 pg/mL. Variables related to PFS or OS were detected by univariate and forward stepwise multivariate Cox's proportional hazards regression models. $P < 0.05$ was considered significant.

## Results

*Clinical features*

The mean age of the 35 advanced melanoma patients

receiving PD-1 inhibitor monotherapy was 61.3 ± 8.0 years, and 20 (57.1%) patients were aged over 60 years. Among all, there were 19 (54.3%) male patients. The number of patients with ECOG PS scores of 0 and 1 was 24 (68.6%) and 11 (31.4%), respectively. The median (interquartile range; IQR) sum of tumor size was 5.0 (3.7-6.9) cm; besides, there were 17 (48.6%) patients with sum of tumor size > 5 cm. Additionally, 8 (22.9%) and 27 (77.1%) patients were assessed as TNM stage III and IV, accordingly. There were 12 (34.3%) patients with abnormal lactate dehydrogenase (LDH). Programmed death-ligand 1 (PD-L1) expression was positive in 28 (80.0%) patients (Table 1).

*Pre-treatment CDC42 level in melanoma patients and its association with clinical features*

Overall, the distribution of pre-treatment CDC42 level among patients was skewed ($P$ = 0.001). Pre-treatment CDC42 level ranged from 335.0 to 2,105.0 pg/mL. The median (IQR) pre-treatment CDC42 level was 706.0 (551.0-1,246.0) pg/mL, and its mean value was 900.5 ± 452.9 pg/mL (Table 2). Pre-treatment CDC42 level was elevated in patients with TNM stage IV (vs. III) ($P$ = 0.050) and patients with abnormal LDH (vs. normal) ($P$ = 0.022). While pre-treatment CDC42 level was not varied in patients with different age, sex, ECOG PS, sum of tumor size, and PD-L1 (all $P$ > 0.050) (Table 3).

*Clinical response*

A respective of 3 (8.6%), 9 (25.7%), 10 (28.6%), and 13 (37.1%) patients were assessed as complete response, partial response, stable disease, and progressive disease. Additionally, the objective response rate and disease control rate were 34.3% and 62.9%, respectively (Table 4).

*Correlation of pre-treatment CDC42 level with clinical response and survival*

Pre-treatment CDC42 level was increased in patients without objective response compared to those with objective response ($P$ = 0.022) (Fig. 1A), and it was also elevated in patients without disease control compared with those with disease control ($P$ = 0.003) (Fig. 1B). Concerning the

Table 1. Clinical characteristics.

| Items | Advanced melanoma patients (N = 35) |
|---|---|
| Age (years), mean ± SD | 61.3 ± 8.0 |
| Age > 60 years, n (%) | 20 (57.1) |
| Male, n (%) | 19 (54.3) |
| ECOG PS score, n (%) | |
| 0 | 24 (68.6) |
| 1 | 11 (31.4) |
| Sum of tumor size (cm), median (IQR) | 5.0 (3.7-6.9) |
| Sum of tumor size > 5 cm, n (%) | 17 (48.6) |
| TNM stage, n (%) | |
| III | 8 (22.9) |
| IV | 27 (77.1) |
| Abnormal LDH, n (%) | 12 (34.3) |
| Positive PD-L1, n (%) | 28 (80.0) |

SD, standard deviation; ECOG PS, Eastern Cooperative Oncology Group Performance Status; IQR, interquartile range; TNM, Tumor-Node-Metastasis; LDH, lactate dehydrogenase; PD-L1, Programmed Death-Ligand 1.

Table 2. Pre-treatment CDC42 level.

| Pre-treatment CDC42 level | Advanced melanoma patients (N = 35) |
|---|---|
| Distribution | Skewness ($P$ = 0.001) |
| Minimum value | 335.0 pg/mL |
| Quartile 1 | 551.0 pg/mL |
| Median value | 706.0 pg/mL |
| Quartile 3 | 1,246.0 pg/mL |
| Maximum value | 2,105.0 pg/mL |
| Mean value | 900.5 pg/mL |
| SD | 452.9 pg/mL |

CDC42, Cell Division Cycle 42; SD, standard deviation.

Table 3. Comparison of pre-treatment CDC42 level in advanced melanoma patients with different clinical characteristics.

| Items | Pre-treatment CDC42 level, median (IQR) | *P* value |
|---|---|---|
| Age | | 0.739 |
| ≤ 60 years | 693.0 (541.0-1,214.0) | |
| > 60 years | 746.0 (561.0-1,351.8) | |
| Sex | | 0.868 |
| Female | 705.5 (599.3-1,172.5) | |
| Male | 706.0 (539.0-1,387.0) | |
| ECOG PS | | 0.414 |
| 0 | 679.0 (543.5-1,238.0) | |
| 1 | 830.0 (646.0-1,587.0) | |
| Sum of tumor size | | 0.235 |
| ≤ 5 cm | 640.0 (540.5-1,222.0) | |
| > 5 cm | 830.0 (633.0-1,361.0) | |
| TNM stage | | 0.050 |
| III | 632.0 (499.3-700.8) | |
| IV | 889.0 (591.0-1,335.0) | |
| LDH | | 0.022 |
| Normal | 634.0 (498.0-1,181.0) | |
| Abnormal | 968.5 (696.3-1,468.8) | |
| PD-L1 | | 0.053 |
| Negative | 926.0 (774.0-1,496.0) | |
| Positive | 679.0 (539.5-1,205.8) | |

CDC42, Cell Division Cycle 42; IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, Tumor-Node-Metastasis; LDH, lactate dehydrogenase; PD-L1, Programmed Death-Ligand 1.

Table 4. Tumor response.

| Items | Advanced melanoma patients (N = 35) |
|---|---|
| Response, No. (%) | |
| CR | 3 (8.6) |
| PR | 9 (25.7) |
| SD | 10 (28.6) |
| PD | 13 (37.1) |
| Objective response (CR+PR), No. (%) | 12 (34.3) |
| Disease control (CR+PR+SD), No. (%) | 22 (62.9) |

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

survival, patients with pre-treatment CDC42 level > 700 pg/mL had a shorter PFS ($P$ = 0.013) compared to those with pre-treatment CDC42 level ≤ 700 pg/mL. Specifically, the 12-month PFS rate in patients with pre-treatment CDC42 level > 700 pg/mL and ≤ 700 pg/mL was 26.7% and 66.2%, accordingly; the 24-month PFS rate of these patients was 8.9% and 55.2%, correspondingly (Fig. 1C). Additionally, patients with pre-treatment CDC42 level > 700 pg/mL displayed a relatively shortened OS (without statistical significance) compared with those with pre-treatment CDC42 level ≤ 700 pg/mL ($P$ = 0.060). The 12-month OS rate in patients with pre-treatment CDC42 level > 700 pg/mL and ≤ 700 pg/mL was 61.1% and 82.5%, respectively. The 24-month OS rate of the two populations was 31.4% and 55.0%, accordingly (Fig. 1D).

*Correlation of post-treatment CDC42 level with clinical response and survival*

Post-treatment CDC42 level was reduced compared with pre-treatment CDC42 level [median (IQR): 480.0 (334.0-810.0) pg/mL vs. 706.0 (551.0-1,246.0) pg/mL, $P$ < 0.001] (Fig. 2). Post-treatment CDC42 level was elevated in patients without objective response compared to those with objective response ($P$ = 0.033) (Fig. 3A), and it was elevated in patients without disease control compared with those with disease control ($P$ = 0.009) (Fig. 3B). In terms

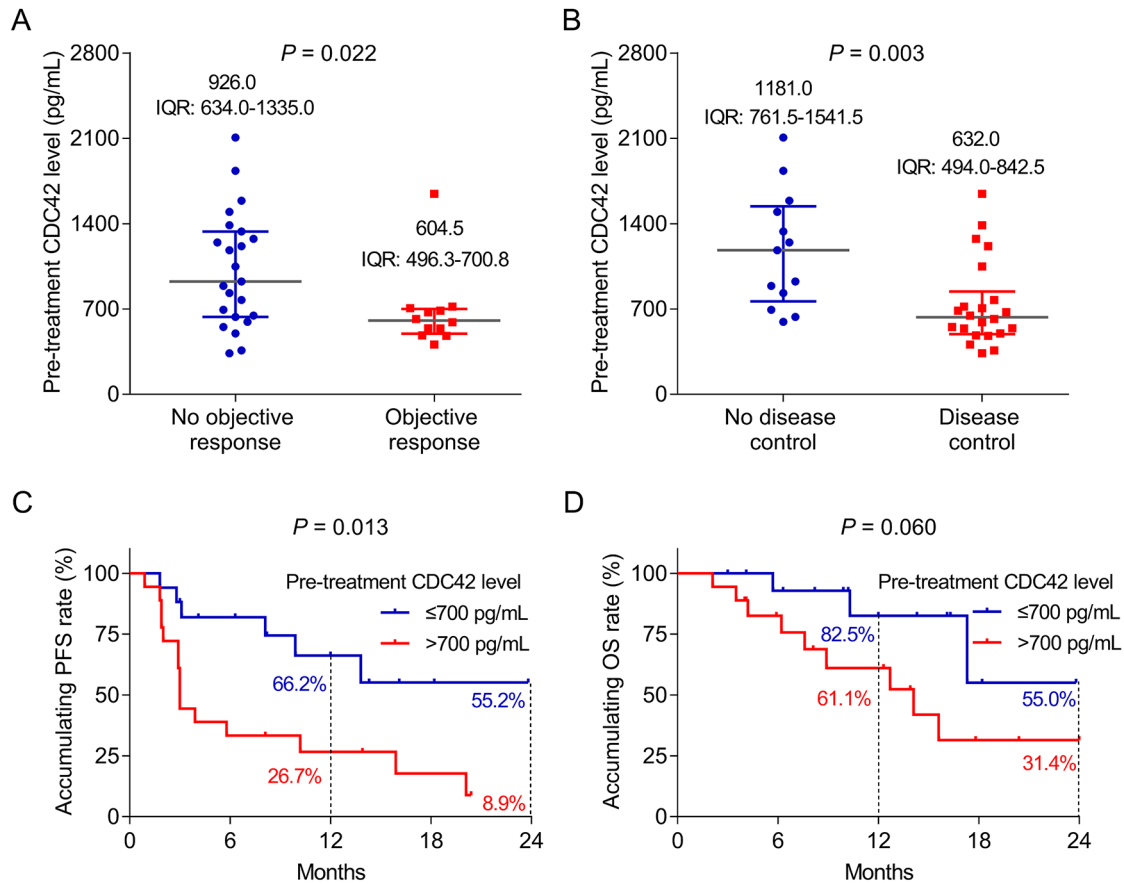


Fig. 1. Linkage of pre-treatment CDC42 level with clinical response and survival.
Comparison of pre-treatment CDC42 level in patients with and without objective response (A) and disease control (B). Comparison of progression-free survival (PFS) (C) and overall survival (OS) (D) in patients with pre-treatment CDC42 level > 700 pg/mL and ≤ 700 pg/mL.

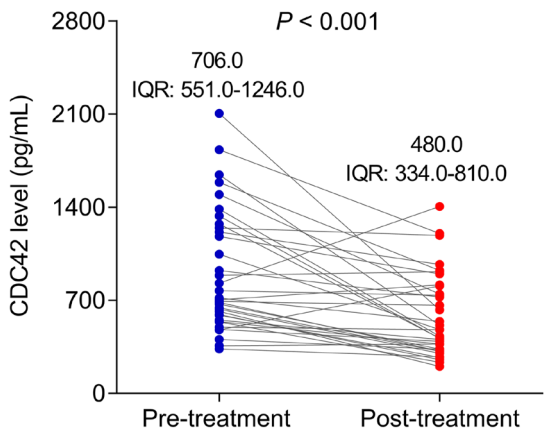


Fig. 2. Post-treatment CDC42 level was decreased compared with pre-treatment CDC42 level.

of survival, patients with post-treatment CDC42 level > 700 pg/mL exhibited shorter PFS ($P$ = 0.010) and OS ($P$ = 0.006) compared with those with post-treatment CDC42 level ≤ 700 pg/mL (Fig. 3C, D). The 12-month PFS rate in patients with post-treatment CDC42 level > 700 pg/mL and ≤ 700 pg/mL was 12.5% and 62.0%, accordingly. The 24-month PFS rate in the two populations was 12.5% and 22.0%, correspondingly (Fig. 3C). The 12-month OS rate of patients with post-treatment CDC42 level > 700 pg/mL and ≤ 700 pg/mL was 42.3% and 88.0%, accordingly, and the 24-month OS rate of the two kinds of patients was 21.2% and 58.1%, respectively (Fig. 3D).

*Factors affecting PFS*

Pre-treatment CDC42 level > 700 pg/mL (vs. ≤ 700 pg/mL) [hazard ratio (HR): 3.131, $P$ = 0.019], post-treatment CDC42 level > 700 pg/mL (vs. ≤ 700 pg/mL) (HR: 2.957, $P$ = 0.015), and TNM stage IV (vs. III) (HR: 5.141, $P$ = 0.030) were related to shorter PFS. PD-L1 positive (vs. negative) (HR: 0.318, $P$ = 0.015) was linked with prolonged PFS. Forward stepwise multivariate model suggested that post-treatment CDC42 level > 700 pg/mL (vs. ≤ 700 pg/mL) (HR: 2.704, $P$ = 0.029) was independently associated with shortened PFS, while PD-L1 positive (vs. negative) (HR: 0.361, $P$ = 0.034) was independently correlated with longer PFS (Table 5).

*Factors affecting OS*

Pre-treatment CDC42 level > 700 pg/mL (vs. ≤ 700

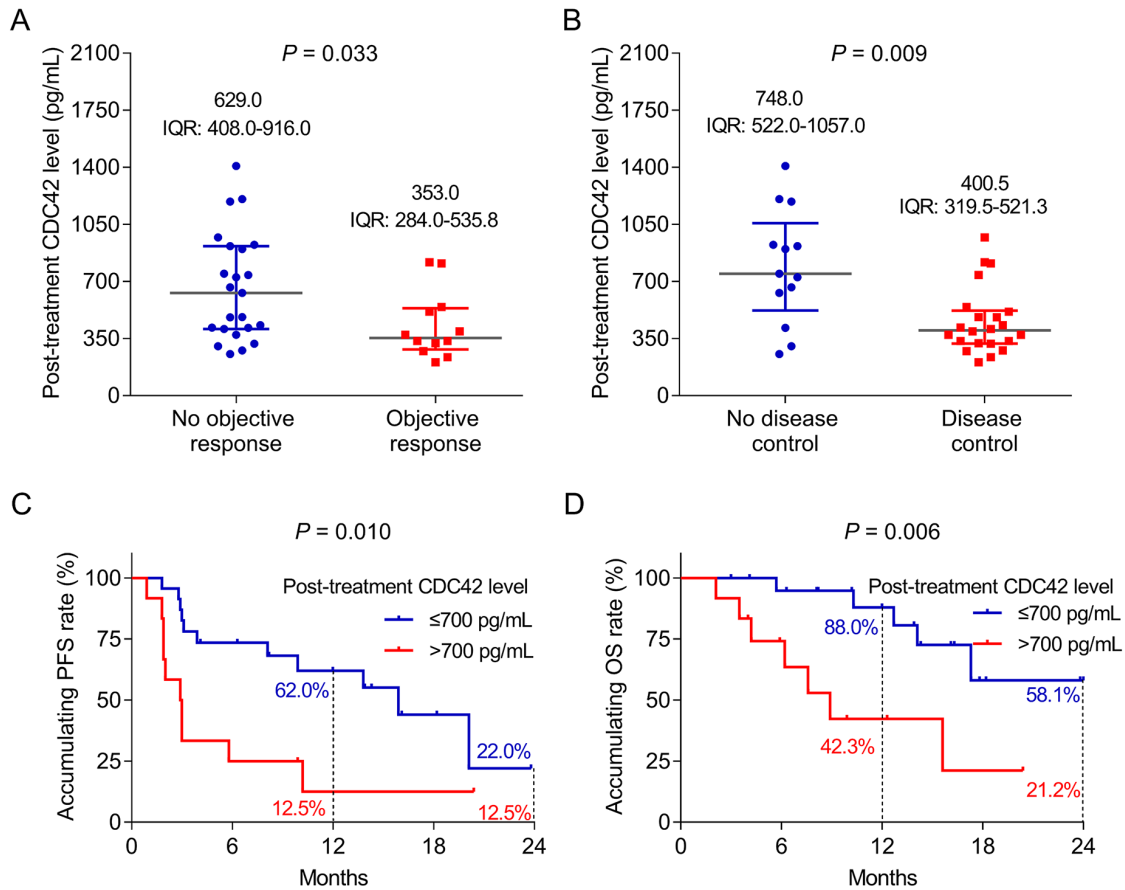


Fig. 3. Linkage of post-treatment CDC42 level with clinical response and survival.
Comparison of post-treatment CDC42 level in patients with and without objective response (A) and disease control (B). Comparison of progression-free survival (PFS) (C) and overall survival (OS) (D) in patients with post-treatment CDC42 level > 700 pg/mL and ≤ 700 pg/mL.

pg/mL) was not associated with OS (HR: 3.272, $P = 0.076$). Post-treatment CDC42 level > 700 pg/mL (vs. ≤ 700 pg/mL) (HR: 4.487, $P = 0.012$) and TNM stage IV (vs. III) (HR: 8.258, $P = 0.047$) were related to shorter OS. According to the forward stepwise multivariate model, post-treatment CDC42 level > 700 pg/mL (vs. ≤ 700 pg/mL) (HR: 7.749, $P = 0.005$), ECOG PS 1 (vs. 0) (HR: 8.015, $P = 0.005$), and TNM stage IV (vs. III) (HR: 36.721, $P = 0.010$) were independently related to worse OS (Table 6).

*Subgroup analyses in patients with different TNM stages*

In advanced melanoma patients with TNM stage III, pre-treatment and post-treatment CDC42 level was not linked with PFS or OS (all $P > 0.050$). Differently, in patients with TNM stage IV, pre-treatment CDC42 level > 700 pg/mL was linked with reduced PFS ($P = 0.020$), but not OS ($P = 0.125$), while post-treatment CDC42 level > 700 pg/mL was associated with shortened PFS ($P = 0.007$) and OS ($P = 0.015$) (Supplementary Table S1).

## Discussion

Recent studies have observed the association of increased CDC42 expression with unfavorable tumor features (Yang et al. 2017; Gao et al. 2022; Yan and Wan 2022). For example, a study reveals that elevated CDC42 level is associated with higher TNM stage and poor tumor differentiation in pancreatic cancer patients (Yang et al. 2017). Another study discloses that higher CDC42 expression is related to abnormal carcinoembryonic antigen in patients with colorectal cancer (Gao et al. 2022). The current study identified that melanoma patients with TNM stage IV (vs. III) and abnormal LDH (vs. normal) had a higher pre-treatment CDC42 level. The probable explanations might be those: (1) CDC42 facilitated tumor growth and metastasis, which resulted in advanced TNM stage (Gershenwald et al. 2017; Maldonado and Dharmawardhane 2018; Huang et al. 2022). Thus, pre-treatment CDC42 level was elevated in melanoma patients at TNM stage IV compared with those at TNM stage III. (2) Abnormal LDH contributed to the proliferation, invasion, and immune escape of tumor cells (Feichtinger and Lang 2019; Claps et al. 2022), meanwhile, the aforementioned malignant behaviors were associated with increased CDC42 expression (Marques et al. 2008; Crosas-Molist et al. 2022). Hence, the pre-treatment CDC42 level was increased in melanoma patients with abnormal LDH compared with those with nor-

Table 5. Univariate and multivariate Cox's proportional hazard regression model for progression-free survival (PFS).

| Items | *P* value | HR | 95% CI |
|---|---|---|---|
| Univariate models | | | |
| Pre-treatment CDC42 level, > 700 pg/mL vs. ≤ 700 pg/mL | 0.019 | 3.131 | 1.206-8.127 |
| Post-treatment CDC42 level, > 700 pg/mL vs. ≤ 700 pg/mL | 0.015 | 2.957 | 1.238-7.064 |
| Age, > 60 years vs. ≤ 60 years | 0.143 | 1.982 | 0.794-4.944 |
| Sex, male vs. female | 0.449 | 0.717 | 0.302-1.698 |
| ECOG PS, 1 vs. 0 | 0.350 | 1.525 | 0.629-3.695 |
| Sum of tumor size, > 5 cm vs. ≤ 5 cm | 0.201 | 1.769 | 0.738-4.243 |
| TNM stage, IV vs. III | 0.030 | 5.141 | 1.171-22.567 |
| LDH, abnormal vs. normal | 0.090 | 2.109 | 0.890-4.997 |
| PD-L1, positive vs. negative | 0.015 | 0.318 | 0.126-0.802 |
| Forward stepwise multivariate model | | | |
| Post-treatment CDC42 level, > 700 pg/mL vs. ≤ 700 pg/mL | 0.029 | 2.704 | 1.106-6.611 |
| PD-L1, positive vs. negative | 0.034 | 0.361 | 0.141-0.924 |

HR, hazard ratio; CI, confidence interval; CDC42, Cell Division Cycle 42; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, Tumor-Node-Metastasis; LDH, lactate dehydrogenase; PD-L1, Programmed Death-Ligand 1.

Table 6. Univariate and multivariate Cox's proportional hazard regression model for overall survival (OS).

| Items | *P* value | HR | 95% CI |
|---|---|---|---|
| Univariate models | | | |
| Pre-treatment CDC42 level, > 700 pg/mL vs. ≤ 700 pg/mL | 0.076 | 3.272 | 0.884-12.106 |
| Post-treatment CDC42 level, > 700 pg/mL vs. ≤ 700 pg/mL | 0.012 | 4.487 | 1.395-14.430 |
| Age, > 60 years vs. ≤ 60 years | 0.170 | 2.500 | 0.674-9.269 |
| Sex, male vs. female | 0.341 | 0.572 | 0.181-1.804 |
| ECOG PS, 1 vs. 0 | 0.098 | 2.640 | 0.837-8.330 |
| Sum of tumor size, > 5 cm vs. ≤ 5 cm | 0.346 | 1.738 | 0.551-5.486 |
| TNM stage, IV vs. III | 0.047 | 8.258 | 1.032-66.055 |
| LDH, abnormal vs. normal | 0.287 | 1.854 | 0.596-5.772 |
| PD-L1, positive vs. negative | 0.072 | 0.346 | 0.109-1.101 |
| Forward stepwise multivariate model | | | |
| Post-treatment CDC42 level, > 700 pg/mL vs. ≤ 700 pg/mL | 0.005 | 7.749 | 1.874-32.046 |
| ECOG PS, 1 vs. 0 | 0.005 | 8.015 | 1.857-34.600 |
| TNM stage, IV vs. III | 0.010 | 36.721 | 2.342-575.849 |

HR, hazard ratio; CI, confidence interval; CDC42, Cell Division Cycle 42; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, Tumor-Node-Metastasis; LDH, Lactate Dehydrogenase; PD-L1, Programmed Death-Ligand 1.

mal.

CDC42 prevents cytotoxic T lymphocyte-induced apoptosis and facilitates immune escape in cancer (Marques et al. 2008; Kalim et al. 2022). For instance, a study illustrates that CDC42 protects tumor cells from cytotoxicity of T lymphocytes (Marques et al. 2008). Another study indicates that CDC42 mediates immune tolerance of tumor cells via regulating Treg cell stability (Kalim et al. 2022). Given the above information, it is hypothesized that increased CDC42 level is associated with unpleasing clinical response towards PD-1 inhibitor. The present study herein identified that the post-treatment CDC42 level was declined compared with pre-treatment CDC42 level in advanced melanoma patients receiving PD-1 inhibitor monotherapy. The possible explanation could be that: Elevated CDC42 facilitated immune escape (Marques et al. 2008), which was reversed after PD-1 inhibitor treatment in advanced melanoma patients, resulting in a declined CDC42 level. Hence, CDC42 level was declined after the administration of PD-1 inhibitor monotherapy in advanced melanoma patients. Furthermore, this study also found that pre-treatment and post-treatment CDC42 level were both associated with unfavorable objective response and disease control in advanced melanoma patients receiving PD-1 inhibitor monotherapy. The potential reasons might be those: (1) CDC42 mediated immune escape in melanoma,

whose elevation adversely affected the effect of PD-1 inhibitor on inhibiting immune escape (Marques et al. 2008; Budimir et al. 2022). (2) Elevated CDC42 might induce drug resistance of tumor cells towards PD-1 inhibitor, leading to unsatisfactory clinical response (Xu et al. 2023). Therefore, CDC42 level was negatively correlated with objective response and disease control in advanced melanoma patients receiving PD-1 inhibitor monotherapy.

Previous studies have identified that increased CDC42 is a predictor of unfavorable survival profile in cancer patients treated with PD-1 inhibitor (Jiang et al. 2023; Xu et al. 2023). Similarly, this study observed that CDC42 level > 700 pg/mL correlated with worse survival in advanced melanoma patients. Furthermore, post-treatment CDC42 level > 700 pg/mL was independently correlated with shorter PFS and OS. The potential explanation might be those: (1) Overexpression of CDC42 potentiated tumor progression (Rathinam et al. 2011), resulting in dismal survival in advanced melanoma patients (Xu et al. 2023). Therefore, CDC42 level > 700 pg/mL predicted worse survival in advanced melanoma patients receiving PD-1 inhibitor monotherapy. (2) Elevated CDC42 level after the administration of PD-1 inhibitor suggested the possibility of tumor cell resistance to PD-1 inhibitor, which might lead to insufficient treatment response and worse survival (Xu et al. 2023). Hence, post-treatment CDC42 level > 700 pg/mL displayed a stronger potency for predicting shorter PFS and OS compared with pre-treatment CDC42 level in advanced melanoma patients receiving PD-1 inhibitor monotherapy.

This study comprehensively enrolled patients with different PD-1 inhibitor monotherapy, including nivolumab, camrelizumab, and pembrolizumab, and suggested the clinical utility of CDC42 as an assistant biomarker for predicting treatment response and outcomes of PD-1 inhibitors in advanced melanoma patients, which was meaningful to improve the management of advanced melanoma. However, unavoidable limitations of the present study were as follows: Firstly, limited by the relatively low incidence of melanoma in China, only 35 advanced melanoma patients were enrolled in this study despite efforts of searching for eligible patients. Thus, further study with a larger sample size is required to verify the findings. Secondly, the present study determined serum CDC42 before and after 2 treatment cycles, while the prognostic value of CDC42 at multiple time points in advanced melanoma patients receiving PD-1 inhibitor monotherapy was uncertain. Thirdly, patients enrolled in this study all received PD-1 inhibitor, and the value of CDC42 on predicting treatment outcomes of other immunotherapies (such as anti-cytotoxic T lymphocyte antigen-4 and PD-L1 inhibitor, etc.) was uncertain (Cuevas and Daud 2018). Lastly, the wide range of follow-up period (2.1-24.0 months) might be due to the heterogeneous survival of advanced melanoma patients, which would cause the bias of the prognostic value of CDC42.

In conclusion, CDC42 reduces after treatment and correlates with advanced TNM stage, abnormal LDH, and worse clinical response in advanced melanoma patients receiving PD-1 inhibitor monotherapy. More importantly, post-treatment CDC42 level > 700 pg/mL potentially predicts shortened PFS and OS in these patients, while further validation in studies with a large sample size and an expanded follow-up duration is required.

## Conflict of Interest

The authors declare no conflict of interest.

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## Supplementary Files

Please find supplementary file(s);
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