Original Article

Association between changes in thioredoxin reductase and other peripheral blood biomarkers with response to PD-1 inhibitor-based combination immunotherapy in non-small cell lung cancer: a retrospective study

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Background: Immunotherapy deeply changed the treatment paradigm of advanced non-small cell lung cancer (NSCLC) in the past years. However, the objective response rate (ORR) after immunotherapy is about 20–30% of NSCLC patients. Therefore, identification of predictive biomarkers is crucial for selecting patients with NSCLC who would most benefit from programmed cell death receptor protein 1 (PD-1) inhibitor-based immunotherapy.

Methods: We retrospectively collected medical records and thioredoxin reductase (TrxR) data from 90 patients with a NSCLC who received PD-1 inhibitor-based combination therapy. Serum biomarkers were also measured at 6- and 12-week post-treatment and compared with their baseline values. Associations between changes in serum biomarkers, clinical characteristics and treatment efficacy were evaluated using univariate tests. The patients who were still alive were followed up remotely by phone or email to assess survival. The association between serum biomarkers and TrxR with overall survival (OS) and progression-free survival (PFS) were assessed by univariate and multivariate Cox proportional hazard regression. Nomogram prediction models were constructed using factors associated with PFS and OS, respectively.

Results: The median follow-up time among the 90 patients was 19.7 (range, 13.6 to 25.8) months. Median PFS and OS were 13.6 [95% confidence interval (CI): 13.5 to 13.7] and 19.7 (95% CI: 13.6 to 25.8) months, respectively. Patients with decreased carcinoembryonic antigen (CEA), albumin (Alb), and TrxR values at 6- and 12-week post-treatment compared to baseline had statistically significantly improved disease remission rates ($P<0.05$). Patients with decreased white blood cell (WBC), neutrophil-to-lymphocyte ratio (NLR), derived NLR (dNLR) at week 6, and decreased Alb, CEA, and lymphocyte-to-monocyte ratio (LMR) at week 12 had statistically significantly increased ORRs ($P<0.05$). According to the univariate and multivariate Cox regression analyses, we included adenocarcinoma, Eastern Cooperative Oncology Group performance status (ECOG PS), and CEA change at week 6 post-treatment as predictors for PFS, and adenocarcinoma, change in absolute lymphocyte count (ALC), and TrxR at week 6 as predictors for OS in the nomogram

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models. Each nomogram was also validated internally using a bootstrap method with 1,000 resamples.

Conclusions: Change in TrxR at 6 weeks post-treatment in combination with other clinical and hematological biomarkers could be used as a predictor for treatment outcome and prognosis in NSCLC patients after PD-1 inhibitor-based combination immunotherapy.

Keywords: Non-small cell lung cancer (NSCLC); immunotherapy; thioredoxin reductase (TrxR); nomogram; survival

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Introduction

Lung cancer was the second most frequently diagnosed cancer and the leading cause of cancer-related death worldwide in 2020 (1). Approximately two-thirds of lung cancer deaths worldwide are attributed to smoking (large variation according to the countries/continent) (1,2). Non-small cell lung cancer (NSCLC) represents is the most common histological subtype of lung cancer (3). Immunotherapy alone or in combination with chemotherapy have showed to significantly increase survival as compared to chemotherapy in patients with advanced NSCLC. Programmed death-ligand 1 (PD-L1), a ligand of programmed cell death receptor protein 1 (PD-1), has been shown to be upregulated in cancer cells and immune cells to inhibit the effector T cells (4,5). Correspondingly, antibodies that inhibit the interaction between PD-1 and PD-L1 have been developed as a therapeutic approach to enhance patients' immune response against tumor cells. However, most of the patients benefit from immunotherapy but not all, some of them can experience significant immunotoxicities, moreover this kind of treatment is expensive and is not always widely available (6,7). By identifying predictive biomarkers effectiveness of PD-1 inhibitor-based therapies could be maximize, leading to healthcare resources optimization.

Currently, the solely biomarker approved to drive the immunotherapy is the PD-L1 expression that has demonstrated a correlation with overall response rate but has strong limitations (8). Several tumor tissue biomarkers (tumor mutational burden, gene expression signatures, presence or absence of oncogenic driver mutation, the density of tumor-infiltrating lymphocytes) and blood biomarkers have proved to affect the clinical outcome of patients when treated with immunotherapy but must be considered still investigational (9). In recent years, several biomarkers, including different types of blood cell counts, circulating tumor markers [interleukin (IL)-8, IL-6, blood tumor mutation burden, etc.], peripheral blood inflammation parameters, and thioredoxin reductase (TrxR), have been identified as potential prognostic factors for immunotherapies in patients with NSCLC (10,11). TrxR is an enzyme and part of the antioxidant reduction (redox) system which plays a crucial role in tumor development. High reactive oxygen species (ROS) level leads to oxidative stress and eventually increases serum TrxR (12). Several animal model studies have demonstrated a link between TrxR levels, cancer progression, and chemotherapy resistance in cancer cells (13-16). There was a tight relationship between TrxR and chemotherapy, which influences the redox process, and most of the PD-1-based immunotherapy performed for patients with advanced NSCLC was based on combination therapy. Therefore, it is important to monitor and analyze TrxR values in the treatment of advanced NSCLC patients. A retrospective population-based study showed that TrxR correlated with the efficacy of chemotherapy for gastric cancer (17). Other cell studies have shown that TrxR can modify immunomodulatory activities while increasing the effectiveness of various growth factors leading to cancer cell growth (18,19). Redox was also found to improve the immune microenvironment of tumors by blocking the thioredoxin (Trx)/TrxR system (20). In the context of immunotherapy, TrxR plays a crucial role in immunoregulation, and the complex mechanisms of action between the tumour microenvironment and immunotherapy drugs remain unclear, and it remains to be determined whether changes in TrxR will affect the efficacy of immunotherapy. Despite that there is evidence that TrxR could predict the response to chemotherapy in patients with gastric (17), hepatocellular (21) and breast cancer (22),

the relationship between PD-1-based immunotherapy and TrxR in NSCLC remains unclear, highlighting the need for further research.

In this study, we aimed to retrospectively evaluate the prognostic value of biomarkers such as TrxR, clinicopathological parameters, and peripheral blood inflammatory factors in patients with advanced NSCLC receiving PD-1-based immunotherapy, association between TrxR and clinical outcomes in particular. We also aimed to develop predictive survival models that may guide clinicians in the use of PD-1-based immunotherapy in NSCLC patients. We present the following article in accordance with the REMARK reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-300/rc).

Methods

Study design and participants

We retrospectively enrolled patients diagnosed with advanced NSCLC who underwent PD-1-based immunotherapy as monotherapy or in combination with chemotherapy and bevacizumab at the Jiangsu Cancer Hospital, China from August 2018 to January 2021. Patients were considered eligible for the clinical study if we have stored peripheral serum samples at baseline, at 6 and 12 weeks after treatment onset. The following patients were excluded: (I) those with comorbidity including severe diabetes mellitus, heart failure, liver, and/or kidney failure; (II) those with major psychiatric disorders; (III) those with a history of other malignancies; and (IV) special populations, such as pregnant and lactating women. The principal endpoints of this study were the definition of the association between serum biomarkers and TrxR with overall survival (OS) and progression-free survival (PFS) assessed by univariate and multivariate Cox proportional hazard regression. Patients' tumor characteristics, including gender, age, smoking status, types of pathology, number of metastatic lesions, epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma viral oncogene homologue (*KRAS*) mutational status, stage, Eastern Cooperative Oncology Group performance status (ECOG PS), and other treatment received (i.e., radiotherapy), were extracted from the electronic medical records. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional Ethics Committee of Jiangsu Cancer hospital. Individual consent for this retrospective analysis was waived.

Assessment of hematological parameters

Hematological parameters, including white blood cell (WBC) count, absolute neutrophil count (ANC), absolute monocyte count (AMC), absolute lymphocyte count (ALC), platelet (PLT) count, lactate dehydrogenase (LDH), hemoglobin (Hb), albumin (Alb), carcinoembryonic antigen (CEA), neutrophil-to-lymphocyte ratio (NLR; ANC/ALC), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR; absolute platelet count/ALC), platelet-to-albumin ratio (PAR; absolute platelet count/Alb), and derived NLR (dNLR), were collected at baseline (before first round of treatment), 6 weeks after 2 cycles of treatment, and 12 weeks after 4 cycles of treatment. The differences in the hematological parameters at weeks 6 and 12 from the baseline values were calculated.

Assay for TrxR activity

Peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA) and then centrifuged at 3,500 rpm for 5 minutes at room temperature within 2 hours of collection. The supernatant was harvested at 4 ℃ and tested immediately. Storage temperature was 4 ℃ while incubation temperature was 37 ℃. We measured TrxR activity with a commercially available TrxR activity colorimetric assay kit (Clairvoyance Health Technology Co., Ltd., Wuhan, China) using a 5,5'-dithiobis (2-nitrobenzoic) acid reduction, as recommended by the manufacturer. Positive and negative controls for the kit were performed for each reaction to monitor the performance of the assay.

Assessment of treatment response and survival

Whole-body computed tomography (CT) scans were performed every 6–8 weeks during treatment and response was assessed by investigators according to investigator asses the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (23). Follow-up was concluded by on January 1st 2021. Objective response rate (ORR) and disease control rate (DCR) were calculated for each patient. The ORR was defined as the sum of complete response (CR) and partial response (PR). The DCR was defined as the sum of CR, PR, and stable disease (SD). PFS and OS status were also assessed. Progression was defined as clinical or radiological progression or death after the initial treatment, whichever occurred first. The patients who, according to their medical records, were still alive at the time of data collection were

followed up remotely by phone or email to assess survival.

Statistical analysis

We first evaluated the biomarker change among patients with advanced NSCLC. The patients' baseline treatment blood parameters were considered as continuous variables. The post-treatment parameters were categorized into two groups based on whether they increased (Up group) or decreased (Down group) compared to the baseline treatment parameter. Continuous variables were first checked for normality by Shapiro-Wilk test, were all found to be not normally distributed, and were therefore expressed as medians and interquartile ranges (IQRs). Categorical variables were expressed as frequencies and percentages. Chi-square test or Fisher's exact test was used to determine the univariate associations between categorical variables with treatment responses. Fisher's test was used if the expected frequency of any categorical variable was less than 5.

Survival analysis was carried out using the Kaplan-Meier method and the log-rank test. Survival status and survival time factors were considered in the survival analysis. Cox proportional hazards regression analysis was conducted to identify potential indicators associated with PFS and/or OS. Factors with a P value less than 0.05 in the univariate Cox regression analysis were further included in a multivariate analysis to identify the factors independently associated with survival. Nomograms to predict the survival probability at 6, 12, and 18 months after treatment were constructed for each identified predictive factor. Each nomogram was also validated internally using a bootstrap method with 1,000 resamples. The predictive ability of each factor was assessed by calculating the concordance index (C-index), with a C-index of 0.5 indicating a completely random prediction and a C-index of 1.0 indicating a perfect prediction. Calibration curves and area under the curve (AUC) were used to assess the correlation between actual outcomes and predicted probabilities. Participants were categorized into high- or low-risk groups based on their predictive risk scores (higher or lower than the median score). The Kaplan-Meier method was used to compare the survival between the high- and low-risk groups for all constructed models.

The patient risk score and clinical characteristics were then tested for normality and homogeneity using the Shapiro-Wilk test and Levene's test, respectively, whereby a P value above 0.05 indicated that the data were normally distributed with equal variance. For patient risk score and clinical characteristics that were normally distributed, analysis of variance (ANOVA) was used to compare the correlation between categorical variables. Conversely, if the distribution was not normal, or the variance was not equal, the non-parametric Kruskal-Wallis H-test was used to compare categorical variables.

We calculated the sample size of the multivariable Cox regression model for OS using the previously reported method (24). Given the widely accepted rule of thumb of 10 events per variable and given there were 3 variables in the final Cox model (25,26), the total number of events expected was 30. Taking into account an approximate 41% 1-year event rate and a 20% lost-to-review rate among the participants, we required a total sample size of at least 91 patients.

Statistical analyses were performed using R software (version 4.0.5; The R Foundation for Statistical Computing, Vienna, Austria) and SPSS 26.0 (IBM Corp., Armonk, NY, USA). A two-sided P value less than 0.05 was considered statistically significant.

Results

Participant characteristics

We retrospectively enrolled a total of 90 patients with advanced NSCLC. The patients' baseline characteristics are presented in *Table 1*. The median age was 63 years, and only 3 cases had an ECOG PS of 2. Three-quarters of them were males and about two-thirds were ever smokers. About 60% of them had adenocarcinoma. All patients received PD-1 inhibitor-based combinations, less than 20% were treated with PD-1 inhibitor given as monotherapy. About two-thirds received immunotherapy in the second-line or third-line setting. After 6 weeks of treatment, TrxR was elevated in 31 patients and had decreased in 59 patients. The median age was 63 years, and only 3 cases had an ECOG PS of 2. The ORR and DCR were 26.7% and 94.4%, respectively. The median follow-up time was 19.7 (range, 13.6 to 25.8) months. The median PFS and OS were 8.5 months (range, 7.1 to 10.0 months; *Figure 1A*) and 15.5 months (range, 13.8 to 17.2 months; *Figure 1B*), respectively.

Relationship between immunotherapy response and peripheral blood parameters of patients with advanced NSCLC

Table 2 summarizes the association between the peripheral blood indicators and treatment response. The variations of these indicators from baseline were calculated, and those

Table 1 Baseline characteristics of NSCLC patients

Characteristics	TrxR at 6-week decreased (n=59)	TrxR at 6-week increased (n=31)	Total (n=90)
Gender			
Male	43 (72.9)	26 (83.9)	69 (76.7)
Female	16 (27.1)	5 (16.1)	21 (23.3)
Age (years)			
≥63	30 (50.8)	15 (48.4)	45 (50.0)
63	29 (49.2)	16 (51.6)	45 (50.0)
ECOG PS			
0	11 (18.6)	3 (9.7)	14 (15.6)
1	45 (76.3)	28 (90.3)	73 (81.1)
2	3 (5.1)	0 (0.0)	3 (3.3)
PD-1 inhibitor			
Pembrolizumab	26 (44.1)	7 (22.6)	33 (36.7)
Sintilimab	27 (45.8)	20 (64.5)	47 (52.2)
Toripalimab	6 (10.2)	4 (12.9)	10 (11.1)
Stage			
Recurrence	11 (18.6)	6 (19.4)	17 (18.9)
IIIB	8 (13.6)	6 (19.4)	14 (15.6)
IV	40 (67.8)	19 (61.3)	59 (65.6)
Types of pathology			
Adenocarcinoma	37 (62.7)	20 (64.5)	57 (63.3)
Squamous	22 (37.3)	11 (35.5)	33 (36.7)
Smoking			
No	20 (33.9)	11 (35.5)	31 (34.4)
Yes	39 (66.1)	20 (64.5)	59 (65.6)
Mutation type			
EGFR	8 (13.6)	8 (25.8)	16 (17.8)
KRAS	3 (5.1)	2 (6.5)	5 (5.6)
Wild-type	48 (81.4)	20 (64.5)	68 (75.6)
Unknown	0 (0.0)	1 (3.2)	1 (1.1)
Degree of differentiation			
Low	49 (83.1)	24 (77.4)	73 (81.1)
Moderate/high	10 (16.9)	7 (22.6)	17 (18.9)
Lines of therapy			
1	22 (37.3)	9 (29.0)	31 (34.4)
2	23 (39.0)	13 (41.9)	36 (40.0)
3	14 (23.7)	9 (29.0)	23 (25.6)

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	TrxR at 6-week decreased (n=59)	TrxR at 6-week increased (n=31)	Total (n=90)
Treatment options			
PD-1 alone	11 (18.6)	5 (16.1)	16 (17.8)
Combination chemotherapy	36 (61.0)	17 (54.8)	53 (58.9)
Combination anti-angiogenic therapy	3 (5.1)	5 (16.1)	8 (8.9)
Both	9 (15.3)	4 (12.9)	13 (14.4)
Radiotherapy			
No	30 (50.8)	15 (48.4)	45 (50.0)
Yes	29 (49.2)	16 (51.6)	45 (50.0)

Data are expressed as n (%). NSCLC, non-small cell lung cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; PD-1, programmed cell death receptor protein 1; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene homologue; TrxR, thioredoxin reductase.

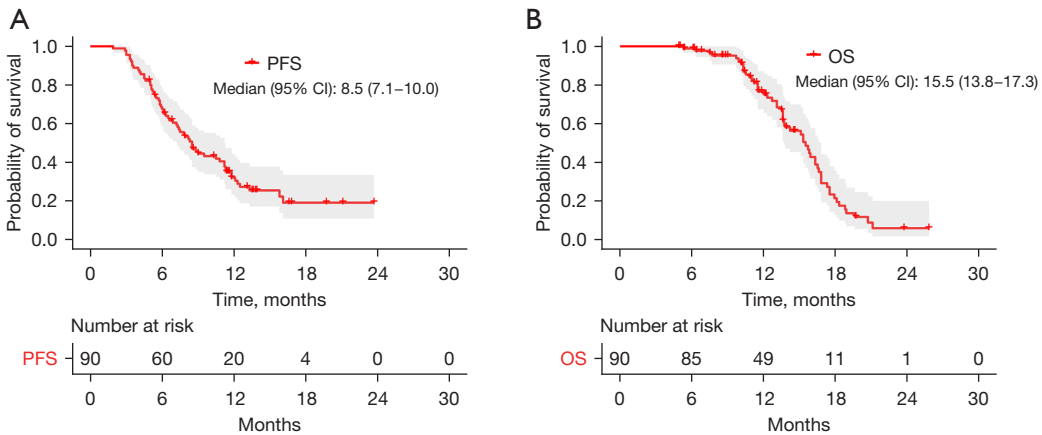

Figure 1 Overall population survival curves: PFS (A), OS (B). PFS, progression-free survival; CI, confidence interval; OS, overall survival.

that conformed to a skewed distribution were expressed as a median and IQR, as shown in Tables S1,S2.

The chi-squared test showed that DCR was associated with decreased $TrxR_{12w}$ (χ^2=3.696; P=0.049), decreased Alb_{12w} (χ^2=3.994; P=0.046), and decreased CEA_{6w} (χ^2=4.695; P=0.030), (*Table 2*), respectively. ORR was associated with WBC_{6w} (χ^2=11.012; P=0.001), ANC_{6w} (χ^2=12.007; P=0.001), NLR_{6w} (χ^2=5.260; P=0.022), $dNLR_{6w}$ (χ^2=4.139; P=0.042), Alb_{12w} (χ^2=5.260; P=0.022), CEA_{12w} (χ^2=5.682; P=0.017), and LMR_{12w} (χ^2=6.173; P=0.013), respectively.

Clinical prediction model for PFS in patients with advanced NSCLC

The univariate and multivariate Cox regression analyses showed that types of pathology [hazard ratio (HR) =2.10; 95% confidence interval (CI): 1.237–3.576; P=0.006], ECOG PS of 1 (HR =0.10; 95% CI: 0.024–0.411; P=0.001), ECOG PS of 0 (HR =0.22; 95% CI: 0.062–0.794; P=0.021), CEA_{6w} (HR =1.81; 95% CI: 1.091–3.003; P=0.022) were significantly associated with a prolonged PFS (*Table 3*). The univariate log-rank tests showed that patients with adenocarcinoma (HR =2.00; 95% CI: 1.200–3.200; P=0.008; *Figure 2A*), ECOG PS of 0 (HR =2.40; 95% CI: 1.200–5.000; P=0.001; *Figure 2B*) and CEA_{6w} decrease (HR =1.80; 95% CI: 1.100–2.900; P=0.025; *Figure 2C*) had a longer PFS (*Figure 2*), respectively. Other variable values that were not statistically significant in association with PFS were listed in Table S3.

The PFS prediction model was validated using

Table 2 The association between patient peripheral blood indicators and treatment response

Variables	DCR			ORR		
	With DCR/total, n/N (%)	χ^2	P value	With ORR/total, n/N (%)	χ^2	P value
Age (years)		5.294	0.021		0.227	0.634
≥63	45/45 (100.0)			13/45 (28.9)		
<63	40/45 (88.9)			11/45 (24.4)		
WBC_{6W}		2.532	0.112		11.012	0.001
Up	37/41 (90.2)			4/41 (9.8)		
Down	48/49 (98.0)			20/49 (40.8)		
ANC_{6W}		0.756	0.385		12.007	0.001
Up	42/46 (91.3)			5/46 (10.9)		
Down	43/44 (97.7)			19/44 (43.2)		
AMC_{6W}		0.000	1.000		0.909	0.340
Up	42/45 (93.3)			14/45 (31.1)		
Down	43/45 (95.6)			10/45 (22.2)		
ALC_{6W}		0.024	0.878		0.009	0.924
Up	39/42 (92.9)			11/42 (26.2)		
Down	46/48 (95.8)			13/48 (27.1)		
EON_{6W}		2.275	0.319		2.656	0.102
Up	32/35 (91.4)			6/35 (17.1)		
Down	53/55 (96.4)			18/55 (32.7)		
PLT_{6W}		0.003	0.959		0.818	0.366
Up	35/37 (94.6)			8/37 (21.6)		
Down	50/53 (94.3)			16/53 (30.2)		
LDH_{6W}		0.447	0.504		0.026	0.873
Up	46/50 (92.0)			13/50 (26.0)		
Down	39/40 (97.5)			11/40 (27.5)		
Hb_{6W}		0.101	0.750		0.010	0.921
Up	32/33 (97.0)			9/33 (27.3)		
Down	53/57 (93.0)			15/57 (26.3)		
Alb_{6W}		0.756	0.385		0.016	0.899
Up	43/44 (97.7)			12/44 (27.3)		
Down	42/46 (91.3)			12/46 (26.1)		
CEA_{6W}		4.695	0.030		2.675	0.102
Up	34/39 (87.2)			7/39 (17.9)		
Down	51/51 (100.0)			17/51 (33.3)		

Table 2 (*continued*)

Table 2 (*continued*)

Variables	DCR			ORR		
	With DCR/total, n/N (%)	χ^2	P value	With ORR/total, n/N (%)	χ^2	P value
$TrxR_{6W}$		1.401	0.237		0.018	0.894
Up	31/31 (100.0)			8/31 (25.8)		
Down	54/59 (91.5)			16/59 (27.1)		
NLR_{6W}		0.591	0.442		5.260	0.022
Up	44/48 (91.7)			8/48 (16.7)		
Down	41/42 (97.6)			16/42 (38.1)		
LMR_{6W}		0.000	1.000		0.227	0.634
Up	42/45 (93.3)			11/45 (24.4)		
Down	43/45 (95.6)			13/45 (28.9)		
PLR_{6W}		0.000	1.000		0.000	1.000
Up	43/45 (95.6)			12/45 (26.7)		
Down	42/45 (93.3)			12/45 (26.7)		
PAR_{6W}		0.000	1.000		2.675	0.102
Up	37/39 (94.9)			7/39 (17.9)		
Down	48/51 (94.1)			17/51 (33.3)		
$dNLR_{6W}$		0.000	1.000		4.139	0.042
Up	43/46 (93.5)			8/46 (17.4)		
Down	42/44 (95.5)			16/44 (36.4)		
PNI_{6W}		0.000	1.000		0.909	0.340
Up	42/45 (93.3)			14/45 (31.1)		
Down	43/45 (95.6)			10/45 (22.2)		
WBC_{12W}		0.000	1.000		0.227	0.634
Up	42/45 (93.3)			11/45 (24.4)		
Down	43/45 (95.6)			13/45 (28.9)		
ANC_{12W}		0.000	1.000		0.365	0.546
Up	43/46 (93.5)			11/46 (23.9)		
Down	42/44 (95.5)			13/44 (29.5)		
AMC_{12W}		1.048	0.306		2.737	0.98
Up	39/43 (90.7)			8/43 (18.6)		
Down	46/47 (97.9)			16/47 (34.0)		
ALC_{12W}		0.221	0.639		0.038	0.846
Up	35/36 (97.2)			10/36 (27.8)		
Down	50/54 (92.6)			14/54 (25.9)		

Table 2 (*continued*)

Table 2 (*continued*)

Variables	DCR			ORR		
	With DCR/total, n/N (%)	χ^2	P value	With ORR/total, n/N (%)	χ^2	P value
EON_{12W}		0.000	1.000		1.068	0.301
Up	35/37 (94.6)			12/37 (32.4)		
Down	50/53 (94.3)			12/53 (22.6)		
PLT_{12W}		0.131	0.717		1.060	0.303
Up	35/38 (92.1)			8/38 (21.1)		
Down	50/52 (96.2)			16/52 (30.8)		
LDH_{12W}		0.000	1.000		0.050	0.824
Up	41/43 (95.3)			11/43 (25.6)		
Down	44/47 (93.6)			13/47 (27.7)		
Hb_{12W}		0.000	1.000		0.000	1.000
Up	43/45 (95.6)			12/45 (26.7)		
Down	42/45 (93.3)			12/45 (26.7)		
Alb_{12W}		3.994	0.046		5.260	0.022
Up	48/48 (100.0)			8/48 (16.7)		
Down	37/42 (88.1)			16/42 (38.1)		
CEA_{12W}		0.000	1.000		5.682	0.017
Up	42/45 (93.3)			7/45 (15.6)		
Down	43/45 (95.6)			17/45 (37.8)		
$TrxR_{12W}$		3.696	0.049		0.299	0.584
Up	48/53 (90.6)			14/53 (26.4)		
Down	37/37 (100.0)			10/37 (27.0)		
NLR_{12W}		0.000	1.000		0.365	0.546
Up	43/46 (93.5)			11/46 (23.9)		
Down	42/44 (95.5)			13/44 (29.5)		
LMR_{12W}		1.158	0.282		6.173	0.013
Up	47/48 (97.9)			18/48 (37.5)		
Down	38/42 (90.5)			6/42 (14.3)		
PLR_{12W}		0.000	1.000		0.065	0.799
Up	44/47 (93.6)			12/47 (25.5)		
Down	43/41 (95.3)			12/43 (27.9)		
PAR_{12W}		1.533	0.216		0.454	0.501
Up	35/39 (89.7)			9/39 (23.1)		
Down	50/51 (98.0)			15/51 (29.4)		

Table 2 (*continued*)

Table 2 (*continued*)

Variables	DCR			ORR		
	With DCR/total, n/N (%)	χ^2	P value	With ORR/total, n/N (%)	χ^2	P value
$dNLR_{12W}$		0.000	1.000		0.490	0.484
Up	44/47 (93.6)			14/47 (29.8)		
Down	41/43 (95.3)			10/43 (23.3)		
PNI_{12W}		0.591	0.442		1.105	0.293
Up	41/42 (97.6)			9/42 (21.4)		
Down	44/48 (91.7)			15/48 (31.3)		

WBC, white blood cell; ANC, absolute neutrophil count; AMC, absolute monocyte count; ALC, absolute lymphocyte count; EON, eosinophil number; PLT, platelet; LDH, lactate dehydrogenase; Hb, hemoglobin; Alb, albumin; CEA, carcinoembryonic antigen; TrxR, thioredoxin reductase; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; PAR, platelet-to-albumin ratio; dNLR, derived NLR; PNI, prognostic nutritional index; DCR, disease control rate; ORR, objective response rate.

Table 3 Results of univariate and multivariate Cox regression analysis for PFS (univariate analysis of the significant variables)

Variables	Progression					
	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Types of pathology	1.953	1.180–3.231	0.009*	2.103	1.237–3.576	0.006*
ECOG PS						
2	1			1		
1	0.096	0.024–0.388	0.001*	0.099	0.024–0.411	0.001*
0	0.177	0.052–0.601	0.006*	0.221	0.062–0.794	0.021*
CEA_{6W}	1.758	1.066–2.900	0.027*	1.810	1.091–3.003	0.022*
PLT_{12W}	1.754	1.044–2.918	0.034*	1.607	0.995–2.703	0.074

*, P<0.05. PFS, progression-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; CEA, carcinoembryonic antigen; PLT, platelet; HR, hazard ratio; CI, confidence interval.

types of pathology, ECOG PS and CEA_{6w} population (*Figure 3A*), and showed a C-index of 0.715 (95% CI: 0.685–0.745) and an AUC of 0.694 (95% CI: 0.667–0.704; *Figure 3B*). The 6- and 12-month calibration curves showed a good correlation between the actual and predicted outcomes (*Figure 3C,3D*). Patients with lower risk scores (50%) had a longer PFS (HR =0.33; 95% CI: 0.20–0.55; P<0.0001; *Figure 3E*). The relationship between risk scores and clinical characteristics were assessed, ECOG PS and best response to treatment were found to be correlated with the model's risk score (*Figure 4*).

Clinical prediction model for OS in patients with advanced NSCLC

Table 4 summarizes the results of the univariate and multivariate Cox regression analyses for OS. The univariate and multivariate Cox regression analyses showed that types of pathology, ALC_{6w} decreased, CEA_{6w} decreased, PLT_{6w} increased, $TrxR_{6w}$ decreased, PLR_{6w} increased, PAR_{6w} increased, WBC_{12w} decreased, ANC_{12w} decreased, PLT_{12w} decreased, PLR_{12w} decreased, and $dNLR_{12w}$ decreased were significantly associated with a prolonged OS. The univariate log-rank tests showed that patients with $TrxR_{6w}$ decreased

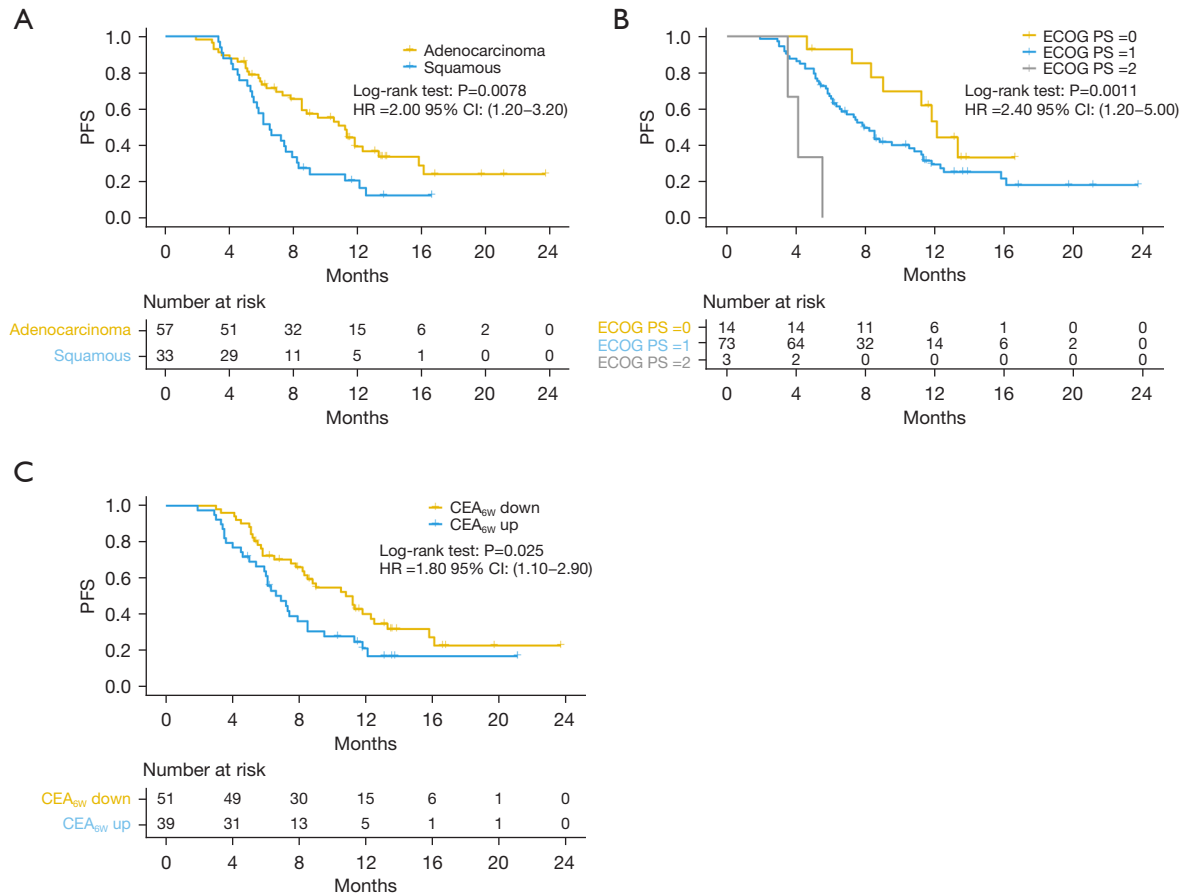

Figure 2 PFS curve. Types of pathology (A), ECOG PS (B), CEA (C). HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; CEA, carcinoembryonic antigen; PFS, progression-free survival.

(HR =3.402; 95% CI: 1.701–6.806; P=0.001; *Figure 5A*), ALC_{6w} decreased (HR =2.182; 95% CI: 1.013–4.700; P=0.046; *Figure 5B*), and adenocarcinoma (HR =1.950; 95% CI: 1.044–3.642; P=0.0036; *Figure 5C*) had a longer OS (*Figure 5*). Other variable values that were not statistically significant in association with OS were listed in Table S3.

The OS prediction model resulted in a C-index of 0.671 (95% CI: 0.626–0.716; *Figure 6A*) and an AUC of 0.561 (95% CI: 0.556–0.574; *Figure 6B*). The 12- and 18-month calibration curves showed a good correlation between the actual and predicted outcomes (*Figure 6C,6D*). After calculating the risk score, 45 cases were classified as having a higher risk score and 45 cases were classified as having a lower risk score. Cases with lower risk scores had a longer OS (HR =0.49; 95% CI: 0.28–0.84; P=0.0089; *Figure 6E*). However, no correlation was found between any of the clinical characteristics and the model's risk score (*Figure 7*).

Discussion

Anti-PD-1-based immunotherapy plays an important role in the treatment of NSCLC (27,28). However, the lack of strong predictive markers of immune checkpoint inhibitor efficacy is still a major issue, with patients exposed to the risk of immune-related adverse events not getting any survival benefit from these agents, without considering the high costs for healthcare systems. This emphasizes the need to identify appropriate immune checkpoint inhibitor biomarkers. The TrxR is a redox system link that is closely associated with tumor development and alteration of the tumor microenvironment leading to disease progression (29,30). Previous studies have identified that CEA, Alb, WBC, ANC, NLR, dNLR, and LMR are associated with immunotherapy response in patients with advanced NSCLC (31-35). However, to our knowledge, this was the

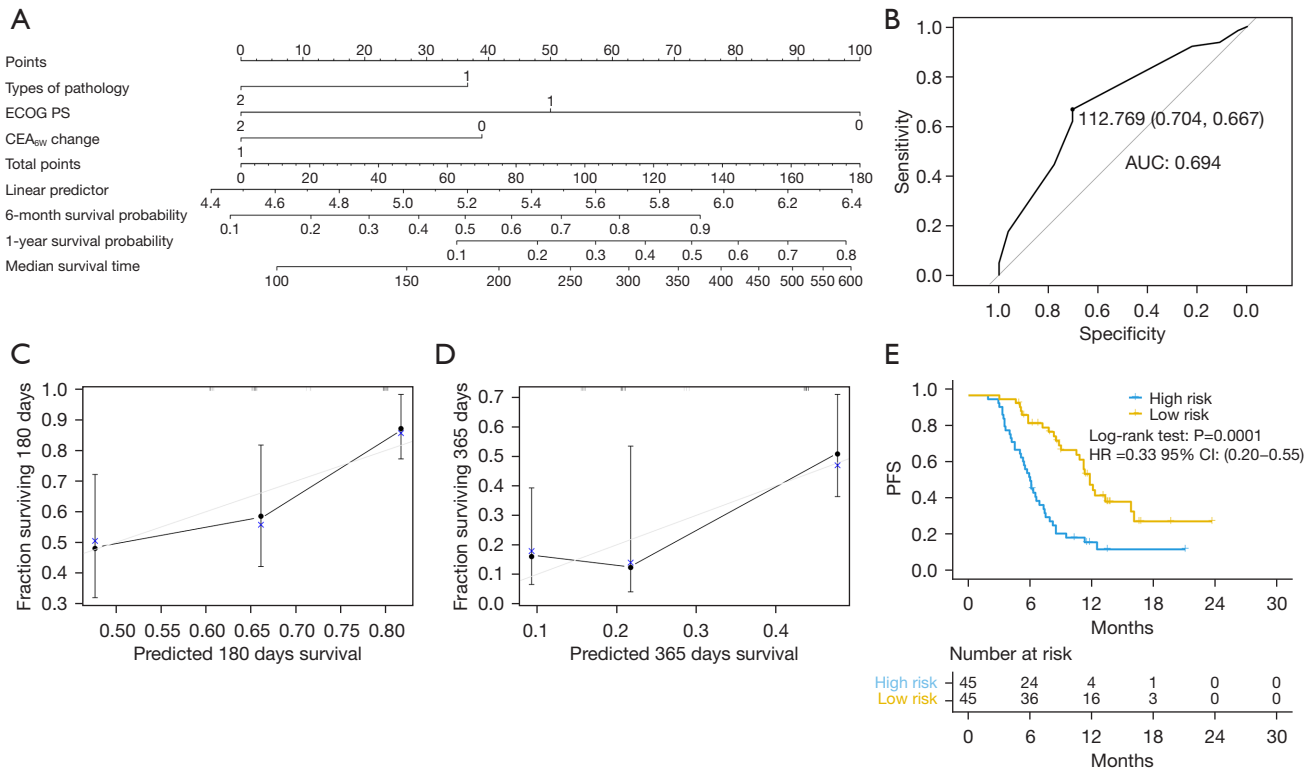

Figure 3 PFS prediction model. Nomogram for predicting survival and calibration curve of the nomogram with PFS. Based on 3 factors, including types of pathology, CEA_{6w} and ECOG PS, nomogram was developed to predict the probability of survival at 6 and 12 months. This probability could be calculated as a function of the total score, as the sum of the scores for each specific variable. Points are assigned to each factor by drawing a line up from the corresponding value to the 'point' line. The total number of points added to each factor was drawn on the 'total points'. Drawing a line down to read the corresponding predicted probability (A). ROC for the model (B). The calibration curve for the nomogram to predict 6-month survival (C). Calibration curve for the nomogram to predict 12-month survival (D). Kaplan-Meier curves using model predicted scores (E). ECOG PS, Eastern Cooperative Oncology Group performance status; CEA, carcinoembryonic antigen; AUC, area under the curve; HR, hazard ratio; CI, confidence interval; PFS, progression-free survival; ROC, receiver operating characteristic curve.

first study to evaluate the association between TrxR and the efficacy of PD-1-based immunotherapy in the management of advanced NSCLC.

Our findings revealed that a decrease in TrxR post-treatment was associated with better treatment outcome and prognosis in NSCLC patients after PD-1 inhibitor-based immunotherapy. Age, change in CEA_{6w}, Alb_{12w}, and $TrxR_{12w}$ were shown to be associated with DCR, while change in WBC_{6w}, ANC_{6w}, NLR_{6w}, $dNLR_{6w}$, Alb_{12w}, CEA_{12w}, and LMR_{12w} were associated with ORR. The cut-off for TrxR were determined by ROC, which were relied on the data we collected and lacked uniform criteria for grouping. In addition, limited by sample size, we did not perform external validation of the validation cohort, only internal validation of the model.

The TrxR is a reduced coenzyme II (NADPH)-dependent dimeric selenase containing the flavin adenine dinucleotide (FAD) structural domains TrxR1, TrxR2, and TrxR3, whose main function is to catalyze NADPH and maintain reduction of the small molecule Trx (18). TrxR, together with Trx and NADPH, forms a potent protein reduction system called the Trx system, which plays an important role in the body's redox regulation and antioxidant defense, cell proliferation, and signal transduction (36,37). Both Trx and TrxR have been found to be overexpressed in a variety of tumor tissues and oversecreted by tumor cells, which in turn stimulates tumor cell growth and prevent apoptosis (38). Given that TrxR can be inhibited by specific drugs, it is considered a

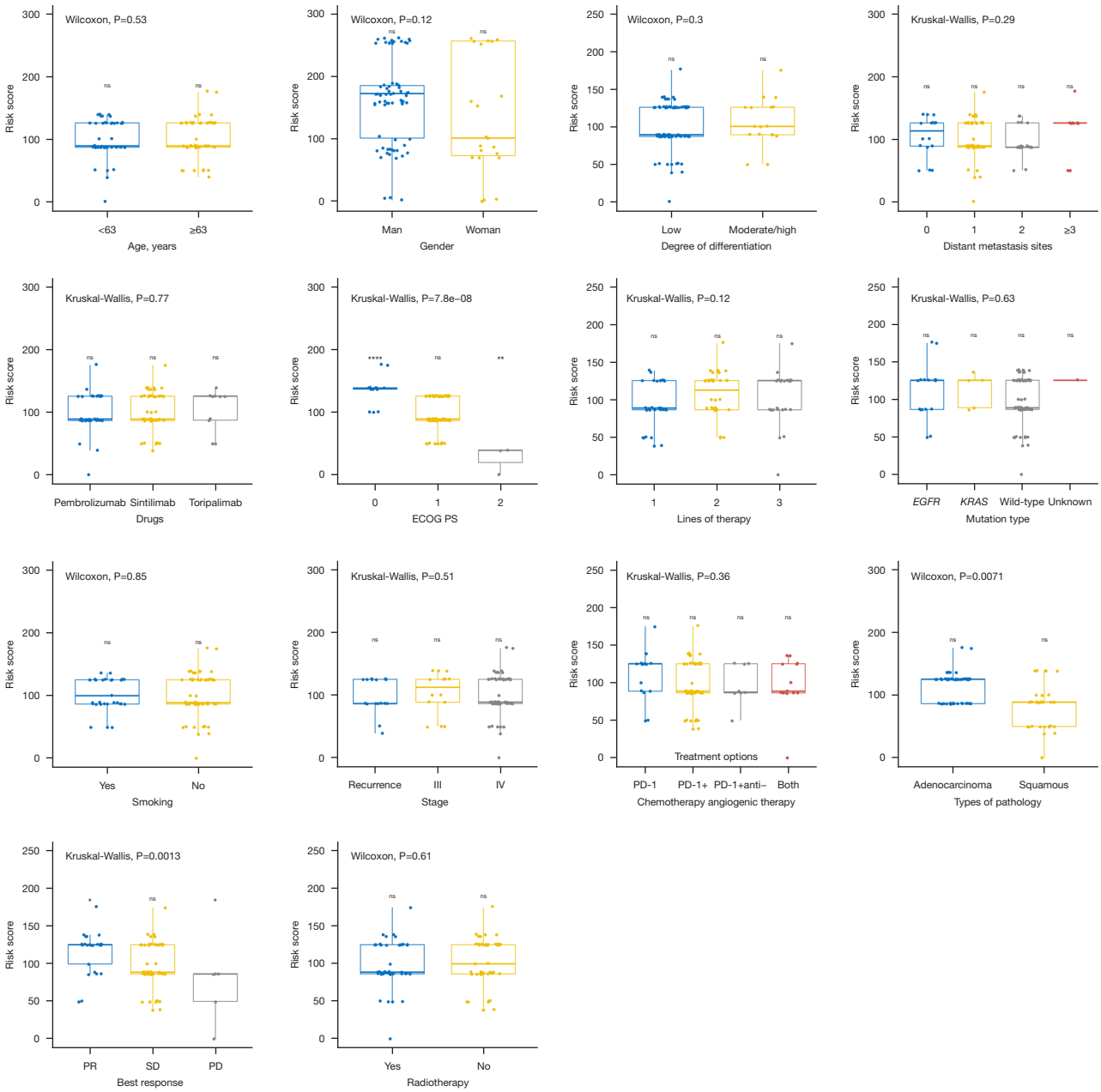

Figure 4 PFS prediction model and clinical characteristics. *, P≤0.05; **, P≤0.01; ***, P≤0.001; ****, P≤0.0001; ns, P>0.05. ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene homologue; PD-1, programmed cell death receptor protein 1; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival.

promising target for cancer research. Furthermore, previous studies have reported that TrxR expression in tumor tissues is higher when compared with normal tissues (39,40). TrxR could be used to monitor abnormal cell proliferation or tumorigenesis in humans, and hence could potentially be used to identify tumors at an early stage (41,42). In lung cancer, TrxR is more frequently expressed in patients with poorly differentiated and larger tumors, and in those with lymph node metastases (43-45). In our study, we explored the association between TrxR, survival outcome and response

Table 4 Results of univariate and multivariate Cox regression analysis for OS (univariate analysis of the significant variables)

Variables	Mortality					
	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Types of pathology	1.824	1.017–3.274	0.044*	1.950	1.044–3.642	0.036*
ALC_{6W}	1.950	1.132–3.360	0.016*	2.182	1.013–4.700	0.046*
CEA_{6W}	1.936	1.121–3.343	0.018*	1.634	0.793–3.369	0.183
PLT_{6W}	0.527	0.299–0.930	0.027*	0.387	0.111–1.351	0.137
$TrxR_{6W}$	2.550	1.409–4.615	0.002*	3.402	1.701–6.806	0.001*
PLR_{6W}	0.437	0.250–0.763	0.004*	0.921	0.322–2.634	0.879
PAR_{6W}	0.571	0.327–0.997	0.049*	2.094	0.552–7.942	0.277
WBC_{12W}	1.733	1.004–2.993	0.049*	1.458	0.444–4.788	0.535
ANC_{12W}	1.864	1.079–3.218	0.025*	0.723	0.186–2.818	0.641
PLT_{12W}	2.528	1.409–4.535	0.002*	2.213	0.921–5.318	0.076
PLR_{12W}	2.289	1.263–4.148	0.006*	1.029	0.439–2.415	0.947
$dNLR_{12W}$	1.801	1.040–3.118	0.036*	1.786	0.693–4.602	0.230

*, P<0.05. OS, overall survival; ALC, absolute lymphocyte count; CEA, carcinoembryonic antigen; PLT, platelet; TrxR, thioredoxin reductase; PLR, platelet-to-lymphocyte ratio; PAR, platelet-to-albumin ratio; WBC, white blood cell; ANC, absolute neutrophil count; dNLR, derived NLR; HR, hazard ratio; CI, confidence interval.

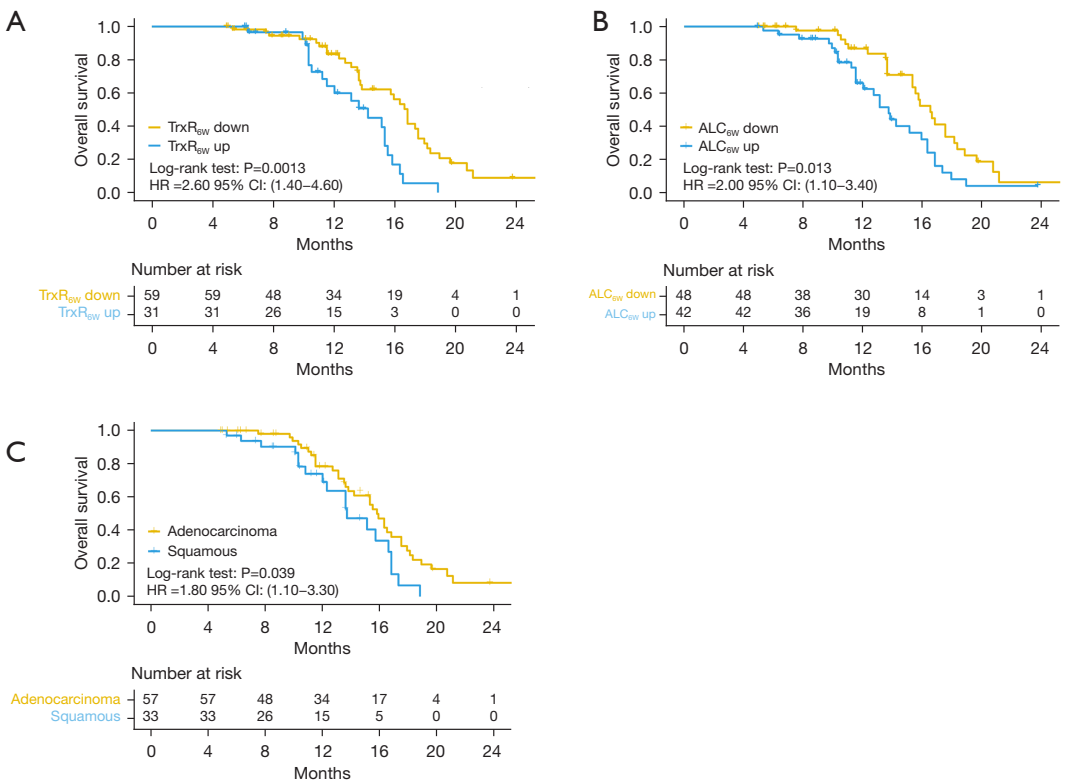

Figure 5 OS curve. TrxR (A), ALC (B), types of pathology (C). TrxR, thioredoxin reductase; HR, hazard ratio; CI, confidence interval; ALC, absolute lymphocyte count; OS, overall survival.

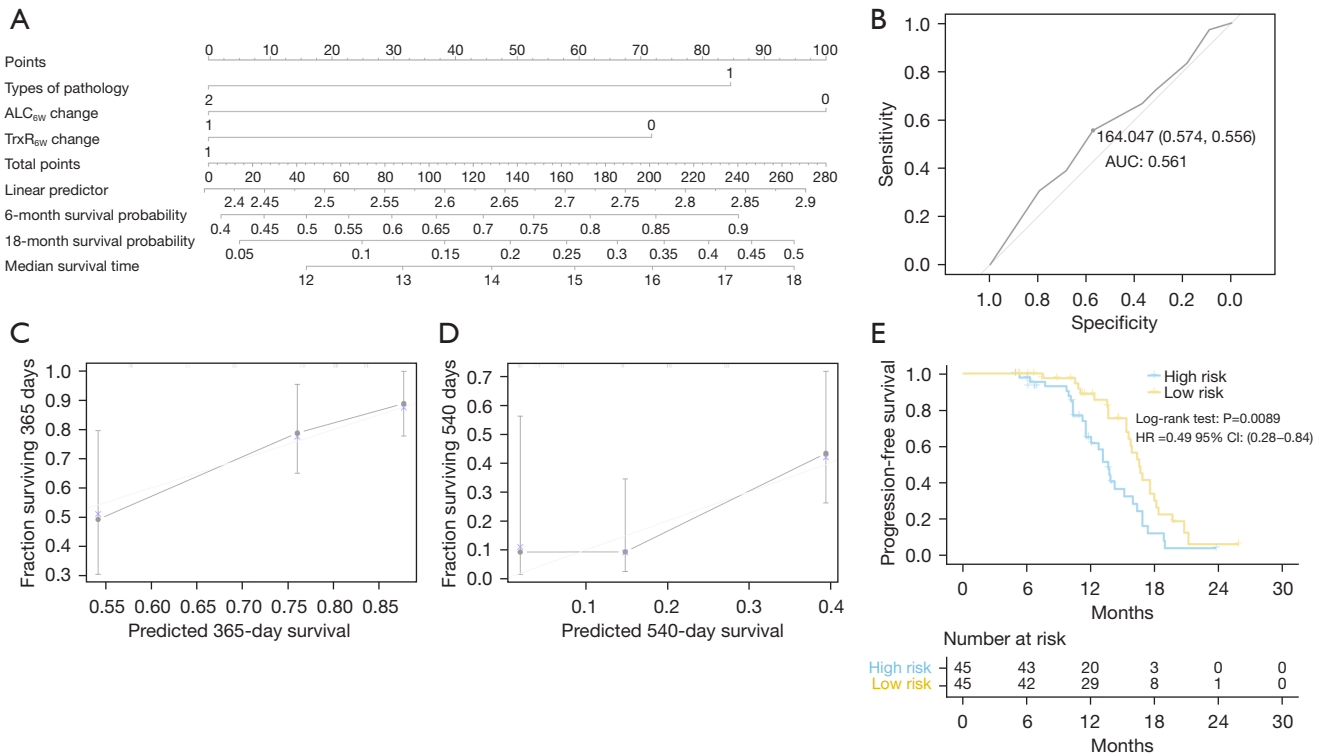

Figure 6 OS prediction model. Nomogram for predicting survival and calibration curves of a nomogram versus OS. The probability of survival at 12 and 18 months was predicted based on 3 factors: types of pathology, ALC_{6w} and $TrxR_{6W}$. As a function of the total points, this probability could be calculated as the sum of the points for each specific variable. Points were assigned to each factor by drawing a line upwards from the corresponding value to the 'point' line. The total number of points added to each factor was drawn on the 'total points' line. Drawing a line down to read the corresponding predicted probability (A). ROC curve for the model (B). Calibration curve of a nomogram to predict survival at 12-month (C). Calibration curve of a nomogram to predict survival at 18-month (D). Kaplan-Meier curves using model predicted scores (E). ALC, absolute lymphocyte count; TrxR, thioredoxin reductase; AUC, area under the curve; HR, hazard ratio; CI, confidence interval; OS, overall survival; ROC, receiver operating characteristic.

to immunotherapy among patients with advanced NSCLC. Patients with decreased TrxR after treatment had a more favorable clinical outcome, which is consistent with previous research in gastric cancer patients (17). Unfortunately, we were not able to assess the relationship between TrxR and diagnosis of advanced NSCLC, as the TrxR levels are not routinely measured at the time of diagnosis in the participating oncology hospitals. Further research on TrxR and NSCLC diagnosis is warranted.

This study also investigated the association between peripheral blood parameters and prognosis treated with PD-1 inhibitor-based immunotherapy in advanced NSCLC patients. The blood parameters associated with PFS or OS in the multivariate Cox regression were used to build clinical prediction models, respectively. We also compared the risk scores with patient prognosis (*Figures 3E,6E*) to provide a more intuitive and effective view of the validity of the model scores. The model scores showed moderate prediction performance for both PFS and OS.

Our study had several limitations. The retrospective study design as well as the small number of cases included in the study limited the statistical power of analyses. In addition, the collection and analysis of TrxR and blood samples may vary between patient area, leading to batch effects that might compromise the validity of our findings. Furthermore, due to the retrospective nature of our study, we could not compare our findings with a control group of patients treated with chemotherapy alone. Another mayor issue is the lack of PD-L1 expression score of included patients. Indeed, although suboptimal, PD-L1 expression showed to some predictive role in NSCLC treated with immune checkpoint inhibitors (46). Moreover,

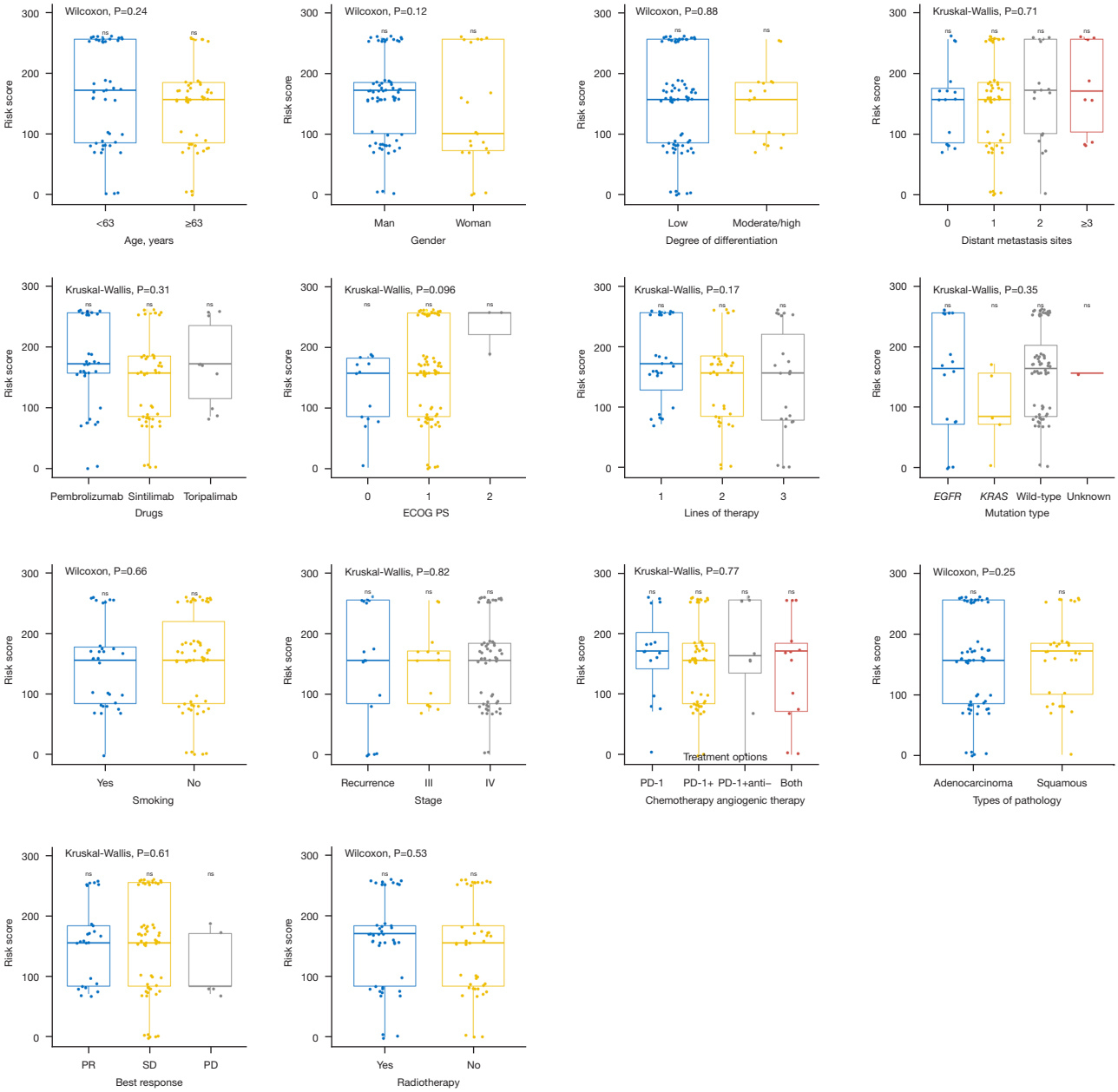

Figure 7 OS prediction model and clinical characteristics. ns, P>0.05. ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene homologue; PD-1, programmed cell death receptor protein 1; PR, partial response; SD, stable disease; PD, progressive disease; OS, overall survival.

our population was tested for EGFR and KRAS mutations only and we could not exclude that some included patients harbor other oncogenic alterations (i.e., anaplastic lymphoma kinase or ROS1 rearrangements) that may be negatively related to immunotherapy efficacy in NSCLC, especially when used as single-agents (47). Although we analyzed the ORR and DCR, the small number of events did not allow us to perform a multivariate regression analysis on treatment responses, which highlights the need for extended monitoring. Our study had a limited sample size, so we could not externally validate the model, and as a result, we will continue to accumulate and build a better

clinical prediction model in subsequent studies. Finally, the 6- and 12-week check points for hematological parameters were arbitrarily chosen, and thus may not be optimal.

Conclusions

In summary, several peripheral blood markers, including TrxR, can be used to evaluate the response to immunotherapy in patients with advanced NSCLC. Therefore, repeated measurements of peripheral blood markers during treatment may help physicians to response to immunotherapy and clinical outcome.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-300/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-300/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-300/coif). GS received payment from Takeda Pharmaceutical Co. Advisory board. PB received honoraria from AstraZeneca, BeiGene, Bristol Meyers Squibb, Roche, Takeda; he received support for virtual meeting registration from Amgen, Daiichi Sankyo; he received institutional research grants from Roche, Pfizer. He did not receive support for the present work. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional Ethics Committee of Jiangsu Cancer hospital. Individual consent for this retrospective analysis was waived.

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