Am J Cancer Res 2025;15(4):1705-1718
www.ajcr.us /ISSN:2156-6976/ajcr0162013

Original Article

Predictive role of peripheral blood indicators in the prognosis of patients with cutaneous squamous cell carcinoma treated with immune checkpoint inhibitors

Xiaoyue Xiao[1,2*], Qianying Yu[1,2*], Bingying Han[2], Min Fu[2], Mingling Chen[1,2]

[1]Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu 610075, Sichuan, China; [2]Chengdu University of Traditional Chinese Medicine, Chengdu 610075, Sichuan, China. [*]Equal contributors.

Received November 14, 2024; Accepted March 25, 2025; Epub April 15, 2025; Published April 30, 2025

Abstract: This study aimed to explore the predictive role of peripheral blood indicators in the prognosis of cutaneous squamous cell carcinoma (cSCC) patients treated with immune checkpoint inhibitors (ICIs). Clinical data of 139 cSCC patients receiving ICIs treatment were retrospectively collected. Peripheral blood indicators, including blood cell counts, neutrophil-to-lymphocyte ratio (NLR), liver and kidney function markers, and inflammation markers, were examined. A binary logistic regression model was used to identify risk factors for non-response to ICIs, and a predictive model was constructed. Additionally, multiple linear regression and Pearson correlation analysis were employed to assess relevant influences and relationships. Results showed that immunotherapy timing, lymphocyte count, NLR, and C-reactive protein (CRP) were influencing factors for non-response to ICIs (all $P<0.05$). The area under the curve (AUC) for these indicators in predicting non-response risk was 0.651 (95% CI: 0.529-0.773), 0.671 (95% CI: 0.542-0.801), 0.775 (95% CI: 0.682-0.868), and 0.717 (95% CI: 0.573-0.861), respectively. The combined AUC of these four factors was 0.878 (95% CI: 0.790-0.966), with sensitivity and specificity of 76.0% and 93.0%, respectively. After internal verification, the constructed model exhibited predicted sensitivity and specificity of 80.00% and 94.29% respectively. Multiple linear regression analysis indicated that these four factors were independent predictors of progression-free survival (PFS) in cSCC patients. Immunotherapy timing, NLR, and CRP were negatively correlated with PFS ($r = -0.235, -0.330, -0.494$), while lymphocyte count was positively correlated with PFS ($r = 0.326$). In conclusion, peripheral blood indicators are valuable for predicting the response to ICIs in cSCC and can influence patients' PFS.

Keywords: Cutaneous squamous cell carcinoma, immune checkpoint inhibitors, peripheral blood indicators, prognosis

Introduction

Cutaneous squamous cell carcinoma (cSCC) is a common skin malignancy accounting for 25% of all skin cancers, with a higher malignancy and metastasis rate than basal cell carcinoma [1]. Most cSCC cases are caused by actinic keratosis due to long-term sunlight exposure. Besides, scars after burns and trauma, and chronic inflammatory ulcers can also induce cSCC. In recent years, the incidence of cSCC has been on the rise [2]. With advancements in medical treatments, immune checkpoint inhibitors (ICIs) have shown great potential in managing cSCC [3]. However, not all patients benefit equally from ICI therapy, and their prognosis can vary widely [4]. Identifying reliable prognostic predictors is crucial for optimizing treatment plans and improving patient outcomes.

Peripheral blood indicators, being simple, accessible, and repeatable, are of great significance in the diagnosis and prognosis evaluation of tumors. Recent studies have increasingly focused on the relationship between peripheral blood indicators and tumor prognosis. For example, the neutrophil-to-lymphocyte ratio (NLR) has been closely linked to the prognosis of various cancers, with a high NLR often indicating a poor prognosis [5-7]. Lymphocyte count is also associated with immune status and tumor prognosis, where higher counts typi-

https://doi.org/10.62347/KZOQ9722

cally correlate with better prognosis [8, 9]. In the context of ICI therapy, peripheral blood markers could serve as important predictors of prognosis in cSCC patients by reflecting immune status, inflammation, and other factors. Markers such as immune cell subsets and inflammatory factors may interact with immune checkpoint inhibitors, influencing treatment response and survival outcome of patients [10, 11].

This study aims to investigate the predictive role of peripheral blood indicators in the prognosis of cSCC patients undergoing ICI treatment. By analyzing various peripheral blood markers, we seek to identify key indicators that can accurately predict patient outcomes and provide a scientific basis for personalized treatment plans, ultimately enhancing treatment effectiveness and patient quality of life.

Materials and methods

Research subjects

Clinical data from 139 cSCC patients undergoing ICIs treatment at the Hospital of Chengdu University of Traditional Chinese Medicine from August 2018 to July 2022 were retrospectively analyzed in this study. Inclusion criteria: (1) cSCC diagnosis confirmed by pathology, imaging, and dermatovenereology [12]; (2) Immunotherapy for ≥2 cycles; (3) Eastern Cooperative Oncology Group-performance status (ECOG-PS) [13] ≤3 points; (4) Presence of at least one measurable or evaluable primary or metastatic lesion on imaging; (5) Complete clinical, pathological, and follow-up data. Exclusion criteria: (1) Multiple primary tumors, either simultaneously or previously; (2) Presence of hematological or immune system diseases that affect hematological indicators.

Another 100 cSCC patients treated with ICIs at our hospital between August 2022 and July 2024 were selected for external validation of the predictive risk model.

This study was approved by the Ethics Committee of Hospital of Chengdu University of Traditional Chinese Medicine.

Collection and detection methods of peripheral blood indicators

Blood routine detection: Before treatment, 2-5 mL of fasting peripheral venous blood was collected from patients. The samples were immediately mix gently to prevent coagulation and stored at 2-8°C for further analysis. Blood cell counts and classifications, including white blood cells, lymphocytes, and neutrophils, were detected using a Mindray BC-6800 automatic hematology analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.), employing methods such as electrical impedance and laser scattering. The neutrophil-to-lymphocyte ratio (NLR) was calculated as the ratio of neutrophils to lymphocytes.

Liver and kidney function detection: Fasting peripheral venous blood was collected before treatment, centrifuged at 3000-4000 r/min for 10-15 minutes to separate serum, which was stored in a refrigerator for subsequent analysis. Liver and kidney function markers (e.g., alanine aminotransferase, aspartate aminotransferase, creatinine, and urea nitrogen) were detected using a Beckman Coulter AU5800 automatic biochemical analyzer (Beckman Coulter Commerce (China) Co., Ltd.) with its corresponding biochemical detection kit, applying the colorimetric method.

Inflammatory marker detection: Before treatment, inflammatory markers, including C-reactive protein (CRP) and procalcitonin (PCT), were measured using a Roche Cobas e 411 automatic electrochemiluminescence immunoanalyzer (Roche Diagnostics (Shanghai) Co., Ltd.) and its respective detection kit.

Prognostic assessment

Follow-up data were obtained from hospital records or telephone calls, with a cutoff date of July 31, 2024. Efficacy was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST v.1.1) [14], which classifies responses as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The objective response rate (ORR) was calculated as the percentage of patients achieving CR and PR after treatment, relative to the total number of patients; the disease control rate (DCR) was calculated as the percentage of patients achieving PR+CR+SD after treatment relative to the total number of cases. Response to ICIs was defined as tumor shrinkage, tumor stability, or symptom improvement (corresponding to CR, PR and SD); while no

response was characterized by tumor progression or aggravated symptoms (corresponding to PD) [15, 16]. Progression-free survival (PFS) was defined as the time from the start of ICIs treatment to the first observation of PD, the last follow-up, or death [17, 18]. All evaluation was conducted by an independent team, not involved in patient treatment, to minimize evaluation bias.

Immune checkpoint inhibitor medication situation

In this study, the main immune checkpoint inhibitor used by the included patients was pembrolizumab (manufacturer: Merck Sharp & Dohme Corp., USA; approval number: National Drug Approval No. SJ20180019). Treatment with pembrolizumab began immediately after diagnosis. The average treatment duration was 7.5 months, with a range from 5 months to 12 months. Regarding drug changes, 129 patients continued with pembrolizumab throughout the treatment period. Ten patients changed their treatment regimen due to disease progression or intolerable adverse reactions, but the type of immune checkpoint inhibitor remained the same. For TNM stage at the time of treatment, stage I patients primarily received adjuvant therapy to reduce the risk of recurrence; stage II patients typically received pembrolizumab monotherapy, while some stage III patients were treated with a combination of immune checkpoint inhibitors and other therapeutic modalities, such as surgery or radiotherapy.

Statistical analysis

Data were analyzed using SPSS 21.0 software. Measurement data conforming to normal distribution were expressed as mean ± standard deviation, and compared using independent sample t-test between two groups. Count data were described by n (%), and the chi-square test or continuity correction was use for data comparison. A binary logistic regression model [19] was used to identify risk factors for non-response to ICIs in cSCC patients and to construct a predictive model. The model's predictive performance was evaluated using receiver operating characteristic (ROC) curves, area under the curve (AUC), sensitivity and specificity. Multiple linear regression [20] analysis and Pearson correlation analysis were used to assess the impact of Immunotherapy timing, lymphocyte count, NLR, and CRP on PFS and to explore the relationships between these factors. A *P*-value of <0.05 was considered statistically significant.

Results

Patient characteristics

The 139 cSCC patients were aged 49-68 years, with an average age of (57.72±3.81) years; the lesion diameter ranged from 1.7-8.6 cm, with an average of (5.28±1.32) cm; the disease duration ranged from 1-4 years, with an average disease course of (2.63±0.68) years. Of the patients, 92 cases (66.19%) were male, 93 cases (66.91%) had lesions on exposed parts, 65 cases (46.76%) had tumors infiltrating into the subcutaneous tissue, 73 cases (52.52%) had moderate to high-grade cSCC. Additionally, 36 cases (25.90%) had a history of cSCC recurrence, and 93 cases (66.91%) had received radiotherapy (**Table 1**).

Analysis of treatment response

Thirteen patients (9.35%) achieved CR, 51 patients (36.69%) achieved PR, 50 patients (35.97%) achieved SD, and 25 patients (17.98%) had PD. The ORR was 46.04%, and the DCR was 82.01%.

Clinicopathological features and peripheral blood index levels of patients with or without response to ICIs

Among patients without response to ICIs, a higher proportion had TNM stage I/II and underwent second-/third-/later-line treatments compared to those with a response ($P<0.05$). No significant differences were observed in other baseline data or pathological characteristics ($P>0.05$). In total, 25 patients did not respond to ICIs, while 114 patients showed a response. Peripheral blood analysis revealed that non-responders had significantly higher NLR and CRP levels and lower lymphocyte counts compared to responders (all $P<0.05$) (**Table 2**).

Logistic regression analysis of factors influencing non-response to ICIs

Taking response to ICIs as the dependent variable (yes = 0, no = 1), logistic regression analy-

Table 1. Baseline characteristics of the included 139 patients

Characteristic	N	Percentage
Gender		
Male	92	66.19%
Female	47	33.81%
Age		
<60 years old	98	70.50%
≥60 years old	41	29.50%
Lesion location		
Exposed	93	66.91%
Unexposed	46	33.09%
Lesion diameter		
<5 cm	58	41.73%
≥5 cm	81	58.27%
Infiltration depth		
Epidermis	32	23.02%
Dermis	42	30.22%
Subcutaneous tissue	65	46.76%
Pathological grade		
Low grade	66	47.48%
Moderate/high grade	73	52.52%
TNM stage		
Stage I/II	98	70.50%
Stage III	41	29.50%
Lymph node metastasis		
Yes	16	11.51%
No	123	88.49%
cSCC recurrence		
Yes	36	25.90%
No	103	74.10%
Radiotherapy		
Yes	93	66.91%
No	46	33.09%
Disease course (years)		
<2 years	65	89.93%
≥2 years	74	10.07%
Immunotherapy timing		
First-line treatment	104	74.82%
Second-/third-/later-line treatment	35	25.18%

sis was performed with the following significant indicators as independent variables: TNM stage (stage I/II = 0, stage III/IV = 1), Immunotherapy timing (first-line = 0, second-line, third-line or later-line treatment = 1), lymphocyte count, NLR, CRP levels. The analysis revealed that Immunotherapy timing, lymphocyte count, NLR, and CRP were influencing factors for non-response to ICIs in cSCC patients (P<0.05) (**Table 3**).

Risk model building, predictive performance, and internal validation

A risk prediction model for non-response to ICIs in cSCC patients was constructed based on significant risk factors: Logit(P) = -4.875 + 1.484 * Immunotherapy timing - 3.071 * lymphocyte count + 0.758 * NLR + 0.259 * CRP. The AUCs of these indicators for predicting non-response to ICIs were: Immunotherapy timing: 0.651 (95% CI: 0.529-0.773); Lymphocyte count: 0.671 (95% CI: 0.542-0.801); NLR: 0.775 (95% CI: 0.682-0.868); and CRP: 0.717 (95% CI: 0.573-0.861). Immunotherapy timing and NLR demonstrated relatively high sensitivities, 60.0% and 88.0%, respectively, while lymphocyte count and NLR showed high specificities of 97.4% and 86.8%, respectively (**Table 4**; **Figure 1**).

Prediction performance and internal validation of the risk model

The model's predictive performance was first evaluated using a training set of 139 patients. Additionally, data from 100 other cSCC patients treated with ICIs were used as an external validation set.

ROC curve analysis: Training set: ROC curve analysis indicated a strong predictive ability, with an AUC of 0.866 (95% CI: 0.773-0.958), suggesting high prediction accuracy in the training set (**Figure 2A**). Validation set: The ROC curve for the validation set also demonstrated good performance, with an AUC of 0.826 (95% CI: 0.726-0.926). Although slightly lower than that of the training set, this result still showed that the model had a certain ability to distinguish between responders and non-responders in new data, highlighting its acceptable generalization capability (**Figure 2B**).

Calibration curve analysis: Training set: The "Apparent" and "Bias-corrected" curves demonstrated the model's prediction accuracy by comparing them to the "Ideal" curve. As shown in **Figure 3A**, the calibration curve for the training set data was closely aligns with the ideal curve within a certain range, indicating good consistency between the model's predicted

Table 2. Comparison of baseline characteristics between patients with and without response to ICIs

Characteristic	Responders (n=25)	Non-responders (n=114)	x^2/t	*P*
Gender			0.045	0.832
Male	17 (68.00%)	75 (65.79%)		
Female	8 (32.00%)	39 (34.21%)		
Age			0.620	0.431
<60 years old	16 (64.00%)	82 (71.93%)		
≥60 years old	9 (36.00%)	32 (28.07%)		
Lesion location			0.357	0.550
Exposed	18 (72.00%)	75 (65.79%)		
Unexposed	7 (28.00%)	39 (34.21%)		
Lesion diameter			0.037	0.847
<5 cm	10 (40.00%)	48 (42.11%)		
≥5 cm	15 (60.00%)	66 (57.89%)		
Infiltration depth			2.826	0.243
Epidermis	8 (32.00%)	24 (21.05%)		
Dermis	9 (36.00%)	33 (28.95%)		
Subcutaneous tissue	8 (32.00%)	57 (50.00%)		
Pathological grade			0.250	0.617
Low grade	13 (52.00%)	53 (46.49%)		
Moderate/high grade	12 (48.00%)	61 (53.51%)		
TNM stage			8.180	0.004
Stage I/II	10 (40.00%)	80 (70.18%)		
Stage III	15 (60.00%)	34 (29.82%)		
Lymph node metastasis			0.068	0.794
Yes	2 (8.00%)	14 (12.28%)		
No	23 (92.00%)	100 (87.72%)		
cSCC recurrence			0.591	0.442
Yes	8 (32.00%)	28 (24.56%)		
No	17 (68.00%)	86 (75.44%)		
Radiotherapy			1.638	0.201
Yes	14 (56.00%)	79 (69.30%)		
No	11 (44.00%)	35 (30.70%)		
Disease course (years)			1.678	0.195
<2 years	13 (52.00%)	75 (65.79%)		
≥2 years	12 (48.00%)	39 (34.21%)		
Immunotherapy timing			7.224	0.007
First-line treatment	11 (44.00%)	82 (71.93%)		
Second-/third-/later-line treatment	14 (56.00%)	32 (28.07%)		
White blood cell ($\times10^9$/L)	6.35±1.27	6.72±1.50	1.146	0.254
Lymphocyte ($\times10^9$/L)	1.02±0.28	1.19±0.31	2.524	0.013
Neutrophil ($\times10^9$/L)	2.35±0.56	2.28±0.73	0.451	0.653
NLR	2.89±1.41	2.00±0.87	4.086	<0.001
Alanine aminotransferase (U/L)	32.55±4.68	30.62±5.61	1.601	0.112
Aspartate aminotransferase (U/L)	28.22±3.54	29.16±3.70	1.159	0.248
Creatinine (μmol/L)	75.16±5.11	73.58±8.42	0.901	0.369
Urea nitrogen (mmol/L)	6.02±1.54	5.79±1.28	0.784	0.435
CRP (mg/L)	24.16±12.39	13.28±3.21	8.281	<0.001
Procalcitonin (μg/mL)	0.33±0.08	0.35±0.06	1.416	0.159

ICI: Immune checkpoint inhibitor; cSCC: cutaneous squamous cell carcinoma; NLR: Neutrophil-to-Lymphocyte Ratio; CRP: C-reactive protein.

Table 3. Logistic regression analysis of factors influencing non-response to ICIs in cSCC patients

Variable	B	SE	Wals	*P*	OR (95% CI)
Step 1					
TNM staging	0.026	0.624	0.002	0.967	1.026 (0.302-3.487)
Immunotherapy timing	1.485	0.628	5.596	0.018	4.415 (1.290-15.110)
Lymphocyte count	-3.070	1.274	5.810	0.016	0.046 (0.004-0.564)
NLR	0.758	0.247	9.411	0.002	2.134 (1.315-3.463)
CRP	0.259	0.075	11.858	0.001	1.296 (1.118-1.502)
Constant	-4.890	1.727	8.017	0.005	0.008
Step 2					
Immunotherapy timing	1.484	0.627	5.596	0.018	4.412 (1.290-15.091)
Lymphocyte count	-3.071	1.273	5.818	0.016	0.046 (0.004-0.562)
NLR	0.758	0.247	9.448	0.002	2.135 (1.316-3.462)
CRP	0.259	0.075	11.939	0.001	1.295 (1.119-1.500)
Constant	-4.875	1.685	8.365	0.004	0.008

ICI: Immune checkpoint inhibitor; cSCC: cutaneous squamous cell carcinoma; NLR: Neutrophil-to-Lymphocyte Ratio; CRP: C-reactive protein.

Table 4. Predictive performance of the constructed risk model

Test result variable	AUC (95% CI)	Standard error	*P*	Sensitivity (%)	Specificity (%)	Optimal cut-off value
Immunotherapy timing	0.651 (0.529-0.773)	0.062	0.018	60.0	70.2	-
Lymphocyte count	0.671 (0.542-0.801)	0.066	0.007	52.0	97.4	18.55
NLR	0.775 (0.682-0.868)	0.047	0.000	52.0	86.8	0.26
CRP	0.717 (0.573-0.861)	0.074	0.001	88.0	45.0	1.96

NLR: Neutrophil-to-Lymphocyte Ratio; CRP: C-reactive protein.

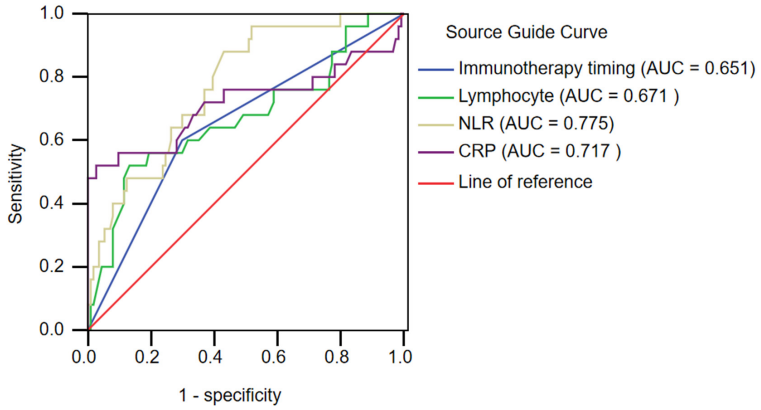

Figure 1. ROC curves for immunotherapy timing, lymphocyte count, NLR and CRP in predicting the non-response to ICIs in cSCC patients. ROC: receiver operating characteristic; NLR: Neutrophil-to-Lymphocyte Ratio; CRP: C-reactive protein; ICIs: Immune checkpoint inhibitors.

probabilities and the actual outcomes (**Figure 3A**). Validation set: Similar to the training set, the calibration curve for the validation set was also closely approached the ideal curve, further confirming the reliability of the model's predicted probabilities in the validation set (**Figure 3B**).

Decision curve analysis: Training set: The decision curve analysis demonstrated that the "Premodel" curve provided a net benefit. Compared with the "All" and "None" curves, the "Premodel" curve was in a relatively high position, indicating that using this model for decision-making could yield a greater net benefit, that is, the model has strong clinical decision-making value in the training set (**Figure 4A**). Validation set: The decision curve of the validation set showed a similar trend. Within a certain risk-threshold range, the "Premodel" curve was also higher than the "All" and "None" curves,

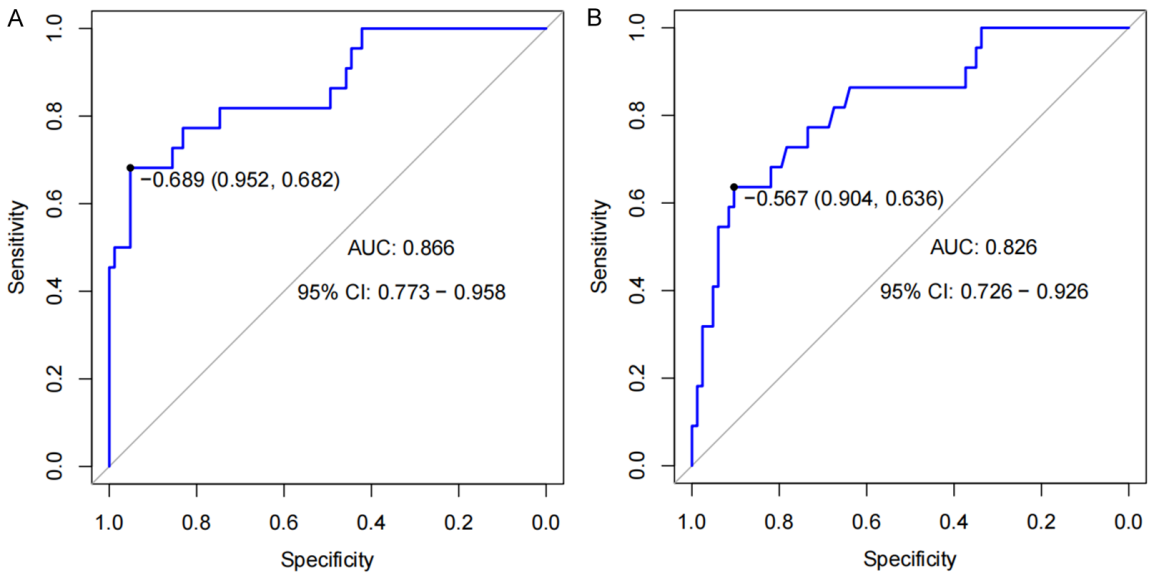

Figure 2. The ROC curves for the constructed predictive model in training set (A) and the validation set (B).

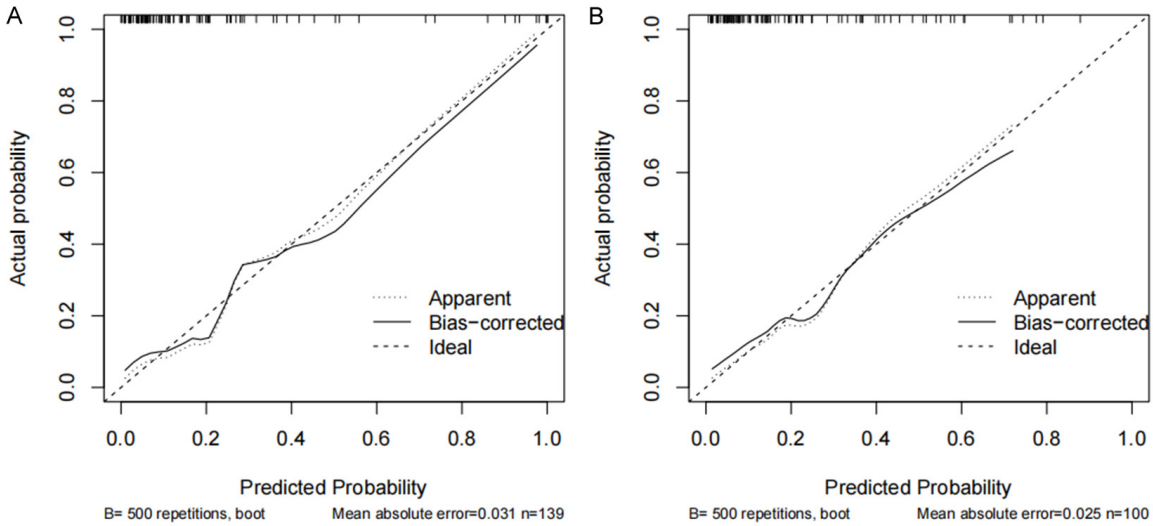

Figure 3. The calibration curves for the constructed predictive model in training set (A) and the validation set (B).

indicating that the model maintains clinical decision-making value in the validation set and can provide a valuable reference for clinical decision-making (**Figure 4B**).

Influence of immunotherapy timing, lymphocyte count, NLR, and CRP, on PFS

Multiple linear regression analysis was performed with PFS of cSCC patients as the dependent variable and immunotherapy timing, lymphocyte count, NLR, and CRP as independent variables. The results showed that immunotherapy timing, lymphocyte count, NLR, and CRP are independent factors influencing PFS in cSCC patients (all $P<0.05$) (**Table 5**). The model summary and ANOVA show that an R value greater than 0.6 (0.630) indicates a good model fit. The *P*-value of Anova for PFS being less than 0.05 implies a linear correlation. The histogram (**Figure 5A**) shows that the residual distribution is roughly higher in the middle and lower on both sides, approximating a normal distribution, which indicates a good model fit for the data. In the scatter plot (**Figure**

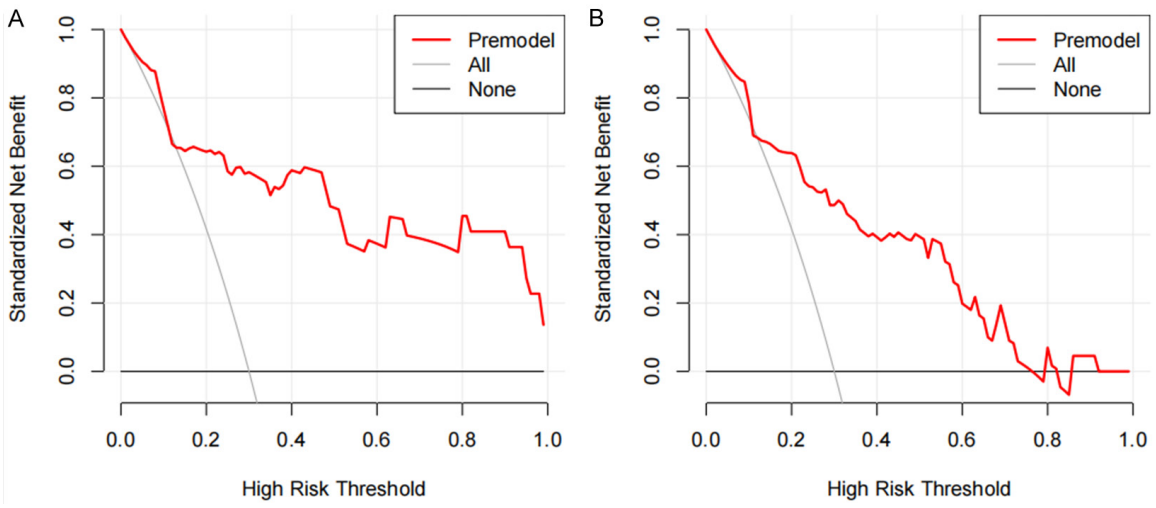

Figure 4. The decision curves for the constructed predictive model in training set (A) and the validation set (B).

Table 5. Influence of Immunotherapy timing, lymphocyte count, NLR, and CRP on PFS

Variable	*β*	SE	*β′*	t	*P*
Constant	-0.217	0.127	-	-1.706	0.040
Immunotherapy timing	0.160	0.054	0.199	2.947	0.004
Lymphocyte count	-0.207	0.083	-0.167	-2.485	0.014
NLR	0.083	0.022	0.268	3.820	<0.001
CRP	0.025	0.004	0.413	5.934	<0.001

β is the unstandardized coefficient, β' is the standardized coefficient, and t is the t-value of multiple linear regression analysis. NLR: Neutrophil-to-Lymphocyte Ratio; CRP: C-reactive protein.

5B), although the scatter points are somewhat dispersed, they generally show a random distribution around a certain horizontal position without an obvious curvilinear trend, suggesting a linear relationship between the independent and dependent variables. Immunotherapy timing, NLR, and CRP were negatively correlated with PFS (r = -0.235, -0.330, -0.494), while lymphocyte count was positively correlated with PFS (r = 0.326) (all $P<0.05$).

Discussion

Immune checkpoint inhibitors (ICIs) have shown potential in the treatment of cSCC [21]. Based on the objective response rate (ORR) and disease control rate (DCR), this treatment has shown some effectiveness. The ORR indicates that nearly half of the patients experienced significant tumor shrinkage or complete disappearance after treatment, which is a positive outcome for cSCC patients and aligns with findings by Grob [22] and Rischin [23]. The DCR suggests that most patient' conditions were controlled, with stable or remitted disease, offering survival benefits and improved quality of life [24, 25]. The distribution of patients in different response states also provides further insight. The relatively high proportion of patients achieving PR and SD indicates that ICIs are effective in controlling disease progression. However, the relatively low proportion of patients achieving CR suggests a need to further optimize treatment strategies to enhance the likelihood of deeper remission. Patients with PD also need closer monitoring to adjust treatment plans promptly [26, 27].

Treatment line may reflect both the stage of disease progression and dynamic changes in the immune system [28, 29]. During first-line treatment, patients typically have a lower tumor burden and a relatively intact immune system, increasing the likelihood of responding to ICIs. As the disease progresses to second-line, third-line, or later treatments, tumors may develop immune escape mechanisms [30, 31]. The immune system of patients with early-stage tumors is often more active, allowing immune cells to effectively target and attack tumor cells

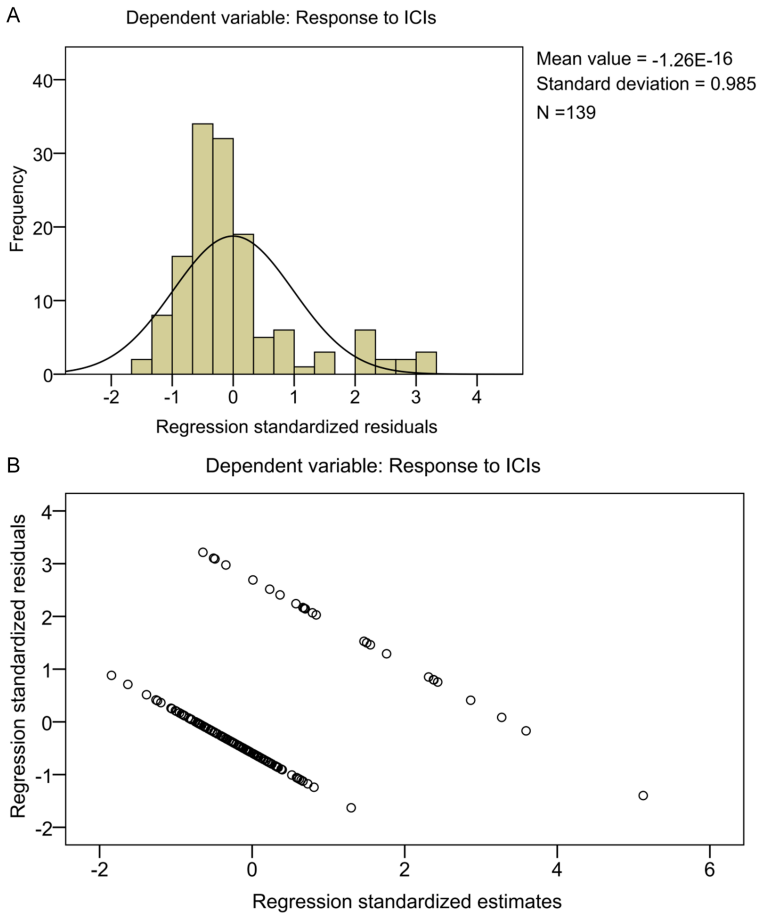

Figure 5. Multiple linear regression analysis of factors influencing progression-free survival (PFS). A. Histogram; B. Scatter plot.

[32, 33]. In contrast, at later stages, the immune system may be severely compromised, reducing the effectiveness of immunotherapy [34].

The non-response of cSCC patients to ICIs may be related to the imbalance in lymphocyte subsets. A study [35] found that SCC cells recruit regulatory T cells (Tregs) into the tumor microenvironment (TME), potentially to evade immune surveillance. Tregs suppress effector T cell activity, and an excessive number of Tregs can impair anti-tumor immunity [36]. Furthermore, some cSCC patients exhibit the expression of new immune checkpoint molecules, which hinder the efficacy of ICIs [37]. The immunosuppressive factors in the TME also affect lymphocyte function, and altered lymphocyte metabolism may contribute to non-responsiveness to ICIs. A study [38] indicates that abnormal lymphocyte metabolism in some cSCC patients may affect their function and survival, thus influencing the efficacy of ICIs.

A high NLR is associated with poor prognosis in cSCC, likely due to several mechanisms. Neutrophils release various tumor-promoting factors, such as vascular endothelial growth factor (VEGF) [39] and matrix metalloproteinases (MMPs) [40], which promote tumor growth, invasion, and angiogenesis. Additionally, neutrophils can also inhibit the activity of lymphocytes, weakening the anti-tumor immune response. Elevated NLR is often indicative of immune system dysfunction [41]. An increase in neutrophils can trigger excessive inflammation, suppressing immune function, while a decrease in lymphocytes implies a weakened anti-tumor ability [42].

CRP was identified as an influencing factor for the non-response to ICIs in cSCC patients. High CRP levels generally indicate a strong inflammatory state, which can lead to the release of inflammatory cells and cytokines [43] that promote tumor progression. Elevated CRP level may also signify immune system dysfunction [44]. The mechanism of ICIs involves activating the immune system to attack tumor cells; however, when CRP levels are too high, the immune system may become over-activated or unbalanced, reducing ICI efficacy. In addition, CRP may interact with the complement system, triggering the complement cascade and producing immunosuppressive fragments that inhibit immune cell activity [45]. Thus, CRP is a potential biomarker for predicting the treatment response of cSCC patients to ICIs.

The developed risk prediction model shows that each individual risk factor has predictive value for the non-response risk of cSCC patients to ICIs. Notably, the combined prediction model, which incorporates all four indicators,

demonstrates a higher AUC than any single indicator, underscoring the advantage of a multi-factorial approach. This combined prediction model provides a more powerful tool for clinicians, enabling early identification of high-risk patients who may not respond to ICIs. Clinicians can then adjust the treatment plans accordingly, such as considering combination therapies or intensifying monitoring. Additionally, this approach supports personalized medicine, allowing for tailored treatment strategies based on individual risk profiles, ultimately improving treatment outcomes and enhancing patient quality of life.

Multiple linear regression analysis showed a significant linear relationship between immunotherapy timing, lymphocytes, NLR, and CRP with PFS. First, the impact of immunotherapy timing on PFS is likely related to differences in the patient's tumor burden, immune system state, and TME at various stages of treatment. Early initiation of immunotherapy may more effectively stimulate the immune system to attack the tumor before immune escape mechanisms become more established, thereby prolonging PFS [46]. A high NLR is associated with a shorter PFS, suggesting that immune imbalance hinders disease control in patients [47, 48]. Hu et al. [49] showed that a high NLR was associated with poor overall survival (OS) and PFS in hypopharyngeal cancer, which aligns with the findings in this study. Elevated CRP levels reflect systemic inflammation, which can promote tumor progression and immune suppression. A high level of CRP is associated with a poor PFS, likely due to an inflammatory microenvironment that undermines the effectiveness of immunotherapy [50, 51]. Lower lymphocytes may imply immune dysfunction, negatively affecting both immunotherapy response and PFS. Similar studies have confirmed that these factors significantly influence immunotherapy outcomes and survival in various tumor types [52-54].

This study still has several limitations. First, its retrospective design may introduce selection and information bias, potentially affecting the reliability of the results. Second, the relatively small sample size may limit statistical power and affect the generalizability of the findings. Third, the study mainly focused on peripheral blood indicators, potentially overlooking other key prognostic factors, such as the gene mutations and other molecular markers in the TME. In addition, variations in detection methods and patients' physiological changes could influence the stability of the results. Finally, this study lacks long-term follow-up data, which may prevent a comprehensive assessment of the long-term prognosis of patients. Future studies should aim to expand the sample size, adopt a prospective design, include additional prognostic factors, and conduct long-term follow-ups to further elucidate the predictive role of peripheral blood indicators in the prognosis of cSCC patients treated with ICIs.

Conclusion

Peripheral blood indicators can effectively predict the treatment response of cSCC patients to ICIs and are also associated with patient PFS. This study confirms the significant role of immunotherapy timing, lymphocytes, NLR, and CRP in tumor immunotherapy. The possible mechanisms include tumor burden, immune system dysfunction, and alterations in the TME. These results offer valuable clinical insights for the treatment of cSCC patients, aiding in the development of personalized treatment plans. Future studies can further explore the specific mechanisms through which these factors influence ICIs response, providing additional evidence to enhance cSCC treatment outcomes.

Disclosure of conflict of interest

None.

Address correspondence to: Mingling Chen, Hospital of Chengdu University of Traditional Chinese Medicine, No. 39, Shierqiao Road, Jinniu District, Chengdu 610075, Sichuan, China. Tel: +86-028-87766014; E-mail: cmlll388@sina.com

References

[1] Nasser N, Nasser Filho N and Lehmkuhl RL. Squamous cell cancer--31-year epidemiological study in a city of south Brazil. An Bras Dermatol 2015; 90: 21-6.
[2] Caudill J, Thomas JE and Burkhart CG. The risk of metastases from squamous cell carcinoma of the skin. Int J Dermatol 2023; 62: 483-486.
[3] Kurosaki T, Mitani S, Tanaka K, Suzuki S, Kanemura H, Haratani K, Fumita S, Iwasa T,

Hayashi H, Yoshida T, Ishikawa K, Kitano M, Otsuki N, Nishimura Y, Doi K and Nakagawa K. Safety and efficacy of cetuximab-containing chemotherapy after immune checkpoint inhibitors for patients with squamous cell carcinoma of the head and neck: a single-center retrospective study. Anticancer Drugs 2021; 32: 95-101.

[4] Mallardo D, Sparano F, Vitale MG, Trojaniello C, Fordellone M, Cioli E, Esposito A, Festino L, Mallardo M, Vanella V, Facchini BA, De Filippi R, Meinardi P, Ottaviano M, Caracò C, Simeone E and Ascierto PA. Impact of cemiplimab treatment duration on clinical outcomes in advanced cutaneous squamous cell carcinoma. Cancer Immunol Immunother 2024; 73: 160.

[5] Zhou J, Wei S, Guo X, Huang Y, Zhang Y, Hong Y, Chen X, Lu M, Zheng F and Zheng C. Correlation between preoperative peripheral blood NLR, PLR, LMR and prognosis of patients with head and neck squamous cell carcinoma. BMC Cancer 2023; 23: 1247.

[6] Deng L, Si Y, Wu Q, Cao Y, Lian S and Li L. Higher neutrophil-to-lymphocyte ratio (NLR) is a preoperative inflammation biomarker of poor prognosis in HIV-infected patients with colorectal cancer: a retrospective study. Can J Gastroenterol Hepatol 2023; 2023: 7966625.

[7] Li X, Zhang Y, Ma W and Li J. An elevated neutrophil-to-lymphocyte ratio predicts poor prognosis in patients with liver cancer after interventional treatments. Biomed Res Int 2022; 2022: 6141317.

[8] Miyamoto N, Inoue H, Inui T, Sasa S, Aoyama M, Okumura K, Toba H, Hino N, Nishisho A, Yukishige S, Kawanaka T, Takizawa H and Tangoku A. Absolute lymphocyte count changes during neoadjuvant chemotherapy are associated with prognosis of human epidermal growth factor receptor 2-positive breast cancer patients. Clin Breast Cancer 2023; 23: e68-e76.

[9] Khouri J, Dima D, Li H, Hansen D, Sidana S, Shune L, Anwer F, Sborov D, Wagner C, Kocoglu MH, Atrash S, Voorhees P, Peres L, Hovanky V, Simmons G, Williams L, Raza S, Afrough A, Anderson LD Jr, Ferreri C, Hashmi H, Davis J, McGuirk J, Goldsmith S, Borogovac A, Lin Y, Midha S, Nadeem O, Locke FL, Baz R, Hamilton B, Alsina M, Sauter C, Patel K and Kaur G. Absolute lymphocyte count and outcomes of multiple myeloma patients treated with idecabtagene vicleucel: the US Myeloma Immunotherapy Consortium real-world experience. Transplant Cell Ther 2024; 30: 790.e1-790.e16.

[10] Xu X, Wang D, Chen W, Li N, Suwinski R, Rossi A, Rosell R, Zhong J and Fan Y. A nomogram model based on peripheral blood lymphocyte subsets to assess the prognosis of non-small cell lung cancer patients treated with immune checkpoint inhibitors. Transl Lung Cancer Res 2021; 10: 4511-4525.

[11] Sakai A, Ebisumoto K, Iijima H, Yamauchi M, Teramura T, Yamazaki A, Watanabe T, Inagi T, Maki D and Okami K. Chemotherapy following immune checkpoint inhibitors in recurrent or metastatic head and neck squamous cell carcinoma: clinical effectiveness and influence of inflammatory and nutritional factors. Discov Oncol 2023; 14: 158.

[12] Veenstra J, Ozog D, Loveless I, Adrianto I, Dimitrion P, Subedi K, Friedman BJ, Zhou L and Mi QS. Distinguishing keratoacanthoma from well-differentiated cutaneous squamous cell carcinoma using single-cell spatial pathology. J Invest Dermatol 2023; 143: 2397-2407, e8.

[13] Carinato H, Burgy M, Ferry R, Fischbach C, Kalish M, Guihard S, Brahimi Y, Flesch H, Bronner G, Schultz P, Frasie V, Thiéry A, Demarchi M, Petit T, Jung AC, Wagner P, Coliat P and Borel C. Weekly paclitaxel, carboplatin, and cetuximab as first-line treatment of recurrent and/or metastatic head and neck squamous cell carcinoma for patients ineligible to cisplatin-based chemotherapy: a retrospective monocentric study in 60 patients. Front Oncol 2021; 11: 714551.

[14] Clingan P, Ladwa R, Brungs D, Harris DL, McGrath M, Arnold S, Coward J, Fourie S, Kurochkin A, Malan DR, Mant A, Sharma V, Shue H, Tazbirkova A, Berciano-Guerrero MA, Charoentum C, Dalle S, Dechaphunkul A, Dudnichenko O, Koralewski P, Lugowska I, Montaudié H, Muñoz-Couselo E, Sriuranpong V, Oliviero J and Desai J. Efficacy and safety of cosibelimab, an anti-PD-L1 antibody, in metastatic cutaneous squamous cell carcinoma. J Immunother Cancer 2023; 11: e007637.

[15] Saba NF, Steuer CE, Ekpenyong A, McCook-Veal A, Magliocca K, Patel M, Schmitt NC, Stokes W, Bates JE, Rudra S, Remick J, McDonald M, Abousaud M, Tan AC, Fadlullah MZH, Chaudhary R, Muzaffar J, Kirtane K, Liu Y, Chen GZ, Shin DM, Teng Y and Chung CH. Pembrolizumab and cabozantinib in recurrent metastatic head and neck squamous cell carcinoma: a phase 2 trial. Nat Med 2023; 29: 880-887.

[16] Cassier PA, Navaridas R, Bellina M, Rama N, Ducarouge B, Hernandez-Vargas H, Delord JP, Lengrand J, Paradisi A, Fattet L, Garin G, Gheit H, Dalban C, Pastushenko I, Neves D, Jelin R, Gadot N, Braissand N, Léon S, Degletagne C, Matias-Guiu X, Devouassoux-Shisheboran M, Mery-Lamarche E, Allard J, Zindy E, Decaestecker C, Salmon I, Perol D, Dolcet X, Ray-Coquard I, Blanpain C, Bernet A and Mehlen P.

Netrin-1 blockade inhibits tumour growth and EMT features in endometrial cancer. Nature 2023; 620: 409-416.
[17] Walia A, Tuia J and Prasad V. Progression-free survival, disease-free survival and other composite end points in oncology: improved reporting is needed. Nat Rev Clin Oncol 2023; 20: 885-895.
[18] Martini A, Fallara G, Ploussard G and Pradere B. Progression-free survival is an adequate endpoint for clinical trials of locally advanced and metastatic urothelial carcinoma. Curr Opin Urol 2022; 32: 500-503.
[19] Song X, Liu X, Liu F and Wang C. Comparison of machine learning and logistic regression models in predicting acute kidney injury: a systematic review and meta-analysis. Int J Med Inform 2021; 151: 104484.
[20] Munn JS, Lanting BA, MacDonald SJ, Somerville LE, Marsh JD, Bryant DM and Chesworth BM. Logistic regression and machine learning models cannot discriminate between satisfied and dissatisfied total knee arthroplasty patients. J Arthroplasty 2022; 37: 267-273.
[21] Burns C, Kubicki S, Nguyen QB, Aboul-Fettouh N, Wilmas KM, Chen OM, Doan HQ, Silapunt S and Migden MR. Advances in cutaneous squamous cell carcinoma management. Cancers (Basel) 2022; 14: 3653.
[22] Grob JJ, Gonzalez R, Basset-Seguin N, Vornicova O, Schachter J, Joshi A, Meyer N, Grange F, Piulats JM, Bauman JR, Zhang P, Gumuscu B, Swaby RF and Hughes BGM. Pembrolizumab monotherapy for recurrent or metastatic cutaneous squamous cell carcinoma: a single-arm phase II trial (KEYNOTE-629). J Clin Oncol 2020; 38: 2916-2925.
[23] Rischin D, Migden MR, Lim AM, Schmults CD, Khushalani NI, Hughes BGM, Schadendorf D, Dunn LA, Hernandez-Aya L, Chang ALS, Modi B, Hauschild A, Ulrich C, Eigentler T, Stein B, Pavlick AC, Geiger JL, Gutzmer R, Alam M, Okoye E, Mathias M, Jankovic V, Stankevich E, Booth J, Li S, Lowy I, Fury MG and Guminski A. Phase 2 study of cemiplimab in patients with metastatic cutaneous squamous cell carcinoma: primary analysis of fixed-dosing, long-term outcome of weight-based dosing. J Immunother Cancer 2020; 8: e000775.
[24] Gross ND, Miller DM, Khushalani NI, Divi V, Ruiz ES, Lipson EJ, Meier F, Su YB, Swiecicki PL, Atlas J, Geiger JL, Hauschild A, Choe JH, Hughes BGM, Schadendorf D, Patel VA, Homsi J, Taube JM, Lim AM, Ferrarotto R, Yoo SY, Mathias M, Han H, Seebach F, Lowy I, Fury MG and Rischin D. Neoadjuvant cemiplimab and surgery for stage II-IV cutaneous squamous-cell carcinoma: follow-up and survival outcomes of a single-arm, multicentre, phase 2 study. Lancet Oncol 2023; 24: 1196-1205.
[25] Liu Y, Cao Z and Wei G. Effects of photodynamic therapy using Red LED-light combined with hypocrellin B on apoptotic signaling in cutaneous squamous cell carcinoma A431 cells. Photodiagnosis Photodyn Ther 2023; 43: 103683.
[26] Ondo AL, Kerner JD, Kerner JV, Ondo IP, Trainor P and Shanler SD. Treatment of cutaneous squamous cell carcinoma in situ with curettage followed by topical imiquimod 5% cream. Dermatol Surg 2021; 47: 609-612.
[27] Zhang X, Bu X, Jia W, Ying Y, Lv S and Jiang G. Near-infrared light-activated oxygen generator a multidynamic photo-nanoplatform for effective anti-cutaneous squamous cell carcinoma treatment. Int J Nanomedicine 2022; 17: 5761-5777.
[28] Vodárek P, Écsiová D, Řezáčová V, Souček O, Šimkovič M, Vokurková D, Belada D, Žák P and Smolej L. A comprehensive assessment of lymphocyte subsets, their prognostic significance, and changes after first-line therapy administration in patients with chronic lymphocytic leukemia. Cancer Med 2023; 12: 6956-6970.
[29] An M, Mehta A, Min BH, Heo YJ, Wright SJ, Parikh M, Bi L, Lee H, Kim TJ, Lee SY, Moon J, Park RJ, Strickland MR, Park WY, Kang WK, Kim KM, Kim ST, Klempner SJ and Lee J. Early immune remodeling steers clinical response to first-line chemoimmunotherapy in advanced gastric cancer. Cancer Discov 2024; 14: 766-785.
[30] Ding Y, Wang Z, Zhou F, Chen C and Qin Y. Associating resistance to immune checkpoint inhibitors with immunological escape in colorectal cancer. Front Oncol 2022; 12: 987302.
[31] Yu C, Hsieh K, Cherry DR, Nehlsen AD, Resende Salgado L, Lazarev S and Sindhu KK. Immune escape in glioblastoma: mechanisms of action and implications for immune checkpoint inhibitors and CAR T-cell therapy. Biology (Basel) 2023; 12: 1528.
[32] Kraehenbuehl L, Weng CH, Eghbali S, Wolchok JD and Merghoub T. Enhancing immunotherapy in cancer by targeting emerging immunomodulatory pathways. Nat Rev Clin Oncol 2022; 19: 37-50.
[33] Mogavero A, Cantale O, Mollica V, Anpalakhan S, Addeo A, Mountzios G, Friedlaender A, Kanesvaran R, Novello S and Banna GL. First-line immunotherapy in non-small cell lung cancer: how to select and where to go. Expert Rev Respir Med 2023; 17: 1191-1206.
[34] Hanna GJ, Dharanesswaran H, Giobbie-Hurder A, Harran JJ, Liao Z, Pai L, Tchekmedyian V, Ruiz ES, Waldman AH, Schmults CD, Riella LV, Lizotte P, Paweletz CP, Chandraker AK, Mu-

rakami N and Silk AW. Cemiplimab for kidney transplant recipients with advanced cutaneous squamous cell carcinoma. J Clin Oncol 2024; 42: 1021-1030.
[35] Schipmann S, Wermker K, Schulze HJ, Kleinheinz J and Brunner G. Cutaneous and oral squamous cell carcinoma-dual immunosuppression via recruitment of FOXP3+ regulatory T cells and endogenous tumour FOXP3 expression? J Craniomaxillofac Surg 2014; 42: 1827-33.
[36] Alvisi G, Puccio S, Roychoudhuri R, Scirgolea C and Lugli E. High-dimensional single-cell profiling of tumor-infiltrating $CD4^+$ regulatory T cells. Methods Mol Biol 2023; 2559: 243-257.
[37] Dany M, Doudican N and Carucci J. The novel checkpoint target lymphocyte-activation gene 3 is highly expressed in cutaneous squamous cell carcinoma. Dermatol Surg 2023; 49: 1112-1115.
[38] Ou Z, Lin S, Qiu J, Ding W, Ren P, Chen D, Wang J, Tong Y, Wu D, Chen A, Deng Y, Cheng M, Peng T, Lu H, Yang H, Wang J, Jin X, Ma D, Xu X, Wang Y, Li J and Wu P. Single-nucleus RNA sequencing and spatial transcriptomics reveal the immunological microenvironment of cervical squamous cell carcinoma. Adv Sci (Weinh) 2022; 9: e2203040.
[39] Yu Y, Jin H, Li L, Zhang X, Zheng C, Gao X, Yang Y and Sun B. An injectable, activated neutrophil-derived exosome mimetics/extracellular matrix hybrid hydrogel with antibacterial activity and wound healing promotion effect for diabetic wound therapy. J Nanobiotechnology 2023; 21: 308.
[40] Modestino L, Cristinziano L, Poto R, Ventrici A, Trocchia M, Ferrari SM, Fallahi P, Paparo SR, Marone G, Antonelli A, Varricchi G and Galdiero MR. Neutrophil extracellular traps and neutrophil-related mediators in human thyroid cancer. Front Immunol 2023; 14: 1167404.
[41] Buonacera A, Stancanelli B, Colaci M and Malatino L. Neutrophil to lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases. Int J Mol Sci 2022; 23: 3636.
[42] Forlani G, Shallak M, Gatta A, Shaik AKB and Accolla RS. The NLR member CIITA: master controller of adaptive and intrinsic immunity and unexpected tool in cancer immunotherapy. Biomed J 2023; 46: 100631.
[43] Rodriguez-Gil JL, Takita C, Wright J, Reis IM, Zhao W, Lally BE and Hu JJ. Inflammatory biomarker C-reactive protein and radiotherapy-induced early adverse skin reactions in patients with breast cancer. Cancer Epidemiol Biomarkers Prev 2014; 23: 1873-83.
[44] Jiang J, Peng Z, Wang J, Chen M, Wan Y, Huang H, Liu Z, Wang J and Hou J. C-reactive protein impairs immune response of CD8+ T cells via FcγRIIb-p38MAPK-ROS axis in multiple myeloma. J Immunother Cancer 2023; 11: e007593.
[45] Sobuś A, Baumert B, Gołąb-Janowska M, Kulig P, Paczkowska E, Łuczkowska K, Rogińska D, Zawiślak A, Milczarek S, Osękowska B, Pawlukowska W, Meller A, Machowska-Sempruch K, Wełnicka A, Nowacki P and Machaliński B. Adjuvant lineage-negative cell therapy as a potential silencer of the complement-mediated immune system in ALS patients. J Clin Med 2021; 10: 5251.
[46] Gonçalves L, Gonçalves D, Esteban-Casanelles T, Barroso T, Soares de Pinho I, Lopes-Brás R, Esperança-Martins M, Patel V, Torres S, Teixeira de Sousa R, Mansinho A and Costa L. Immunotherapy around the clock: impact of infusion timing on stage IV melanoma outcomes. Cells 2023; 12: 2068.
[47] Naik MD, Kataria SP, Kumar G and Singh R. Correlation of prognostic markers {CEA, Ca19.9, Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR)} with progression free survival (PFS) in advanced/metastatic gallbladder carcinoma. J Assoc Physicians India 2022; 70: 11-12.
[48] Chen D, Xu J, Zhao Y, Han B and Zhong R. Prognostic value of pretreatment procalcitonin and neutrophil-lymphocyte ratio in extensive-stage small-cell lung cancer. Cancer Biol Ther 2024; 25: 2331273.
[49] Hu X, Tian T, Zhang X, Sun Q, Chen Y and Jiang W. Neutrophil-to-lymphocyte and hypopharyngeal cancer prognosis: system review and meta-analysis. Head Neck 2023; 45: 492-502.
[50] Laino AS, Woods D, Vassallo M, Qian X, Tang H, Wind-Rotolo M and Weber J. Serum interleukin-6 and C-reactive protein are associated with survival in melanoma patients receiving immune checkpoint inhibition. J Immunother Cancer 2020; 8: e000842.
[51] Gauci ML, Boudou P, Baroudjian B, Vidal-Trecan T, Da Meda L, Madelaine-Chambrin I, Basset-Seguin N, Bagot M, Pages C, Mourah S, Resche-Rigon M, Pinel S, Sassier M, Rouby F, Eftekhari P, Lebbé C and Gautier JF. Occurrence of type 1 and type 2 diabetes in patients treated with immunotherapy (anti-PD-1 and/or anti-CTLA-4) for metastatic melanoma: a retrospective study. Cancer Immunol Immunother 2018; 67: 1197-1208.
[52] Duchemann B, Naigeon M, Auclin E, Ferrara R, Cassard L, Jouniaux JM, Boselli L, Grivel J, Desnoyer A, Danlos FX, Mezquita L, Caramella C, Marabelle A, Besse B and Chaput N. CD8+PD-1+ to CD4+PD-1+ ratio (PERLS) is associated with prognosis of patients with advanced NSCLC treated with PD-(L)1 blockers. J Immunother Cancer 2022; 10: e004012.

[53] Chen X, Zhang W, Qian D, Guan Y, Wang Y, Zhang H, Er P, Yan C, Li Y, Ren X, Pang Q and Wang P. Chemoradiotherapy-induced CD4+ and CD8+ T-cell alterations to predict patient outcomes in esophageal squamous cell carcinoma. Front Oncol 2019; 9: 73.

[54] Zhang G, Liu A, Yang Y, Xia Y, Li W, Liu Y, Zhang J, Cui Q, Wang D, Liu X, Guo Y, Chen H and Yu J. Clinical predictive value of naïve and memory T cells in advanced NSCLC. Front Immunol 2022; 13: 996348.