Table S1 Baseline clinical characteristics and laboratory parameters of patients

Characteristic	All (n = 357)	Training dataset (n = 214)	Validation dataset (n=143)
Age			
Median (IRQ)	65.00 (58-70)	65 (58-71)	64 (58-70)
Gender, n (%)			
Male	293 (82.1)	185 (86.4)	108 (75.5)
female	64 (17.9)	29 (13.6)	35 (24.5)
Smoking status, n (%)			
Yes	178 (49.9)	112 (52.3)	66 (46.2)
No	179 (50.1)	102 (47.7)	77 (53.8)
Gene mutation			
Yes	52 (14.6)	33 (15.4)	19 (13.3)
No	305 (85.4)	181 (84.6)	124 (86.7)
ECOG status, n (%)			
0-1	271 (75.9)	166 (77.6)	105 (73.4)
≥2	86 (24.1)	48 (22.4)	38 (26.6)
Clinical stage, n (%)			
I II	92 (25.8)	55 (25.7)	37 (25.9)
IV	265 (74.2)	159 (74.3)	106 (74.1)
BMI (kg/m^2)			
Median (IQR)	22.10 (20.03-23.92)	22.01 (19.8 -23.9)	22.21 (20.1-24.0)
Histology, n (%)			
Adenocarcinoma	173 (48.5)	103 (48.1)	70 (49.0)
Squamous cell carcinoma	184 (51.5)	111 (51.9)	73 (51.0)
Treatment lines, n (%)			
1	187 (52.4)	114 (53.5)	73 (51.0)
2	103 (28.9)	58 (27.2)	45 (31.5)
3	53 (14.8)	32 (14.9)	21 (14.7)
4	14 (3.9)	10 (4.7)	4 (2.8)
Medicine			
Monotherapy	110(30.8)	67 (31.3)	43 (30.1)
Combination therapy	247(69.2)	147 (68.7)	100 (69.9)
irAEs (≥G2), n (%)			
Yes	79 (22.1)	45 (21.0)	34 (23.8)
No	278 (77.9)	169 (79.0)	109 (76.2)
Laboratory parameters, (IRQ)			
WBC	6.60 (5.40, 8.10)	6.70 (5.60, 8.10)	6.20 (5.10, 8.05)
ALC	1.29 (0.97, 1.60)	1.30 (0.98, 1.66)	1.26 (0.94, 1.52)
AMC	0.50 (0.39, 0.65)	0.50 (0.40, 0.65)	0.49 (0.37, 0.66)
ANC	4.65 (3.42, 5.78)	4.65 (3.56, 5.71)	4.65 (3.21, 5.87)
AEC	0.12 (0.07, 0.24)	0.13 (0.07, 0.26)	0.10 (0.06, 0.20)

LDH	216.00 (180.00, 233.00)	223.50 (179.00, 238.75)	210.00 (181.50, 229.12)
CRP	10.70 (3.00, 33.20)	11.65 (3.35, 30.65)	10.50 (2.10, 33.75)
NSE	16.80 (12.65, 20.43)	16.70 (12.69, 20.43)	17.20 (12.57, 20.43)
SCC	1.40 (0.81, 2.92)	1.45 (0.85, 2.92)	1.30 (0.80, 2.92)
ProGRP	50.10 (37.86, 81.40)	52.04 (39.36, 78.18)	47.12 (35.60, 82.36)
CYFRA21-1	7.14 (3.57, 14.17)	7.02 (3.83, 14.17)	7.22 (3.34, 14.17)
CA125	31.74 (14.20, 79.17)	32.59 (15.35, 77.63)	31.53 (12.35, 79.17)
CEA	5.21 (2.85, 18.08)	5.40 (2.82, 17.62)	4.89 (2.96, 18.52)
NLR	3.64 (2.50, 5.30)	3.65 (2.59, 5.42)	3.64 (2.46, 4.98)
PLR	173.10 (135.71, 254.95)	173.59 (134.11, 243.22)	172.90 (141.05, 261.39)
PNI	45.70 (40.40, 48.80)	46.05 (40.74, 49.04)	44.7 (39.52, 48.62)
LMR	2.62 (1.81, 3.34)	62 (1.83, 3.41)	2.57 (1.79, 3.26)
dNLR	2.26 (1.67, 3.13)	2.28 (1.67, 3.07)	2.24 (1.67, 3.18)
SII	822.56 (536.28,1385.13)	821.78 (556.71,1326.36)	829.62 (520.43,1437.52)

Table S2 Subgroup analysis of irAEs risk prediction model

Subgroup	Number of irAEs, n%	HR (95%CI)	*P* value
Therapy Medicine, n			
Monotherapy, n=110	25, 22.7%	4.24 (1.825-9.851)	0.001
combination therapy, n=247	54, 21.9%	3.33 (1.894-5.848)	<0.0001
TNM, n			
III, n=92	18, 19.6%	5.67 (1.859-17.3)	0.002
IV, n=265	61, 23.0%	3.19 (1.891-5.37)	<0.0001
Treatment lines, n			
Line 1, n=187	41, 21.8%	2.47 (1.328-4.591)	0.004
Line 2, n=103	26, 25%	6.04 (2.27-16.07)	<0.0001
Line 3, n=53	11, 20.8%	3.99 (1.132-14.09)	0.031
Line 4, n=14	1, 8.3%	/	/
Surgery, n			
Yes, n=90	15, 16.7%	3.74 (1.261-11.101)	0.017
No, n=267	64, 23.9%	3.6 (2.141-6.046)	<0.0001

Materials and Methods

Research design

We retrospectively studied 357 patients with advanced NSCLC (stage III or IV) from Sir Run Run Shaw Hospital, Zhejiang University School of Medicine between January 2017 and August 2021, splitting the population into a training dataset and a validation dataset in a 6:4 ratio according to temporal order, to construct and validate the irAEs risk prediction model that predicts the risk of developing grade ≥2 irAEs after treatment with PD-1/PD-L1 inhibitors. This study was approved by the Ethics Committee of Sir Run Run Shaw Hospital [2019 Scientific Research Ethics (20190211–55)]. And the project complies with the 2013 revised Declaration of Helsinki.

Patient eligibility

Patients with NSCLC who had received at least one treatment with PD-1/PD-L1 inhibitors were selected. The inclusion criteria were as follows: 1) All patients were pathologically confirmed with unresectable stage III and IV NSCLC or postoperative recurrent NSCLC patients. 2)aged ≥18 years.3) The patient has received at least one cycle (1 or more doses) of PD-1/PD-L1 inhibitor treatment and has completed at least one follow-up visit. 4) The medication regimens include PD-1/PD-L1 inhibitor monotherapy, combined with chemotherapy or antiangiogenic therapy. The exclusion criteria were as follows: 1) History of previous treatment with PD-1/PD-L1 inhibitors and/or study medication at other hospitals prior to the time of study inclusion. 2) Patients with any of the following diseases: autoimmune disease; interstitial lung disease; bone marrow hematopoietic, hepatic, or renal insufficiency; patients who have received systemic glucocorticoids or other immunosuppressive therapy; severe comorbidities such as stroke, myocardial infarction, and severe infections. 3) Patients who have taken any medication that may interfere with hematological indicators within one week prior to the baseline time; 4) Patients with no documented hematological indicators in the week prior to baseline time;5) Patients who have received anti-cancer treatment for other malignancies. 6) Adverse reactions that are judged by clinicians to be non-immune-related.

The definitions of primary and secondary outcomes

The primary outcome was defined as the occurrence of irAEs: the time from the date of initiation of treatment with PD-1/PD-L1 inhibitors to the first reported irAEs (grade≥2); irAEs were immunotherapy-related adverse reactions clearly diagnosed by clinicians, including skin disease, enteritis, nephritis, pneumonitis, neurotoxicity, endocrine toxicities, Hepatitis, blood disorders. The severity grading of irAEs was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (if 2 or more irAEs were present, the severe one was selected)[1]. Only irAEs with grade ≥2 were included in this study. Secondary outcomes were progression-free survival (PFS) and overall survival (OS). PFS was defined as the time from initiation of PD-1/PD-L1 inhibitors to disease progression or death from any cause, and OS was defined as the time from initiation of PD-1/PD-L1 inhibitors to death from any cause.

Data collection

The baseline clinical characteristics and laboratory peripheral blood parameters of patients within one week before PD-1/PD-L1 inhibitors were obtained from the electronic medical record. The first researcher collects the data， and then second researcher checks it. Baseline clinical characteristics include age, sex, smoking history, Eastern Cooperative Oncology Group (ECOG-PS), clinical stage, pathological types (squamous carcinoma, adenocarcinoma), number of previous treatment lines, number of metastatic sites. Laboratory peripheral blood parameters include white blood cell count (WBC), absolute lymphocyte count (ALC), absolute monocyte count (AMC),

absolute neutrophil count (ANC), absolute eosinophil count (AEC), lactate dehydrogenase (LDH), C-reactive protein (CRP), neuron-specific enolase (NSE), squamous cell carcinoma (SCC), pro-gastrin-releasing peptide (ProGRP), cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), cancer antigen-125 (CA125), carcinoembryonic antigen (CEA), dNLR:ANC/(WBC-ANC), NLR:ANC/ALC,PLR:PLT/ALC, LMR:ALC/AMC, PNI:albumi+5×ALC, and SII:PLT×ANC/ALC. Clinical follow-up was conducted before administration of each PD-1/PD-L1 inhibitor, and the follow-up was conducted every 3–8 weeks according to the condition. The tumor staging criteria are the 7th edition of the International Association for the Study of Lung Cancer TNM system [2]. Disease progression was assessed by clinicians based on clinical examination and imaging findings with reference to the Response Evaluation Criteria in Solid Tumors(RECIST) version 1.1[3].

Statistical analysis

All continuous variables satisfying the normal distribution were expressed as mean standard deviation, and comparisons between groups were made using the student T-Test; continuous variables with non-normal distribution were expressed as median (interquartile spacing), and comparisons between groups were made using the Mann-Whitney U test. Categorical variables were expressed as frequencies (percentages), and comparisons between groups were made using the chi-square test or Fisher's exact test. To identify the risk factors of irAEs in NSCLC, we used the survminer R package to seek the optimal cut-off values for all continuous variables. Then, they were transformed into dichotomous variables for further analysis. In addition, univariate COX regression analysis of irAEs (≥2 levels) was performed, and predictor variables with P values <0.05 were included in the multifactor regression model. The predictor variables with statistically significant (P<0.05) values in the multifactorial COX regression model were selected to construct the risk prediction model based on the regression coefficients. The cutoff values of the risk prediction model was determined based on the best discrimination of the occurrence of irAEs using the survminer R package, and the population was divided into a high-risk population and a low-risk population according to the cutoff values. The discriminative power and calibration of the prediction model were assessed using the receiver operating characteristic (ROC) curve and calibration curve in the training and validation sets, respectively. The correlation between the prediction models and clinical prognosis (PFS, OS) was assessed by Kaplan-Meier method (log-rank test).

All probability values were two-sided, and P values <0.05 were considered statistically significant differences. All statistical analyses were performed using SPSS version 23.0 and the R program (version 4.1.0).

References

1. NCI. National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0.

2. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol.* Aug 2007;2(8):706-714.

3. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* Jan 2009;45(2):228-247.