Original Article

# Novel risk scoring system for immune checkpoint inhibitors treatment in non-small cell lung cancer

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**Background:** Immune checkpoint inhibitor (ICI)-based immunotherapy has improved the clinical outcome of non-small cell lung cancer (NSCLC). However, current indicators, such as programmed cell death-ligand 1 (PD-L1) expression in tumors or tumor mutational burden (TMB), are not considered ideal biomarkers for prognosis. Thus, there is an urgent requirement for a comprehensive risk scoring system.

**Methods:** In this study, we enrolled 464 NSCLC patients who received ICIs between March 2017 and January 2020 at four clinical centers. Univariate and multivariate (the logistic and the Cox regression) analyses were conducted to screen clinically relevant variables. Significant parameters (P<0.05) including absolute lymphocyte count (ALC, L), Eastern Cooperative Oncology Group Performance Status (ECOG PS, E) and lung/pleural metastasis (M) were selected for LEM score. Weighted values based on odds ratio and hazard ratio of multiple analyses were assigned to each parameter. LEM score was the sum of weighted values of each variable (Good, 0-1; Intermediate, 2-3; Poor, 4-6). Kaplan-Meier curves were used to evaluate the association between LEM score and progression-free survival (PFS).

**Results:** In total, 258 patients were pooled and stratified into three risk categories based on the LEM score. Objective response rate (ORR) was significantly higher in the good-risk group compared with the poor-risk group [55.9% *vs.* 7.3%, odds ratio (OR), 0.023; 95% confidence interval (CI), 0.005–0.099; P<0.001]. Patients with good risk [hazard ratio (HR), 0.130; 95% CI, 0.084–0.203; median PFS, 12.5 months; P<0.001] or intermediate risk (HR, 0.330; 95% CI, 0.222–0.490; median PFS, 4.2 months; P<0.001) had longer PFS than those with poor risk (median PFS, 2.1 months). DNA sequencing was performed in 41 patients [no durable benefit (NDB): n=29; durable clinical benefit (DCB): n=12] and epidermal growth factor receptor (EGFR) mutations were enriched in samples of the NDB group *vs.* the DCB group (11/29 *vs.* 1/12; Fisher's exact P=0.073; OR, 6.722; 95% CI, 0.760–59.479). Additionally, patients with EGFR mutations had higher LEM scores than those with wild-type EGFR.

**Conclusions:** In conclusion, the LEM score provided a potential prognostic biomarker for NSCLC patients treated with ICIs.

**Keywords:** Non-small cell lung cancer (NSCLC); immune checkpoint inhibitors (ICIs); LEM; risk scoring system; prognostic biomarker

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## Introduction

In the past decade, immune checkpoint inhibitors (ICIs) have dramatically altered the management of non-small-cell lung cancer (NSCLC) (1-3). Consequently, programmed cell death-1 (PD-1) inhibitors have proven more effective than conventional chemotherapy for the treatment of metastatic NSCLC (3-6). Additionally, the combination of PD-1 inhibitors and chemotherapy has also resulted in improved outcomes of NSCLC (7,8).

Despite the advances in ICIs therapy, some patients do not respond to ICIs. Some of the diagnostic tests performed for ICIs include Programmed cell death ligand-1 (PD-L1) immunohistochemistry (IHC) and tumor mutational burden (TMB); however, these have proven to be imperfect biomarkers. Studies have shown that several patients without PD-L1 expression did respond to ICIs, while those with PD-L1 expression did not (3,9). TMB is another potential indicator, which represents the number of somatic mutations detected by DNA sequencing (10). The lack of uniform methodology limits its widespread application. Therefore, there is an urgent need to develop a robust and reproducible scoring system to predict ICIs' response.

Several studies have tried to predict ICIs' response using various parameters, such as clinical features [e.g., metastatic site (11), computational image-based features (12,13)], laboratory parameters [e.g., neutrophil-to-lymphocyte ratio (NLR) (14-16), lactate dehydrogenase (LDH) (17), tumor markers (18)], and genetic landscape (19,20). Most of these parameters have yielded a poor performance due to lack of comprehensive evaluation in risk stratification. Recent studies have shown that the anti-tumor response to ICIs is a complicated process involving several factors. Previous studies have developed various prognostic models for prognostic evaluation in ICIs therapy. For example, a lung immune prognostic index (LIPI) combining derived NLR (dNLR) and LDH (21), as well as a risk scoring criteria including monocyte-to-lymphocyte ratio (MLR), sites of metastasis, and nutritional index-body mass index (BMI) (22) were respectively developed for NSCLC and metastatic renal cell cancer (mRCC) patients treated with ICIs.

Thus, we generated a novel risk scoring system for ICIs treatment in NSCLC. This index was labeled "LEM" and included absolute lymphocyte count (ALC) $<1.5\times10^9$/L, Eastern Cooperative Oncology Group Performance Status (ECOG PS) ≥2, and lung/pleura metastasis. Patients who received PD-1 inhibitors were stratified into three risk stratifications (good, intermediate, and poor) based on the LEM score. Here, we developed this scoring system to explore the association between LEM score and clinical outcome.

We present the following article in accordance with the TRIPOD reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-832).

## Methods

### *Patient selection*

This study retrospectively screened 464 metastatic NSCLC patients who had been treated with PD-1 inhibitors [pembrolizumab (Merck Sharp & Dohme), nivolumab (Bristol-Myers Squibb), sintilimab (Innovent), or toripalimab (Topalliance)] between March 2017 and January 2020 at four clinical centers. We excluded the patients who received initial PD-1 immunotherapy at out hospital but was lost-to-follow-up thereafter (n=110) and those who do not receive sufficient sessions for evaluation at the time of study (n=79). The final analysis included 258 patients. Data from Jingling Hospital (n=87) were used to develop the risk scoring system, and the remaining data were used for validation (Figure S1).

Prior to treatment, we obtained clinical information and routine laboratory test records. Laboratory tests included blood platelet count, lymphocyte count, neutrophil count, NLR, levels of albumin, globulin, LDH and albumin to globulin ratio (A/G). Clinical data included demographic information, ECOG PS, TNM stage, metastatic site, and the number of prior therapies. PD-L1 (Dako 22C3) IHC staining was performed at the pathology department of each center. Response and progression were evaluated based on the RECIST v1.1 criterion (23). Patients were stratified as a durable clinical benefit (DCB: partial or stable response lasting >6 months) and no durable benefit (NDB) groups based on the published metrics (19,24). The primary endpoint was progression-free survival (PFS) (time from

 

initial ICIs administration to confirmed progressive disease radiologically or death due to any cause). We also measured other objectives including treatment efficacy (DCB/NDB), objective response rate (ORR) and one-year overall survival (OS) (time from initial ICIs administration to death due to any cause) rate.

Sintilimab and toripalimab are both domestic PD-1 inhibitors which have not yet been approved by FDA for NSCLC treatment. In the study, patients who received sintilimab or toripalimab were undergoing approved clinical trials. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the local ethics committee of Jinling Hospital (registration ID. 2017NZHX-022). Informed consent from individuals was waived based on the retrospective nature of this study.

### *DNA sequencing and data sources*

Tumor tissue and corresponding blood samples (n=41) collected from Jinling Hospital were sent to a gene company for DNA extraction and sequencing. DNA was extracted from the blood samples, and formalin-fixed paraffin embedded (FFPE) slices were prepared. Deep sequencing (139 genes, 10,000×) based on Illumina Hiseq2000 system platform (Illumina, USA) was used to detect gene alterations, such as missense mutation, insertion, deletion, copy number variation, etc. TMB was expressed as the number of non-synonymous mutations.

To further explore the genetic factors that influenced the clinical efficacy of ICIs, we compared the gene expression data with Helmann (19) (MSK, Cancer Cell 2018) (n=75) and Rizvi (25) (MSKCC, J Clin Oncol 2018) (n=240). The genomic information of NSCLC cohorts treated with ICIs was shared in the cBioPortal (www. cbioportal.org).

### *Statistical analysis*

The Mann-Whitney *U* test was used for univariate analyses. The logistic regression model and the Cox regression model were used to evaluate the association between characteristics and best response/PFS. After multivariate analyses, factors of statistical significance were included in the risk scoring system, and the weight values of all indices were determined based on the odds ratio (OR) for response and hazard ratio (HR) for PFS. Survival estimates were generated by Kaplan-Meier analysis, and the log-rank test was used to compare the differences in PFS among subgroups. Statistical analysis was done using the SPSS software (v22.0, SPSS, Inc., Chicago) and expressed using GraphPad Prism 5 and R 3.3.2 software. A two-sided P<0.05 was considered statistically significant.

## Results

### *Baseline clinical features of the cohort*

*Table 1* summarizes the baseline characteristics of 258 NSCLC patients. In the pooled cohort, the median age of patients was 61 years (range, 38–81); 200 (77.5%) patients were male; 150 (58.1%) patients had a history of smoking. Of the 258 NSCLC patients, 88 (34.1%) had the squamous subtype, and 170 (65.9%) had the non-squamous subtype. Most patients (81.4%) were in good physical health with ECOG PS <2. Approximately 121 (47.6%) patients had contralateral lung or pleura metastasis, while 27 (10.6%) had liver metastasis. Additionally, 150 (58.1%) patients received PD-1 inhibitors as the first-line or second-line of treatment. Of the 258 patients enrolled in this study, 54 were treated with PD-1 inhibitor monotherapy, while others received a combination treatment of PD-1 inhibitor and chemotherapy.

### *Univariate and multivariate analyses of baseline characteristics*

The univariate analyses of routine laboratory parameters revealed that there was a significant difference in the levels of ALC [(1.191±0.068) $\times 10^9$/L *vs.* (1.787±0.122) $\times 10^9$/L; P<0.001] (Figure S2A), NLR (5.410±0.679 *vs.* 3.717±0.373; P<0.05) (Figure S2B), albumin level (35.48±0.637 *vs.* 37.40±0.768 g/L; P<0.05) (Figure S2C), and A/G ratio (1.185±0.047 *vs.* 1.309±0.036; P<0.05) (Figure S2D) in the DCB and NDB groups. However, we found no significant difference for other parameters among the groups (Figure S3).

After univariate analyses, marginally significant (P<0.1) factors and demographic characteristics were included for multivariate analyses (*Table 2*). The ORR was 36.8% implying that of the 87 patients, 32 responded. Response to ICIs treatment was associated with ECOG PS [OR, 5.242; 95% confidence interval (CI), 1.056–26.016; P=0.043], ALC (OR, 0.197; 95% CI, 0.071–0.545; P=0.002), and lung/pleura metastasis (OR, 3.638; 95% CI, 1.090–12.143; P=0.036). Cox regression model revealed that inferior PFS was associated with ECOG PS ≥2 (HR, 2.312; 95% CI,

 

**Table 1** Baseline characteristics of patients

| Characteristics | Test set, N=87 (%) | Validation set, N=171 (%) | Pooled cohort, N=258 (%) |
|---|---|---|---|
| Age (years) | | | |
| <65 | 50 (57.5) | 99 (57.9) | 149 (57.8) |
| ≥65 | 37 (42.5) | 72 (42.1) | 109 (42.2) |
| Gender | | | |
| Male | 64 (73.6) | 136 (79.5) | 200 (77.5) |
| Female | 23 (26.4) | 35 (20.5) | 58 (22.5) |
| Smoking status | | | |
| Smoker | 56 (64.4) | 94 (55.0) | 150 (58.1) |
| Non-smoker | 31 (35.6) | 77 (45.0) | 108 (41.9) |
| Histology | | | |
| Squamous | 28 (32.2) | 60 (35.1) | 88 (34.1) |
| Non-squamous | 59 (67.8) | 111 (64.9) | 170 (65.9) |
| ECOG PS | | | |
| <2 | 56 (64.4) | 154 (90.1) | 210 (81.4) |
| ≥2 | 31 (35.6) | 17 (9.9) | 48 (18.6) |
| TNM stage | | | |
| III | 21 (24.1) | 35 (20.5) | 56 (21.7) |
| IV | 66 (75.9) | 136 (79.5) | 202 (78.3) |
| Metastatic site | | | |
| Lymph node | 79 (90.8) | 163 (95.3) | 242 (93.8) |
| Lung/pleura | 38 (45.2) | 83 (48.5) | 121 (47.6) |
| Brain | 18 (21.4) | 25 (14.6) | 43 (16.9) |
| Liver | 7 (8.3) | 20 (11.7) | 27 (10.6) |
| Prior systemic therapy | | | |
| <2 | 48 (55.2) | 102 (59.6) | 150 (58.1) |
| ≥2 | 39 (44.8) | 69 (40.4) | 108 (41.9) |
| Type of treatment | | | |
| PD-1 inhibitor | 29 (33.3) | 25 (14.6) | 54 (20.9) |
| PD-1 inhibitor + Chemotherapy | 58 (66.7) | 146 (85.4) | 204 (79.1) |
| PD-1 inhibitor | | | |
| Pembrolizumab | 33 (37.9) | 76 (44.4) | 109 (42.2) |
| Nivolumab | 16 (18.4) | 49 (28.7) | 65 (25.2) |
| Sintilimab | 38 (43.7) | 31 (18.1) | 69 (26.7) |
| Toripalimab | 0 (0) | 15 (8.8) | 15 (5.9) |

**Table 1** (*continued*)

**Table 1** (*continued*)

| Characteristics | Test set, N=87 (%) | Validation set, N=171 (%) | Pooled cohort, N=258 (%) |
|---|---|---|---|
| Molecular alteration | | | |
| EGFR mutation | 14 (18.4) | 18 (15.9) | 32 (16.9) |
| ALK rearrangement | 2 (2.6) | 5 (4.4) | 7 (3.7) |
| Unknown | 10 (11.5) | 58 (33.9) | 69 (26.7) |
| PD-L1 status | | | |
| Negative | 13 (14.9) | 21 (12.3) | 34 (13.2) |
| Positive | 26 (29.9) | 34 (19.9) | 60 (23.2) |
| Unknown | 48 (55.2) | 116 (67.8) | 164 (63.6) |
| Best response | | | |
| CR+PR | 32 (36.8) | 68 (39.8) | 100 (38.8) |
| SD+PD | 55 (63.2) | 103 (60.2) | 158 (61.2) |
| PFS, median, month (95% CI) | 5.125 (3.834–6.416) | 6.637 (3.643–9.631) | 5.585 (3.971–7.199) |

ALK, anaplastic lymphoma kinase; CI, confidence interval; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; HR, hazard ratio; PD, progression disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

**Table 2** Multiple logistic regression analysis for response and Cox regression analysis for progression-free survival (PFS) in the test set

| Characteristics (reference) | OR for Response (95% CI) | P value | HR for PFS (95% CI) | P value |
|---|---|---|---|---|
| Age (≥65) | 0.521 (0.146–1.862) | 0.315 | 0.748 (0.423–1.325) | 0.320 |
| Gender (female) | 0.233 (0.035–1.542) | 0.131 | 0.431 (0.175–1.062) | 0.067 |
| Smoking status (smoker) | 0.293 (0.049–1.742) | 0.177 | 0.704 (0.320–1.546) | 0.382 |
| Histology (non-squamous) | 1.537 (0.455–5.196) | 0.489 | 1.104 (0.608–2.003) | 0.745 |
| ALC ($\times 10^9$/L) | 0.197 (0.071–0.545) | 0.002** | 0.561 (0.341–0.922) | 0.023* |
| Albumin (g/L) | 0.894 (0.780–1.025) | 0.107 | 0.973 (0.911–1.038) | 0.407 |
| ECOG PS (≥2) | 5.242 (1.056–26.016) | 0.043* | 2.312 (1.183–4.516) | 0.014* |
| Metastatic site | | | | |
| Lung/pleura | 3.638 (1.090–12.143) | 0.036* | 2.019 (1.063–3.836) | 0.032* |
| Liver | 2.389(0.368–15.510) | 0.362 | 0.950 (0.396–2.280) | 0.908 |
| Brain | 1.670 (0.333–8.390) | 0.533 | 0.943 (0.474–1.876) | 0.867 |
| Prior systemic therapy (≥2) | 0.852 (0.205–3.536) | 0.825 | 1.332 (0.690–2.572) | 0.393 |

ALC, absolute lymphocyte count; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio; OR, odds ratio; PFS, progression-free survival; *, P<0.05; **, P<0.01.

1.183–4.516; P=0.014) and lung/pleura metastasis (HR, 2.019; 95% CI, 1.063–3.836; P=0.032). However, patients with higher ALC had significantly longer PFS (HR, 0.561; 95% CI, 0.341–0.922; P=0.023).

### *Analyses of the LEM risk scoring system*

The final selected variables were ALC, ECOG PS and lung/pleura metastasis. Relative weights were based on odds ratio and hazard ratio of multivariate analyses (high HR/OR:

 

**Table 3** The LEM prognostic scoring system and risk stratification construction

| Variable | Absolute lymphocyte count (×10$^9$/L) | | Lung/pleura metastasis | | ECOG PS | |
|---|---|---|---|---|---|---|
| | <1.5 | ≥1.5 | Positive | Negative | ≥2 | <2 |
| Weighted value | 1 | 0 | 2 | 0 | 3 | 0 |
| Risk stratification (LEM score) | Good [0-1]; intermediate [2-3]; poor [4-6] | | | | | |

Weighted values based on odds ratio and hazard ratio of multiple analyses (high HR/OR: weighted value =3; intermediate HR/OR: weighted value =2; low HR/OR: weighted value =1) were assigned to each parameter. LEM score was the sum of weighted values of each variable. ECOG PS, Eastern Cooperative Oncology Group Performance Status.

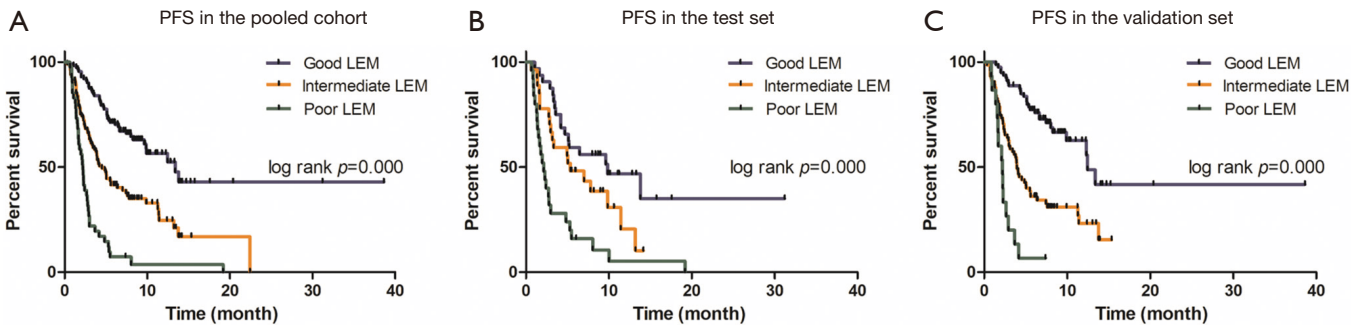


**Figure 1** Progression-free survival (PFS) based on the LEM score. (A) The pooled cohort (P<0.001); (B) The test set (P<0.001); (C) The validation set (P<0.001). LEM score: Good: 0–1; Intermediate: 2–3; Poor: 4–6.

weighted value =3; intermediate HR/OR: weighted value =2; low HR/OR: weighted value =1). Weighted values were assigned to each parameter, and LEM score was the sum of weighted values of each variable (*Table 3*).

In the test set, based on the LEM score, patients were divided into three risk groups [good (37.9%), intermediate (32.2%), and poor (29.9%)]. We found that a good risk (HR, 0.216; 95% CI, 0.117–0.398; median PFS, 9.9 months; P<0.001) and an intermediate risk (HR, 0.322; 95% CI, 0.176–0.591; median PFS, 7.0 months; P<0.001) was associated with longer PFS compared with a poor risk (median PFS, 2.1 months). The validation set further verified these results [good (45.6%), intermediate (45.6%), and poor (8.8%)]. Patients with good risk (HR, 0.076; 95% CI, 0.038–0.150; median PFS, 12.5 months; P<0.001) or intermediate risk (HR, 0.240; 95% CI, 0.130–0.443; median PFS, 3.9 months; P<0.001) trended toward longer PFS than those with poor risk (median PFS, 2.1 months) (*Figure 1* and *Table 4*). Further subgroup analyses based on smoking status, age, and histology revealed similar association between LEM score and PFS (log-rank P<0.001) (*Figure 2*). Figure S4 also shows the one-year OS rate based on the LEM score.

We found that LEM score was associated with ORR (P<0.001), and ORR ranged from 7.7% (for poor risk) to 54.5% (for good risk) in the test set. The pooled cohort also showed significant difference in ORR: 7.3% (for poor risk) *vs.* 55.9% (for good risk) (OR, 0.023; 95% CI, 0.005–0.099; P<0.001) and 33.0% (for intermediate risk) (OR, 0.078; 95% CI, 0.005–0.099; P<0.001) (Table S1).

### *Association between gene mutation and LEM score*

Tumor tissue from 41 patients (NDB: n=29; DCB: n=12) were processed for DNA sequencing. We limited our research to 34 genes which were either highly deleterious or had mutations in at least three patients. These variants were used for further analysis. *Figure 3* shows the gene profiles (frameshift insertion or deletion, splice-site, or missense mutation) for the two groups, and Table S2 shows the corresponding LEM scores.

The most frequent mutations detected were TP53 (n=27, 65.9%), EGFR (n=12, 29.3%), KRAS (n=11, 26.8%), and PIK3CA (n=8, 19.5%). The distribution of genetic alterations in KRAS (NDB: 6/29 *vs.* DCB: 5/12), PIK3CA (NDB: 6/29 *vs.* DCB: 2/12) and TP53 (NDB:

 

**Table 4** Progression-free Survival (PFS) based on the LEM score in the test set, validation set, and pooled cohort

| Characteristic | Risk stratification | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Test set (n=87) | | | Validation set (n=171) | | | Pooled cohort (n=258) | | | |
| | Good (n=33) | Intermediate (n=28) | Poor (n=26) | Good (n=78) | Intermediate (n=78) | Poor (n=15) | Good (n=111) | Intermediate (n=106) | Poor (n=41) |
| HR (95% CI) | 0.216 (0.117–0.398) | 0.322 (0.176–0.591) | 1 [Reference] | 0.076 (0.038–0.150) | 0.240 (0.130–0.443) | 1 [Reference] | 0.130 (0.084–0.203) | 0.330 (0.222–0.490) | 1 [Reference] |
| Median PFS, month (95% CI) | 9.856 (6.064–13.648) | 6.998 (1.001–12.994) | 2.136 (1.397–2.874) | 12.452 (10.907–13.996) | 3.943 (3.125–4.760) | 2.103 (1.439–2.766) | 12.452 (8.409–16.495) | 4.205 (3.211–5.200) | 2.136 (1.600–2.671) |
| P value | P<0.001 | | | P<0.001 | | | P<0.001 | | |

LEM score: Good: 0–1; Intermediate: 2–3; Poor: 4–6. CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

20/29 *vs.* DCB: 7/12) was similar for the NDB/DCB group. Nevertheless, mutations in EGFR (ex19del: 6/12; ex20ins: 5/12; ex21L858R: 1/12) were enriched in samples of the NDB group *vs.* the DCB group with negligible significance (11/29 *vs.* 1/12; Fisher's exact P=0.073; OR, 6.722; 95% CI, 0.760–59.479). Here, mutations in ARID2, CCNE1, CDKN2A, MET, PKHD1, SETD and RAF1 were all observed in the NDB group. Additionally, mutations in LRP1B, NTRK3 and TERT in the DCB group were more than that in the NDB group.

Next, we cross-validated our data with the published datasets to verify our findings. Datasets from Rizvi (25) (n=240) and Helmann (19) (n=75) contained both genomic data and clinical response (NDB *vs.* DCB) to ICIs treatment in NSCLC. It showed that EGFR mutations were related to ICIs treatment response in both our cohort (P=0.004) as well as datasets of Rizvi and Helmann (P=0.036). Additionally, in Rizvi and Helmann's dataset, mutations in AR (P=0.036), FAT1 (P=0.036) and KMT2C (P=0.036) trended toward DCB, while in our cohort, TP53 mutations were associated with NDB (P=0.029) (*Figure 4A*). Due to the small sample size of our study, we further combined our data with these two datasets. Mutations in FAT1 (OR, 0.502; P=0.087), FBXW7 (OR, 0.342; P=0.087), KMT2C (OR, 0.5; P=0.075), and STK11 (OR, 1.766; P=0.082) were associated with treatment response with negligible significance; however, EGFR mutation (OR, 3.149; P=0.001) showed statistically significance (*Figure 4B*).

We further explored the association between individual EGFR mutations and LEM scores. Data derived from our pooled set showed that LEM scores of patients with EGFR mutations had a higher LEM score than those with wildtype EGFR [$(3.000\pm0.359)\times10^9$/L *vs.* $(2.182\pm0.142)\times10^9$/L; P=0.025] (*Figure 4C*), while there was no difference among EGFR mutation sites (Ex19del, Ex20ins and Ex21L858R) (*Figure 4D*).

## Discussion

ICIs therapy is considered a milestone in the history of NSCLC treatment. However, only some patients are benefitted due to the lack of comprehensive biomarkers (10,26). Hence, there is an urgent need to develop a risk scoring system to stratify NSCLC patients. Thus, our study retrospectively investigated factors associated with ICIs treatment response to establish and verify a novel risk scoring system in four centers. Based on the results of univariate and multivariate analyses, the LEM score

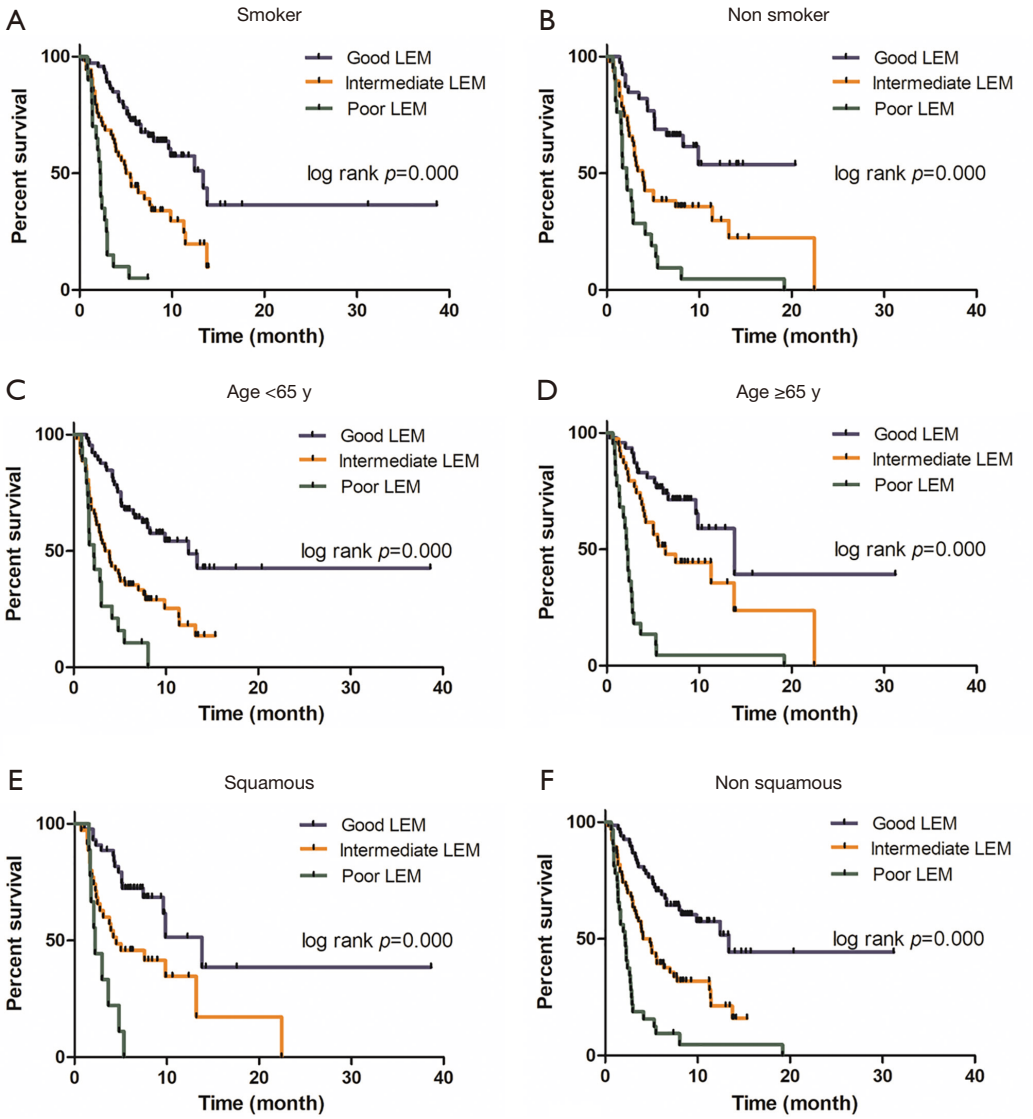


**Figure 2** Subgroup analyses of progression-free survival (PFS) based on the LEM score in the pooled cohort based on the (A,B) smoking status (smoker *vs.* non-smoker, P<0.001); (C,D) age (<65 *vs.* ≥65, P<0.001) and (E,F) histology (squamous *vs.* non-squamous, P<0.001). LEM score: Good: 0–1; Intermediate: 2–3; Poor: 4–6.

included ALC (L), ECOG PS (E), and lung/pleural metastasis status (M). A higher LEM score was associated with limited response and inferior PFS, as well as EGFR mutation. Therefore, the LEM score could act as a pre-treatment guide for optimization and candidate selection for ICIs therapy in NSCLC.

Metastatic sites are known to influence the efficacy of cancer treatment by formatting specific tumor microenvironment (TME) (27). Previous studies have shown the association between liver metastasis and a lower ICIs treatment response rate (11,28). Other studies have also shown that NSCLC patients with pleural metastasis experience more serious adverse events (SAEs), exhibit a limited response to ICIs (29), and have poor prognosis (30,31). In this study, we observed that lung/pleura metastases but not liver or brain metastasis influenced ICIs treatment outcomes. Lung/pleura was the most common metastatic site of advanced NSCLC (32). Additionally, patients with EGFR mutations and anaplastic lymphoma kinase (ALK) rearrangements were more likely to have

 

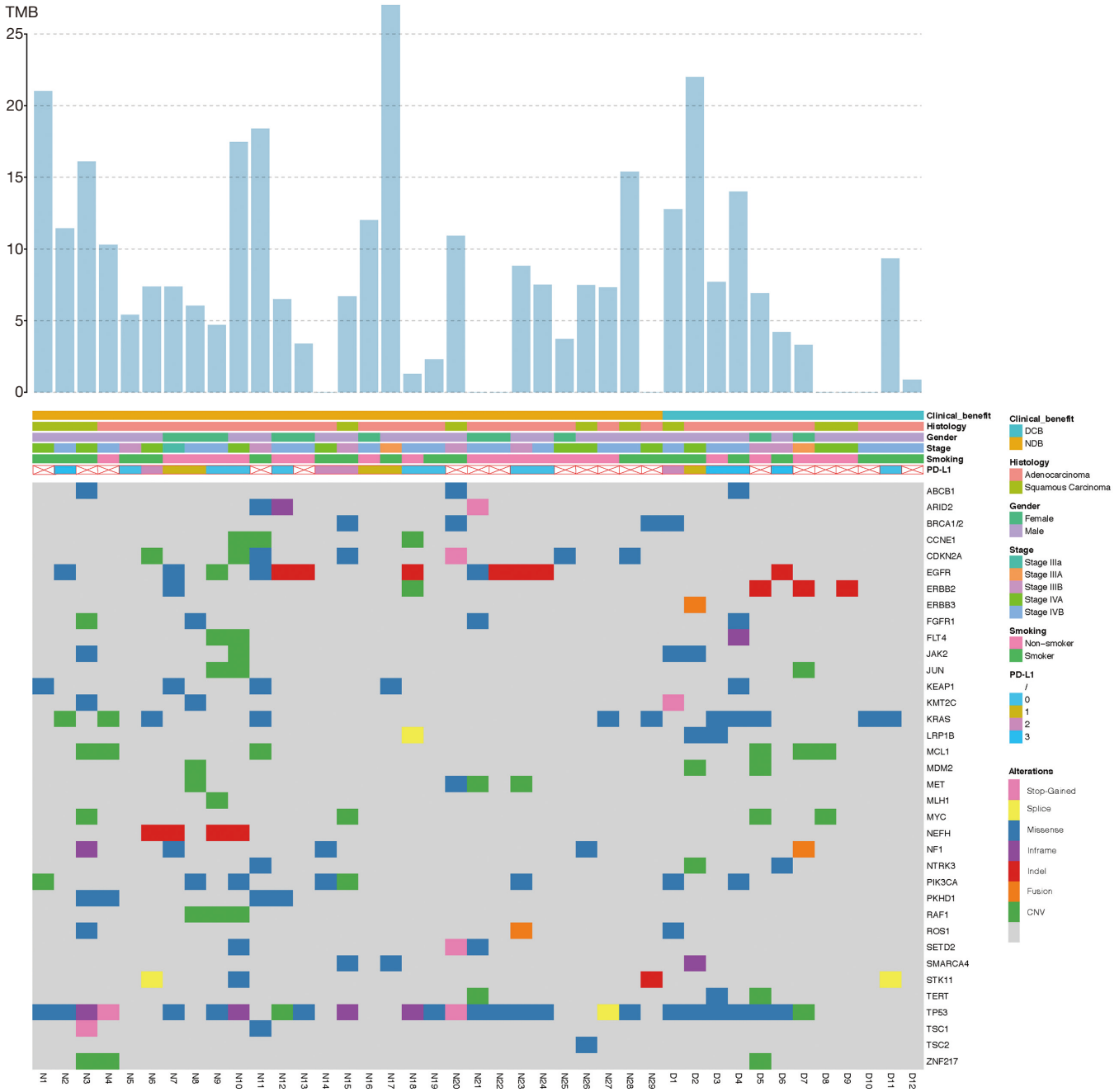


**Figure 3** Gene alterations landscape of patients in the NDB and DCB groups. Thirty-four genes are shown, which were highly deleterious or had variants in at least three patients. Each column represents one patient. TMB value and clinical features are shown at the top. NDB (N): no durable benefit; DCB (D): durable clinical benefit.

metastasis to pleura and lung, respectively (33-35). The results of clinical trials, as well as our study, confirmed that EGFR mutations had a negative impact on ICIs treatment response (36). This might partially explain why lung/pleura metastasis was a negative factor in the current risk scoring system.

Systemic inflammatory and immune status are known to impact the efficacy of cancer treatments. Several blood parameters are used as ICIs biomarkers. Here, we found a correlation between ALC and ICIs treatment response.

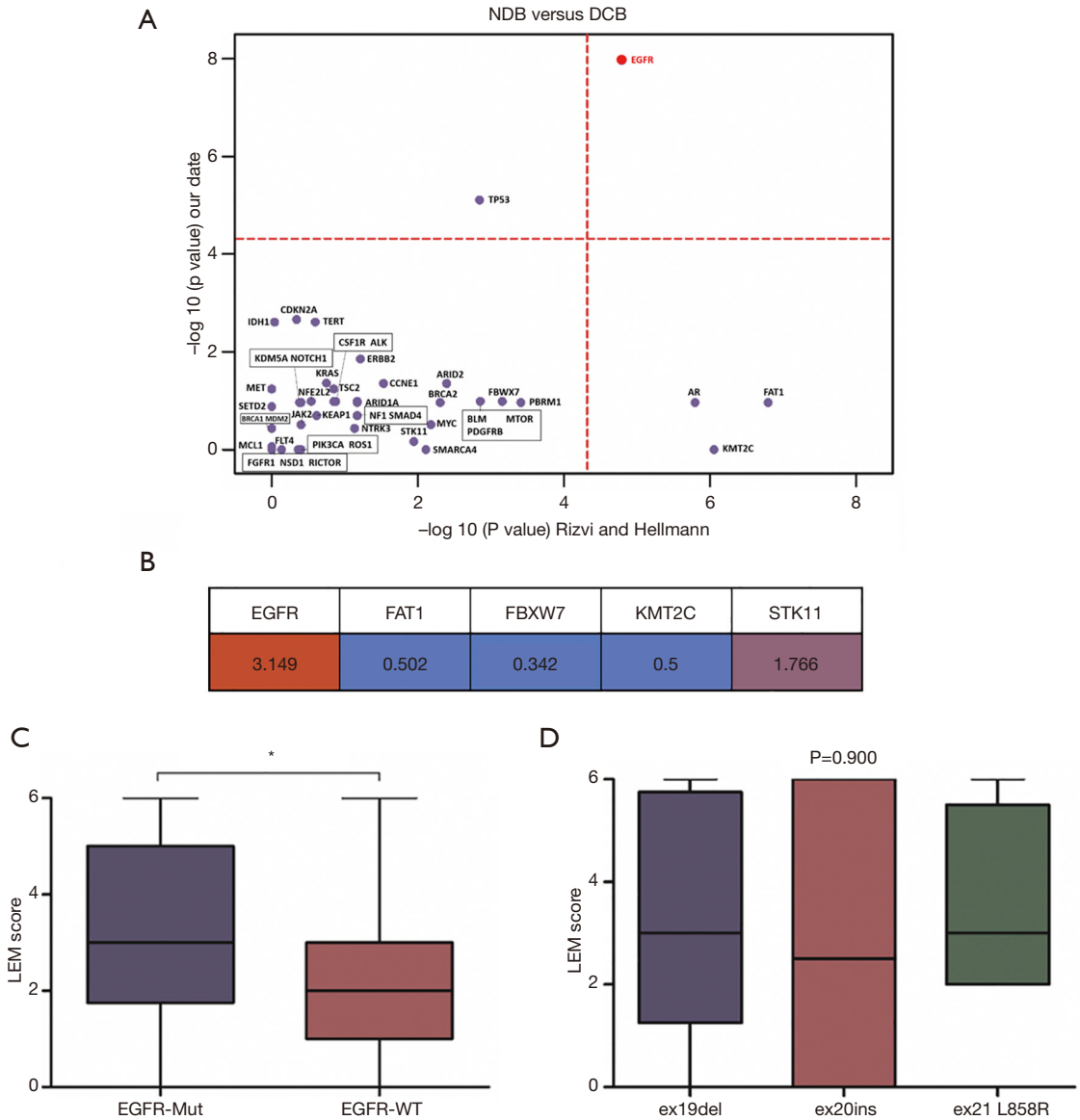


**Figure 4** Gene alteration distribution associated with ICIs treatment response. (A) The comparison of gene alterations that were differentially expressed in the NDB/DCB group between our data and Rizvi and Hellmann datasets based on -log10 (P value). Red lines indicate P<0.05. (B) OR values of gene alterations that were differentially expressed in the NDB/DCB group with negligible significance (FAT1, FBXW7, KMT2C, STK11 P<0.1; EGFR P<0.05) in combined datasets. Red and blue colors color indicate negative and positive factors for ICIs; (C) LEM scores differed between EGFR-mutated/wildtype groups, *, P<0.05. (D) LEM scores showed no significant difference among the EGFR mutation sites. Mut, mutated; WT, wildtype; ex19del: exon 19 deletions; ex20ins: exon 20 insertions; ex21L858R: exon 21 L858R.

PD-1 inhibitors are known to enhance the anti-tumor immunity of T lymphocytes by blocking PD-1 protein, which is expressed on the cell surface. The ICIs treatment focuses on advanced melanoma due to the presence of abundant lymphocytes (37). Thus, reduced levels of circulating lymphocytes might lead to a decrease in tumor-infiltrating T lymphocytes (TIL) as well as an imbalance in Th-1 and Th-2 phenotypes (38,39). Additionally, potential inflammatory biomarkers, including NLR, albumin, LDH, C-reactive protein (CRP), neutrophils, and platelets, have

 

been shown to be associated with ICIs treatment response (40,41). However, in our test cohort, we observed that NLR, albumin and A/G ratio rather than LDH, neutrophils, and platelets were associated with treatment outcomes. This difference might be attributed to the potential bias caused by the confounding factors in real-world data analysis.

Previous studies have shown that PD-L1 and TMB re correlated to clinical benefits of ICIs (2,8,42). Hu-Lieskovan *et al.* (43) explored the association between PD-L1/TMB and benefit from pembrolizumab and discovered that PD-L1 was related to ORR [OR, 0.96 (0.93–0.99), P=0.007] and PFS [HR, 0.98 (0.96–0.99), P=0.002], while no such association was found for TMB. However, our novel scoring system showed great predictive value for both ORR [good risk: OR, 0.023 (0.005–0.099); P<0.001] and PFS [good risk: HR, 0.130 (0.084–0.203); P<0.001] even in the absence of PD-L1 and TMB.

Single parameters are known to have limited performance as a prognostic predictor. Previous studies have explored risk stratification score for patients treated with ICIs. Mezquita *et al.* (21) developed a prognostic index, LIPI, based on a multicenter retrospective study with a total of 466 ICIs-treated NSCLC. This index based on dNLR greater than 3 and LDH greater than upper limit of normal (ULN) was correlated with worse outcomes for ICIs (good, 0 factor; intermediate, 1 factor; poor, 2 factors). Furthermore, it was further verified by Kazandjian (44) and Sorich (45). Mazzaschi *et al.* (46) also generated an Immune effector score ($I_{eff}S$) featuring high soluble PD-L1 (sPD-L1) and low $CD8^+PD\text{-}1^+$ and NK cells levels, which outperformed LIPI in the prognostic power. It even showed remarkable impact of $I_{eff}S$ and LIPI integration on survival outcome. Kasahara *et al.* (47) employed Glasgow prognostic score (GPS), which contained CRP and albumin, to predict the efficacy of treatment with PD-1 inhibitors. These retrospective studies had certain limitations regarding the lack of comprehensive clinical and pathological data. Martini *et al.* (22) also developed a risk scoring criteria for patients with mRCC who were treated with ICIs. These criteria included MLR, sites of metastasis and nutritional index-BMI. Patients were also categorized into 4 groups (good, intermediate, poor, very poor). It turned out to be an effective way to predict survival in mRCC patients receiving ICIs. Also, another study (48) indicated that the ECOG PS, which reflected overall performance status, was better than BMI for risk stratification of survival in patients with metastatic cancer.

Our study had several limitations. First, it was a retrospective study based on real-world data; thus, there was scope for potential bias due to loss to follow-up or missing data. For example, in our study, PD-L1 IHC status and TMB were not routinely tested in our study, especially for those who underwent posterior–line ICIs treatment. Second, we chose a one-year OS rate rather than OS as the observe objective due to insufficient follow-up data. In future studies, we would investigate the association between LEM score and OS. Finally, only a few patients (41/258) had tumor sequencing data. Further efforts are needed to develop a more comprehensive index combining genomic and clinical variables to predict response to ICIs treatment.

## Conclusions

Thus, the LEM score is a novel risk scoring system consisting of ALC (L), ECOG PS (E), and lung/pleural metastasis (M). It could act as a potential prognostic biomarker of ORR and PFS for patients treated with ICIs in NSCLC. Further large-sample studies are required to externally validate the LEM score.

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*Research*. The other authors have no conflicts of interest to declare.

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