Original Article

Clinical-genomic nomogram for predicting sensitivity to second-line immunotherapy for advanced non-small cell lung cancer

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Background: The introduction of immune checkpoint inhibitors (ICIs) has significantly improved the outcomes of patients with advanced non-small cell lung cancer (NSCLC). However, ICIs only benefit a subset of patients. The study aimed to identify genomic biomarkers and construct models to predict the response to second-line ICI therapy.

Methods: We retrospectively collected clinical data and genetic testing results from patients with NSCLC treated with second-line ICI at a single medical center between August 2018 and June 2021. We reanalyzed the raw sequence data of clinical genetic testing and defined the common detection region among the different testing panels. Immunotherapy sensitivity was evaluated using the immune-based Response Evaluation Criteria in Solid Tumors.

Results: We included 102 patients as a training cohort and 46 as a test cohort. In the training cohort, we examined the relationship between ICI response and the mutation status of 343 genes. Mutations in the *EGFR* gene were significantly more common in the resistant group than in the sensitive group (41.0% *vs.* 20.6%; P=0.04), while mutations in the *EP300* gene were associated with greater sensitivity to ICIs (39.7% *vs.* 15.4%; P=0.01). A nomogram was built based on clinical variables, genomic data, and programmed death-ligand 1 (PD-L1) expression. The total nomogram points were significantly higher in the sensitive group than in the resistance group in both cohorts, and the areas under the receiver operating characteristic curve were 0.780 in the training cohort and 0.720 in the test cohort. The higher nomogram points also indicated better progression-free survival.

Conclusions: Based on real-world clinical settings, the clinical genomic nomogram, which involved limited input variables that were economical and easy to obtain, demonstrated a good ability to predict the response to second-line ICI treatment in advanced NSCLC.

Keywords: Immune checkpoint inhibitors (ICIs); advanced non-small cell lung cancer (advanced NSCLC); immunotherapy sensitivity; somatic mutations; nomogram

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Introduction

Historically, the prognosis of patients with advanced non-small cell lung cancer (NSCLC) has been poor, whose median survival time was no more than 6 months. The advent of immune checkpoint inhibitor (ICIs) immunotherapy, including programmed cell death 1 (PD-1) inhibitors and programmed death-ligand 1 (PD-L1) inhibitors, has dramatically altered this landscape (1). Thus far, a range of ICIs, including pembrolizumab, nivolumab, and sintilimab, have received approval from the National Medical Products Administration in China (2,3).

Immunotherapy is the recommended first-line treatment for patients with advanced NSCLC without driver gene mutations (4). In the second-line treatment and beyond, all patients with advanced NSCLC may benefit from immunotherapy (5). Although anti-PD-1/PD-L1 therapy has proven successful, it benefits only a limited subset of patients, with the objective response rate (ORR) ranging from 20% to 40% in unselected patient groups (6).

Consequently, significant efforts have been directed toward identifying biomarkers capable of predicting the response to PD-L1 blockade-based ICI therapy before its initiation (7,8).

Highlight box

Key findings

- Based on real-world clinical settings, we built and validated a clinical-genomic nomogram, which demonstrated good performance in predicting response to second-line immune checkpoint inhibitors (ICIs) in patients with advanced non-small cell lung cancer (NSCLC).

What is known and what is new?

- Certain somatic mutations within tumors can influence immune evasion and microenvironment and, consequently, the effectiveness of ICI treatment in NSCLC.
- Based on the somatic mutations related to the response to second-line ICI-based therapy, we constructed and validated a clinical-genomic nomogram for clinical prognostication.

What is the implication, and what should change now?

- The clinical-genomic nomogram that consisted of limited but economical and easy-to-obtain input variables could provide improved individualized ICI treatment in patients with NSCLC.

The most widely recognized biomarkers for ICI therapy currently include PD-L1 expression status (9,10), microsatellite instability (MSI) status (11), and tumor mutational burden (TMB) (12). However, these biomarkers were insufficient for identifying all potential responders of ICIs.

The application of next-generation sequencing (NGS) for genomic diagnostic testing has recently introduced new biomarkers into the field. Mutations in the cancer genome may generate novel antigens that enable $CD8^+$ T cells to target and eliminate tumor cells (13,14). Research indicates that certain somatic mutations within tumors can influence immune evasion and microenvironment and, consequently, the effectiveness of ICI treatment in NSCLC (15-21). However, the results related to the prediction of response to ICI treatment often conflict, possibly due to the high heterogeneity of tumor genomes and clinical factors (22). Based on the widely available NGS genomic panel for clinical application, we hope to find a simple but reliable method for predicting sensitivity to ICIs.

Our study aimed to establish a simple model to predict the efficacy of initial immunotherapy. This study included NSCLC patients from August 2018 to June 2021, when chemotherapy was the standard first-line treatment for patients with advanced NSCLC with non-sensitive mutations. Since most patients started immunotherapy in the second line at that time, we limited the included patients to those receiving second-line treatment. In this study, we retrospectively collected clinical data and genetic testing results of patients with NSCLC treated with second-line immunotherapy at a single medical center. According to the somatic mutations related to the response to ICI-based treatment, we constructed and validated a clinical-genomic nomogram for clinical prognostication. We present this article in accordance with the TRIPOD reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2024-1249/rc).

Methods

Patients

We included patients with advanced NSCLC at Shandong Cancer Hospital and Institute between August 2018 and

June 2021.

The inclusion criteria were as follows: (I) age between 18 and 75 years; (II) stage IIIB–IV NSCLC according to The International Association for the Study of Lung Cancer (IASLC) eight edition, with the pathological type being lung squamous cell carcinoma (LUSC) or lung adenocarcinoma (LUAD); (III) chemotherapy-based first-line treatment regimens; (IV) stable tumor status after first-line treatment for at least 3 months; (V) administration of immunotherapy in second-line treatment with a duration of least four cycles; (VI) tumor-normal-paired genetic testing performed before immunotherapy, with available raw sequence data (fastq); and (VII) measurable lesions evaluated according to immune-based Response Evaluation Criteria in Solid Tumors (iRECIST).

The genetic testing included a 654-gene panel, a 457-gene panel, a 363-gene panel, a 180-gene panel, and a 63-gene panel. When dividing the test set, we have the following two considerations: (I) all patients with the large NGS panel (654 genes, 457 genes, 363 genes) would be used as the exploration and training set for building the model (available online: https://cdn.amegroups.cn/static/public/tlcr-2024-1249-1.xlsx); (II) according to the genes included in the nomogram model, we select appropriate small panel patients as test sets to guarantee the independence of the test set. In the initial patient selection, we considered a variety of small NGS panel cohorts, in addition to 180-gene and 63-gene panels, there were patients with 18-gene, 12-gene, 8-gene, etc. Only patients with 180-gene and 63-gene panels were selected to form the test set because only these panels included all significant genes (*EGFR* and *EP300*), while other panels were excluded because they did not include *EP300*. If the significant genes changed, the selection of the test cohort would also change.

Due to the small number of patients, we excluded the following subgroups to improve the homogeneity of the patient population: patients receiving immunotherapy alone, patients receiving first-line immunotherapy, and pathological types other than LUSC and LUAD. In the involved cohort, the second-line treatment mode for most patients was ICIs combined with chemotherapy or ICIs combined with radiotherapy. Other treatment modes included: ICIs + anti-vascular therapy (such as anlotinib, bevacizumab), ICIs + chemotherapy + anti-vascular therapy, ICIs + local ablation therapy, ICIs + chemotherapy + local therapy, etc. There were fewer people using these treatment modes, so classified as other categories.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Board of Shandong Cancer Hospital and Institute (No. SDTHEC2024003156) and the requirement for individual consent was waived due to the retrospective nature of the analysis.

Clinical data collection

Clinical and pathological characteristics and treatment process data were collected from institutional medical records. Progression-free survival (PFS) was defined as the time from initiation of immunotherapy until clinical or radiographic progression or death from any cause. The sensitivity of immunotherapy was evaluated using the iRECIST (23), and the patients identified with immune confirmed progressive disease (iCPD) were regarded as resistant to immunotherapy. Since only patients who received second-line immunotherapy or beyond were included, we did not find any patients who achieved immune complete response (iCR).

The expression of PD-L1 was evaluated with immunohistochemistry using the tumor proliferation score (TPS), defined as the percentage of cells stained positive for PD-L1 among all tumor cells. A less than 1% TPS value was categorized as low PD-L1 expression, 1% to 50% as median PD-L1 expression, and more than 50% as high expression.

Bioinformatics analysis

We reanalyzed the raw data (fastq) of the NGS results. The fastq files were subjected to quality control via fastp (v0.23) and mapped to genome (hg38.p13) with BWA (v. 0.7) software and processed by GATK package (v. 4.1) according to the recommended practice. The somatic mutations were called with Mutect2 and annotated with ANNOVAR (v. 4.0) (24). The mutational sites below 2% of frequency were filtered out in the downstream analysis.

To determine the intersection between different detection panels, we used bamCoverage in deepTools (v. 3.5) (25) to extract the genome coverage of the detection samples (bed files) and used the intersect command in bedtools (v. 2.31) (26) to intersect the bed files of different samples. We defined effective coverage region as the genomic area with coverage depth exceeding 20× in the pair of tumor-normal samples, and the common effective coverage region shared by more than 90% of samples was considered the common detection region.

Based on the coverage of the detection panels, we placed patients with more covered genes detected in the training cohort and patients with fewer covered genes as the test cohort.

Statistical analysis

We used the Fisher exact test for the comparison of categorical variables between groups. The hazard ratio (HR) was calculated using the Cox proportional hazards model, and survival curves were plotted using the Kaplan-Meier method. The logistic model was fitted and represented by a nomogram. Decision curve analysis (DCA) was used to evaluate and compare prognostication models. For all statistical tests, a two-sided P value of <0.05 was considered significant. Due to the small sample size, we did not conduct significance adjustments in multiple testing of genomic comparisons in the training set. All statistical analyses were performed using R version 4.3 (The R Project for Statistical Computing).

Results

Patients

We examined 102 patients with available test results from large genomic panels (654-gene panel, 457 gene-panel, or 363-gene panel) and 46 patients with results from small genomic panels (180-gene panel or 63-gene panel); in the subsequent analysis, these patients were the training cohort (n=102) and test cohort (n=46) respectively.

The first-line treatment regimens of the patients included in this study were all chemotherapy-based. Among them, most adenocarcinoma patients used the PC (paclitaxel + carboplatin) regimen, and most squamous cell carcinoma patients used the TP (paclitaxel + cisplatin) or DP (docetaxel + cisplatin) regimen. Some patients combined anti-vascular therapy, TKI targeted therapy, or local therapy. Since the first-line treatment patterns of the included patients were relatively homogeneous, the first-line treatment status had little effect on the sensitivity to second-line immunotherapy in our cohort. We also analyzed the efficacy in different second-line treatment mode and no significant difference was found between the combination therapy mode and ICIs sensitivity (Table S1, P=0.709).

At the beginning of ICI treatment, 64 patients (62.7%) in the training cohort were diagnosed with LUAD and 83 (81.4%) with stage IV disease. During ICI treatment, most patients received combined chemotherapy (54.9% in the training cohort and 58.7% in the test cohort) or radiotherapy (19.6% in the training cohort and 17.4% in the test cohort). High PD-L1 expression (TPS >50%) was detected in 31.4% patients in the training cohort and 32.6% in the test cohort (*Table 1*).

ICI sensitivity-related mutational profile

To evaluate the ICI sensitivity-related mutational profile, we first identified the commonly detected genomic regions among the three gene detection panels (654 genes, 457 genes, 363 genes) in the training cohort, and finally obtained 343 commonly detected genes (available online: https://cdn.amegroups.cn/static/public/tlcr-2024-1249-1.xlsx). To avoid the heterogeneity caused by different detection panels, we only involved these 343 genes in the subsequent analysis. The most frequently mutated genes included *TP53* (60.8%), *EP300* (30.4%), *EGFR* (28.4%), *MSH6* (22.5%), *BRIP1* (18.6%), *RB1* (16.7%), *PTCH* (15.7%), *AXL* (14.7%), *ARID1A* (13.7%), and *NOTCH1* (13.7%). It was observed that although most mutations were missense mutations, some genes, including *EP300* and *MSH6*, mainly presented frame-shift insertion/deletions, which indicated that they were mainly loss-of-function mutations (*Figure 1A*).

Among the 343 commonly detected genes in the training cohort, the median number of mutated genes was 11. In the sensitive group, the median number of mutated genes was 13, while in the resistant group, the median number was 9 (P=0.05, Wilcoxon rank-sum test). Since the calculation of TMB value can be highly influenced by the panel design, especially small testing panels commonly used in clinical practice (27), to avoid possible systematic bias and misleading conclusions, we did not incorporate TMB in the subsequent analysis.

Most genes had higher mutational frequencies in the sensitive group than in the resistant group, including *EP300* (39.7% *vs.* 15.4%; P=0.014), *MSH6* (25.4% *vs.* 17.9%), *ARID1A* (17.5% *vs.* 7.7%), *BRCA2* (14.3% *vs.* 7.7%), *CREBBP* (12.7% *vs.* 5.1%), *MET* (12.7% *vs.* 2.6%), and *ATR* (12.7% *vs.* 2.6%). However, *EGFR* had a higher mutation frequency in the resistant group (41.0% *vs.* 20.6%; P=0.04; *Figure 1B*).

We further examined the survival data in training cohort. The sensitive group had significantly better PFS than the resistant group [HR =0.06, 95% confidence interval (CI): 0.03–0.15; *Figure 1C*]. Consistent with the results of intergroup mutational comparison, the prognosis of patients

Table 1 Characteristics of patients in the training and test cohort

Characteristics	Training cohort (N=102)	Test cohort (N=46)
Sex, n (%)		
Female	40 (39.2)	19 (41.3)
Male	62 (60.8)	27 (58.7)
Age (years), n (%)		
<60	47 (46.1)	20 (43.5)
≥60	55 (53.9)	26 (56.5)
Stage, n (%)		
III	19 (18.6)	7 (15.2)
IV	83 (81.4)	39 (84.8)
Metastasis, n (%)		
None	19 (18.6)	7 (15.2)
Liver	23 (22.5)	8 (17.4)
Bone	25 (24.5)	9 (19.6)
Brain	20 (19.6)	12 (26.1)
Multi-organ	15 (14.7)	10 (21.7)
PD-L1 expression, n (%)		
Low	35 (34.3)	18 (39.1)
Median	35 (34.3)	13 (28.3)
High	32 (31.4)	15 (32.6)
Histology, n (%)		
LUSC	38 (37.3)	14 (30.4)
LUAD	64 (62.7)	32 (69.6)
Combined therapy, n (%)		
Chemotherapy	56 (54.9)	27 (58.7)
Radiotherapy	20 (19.6)	8 (17.4)
Other	26 (25.5)	11 (23.9)
ICI drug, n (%)		
Sintilimab	41 (40.2)	18 (39.1)
Pembrolizumab	11 (10.8)	6 (13.0)
Nivolumab	6 (5.9)	2 (4.3)
Triprolizumab	29 (28.4)	11 (23.9)
Carilizumab	15 (14.7)	9 (19.6)
Response, n (%)		
Sensitive	63 (61.8)	19 (41.3)
Resistant	39 (38.2)	27 (58.7)

ICI, immune checkpoint inhibitor; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; PD-L1, programmed death-ligand 1.

with *EGFR* mutations was significantly worse (HR =2.15, 95% CI: 1.17–3.96), while those with *EP300* mutations had significantly better PFS (HR =0.46, 95% CI: 0.22–0.95; *Figure 1D*).

Prediction of ICI sensitivity

Base on the genetic findings of *EGFR* and *EP300*, we built a model to predict ICI sensitivity. We first constructed separated logistic prediction models based on basic clinical characteristics [including stage, sex, age, and histology type (clinical variable model)], PD-L1 expression status (PD-L1 model), or *EGFR* and *EP300* mutation status (mutation model) in the training cohort. Because smoking status was highly correlated with gender (Table S2, P<0.001), to avoid the instability of the nomogram model caused by multicollinearity, we excluded the smoking status variable after including sex.

In the internal evaluation of discrimination ability, the concordance index (C-index) values of the clinical variable, PD-L1, and mutation models, which were equivalent to the area under the curve (AUC) value in binary classification prediction, were 0.717, 0.652, and 0.688, respectively (*Figure 2A*). If we included all seven predictor variables (including stage, sex, age, and histology type, PD-L1 expression status, *EGFR* and *EP300* mutation status) in model building (full model), the C-index values of internal evaluation reached 0.780 (*Figure 2A*). According to DCA, the net benefit curve of the full model was also superior to that of each of separated prediction models (clinical variable, PD-L1, and mutation models). Therefore, we used the full model for prediction in the subsequent analysis.

To render the linear predictive model easy to interpret and use, we constructed a corresponding nomogram of the full model (*Figure 2B*). Based on the nomogram, we found that wild-type *EGFR*, mutant *EP300* mutation, stage III during ICI treatment, male gender, an age less than 60 years old, LUSC pathological type, and high PD-L1 expression were all associated with a higher probability of sensitivity to ICI treatment. Among the above seven predictive variables, high expression of PDL1 had the highest score, followed by *EP300* mutation status and *EGFR* mutation status, which indicated that the above three factors were the main contributors to the prediction results.

Validation of nomogram

Since the genetic test panels in our test cohort (n=46)

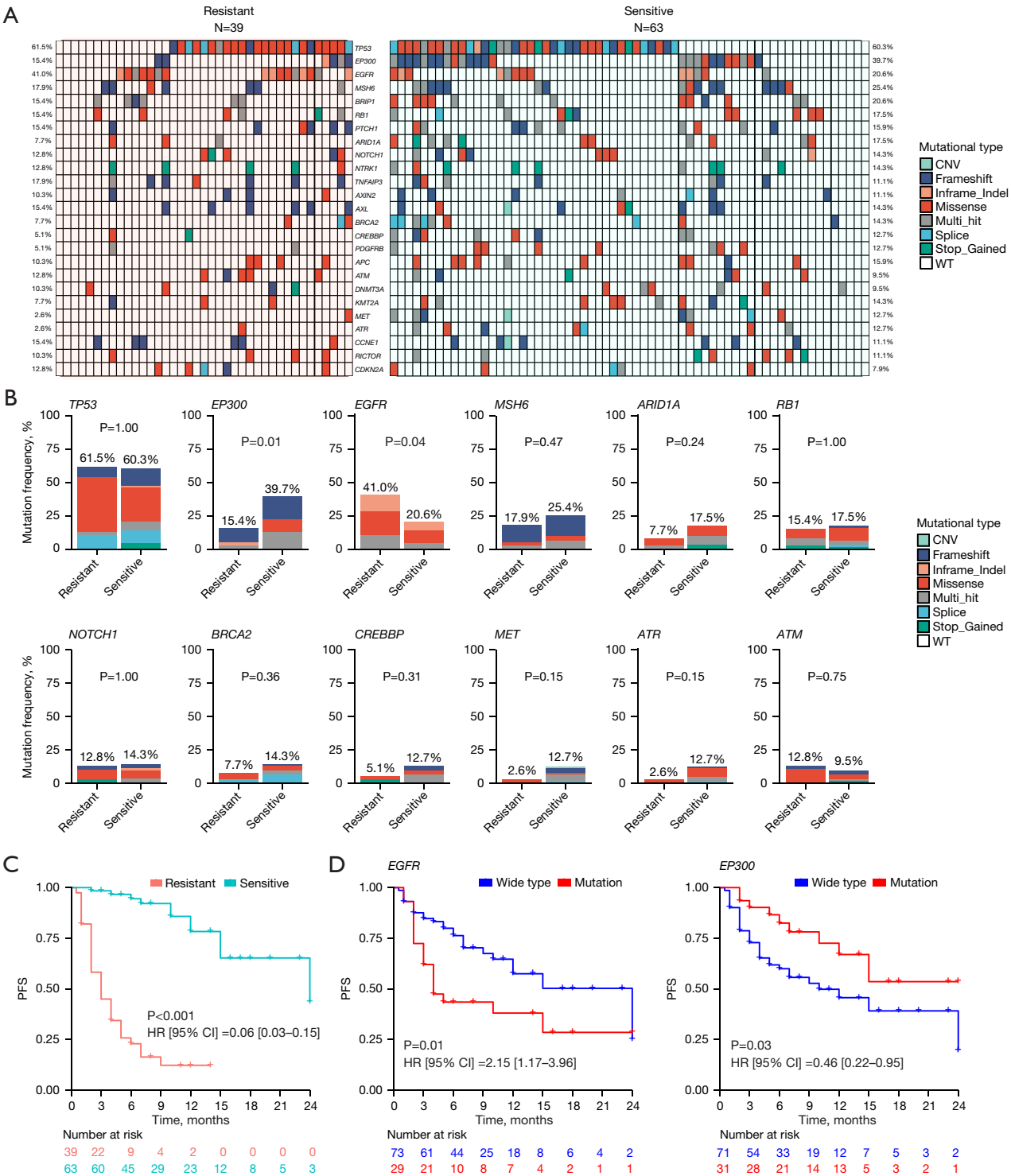

Figure 1 ICI sensitivity-related mutational profile in the training cohort. (A) Comparative heatmap of mutational status of the 25 most common mutated genes in the resistant group (left) and sensitive group (right). (B) Comparative bar plots of mutational frequency for the resistant group and sensitive group. (C) Kaplan-Meier survival plot of PFS for the resistant group and sensitive group. (D) Kaplan-Meier survival plot of PFS for patients with mutated and wild-type *EGFR* and *EP300*. CNV, copy number variants; HR, hazard ratio; ICI, immune checkpoint inhibitor; PFS, progression-free survival; WT, wild type.

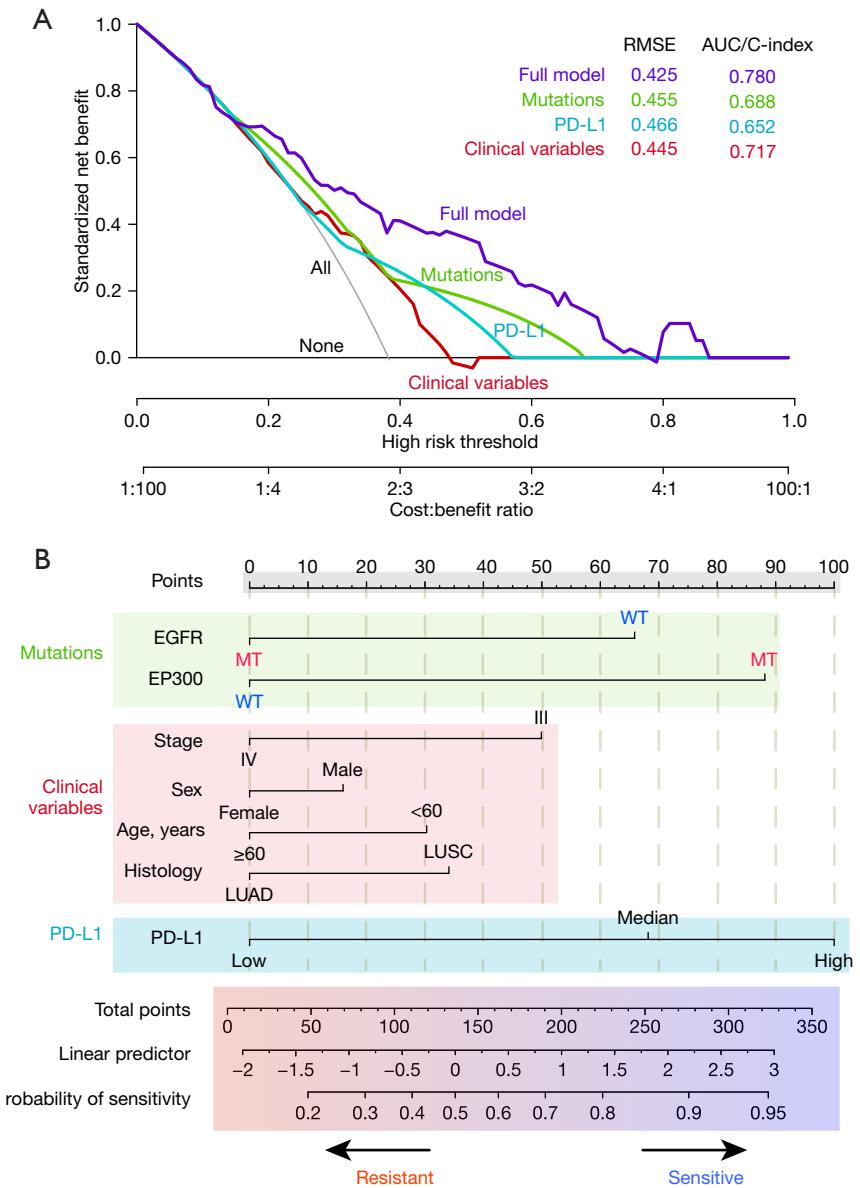

Figure 2 Prognostication model for sensitivity to ICIs. (A) DCA of the different logistic prognostication models. The RMSE and C-index values of the internal evaluations are labelled. (B) Nomogram of the full model. AUC, area under the curve; DCA, decision curve analysis; EGFR, epidermal growth factor receptor; ICIs, immune checkpoint inhibitors; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MT, mutant; PD-L1, programmed death-ligand 1; RMSE, root mean square error; WT, wild type.

included *EGFR* and *EP300*, the nomogram points could be calculated in both the training and test cohorts to verify the prognostic model. In the training cohort, the median nomogram points of the sensitive group and resistant group were 196.2 and 130.3, respectively (P<0.001); meanwhile, in the test cohort, the median nomogram points of the sensitive group and the resistant group were 184.1 and 148.6, respectively (P=0.01; *Figure 3A*). We evaluated the predictive performance of the nomogram using the receiver operating characteristic (ROC) curve (*Figure 3B*). In the training set, the area under the ROC curve was 0.780 (95% CI: 0.687–0.874), and in the test cohort, it was 0.720 (95% CI: 0.557–0.883). These results indicated that the nomogram had good prognostication capability in the

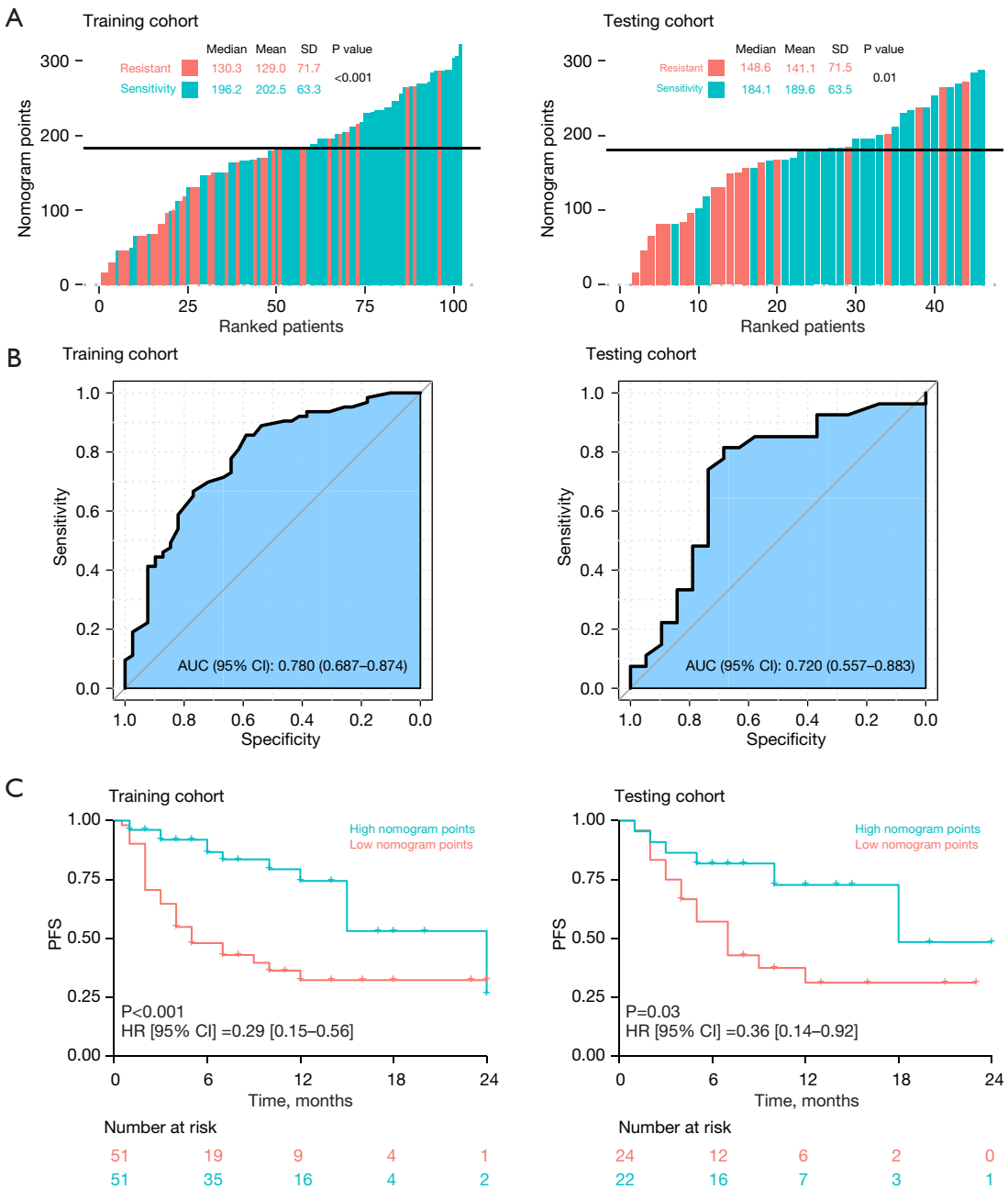

Figure 3 Validation of the nomogram. (A) Distribution of total nomogram points in the training cohort (n=102) and test cohort (n=46). (B) ROC curve of nomogram points in the training cohort and test cohort. (C) Kaplan-Meier survival plot of PFS for patients with a high nomogram score and a low nomogram score in the training cohort and test cohort. AUC, area under the curve; HR, hazard ratio; PFS, progression-free survival; ROC, receiver operating characteristic; SD, standard deviation.

independent cohort.

We further evaluated the prognostic value of nomogram points (*Figure 3C*). Both the training cohort and test cohort presented high nomogram scores, suggesting a significantly improved PFS (training cohort: HR =0.29, P<0.001; test cohort: HR =0.36, P=0.03), which indicated that the nomogram score could also be used to predict the prognostic outcomes in ICI treatment.

Discussion

Although ICI therapy has significantly improved patient survival in advanced NSCLC and dramatically changed the treatment landscape in routine clinical practice, only a portion of these patients demonstrate substantial response to treatment. Therefore, accurately predicting the efficacy of ICIs and identifying patients who will respond well to therapy is crucial for optimizing clinical practice. In this study, we examined the second-line ICI treatment response-related genomic landscape in advanced NSCLC and developed an integrated prognostication model for convenient clinical utilization in selecting highly sensitive patients.

Among the commonly detected genes in test panels, we found that the mutational status of *EGFR* and *EP300* was significantly associated with ICI response. Multiple reports have shown that *EGFR* mutations are associated with elevated PD-L1 expression and an immunosuppressive tumor microenvironment (28-30). In large immunotherapy-treated NSCLC cohorts, some types of *EGFR* mutations have also been confirmed to be significantly associated with resistance to ICI treatment (31-34). In our cohort, most *EGFR* mutations were non-drug sensitive mutations, such as H835R, G719S, E734K, etc. The first-line treatment for all involved patients was chemotherapy-based, and we did not included patients treated with TKI alone in first line. These inclusion criteria exclude the influence of first-line treatment mode as much as possible.

EP300 encodes the p300 protein, a homologous molecule to CBP (encoded by *CREBBP*), and both are histone acetylases critical to maintaining chromatin stability. In our study, both *EP300* and *CREBBP* presented a higher mutation frequency in the sensitive group. A previous study shown that the mutational status of *EP300* is associated with TMB and benefits from immunotherapy in bladder cancer (35). The loss-of-function mutations in CBP/p300 could also impair the function of regulatory T cells and promote antitumor immunity (36,37). In addition, the *EP300* and *CREBBP* mutations have also been associated with poor prognosis and resistance to chemotherapy or radiotherapy in many tumors (38-41). In our study, we did not incorporate TMB in the model to avoid possible systematic bias given that the TMB value could be highly influenced by the panel design. The mutational status of *EP300* might act as a surrogate for TMB in the nomogram model.

Many studies on the genomic-based prediction of immunotherapy efficacy have been published thus far (42-47). Most of this research has been based on a wide range of genetic tests or multiomics data. For example, Pan *et al.* developed a genetic signature based on 52 candidate genes to predict the immunotherapy benefits in patients with NSCLC (45), and Anagnostou *et al.* constructed a multimodal genomic feature model, including TMB, mutational status, mutational signature, and human leukocyte antigen (HLA) status, to predict the outcome of ICI therapy in patients with NSCLC (43). In cases of NSCLC with acquired resistance to ICIs, Ricciuti *et al.* identified resistance-related loss-of-function mutations in *STK11*, *B2M*, *APC*, *MTOR*, and *KEAP1* (48).

Although complex models might better reflect the heterogeneity of patients, they also face higher risks of overfitting in smaller-sized training cohorts and reduced interpretability. Meanwhile, models based on many input variables are difficult to apply in routine clinical genomic testing. Our model of nomogram was based on real-world clinical settings and involved a limited number of input variables that were economical and easy to obtain but offered reliable predictions results.

Our study, being a single-institution retrospective analysis, has some inherent limitations. First, the relatively small sample size limits the power of the conclusions, especially as it concerns the analysis of multiple clinical and genomic factors. Second, we only used genomic data from a clinical testing panel and lacked full-genome sequence data, which might have introduced biases related to the detection range. Moreover, due to limitations in real-world samples, our cohort did not include patients who had undergone first-line immunotherapy, which limits the applicability of our model.

Conclusions

The mutation status of *EGFR* and *EP300* is associated with the therapeutic sensitivity of ICIs. The integrated clinical-genomic nomogram model based on *EGFR* and *EP300*, designed for easy clinical utilization, demonstrated good performance in predicting the outcome of ICI therapy in patients with NSCLC.

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None.

Footnote

Reporting Checklist: The authors have completed the

TRIPOD reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2024-1249/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2024-1249/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-2024-1249/coif). The 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 the Research Ethics Board of Shandong Cancer Hospital and Institute (No. SDTHEC2024003156) and the requirement for individual consent was waived due to the retrospective nature of the analysis.

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