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A prospective multi-cohort study identifies and validates a 5-gene peripheral blood signature predictive of immunotherapy response in non-small cell lung cancer

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Abstract

Background Immune checkpoint inhibitors (ICIs) have revolutionized the treatment landscape for non-small cell lung cancer (NSCLC). The variability in patient responses necessitates a blood-based, multi-cohort gene signature to predict ICI response in NSCLC.

Methods We performed transcriptomic profiling of peripheral blood mononuclear cell (PBMC) and buffy coat (BC) samples from three independent cohorts of NSCLC patients treated with ICIs: a retrospective cohort (PMBCR, $n=59$), a retrospective validation cohort (BC, $n=44$), and a prospective validation cohort (PBMCP, $n=42$). We identified a 5-gene signature (UQCRB, NDUFA3, CDKN2D, FMNL1-DT, and APOL3) predictive of ICI response and validated its clinical utility in the prospective PBMCP cohort. Response was evaluated using RECIST criteria, and patients were followed up for progression-free survival (PFS) and overall survival (OS).

Results In the prospective PBMCP cohort, the 5-gene signature demonstrated high accuracy in stratifying patients into responders and non-responders (AUC = 0.89, 95% CI: 0.80–0.99). Predicted responders exhibited significantly longer PFS compared to predicted non-responders (median: 13.8 months vs. 4.2 months, HR = 0.21, 95% CI: 0.07–0.58, $p=0.005$).

Conclusion Our study confirms a 5-gene signature as a key biomarker for ICI response in NSCLC, enhancing treatment precision.

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To the editor

Introduction

The initiation of immunotherapeutic strategies, especially the deployment of Anti-PD(L)-1 and CTLA-4 checkpoint blockade, has precipitated a paradigm shift in the therapeutic landscape of non-small cell lung cancer (NSCLC), which constitutes approximately 85% of lung cancer incidences. Emblematic of this advancement, Pembrolizumab markedly prolonged median progression-free survival to 10.3 months over the 6.0 months observed with traditional chemotherapy, as delineated in the landmark KEYNOTE-024 trial [1]. Though immunotherapy heralds a new era in NSCLC treatment with response rates spanning from 14.8 to 44.8%, its efficacy is not uniform across all patients. This variability, coupled with potential adverse effects and toxicities, accentuates the imperative for identifying precise and reliable predictive biomarkers [1; 2; 3]. While PD-L1 expression and tumor mutational burden (TMB) have been explored as predictive biomarkers for immune checkpoint inhibitor (ICI) efficacy, their reliability is notably inconsistent. The KEYNOTE-010 trial underscored this, revealing that high PD-L1 expression (≥50%) correlated with a 44.8% response rate to Pembrolizumab, yet lower expression significantly reduced effectiveness [4]. Critically, McGrail et al.'s 2021 study highlighted the limitations of TMB as a universal predictor, demonstrating that high TMB does not uniformly forecast ICI response across various cancer types [5].

The limitations of current predictive markers stem from their reliance on invasive tumor biopsies, which are not only challenging to obtain repeatedly but also fail to capture the tumor's spatial and temporal heterogeneity. This has led researchers to explore non-invasive alternatives that can provide a holistic view of the tumor-immune interaction. Peripheral blood, as a mirror to the body's systemic response, offers an unparalleled opportunity to monitor the dynamic changes associated with therapy response.

In this study, we aimed to identify and validate a peripheral blood-based gene signature for predicting ICI response in NSCLC through a multi-cohort approach. We hypothesized that a gene signature derived from peripheral blood mononuclear cells (PBMCs) and buffy coat (BC) samples could serve as a robust predictive biomarker for ICI response, enabling personalized treatment decisions and improving clinical outcomes.

To test this hypothesis, we first conducted a retrospective analysis of PBMC and BC samples from two cohorts of NSCLC patients treated with ICIs (PMBCR and BC cohorts) to identify a gene signature predictive of ICI response. We then prospectively validated the clinical utility of this signature in an independent cohort of NSCLC patients treated with pembrolizumab plus carboplatin and pemetrexed (PBMCP cohort). By combining retrospective discovery and prospective validation, we sought to develop a robust and clinically applicable predictive biomarker for precision immunotherapy in NSCLC.

Results

Patient characteristics

In this multi-cohort study, we employed a systematic approach to identify and validate a gene signature predictive of ICI response in NSCLC (Supplementary Figure S1). The study design encompassed retrospective discovery in two cohorts (PMBCR and BC) and prospective validation in an independent cohort (PBMCP). A total of 145 NSCLC patients were included in this study, with 59 in the PMBCR (WHTJ) cohort, 44 in the BC (RUMC) cohort, and 42 in the PBMCP (WHTJ) cohort. All patients received treatment with pembrolizumab, carboplatin, and pemetrexed. The median age was 62, 68, and 62 years in the PMBCR, BC, and PBMCP cohorts, respectively. The majority of patients were male (77.9% in PMBCR, 68.2% in BC, and 71.4% in PBMCP). The median progression-free survival (PFS) time was 190, 213, and 260.5 days, and the median follow-up time was 478, 520.5, and 535.5 days in the PMBCR, BC, and PBMCP cohorts, respectively. Smoking history was present in 55.9%, 95.5%, and 54.8% of patients in the PMBCR, BC, and PBMCP cohorts, respectively. Adenocarcinoma was the predominant pathological type across all cohorts. Most patients had stage IV disease (52.5% in PMBCR, 100% in BC, and 73.8% in PBMCP). The response rates were 47.5%, 22.7%, and 54.8% in the PMBCR, BC, and PBMCP cohorts, respectively (Supplementary Table 1).

A novel 5-gene signature emerges as a robust predictor of ICI response in NSCLC

Through retrospective analysis of the PMBCR and BC cohorts, we identified 5 genes (UQCRB, NDUFA3, CDKN2D, FMNL1-DT, and APOL3) that contributed the most to differentiating responders from non-responders in both datasets (Supplementary Figure S2 and Supplementary Table 5). Based on these 5 genes, we established a 5-gene signature and calculated a meta-score for each patient. The distribution of meta-scores for responders and non-responders in the PMBCR and BC cohorts is shown in Fig. 1A and B, respectively.

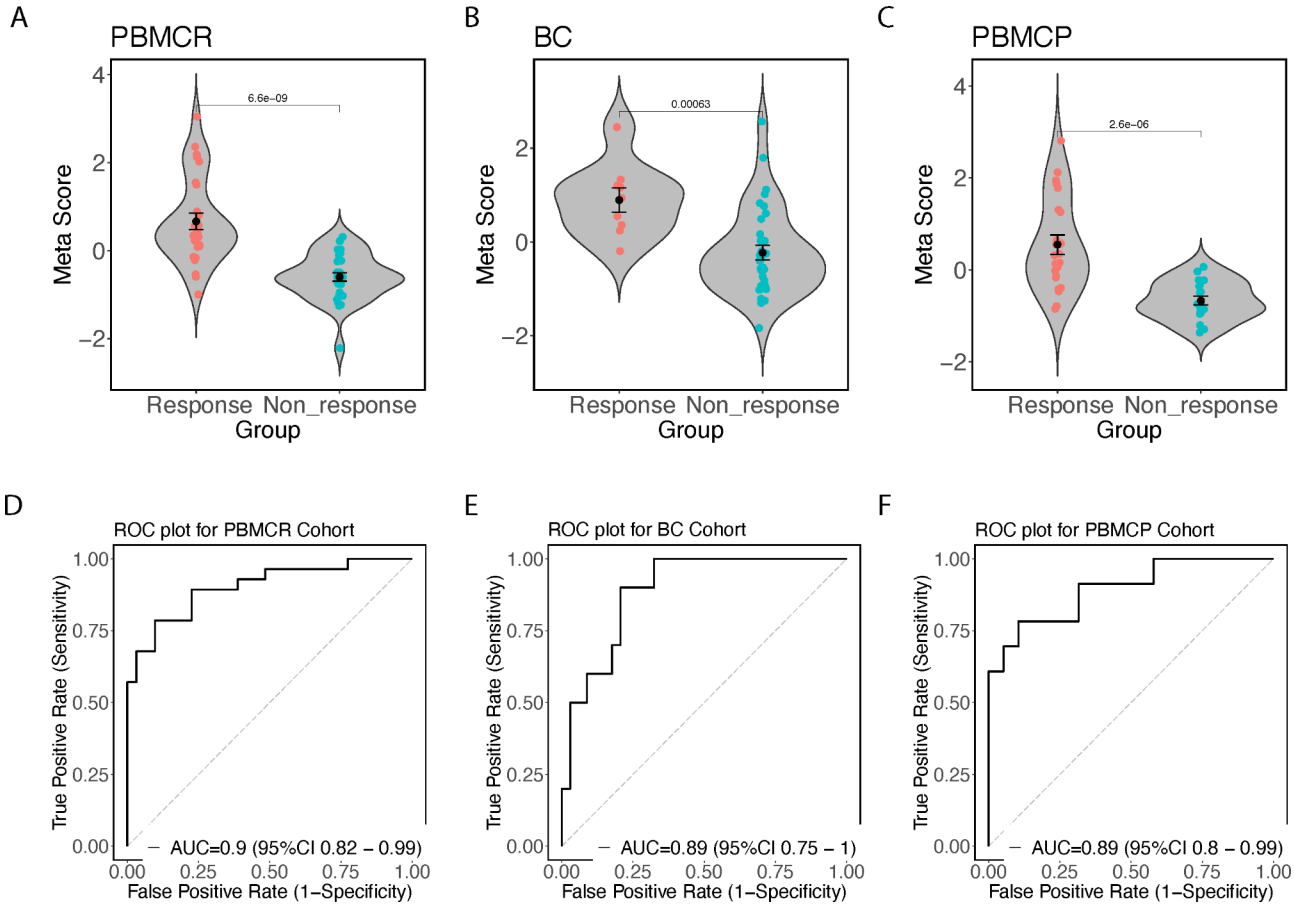

Fig. 1 Transcriptomic Analysis Outcomes. **A**: Meta-score distribution of the 5-gene signature inresponders and non-responders of the PMBCR (WHTJ) training cohort. **B**: Meta-score distribution of the 5-genesignature in responders and non-responders of the BC(RUMC)validation cohort. **C** Meta-score distribution of the 5-gene signature in responders and non-responders of the PBMCP (WHTJ) prospective validation cohort. **D**: ROCcurve analysis demonstrating the predictive performance of the 5-gene signature in the PMBCR cohort (AUC=0.90,95% CI: 0.82-0.99). E: ROC curve analysis confirming the predictive value of the 5-gene signature in the BC(RUMC) validation cohort (AUC=0.89, 95% CI: 0.75-1.00). F: ROC curve analysis validating the predictive accuracyof the 5-gene signature in the prospective PBMCP cohort (AUC=0.89, 95% CI: 0.80-0.99)

Retrospective cohorts validate the strong predictive power of the 5-gene signature

To validate the predictive performance of the 5-gene signature, we performed ROC curve analysis in both cohorts. The signature achieved an AUC of 0.90 (95% CI: 0.82–0.99) in the PMBCR cohort (Fig. 1D) and an AUC of 0.89 (95% CI: 0.75-1.00) in the BC cohort (Fig. 1E), demonstrating its robust ability to distinguish responders from non-responders.We further compared the 5-gene signature with other published gene markers (Supplementary Tables 2 & 3). The results showed that the 5-gene signature exhibited the highest stability and predictive accuracy among all the compared markers.

Examining the 5-gene signature in other cancer types

To evaluate whether the 5-gene signature is specific to NSCLC, we analyzed its predictive performance in other cancer types. We collected Whole-blood RNA transcriptome sequencing data from Renal Cell Carcinoma patients and applied our 5-gene predictive model [6]. The results indicated a poor predictive performance, with an AUC of 0.42 (95% CI: 0.22−0.62), as shown in Supplementary Figure S3. Furthermore, we compared our 5-gene signature with predictive models used in melanoma and bladder cancer and found no overlap between the genes involved [7; 8]. This suggests that our 5-gene signature demonstrates strong specificity to NSCLC, and may not be broadly applicable across other cancer types.

Prospective validation solidifies the clinical utility of the 5-gene signature in guiding ICI treatment decisions

To assess the clinical utility of the 5-gene signature, we conducted a prospective study in the PBMCP cohort. The meta-scores for responders and non-responders in the PBMCP cohort were calculated (Fig. 1C), and ROC curve analysis revealed an AUC of 0.89 (95% CI: 0.80–0.99) (Fig. 1F), confirming the signature's predictive value in a prospective setting.

5-Gene signature serves as a reliable prognostic marker for survival outcomes in NSCLC patients receiving ICI therapy

We performed survival analysis based on the predicted response status determined by the 5-gene signature. In the PMBCR cohort, patients predicted as responders had significantly longer progression-free survival (PFS) compared to those predicted as non-responders ($P<0.001$) (Fig. 2A). Similarly, in the BC cohort, both PFS and overall survival (OS) were significantly longer in the predicted responder group compared to the predicted non-responder group ($P<0.001$) (Fig. 2C and D). The prospective PBMCP cohort also demonstrated significantly improved PFS in the predicted responder group ($P<0.001$) (Fig. 2B), further validating the prognostic value of the 5-gene signature.

5-gene signature proves superior to clinical factors in predicting ICI response, highlighting its independent predictive value

To investigate the relationship between clinical factors and the 5-gene signature, we performed multivariate logistic regression analysis. The results showed that the 5-gene signature was the most significant predictor of ICI response among all clinical factors ($P<0.001$). Smoking history was also found to be significantly associated with ICI response ($P<0.05$) (Supplementary Table 4).

Conclusion

This study affirms the clinical relevance of a 5-gene signature in predicting the efficacy of pembrolizumab combined with carboplatin and pemetrexed in advanced NSCLC. Demonstrating robust accuracy in distinguishing responders from non-responders, this gene signature notably improves prediction of overall response rates and progression-free survival. Such precise stratification

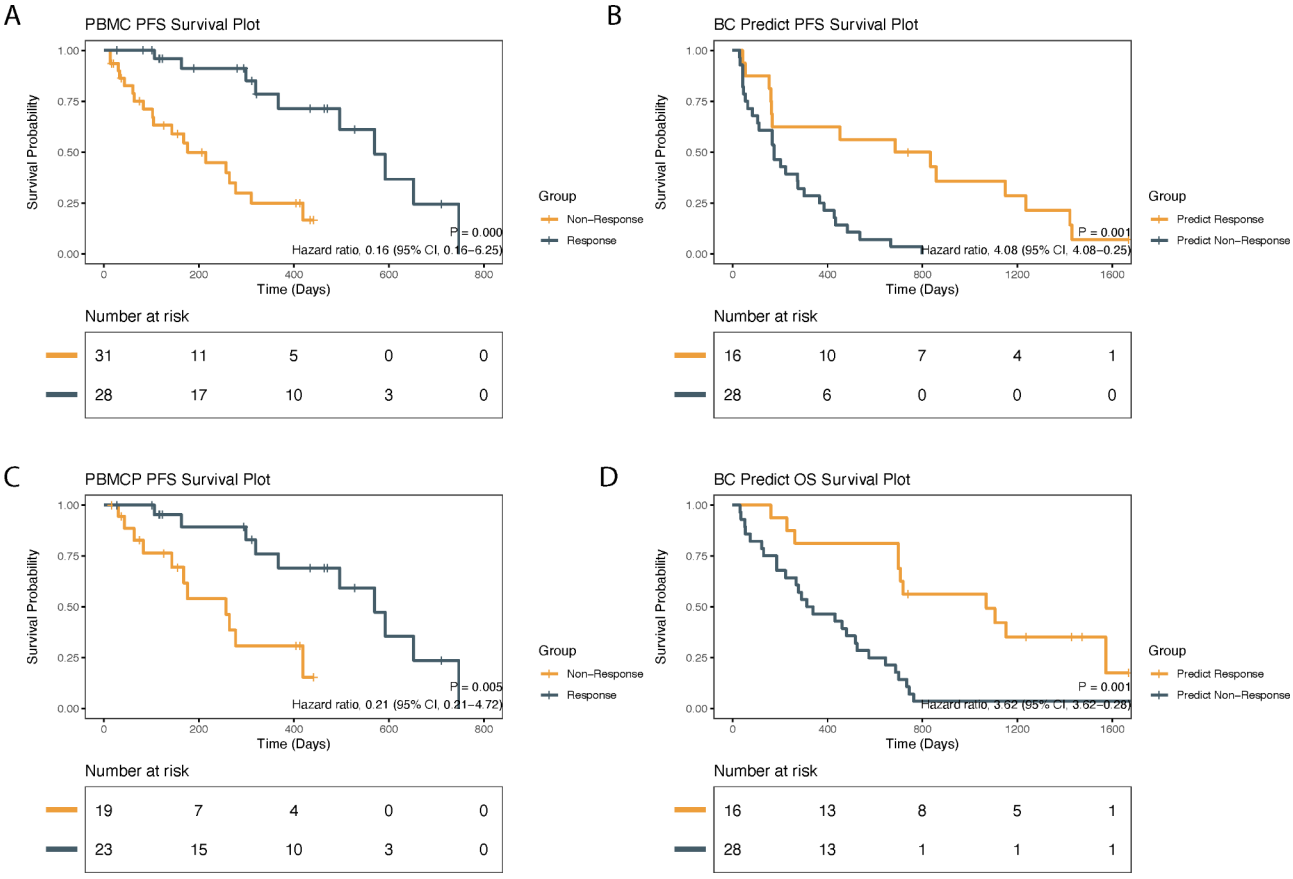

Fig. 2 Survival Analysis Demonstrating the Prognostic Value of the 5-Gene Signature. **A**: Kaplan-Meiercurves for progression-free survival (PFS) in the PMBCR cohort, revealing a significant survival benefit for patientspredicted as responders by the 5-gene signature compared to predicted non-responders (P < 0.001). **B**: Kaplan-Meier curves for PFS in the prospective PBMCP cohort, confirming the prognostic value of the 5-gene signature,with predicted responders exhibiting significantly longer PFS than predicted non-responders (P < 0.001). **C**: Kaplan-Meier curves for PFS in the BC cohort, demonstrating a significant survival advantage for predicted respondersover predicted non-responders based on the 5-gene signature (P < 0.001). **D**: Kaplan-Meier curves for overallsurvival (OS) in the BC cohort, showing a significant survival benefit for patients predicted as responders by the 5-gene signature compared to predicted non-responders (P < 0.001)

underscores its value in guiding treatment decisions, enhancing the personalization of ICI-based therapies.

The 5-gene signature, encompassing UQCRB, NDUFA3, CDKN2D, FMNL1-DT, and APOL3, integrates critical aspects of immune response and tumor biology, potentially optimizing therapeutic outcomes. These genes contribute to various biological functions, from mitochondrial activity and energy metabolism to cell cycle regulation and immune surveillance, thus fostering a conducive environment for effective immunotherapy. This interplay underscores the potential of this signature to refine clinical strategies for NSCLC patients, supporting broader clinical adoption.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12943-024-02160-2.

Supplementary Material 1

Author contributions

S.C Q.C and Y.D conceived and designed the study. S.C., Y.F. ,F.F.L, Z.G.L, and C.K.D. acquired the data. S.C., J.A.B., A.A.G., F.F.L, Z.G.L, and M.N. analyzed and interpreted the data. S.C., M.J. Z.G.L, and H.Y. developed the new software used in the work. S.C., T.G. F.F.L, Z.G.L, and J.W. drafted the manuscript and made substantial revisions. S.C., Z.L., X.W.,and H.W. provided critical feedback and helped shape the research, analysis, and manuscript. S.C., and Q.C. supervised the project. Y.D. managed the overall project and ensured the integrity of the work. All authors approved the submitted version and any substantially modified version that involves their contribution to the study.

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Data availability

The datasets analyzed in the current study are available upon reasonable request from the corresponding author.

Declarations

Ethical approval

The study was conducted in accordance with the ethical principles in the Declaration of Helsinki and was approved by the Institutional Review Board of Wuhan Tongji Hospital (WHTJ, China, TJ-IRB20200731) and Rush University Medical Center (RUMC, USA). Written informed consent was obtained from all participants.

Competing interests

The authors declare no competing interests.

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