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FAT1 Mutation as a Predictive Biomarker of Non-Durable Response to Immune Checkpoint Blockade in Non-Small Cell Lung Cancer

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ABSTRACT

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of non-small cell lung cancer (NSCLC), offering durable benefits in a subset of patients. Nevertheless, only about one-fifth of individuals respond effectively, leaving resistance to ICI therapy as a critical barrier to improved outcomes. To explore the genomic factors underlying limited therapeutic benefit, four publicly available NSCLC cohorts (n = 2,986) were analyzed. From the Rizvi cohort, 158 patients exhibiting no durable clinical benefit (NDB) following ICI treatment were identified. Potential NDB-associated mutations were detected through univariate and multivariate Cox regression analyses. In addition, PD-L1 expression, tumor mutation burden (TMB), neoantigen load, tumor-infiltrating lymphocytes, and immune-related gene profiles were examined to clarify immune microenvironmental differences. A composite predictive model was subsequently constructed to estimate ICI treatment efficacy. Mutations in *FAT1* and *KEAP1* were enriched in NDB cases. Further analyses revealed that *FAT1* mutation was specifically linked to diminished response to ICI therapy, whereas *KEAP1* mutation functioned primarily as a prognostic rather than predictive factor. *FAT1*-mutant tumors displayed elevated TMB yet reduced immune infiltration, particularly of $CD8^+$ T cells. Incorporating PD-L1 expression, TMB, smoking history, treatment regimen, therapy type, and *FAT1* mutation status, the resulting prognostic model achieved high predictive accuracy (AUC = 0.763 for 6-month survival; AUC = 0.871 for 12-month survival). The presence of *FAT1* mutation may signify resistance to ICI therapy and serve as a predictive biomarker in NSCLC. The proposed *FAT1*-based predictive framework may aid in identifying patients more likely to benefit from immunotherapy, contributing to precision treatment approaches.

Keywords: Non-small cell lung cancer, Immune checkpoint inhibition, FAT1, KEAP1, PD-1/PD-L1 blockade, Anti-CTLA-4

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Introduction

Lung cancer continues to rank as the most prevalent malignancy and remains the foremost cause of cancer-associated mortality across the globe [1]. Among its subtypes, non-small cell lung cancer (NSCLC) constitutes nearly 80–85% of all cases [2]. Although targeted therapies have markedly improved outcomes for patients with defined oncogenic drivers, these actionable mutations are present in only a limited fraction of individuals [3].

The advent of immunotherapy has reshaped the treatment landscape of NSCLC, particularly with the introduction of immune checkpoint inhibitors (ICIs) directed against programmed cell death protein 1 (PD-1) and its ligand PD-L1. These agents have demonstrated considerable success, especially in tumors lacking targetable alterations. Clinical trials have verified that immune checkpoint blockade (ICB) provides meaningful benefit in several contexts—ranging from first- and second-line therapy for advanced NSCLC to consolidation regimens in locally advanced disease and neoadjuvant therapy for early-stage cases [4, 5].

Nevertheless, only a minority of patients—approximately 20–30%—achieve a lasting clinical response to anti–PD-1/PD-L1 therapy [4, 5]. The diversity of treatment outcomes underscores the complexity of immune response

in NSCLC. Some individuals experience atypical patterns such as pseudoprogression, characterized by a temporary tumor increase prior to regression, or hyperprogression, marked by rapid disease acceleration [6]. Despite such variability, non-responsiveness to ICB remains a major obstacle in thoracic oncology. Determining which patients are unlikely to benefit from immunotherapy is therefore crucial for minimizing overtreatment, improving cost-effectiveness, and personalizing clinical decision-making.

A variety of potential biomarkers—including PD-L1 expression [7, 8], tumor mutation burden (TMB) [9], neoantigen abundance [10], the density of immune and stromal infiltrates [11], and immune-related transcriptional signatures [12]—have been investigated to predict ICB efficacy. Yet, each of these factors alone provides limited discriminatory power. To address this gap, the present study systematically analyzed public genomic datasets to delineate the molecular characteristics and potential mechanisms underlying poor immunotherapy response in NSCLC patients showing no durable clinical benefit (NDB) [13, 14].

Materials and Methods

Data sources

Genomic and transcriptomic profiles from a total of 1,144 NSCLC samples were collected from *The Cancer Genome Atlas (TCGA)* via the cBioPortal interface (http://www.cbioportal.org/, accessed on 20 September 2020) [14]. RNA sequencing data corresponding to 515 lung adenocarcinoma (LUAD) and 501 lung squamous cell carcinoma (LUSC) cases were downloaded directly from the TCGA portal (https://portal.gdc.cancer.gov/, accessed on 21 September 2020).

To expand the analysis, three additional cohorts from the Memorial Sloan Kettering Cancer Center (MSKCC)—the Zehir [15] (n = 1,567), Rizvi [13] (n = 240), and Naiyer [16] (n = 35) datasets—were obtained through cBioPortal under identical access dates. Summary statistics for all datasets are presented in **Table 1**. Neoantigen loads for TCGA samples were extracted from *The Cancer Immunome Atlas* (https://tcia.at/home, accessed on 24 September 2020).

In this study, durable clinical benefit (DCB) was defined as a partial response or stable disease lasting more than six months, whereas patients who failed to achieve such outcomes were classified as having no durable clinical benefit (NDB).

Table 1. Clinical characteristics of the included cohorts.

Characteristic		TCGA Cohort n = 1144	Rizvi Cohort n = 240	Naiyer Cohort n = 35			Zehir Cohort n = 1567	Characteristic
				DCB	NDB	NA		
Gender	Male	673 (59%)	32 (13%)	79 (33%)	7 (3%)	16 (46%)	681 (43%)	
	Female	468 (41%)	37 (15%)	79 (33%)	6 (3%)	19 (54%)	886 (57%)	
Age	>60	695 (71%)	39 (16%)	53 (22%)	12 (5%)	19 (54%)	NA	
	≤60	253 (26%)	30 (13%)	105 (44%)	1 (0.4%)	16 (46%)	NA	
Smoking status	Ever	976 (85%)	60 (26%)	123 (80%)	10 (4%)	30 (86%)	972 (62%)	
	Never	111 (10%)	9 (4%)	35 (20%)	3 (1%)	5 (14%)	334 (21%)	
	Unknown	57 (5%)	0	0	0	0	261 (17%)	
Histology	AD	660 (58%)	56 (23%)	124 (6%)	7 (3%)	30 (86%)	1268 (81%)	
	SCC	484 (42%)	9 (4%)	22 (9%)	3 (1%)	4 (11%)	163 (10%)	
	Others	0	5 (2%)	12 (5%)	3 (1%)	1 (3%)	136 (9%)	
ICBs	PD1/PDL1	NA	53 (22%)	142 (59%)	11 (5%)	35 (100%)	NA	
	Combination	NA	16 (7%)	16 (7%)	2 (0.8%)	0	NA	

Cox proportional hazards modeling

From the Rizvi cohort, 158 NSCLC patients exhibiting no durable clinical benefit (NDB) following immunotherapy were selected for further analysis. The available next-generation sequencing (NGS) results were level 3 processed data, and mutation information was extracted from Mutation Annotation Format (MAF) files using Perl scripts. Genes with a mutation frequency exceeding 5% were identified for subsequent evaluation

through univariate Cox regression. Variables meeting the threshold of $p < 0.20$ were then entered into a multivariate Cox proportional hazards model. Only those with $p < 0.05$ in multivariate analysis were retained for additional investigation.
Univariate Cox regression was subsequently performed for the selected variables along with key clinical factors from the Rizvi cohort, such as age and sex. Significant predictors were incorporated into a multivariate model, which was internally validated through the construction of a nomogram and corresponding calibration curve. Individual risk scores were derived from regression coefficients, and patients were classified into high- or low-risk groups based on the median value. External validation was carried out using the Naiyer cohort.

Propensity score matching and survival analysis
To reduce potential baseline imbalances, propensity score matching was performed on the Zehir cohort using the "matchit" R package, applying a 1:3 nearest-neighbor matching approach. Patients were stratified according to the presence or absence of specific gene mutations before matching. Survival outcomes in the matched cohorts were subsequently analyzed using the "survminer" package, and Kaplan–Meier survival curves were plotted to visualize differences between groups.

Evaluation of tumor mutation burden (TMB)
Tumor mutation burden (TMB) was defined as the total number of somatic, nonsynonymous mutations—including single-nucleotide variants, insertions, and deletions—per coding region of the genome. In the TCGA cohort, TMB was derived from whole-exome sequencing (WES), whereas in the Rizvi cohort, values were calculated using NGS-based targeted sequencing. Specifically, 56 NSCLC samples were analyzed with a 341-gene panel, 164 samples with a 410-gene panel, and 20 samples with a 468-gene panel.

mRNA expression profiling
Given that responsiveness to ICB therapy is influenced by the immune composition of the tumor microenvironment—including T-cell effector functions, interferon-gamma (IFN-γ) signaling, and immune receptor expression [17, 18]—we examined the relationship between gene mutations and immune-related transcriptomic profiles. This analysis included 1,026 TCGA samples for which both RNA-seq and DNA-seq data were available. The immune gene set, comprising 47 genes, was curated from a previously published study that characterized genes associated with activated T cells, cytolytic activity, and IFN-γ production [19].

Functional enrichment analysis
Functional pathway enrichment was conducted using the "clusterProfiler" R package to perform Gene Set Enrichment Analysis (GSEA), comparing wild-type tumors with those harboring *FAT1* or *KEAP1* mutations. A false discovery rate (FDR) < 0.25 was considered statistically significant. Immune and stromal cell-type enrichment across 64 categories in the TCGA dataset was estimated using XCell-based deconvolution implemented through the TIMER2.0 platform (http://timer.comp-genomics.org/, accessed on 20 September 2020) [20].

Statistical analyses
All statistical procedures were conducted in R software version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). The "ggplot2," "rms," "foreign," "survivalROC," and "survival" packages were employed for data visualization and statistical modeling. Survival analyses were performed using the "survival" and "survminer" packages. Two-group comparisons were carried out using independent-sample *t*-tests, while the Wilcoxon rank-sum test was applied for multiple-group comparisons. Correlation analyses were performed using Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$.

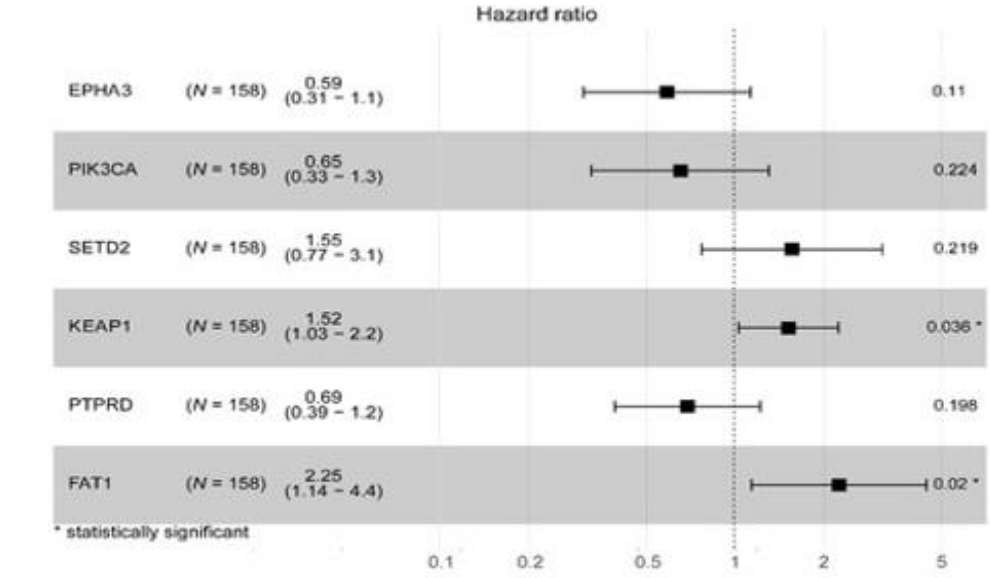
Results and Discussion

Clinical characteristics of included cohorts
Four independent cohorts were analyzed in this study, and their baseline characteristics are summarized in **Table 1**. In the Naiyer cohort, all patients received either anti–PD-1 or anti–PD-L1 therapy. Within the Rizvi cohort, 206 NSCLC patients were treated with anti–PD-1/PD-L1 inhibitors alone, while 34 received combination therapy with

anti–PD-1/PD-L1 and anti–CTLA-4 antibodies. Among these, 142 and 16 patients, respectively, exhibited no durable clinical benefit (NDB) following treatment **(Table 1)**.

Identification of genes associated with NDB to immunotherapy

Analysis of the Rizvi cohort (n = 158 NDB patients) identified genes with mutation frequencies greater than 5%, which were subsequently subjected to univariate and multivariate Cox regression. Variables with $p < 0.20$ in univariate analysis were included in the multivariate model. As illustrated in **Figure 1a**, mutations in *KEAP1* and *FAT1* were significantly correlated with poor prognosis among NSCLC patients experiencing NDB after ICB therapy, suggesting these alterations may contribute to primary resistance mechanisms. Interestingly, *KEAP1* mutations—but not *FAT1*—were also associated with unfavorable survival outcomes in NSCLC patients who did not receive immunotherapy **(Figures 1b and 1c)**.



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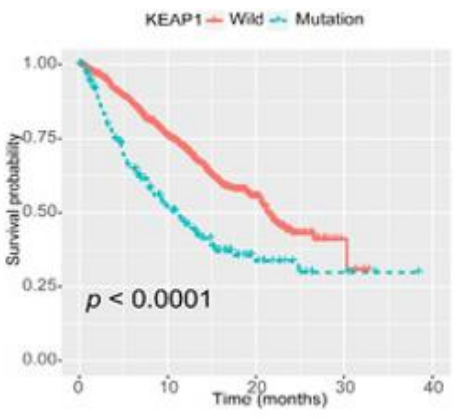

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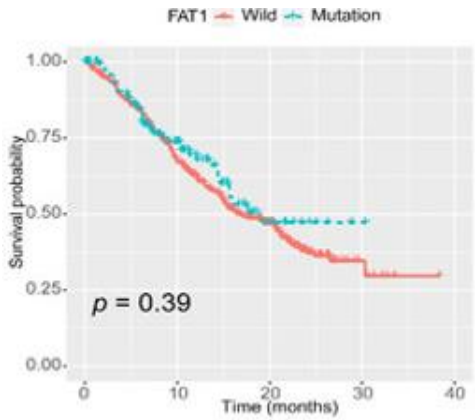


c)

Figure 1. (a) Cox proportional hazards analysis examining how KEAP1 and FAT1 mutations relate to prognosis in patients without durable clinical benefit (NDB) following immune checkpoint blockade (ICB). (b) Overall survival (OS) curves according to KEAP1 mutation status in the Zehir cohort. (c) Overall survival (OS) curves according to FAT1 mutation status in the Zehir cohort.

Association between immune characteristics and KEAP1/FAT1 mutations in NSCLC

To investigate why NSCLC patients harboring KEAP1 or FAT1 mutations exhibit poor responses to ICB therapy, we analyzed the TCGA dataset for immunological differences between mutant and wild-type tumors. The comparison included indicators such as immune cell infiltration, neoantigen count, tumor mutational burden (TMB), PD-L1 expression, and immune-regulatory gene expression.

Tumors carrying KEAP1 mutations showed a clear reduction in PD-L1 expression compared to wild-type tumors ($p < 0.001$; **(Figure 2a)**). In contrast, TMB values were markedly higher in both KEAP1- and FAT1-mutant tumors than in their wild-type counterparts ($p < 0.001$; **(Figure 2b)**). Likewise, tumors with KEAP1 mutations displayed an increased neoantigen burden ($p < 0.001$; **(Figure 2b)**).

Meanwhile, FAT1 mutations were associated with notably lower $CD8^+$ T-cell infiltration ($p = 0.005$; **(Figure 2a)**). Although these genetic alterations were linked to greater mutational load and more complex tumor antigens, many immune-related genes were simultaneously downregulated. This imbalance may contribute to immune suppression or escape**,** providing a potential explanation for the diminished ICB responsiveness observed in patients with FAT1 or KEAP1 mutations.

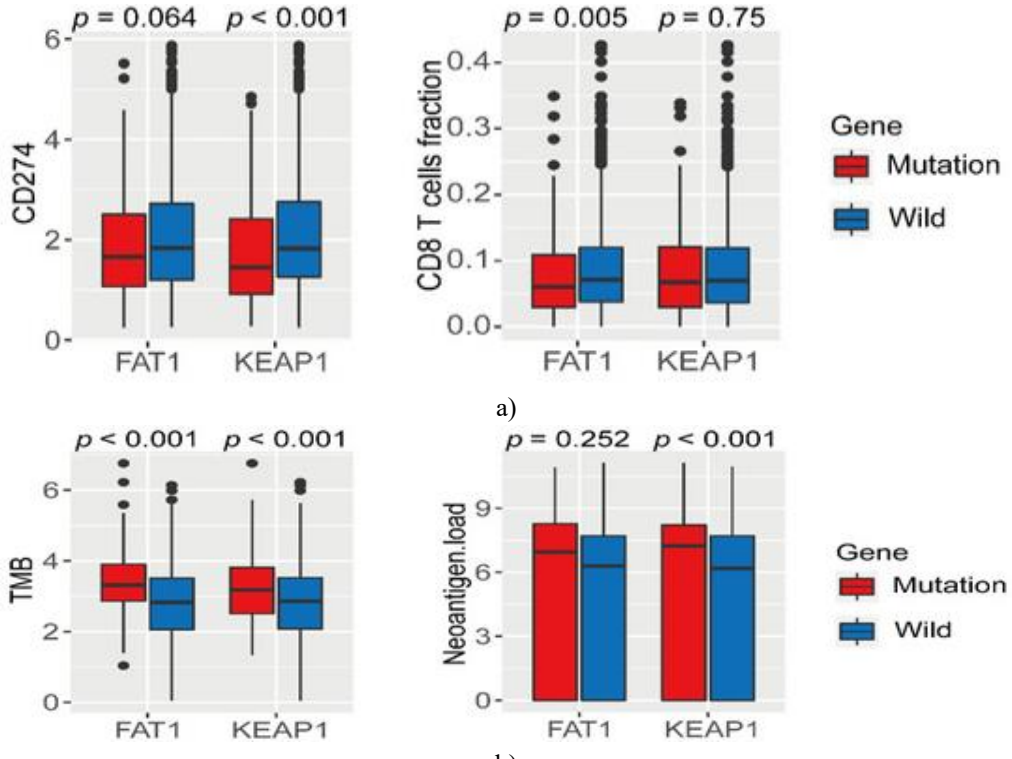
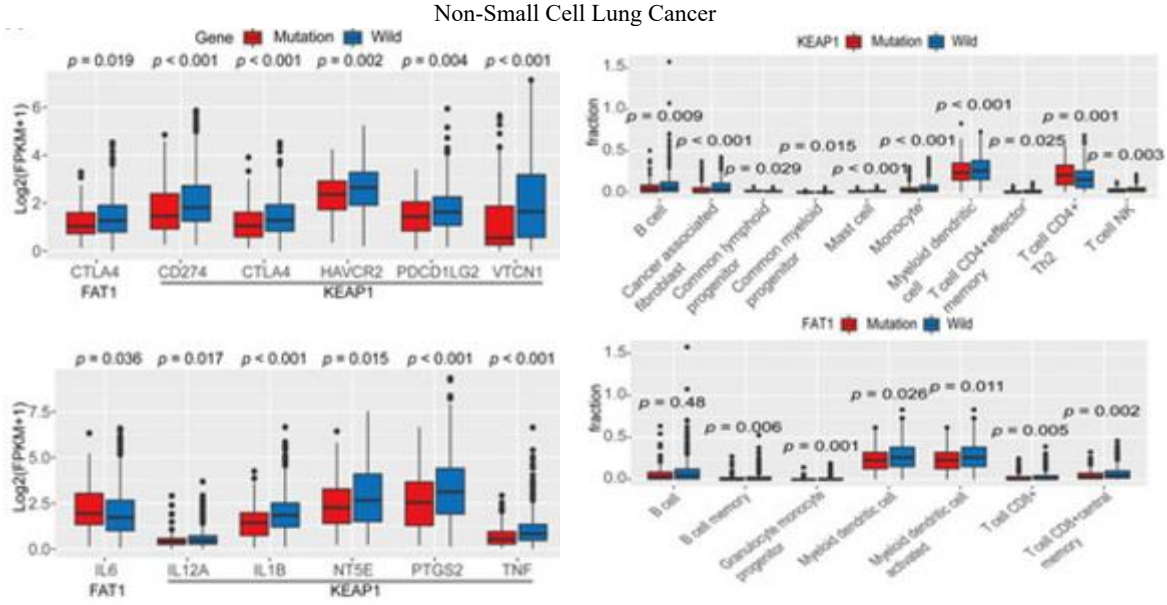


Figure 2. Correlation between gene mutation and status of common biological indicators in patients treated with immune checkpoint blockade (ICB). (**a**) Left: mRNA expression of CD274, right: CD8+ T cell infiltration; (**b**) left: tumor mutation load, right: neoantigen load.

In NSCLC tumors carrying KEAP1 mutations, most immune-related lymphocyte populations—specifically eight subsets—showed reduced infiltration compared to wild-type tumors. The only exceptions were common lymphoid progenitor cells and Th2 $CD4^+$ T cells, both of which displayed higher infiltration levels **(Figure 3b)**. Similarly, tumors with FAT1 mutations demonstrated diminished infiltration across seven types of immune-associated cells when contrasted with FAT1 wild-type cases **(Figure 3b)**.

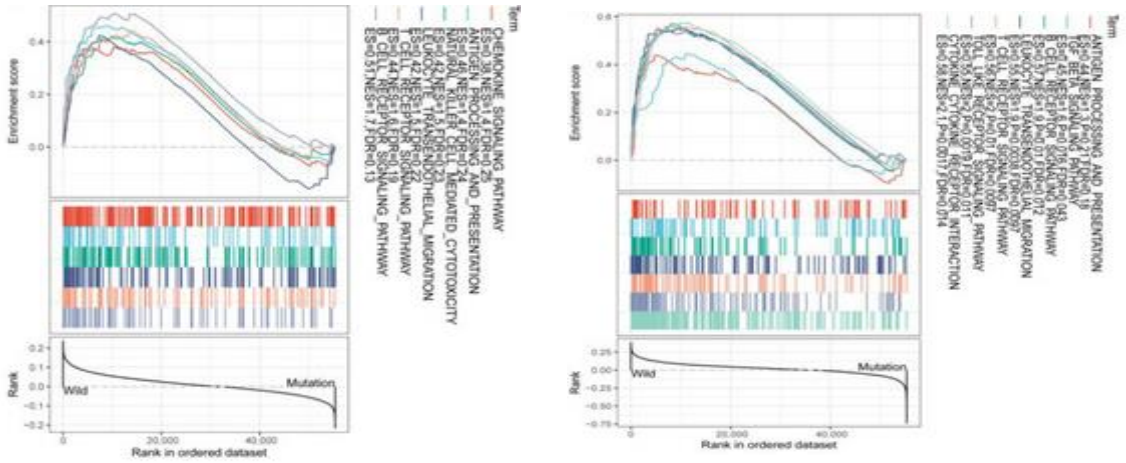


a) b)

Figure 3. Expression of immune-regulatory genes and status of immune cell infiltration. (a) Expression of immune checkpoint genes (upper); status of immune cell infiltration induced by mutation in KEAP1 (lower); (b) expression of tumor immune microenvironment genes (upper); status of immune cell infiltration induced by mutation in FAT1 (lower).

Figure 3 depicts both the expression patterns of immune-related genes and the landscape of immune cell infiltration in NSCLC tumors. Panel A highlights immune checkpoint gene expression alongside the immune cell composition in tumors with KEAP1 mutations, while panel B illustrates the expression of tumor microenvironment–related genes and the corresponding immune infiltration in FAT1-mutant tumors.

To determine how KEAP1 and FAT1 alterations affect the immune environment, we analyzed 1,026 NSCLC samples from TCGA with paired RNA-seq and DNA-seq data. Using gene set enrichment analysis, we compared tumors carrying these mutations to their wild-type counterparts. FAT1-mutant tumors showed widespread downregulation of pathways involved in chemokine signaling, antigen presentation, natural killer cell cytotoxicity, leukocyte migration, and T- and B-cell receptor signaling **(Figure 4a)**. KEAP1-mutant tumors exhibited similar disturbances, with reduced activity in antigen presentation, TGF-β signaling, leukocyte migration, T- and B-cell receptor pathways, toll-like receptor signaling, and cytokine–receptor interactions **(Figure 4b)**. These findings indicate that mutations in KEAP1 or FAT1 are linked to broad suppression of immune-related pathways, which may contribute to impaired antitumor immune responses in affected NSCLC cases.



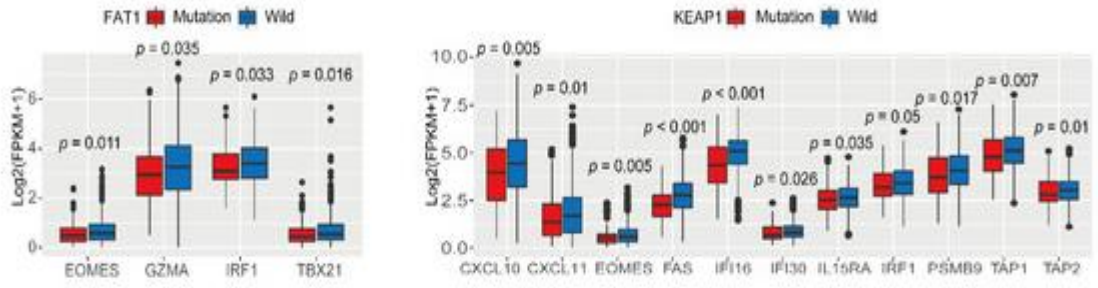
a) b)

Figure 4. Gene Set Enrichment Analysis (GSEA). (**a**) GSEA between the wild-type and FAT1 mutation groups. (**b**) GSEA between the wild-type and KEAP1 mutation groups.

Examination of NSCLC tumors carrying FAT1 mutations revealed a broad suppression of genes involved in immune regulation. Compared with tumors without this mutation, FAT1-mutant cases exhibited reduced activity in multiple pathways, including checkpoint regulation (for example, CTLA-4), signaling components of the tumor microenvironment such as IL6, molecules associated with T-cell effector functions and interferon-γ responses (including EOMES, GZMA, GZMB, IRF1, and TBX21), and numerous genes related to T-cell receptor signaling (CD3G, CD3D, CD4, CD8A, CD74, GRAP2, IKZF3, LCK, and TIGIT).

Similarly, KEAP1-mutant tumors showed decreased expression across a range of immune-related genes. This encompassed checkpoint regulators such as CD274, CTLA-4, HAVCR2, PDCD1LG2, and VTCN1, tumor microenvironment mediators including IL1B, IL12A, NT5E, PTGS2, and TNF, effector T-cell and IFN-γ–associated genes such as CXCL9, CXCL10, CXCL11, GZMA, GZMB, IFNG, IRF1, PSMB9, and TAP1, and several T-cell receptor–linked genes including CCL5, CD3G, CD3D, CD4, CD74, GRAP2, IKZF3, IL2RB, LCK, and TIGIT.

Taken together, these results suggest that mutations in FAT1 and KEAP1 coincide with extensive downregulation of immune signaling and effector pathways, which may hinder effective antitumor immune responses and contribute to reduced efficacy of immune checkpoint blockade therapies.



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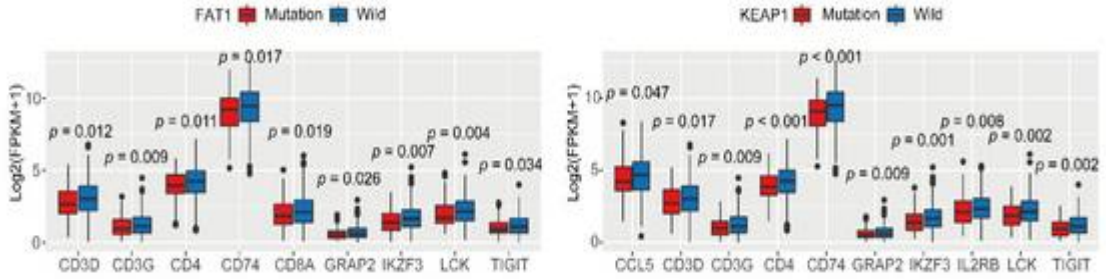


b)

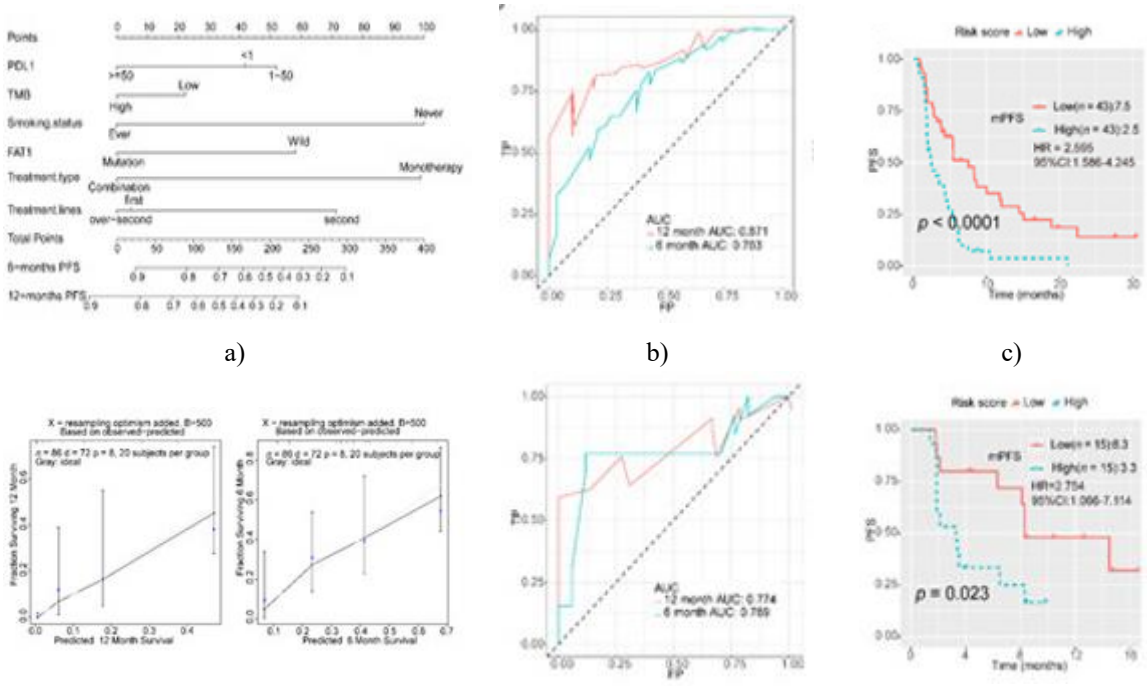
Figure 5. Expression of immune-regulatory genes. (**a**) T-cell effector and IFN-γ-associated gene signatures; (**b**) T-cell receptor (TCR)-related genes.

Prognostic modeling combining FAT1 mutation and clinical factors for ICB-treated NSCLC

To develop a predictive model for outcomes following immune checkpoint blockade, six variables were incorporated based on univariate Cox regression analysis: PD-L1 expression, tumor mutational burden, smoking history, treatment regimen, treatment type, and FAT1 mutation status **(Figure 6a)**. The performance of the resulting prognostic model was evaluated using receiver operating characteristic analysis, which demonstrated strong predictive capability, with area under the curve values of 0.763 for six-month survival and 0.871 for twelve-month survival **(Figure 6b)**.

Internal validation of the model was performed using a calibration curve for the nomogram, confirming its reliability **(Figure 6d)**. Survival analysis indicated that patients classified as low-risk exhibited significantly longer progression-free survival compared with high-risk patients (2.5 months vs. 7.5 months; $p < 0.001$; hazard ratio 2.595; 95% confidence interval 1.586–4.245; **(Figure 6c)**).

External validation using the Naiyer cohort further supported the robustness of the model. The ROC analysis in this independent dataset showed consistent predictive performance, with six- and twelve-month AUC values of 0.769 and 0.774, respectively **(Figure 6e)**. Progression-free survival was also significantly improved in the low-risk group compared with the high-risk group (3.3 months vs. 8.3 months; p = 0.023; hazard ratio 2.754; 95% confidence interval 1.066–7.114; **(Figure 6f)**).



a) b) c)

d) e) f)

Figure 6. Development of an integrated prognostic model. (a) Nomogram incorporating PD-L1 expression, tumor mutation burden, smoking history, treatment regimen, treatment type, and FAT1 mutation status in the Rizvi cohort. (b) ROC curves assessing the nomogram's ability to predict progression-free survival (PFS) in the Rizvi cohort. (c) Kaplan–Meier curves for PFS in the Rizvi cohort based on risk scores derived from the nomogram. (d) Calibration plots for 6- and 12-month PFS predictions in the Rizvi cohort. (e) ROC curves evaluating the model in the Naiyer cohort. (f) Kaplan–Meier curves for PFS in the Naiyer cohort stratified by risk score.

In this study, we investigated mutations in NSCLC patients exhibiting no durable benefit (NDB) from immune checkpoint blockade (ICB) using multiple cohorts from public databases. Our analyses indicated that FAT1 mutations may serve as a predictive biomarker for ICB efficacy, whereas KEAP1 mutations act as prognostic indicators but are not predictive of immunotherapy response. Previous studies have suggested that factors such as tumor mutational burden (TMB), PD-L1 expression, neoantigen load, CD8+ T-cell infiltration, and immune-regulatory mRNA expression can influence the effectiveness of immunotherapy [21]. Therefore, to elucidate the mechanisms underlying poor ICB response in NDB NSCLC patients, we systematically assessed these immunophenotypic indicators in patients harboring KEAP1 or FAT1 mutations. We observed that KEAP1-mutant tumors displayed lower PD-L1 expression relative to wild-type tumors. Tumors with KEAP1 or FAT1 mutations generally exhibited higher TMB and neoantigen loads but lower expression of immune-related genes and reduced CD8+ T-cell infiltration compared to wild-type tumors. This deficiency in immune cell infiltration and diminished immune gene expression may contribute to the poor therapeutic response to ICB, highlighting the need for caution in treating NSCLC patients with these mutations.

In contrast to prior reports, such as H. Rizvi *et al*. [13], which associated STK11 mutations with NDB and suggested that low TMB contributes to poor outcomes, our analysis of frequently mutated genes (mutation frequency >5%) found no significant relationship between STK11 mutations and prognosis in NDB NSCLC patients receiving ICB. Instead, KEAP1 and FAT1 mutations were significantly linked to worse outcomes, suggesting their role in primary resistance to ICB. Further analysis revealed that KEAP1 mutations—but not FAT1—also correlated with poorer prognosis in NSCLC patients who did not receive immunotherapy, consistent

with previous studies indicating that KEAP1 (and STK11) are prognostic rather than predictive biomarkers [16, 22, 23]. Notably, a prior study suggested FAT1-mutated NSCLC may exhibit improved response to anti-PD-L1 therapy [24], which contrasts with our findings; this discrepancy likely arises from differences in patient populations, as our study focused specifically on NDB patients, whereas earlier studies included all NSCLC patients receiving ICB. Additionally, FAT1 deletion has been linked to enhanced epithelial-mesenchymal transition, tumor stemness, and metastatic potential in lung squamous cell carcinoma, potentially explaining the reduced efficacy of ICB in FAT1-mutant NDB cases [25].

Using Cox regression analyses, we confirmed FAT1 as a predictive biomarker for ICB response. Subsequently, we integrated FAT1 mutation status with PD-L1 expression, TMB, smoking history, treatment regimen, and treatment type to develop a multivariate prognostic model for ICB efficacy, which was validated in an independent cohort. A nomogram based on this model demonstrated high predictive accuracy, with an AUC of 0.871 for 12-month progression-free survival (PFS). Risk scores derived from this model effectively stratified patients into low- and high-risk groups, with the low-risk group showing significantly longer PFS. This nomogram may assist clinicians in anticipating ICB treatment outcomes in NSCLC.

However, this study has several limitations. First, as a retrospective analysis of public databases, some clinical information—including performance status, prior therapies, and post-progression treatments—was unavailable, potentially introducing bias. Second, the cohort sizes were relatively small. Finally, our analysis was limited to high-frequency mutations (>5%) assessed via targeted NGS panels rather than whole-exome sequencing, leaving out potentially relevant low-frequency variants.

Conclusion

Our findings suggest that FAT1 mutations may serve as predictive biomarkers for NSCLC patients who fail to achieve durable benefit from ICB therapy. The FAT1-based prognostic model developed in this study could aid in identifying patients most likely to respond to ICB, supporting a more personalized approach to immunotherapy.

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Conflict of Interest: None

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Ethics Statement: None

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