Article's History: Received: 30.04.2025; Revised: 31.07.2025; Accepted: 02.09.2025

Bulletin of Medical and Biological Research

Vol. 7, No. 3
2025

DOI: 10.63341/bmbr/3.2025.26

Journal homepage: https://bmbr.com.ua/en

UDC 616.24-006.6

Molecular and immune predictors of survival in lung squamous cell carcinoma: A TCGA-based analysis

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Abstract. Lung squamous cell carcinoma remains one of the most aggressive forms of non-small cell lung cancer with limited options for personalised therapy, highlighting the need for new prognostic biomarkers. The purpose of this study was to analyse the impact of somatic mutations and their co-mutations in key oncogenes (TP53, TTN, PIK3CA, and KEAP1/NFE2L2) on the survival of patients with lung squamous cell carcinoma, considering the tumour immune subtype. Data from open-access oncology repositories (TCGA, UCSC Xena, cBioPortal) were used for 419 patients with complete clinical, immune, and mutational profiles. Patients were stratified by tumour immune activity (high/intermediate vs low subtype), and the mutational analysis included both individual genes and co-mutations. Survival was assessed using the Kaplan-Meier method with log-rank testing. It was found that TP53 mutations were significantly associated with improved survival in both the high/intermediate immune subtype group (median 57.9 vs 27.8 months, $p = 0.0141$) and the low immune subtype group ($p = 0.0361$). TTN mutations showed a positive trend in the high/intermediate group ($p = 0.0582$) and a statistically significant association with survival in the low immune activity group ($p = 0.0123$). The strongest effect was observed for the TP53+TTN co-mutation, which significantly improved survival in both immune subtypes (high/intermediate: $p = 0.0065$; low: $p = 0.0006$). In contrast, PIK3CA and KEAP1/NFE2L2 mutations and their combinations did not show a statistically significant impact on survival. Cluster analysis of the mutational profile revealed two primary patterns, with TP53 and TTN mutations tending to cluster, though no clear visual association with survival status was observed. Thus, the mutational status of TP53 and TTN, particularly their co-mutation, has substantial prognostic value in patients with lung squamous cell carcinoma, especially when considered alongside immune microenvironment characteristics. Isolated PIK3CA and KEAP1/NFE2L2 mutations showed no significant effect. These results emphasised the importance of integrating mutational and immune profiling to guide personalised treatment strategies

Keywords: TP53; TTN; PIK3CA; KEAP1/NFE2L2; lung cancer; co-mutations

INTRODUCTION

Lung squamous cell carcinoma (LUSC) is an aggressive histological subtype of non-small cell lung cancer, associated with high mortality rates and limited options for targeted treatment. Although immune checkpoint inhibitors have significantly transformed the therapeutic landscape, clinical outcomes in LUSC remain highly variable. This variability is partly explained by the complex interplay between the tumour's molecular background and its immune microenvironment. Consequently, identifying robust prognostic biomarkers is essential for improving patient stratification and developing personalised therapeutic approaches, especially in the context of immunotherapy resistance.

Increasing scientific attention has been devoted to investigating the role of somatic mutations in shaping the

Suggested Citation:
Moskalenko Yu, Kovchun V. Molecular and immune predictors of survival in lung squamous cell carcinoma: A TCGA-based analysis. Bull Med Biol Res. 2025;7(3):26–36. DOI: 10.63341/bmbr/3.2025.26

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tumour microenvironment and determining survival outcomes in patients with LUSC. J. Zhao *et al.* [1] described the immune infiltration landscape in LUSC and emphasised the impact of key oncogenic mutations on either enhancing or suppressing anti-tumour immune responses. Their findings underscored the close relationship between genetic alterations and the immunological behaviour of tumours. In a comparative mutational analysis conducted by C. Pop-Bica *et al.* [2], TP53 (tumour protein p53) and TTN (titin) mutations were identified as dominant events in LUSC, suggesting their relevance as molecular drivers. These mutations were among the most frequent and may play a central role in the initiation and progression of the disease. Y. Xu *et al.* [3] examined the prognostic significance of genomic variation, tumour mutational burden (TMB), and programmed death-ligand 1 (PD-L1) expression in LUSC patients treated with immunotherapy. Their study showed a positive association between tumour protein p53 (TP53) mutations and favourable treatment response. P.K. Paik *et al.* [4] explored Kelch-like ECH-associated protein 1/nuclear factor erythroid 2-related factor 2 (KEAP1/NFE2L2) mutations as potential therapeutic targets. Their trial involving the target of rapamycin complex 1/2 (TORC1/2) inhibitor TAK-228 revealed only limited clinical activity, but highlighted the need for combination treatment strategies in these molecular subsets. Meanwhile, D. Wang *et al.* [5] developed a prognostic signature based on TMB, which correlated with survival and immunotherapy efficacy in LUSC, particularly in patients with TTN mutations.

X. Deng *et al.* [6] investigated pyroptosis-related genes and demonstrated their influence on the immune composition of the tumour microenvironment. Their study provided evidence that specific genomic alterations can shape distinct immune phenotypes in LUSC. J.A. Hellyer *et al.* [7] similarly confirmed that KEAP1/NFE2L2 mutations are associated with "immunologically silent" microenvironments and reduced response to PD-1 blockade. These data highlighted the immunosuppressive potential of NRF2-driven signalling pathways. C. Su *et al.* [8] identified circulating TTN mutations in blood as potential markers of immunotherapy response. The researchers showed that TTN mutations increase tumour antigenicity and promote immunogenic microenvironments, thereby enhancing the likelihood of immunotherapeutic benefit. Overall, findings from international studies emphasise the prognostic importance of TP53, TTN, KEAP1/NFE2L2, and PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) mutations in LUSC and their influence on treatment response. However, most available data rely either on mutational analysis alone or on isolated assessments of immune phenotype, without integrating both dimensions. This limits the clinical applicability of current evidence in real-world settings.

Despite the limited number of studies on this topic in Ukraine, a research group has already been formed in the scientific community that is actively working in this area. The Ukrainian cohort study by D. Kozakov *et al.* [9] investigated the relationship between genetic alterations and the immune contexture of NSCLC, laying the groundwork for exploring the link between genomic profiles and immune activation in lung cancer. Their findings provided an important national perspective and contribute to the global understanding of tumour-immune interactions. Additionally, in a related review by O. Sulaieva *et al.* [10], the role of molecular biomarkers in guiding personalised management of NSCLC was discussed, emphasising the relevance of integrated molecular and immune profiling. These studies mark an important step toward establishing a national framework for translational research in lung cancer.

The purpose of this study was to analyse the association between somatic mutations and co-mutations in TP53, TTN, PIK3CA, and KEAP1/NFE2L2 and overall survival in patients with lung squamous cell carcinoma, taking into account the tumour's immune subtype.

MATERIALS AND METHODS

Patient selection criteria and procedure. This retrospective cohort study was conducted between January and March 2025 at the Educational and Scientific Medical Institute of Sumy State University (Sumy, Ukraine). The analysis was based on de-identified data obtained from publicly accessible oncological repositories: The Cancer Genome Atlas (TCGA) [11], the UCSC Xena Browser [12], and cBioPortal [13]. These platforms provided comprehensive clinical, immunological, and genomic information on patients with various malignancies, including LUSC. The initial dataset comprised of 504 patients diagnosed with histologically confirmed LUSC and enrolled in the TCGA-LUSC project. To ensure the completeness and reliability of the data, specific inclusion and exclusion criteria were applied. Eligible patients were aged 18 years or older, had complete clinical information (including age, sex, tumour stage, overall survival time, and status), a defined tumour immune subtype according to the Pan-Cancer immune classification by V. Thorsson *et al.* [14], and sequencing data for four key genes: TP53, TTN, PIK3CA, and KEAP1/NFE2L2. Patients were excluded if they lacked valid entries for age, survival status, or follow-up time (n = 44), if immune subtype data were unavailable (n = 33), or if mutation data were missing (n = 8). The final cohort included 419 patients who fulfilled all eligibility criteria and had a complete set of clinical, immunological, and genomic variables.

The mean age of the study cohort was 67.0 ± 8.53 years (range: 39-84), and 74.7% of patients were male. Most patients were diagnosed at early stages: 43.2% were stage 1 and 29.6% were stage 2. Distribution by immune subtype revealed that 41.3% of patients (n = 173) belonged to the high or intermediate immune subtype group (C2, C3, C6), while 58.7% (n = 246) were classified as low immune subtype (C1, C4). Regarding mutational status, TP53 mutations were detected in 90.0% of patients (n = 377), TTN mutations in 85.0% (n = 356), and PIK3CA mutations in 13.4% (n = 56). KEAP1 and NFE2L2 mutations were combined into a single variable due to their functional overlap and mutual exclusivity, and were present in 27.9% of cases (n = 117). The most common co-mutation was TTN+TP53, observed in 76.1% of patients (n = 319), while TP53 + PIK3CA was found in 11.5% (n = 48). These frequencies did not significantly differ between immune subtypes, except for KEAP1/NFE2L2 mutations, which were more prevalent in the low immune subtype group (p = 0.012).

All datasets were fully anonymised and complied with ethical standards for secondary data analysis. Thus, individual informed consent was not required. Nevertheless, the study adhered to the principles of the Declaration of Helsinki [15], ensuring the protection of human dignity,

privacy, and data integrity. The research protocol was reviewed and approved by the Bioethics Committee for Experimental and Clinical Studies at the Educational and Scientific Medical Institute of Sumy State University (protocol No. 3/12, approved on December 17, 2024). Limitations of the study included its retrospective design and reliance on public datasets, which may contain incomplete or heterogeneous information. The lack of treatment data also limited interpretability, particularly regarding immunotherapy effects. Additionally, the small number of cases with high immune activity reduced the statistical power of subgroup comparisons. External validation using independent cohorts with detailed clinical data was necessary to confirm these results.

Methods of statistical data analysis. All statistical analyses were performed using Stata software suite, version 19.5 (StataCorp LLC, College Station, TX, USA). Prior to analysis, all datasets were examined for completeness, inconsistencies, and outliers. Continuous variables were assessed for normality using visual inspection of histograms and the Shapiro-Wilk test. Given the non-parametric distribution of age, results are presented as median and interquartile range (IQR), and comparisons between age groups were conducted using the Mann-Whitney U-test. Categorical variables, including sex, tumour stage, mutational status, and immune subtype, were expressed as absolute counts and percentages. Their distribution across groups was compared using Pearson's chi-square test (χ^2), with p-values <0.05 considered statistically significant.

Overall survival was the primary endpoint and was defined as the time from diagnosis to death or last follow-up. Survival curves were estimated using the Kaplan-Meier method, and differences between groups were evaluated using the log-rank test. Separate survival analyses were performed for each gene (TP53, TTN, PIK3CA, and KEAP1/NFE2L2) and for co-mutations (TP53+TTN and TP53 + PIK-3CA) within both high/intermediate and low immune subtypes to explore potential interaction effects between mutational status and immune phenotype. To visualise mutational patterns and potential patient clusters, a binary matrix was created to reflect the presence or absence of mutations in each gene. This matrix was used to generate a clustered heatmap using Ward's method for hierarchical clustering. Patients were colour-coded by survival status to explore possible associations between mutation clusters and outcomes. Although this was an exploratory, descriptive component, it provided additional insights into the molecular heterogeneity of LUSC. No multiple comparison correction was applied, given the hypothesis-driven nature of the analysis. Nonetheless, all interpretations were contextualised with caution.

RESULTS AND DISCUSSION

Comparative analysis revealed no statistically significant differences in the frequencies of TP53 (p = 0.910), TTN (p = 0.779), or PIK3CA (p = 0.584) mutations between high/medium and low immune subtypes. However, KEAP1/NFE2L2 mutations were significantly more frequent in the low immune subtype group (21.4% vs 32.5%, p = 0.012), suggesting a potential link with an immunosuppressive tumour environment. Co-mutations of TP53 + TTN and TP53+PIK3CA were similarly distributed across subtypes (p = 0.594 and p = 0.713, respectively), indicating that their occurrence is independent of immune phenotype. These findings highlighted that among all examined mutations, only KEAP1/NFE2L2 status may partially correlate with immunological features of the tumour (Table 1).

Table 1. Associations between clinical, molecular-genetic features and immune subtypes in patients with lung squamous cell carcinoma

Variables	Total, n = 419	High / medium immune subtype, n = 173	Low immune subtype, n = 246	p-value
Median age (years, range)	67.0±8.53 (39-84)	66.3±8.68 (40-84)	67.4±8.40 (39-84)	0.2497
Sex:				
Female	106 (25.3)	49 (28.3)	57 (23.2)	0.232
Male	313 (74.7)	124 (71.7)	189 (76.8)	
Stage:				
I	181 (43.2)	74 (42.8)	107 (43.5)	0.977
II	124 (29.6)	53 (30.6)	71 (28.9)	
III	58 (13.8)	22 (12.7)	36 (14.6)	
IV	5 (1.2)	2 (1.2)	3 (1.2)	
Unknown	51 (12.2)	22 (12.7)	29 (11.8)	
Mutation TTN:				
Present	356 (85.0)	148 (85.5)	208 (84.6)	0.779
Absent	63 (15.0)	25 (14.5)	38 (15.4)	
Mutation TP53:				
Present	377 (90.0)	156 (90.2)	221 (89.8)	0.910
Absent	42 (10.0)	17 (9.8)	25 (10.2)	
Mutation PIK3CA:				
Present	56 (13.4)	25 (14.5)	31 (12.6)	0.584
Absent	363 (86.6)	148 (85.5)	215 (87.4)	
Mutation KEAP1/NFE2L2:				0.012
Present	117 (27.9)	37 (21.4)	80 (32.5)	
Absent	302 (72.1)	136 (78.6)	166 (67.5)	

Table 1. Continued

Variables	Total, n = 419	High / medium immune subtype, n = 173	Low immune subtype, n = 246	p-value
Co-mutation TTN+TP53:				0.594
Present	319 (76.1)	134 (77.5)	185 (75.2)	
Absent	100 (23.9)	39 (22.5)	61 (24.8)	
Co-mutation TP53+PIK3CA:				0.713
Present	48 (11.5)	21 (12.1)	27 (11.0)	
Absent	371 (88.5)	152 (87.9)	219 (89.0)	

Source: The Cancer Genome Atlas (TCGA) [11], UCSC Xena [12], and cBioPortal [13]

In summary, comparative analysis showed that the majority of gene mutations and co-mutations occurred independently of immune subtype classification. The only statistically significant difference was observed for KEAP1/NFE2L2 alterations, which were more common in tumours with low immune activity. These findings suggest a potential association between oxidative stress-related mutations and an immunosuppressive tumour microenvironment, warranting further investigation into their biological role in immune escape mechanisms. A comparison of patient survival based on immune subtypes revealed that the median overall survival was 57.1 months in the high/medium immune activity group, compared to 44.9 months in the low immune activity group. Despite a trend toward better survival in patients with higher immune activity, the difference between the groups was not statistically significant (Log-rank test: $\chi^2(1) = 1.14$, $p = 0.2854$). These results indicate that immune subtype did not have a significant impact on overall survival in this cohort of patients with lung squamous cell carcinoma (Fig. 1).

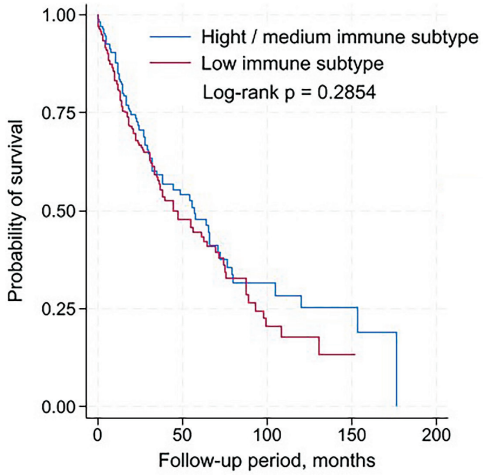

Figure 1. Kaplan-Meier survival curves for patients with lung squamous cell carcinoma according to tumour immune subtype
Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

Among patients with lung squamous cell carcinoma classified as having high/medium immune subtypes, stratification was performed based on the presence of mutations in TP53, TTN, PIK3CA, KEAP1/NFE2L2, and their combinations. Kaplan-Meier analysis with log-rank testing revealed no statistically significant differences in survival associated with PIK3CA mutations ($p = 0.2913$), KEAP1/NFE2L2 mutations ($p = 0.3796$), or the TP53+PIK3CA co-mutation ($p = 0.8296$). In contrast, patients harbouring TP53 mutations demonstrated significantly better overall survival compared to those without such mutations (median survival 57.9 vs 27.8 months, $p = 0.0141$). Similarly, TTN mutations were associated with a trend toward improved survival (median 57.9 vs 27.8 months), though this difference did not reach statistical significance ($p = 0.0582$). The most pronounced effect was observed for the TTN+TP53 co-mutation, which was significantly associated with better survival compared to patients lacking this co-mutation (median survival 63.7 vs 27.8 months, $p = 0.0065$) (Fig. 2).

Among patients with lung squamous cell carcinoma classified as having a low immune subtype, no statistically significant differences in survival were observed across groups with mutations in KEAP1/NFE2L2 ($p = 0.8091$), PIK3CA ($p = 0.5478$), or the TP53+PIK3CA co-mutation ($p = 0.7477$), indicating a lack of prognostic impact for these alterations in this cohort. In contrast, the presence of TP53 mutations was significantly associated with improved overall survival ($p = 0.0361$), as was the presence of TTN mutations ($p = 0.0123$). The strongest prognostic effect was observed in patients with co-mutations in TTN and TP53, who demonstrated the highest survival rates ($p = 0.0006$). Overall, these findings suggest that mutations in TP53 and

TTN – particularly in combination – may serve as favourable prognostic markers in patients with lung squamous cell carcinoma, in contrast to isolated alterations in PIK3CA or KEAP1/NFE2L2 (Fig. 3).

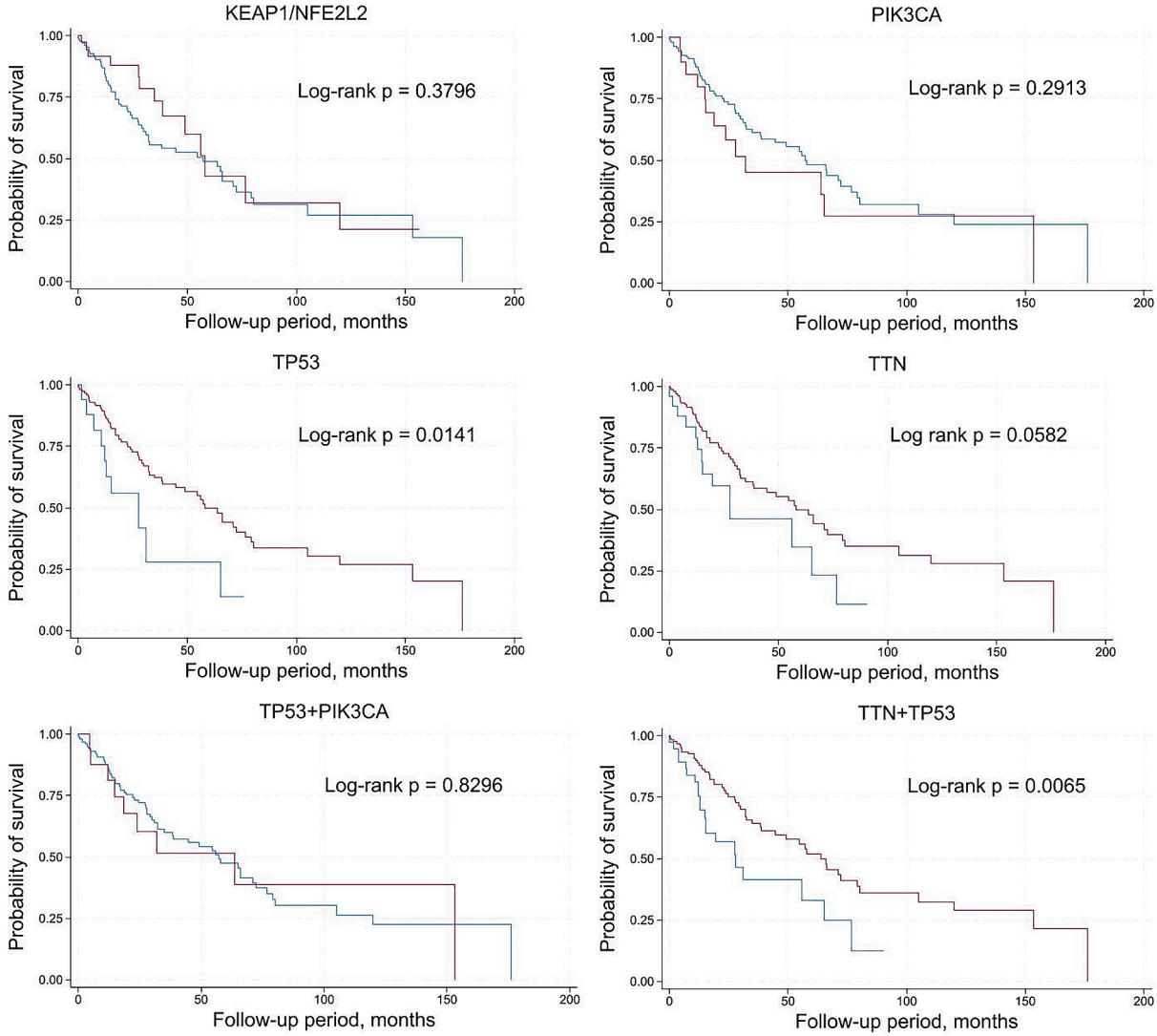

Figure 2. Kaplan-Meier survival curves according to mutation status in patients with lung squamous cell carcinoma and high/medium immune subtype

Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

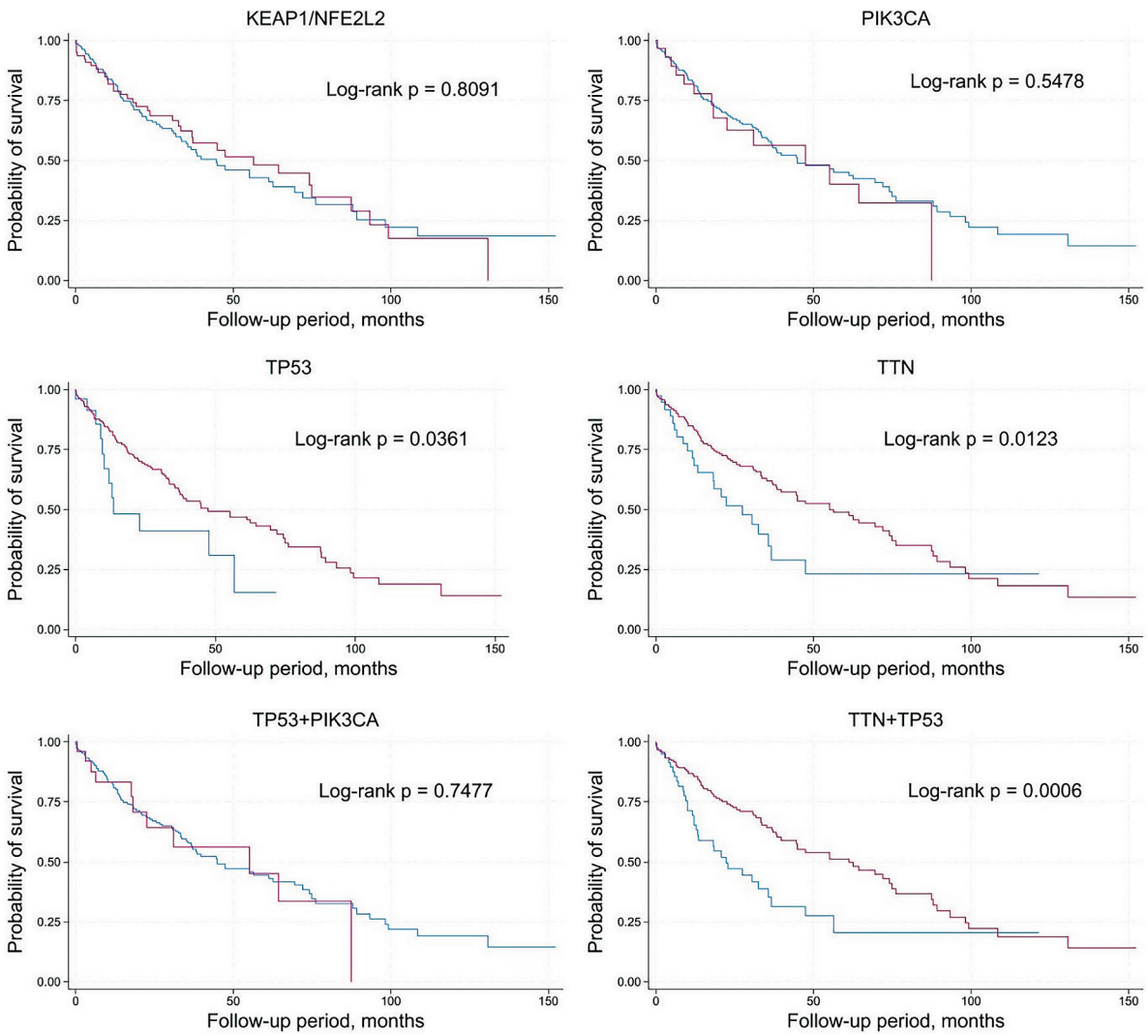

Figure 3. Kaplan-Meier survival curves according to mutation status in patients with lung squamous cell carcinoma and low immune subtype

Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

In conclusion, survival analysis revealed that individual mutations such as KEAP1/NFE2L2 and TTN, and specific co-mutation profiles, have prognostic relevance in LUSC. The effect of these mutations on overall survival was further modulated by immune subtype, emphasising the importance of integrating molecular and immunological features when assessing patient outcomes. To investigate the relationships between somatic mutations in key genes among patients with lung squamous cell carcinoma, a clustered heatmap was generated based on mutations in TTN, TP53, PIK3CA, and KEAP1/NFE2L2 (Fig. 4).

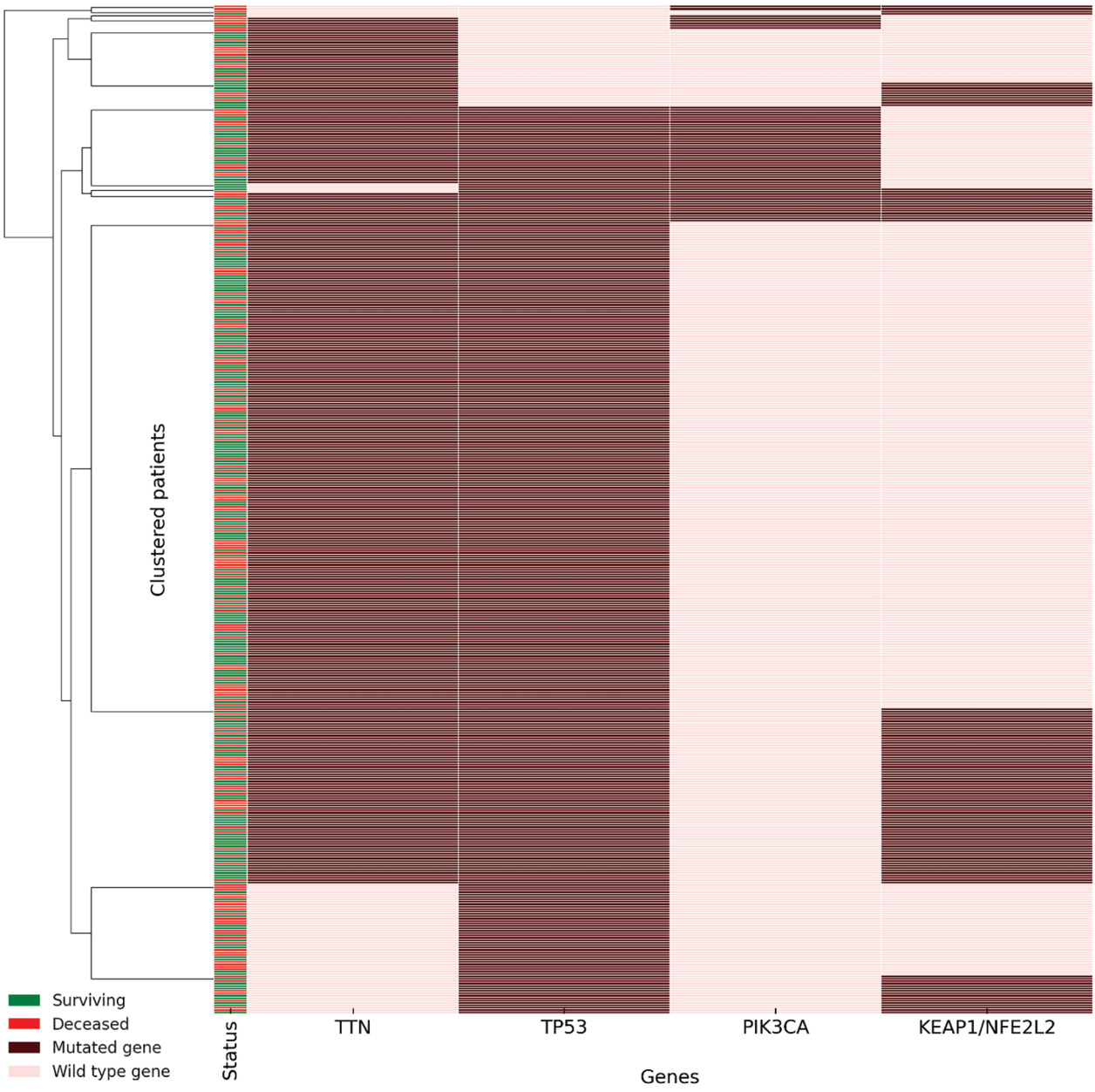

Figure 4. Clustered heatmap of mutations in patients with lung squamous cell carcinoma
Source: TCGA [11], UCSC Xena [12], and cBioPortal [13]

During the analysis, notable heterogeneity in the mutational landscape of LUSC was observed, allowing for the tentative identification of two major patient clusters based on shared mutational features. Mutations in TTN and TP53 were the most prevalent, occurring in over 76% of cases, and frequently co-occurred. This co-occurrence appears to define a substantial subgroup of patients with potentially common molecular drivers. In contrast, PIK3CA and KEAP1/NFE2L2 mutations showed a more scattered distribution, appearing independently across patients without consistent co-localisation. To explore possible prognostic implications, patients were colour-coded based on survival status (green for surviving, red for deceased). However, no distinct spatial clustering of deceased individuals was evident on the heatmap. Patients with poor outcomes were distributed throughout both clusters, suggesting that mutational status alone does not fully account for prognosis. The lack of visually dominant patterns linking mutational combinations to survival highlights the multifactorial nature of disease progression in LUSC. These findings emphasised the structural complexity and individual variability of mutational alterations in LUSC. While TTN and TP53 mutations may represent shared early events in tumorigenesis, mutations in KEAP1/NFE2L2 – which are known to affect oxidative stress pathways – may contribute to an immunosuppressive microenvironment. This underscores the necessity for integrated molecular-immunological approaches and further stratified analyses to better characterise prognostic subgroups and guide personalised treatment strategies.

The analysis of the mutational landscape in LUSC revealed intricate interactions between genetic alterations

and the tumour immune microenvironment. A prominent finding was the association between TP53 and TTN co-mutations and improved overall survival, particularly in patients with high or intermediate levels of immune infiltration. This observation was consistent with findings by K. Ying *et al.* [16], who demonstrated that TP53 + TTN co-mutations are predictive of enhanced immunotherapy responses in LUSC, likely due to increased tumour mutational burden and elevated neoantigen load. S. Zou *et al.* [17] further confirmed the prognostic value of TTN mutations, showing their association with prolonged survival and potential to stratify patients for immune checkpoint blockade. These findings underlined the relevance of TTN as a candidate biomarker for immunotherapy response prediction. X. Xie *et al.* [18] attributed this association to the ability of TTN mutations to drive immune activation, including the upregulation of cytotoxic T-cell markers and pro-inflammatory chemokines. Such immunogenic changes may explain the enhanced sensitivity of TTN-mutant tumours to immune checkpoint inhibition observed in clinical settings. Consistent with these studies, authors' analysis demonstrated that TTN mutations – particularly in combination with TP53 – were associated with significantly better overall survival, reinforcing their potential role in patient stratification. These immunostimulatory effects may contribute to reshaping the tumour microenvironment, thereby increasing the probability of durable response to immune checkpoint inhibitors in patients harbouring such alterations.

TP53, a canonical tumour suppressor gene, plays a central role in maintaining genomic integrity. Z. Fan *et al.* [19] reported that TP53-mutant tumours display distinct transcriptional programs associated with immune activation, including interferon signalling. These molecular features may explain why patients harbouring TP53 mutations tend to exhibit better immunotherapy outcomes. Moreover, J. Yu *et al*. [20] emphasised the importance of TP53 co-status with LRP1B, indicating that the absence of mutations in both genes correlates with favourable prognosis in LUSC patients treated with PD-L1 inhibitors. Collectively, these findings support the notion that the mutational background of tumours directly influences the efficacy of immunotherapies. These insights also imply that biomarker-driven patient stratification should account for not only single-gene alterations but also specific mutation combinations.

Importantly, the observed benefit of TP53 + TTN co-mutations extended, albeit to a lesser extent, to patients within the low immune subtype group. This suggests that even in immunologically "cold" tumours, the underlying genomic architecture may confer some degree of immunogenicity. L. Yang *et al.* [21] underscored the relevance of integrating immune cell infiltration profiles with molecular subtyping to better predict therapy responses, reinforcing the concept that both immune and genetic features must be jointly considered. A similar view was presented by J. Wang *et al*. [22], who proposed that specific autophagy-related gene signatures modulate immune infiltration and may underlie differences in survival among molecular subtypes of LUSC. Such findings collectively point to the necessity of combining mutational and immunologic parameters when developing predictive models for immunotherapy outcomes.

In contrast, mutations in the KEAP1/NFE2L2 pathway were predominantly observed in tumours with low immune activity. P.K. Paik *et al.* [4] demonstrated that alterations in this axis contribute to resistance against TORC1/2 inhibitors and promote tumour progression by suppressing oxidative stress responses. These findings highlighted the complexity of redox signalling in NSCLC and the challenges in targeting this pathway. J.A. Hellyer *et al.* [7] explored the clinical relevance of KEAP1-NFE2L2 mutations and linked them to immune silencing and reduced efficacy of immune checkpoint inhibitors. This further emphasised the immunosuppressive role of the KEAP1/NRF2 pathway. Supporting this, M. Sánchez-Ortega *et al.* [23] showed that wild-type NRF2/KEAP1 tumours exhibit higher oxidative stress susceptibility, suggesting a potential therapeutic window that is absent in mutant tumours. This finding underscored the therapeutic relevance of redox imbalance and highlighted the importance of molecular subtyping when designing targeted interventions. In the current cohort, the higher prevalence of these mutations among low immune subtype patients aligns with a model of immune evasion mediated by aberrant antioxidant regulation. These insights further confirm that KEAP1/NFE2L2 mutations not only affect tumour metabolism but also alter immune escape dynamics.

Mutations in PIK3CA, although not statistically significant in this analysis, have been the subject of extensive investigation. Q. Huang *et al.* [24] characterised the mutational landscape of PIK3CA in Chinese patients and reported substantial heterogeneity in mutation co-occurrence patterns. S. Cokpinar *et al.* [25] also highlighted the variable prognostic role of PIK3CA, emphasising that its clinical implications may depend on the presence of co-mutations such as TP53. In the present study, PIK3CA mutations exhibited a dispersed distribution across immune subtypes and were not linked to survival outcomes. However, their potential role as modifiers in polygenic contexts warrants further exploration. The interaction between PIK3CA and immune signalling pathways remains an open area of research, with implications for therapy resistance and immune modulation.

Cluster analysis of the mutational heatmap provided additional insights. Two main clusters of patients emerged, reflecting distinct mutation combinations and partially correlating with survival status. C. Su *et al*. [8] demonstrated that circulating TTN mutations can serve as dynamic markers for predicting responses to immunotherapy in advanced NSCLC. Similarly, D. Kozakov *et al.* [9] examined Ukrainian patients with NSCLC and concluded that specific mutation patterns shape the immune milieu of the tumour, impacting clinical trajectories. These findings reinforce the concept that the mutational composition of tumours shapes immune interactions and may serve as a basis for patient stratification. Moreover, they support the incorporation of genomic clustering analyses in the interpretation of immune-related phenotypes.

From a broader perspective, the interplay between immune contexture and genomic alterations in LUSC reflects a multifactorial and dynamic landscape. In their seminal study, V. Thorsson *et al.* [14] proposed six pan-cancer immune subtypes based on transcriptomic features, highlighting differences in leukocyte infiltration, antigen

presentation, and expression of immune checkpoints. The current results were congruent with this framework, particularly the observed heterogeneity among tumours with similar mutational loads but divergent immune infiltration patterns. Integrating such immune classification systems into genomic profiling may enhance the precision of personalised therapies. This approach may be especially relevant in tumours that lack conventional biomarkers but exhibit atypical immune behaviour [26].

Several additional studies included in the reference list provide complementary perspectives. For instance, X. Deng *et al.* [6] utilised pyroptosis-related genes to model immunotherapy efficacy, demonstrating that cell death pathways intersect with immune signalling in LUSC. Their model highlighted the relevance of immune cell infiltration patterns in shaping treatment response. D. Wang *et al.* [5] employed a multi-omics approach to build a prognostic signature incorporating TMB and gene expression, further supporting the integration of multiple data layers. This approach underscored the importance of combining genomic and transcriptomic variables to improve prognostic accuracy. W. Tao *et al.* [27] reviewed the predictive value of omics-derived markers in immunotherapy, advocating for combinatorial models over single biomarkers. They emphasised that such models offer greater robustness in real-world clinical settings. The researchers' conclusions were consistent with this integrative perspective, as it has been demonstrated that combined consideration of mutation and immune profiles improves the identification of prognostically significant subgroups in LUSC. Together, these studies reinforce the current findings and advocate for broader, systems-level approaches to immuno-oncology in LUSC.

In terms of clinical translation, these insights suggest that patient selection for immunotherapy in LUSC should move beyond PD-L1 status and encompass genomic features such as TTN and TP53 mutations, KEAP1/NFE2L2 status, and multi-parameter immune profiling. C. Pop-Bica *et al.* [2] noted the importance of comprehensive genomic analysis via NGS in both NSCLC and SCLC, stressing that divergent mutation profiles require tailored approaches. Their findings also highlighted the potential of genomic profiling to inform histology-specific therapeutic decisions. Y. Xu *et al.* [3] and A. Burlaka [28] provided evidence that PD-L1 expression alone does not capture the complexity of immune responsiveness, reinforcing the need for integrative biomarkers. Moreover, their study showed that a combination of high TMB and TP53 mutation was associated with improved clinical outcomes in patients receiving immunotherapy. These observations were consistent with authors' findings, which indicated that TP53 co-mutations – particularly with TTN – may enhance survival specifically in immune-inflamed tumour phenotypes. Overall, expanding the biomarker panel beyond PD-L1 may improve therapeutic outcomes by identifying subgroups most likely to benefit from immunotherapy.

Despite considerable progress in elucidating the genomic and immunological features of LUSC, few studies have integrated these parameters in a comprehensive survival-based analysis. Unlike previous research, which primarily focused either on mutational frequency or immune classification, the current study examined the prognostic impact of both individual and concomitant mutations in relation to immune tumour subtypes. This integrated approach offers a broader understanding of how specific genetic alterations interact with the immune contexture to influence patient outcomes. By stratifying survival analyses based on immune subtype, the research provided new insights into the heterogeneity of LUSC and highlighted potential avenues for improving risk stratification and developing personalised therapy.

CONCLUSIONS

In this study, a comprehensive analysis of the mutational profile, immune subtypes, and overall survival was conducted in 419 patients with LUSC using data from TCGA. Particular attention was given to four frequently mutated genes – TP53, TTN, PIK3CA, and KEAP1/NFE2L2 – which have been implicated in oncogenesis and immune regulation. Mutations in TP53 and TTN, both individually and in combination, demonstrated significant prognostic impact, especially among patients with high or intermediate immune activity in the tumour microenvironment. The highest overall survival was observed in patients with TP53+TTN co-mutations, with a median survival of 63.7 months compared to 27.8 months in patients without such alterations ($p = 0.0065$). A similar trend was observed in the subgroup with low immune activity, where patients harbouring TP53+TTN co-mutations also demonstrated the most favourable survival outcomes ($p = 0.0006$). In contrast, individual mutations in PIK3CA and KEAP1/NFE2L2 showed no statistically significant association with prognosis, suggesting limited prognostic relevance when considered in isolation from the broader genomic context. KEAP1/NFE2L2 was the only mutation significantly associated with immune subtype. It was more frequently observed in tumours with low immune activity (32.5% vs 21.4%, $p = 0.012$), indicating a potential role in shaping an immunosuppressive tumour microenvironment through dysregulation of oxidative stress pathways. Cluster analysis further confirmed the high frequency of TP53 and TTN co-mutations but did not reveal any distinct visual patterns related to survival outcomes. These findings highlighted the molecular heterogeneity of LUSC and underscore the necessity for a multifactorial approach to risk stratification and the development of integrated, personalised therapeutic strategies based on both genomic and immunological characteristics, which may be considered in the future.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Молекулярні та імунні предиктори виживаності при плоскоклітинній карциномі легень: аналіз даних TCGA

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Анотація. Плоскоклітинна карцинома легень залишається однією з найбільш агресивних форм недрібноклітинного раку з обмеженими можливостями для персоналізованого лікування, що зумовлює потребу у нових прогностичних біомаркерах. Мета дослідження – проаналізувати вплив соматичних мутацій та їх ко-мутацій у ключових онкогенах (TP53, TTN, PIK3CA та KEAP1/NFE2L2) на виживаність хворих на плоскоклітинну карциному легень з урахуванням імунного підтипу пухлини. Було використано дані відкритих онкологічних репозиторіїв (TCGA, UCSC Xena, cBioPortal) для 419 пацієнтів з повним клінічним, імунним та мутаційним профілем. Пацієнтів стратифікували за рівнем імунної активності пухлини (високий/середній або низький підтип), а аналіз мутацій охоплював окремі гени та ко-мутації. Виживаність оцінювали за методом Каплана-Майєра з лог-ранговим тестом. Було встановлено, що мутація TP53 асоціюється з достовірно кращою виживаністю як у групі з високою/середньою імунною активністю (медіана 57,9 проти 27,8 міс., p = 0,0141), так і в групі з низькою імунною активністю (p = 0,0361). Мутація TTN продемонструвала позитивну тенденцію у групі з високою/середньою активністю (p = 0,0582) та достовірний зв'язок з виживаністю у групі з низькою активністю (p = 0,0123). Найсильніший ефект виявлено для ко-мутації TP53 + TTN, яка значуще покращувала виживаність у пацієнтів з обома імунними підтипами (високий/середній: p = 0,0065; низький: p = 0,0006). Водночас мутації PIK3CA, KEAP1/NFE2L2 та їх комбінації не продемонстрували статистично значущого впливу на виживаність. Кластерний аналіз мутаційного профілю виявив два основні патерни, де TP53 і TTN мутації мали тенденцію до кластеризації. Водночас не було виявлено чіткої візуальної асоціації між мутаційними підтипами та статусом виживаності. Таким чином, мутаційний статус генів TP53 і TTN, а також їх ко-мутація TP53+TTN мають суттєве прогностичне значення у пацієнтів з плоскоклітинною карциномою легень, особливо у поєднанні з характеристиками імунного мікрооточення. Натомість ізольовані мутації в PIK3CA та KEAP1/NFE2L2 не мали достовірного впливу на виживаність. Отримані результати підкреслили важливість інтеграції мутаційного та імунного профілю для формування персоналізованих стратегій лікування

Ключові слова: TP53; TTN; PIK3CA; KEAP1/NFE2L2; рак легень; ко-мутації