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Prognostic effect of coexisting TP53 and ZFHX3 mutations in non-small cell lung cancer patients treated with immune checkpoint inhibitors

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Abstract

In recent years, immune checkpoint inhibitor (ICI) therapy has revolutionized the treatment of patients with advanced-stage non-small cell lung cancer (NSCLC). The relationship between TP53 mutation and prognosis of non-small cell lung cancer (NSCLC) remains controversial. We aimed to identify advanced-stage NSCLC patients harboring TP53 mutation who would benefit from ICI treatment. Gene mutations and tumor mutational burden (TMB) data of NSCLC patients who received at least one dose of ICI therapy at the Memorial Sloan Kettering Cancer Center between 2013 and 2017 were extracted from the cBioPortal online platform. Gene clustering analyses were performed for patients with short and long overall survival (OS). The top ten significantly different mutated genes were identified. Furthermore, we analyzed the different OS of coexisting TP53 and other significantly different mutated genes to identify NSCLC patients with TP53 mutations who would benefit from immunotherapy. A total of 350 patients were enrolled in the study. Of these a total of 219 (62.6%) patients were found to harbor TP53 mutations, whereas 131 (37.4%) had wild-type TP53. There was no statistically significant difference in OS between TP53 mutated or wild-type NSCLC patients who underwent ICI treatment. However, coexisting TP53 and ZFHX3 mutations were independent prognostic factors. Higher somatic TMB (highest 20% in each histology) and combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapy were also associated with longer OS in multivariate analysis. Coexisting TP53 and ZFHX3 mutations are independent prognostic factors for advanced-stage NSCLC patients undergoing ICI treatment. These findings could help identify patients harboring TP53 mutations that would benefit from ICI treatment.

1 | INTRODUCTION

Lung cancer is one of the most common aggressive malignancies and a leading cause of cancer-related mortality worldwide.[1] In recent years, immune checkpoint inhibitor (ICI) therapy has revolutionized the treatment of patients with advanced-stage cancers, notably in stage III B or IV non-small cell lung cancer (NSCLC). Programmed cell death-1 (PD-1) inhibitors, such as pembrolizumab or nivolumab, and programmed cell death ligand-1 (PD-L1) inhibitors, such as

atezolizumab and durvalumab, have significantly improved the overall survival (OS) of patients with advanced-stage NSCLC compared to standard platinum-based chemotherapy.[2-5] However, only approximately 17% of these patients truly benefit from these drugs as monotherapy.[6] Several immune-related biomarkers, such as intratumoural PD-L1 expression,[7] tumour mutational burden (TMB),[8] DNA mismatch repair (MMR) deficiency[9] and intensity of $CD8^+$ T cell infiltrates,[10] are used to predict ICI efficacy. However, to the best of our knowledge, they are still far from satisfactory.[11] Therefore, more accurate markers are needed to predict the efficacy of immunotherapy.

Mutations in the tumour suppressor gene, TP53, occur in approximately 50% of patients with NSCLC.[12] TP53 mutations play a key role in carcinogenesis by disrupting various cellular processes including cell cycle, apoptosis, DNA repair, cellular senescence and autophagy.[13] TP53 may also influence the ability of cancer cells to escape immune detection.[14] Some TP53 mutations, such as nondisruptive mutations, are an independent prognostic factor for shorter survival in advanced-stage NSCLC.[15] TP53 mutation has been reported to be a potential predictive marker in NSCLC patients undergoing ICI therapy. In a recent study, Dong et al retrospectively analysed 30 advanced-stage NSCLC patients treated with pembrolizumab. Patients with TP53 mutation had significantly prolonged progression-free survival (PFS) compared to patients with wild-type TP53 (14.5 vs 3.5 months, $P = .042$).[16] In another study, Assoun et al retrospectively analysed 72 advanced-stage NSCLC patients treated with PD-1 blockers and found that the median OS was significantly longer in the TP53-mutated group than in the TP53 wild-type group (18.1 vs 8.1 months, $P = .04$).[17] However, the sample sizes were relatively small in these studies.

A recent meta-analysis of 19 studies involving a total of 6,084 patients with NSCLC found that TP53 mutations were associated with poor clinical outcomes. When the TP53-mutated group (n = 1406) was compared to the wild-type group (lacking TP53 mutations; n = 1965), the wild-type group was associated with a significantly higher OS rate (hazard ratio [HR], 1.26; 95% confidence interval [CI] 1.12-1.41, $P < .001$].[18] Although most patients in this meta-analysis underwent surgical resection and only a few patients were stage III-IV, the results were contrary to those in the reports mentioned above.[16,17]

In this study, original data pertaining to 350 NSCLC patients who received immunotherapy were downloaded from cBioPortal. The cBioPortal (www.cbioportal.org) online platform is an open-access website resource for researchers to explore, visualize and analyse multidimensional cancer genomics data.[19] The correlation between TP53 mutations and prognosis of these patients was evaluated. Statistical analysis suggested that there was no survival difference between TP53 wild-type and TP53 mutation groups. To identify NSCLC patients with TP53 mutations that would benefit from immunotherapy, we performed cluster analysis of gene mutations based on OS. The top ten significantly different mutated genes were identified, and the correlation of these genes with the prognosis of NSCLC patients was identified. We found that coexisting mutations in TP53 and zinc finger homeobox 3 (ZFHX3) predicted the best OS benefit.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study was approved by the Institutional Review Board of the Shandong Provincial Hospital Affiliated to Shandong First Medical University. This study is based on published data. In the original publication, it is stated that all included patients gave their informed consents.[20]

2.2 | Patients and data collection

In this study, cBioPortal was used to access NSCLC data. Gene mutation and TMB data of NSCLC patients who received at least one dose of ICI therapy at the Memorial Sloan Kettering Cancer Center between 2013 and 2017 were extracted.[20] Tumours from the primary or metastatic sites were profiled using next-generation sequencing. Most patients had stage IV or metastatic disease, and few patients had locoregionally recurrent disease.

2.3 | Gene clustering analysis

Genetic mutations in the cBioPortal online platform were analysed. Gene clustering analysis was performed between patients with short and long OS, with a cut-off value of 10 months. The top ten significantly different mutated genes were identified. Furthermore, we analysed the differences in OS of coexisting TP53 and other significantly different mutated genes in the long OS group to identify NSCLC patients with TP53 mutations that would benefit most from immunotherapy.

2.4 | Statistical analysis

All statistical analyses were performed using SPSS (version 22.0, SPSS Inc, IL, USA). We used Student's *t* test to compare the differences between continuous variables and chi-squared test for categorical variables. Genetic mutations in TP53 and other genes, and their association with OS in NSCLC patients are displayed as Kaplan-Meier plots. A

log-rank test was performed to identify the significance of the differences between the OS curves. A Cox proportional hazards model was used for multivariate analysis. P value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

The patient clinical characteristics are presented in Table 1. The median age at which sequencing was reported was 67 years (range, 31-90 years). The patient group comprised of 180 women and 170 men. Pathologic analysis included 271 adenocarcinoma, 45 squamous cell carcinoma, 13 poorly differentiated NSCLC, 8 large cell neuroendocrine carcinoma and 13 other pathologic types. The tumours profiled by next-generation sequencing were from primary (171/350, 48.9%) or metastatic sites (179/350, 51.1%). In total, 329 patients (94.0%) received anti-PD-1 or PD-L1, and 21 (6.0%) received a combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapies. To calculate TMB, the total number of somatic nonsynonymous mutations was normalized to the total number of sequenced megabases. The median TMB score for the 350 patients was 6.9 (range, 0-100.4). The median TMB score for patients with adenocarcinoma, squamous cell carcinoma and other pathologic types was 6.9 (range, 0-100.4), 6.7 (range, 0-34.6) and 8.8 (range, 0.88-50.2) respectively ($P = .541$).

TABLE 1 Patient clinical characteristics

Variable	N (%)
Age	
Median (range)	67 (31-90)
Gender	
Male	170 (48.6)
Female	180 (51.4)
Histology	
Adenocarcinoma	271 (77.4)
Squamous cell carcinoma	45 (12.9)
Others	34 (9.7)
Sample type	
Primary	171 (48.9)
Metastasis	179 (51.1)
Drug class	
PD-1/PD-L1	329 (94.0)
Combination	21 (6.0)
TMB score	
Median (range)	6.9 (0-100.4)

3.2 | Relationship between TP53 mutation and prognosis

A total of 219 (62.6%) patients harboured TP53 mutations and 131 (37.4%) carried wild-type TP53. TP53 mutations were more common in squamous cell carcinomas than in adenocarcinomas (Table 2). The median OS was 10 months (range, 0-57 months). A total of 219 (62.6%) deaths were reported during the follow-up period, and 131 (37.4%) patients were alive when censored at the most recent follow-up. The cumulative 5-year OS rate was 24.6%. The median OS of TP53 mutation and TP53 wild-type groups was 10 (95% CI: 7.4-12.6) and 14 (95% CI: 9.4-18.7) months respectively. There was no significant difference in OS between the two groups ($P = .258$) (Figure 1).

3.3 | Cluster analysis of gene mutations according to OS

To identify NSCLC patients with TP53 mutations that would benefit from immunotherapy, we performed cluster analysis of gene mutations according to OS. The cut-off OS was set at 10 months because the median OS of the 350 NSCLC patients was 10 months. Genes with the top ten mutation frequencies were identified. It was found that TP53, KEAP1, STK11 and REX2 were clustered in the group with short OS, whereas KRAS, PTPRD, EPHA3, EGFR, ZFHX3 and PTPRT were clustered in the group with long OS (Figure 2A,B). We then analysed the differences in OS of coexisting TP53 and the other six genes clustered in the long OS and found that coexisting TP53 and ZFHX3 mutations had the longest OS ($P = .040$) (Figure 2C).

3.4 | Relationship between coexisting TP53 and ZFHX3 mutations and prognosis

Next, the differences in OS between the three groups of patients identified according to TP53 and ZFHX3 mutation status were analysed: double-mutant tumours (TP53 (+)/ZFHX3 (+)), double wild-type tumours (TP53 (−)/ZFHX3 (−)), and TP53 mutant and ZFHX3 wild-type tumours (TP53 (+)/ZFHX3 (−)). The TP53 wild-type and ZFHX3 mutant groups were not included in the analysis because there were only five patients with this genotype. The median OS was not reached in the TP53 (+)/ZFHX3 (+) group. The median OS of TP53 (+)/ZFHX3 (−) group and TP53 (−)/ZFHX3 (−) group was 8 (95% CI: 5.7-10.3) and 13 (95% CI: 9.4-18.6) months respectively. Patients in the TP53 (+)/ZFHX3 (+) group had a significantly longer OS than those in the TP53 (+)/ZFHX3 (−) group ($P < .001$) and TP53 (−)/ZFHX3 (−) groups ($P = .003$) (Figure 3). Multivariate analysis

TABLE 2 Patient clinical characteristics according to TP53 mutation status

Variable	No. (%) of patients		*P* value
	TP53 mutation (n = 219)	TP53 wild type (n = 131)	
Age, mean ±SD	65.1 ± 10.8	66.8 ± 10.5	.158
Gender			
Male	113 (51.6)	57 (43.5)	.143
Female	106 (48.4)	74 (56.5)	
Histology			
Ad	157 (71.7)	114 (87.0)	.004
SQC	36 (16.4)	9 (6.9)	
Others	26 (11.9)	8 (6.1)	
Sample type			
Primary	103 (47.0)	68 (51.9)	.377
Metastasis	116 (53.0)	63 (48.1)	
Drug class			
PD-1/PD-L1	209 (95.4)	120 (91.6)	.114
Combination	10 (4.6)	11 (8.4)	
TMB score	12.3 ± 11.5	5.7 ± 4.4	<.001

Abbreviations: Ad, adenocarcinoma; SQC, squamous cell carcinoma.

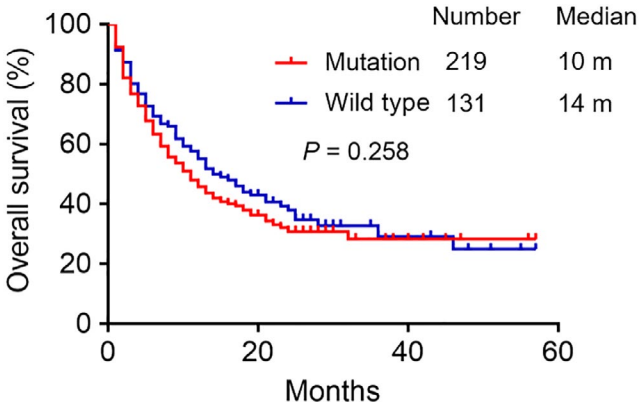

FIGURE 1 Kaplan-Meier survival curve for TP53 mutation vs TP53 wild-type group in NSCLC patients treated with ICIs. There was no significant difference in OS ($P = .258$) between the two arms. ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer; OS, overall survival

demonstrated that coexisting TP53 and ZFHX3 mutations were independent prognostic factors for advanced-stage NSCLC treated with ICI. Higher somatic TMB (highest 20% in each histology) and combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapy were also associated with long OS in multivariate analysis (Table 3). The results showed that patients with coexisting TP53 and ZFHX3 mutations had the longest OS compared to the other groups.

4 | DISCUSSION

In the present study, the prognostic effect of TP53 mutations in NSCLC patients treated with ICIs was analysed using publicly available databases. There was no statistically significant difference in OS between the TP53 gene mutation and wild-type groups. However, coexisting TP53 and ZFHX3 mutations are independent prognostic factors in advanced-stage NSCLC treated with ICIs. Higher somatic TMB (highest 20% in each histology) and combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapy were also associated with longer OS.

Several studies have reported that TP53 gene mutations are an indicator of poor prognosis in patients with NSCLC. In a meta-analysis, Gu et al analysed 19 studies that involved a total of 6,084 patients with NSCLC and examined the association between TP53 mutations and clinical outcomes. When the TP53 mutation group (n = 1406) was compared to the wild-type group (lacking TP53 mutations; n = 1965), the wild-type group was associated with a significantly higher OS rate (HR, 1.26; 95% CI 1.12-1.41, $P < .001$).[18] In their bioinformatic analysis, Wang et al extensively analysed TP53 mutations, gene expression and clinical data from 33 TCGA cancer type-specific datasets. They identified that lung adenocarcinoma patients with TP53 mutations had significantly worse OS prognosis compared to those without TP53 mutations.[21] However, in our study, TP53 wild-type group was not correlated with better OS compared to TP53 gene mutation. In the meta-analysis by Gu et al, most of the patients were stage I-IIIA and had undergone surgery. Similarly, in the bioinformatic analysis by Wang et al, most of the patients were stage I-III. However, in our study, all patients were at an advanced stage and were treated with ICIs. The composition of NSCLC patients and the treatment received may be the reason for the differences in the results.

In a recent study, Assoun et al retrospectively analysed 72 advanced-stage NSCLC patients treated with PD-1 blockers and found that the median OS was significantly longer in TP53-mutated group than that in TP53 wild-type group (18.1 vs 8.1 months, $P = .04$).[17] In our study, we did not draw the same conclusions. First, 94.0% of our patients received PD-1 or PD-L1 blockade immunotherapy. However, in the study by Assoun et al, 83% of the patients received PD-1 blockade immunotherapy, and none of the patients received PD-L1 blockade immunotherapy. Duan et al collected 19 randomized clinical trials involving 11 379 patients with several types of cancer and reported that anti-PD-1 exhibited superior OS (HR, 0.73; 95% CI, 0.65-0.86; $P < .001$) and PFS (HR, 0.73; 95% CI, 0.56-0.96; $P = .02$) compared with anti-PD-L1 blockade.[22] In lung cancer patients with previously untreated metastatic, squamous NSCLC, the addition of pembrolizumab to chemotherapy with carboplatin plus paclitaxel or nab-paclitaxel resulted in significantly longer OS

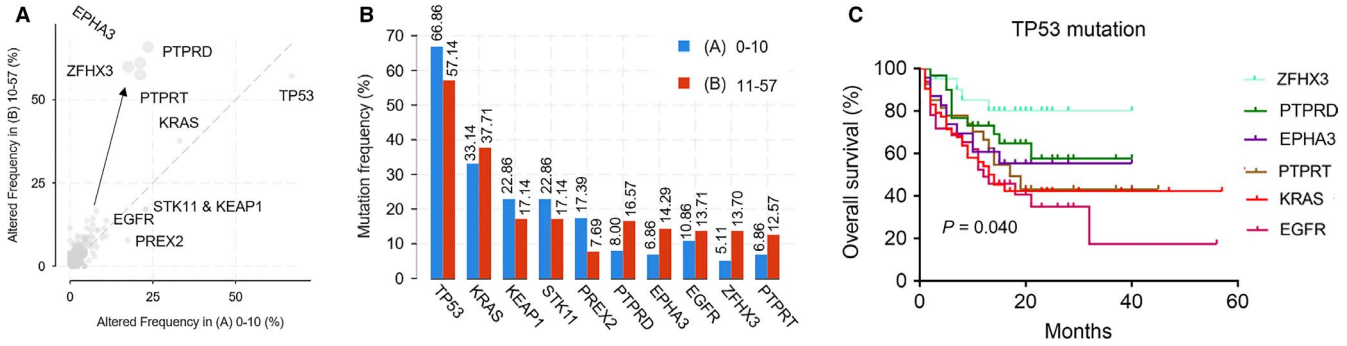

FIGURE 2 Cluster analysis of gene mutations according to OS in NSCLC patients treated with ICIs. The cut-off of OS was set at 10 mo. (A) Genes with the top 10 mutation frequency. It was found that Tp53, KEAP1, STK11 and REX2 were clustered in the groups with shorter OS, while KRAS, PTPRD, EPHA3, EGFR, ZFHX3 and PTPRT were clustered in the groups with longer OS (The arrow in the figure means magnification of the four genes at the beginning of the arrow. It could better label these four genes). (B) Detailed mutation frequency of the top 10 genes. (C) Different OS of coexisting TP53 and other 6 genes clustered in the longer OS. Coexisting TP53 and ZFHX3 mutations had the longest OS ($P = .040$). ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer; OS, overall survival

and PFS than chemotherapy alone.[23] However, in the corresponding PD-L1 blockade study, no OS benefit was achieved when atezolizumab was added to platinum-based chemotherapy.[24] Therefore, different medications may affect the OS. Second, distinct EGFR subtypes have different outcomes with immune checkpoint blockade. EGFR-mutant lung tumours generally show low response to ICIs.[25] There may be significant differences in EGFR subtypes between the two groups. Unfortunately, the composition of EGFR subtypes was not clear in either group. Third, the sample size used in the study by Assoun et al was relatively small. The limited sample size may have resulted in bias due to lack of statistical power. Dong et al retrospectively analysed 30 advanced-stage NSCLC patients treated with pembrolizumab. Patients with TP53 mutations had a significantly prolonged PFS compared with wild-type patients (14.5 vs 3.5 months, $P = .042$).[16] However, in our study, PFS was not studied. Hence, the relationship between TP53 mutations and PFS could not be inferred.

Recently, TMB, an indirect measure of tumour-derived neoantigens, has emerged as a promising biomarker for the stratification of ICI patients. In our study, higher somatic TMB (highest 20% in each histology) was associated with longer OS. The prognostic significance of TMB in ICI treatment of NSCLC has been supported by several reports.[20,26,27] Alborelli et al evaluated the predictive power of TMB in 76 NSCLC patients treated with ICIs. TMB was significantly higher in patients with durable clinical benefits than in patients with no durable benefit. Patients with high TMB showed significantly longer OS and PFS (log-rank test, $P = .0197$ and .0014 for OS and PFS respectively).[26] Gandara et al retrospectively analysed two large randomized trials as test and validation studies, and found that higher TMB in plasma (TMB $\geq$ 24 and TMB $\geq$ 26), which was distinct from that in tissue-based approaches, was associated with longer OS in NSCLC patients treated with atezolizumab.[27] However, the appropriate cut-off values of TMB vary between studies. Even in the same study, different cut-off values lead to different conclusions.[28] Therefore, further trials are needed to determine the optimal cut-off value.

In our study, a combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapies was associated with longer OS. Anti-CTLA-4 and anti-PD-1/PD-L1 agents act on different parts of the cancer immunity cycle. Combination of these drugs is synergetic and may help overcome resistance to single-drug immunotherapy. Previous studies on NSCLC and other tumour types have indicated that the combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapy is associated with higher clinical activity than single-drug treatment.[29-31] Among previously untreated patients with metastatic melanoma, nivolumab alone or in combination with ipilimumab resulted in significantly longer PFS than ipilimumab alone. In patients with PD-L1 negative tumours, the combination of PD-1 and CTLA-4 blockade was more effective than either agent alone.[30] In NSCLC, first-line nivolumab plus ipilimumab had a tolerable safety profile and showed encouraging clinical activity characterized including high response rate and durable response.[29] Phase 3 studies to further assess this combination are ongoing. However, the patients were stratified into PD-1/PD-L1 and combination groups. The PD-1/PD-L1 group could not be further stratified as PL-1 alone or PD-L1 alone from the cBioPortal online platform. In addition, the dose and duration of immunotherapy for each patient were not known from the cBioPortal online platform.

ZFHX3, a large transcription factor containing four homeodomains, 23 zinc finger domains, and multiple other motifs, was originally identified as ATBF1 that represses transcription of alpha-fetoprotein (AFP) by binding to its promoter.[32,33] The *ZFHX3* gene is encoded at locus 16q22.3-q23.1[34] and is likely a tumour suppressor in human cancer. ZFHX3 mutations are frequent in prostate cancer, especially with high-grade tumours, and ZFHX3 functionally suppresses cell proliferation.[35]

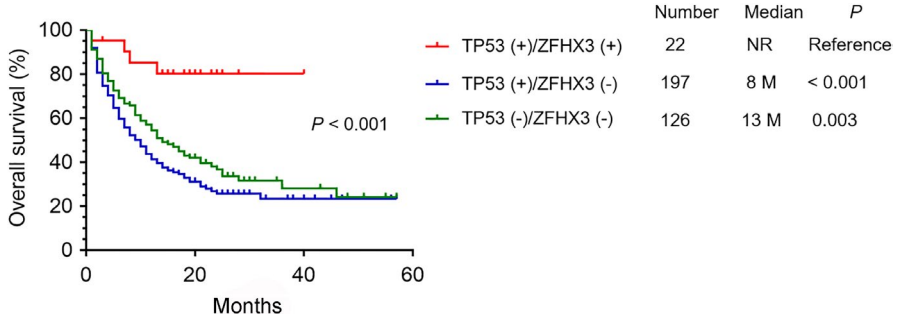

FIGURE 3 Kaplan-Meier survival curve for NSCLC patients treated with ICIs according to different TP53 and ZFHX3 mutation status. Patients in TP53 (+)/ZFHX3 (+) group had a significantly longer OS than those in TP53 (+)/ZFHX3 (−) group ($P < .001$) and TP53 (−)/ZFHX3 (−) group ($P = .003$). ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer; OS, overall survival; +, mutation; −, wild-type

TABLE 3 Cox regression analysis for estimating the risk factors of overall survival

	Univariate			Multivariate		
Variables	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age			.983			NS
≤67	Ref					
>67	0.997	0.761-1.306	.983			
Gender			.426			NS
Male	Ref					
Female	0.894	0.678-1.179	.426			
Histology			.723			NS
Ad	Ref					
SQC	1.118	0.763-1.638	.569			
Others	1.176	0.722-1.916	.515			
Sample type			.628			NS
Primary	Ref					
Metastasis	1.069	0.817-1.398	.628			
Drug Class			.008			.007
PD-1/PD-L1	Ref			Ref		
Combination	0.398	0.201-0.788	.008	0.395	0.201-0.779	.007
TMB score			.001			.001
>14	Ref			Ref		
≤14	2.003	1.336-3.004	.001	1.969	1.321-2.935	.001
Mutation group			.004			.002
TP53 (+)/ ZFHX3 (+)	Ref			Ref		
TP53 (+)/ ZFHX3 (−)	4.246	1.552-11.616	.005	4.307	1.575-11.773	.004
TP53 (−)/ ZFHX3 (−)	2.982	1.066-8.340	.037	2.929	1.051-8.165	.040

Abbreviations: Ad, adenocarcinoma; HR, Hazard ratio; NS, $P > .05$ on univariate analysis; Ref, Reference; SQC, squamous cell carcinoma.

ZFHX3 is also frequently mutated in endometrial carcinoma, and defects in ZFHX3 are associated with poor outcomes.[36] Zhou et al used a 583 gene panel to analyse the mutational spectrum of tumours collected from 98 lung adenocarcinoma patients and identified ZFHX3 mutations in 19% of patients.[37] The 5-year OS rate among patients weakly expressing ZFHX3 was significantly lower than that of patients expressing higher levels of ZFHX3 ($P < .0001$). Multivariate Cox proportional

hazard analyses revealed that lower ZFHX3 expression (HR, 4.42; 95% CI, 2.09-8.92; $P = .0002$) was an independent factor affecting 5-year OS. Suppression of ZFHX3 expression in tumour cells decreased the survival rate of patients with NSCLC.[38] In our study, the ZFHX3 mutation predicted better OS in NSCLC patients treated with ICIs.

5 | CONCLUSION

In conclusion, there was no statistically significant difference in OS between TP53 mutation and TP53 wild-type groups in advanced-stage NSCLC patients receiving ICI treatment. The role of TP53 as a prognostic marker for immunotherapy remains unclear. However, coexisting TP53 and ZFHX3 mutations are independent prognostic factors for advanced-stage NSCLC patients undergoing ICI treatment. The results of the present study may help in the identification of advanced-stage NSCLC patients harbouring TP53 mutations that would benefit from ICI treatment. Unfortunately, we could not validate this conclusion in our cohort. More comprehensive studies need to be conducted in future.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65:87-108.
2. Antonia SJ, Villegas A, Daniel D, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med*. 2018;379(24):2342-2350.
3. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373:123-135.
4. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet*. 2016;387(10027):1540-1550.
5. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med*. 2018;378(24):2288-2301.
6. Wang H, Gao J, Zhang R, Li M, Peng Z, Wang H. Molecular and immune characteristics for lung adenocarcinoma patients with CMTM6 overexpression. *Int Immunopharmacol*. 2020;83:106478.
7. Shukuya T, Carbone DP. Predictive markers for the efficacy of anti-PD-1/PD-L1 antibodies in lung cancer. *J Thorac Oncol*. 2016;11(7):976-988.
8. Hendriks LE, Rouleau E, Besse B. Clinical utility of tumor mutational burden in patients with non-small cell lung cancer treated with immunotherapy. *Transl Lung Cancer Res*. 2018;7(6):647-660.
9. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-413.
10. Tang H, Wang Y, Chlewicki LK, et al. Facilitating T cell infiltration in tumor microenvironment overcomes resistance to PD-L1 blockade. *Cancer Cell*. 2016;30(3):500.
11. Wcm D, Fenchel K, Dale SP. Programmed cell death ligand-1 (PD-L1) as a biomarker for non-small cell lung cancer (NSCLC) treatment-are we barking up the wrong tree. *Transl Lung Cancer Res*. 2018;7(S3):S275-S279.
12. Deben C, Deschoolmeester V, Lardon F, Rolfo C, Pauwels P. TP53 and MDM2 genetic alterations in non-small cell lung cancer: Evaluating their prognostic and predictive value. *Crit Rev Oncol Hematol*. 2016;99:63-73.
13. Zilfou JT, Lowe SW. Tumor suppressive functions of p53. *Cold Spring Harb Perspect Biol*. 2009;1(5):a001883.
14. Zitvogel L, Kroemer GCANCER. A p53-regulated immune checkpoint relevant to cancer. *Science*. 2015;349(6247):476-477.
15. Molina-Vila MA, Bertran-Alamillo J, Gascó A, et al. Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. *Clin Cancer Res*. 2014;20(17):4647-4659.
16. Dong ZY, Zhong WZ, Zhang XC, et al. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. *Clin Cancer Res*. 2017;23(12):3012-3024.
17. Assoun S, Theou-Anton N, Nguenang M, et al. Association of TP53 mutations with response and longer survival under immune checkpoint inhibitors in advanced non-small-cell lung cancer. *Lung Cancer*. 2019;132:65-71.
18. Gu J, Zhou Y, Huang L, et al. TP53 mutation is associated with a poor clinical outcome for non-small cell lung cancer: evidence from a meta-analysis. *Mol Clin Oncol*. 2016;5(6):705-713.
19. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1.
20. Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet*. 2019;51(2):202-206.
21. Wang X, Sun Q. TP53 mutations, expression and interaction networks in human cancers. *Oncotarget*. 2017;8(1):624-643.
22. Duan J, Cui L, Zhao X, et al. Use of immunotherapy with programmed cell death 1 vs programmed cell death ligand 1 inhibitors in patients with cancer: a systematic review and meta-analysis. *JAMA Oncol*. 2019;6(3):375-384.
23. Paz-Ares L, Luft A, Vicente D, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med*. 2018;379(21):2040-2051.
24. Jotte R, Cappuzzo F, Vynnychenko I, et al. Atezolizumab in combination with carboplatin and nab-paclitaxel in advanced squamous

NSCLC (IMpower131): results from a randomized phase III trial. *J Thorac Oncol*. 2020;15(8):1351-1360.
25. Hastings K, Yu HA, Wei W, et al. EGFR mutation subtypes and response to immune checkpoint blockade treatment in non-small-cell lung cancer. *Ann Oncol*. 2019;30(8):1311-1320.
26. Alborelli I, Leonards K, Rothschild SI, et al. Tumor mutational burden assessed by targeted NGS predicts clinical benefit from immune checkpoint inhibitors in non-small cell lung cancer. *J Pathol*. 2020;250(1):19-29.
27. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med*. 2018;24(9):1441-1448.
28. Rizvi H, Sanchez-Vega F, La K, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *J Clin Oncol*. 2018;36(7):633-641.
29. Hellmann MD, Rizvi NA, Goldman JW, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. *Lancet Oncol*. 2017;18(1):31-41.
30. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373(1):23-34.
31. Swanson MS, Sinha UK. Rationale for combined blockade of PD-1 and CTLA-4 in advanced head and neck squamous cell cancer—review of current data. *Oral Oncol*. 2015;51(1):12-15.
32. Miura Y, Tam T, Ido A, et al. Cloning and characterization of an ATBF1 isoform that expresses in a neuronal differentiation-dependent manner. *J Biol Chem*. 1995;270(45):26840-26848.
33. Morinaga T, Yasuda H, Hashimoto T, Higashio K, Tamaoki T. A human alpha-fetoprotein enhancer-binding protein, ATBF1, contains four homeodomains and seventeen zinc fingers. *Mol Cell Biol*. 1991;11(12):6041-6049.
34. Yamada K, Yoshida MC, Scheidl T, Miura Y, Tamaoki T. Assignment of the human ATBF1 transcription factor gene to chromosome 16q22.3-q23.1. *Genomics*. 1995;29(2):552-553.
35. Sun X, Frierson HF, Chen C, et al. Frequent somatic mutations of the transcription factor ATBF1 in human prostate cancer. *Nat Genet*. 2005;37:407-412.
36. Walker CJ, Miranda MA, O'Hern MJ, et al. Patterns of CTCF and ZFHX3 mutation and associated outcomes in endometrial cancer. *J Natl Cancer Inst*. 2015;107(11):djv249.
37. Zhou X, Xu X, Tian Z, Xu WY, Cui Y. Mutational profiling of lung adenocarcinoma in China detected by next-generation sequencing. *J Cancer Res Clin Oncol*. 2020;146(9):2277-2287.
38. Minamiya Y, Saito H, Ito M, et al. Suppression of Zinc Finger Homeobox 3 expression in tumor cells decreases the survival rate among non-small cell lung cancer patients. *Cancer Biomark*. 2012;11(4):139-146.

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