Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(7): 147
https://doi.org/10.31083/j.fbl2807147

Original Research

TP53 Exon 5 Mutation Indicates Poor Progression-Free Survival for Patients with Stage IV NSCLC

Huijing Feng[1,2,3,4,5,6,7,†], Huiru Xu[1,2,3,4,5,6,†], Xiuhuan Shi[1,†], Guobin Ding[8], Cihui Yan[1], Linhan Li[9], Zuoyi Jian[9], Xuejing Yang[6,7], Hongxia Guo[6,7], Feng Li[10,*], Junping Zhang[6,7,*], Xiubao Ren[1,2,3,4,5,11,*]

[1]Cancer Institute and Hospital, Department of Immunology, Tianjin Medical University, 300060 Tianjin, China
[2]National Clinical Research Center for Cancer, 300060 Tianjin, China
[3]Key Laboratory of Cancer Prevention and Therapy, 300060 Tianjin, China
[4]Tianjin's Clinical Research Center for Cancer, 300060 Tianjin, China
[5]Key Laboratory of Cancer Immunology and Biotherapy, 300060 Tianjin, China
[6]Department of Thoracic Oncology, Cancer Center, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, 030032 Taiyuan, Shanxi, China
[7]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 430030 Wuhan, Hubei, China
[8]Institute of Biotechnology, The Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Shanxi University, 030006 Taiyuan, Shanxi, China
[9]Genetron Health Inc, 102206 Beijing, China
[10]Department of Molecular Biology, Shanxi Cancer Hospital; Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences; Cancer Hospital Affiliated to Shanxi Medical University, 030012 Taiyuan, Shanxi, China
[11]Department of Biotherapy, Cancer Institute and Hospital, 300060 Tianjin, China
*Correspondence: lifenglover@sina.com (Feng Li); Junpingzhang@yeah.net (Junping Zhang); renxiubao@tjmuch.com (Xiubao Ren)
[†]These authors contributed equally.

Academic Editor: Liang-Chuan Lai
Submitted: 17 December 2022 Revised: 30 January 2023 Accepted: 7 February 2023 Published: 24 July 2023

Abstract

Background: Genetic mutations are quite common in non-small cell lung cancer (NSCLC), however, their prognostic value remains controversial. **Methods**: This study explored the mutational landscape of tumor samples from patients with advanced NSCLC by next-generation sequencing (NGS). A total of 101 NSCLC patients in stage III or IV receiving first-line treatment were included. **Results**: *TP53* mutation was the most frequent genetic alteration in NSCLC tumors (68%), followed by *EGFR* (49%), *CDKN2A* (12%), *LRP1B* (9%), and *FAT3* (9%) mutations. Among 85 patients with stage IV NSCLC, first-line targeted therapy remarkably prolonged progression-free survival (PFS) of patients compared with first-line chemotherapy (p = 0.0028). Among 65 patients with stage IV NSCLC whose tumors harbored *EGFR*, *ALK*, *ROS*, or *BRAF* mutations, first-line targeted therapy substantially prolonged the PFS of patients (p = 0.0027). In patients with *TP53* mutations who received first-line targeted therapy or chemotherapy, missense mutation was the most common mutation type (36/78), and exon 5 represented the most common mutated site (16/78). **Conclusions**: *TP53* mutation in exon 5 could independently predict poor PFS of patients with stage IV NSCLC after the first- line treatment. Moreover, mutations in *TP53* exon 5 and *LRP1B* were associated with shorter PFS of such patients whether after first-line chemotherapy or targeted therapy, respectively. Thus, these patients should be given immunotherapy or immunochemotherapy.

Keywords: NSCLC; *TP53* mutation; PFS; targeted sequencing; first-line treatment

1. Introduction

Lung cancer is the most common malignancy worldwide, with non-small cell lung cancer (NSCLC) accounting for 85% of all cases [1,2]. Despite the steady decline in mortality and rapid increase in survival in recent years, specifically for NSCLC, lung cancer remains the most deadly cancer in both men and women, with a 5-year overall survival of 21% for all stages combined [1].

Oncogenic driver mutations are responsible for the initiation and development of NSCLC. Clinical efficacy of targeting oncogenic driver genes such as epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*), and B-Raf proto-oncogene (*BRAF*) has been confirmed in NSCLC treatment [3–6]. So far, several treatment guidelines have been proposed for NSCLC patients with targetable driver mutations [7,8]. However, the prognostic value of those mutations remains controversial. Therefore, a better understanding of the mutational landscape and the prognostic value of the mutations in NSCLC may provide valuable information for risk stratification and refining of the treatment strategies.

Next-generation sequencing (NGS) has been widely used to detect germline and somatic mutations in human cancers, showing advantages compared with conventional

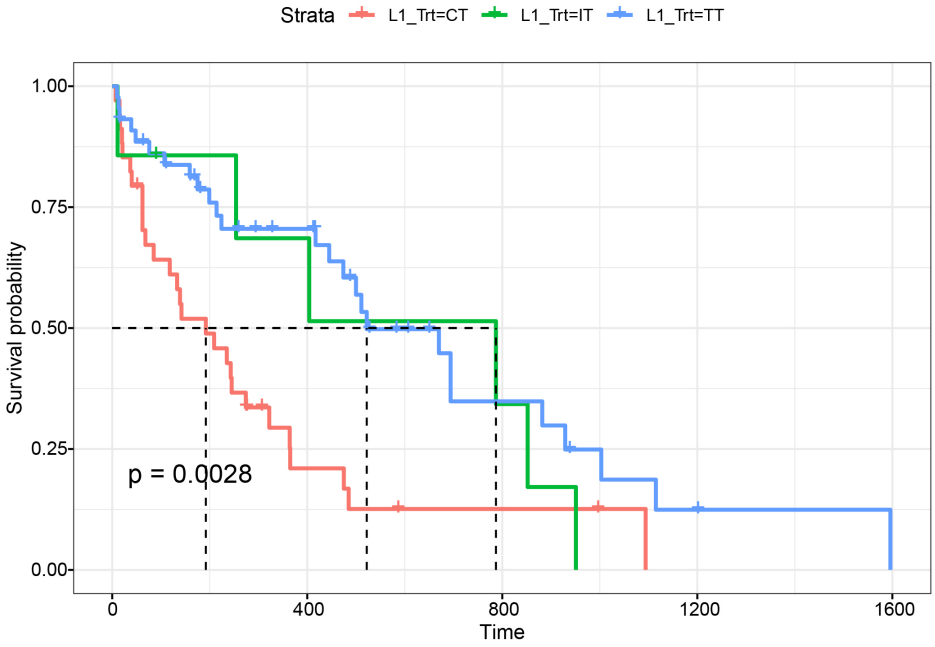

Fig. 1. Kaplan-Meier curves comparing PFS in stage IV NSCLC patients receiving different first-line therapies. A total of 85 patients with stage IV NSCLC were stratified into first-line chemotherapy (n = 34), targeted therapy (n = 44), and immunotherapy (n = 7) groups. Kaplan-Meier curves were generated to evaluate the association of first-line treatment with progression-free survival (PFS) of patients.

genomic sequencing methods. Previous studies have shown that some somatic mutations are strongly associated with the survival of NSCLC patients, providing valuable information for clinical management of NSCLC patients. For example, it was found that the combination of 6 hotspot mutations of *EGFR*, *PIK3CA*, and tumor suppressor protein p53 (*TP53*) predicts poor survival of NSCLC patients [9]. The presence of specific DDR gene alterations was associated with worse prognosis of advanced NSCLC [10]. In addition, studies found that the tumor mutation burden, i.e., the approximate amount of gene mutation that occurs in the genome of a cancer cell, can predict the response of NSCLC to immunotherapeutic agents [11]. Therefore, using NGS to detect somatic mutations and identify prognostic indicators may provide important information to guide treatment selection and improve outcomes for NSCLC management.

TP53 is the most frequently mutated gene in human cancer, with a frequency of 50–60% [12]. Although many studies have shown that *TP53* mutations are associated with worse prognoses in cancer carriers, the prognostic value of *TP53* mutation in NSCLC remains somewhat controversial [13,14] due to different mutated exons and different cancer stages [15–17]. *TP53* contains 11 exons, and most *TP53* mutations occur in exons 5–8 spanning 540 nucleotides that encode the DNA-binding domain of p53 protein [13]. Previously, all p53 mutants have been considered functionally equal; however, increasing evidence has revealed that mutations occurring in different positions of a single gene, e.g., *TP53,* could have a different effect on the prognosis of cancer [18–20], thus emphasizing the importance of stratifying NSCLC patients according to the classification of detailed gene mutations.

In this study, we analyzed the effect of different first-line treatment strategies for advanced NSCLC patients. To factors that significantly associated with PFS of patients we carried out a detailed stratification of patients accoding to their TNM stages and genomic alterations. We then investigated the associations of high-frequency mutations with PFS of unresectable stage IV NSCLC patients receiving different first-line treatment regimens. Our results suggested that mutations in exon 5 of *TP53* and low-density lipoprotein receptor-related protein 1B (*LRP1B*) were potential adverse prognostic indicators for stage IV NSCLC.

2. Materials and Methods

2.1 Sample Collection

A total of 101 patients diagnosed with NSCLC at Shanxi Bethune Hospital (Taiyuan, Shanxi, China) were recruited for the present study. Formalin-fixed, paraffin-embedded tumor samples were collected between May 2013 and May 2021. Matched white blood cell samples were collected as reference controls for each tumor sample. Tumors were staged according to the 8th edition of the AJCC staging system [21]. Sixteen patients with stage III diseases received surgery and postoperative adjuvant chemotherapy, whereas 85 with unresectable stage IV diseases underwent first-line treat-

ment according to their molecular signatures, which was in accordance with the Guidelines of the Chinese Society of Clinical Oncology (CSCO) Non-Small Cell Lung Cancer (http://meeting.csco.org.cn/MUser/CscoPeriodical/1/2?keyword=&leibie=&pyear=0&loc=E9317455E99FF2AE) [22]. Driver gene *EGFR/ALK/ROS/BRAF* mutations were detected in 65 patients with stage IV diseases. Of these patients, 44 received matched targeted-therapy, while the other 21 received chemotherapy (n = 34) or immunotherapy (n = 7) due to various causes.

This study was approved by the Ethics Committee of Shanxi Bethune Hospital (No. YXLL-2022-045) and conducted according to the Declaration of Helsinki. Informed consent was obtained from all patients.

2.2 DNA Extraction

DNA sequencing was performed as previously described [23]. Total genome DNA was isolated from paraffin-embedded tumor samples and matched white blood cells (germline mutations excluded) using a DNA extraction kit from Qiagen (Hilden, Germany) or Life Technologies (Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA concentration and purity were determined using NanoDrop 2000 (Agilent Technologies, Santa Clara, CA, USA) and Qubit 2.0. Genomic DNA was sonicated into 150–200 bp fragments using Covaris S220 focused ultrasonicator (Covaris, Woburn, MA, USA).

2.3 Library Construction and Sequencing

As previously described [23], a DNA library was constructed using a KAPA Hyper Prep kit (Kapa Biosystems, Woburn, MA, USA) according to the manufacturer's instructions. A panel of 63 genes (Genetron Health, product catalog identifier: fwa-p180-cancer) used in this study is summarized in **Supplementary Table 1**. The DNA library was enriched for regions of the custom-designed captured probe manufactured by Agilent. A total of 750 ng DNA library was incubated with two different hybridization reagents and blocking agents in a SureSelectXT target enrichment system (Agilent Technologies). The enriched library was amplified using P5/P7primer. The quality and quantity of the DNA library were assessed using 2200 Bioanalyzer, Qbit3 (Thermo Fisher Scientific, Waltham, MA, USA), and a qPCR NGS library quantification kit (Agilent Technologies). The library was sequenced using a 150-bp paired-end strategy on the NovaSeq system (Illumina, USA) with a depth of 3500 $\times$ targeting the mutation hotspots of 63 genes.

2.4 Survival Analysis

A total of 78 patients with stage IV NSCLC who received first-line targeted therapy or chemotherapy were included in the analysis. Kaplan-Meier curve analysis was used to assess the associations of variables of interest with the PFS of patients. The patients with stage III diseases with follow-ups $<$180 days were excluded. To investigate the effects of specific gene mutations on prognosis, patients who received first-line immunotherapy were excluded due to the small sample size. A p-value < 0.05 was considered statistically significant.

Table 1. Clinical characteristics of patients.

Characteristics	Value
Total, n	101
Sex n (%)	
Female, n (%)	43 (42.6)
Male, n (%)	58 (57.4)
Age, median (range)	62.00 (55.00, 68.00)
Smoking	
Non-smokers, n (%)	64 (63.4)
Smokers, n (%)	37 (36.6)
Primary site	
Both	2 (2.0)
Left, n (%)	37 (36.6)
Right, n (%)	62 (61.4)
Pathology	
Adenocarcinoma, n (%)	87 (86.1)
Squamous cell carcinoma, n (%)	14 (13.9)
Stage	
IIIA, n (%)	11 (10.9)
IIIB, n (%)	3 (3.0)
IIIC, n (%)	2 (2.0)
IVA, n (%)	72 (71.3)
IVB, n (%)	13 (12.9)
Brain metastasis	
No, n (%)	80 (79.2)
Yes, n (%)	21 (20.8)
Bone metastasis	
No, n (%)	52 (51.5)
Yes, n (%)	49 (48.5)
Therapy	
First-line therapy, n (%)	46 (45.5)
Second-line therapy, n (%)	23 (22.8)
Third-line therapy, n (%)	32 (31.7)
First-line therapy	
Chemotherapy, n (%)	45 (44.6)
Immunotherapy, n (%)	7 (6.9)
Targeted therapy, n (%)	49 (48.5)
Second-line Therapy	
Chemotherapy, n (%)	14 (13.9)
Immunotherapy, n (%)	10 (9.9)
None, n (%)	46 (45.5)
Targeted therapy, n (%)	31 (30.7)
Third-line Therapy	
Chemotherapy, n (%)	3 (3.0)
Immunotherapy, n (%)	4 (4.0)
None, n (%)	69 (68.3)
Targeted therapy, n (%)	25 (24.8)

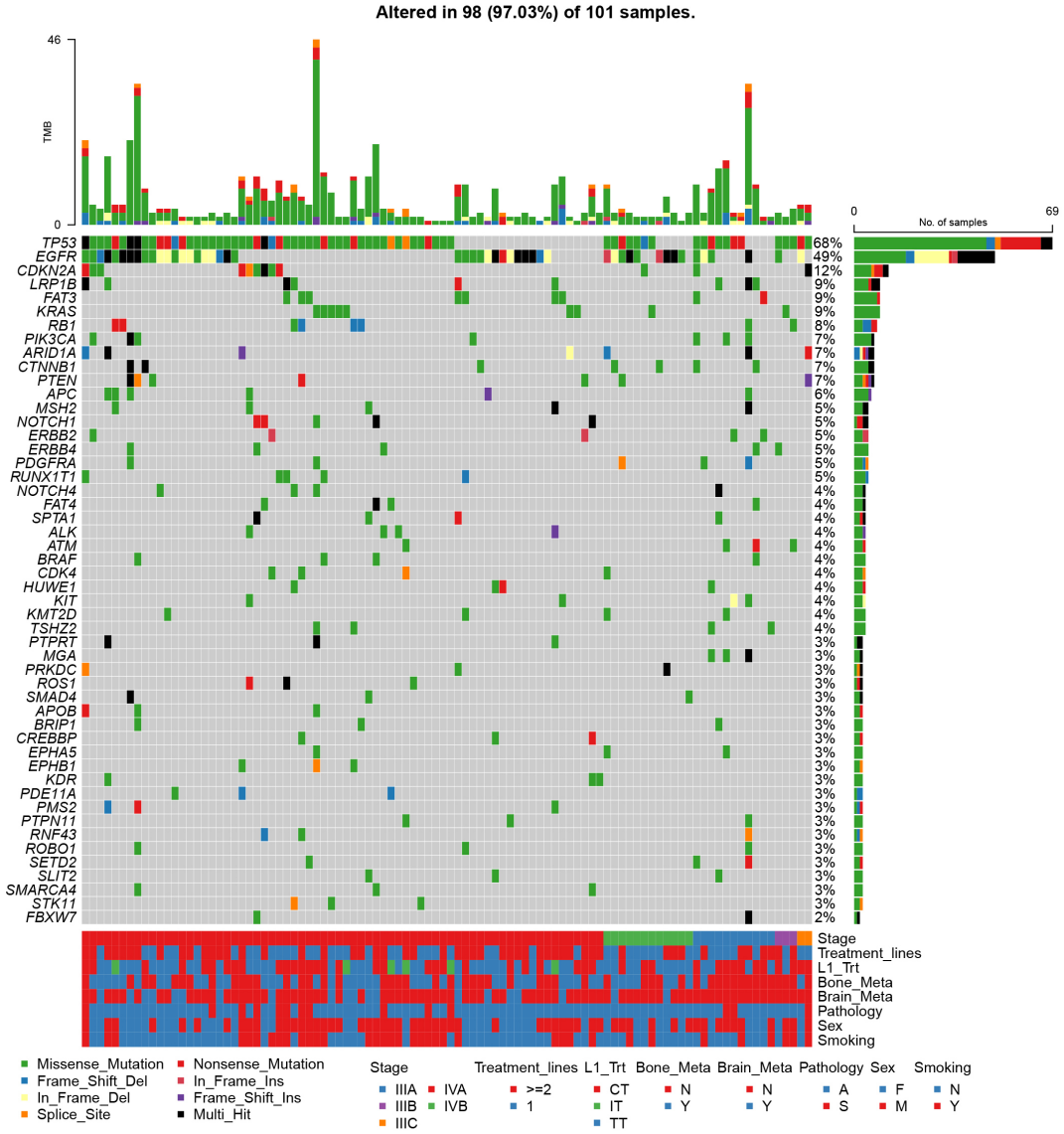

Fig. 2. Mutation landscape of patients with stage III or IV non-small cell lung cancer (NSCLC) (n = 101). Each horizontal line represents a single gene, and each vertical line represents different samples. Different colors indicate the type of mutations. Representative clinical features of the patients, such as tumor stage, metastasis status, and sex, are shown at the bottom of the plot. N, no; Y, yes; F, female; M, male; A, adenocarcinoma; S, squamous cell carcinoma; CT, chemotherapy; IT, immunotherapy; TT, targeted therapy.

2.5 Statistical Analysis

Statistical analysis was performed using R (version 4.1.2, the R Foundation for Statistical Computing, Vienna, Austria) and RStudio (version 1.4.1717, Posit Community portal, Washington D.C., USA). Mutation interactions (co-mutations and mutual exclusivity) were identified by performing Fisher's exact test on pairs of genes. The Cox proportional hazards model was used for multivariate survival analysis. Variables with a p-value < 0.1 in the univariate analysis were included in the multivariate analysis. Schoenfeld residuals were used to check the proportional hazards assumption. All tests were two-sided. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Clinicopathologic and Molecular Features of Patients

A total of 101 patients, 43 females and 58 males (a mean age of 62 years) with stage III (n = 16) or unresectable stage IV (n = 85) NSCLC were included in this study. The clinicopathologic and molecular features of patients are summarized in Table 1. A total of 49 patients received first-line targeted therapy (e.g., gefitinib, osimertinib, and entrectinib), 45 patients received first-line chemotherapy, and 7 patients received first-line immunotherapy (e.g., pembrolizumab, sintilimab, and atezolizumab).

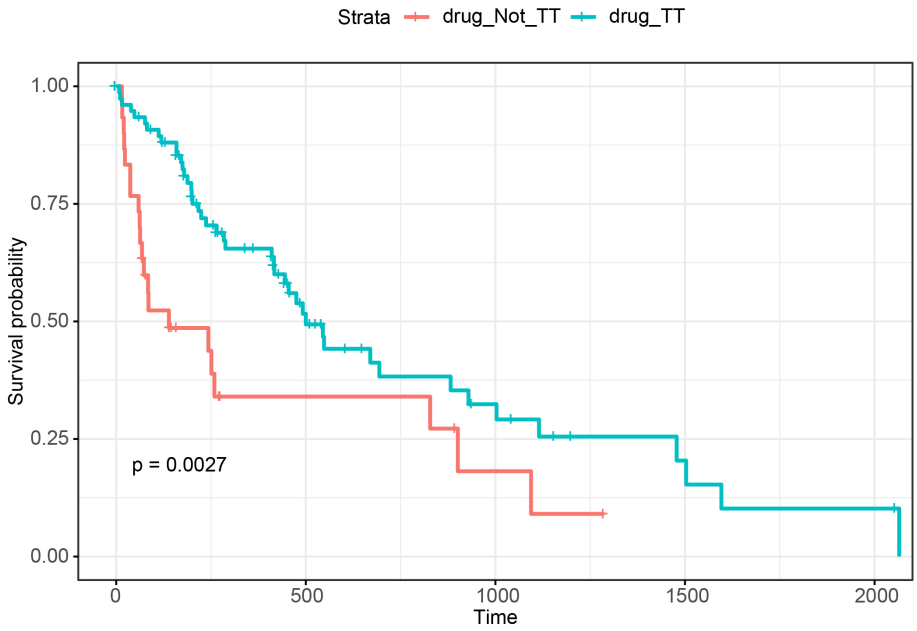

Fig. 3. Comparison of PFS of stage IV NSCLC patients receiving matched targeted therapy and non-targeted therapy. A total of 65 patients with *EGFR/ALK/ROS/BRAF* mutations in the tumor were analyzed, including 44 patients receiving matched targeted therapy and 21 receiving non-targeted therapy. Kaplan-Meier curves were generated to evaluate the association of first-line treatment with the PFS of patients.

3.2 First-Line Targeted Therapy Improves the PFS of Patients with NSCLC

To explore the degree of survival benefit of patients receiving first-line targeted therapy, we conducted Kaplan-Meier curve analysis in patients with stage IV NSCLC (n = 85) who were subsequently stratified into chemotherapy (n = 34), targeted therapy (n = 44), and immunotherapy (n = 7) groups. Kaplan-Meier curve analysis showed that first-line targeted therapy remarkably prolonged PFS of patients compared with first-line chemotherapy (522 days vs. 192 days, $p = 0.0028$). The first-line immunotherapy group was precluded from statistical analysis due to the small sample size (Fig. 1).

3.3 Matched Targeted Therapy Based on Genomic Variation can Significantly Prolong PFS of Advanced NSCLC Patients

In order to identify somatic mutations associated with advanced NSCLC and the survival of these patients, we performed NGS of tumor tissue and matched white blood cell samples from 101 NSCLC patients in stage III or IV, finding that 97.03% (98/101) of tumor samples carried gene mutations, with *TP53* mutation representing the most frequent alteration (68%, 69/101), followed by *EGFR* (49%), *CDKN2A* (12%), *LRP1B* (9%), and *FAT3* (9%) mutations (Fig. 2). We also observed significant concurrent mutations in 13 gene pairs, among which *KRAS/EGFR* were mutually exclusive mutations ($p < 0.05$), whereas the other 12 gene pairs, such as *APC/MSH2*, *TP53/CDKN2A*, and *ARID1A/LRP1B*, were co-mutations ($p < 0.05$) (**Supplementary Fig. 1**). KEGG analysis revealed that the mutated genes were mainly enriched in the *TP53*, cell cycle, and RTK-RAS signaling pathways (**Supplementary Fig. 2A**). However, these pathways were not significantly associated with the PFS of patients (**Supplementary Fig. 2B**).

In order to personalize stratification patients more accurately, we further performed survival analysis of stage III and IV NSCLC patients, respectively. The results showed that only stage IV patients statistically significantly benefit from molecular typing in our cohort. Analysis of the 65 stage IV patients whose tumors harbored driver gene *EGFR/ALK/ROS/BRAF* alterations showed that compared with non-targeted therapy (n = 21), matched targeted therapy (n = 44) significantly prolonged PFS of patients harboring these mutations (500 days vs. 139 days, $p = 0.0027$; Fig. 3). These data suggest that first-line targeted therapy improves the prognosis of NSCLC patients harboring druggable mutations, highlighting the importance of identifying genetic mutations by NGS in direct therapeutic decision-making.

3.4 TP53 Mutation in Exon 5 Predicts Poor PFS in Stage IV NSCLC Patients

Previous studies have shown that different positions of *TP53* mutation have a differential effect on the prognosis of cancer [18–20]. According to those findings we grouped patients more detailed based on their somatic *TP53* mutation subtypes. As shown in Fig. 4A, among 101 NSCLC patients, the frequency of *TP53* mutation was 68% (69/101).

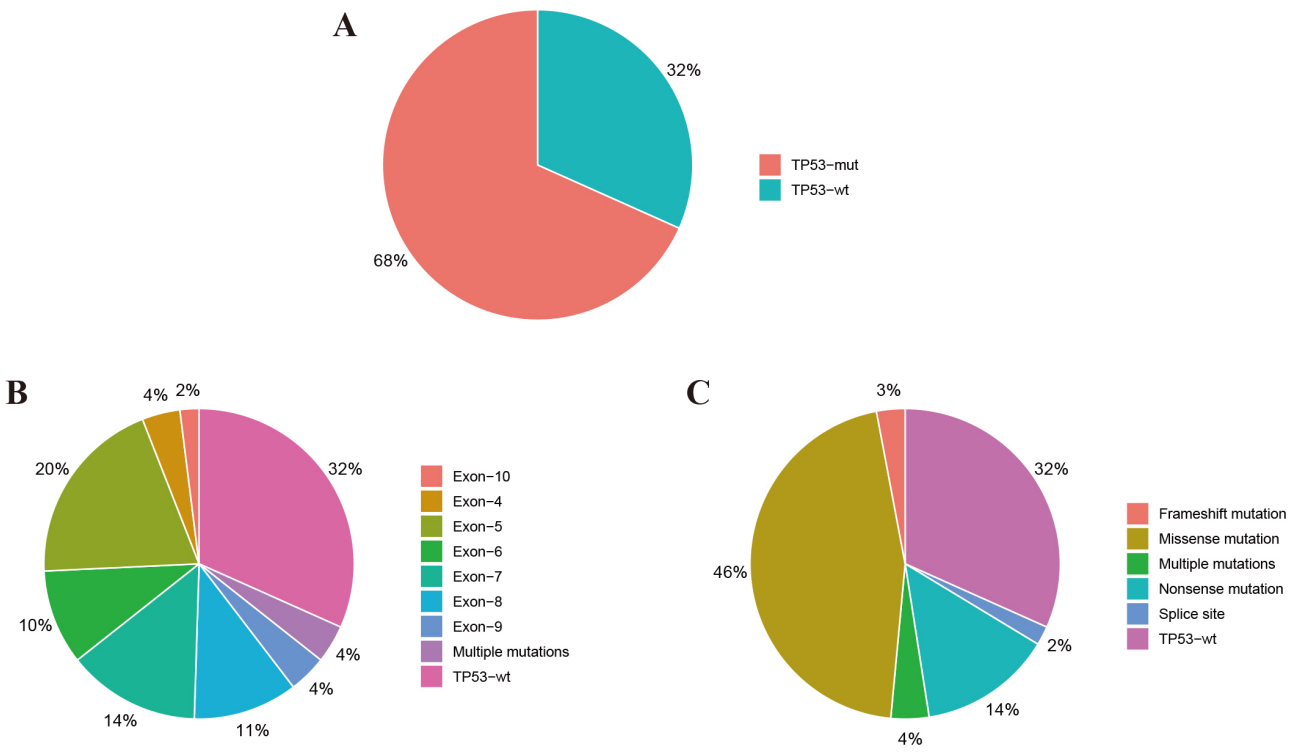

Fig. 4. *TP53* mutation profiles of NSCLC patients. (A) Frequency of *TP53* mutation in 101 patients with NSCLC. (B) Composition of mutation types in 69 NSCLC patients with *TP53* mutation. (C) Distribution of *TP53* mutation subtypes in 101 patients with NSCLC.

We conducted a more thorough analysis of *TP53* mutations in 78 patients receiving first-line chemotherapy (n = 34) or targeted therapy (n = 44). The first-line immunotherapy group was excluded from statistical analysis due to the small sample size (n = 7). The most common mutation type was a missense mutation (46%, 36/78), followed by nonsense mutation (14%, 11/78) and multiple mutations (4%, 3/78) (Fig. 4B). The most common mutated site was exon 5 (20%, 16/78), followed by exon 7 (14%, 11/78), exon 8 (11%, 9/78), and exon 6 (10%, 8/78) (Fig. 4C). Mutations in exons 5–8 accounted for 65% of all *TP53* mutations.

Then, the prognostic value of popular mutations were further evaluated in this study. We carried out univariate cox regression analysis to test the association between gene altaeration and PFS. Those mutations significant associated with PFS identified in the univariate analysis were then selected to conduct multivariate Cox regression analysis, which showed that mutations in *TP53* exon 5 (p = 0.033, hazard ratio (HR) = 2.3 (95% CI [1.069–5.1])), *EP300* (p = 0.005, HR = 23.8 (95% CI [2.654–212.5])), and *PTPN11* ($p < 0.001$, HR = 54.4 (95% CI [5.237–565.6])) could independently predict poor PFS of NSCLC patients after the first-line treatment (Fig. 5). *EP300* (n = 1) and *PTPN11* (n = 2) mutations were excluded from further studies due to small, mutated sample sizes.

3.5 Survival Analysis of Patients with TP53 Exon 5 Mutations

We further performed survival analysis on the 78 patients. As shown in Fig. 6A, patients with *TP53* mutation in exon 5 (n = 13) had shorter PFS than those with wild-type *TP53* or non-exon 5 mutations (n = 65) after first-line treatment (p = 0.0218, HR = 2.22). Among 34 patients receiving first-line chemotherapy, patients with *TP53* mutation in exon 5 (n = 7) had shorter PFS after first-line treatment than those with wild-type *TP53* or non-exon 5 mutations (n = 27) (p = 0.00616, HR = 3.37; Fig. 6B), which indicated that *TP53* mutation in exon 5 was an adverse indicator for the prognosis of advanced NSCLC after first-line treatment or first-line chemotherapy. This further suggests that NSCLC patients with *TP53* exon 5 mutations are insensitive to first-line chemotherapy and could hardly obtain survival benefits from first-line chemotherapy.

The association of *LRP1B* mutation with PFS of patients was also assessed since *LRP1B* was one of the most frequently altered genes in the cohort of this study. As shown in Fig. 7A, similar to *TP53* mutation, *LRP1B* mutation was significantly associated with poor PFS of NSCLC patients (n = 78) after first-line treatment (p = 0.0212, HR = 2.9). Furthermore, of the 44 patients receiving first-line targeted therapy, patients harboring *LRP1B* (n = 2) had significantly shorter PFS than those with wildtype *LRP1B* (n = 42) (p = 0.0414, HR = 4.21) (Fig. 7B). These data suggests that patients with *LRP1B* mutation are insensitive to first-

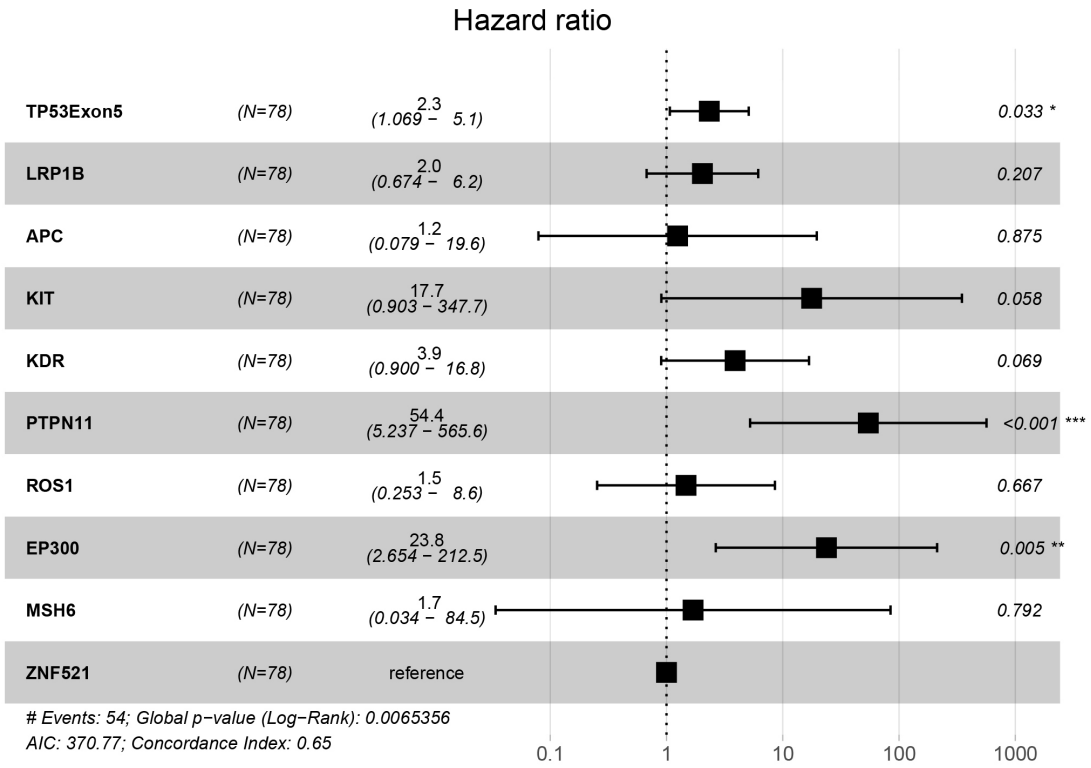

Fig. 5. Multivariate Cox regression analysis of the association between gene mutation and PFS after the first-line treatment. Multivariate Cox regression analysis was performed for 78 patients with stage IV NSCLC, including 34 patients receiving first-line chemotherapy and 44 patients receiving first-line targeted therapy. A forest plot showed that *TP53* exon 5, *EP300*, and *PTPN11* could independently predict poor PFS after the first-line treatment. * indicated statistically significant; ** and *** indicated very significant.

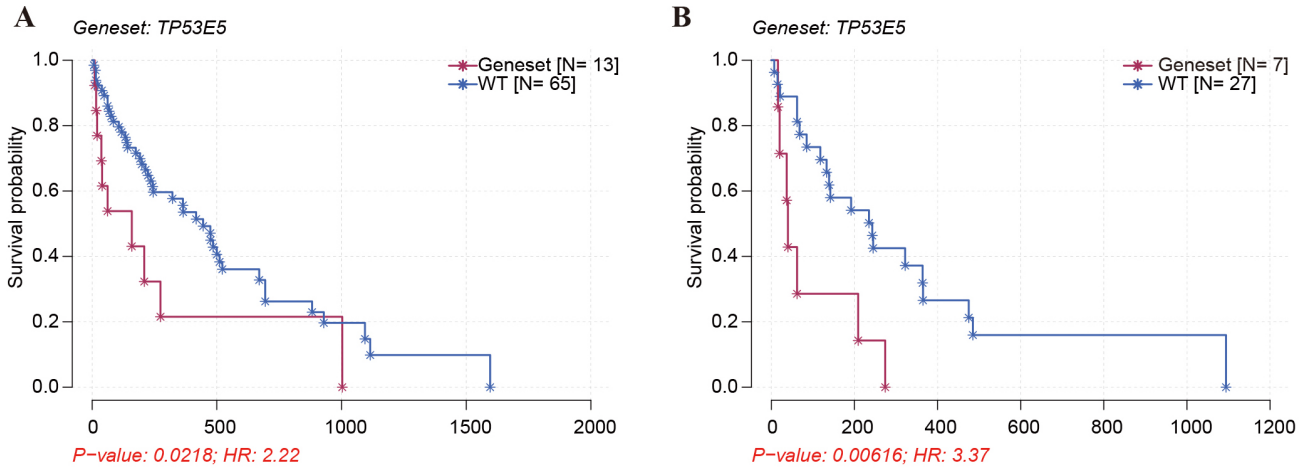

Fig. 6. The association of *TP53* exon 5 mutation with the PFS of stage IV NSCLC patients. (A) Kaplan-Meier survival analysis was carried out to assess the association of *TP53* exon 5 mutation with the PFS of stage IV NSCLC patients receiving first-line treatment. (B) Kaplan-Meier survival analysis was conducted to assess the association of *TP53* exon 5 mutation with the PFS of NSCLC patients after first-line chemotherapy.

line targeted therapy and could hardly achieve a survival benefit from first-line targeted therapy.

4. Discussion

In the present study, we sought to identify somatic mutations associated with PFS in patients with advanced NSCLC after first-line treatment. Analysis of tumor tissue samples from 101 NSCLC patients in stages III and IV us-

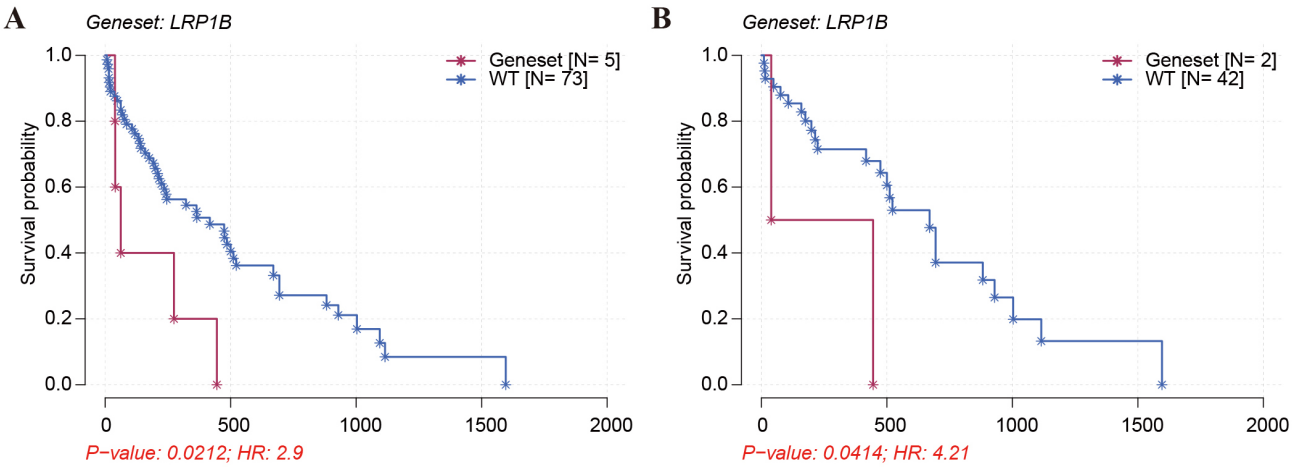

Fig. 7. Kaplan–Meier curve showing the difference in PFS among patients harboring *LRP1B* mutation or not. (A) Kaplan-Meier survival analysis was carried out to assess the association of *LRP1B* mutation with the PFS of patients with stage IV NSCLC, including 5 patients with and 73 patients without *LRP1B* mutation. (B) Kaplan-Meier survival analysis was conducted to assess the association of *LRP1B* mutation with the PFS of NSCLC patients after first-line targeted therapy, including 2 patients with and 42 patients without *LRP1B* mutation.

ing NGS revealed that *TP53* was the most frequently mutated gene, with exon 5 representing the most common mutated site. In patients with stage IV NSCLC, *TP53* mutation in exon 5 was an independent predictor of poor PFS after the first-line treatment. Furthermore, mutations in *TP53* exon 5 and *LRP1B* were associated with worse PFS after first-line chemotherapy and targeted therapy, respectively. These results suggested that *TP53* exon 5 and *LRP1B* mutations are negative prognostic indicators for advanced NSCLC.

The NGS data may guide the treatment options to improve clinical outcomes. Cao *et al.* [24] have demonstrated that after identifying driver mutations by NGS, patients receiving matched targeted therapy achieve significantly longer PFS than those receiving non-targeted therapy. A recent meta-analysis, which included 11 studies and 2874 participants, has shown that *ALK* inhibitor targeted therapy causes a significant increase in PFS of patients with *ALK*-rearranged NSCLC compared with chemotherapy [25]. Similarly, in the present study, the first-line targeted therapy remarkably prolonged PFS of patients compared with first-line chemotherapy, regardless of the mutation status. In patients who harbored driver gene *EGFR/ALK/ROS/BRAF* mutations matched targeted therapy substantially prolonged the PFS of patients compared with non-targeted therapy. Thus, NGS data can indeed inform therapy decisions in patients with advanced NSCLC.

TP53 is the most frequently altered gene in NSCLC, occurring in 30–65% of cases [15,26,27]. The prevalence rate of *TP53* mutation in this study was 68%. About 80–90% of *TP53* mutations encode missense proteins with a reduced capacity to bind to a specific DNA sequence that regulates gene transcription [12,28]. This study found that missense mutation was the most common type of *TP53* mutation, found in 46% of patients. *TP53* mutations frequently occur in the 'hot-spot' region of exons 5–8 encoding the DNA-binding domain [20,29–31]. In the present study, mutations in exons 5–8 accounted for 65% of all *TP53* mutations, and exon 5 was the most common mutated location. Vega *et al.* [32] explored the clinical significance of *TP53* exon 5 mutation, demonstrating that patients with squamous cell lung tumors carrying *TP53* mutations in exon 5 exhibit worse prognoses than those carrying mutations in other locations. Nonetheless, since then, many studies have focused on the prognostic value of *TP53* exon 8 mutations, revealing that *TP53* mutations affecting exon 8 but not exon 5 indicate poor prognosis in NSCLC patients [20,26,33]. In the current study, multivariate Cox regression analysis showed that *TP53* exon 5 mutation was an independent predictor for worse PFS in patients with stage IV NSCLC after the first-line treatment. Moreover, *TP53* mutation in exon 5 was correlated with poor PFS after first-line treatment regardless of the treatment option, suggesting that mutation in exon 5 might identify a subgroup of NSCLC patients with unfavorable prognoses.

With comprehensive genomic profiling, concurrent genetic alterations have been discovered in human cancers. The presence of *TP53* co-mutations can identify high-risk *ALK*$^{+}$-NSCLC cases with earlier treatment failure and shorter survival, regardless of the therapy [34]. *EGFR* mutations are considered mutually exclusive with other oncogenic drivers [35,36]. In the present study, we found that *KRAS/EGFR* were mutually exclusive mutations, whereas the other 12 gene pairs were co-mutations in stage IV NSCLC, including *TP53/CDKN2A*. *CDKN2A* (cyclin-dependent kinase inhibitor 2A) encodes tumor suppressors p16^{INK4a} and p19^{ARF}. Mutations in *TP53* and in-

activation of *CDKN2A* are commonly observed and highly involved in lung squamous cell carcinoma that accounts for 20–30% of NSCLCs [30]. A recent meta-analysis has demonstrated that concurrent *TP53* mutation is a negative prognostic factor that is correlated with poor outcomes in patients with *EGFR*- or *ALK*-targeted therapy in advanced NSCLC [37]. *TP53* mutation is also involved in primary resistance to *EGFR*-targeted therapy in patients with advanced NSCLC [37]. These reports suggest that *TP53* may play an important role in resistance mechanisms against targeted therapy. In this study, we observed co-mutations of *TP53* with *EGFR* and *ALK* in some cases, despite the lack of statistical significance, which could be due to the small sample size.

LRP1B encodes low-density lipoprotein receptor-related protein 1b, which is frequently mutated in NSCLC and acts as a putative tumor suppressor [38,39]. In this study, we observed *LRP1B* mutation in 9% of patients with advanced NSCLC, which was remarkably lower than that observed in other NSCLC cohorts, possibly because the samples being investigated here only include stage III&IV NSCLC patients [40,41]. Accumulating evidence has suggested that *LRP1B* alterations are correlated with high herterogeineity of tumors which may lead to poor prognosis [40]. Multiple studies have shown that *LRP1B* mutation improved outcomes of immunotherapy in patients with different types of cancer, including NSCLC [40,42–47]. One possible mechanism is that NSCLC patients with somatic mutation of *LRP1B* usually have higer tumor mutation burden which postitively associated with immunotherapy [40]. We found that patients with *LRP1B* mutation had significantly shorter PFS compared to those with wild-type *LRP1B* after first-line targeted therapy, which suggests that NSCLC patients with *LRP1B* mutation may benefit from immunotherapy rather than targeted therapy.

The present study has some limitations that need to be addressed in the future. First, 7 patients who received first-line immunotherapy were excluded from the correlation analysis between *TP53* mutation and PFS due to the small sample size. To investigate the prognostic values of immunotherapy-related genes such as *LRP1B*, larger sample size is needed. Second, the predictive potential of *TP53* exon 5 mutation in the prognosis of NSCLC patients should be tested in large validation cohorts. Long-term follow-up data are also needed to investigate whether *TP53* exon 5 mutations correlate with the overall survival of the patients. Third, although many patients have received second and third-line therapies, numerous confounding factors such as loss of follow-up prevented us from data collection and analysis.

5. Conclusions

We used NGS to characterize the mutational landscape in tumor samples from patients with stages III or IV NSCLC. Mutations in *TP53* exon 5 and *LRP1B* resulted in being negative prognostic factors for patients with stage IV NSCLC after first-line chemotherapy and targeted therapy, respectively. According to these findings, immunotherapy or immunochemotherapy are more recommended treatment stratigies for advanced NSCLC patients with somatic mutations in *TP53* exon 5 and *LRP1B*. These findings reinforce the importance of genetic testing to guide first-line therapy for NSCLC management.

Abbreviations

ALK, anaplastic lymphoma kinase; *BRAF*, B-Raf proto-oncogene; *EGFR*, epidermal growth factor receptor; *LRP1B*, low-density lipoprotein receptor-related protein 1B; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PFS, progression-free survival; *ROS1*, c-ros oncogene 1; *TP53*, tumor suppressor protein p53.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

XR, JZ and FL contributed to the conception, design, Supervision, and guidance of the study; HF and HX, performed the data collection and analyses and wrote the manuscript; GD, CY and LL assisted in project design, guided the statistical analysis, and reviewed the manuscript; ZJ assisted in bioinformatic analysis; XY and HG contributed to data collection and collation; XS helped perform the analysis with constructive discussions. XR, JZ and FL critically reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Shanxi Bethune Hospital (No. YXLL-2022-045) and conducted according to the Declaration of Helsinki. As this was a retrospective study, the requirement for informed consent was waived.

Acknowledgment

This work was supported by Cancer Biobank of Tianjin Medical University Cancer Institute and Hospital.

Funding

This work was supported by grants from the Health Commission of Shanxi Provincial [grant number 2019014] and the CAPTRA-Lung Scientific research fund of Beijing Cancer Prevention and treatment Society [grant number CAPTRALung2022010].

Conflict of Interest

LL and ZJ are both Medical Advisors of Genetron Health Inc and they declare no conflicts of interest. Other authors declare no potential conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2807147.

References

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians. 2021; 71: 7–33.

[2] Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. Translational Lung Cancer Research. 2016; 5: 288–300.

[3] Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, *et al*. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. The New England Journal of Medicine. 2014; 371: 2167–2177.

[4] Shaw AT, Ou SHI, Bang YJ, Camidge DR, Solomon BJ, Salgia R, *et al*. Crizotinib in ROS1-rearranged non-small-cell lung cancer. The New England Journal of Medicine. 2014; 371: 1963–1971.

[5] Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, *et al*. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. The New England Journal of Medicine. 2018; 378: 113–125.

[6] Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, *et al*. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. Lancet. 2015; 386: 444–451.

[7] Yu L, Lai Q, Gou L, Feng J, Yang J. Opportunities and obstacles of targeted therapy and immunotherapy in small cell lung cancer. Journal of Drug Targeting. 2021; 29: 1–11.

[8] Peters S, Reck M, Smit EF, Mok T, Hellmann MD. How to make the best use of immunotherapy as first-line treatment of advanced/metastatic non-small-cell lung cancer. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2019; 30: 884–896.

[9] Zhang W, Lin X, Li X, Wang M, Sun W, Han X, *et al*. Survival prediction model for non-small cell lung cancer based on somatic mutations. The Journal of Gene Medicine. 2020; 22: e3206.

[10] Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, *et al*. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clinical Cancer Research: an Official Journal of the American Association for Cancer Research. 2008; 14: 4275–4283.

[11] Evans M, O'Sullivan B, Smith M, Taniere P. Predictive markers for anti-PD-1/PD-L1 therapy in non-small cell lung cancer-where are we? Translational Lung Cancer Research. 2018; 7: 682–690.

[12] Baugh EH, Ke H, Levine AJ, Bonneau RA, Chan CS. Why are there hotspot mutations in the TP53 gene in human cancers? Cell Death and Differentiation. 2018; 25: 154–160.

[13] Gu J, Zhou Y, Huang L, Ou W, Wu J, Li S, *et al*. TP53 mutation is associated with a poor clinical outcome for non-small cell lung cancer: Evidence from a meta-analysis. Molecular and Clinical Oncology. 2016; 5: 705–713.

[14] Scoccianti C, Vesin A, Martel G, Olivier M, Brambilla E, Timsit JF, *et al*. Prognostic value of TP53, KRAS and EGFR mutations in nonsmall cell lung cancer: the EUELC cohort. The European Respiratory Journal. 2012; 40: 177–184.

[15] Jiao XD, Qin BD, You P, Cai J, Zang YS. The prognostic value of TP53 and its correlation with EGFR mutation in advanced non-small cell lung cancer, an analysis based on cBioPortal data base. Lung Cancer. 2018; 123: 70–75.

[16] Yi M, Li A, Zhou L, Chu Q, Song Y, Wu K. The global burden and attributable risk factor analysis of acute myeloid leukemia in 195 countries and territories from 1990 to 2017: estimates based on the global burden of disease study 2017. Journal of Hematology & Oncology. 2020; 13: 72.

[17] Lee SY, Jeon HS, Hwangbo Y, Jeong JY, Park JY, Lee EJ, *et al*. The influence of TP53 mutations on the prognosis of patients with early stage non-small cell lung cancer may depend on the intratumor heterogeneity of the mutations. Molecular Carcinogenesis. 2015; 54: 93–101.

[18] Sabapathy K, Lane DP. Therapeutic targeting of p53: all mutants are equal, but some mutants are more equal than others. Nature Reviews. Clinical Oncology. 2018; 15: 13–30.

[19] Petitjean A, Achatz MIW, Borresen-Dale AL, Hainaut P, Olivier M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene. 2007; 26: 2157–2165.

[20] Canale M, Petracci E, Delmonte A, Chiadini E, Dazzi C, Papi M, *et al*. Impact of *TP53* Mutations on Outcome in *EGFR*-Mutated Patients Treated with First-Line Tyrosine Kinase Inhibitors. Clinical Cancer Research: an Official Journal of the American Association for Cancer Research. 2017; 23: 2195–2202.

[21] Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, *et al*. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA: A Cancer Journal for Clinicians. 2017; 67: 93–99.

[22] Non Small Cell Lung Cancer, NCCN Clinical Practice Guidelines in Oncology. 2020. Available at: https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1450 (Accessed: 15 December 2020).

[23] Fu J, Guo W, Yan C, Lv Z, Wang Y, Wang Z, *et al*. Combining targeted sequencing and ultra-low-pass whole-genome sequencing for accurate somatic copy number alteration detection. Functional & Integrative Genomics. 2021; 21: 161–169.

[24] Cao L, Long L, Li M, Yang H, Deng P, Mao X, *et al*. The utilization of next-generation sequencing to detect somatic mutations and predict clinical prognosis of Chinese non-small cell lung cancer patients. OncoTargets and Therapy. 2018; 11: 2637–2646.

[25] Cameron LB, Hitchen N, Chandran E, Morris T, Manser R, Solomon BJ, *et al*. Targeted therapy for advanced anaplastic lymphoma kinase (*ALK*)-rearranged non-small cell lung cancer. The Cochrane Database of Systematic Reviews. 2022; 1: CD013453.

[26] Canale M, Petracci E, Delmonte A, Bronte G, Chiadini E, Ludovini V, *et al*. Concomitant *TP53* Mutation Confers Worse Prognosis in *EGFR*-Mutated Non-Small Cell Lung Cancer Patients Treated with TKIs. Journal of Clinical Medicine. 2020; 9: 1047.

[27] Fan Z, Zhang Q, Feng L, Wang L, Zhou X, Han J, *et al*. Genomic landscape and prognosis of patients with *TP53*-mutated non-small cell lung cancer. Annals of Translational Medicine. 2022; 10: 188.

[28] Zhou X, Hao Q, Lu H. Mutant p53 in cancer therapy-the barrier or the path. Journal of Molecular Cell Biology. 2019; 11: 293–305.

[29] Bria E, Pilotto S, Amato E, Fassan M, Novello S, Peretti U, *et al*. Molecular heterogeneity assessment by next-generation se-

quencing and response to gefitinib of EGFR mutant advanced lung adenocarcinoma. Oncotarget. 2015; 6: 12783–12795.
[30] Wang P, Wang F, He H, Chen Y, Lin H, Chen P, *et al*. TP53 and CDKN2A mutations in patients with early-stage lung squamous cell carcinoma: an analysis of the correlations and prognostic outcomes. Annals of Translational Medicine. 2021; 9: 1330.
[31] Chien WP, Wong RH, Cheng YW, Chen CY, Lee H. Associations of MDM2 SNP309, transcriptional activity, mRNA expression, and survival in stage I non-small-cell lung cancer patients with wild-type p53 tumors. Annals of Surgical Oncology. 2010; 17: 1194–1202.
[32] Vega FJ, Iniesta P, Caldés T, Sanchez A, López JA, de Juan C, *et al*. p53 exon 5 mutations as a prognostic indicator of shortened survival in non-small-cell lung cancer. British Journal of Cancer. 1997; 76: 44–51.
[33] Liu Y, Xu F, Wang Y, Wu Q, Wang B, Yao Y, *et al*. Mutations in exon 8 of *TP53* are associated with shorter survival in patients with advanced lung cancer. Oncology Letters. 2019; 18: 3159–3169.
[34] Elsayed M, Christopoulos P. Therapeutic Sequencing in ALK$^+$ NSCLC. Pharmaceuticals. 2021; 14: 80.
[35] Guo Y, Song J, Wang Y, Huang L, Sun L, Zhao J, *et al*. Concurrent Genetic Alterations and Other Biomarkers Predict Treatment Efficacy of EGFR-TKIs in EGFR-Mutant Non-Small Cell Lung Cancer: A Review. Frontiers in Oncology. 2020; 10: 610923.
[36] Skoulidis F, Heymach JV. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nature Reviews. Cancer. 2019; 19: 495–509.
[37] Qin K, Hou H, Liang Y, Zhang X. Prognostic value of TP53 concurrent mutations for EGFR- TKIs and ALK-TKIs based targeted therapy in advanced non-small cell lung cancer: a meta-analysis. BMC Cancer. 2020; 20: 328.
[38] Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, *et al*. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008; 455: 1069–1075.
[39] Liu CX, Musco S, Lisitsina NM, Yaklichkin SY, Lisitsyn NA. Genomic organization of a new candidate tumor suppressor gene, LRP1B. Genomics. 2000; 69: 271–274.
[40] Chen H, Chong W, Wu Q, Yao Y, Mao M, Wang X. Association of *LRP1B* Mutation With Tumor Mutation Burden and Outcomes in Melanoma and Non-small Cell Lung Cancer Patients Treated With Immune Check-Point Blockades. Frontiers in Immunology. 2019; 10: 1113.
[41] Jin Y, Chen YM, Hu X, Tang HR, Yu XM, Fan Y, *et al*. Analysis of the feasibility and prognostic value of circulating tumor DNA in detecting gene mutations in small cell lung cancer. Zhonghua Yi Xue Za Zhi. 2020; 100: 3614–3621. (In Chinese)
[42] Brown LC, Tucker MD, Sedhom R, Schwartz EB, Zhu J, Kao C, *et al*. *LRP1B* mutations are associated with favorable outcomes to immune checkpoint inhibitors across multiple cancer types. Journal for Immunotherapy of Cancer. 2021; 9: e001792.
[43] Johnson DB, Frampton GM, Rioth MJ, Yusko E, Xu Y, Guo X, *et al*. Targeted Next Generation Sequencing Identifies Markers of Response to PD-1 Blockade. Cancer Immunology Research. 2016; 4: 959–967.
[44] Ho WJ, Rooper L, Sagorsky S, Kang H. A robust response to combination immune checkpoint inhibitor therapy in HPV-related small cell cancer: a case report. Journal for Immunotherapy of Cancer. 2018; 6: 33.
[45] Domingo-Musibay E, Murugan P, Giubellino A, Sharma S, Steinberger D, Yuan J, *et al*. Near complete response to Pembrolizumab in microsatellite-stable metastatic sebaceous carcinoma. Journal for Immunotherapy of Cancer. 2018; 6: 58.
[46] Tucker MD, Zhu J, Marin D, Gupta RT, Gupta S, Berry WR, *et al*. Pembrolizumab in men with heavily treated metastatic castrate-resistant prostate cancer. Cancer Medicine. 2019; 8: 4644–4655.
[47] Ren X. Cancer immunology and immunotherapy. Cancer Biology & Medicine. 2021; 18: 931–933.