Original Article

http://www.jcpjournal.org
pISSN 2288-3649 · eISSN 2288-3657
https://doi.org/10.15430/JCP.2023.28.4.175

JCP

Prognostic Significance of Tumor Mutation Burden among Patients with Non-small Cell Lung Cancer Who Received Platinum-based Adjuvant Chemotherapy: An Exploratory Study

Wei-Xi Shen[1], Guang-Hua Li[2], Yu-Jia Li[2], Peng-Fei Zhang[2], Jia-Xing Yu[2], Di Shang[1], Qiu-Shi Wang[2]

[1]Department of Oncology, The Second Affiliated Hospital of Harbin Medical University, Harbin, [2]Department of Thoracic Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin, China

This study aimed to investigate the prognostic significance of tumor mutation burden (TMB) among patients with non-small cell lung cancer (NSCLC) who received platinum-based adjuvant chemotherapy. Tumor tissue specimens after surgical resection were collected for DNA extraction. Somatic mutation detection and TMB analysis were conducted using next-generation sequencing (NGS). Recurrence status of the patients was assessed in the hospital during the adjuvant chemotherapy period, and long-term survival data of patients were obtained by telephone follow-up. Univariate analysis between TMB status and prognosis was carried out by survival analysis. A retrospective review of 78 patients with non-squamous NSCLC who received platinum-based adjuvant chemotherapy showed a median disease-free survival of 3.6 years and median overall survival (OS) of 5.3 years. NGS analysis exhibited that the most common mutated somatic genes among the 78 patients were tumor suppressor protein p53 (TP53), epidermal growth factor receptor, low-density lipoprotein receptor related protein 1B, DNA methyltransferase 3 alpha and FAT atypical cadherin 3, and their prevalence was 56.4%, 48.7%, 37.2%, 30.7%, and 25.6%, respectively. TMB status was divided into TMB-L (≤ 4.5/Mb) and TMB-H (> 4.5/Mb) based on the median TMB threshold. Relevance of TMB to prognosis suggested that the median OS of patients with TMB-L was significantly longer than that of patients with TMB-H (NR vs. 4.6, $P = 0.014$). Higher TMB status conferred a worse implication on OS among patients with non-squamous NSCLC who received platinum-based adjuvant chemotherapy.

Key Words Non-small cell lung cancer, Platinum, Adjuvant chemotherapy, Prognosis

INTRODUCTION

Lung cancer is reported to be the most common solid malignancy in both male and female with the highest morbidity and mortality all over the world. It is estimated that there are approximately 2.1 million new cases and 1.77 million new deaths globally [1]. Similarly, approximately 815,000 new cases and 715,000 new deaths of lung cancer are reported in China recently [2]. As the most common subtype, non-small cell lung cancer (NSCLC) accounts for approximately 85% in lung cancer [3]. Currently, there are around 693,000 new cases and 608,000 new deaths of NSCLC in China. Still many patients are presented with locally advanced or metastatic disease at time of diagnosis and miss the opportunity for surgical resection [4]. Consequently, only approximately 20% of patients can receive surgical treatment. Even after surgical resection, considerable proportions of patients might experience recurrence [5]. The platinum-based regimens became the standard of care for NSCLC patients with stage II and III as postoperative adjuvant regimen that dramatically attenuated the risk of recurrence, which was proven to improve the 5-year survival rate of 5% [6].

Cisplatin combined with vinorelbine was widely used as adjuvant therapy for patients with NSCLC who underwent surgical resection initially [7]. Unfortunately, some patients are subjected to hematological adverse reactions, resulting in poor compliance of the regimen and worse completion cycle of adjuvant regimen [8]. Platinum combination of pemetrexed and docetaxel have been found to be safe and is widely used in adjuvant chemotherapy [9]. Nonetheless, the 5-year

Received August 14, 2023, Revised September 24, 2023, Accepted September 27, 2023
Correspondence to Qiu-Shi Wang, E-mail: qiushiwang2005@163.com, https://orcid.org/0000-0001-7221-9798

survival rates ranged from 36% to 66% for NSCLC patients with stage Ib and IIIa who received platinum-based adjuvant chemotherapy [10]. Noteworthily, in addition to pathological staging that served as the most important prognostic factor, a plethora of potential markers were considered to be of clinical significance for the assessment of efficacy and prognosis for platinum-based adjuvant chemotherapy [11].

Tumor mutation burden (TMB) was is defined as the density of the distribution of non-synonymous mutations in the protein coding region, which was is calculated by proportion between the total number of non-synonymous mutation in the protein coding region and the total length of the protein coding region with the unit of the number of mutations/MB [12]. Interestingly, recent years had witnessed great progress of immune checkpoint inhibitors, which brought new insights into the strategy of anti-tumor therapy [13]. However, immune checkpoint inhibitors were are only useful in part of the patients with the objective response rate of approximately 20% [14], which suggested suggests that potential biomarkers such as programmed death ligand 1 (PD-L1) expression and TMB were are of urgent value to be explored for PD-1/PD-L1 checkpoint blockade therapy clinically [15]. The previous study exhibited that higher TMB was associated with more neoantigens produced by tumor cells, increasing the chances for T cell recognition, which might efficaciously augment the probability of being recognized by the immune system [16]. Once PD-1 inhibitors activate the body's own anti-tumor immune response, the probability of killing tumor cells was amazing and the efficacy of PD-1/PD-L1 inhibitors was outstanding [17]. However, the prediction value of TMB is controversial and needs to be adequately investigated. The standard method for TMB testing is scanty, and it is unclear whether the detection of mutations is whole exome sequencing (WES) [18]. More importantly, the correlation between TMB status and the prognosis of patients with postoperative NSCLC administered with platinum-based adjuvant chemotherapy instead of immunotherapy is still unknown and whether TMB status can be used as a potential biomarker for the surgical resection patients with NSCLC who received conventional adjuvant therapy is unclear [19]. Interestingly, a previous study initiated by Devarakonda and colleagues [20] in the adjuvant treatment of NSCLC demonstrated that NSCLC patients with higher TMB who underwent surgical resection exhibited superior disease-free survival (DFS) and overall survival (OS). However, the clinical significance of TMB status among Chinese postoperative patients with NSCLC who received platinum-based conventional adjuvant chemotherapy has not been explored yet.

The aim of this study was to identify the somatic gene mutation profile and clinical implication of TMB among patients with NSCLC who underwent surgical resection and received platinum-based adjuvant chemotherapy.

MATERIALS AND METHODS

Study design and eligibility criteria

Given that platinum-based adjuvant chemotherapy was widely used, and considerable patients with NSCLC were treated with platinum-based adjuvant chemotherapy in clinical practice, this study was designed as a single center retrospective research. Consequently, patients diagnosed with NSCLC who received surgical resection in the Department of Thoracic Surgery of the Second Affiliated Hospital of Harbin Medical University from January 2011 to December 2021 were included in our study consecutively. Main eligibility criteria included: (1) age ≥18 years; (2) eastern cooperative oncology group (ECOG) performance status (PS) score of 0-2 score; (3) clinically diagnosed of NSCLC and subsequent surgical resection; (4) adequate hematological availability for receiving adjuvant chemotherapy; (5) pathological staging of II or IIIa; (6) platinum-based adjuvant chemotherapy practiced. The main exclusion criteria were: (1) concomitant occurrence of another tumor or serious diseases that might shorten the survival of the patients; (2) tumor tissue for TMB analysis not available; (3) patients who failed to receive surgical resection or platinum-based adjuvant chemotherapy; (4) patients diagnosed of squamous cell lung carcinoma. The flow chart of this retrospective study was illustrated in Figure 1. Eventually, a total of 78 patients with NSCLC who met the eligibility criteria participated in this study retrospectively. The primary analysis of present study was the relevance of TMB status to the prognosis of the patients. This study was approved by the ethics committee of the Second Affiliated Hospital of Harbin Medical University (approved number: KY-2020156). Informed consent was signed by each enrolled patient in accordance with the recommendation of the declaration of Helsinki.

Adjuvant chemotherapy regimens of the patients after surgery

All the 78 patients with non-squamous NSCLC who participated in this study were treated with clinically conventional platinum-based adjuvant chemotherapy, and the usage and dosage were as follows: 4 to 6 weeks after surgery according to the physical recovery status of the patients, cisplatin 75 mg/m^2 or carboplatin AUC5 on the first day and combined with pemetrexed 500 mg/m^2, docetaxel 60 to 75 mg/m^2 or vinorelbine 25 to 30 mg/m^2 on the first day, every 21 days was deemed as one cycle; adjuvant chemotherapy was given for 4 cycles or depended on the actual situation of the patients. Each patient was followed up from the administration of adjuvant chemotherapy until disease recurrence or death.

Collection of tumor tissue specimen and sequencing analysis of targeted DNA

Postoperative tumor tissue specimens were collected from the patients with NSCLC during the surgical resection, and tissue specimens were stored in liquid nitrogen subsequently.

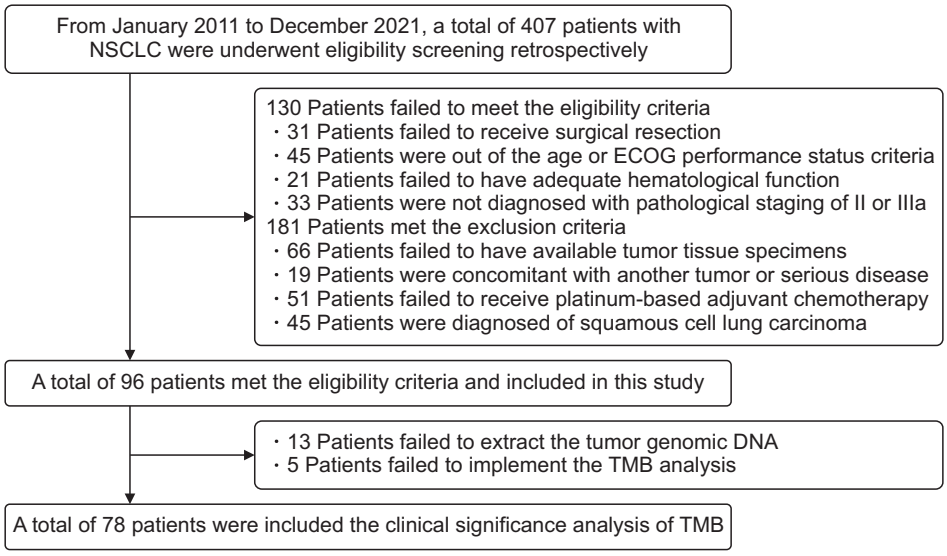

Figure 1. Flow chart of our retrospective study with the 78 postoperative non-squamous non-small cell lung cancer (NSCLC) patients who received platinum-based adjuvant chemotherapy. ECOG, eastern cooperative oncology group; TMB, tumor mutation burden.

Accordingly, DNA was extracted using a DNA FFPE tissue kit (QIAGEN) from the postoperative tumor tissue specimen, and the DNA concentration was measured by the Qubitds DNA assay to make sure that A260/A280 was within the range of 1.8 to 2.0. Simultaneously, approximately 5 mL of peripheral blood was collected and incubated at room temperature for 2 hours. Then the supernatant was transferred to 15-mL centrifuge tubes and centrifuged to obtain the supernatant. Circulating cfDNA was recovered from 4 to 5 mL of plasma by using the QIAamp Circulating Nucleic Acid kit (Qiagen) accordingly. And then approximately Approximately 50 ng of cell free DNA was required for construction of an NGS library.

Targeted DNA sequencing was implemented when the DNA from tumor tissue specimens was extracted. DNA was profiled by using a capture-based targeted sequencing panel (Burning Rock Biotech), targeting 520 cancer-related genes, and spanned 1.67 M of Human genomic regions. More detailed genes were available from the previous study [21]. The somatic alterations in exons of coding regions and the adjacent 20-bp length of both upstream and downstream sequences were included in the calculation of TMB. Fragments of 200 to 400-bp sizes were selected with beads (Agencourt AMPure XP kit; Beckman Coulter), followed by hybridization with the capture probes baits, hybrid selection with magnetic beads, and PCR amplification. Probe hybridization and high-throughput sequencing were used to detect hot-spot mutation regions (exon, intron or promoter regions) of related genes, thus detecting the mutations, amplification and rearrangement (fusion) of genes relevant to cancer comprehensively and accurately.

TMB analysis

The detailed methods of sequencing data analysis were reported previously [22]. The somatic alterations in exons of coding regions and the adjacent 20-bp length of both upstream and downstream sequences were included in the calculation of TMB, which was the TMB region covered. The fusion, copy number variation and non-coding region mutation were not counted as TMB. Furthermore, synonymous mutations were also taken into consideration in order to reduce sampling noise. Additionally, analysis of cell free DNA from plasma was used to filter germline mutation (for instance: genetic polymorphisms, etc.). Besides, it should be noted that TMB status was divided into TMB the high level group (TMB-H) and TMB the low level group (TMB-L) according to the median TMB threshold value.

Statistical analysis

SPSS version 25.0 (IBM Corp.) was used to implement the statistical analysis in this study. In the analysis of baseline characteristics, the correlation between proportions variable and TMB status was analyzed using the chi-square test, and the Fisher's exact test was introduced when data was rare. The association analysis between continuous variable and TMB status was carried out using the Mann–Whitney U nonparametric test. In terms of the survival analysis, Kaplan–Meier survival curves were produced using Stata 14.0 (Stata Co.) to compare the differences in OS and DFS according to TMB status. The log-rank test was used to compare the survival differences. DFS was defined as the interval from the completion of surgical resection to disease recurrence or death from any cause, whichever occurred first. OS was defined from the time of the completion for surgical resection to patients' death from any cause those without death event by the end of the study follow-up, survival end points were censored at the date of last follow-up. Additionally, Cox multivariable analysis was introduced for measuring OS. Statistical significance was accepted when $P < 0.05$.

RESULTS

Baseline characteristics

Baseline clinical characteristics of 78 patients with non-squamous NSCLC who underwent surgical resection in this retrospective study exhibited in Table 1. The median age of the patients was 63 years (range: 21-78 years). The numbers of male and female patients were 37 and 41, respectively. ECOG PS of 0 and 1-2 score was noted in 49 patients and 29 patients, respectively. 52.5% patients were exposure to cigarette previously. Majority histological type of the patients was lung adenocarcinoma (91.0%). Additionally, pathological staging of II and IIIa was observed in 51 and 27 patients, respectively. Interestingly, of the epidermal growth factor receptor (EGFR) positive patients, a total of 38 patients (48.7%) had EGFR positive mutation (in addition to the common EGFR exon 18 to 21 mutations, other exon mutations in the EGFR gene were also defined as EGFR positive mutations). Regarding the surgical resection type, lobectomy and pneumonectomy were seen in 61 and 17 patients, respectively. Furthermore, cisplatin-based adjuvant regimen was noted in 53 patients and carboplatin-based regimen was observed in 25 patients. Regimens of platinum combined with pemetrexed, docetaxel, and vinorelbine were given to 41, 19, and 18 patients, respectively. The median cycle of adjuvant chemotherapy was 3 cycles with the range of 1 to 5 cycles.

The TMB profile among the 78 patients with non-squamous NSCLC was illustrated in Figure 2. The median TMB in the 78 patients was 4.3/Mb (range: 1.5-45.2/Mb). As described previously, TMB status was divided into two groups according to the median TMB threshold value. Therefore, patients with TMB ranging from 1.5 to 4.3/Mb were classified as the TMB-L and those with TMB ranging from 4.3 to 45.2/Mb were classified as TMB-H. The prevalence of TMB-L and

Table 1. Comparison of baseline characteristics of the 78 patients with non-squamous non-small cell lung cancer according to the tumor mutation burden status

Baseline characteristics	Total	TMB status		χ^2	*P*-value
		TMB-H (n = 38)	TMB-L (n = 40)		
Age (yr)				NA	0.617
Median (range)	63 (21-78)	63 (25-78)	63 (21-77)		
Sex				0.216	0.642
Male	37 (47.4)	17 (44.7)	20 (50.0)		
Female	41 (52.6)	21 (55.3)	20 (50.0)		
ECOG PS score				0.004	0.952
0	49 (62.8)	24 (63.2)	25 (62.5)		
1-2	29 (37.2)	14 (36.8)	15 (37.5)		
Smoking history				0.000	0.991
Yes	41 (52.5)	20 (52.6)	21 (52.5)		
No	37 (47.4)	18 (47.4)	19 (47.5)		
Histological type					
Adenocarcinoma	71 (91.0)	35 (92.1)	36 (90.0)	0.106	0.745
Other type	7 (9.0)	3 (7.9)	4 (10.0)		
Pathological staging				0.773	0.379
II	51 (65.4)	23 (60.5)	28 (70.0)		
IIIa	27 (34.6)	15 (39.5)	12 (30.0)		
EGFR gene mutation status				0.470	0.493
Positive	38 (48.7)	17 (44.7)	21 (52.5)		
Negative	40 (51.3)	21 (55.3)	19 (47.5)		
Surgical resection type				0.889	0.346
Lobectomy	61 (78.2)	28 (73.7)	33 (82.5)		
Pneumonectomy	17 (21.8)	10 (26.3)	7 (17.5)		
Adjuvant chemotherapy regimens				0.008	0.931
Cisplatin-based regimen	53 (67.9)	26 (68.4)	27 (67.5)		
Carboplatin-based regimen	25 (32.1)	12 (31.6)	13 (32.5)		
Adjuvant chemotherapy regimens				0.248	0.883
Platinum combined with pemetrexed	41 (52.6)	20 (52.6)	21 (52.5)		
Platinum combined with docetaxel	19 (24.4)	10 (26.3)	9 (22.5)		
Platinum combined with vinorelbine	18 (23.1)	8 (21.1)	10 (25.0)		
Cycles of adjuvant chemotherapy				NA	0.621
Median (range)	3 (1-5)	3 (1-5)	3 (1-5)		

Values are presented as number (%). TMB, tumor mutation burden; TMB-H, TMB the high level group; TMB-L, TMB the low level group; ECOG, eastern cooperative oncology group; PS, performance status; EGFR, epidermal growth factor receptor; NA, not available.

TMB-H were observed in 40 and 38 patients, respectively. As showed in Table 1, patients with TMB-L and TMB-H shared the similar baseline characteristics and no statistically significant difference was found ($P > 0.05$).

Somatic gene mutation profile analysis

The present study further explored the somatic gene mutation profile in the 78 patients with non-squamous NSCLC. As described in the method part, the somatic gene mutation analysis of this study was based on the NGS sequencing platform, and a total of 520 cancer-related genes were selected and analyzed. The somatic gene mutation profile is displayed in Figure 3. The most common (mutation incidence rate > 20%) cancer-related mutated genes among the 78 patients with non-squamous NSCLC are TP53, EGFR, LRP1B, DNMT3A, FAT3, KRAS, and SPTA1 with the mutation frequency was 56.4%, 48.7%, 37.2%, 30.7%, 25.6%, 24.4%, and 21.8%, respectively. Furthermore, the other somatic genes were KEAP1, NF1, STK11, SETD2, PIK3CA, PTPRD, SMARCA4, KMT2D, MYC, ATM, MAP3K13, STAT3, and KMT2A, with the mutation frequency of > 10%. Additionally, it should be noted that numerous genes were observed with comparatively low frequency of < 10%. All the somatic genes with the frequency > 3.8% are illustrated in Figure 3. Somatic genes with a mutation frequency of < 3% were not exhibited in Figure 3.

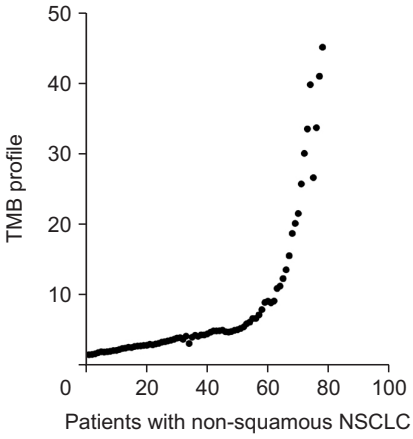

Figure 2. The tumor mutation burden (TMB) profile of the 78 postoperative patients with non-squamous non-small cell lung cancer (NSCLC).

Clinical implication of TMB

All the 78 patients included in this study were available for prognostic assessment, and the last follow-up date of this study was May 2021. The median follow-up duration from the patients included in this study to last follow-up date was 4.8 years (follow-up range: 0.25-8 years). Prognostic data of the 78 patients with non-squamous NSCLC is illustrated in Figure 4. The median DFS of the 78 patients was 3.6 years (95% CI, 2.63-4.57), and the median OS of the 78 patients was 5.3 years (95% CI, 4.37-6.23).

As described in the method part, TMB-L and TMB-H were observed in 40 and 38 patients, respectively. The relevance of TMB to prognosis was implemented in this study. The correlation between TMB and DFS was exhibited in Figure 5, the median DFS of patients with TMB-L and TMB-H was 4.6

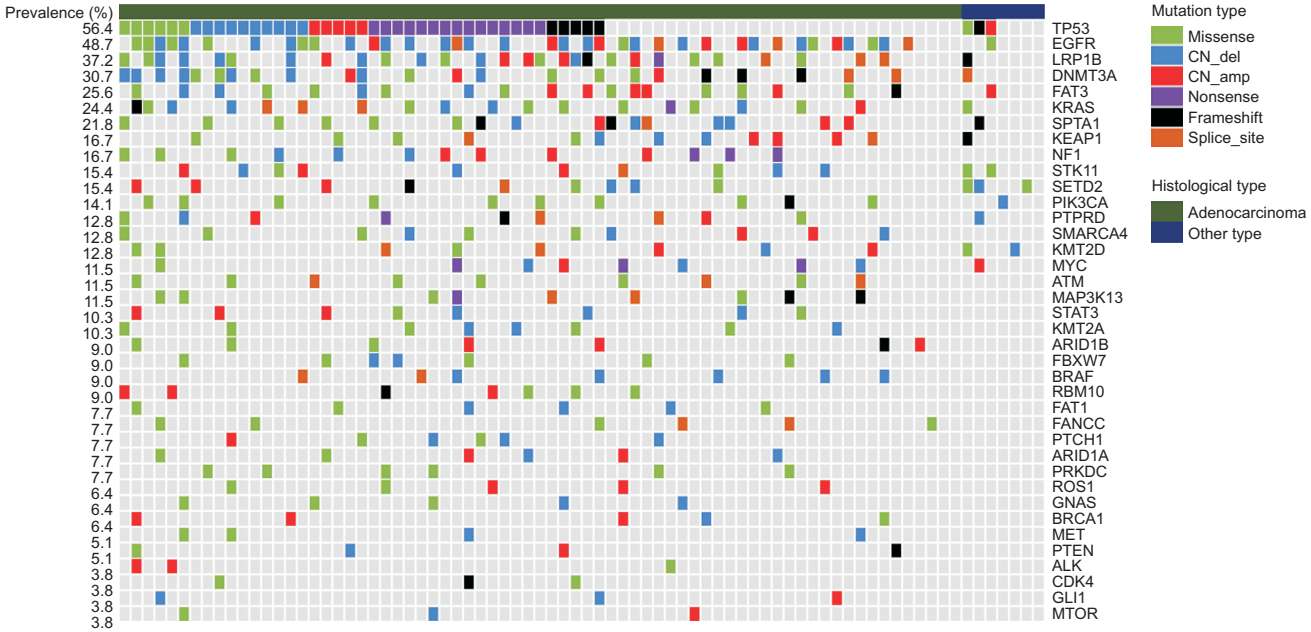

Figure 3. The somatic mutation spectrum of the 78 postoperative patients with non-squamous non-small cell lung cancer identified by targeted next generation sequencing.

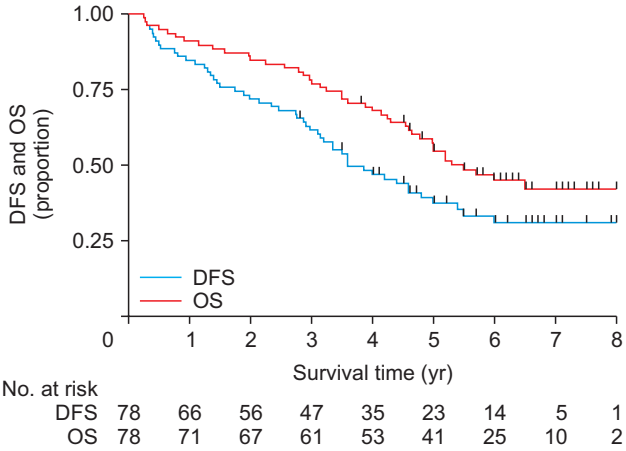

Figure 4. The disease-free survival (DFS) and overall survival (OS) curve of the 78 patients with non-squamous non-small cell lung cancer.

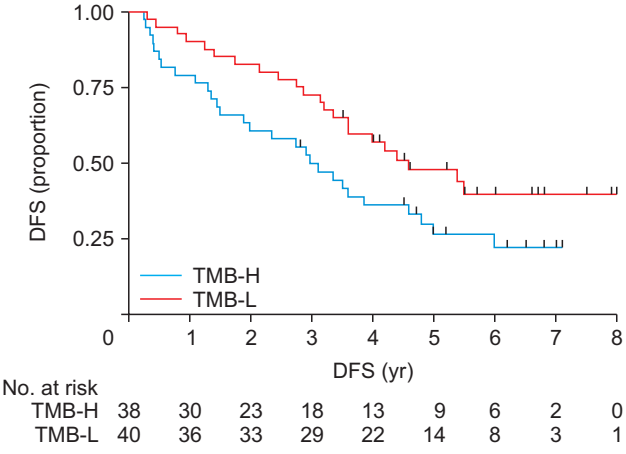

Figure 5. Comparison of the disease-free survival (DFS) in the 78 patients with non-squamous non-small cell lung cancer according to the tumor mutation burden (TMB) status. TMB-L, TMB the low level group; TMB-H, TMB the high level group.

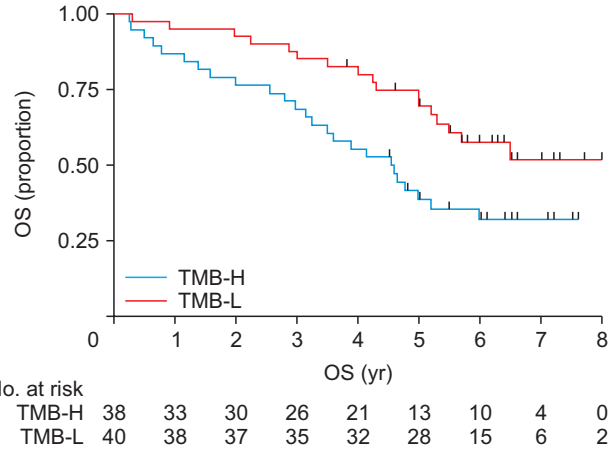

Figure 6. Comparison of the overall survival (OS) in the 78 patients with non-squamous NSCLC according to the TMB status. NSCLC, non-small cell lung cancer; TMB, tumor mutation burden; TMB-L, TMB the low level group; TMB-H, TMB the high level group; NR, not reached; NA, not available.

years (95% CI, 2.88-6.32) and 3.0 years (95% CI, 2.11-3.85), respectively, which showed a marginally statistical significant (χ^2 = 4.11, P = 0.045).

Association between TMB status and OS is illustrated in Figure 6; the median OS of patients with TMB-L and TMB-H was not reached (NR) (95% CI, not available [NA]) and 4.6 years (95% CI, 3.44-5.66), respectively. The difference was statistically significant (χ^2 = 6.04, P = 0.014). Furthermore, in order to identify the clinical significance of TMB status independently, a multivariate Cox regression analysis was introduced for OS. Univariate analysis between clinical characteristics (e.g., age, gender, ECOG PS score, smoking history, histological type, pathological staging, EGFR gene mutation status, surgical resection type, adjuvant chemotherapy regimen and TMB status, etc.), and OS was analyzed separately. As shown in Table 2, age, ECOG PS score, pathological staging, and TMB status were of positive relevance to OS in the univariate analysis. Consequently, these characteristics were taken into consideration in the multivariate Cox regression analysis accordingly, and the results are presented in Table 2, TMB status was an independent factor for OS (hazard ratio [HR] = 0.74; 95% CI, 0.45-0.92; P = 0.025) after multivariate adjustment. Other statistically significant variables in the multivariate Cox analysis were are age (HR = 0.73, P = 0.019), ECOG PS score (HR = 0.71, P = 0.015) and pathological staging (HR = 0.64, P = 0.009), which demonstrated that patients of < 63 years had longer OS than that of patients with ≥ 63 years (median OS: NR vs. 4.4 years), patients with ECOG 0 score conferred superior OS compared with that of patients with 1-2 score (median OS: NR vs. 4.2 years) and patients with pathological staging of II were associated with better OS than that of patients with pathological staging of IIIa (median OS: NR vs. 3.9 years).

DISCUSSION

NSCLC was defined as a highly heterogeneous malignant tumor [23]. Recent years have witnessed remarkable progress regarding the NSCLC treatment, which made the field of advanced NSCLC enter the age of chemotherapy-free therapy preliminarily [24]. However, platinum-based adjuvant chemotherapy still plays an essential role in the field of early-stage NSCLC, and the immune checkpoint inhibitors are still under exploration as adjuvant therapy with limited results being reported [25]. Consequently, it is necessary to identify the efficacious patients and the potential biomarkers to predict the prognosis of platinum-based adjuvant chemotherapy.

The real-world prognostic evidence of the 78 patients with non-squamous NSCLC who received platinum-based adjuvant chemotherapy in this study suggests that the median

Table 2. Univariate and multivariate analyses for overall survival among 78 patients with non-squamous non-small cell lung cancer

Baseline characteristics	Median OS (95% CI)	*P* (univariate analysis)	Multivariate analysis	
			HR (95% CI)	*P*-value
Age		0.009	0.73 (0.44-0.91)	0.019
<63	NR (NA)			
≥63	4.4 (3.21-5.59)			
Sex		0.415		
Male	5.0 (4.24-6.76)			
Female	5.8 (4.87-6.73)			
ECOG PS score		0.011	0.71 (0.39-0.89)	0.015
0	NR (NA)			
1-2	4.2 (3.15-5.25)			
Smoking history		0.732		
Yes	5.3 (4.22-6.38)			
No	5.3 (4.19-6.41)			
Histological type		0.435		
Adenocarcinoma	5.5 (4.41-6.59)			
Other type	5.3 (4.11-6.49)			
Pathological staging		0.007	0.64 (0.32-0.81)	0.009
II	NR (NA)			
IIIa	3.9 (3.02-4.78)			
EGFR gene mutation status		0.515		
Positive	5.5 (4.49-6.51)			
Negative	5.1 (4.17-6.03)			
Surgical resection type		0.612		
Lobectomy	5.5 (4.31-6.69)			
Pneumonectomy	5.3 (4.22-6.38)			
Adjuvant chemotherapy regimens		0.415		
Cisplatin-based regimen	5.3 (4.49-6.11)			
Carboplatin-based regimen	5.3 (4.21-6.39)			
TMB status		0.014	0.74 (0.45-0.92)	0.025
TMB-L	NR (NA)			
TMB-H	4.6 (3.44-5.66)			

OS, overall survival; HR, hazard ratio; ECOG, eastern cooperative oncology group; PS, performance status; EGFR, epidermal growth factor receptor; TMB, tumor mutation burden; TMB-L, TMB the low level group; TMB-H, TMB the high level group; NR, not reached; NA, not available.

DFS is 3.6 years and the median OS is 5.3 years. DFS results of this study are inferior to the median DFS (3.8 years) in the Phase III study of postoperative patients with NSCLC treated with cisplatin-based adjuvant chemotherapy in the International Collaboration of Adjuvant Trials for Lung Cancer [26]. We speculate that the reason could be attributed to the retrospective design of our study.

Obviously, the management of patients was poor and insufficient in the retrospective study when compared with the phase III clinical trials, which was also confirmed in the other retrospective study conducted previously [27]. It should be noted that considerable patients with the ECOG PS 2 score was included in our study (20%). However, only a small number of patients with the ECOG PS 2 score (7.7%) was included in the phase III clinical trial, and 35% of patients in that study were of stage I. Furthermore, multivariate Cox analysis of our study suggested that patients with ECOG 1-2 score conferred worse prognosis compared with those with 0 score, thus contributing to the comparatively superior DFS of the phase III clinical trial. On the other hand, the median OS in our study was 5.3 years, which was relatively longer than that observed in the clinical trial (median OS = 4.4 years). We thought the reason could be attributed to the tendency that numerous PD-1/PD-L1 inhibitors were licensed in China since 2018, postoperative patients with NSCLC stood a good chance to select immunotherapy in the subsequent treatment when relapsed, which was effective and provided the patients with survival benefits dramatically [28]. Additionally, the clinical significance of age, ECOG score and pathological staging for OS identified in this study was consistent with the conclusion made in the study previously [29].

The somatic mutation and TMB analysis in our study was were based on the targeted next-generation sequencing technology, which was proven to be an accurate and clinically available approach for measuring targeted gene mutation with a greater depth and less time compared with WES [30]. Consequently, it was a useful and crucial method for the somatic mutation detection and TMB analysis in view of the

wide genetic heterogeneity of NSCLC [31]. Interestingly, the results of somatic mutation based on NGS analysis in this study suggested that TP53 gene was of the highest mutation frequency in postsurgical NSCLC tissue (56.4%), which was basically consistent with the results of the research implemented by the Singal and colleagues [32] that investigated the clinical significance of TMB in patients with NSCLC. Similarly, the most common somatic gene in Singal et al. [32] study was also the TP53 gene with the mutation frequency of 53%. These results demonstrated the essential function of TP53 gene in tumorigenesis.

TP53 was the most common tumor suppressors suppressor found in multiple human cancers that regulated regulates cell division by keeping cells from growing and proliferating, thus playing an important regulatory role in the development and recurrence of NSCLC [33]. Mutations in TP53 contributed leads to the loss of intracellular p53 protein involved in abnormal apoptotic progression, inhibition of angiogenesis and oncogenic signaling [34]. TP53 mutation was found to be a negative prognostic factor for OS [35]. Furthermore, the somatic gene mutation profile in our study also highlighted that genes encoding EGFR, LRP1B, DNMT3A, FAT3, KRAS and SPTA1 second only to TP53 were closely related to the occurrence and tumorigenesis of NSCLC, and the mutation frequencies were 48.7%, 37.2%, 30.7%, 25.6%, 24.4%, and 21.8%, respectively. This mutation profile was consistent with previous research initiated by Lin and colleagues [17]. Their study also used the NGS technique to extract DNA from the frozen or paraffin-embedded tumor tissue to analyze the somatic mutation genes related; which exhibited that frozen or paraffin-embedded tumor tissue was reliable to perform somatic gene mutation analysis [36]. Additionally, our study also found involvement of KEAP1 and NF1 in NSCLC with the prevalence of 16.7% and 11.5%, indicating that the development of NSCLC is heterogeneous to some extent and that some rare mutated gene might contribute to the occurrence of NSCLC to some extent [37].

TMB results suggested that the overall somatic mutation burden of 78 patients was comparatively low with the median TMB of 4.3/MB (range: 1.5-45.2/MB), which was relatively lower than that observed in previous postoperative patients with NSCLC conducted by the Devarakonda and colleagues (the median TMB was 5.7/MB) [20]. This discrepancy could be attributed to the fact that our study detected only 520 genes involved in the development of tumor whilst Devarakonda et al.'s [20] study included a total of 1,538 genes for TMB analysis. Besides, according to a previous study that explored the genetic mutation burden among various solid tumors [38], TMB was highest in patients with melanoma and lung cancer, approximately 10/Mb and the TMB in non-squamous NSCLC was about 7/Mb, which were basically consistent with the results of TMB in our study. All the TMB data preliminarily highlighted that TMB in patients with non-squamous NSCLC might be lower than that in patients with squamous cell lung carcinoma.

Interestingly, the reason why we divided TMB status into two groups according to the median TMB threshold value rather than 10 or 20 per Mb was due to the fact that there was no lack of a uniform threshold for TMB clinical evaluation currently [39]. Furthermore, the relevance of TMB status to prognosis of the patients in our study demonstrated that the median OS of patients with TMB-H was inferior to that of patients with TMB-L, which was in concert with the results of the study initiated by Nie and colleagues [40]. A total of 425 patients with advanced NSCLC who received docetaxel monotherapy were participated in their study, and the association between TMB status in blood samples and prognosis using NGS sequencing was analyzed. The results exhibited that lower TMB levels in the blood samples were significantly associated with longer OS and DFS. A recent research also suggested association of higher TMB with worse prognosis when receiving conventional adjuvant chemotherapy, which was in line with the results of our study [22]. Furthermore, the TMB threshold in the above study was the same as that in our study with the median threshold to distinguish TMB high and TMB low. Obviously, considerable studies concluded that patients with TMB-L conferred comparatively superior clinical outcomes among patients with early-stage or advanced-stage when they were treated with conventional chemotherapy [20,32,41]. Collectively, our study preliminarily suggest that patients with non-squamous NSCLC of higher TMB experience worse prognosis following platinum-based adjuvant chemotherapy. And the Such conclusion needed to be validated in large-scale clinical trials subsequently.

From the objective view, limitations in our study inevitably. Firstly, the sample size small, and clinical significance of TMB among patients with non-squamous NSCLC still should be evaluated in a larger population. Secondly, the TMB profile in our study is limited to only 520 genes related to tumorigenesis and development, and a deeper evaluation in more genes is needed.

In conclusion, our present study provided valid data for the mutation landscape profile among Chinese patients with NSCLC with some novel somatic mutation genes presented specifically. Distinct from the results of previous studies, we found that a higher TMB level was associated worse prognosis among patients who received platinum-based adjuvant chemotherapy. Furthermore, our study provided real-world prognostic evidence for patients with NSCLC who received platinum-based adjuvant chemotherapy, and the exploratory analysis suggests that higher TMB status confers a worse implication on OS among patients with non-squamous NSCLC treated with platinum-based adjuvant chemotherapy. Therefore, TMB status might be used as a potential biomarker to predict the prognosis of patients with NSCLC who received platinum-based adjuvant chemotherapy.

ACKNOWLEDGMENTS

The authors would like to express our sincere gratitude to the patients and their relatives for agreeing to participate in this work.

FUNDING

None.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ORCID

Wei-Xi Shen, https://orcid.org/0000-0003-4081-3711
Guang-Hua Li, https://orcid.org/0000-0002-8549-5473
Yu-Jia Li, https://orcid.org/0000-0002-8135-2168
Peng-Fei Zhang, https://orcid.org/0009-0006-9112-4456
Jia-Xing Yu, https://orcid.org/0009-0009-5128-8351
Di Shang, https://orcid.org/0009-0006-8958-6600
Qiu-Shi Wang, https://orcid.org/0000-0001-7221-9798

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424. Erratum in: CA Cancer J Clin 2020;70:313.
2. Guo H, Li X, Li W, Wu J, Wang S, Wei J. Climatic modification effects on the association between PM1 and lung cancer incidence in China. BMC Public Health 2021;21:880.
3. Lv M, Li X, Tian W, Yang H, Zhou B. ADGRD1 as a potential prognostic and immunological biomarker in non-small-cell lung cancer. Biomed Res Int 2022;2022:5699892.
4. Jahanzeb M, Lin HM, Pan X, Yin Y, Baumann P, Langer CJ. Immunotherapy treatment patterns and outcomes among ALK-positive patients with non-small-cell lung cancer. Clin Lung Cancer 2021;22:49-57.
5. Katsurada N, Tachihara M, Hatakeyama Y, Koyama K, Yumura M, Kiriu T, et al. Feasibility study of adjuvant chemotherapy with carboplatin and nab-paclitaxel for completely resected NSCLC. Cancer Manag Res 2020;12:777-82.
6. Pirker R, Filipits M. Adjuvant therapy in patients with completely resected non-small-cell lung cancer: current status and perspectives. Clin Lung Cancer 2019;20:1-6.
7. Douillard JY, Rosell R, De Lena M, Carpagnano F, Ramlau R, Gonzáles-Larriba JL, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719-27. Erratum in: Lancet Oncol 2006;7:797.
8. Kreuter M, Vansteenkiste J, Fischer JR, Eberhardt W, Zabeck H, Kollmeier J, et al. Randomized phase 2 trial on refinement of early-stage NSCLC adjuvant chemotherapy with cisplatin and pemetrexed versus cisplatin and vinorelbine: the TREAT study. Ann Oncol 2013;24:986-92.
9. Yamamoto N, Kenmotsu H, Yamanaka T, Nakamura S, Tsuboi M. Randomized phase III study of cisplatin with pemetrexed and cisplatin with vinorelbine for completely resected nonsquamous non-small-cell lung cancer: the JIPANG study protocol. Clin Lung Cancer 2018;19:e1-3.
10. Okamoto T, Yano T, Shimokawa M, Takeo S, Yamazaki K, Sugio K, et al. A phase II randomized trial of adjuvant chemotherapy with S-1 versus S-1 plus cisplatin for completely resected pathological stage II/IIIA non-small cell lung cancer. Lung Cancer 2018;124:255-9.
11. Kusuhara S, Igawa S, Ichinoe M, Nagashio R, Kuchitsu Y, Hiyoshi Y, et al. Prognostic significance of galectin-3 expression in patients with resected NSCLC treated with platinum-based adjuvant chemotherapy. Thorac Cancer 2021;12:1570-8.
12. Steuer CE, Ramalingam SS. Tumor mutation burden: leading immunotherapy to the era of precision medicine? J Clin Oncol 2018;36:631-2.
13. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annu Rev Pathol 2021;16:223-49.
14. Guzik K, Tomala M, Muszak D, Konieczny M, Hec A, Błaszkiewicz U, et al. Development of the inhibitors that target the PD-1/PD-L1 interaction-a brief look at progress on small molecules, peptides and macrocycles. Molecules 2019;24:2071.
15. Berland L, Heeke S, Humbert O, Macocco A, Long-Mira E, Lassalle S, et al. Current views on tumor mutational burden in patients with non-small cell lung cancer treated by immune checkpoint inhibitors. J Thorac Dis 2019;11(Suppl 1):S71-80.
16. Efremova M, Finotello F, Rieder D, Trajanoski Z. Neoantigens generated by individual mutations and their role in cancer immunity and immunotherapy. Front Immunol 2017;8:1679.
17. Lin C, Shi X, Zhao J, He Q, Fan Y, Xu W, et al. Tumor mutation burden correlates with efficacy of chemotherapy/targeted therapy in advanced non-small cell lung cancer. Front Oncol 2020;10:480.
18. Jardim DL, Goodman A, de Melo Gagliato D, Kurzrock R. The challenges of tumor mutational burden as an immunotherapy biomarker. Cancer Cell 2021;39:154-73.
19. Owada-Ozaki Y, Muto S, Takagi H, Inoue T, Watanabe Y, Fukuhara M, et al. Prognostic impact of tumor mutation burden in patients with completely resected non-small cell lung cancer: brief report. J Thorac Oncol 2018;13:1217-21.
20. Devarakonda S, Rotolo F, Tsao MS, Lanc I, Brambilla E, Masood A, et al. Tumor mutation burden as a biomarker in resected non-small-cell lung cancer. J Clin Oncol 2018;36:2995-3006.
21. Jiao XD, Zhang XC, Qin BD, Liu D, Liu L, Ni JJ, et al. Tumor mutation burden in Chinese cancer patients and the underlying driving pathways of high tumor mutation burden across different cancer types. Ann Transl Med 2020;8:860.

22. Xu LB, Zhao ZG, Xu SF, Zhang XX, Liu T, Jing CY, et al. The landscape of gene mutations and clinical significance of tumor mutation burden in patients with soft tissue sarcoma who underwent surgical resection and received conventional adjuvant therapy. Int J Biol Markers 2020;35:14-22.
23. Fu Y, Zhuang X, Xia X, Li X, Xiao K, Liu X. Correlation between promoter hypomethylation and increased expression of syncytin-1 in non-small cell lung cancer. Int J Gen Med 2021;14: 957-65.
24. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet 2019;393:1819-30.
25. Vansteenkiste J, Wauters E, Reymen B, Ackermann CJ, Peters S, De Ruysscher D. Current status of immune checkpoint inhibition in early-stage NSCLC. Ann Oncol 2019;30:1244-53.
26. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351-60.
27. Zhong Y, Wei Q, Lu Y, Tang X, Wang Z, Chen L. Efficacy and safety of anlotinib in patients with advanced non-small cell lung cancer. J Thorac Dis 2020;12:6016-22.
28. Qu J, Mei Q, Liu L, Cheng T, Wang P, Chen L, et al. The progress and challenge of anti-PD-1/PD-L1 immunotherapy in treating non-small cell lung cancer. Ther Adv Med Oncol 2021; 13:1758835921992968.
29. Soh J, Toyooka S, Okumura N, Nakamura H, Nakata M, Yamashita M, et al. Impact of pathological stage and histological subtype on clinical outcome of adjuvant chemotherapy of paclitaxel plus carboplatin versus oral uracil-tegafur for non-small cell lung cancer: subanalysis of SLCG0401 trial. Int J Clin Oncol 2019;24:1367-76.
30. Borówka P, Pułaski Ł, Marciniak B, Borowska-Strugińska B, Dziadek J, Żądzińska E, et al. Screening methods for detection of ancient Mycobacterium tuberculosis complex fingerprints in next-generation sequencing data derived from skeletal samples. Gigascience 2019;8:giz065.
31. Zhang H, Deng YM, Chen ZC, Tang YC, Yang S, Zhang SD, et al. Clinical significance of tumor mutation burden and DNA damage repair in advanced stage non-small cell lung cancer patients. Eur Rev Med Pharmacol Sci 2020;24:7664-72.
32. Singal G, Miller PG, Agarwala V, Li G, Kaushik G, Backenroth D, et al. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. JAMA 2019;321:1391-9.
33. Tanimoto A, Matsumoto S, Takeuchi S, Arai S, Fukuda K, Nishiyama A, et al. Proteasome inhibition overcomes ALK-TKI resistance in *ALK*-rearranged/*TP53*-mutant NSCLC via Noxa expression. Clin Cancer Res 2021;27:1410-20.
34. Li XM, Li WF, Lin JT, Yan HH, Tu HY, Chen HJ, et al. Predictive and prognostic potential of TP53 in patients with advanced non-small-cell lung cancer treated with EGFR-TKI: analysis of a phase III randomized clinical trial (CTONG 0901). Clin Lung Cancer 2021;22:100-9.e3.
35. 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;123:70-5.
36. Janaki N, Harbhajanka A, Michael CW, Bomeisl P, Wasman J, Atchley M, et al. Comparison of cytocentrifugation supernatant fluid and formalin-fixed paraffin-embedded tissue for targeted next-generation sequencing. Cancer Cytopathol 2019;127:297-305.
37. Frank R, Scheffler M, Merkelbach-Bruse S, Ihle MA, Kron A, Rauer M, et al. Clinical and pathological characteristics of *KEAP1*- and *NFE2L2*-mutated non-small cell lung carcinoma (NSCLC). Clin Cancer Res 2018;24:3087-96.
38. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499:214-8.
39. Greillier L, Tomasini P, Barlesi F. The clinical utility of tumor mutational burden in non-small cell lung cancer. Transl Lung Cancer Res 2018;7:639-46.
40. Nie W, Qian J, Xu MD, Gu K, Qian FF, Lu J, et al. Prognostic and predictive value of blood tumor mutational burden in patients with lung cancer treated with docetaxel. J Natl Compr Canc Netw 2020;18:582-9.
41. Sakai K, Tsuboi M, Kenmotsu H, Yamanaka T, Takahashi T, Goto K, et al. Tumor mutation burden as a biomarker for lung cancer patients treated with pemetrexed and cisplatin (the JIPANG-TR). Cancer Sci 2021;112:388-96.