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Evaluate the Prognosis of *MYC/TP53* Comutation in Chinese Patients with EGFR-Positive Advanced NSCLC Using Next-Generation Sequencing: A Retrospective Study

Jin Cao, MS[1], Juan J Gu, MD, PhD[1,2,3], Yichen Liang, MD, PhD[2,3], and Buhai Wang, MD, PhD[1,2,3]

Abstract

Purpose: The purpose of this study was to investigate the effect of *MYC* and *TP53* comutations on the clinical efficacy of EGFR tyrosine kinase inhibitors (TKIs) in Chinese patients with advanced EGFR-positive nonsmall-cell lung cancer (NSCLC). **Patients and methods:** Tissue samples and information from 65 patients with advanced NSCLC in Northern Jiangsu People's Hospital were collected and analyzed by next-generation sequencing (NGS). Progression-free survival (PFS) and total survival (OS) were the main endpoints, and the objective response rate (ORR) and disease control rate (DCR) were the secondary endpoints. **Result**: Among 65 patients, 17 had *TP53* and *MYC* wild-type mutations (*WT/WT*), 36 had *TP53* mutant and *MYC* wild-type mutations (*TP53/WT*), and 12 had coexisting *MYC/TP53* mutations (*MYC/TP53*). When 12 patients with *MYC/TP53* comutation were compared with the other two groups (*TP53/WT*, *WT/WT*), mPFS and mOS are significantly lower than those in the other two groups (mPFS: 4.1 months vs 6.0 months, 12.3 months, HR: 0.769, 95% CI: 4.592-7.608, $P = .047$. mOS: 14.6 months vs 24.1 months, 31.5 months, HR: 3.170, 95% CI: 18.786-31.214, $P < .001$), and the ORR, DCR of patients with *MYC/TP53* comutation was lower than that of the other two groups (ORR, 25% vs 44.4%, 70.6%, $P = .045$. DCR, 58.3% vs 72.2%, 82.4%, $P = .365$). **Conclusion:** Patients with *MYC/TP53* comutations with EGFR-positive advanced NSCLC are more likely to develop drug resistance after early treatment with EGFR-TKIs and have a worse clinical outcome.

Keywords

MYC, EGFR, non-small-cell lung cancer (NSCLC), *TP53*, comutation, EGFR tyrosine kinase inhibitors (TKIs), next-Generation sequencing (NGS)

Abbreviations

NSCLC, Nonsmall-cell lung cancer; NGS, next-generation sequencing; ECOG PS, eastern cooperative oncology group performance status; TKIs, tyrosine kinase inhibitors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; ADK, adenocarcinoma; CI, confidence interval; HR, hazard ratio; *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no *MYC* mutation; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

[1] Medical College, Yangzhou University, Yangzhou, Jiangsu, China
[2] Institute of Oncology, Northern Jiangsu People's Hospital, Yangzhou, Jiangsu, China
[3] Department of Oncology, Northern Jiangsu People's Hospital, Yangzhou, Jiangsu, China

Corresponding Author:
Buhai Wang, MD, PhD, Medical College, Yangzhou University, Yangzhou, Jiangsu, 225000, China.
Email: wbhself@sina.com

Yichen Liang, MD, PhD, Institute of Oncology, Northern Jiangsu People's Hospital, Yangzhou, Jiangsu, 225000, China.
Email: francesyichen1@163.com

Introduction

Lung cancer is one of the most common malignant tumors in the world, and its morbidity and mortality rank first among all malignant tumors.[1] Approximately 80% of lung cancer types are nonsmall-cell lung cancer (NSCLC), and more than 50% of NSCLC patients have adenocarcinomas.[2] Although chemotherapy still plays a dominant role in NSCLC, the OS of most patients is less than a year after chemotherapy, and chemotherapy has a high grade 3-5 toxicity. Most patients stop treatment because they cannot tolerate the toxicity of chemotherapy.[3]

However, in the past decade, molecular targeted therapy based on patient genomes has made a major breakthrough and completely changed the prospects of treatment for advanced NSCLC.[4] Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are the first-line treatment choice for advanced NSCLC, and advanced NSCLC has the most common form of EGFR mutation: deletion of exon 19 or exon 21 L858R.[5] Gefitinib, erlotinib, icotinib, osimertinib, and other targeted drugs have become first-line treatment standards for EGFR mutations in advanced NSCLC.[6] However, drug resistance is inevitable in EGFR-TKIs, and a number of clinical trials have shown that[7–9] approximately 5% to 10% of NSCLC patients with EGFR-sensitive mutations are mainly resistant to EGFR-TKI therapy. Meanwhile, some mechanisms of anti-EGFR-TKIs have been investigated, such as EGFR T790M mutation, MET gene amplification, PIK3CA mutation, ERBB2 amplification, and so on.

MYC is an out-of-control oncogene in human cancer and was first discovered in 1983.[10] *MYC* is one of the most highly amplified oncogenes in many human cancers,[11] and it supports tumorigenesis and progression and is often found in cervical, colon, breast, gastric, and lung cancer.[12] *MYC* is one of the most commonly amplified genes in lung cancer,[13] and some studies have shown that amplification of the *MYC* gene is related to the loss of cell differentiation.[12] Other studies have shown that *MYC* gene amplification is associated with poor prognosis in NSCLC and small cell lung cancer (SCLC).[14–16] Some studies have shown that *MYC* family genes, including *MYC*, *MYCL*, and *MYCN*, as carcinogenic drivers may constitute new therapeutic targets in small cell lung cancer.[17] However, there is no approved treatment for this kind of mutation. Similarly, *TP53* is the most frequently altered gene in human cancer.[18] Many studies have shown that *TP53* mutation is a negative prognostic factor for the prognosis of patients with NSCLC and may lead to drug resistance to EGFR-TKIs.[19,20] However, there are many different kinds of *TP53* mutations, and the effect of each mutation on prognosis is heterogeneous.[3]

The relationship between *MYC* and *TP53* genes was first proposed Gazzeri et al.[21] used Southern and Northern blotting techniques and found that there may be a correlation between *TP53* mutation and *MYC* activation in NSCLC, but the correlation was not significant. At the same time, *TP53* and *MYC* gene changes are very important, representing independent factors in the development of lung cancer. In recent reports, researchers have observed a highly significant co-occurrence of *MYC* gene amplification and *TP53* gene mutations in breast cancer and later in NSCLC.[11] However, the prognosis and predictive value of *MYC/TP53* comutation in patients with EGFR mutation-positive advanced NSCLC treated with oral EGFR-TKIs are still unclear, although some studies have suggested that *MYC* gene amplification is associated with possible EGFR-TKI resistance.[14,16] However, there is not a large amount of clinical data to confirm this hypothesis. For this reason, we studied the correlation between *MYC/TP53* comutation and the therapeutic effect of EGFR-TKIs in Chinese patients with late EGFR mutation-positive NSCLC to determine whether *MYC/TP53* comutation indicates a poor prognosis and whether it is related to primary drug resistance to EGFR-TKIs.

Materials and Methods

Patients Recruitment and Sample Collection

The study is a single center retrospective study, a total of 65 patients with stage IIIA-IV NSCLC diagnosed in the Department of Oncology, Northern Jiangsu People's Hospital from 2016-08 to 2020-10 were retrospectively collected, all the patients enrolled signed a written informed consent form that their tissue samples were analyzed by next-generation sequencing (NGS) and the study have obtained approval from the Ethics Committee of Northern Jiangsu People's Hospital (Approval: ID2017005). The reporting of this study conforms to STROBE guidelines.[22] The inclusion criteria were as follows: (1) EGFR positive patients were analyzed by NGS. (2) Eastern Cooperative Oncology Group (ECOG) score[23] 0-2 points, and there were no serious heart, lung, and other basic diseases. (3) NSCLC staged according to CSCO guidelines as stage IIIA-IV. (4) Oral EGFR-TKI therapy was used at all times throughout the course of the disease, including three kinds of EGFR-TKI drugs gefitinib, alphatinib, or osimertinib. Exclusion criteria: (1) Patients with wild-type EGFR; (2) The general situation was poor, ECOG > 2; (3) Age > 75 years; (4) Patients who were not treated with TKI were selected.

Sample Collection and the Evaluation of Clinical Efficacy

The patient underwent imaging examination and at least one repeated radiological examination. Baseline characteristics of patients (age, sex, smoking history, histology, Eastern Cancer Cooperation Group (ECOG) performance status, current survival status, etc.) and results after continuous and regular TKI treatment were obtained using medical and radiological records and patient follow-up information. The significance of regular review during the administration of EGFR-TKIs is that patients receive chest and abdominal CT, brain MRI, and other imaging examinations every 2-3 months to evaluate the potential therapeutic effect of EGFR-TKIs, according to the RECIST (solid tumor efficacy evaluation criteria)[24] standard to determine the clinical effect. To ensure the privacy of patients, all patient details have been deidentified. The main

endpoints of this study were to determine the difference in PFS or OS among the three groups of patients after EGFR-TKI treatment according to the mutation status of *MYC* and *TP53*: double wild-type tumors (*WT/WT*), *MYC* wild-type and *TP53* mutant tumors (*TP53/WT*) and double mutant tumors (*MYC/TP53*). The PFS of EGFR-positive NSCLC patients after EGFR-TKI treatment was the time from the beginning of oral EGFR-TKI treatment to disease progression, and OS was the time from oral EGFR-TKI treatment to death or the last follow-up time in September 2021. Secondary endpoints included ORR and DCR. ORR is the proportion of patients whose tumor size reduced to a predetermined value and can maintain the minimum time limit, which is the sum of complete response (CR) and partial response (PR), ORR = CR + PR. DCR is the percentage of evaluable cases with response (PR + CR) and stable disease (SD) after treatment, DCR = CR + PR + SD.

Targeted Panel NGS

A total of 168 genes closely related to the pathogenesis and targeted therapy of lung cancer were extracted from tumor tissue and plasma. The important exon regions of 168 genes and the hot intron regions of eight genes were detected by probe hybridization and high-throughput sequencing. Comprehensive and accurate detection of lung cancer-related gene mutation, copy number variation and rearrangement (fusion) and other mutations was performed. For detection, target area probe capture technology and second-generation high-throughput sequencing technology (NGS) based on the Illumina sequencing platform were used to analyze the samples. This technology was independently developed, analyzed and verified by Burning Stone Medicine. The Institute of Burning Stone Medicine has completed the verification of the technical platform according to CLIA'88 and relevant technical guidelines at home and abroad and has passed the interroom quality assessment of high-throughput sequencing for tumor diagnosis and treatment in the Clinical Test Center of the Health Commission. This test can cover the single nucleotide variation (SNV), short fragment insertion or deletion variation (INDEL), gene copy number variation (CNV) and gene rearrangement (rearrangement/fusion) in the capture exons and +/−20 bp flanking region of each exon. The evidence used to distinguish between benign and malignant variants comes from OncoDB,[25] the internal database of burning stone, and refers to public databases such as NCCN guidelines,[26] ACMG guidelines,[27] and OncoKB.[28]

Table 1. Baseline Characteristics.

Characteristics	Patients (n = 65)	Percentage
Gender		
Male	33	50.8
Female	32	49.2
Age in years		
<60	31	47.7
≥60	34	52.3
Smoking status		
Smoker	38	58.5
Nonsmoker	27	41.5
Stage		
IIIA	9	13.9
IIIB	6	9.3
IV	50	76.9
Histology		
Adenocarcinoma	63	96.9
Nonadenocarcinoma	2	3.1
ECOG PS		
0-1	46	70.8
2	19	29.2
EGFR mutation		
19	41	63.1
21 L858R	24	36.9

DNA Extraction Information

Using the DNeasy Blood and Tissue kit (Qiagen) and QIAamp DNA FFPE Tissue Kit (Qiagen) in accordance with the manufacturer's instructions, genomic DNA was extracted from fresh tissue and FFPE samples.[29] Tissue DNA was sheared using Covaris M220 (Covaris, MA, USA), followed by end repair, phosphorylation and adaptor ligation. Fragments between 200 and 400 bp from the sheared tissue DNA were purified (Agencourt AMPure XP Kit, Beckman Coulter, CA, USA), followed by hybridization with capture probes baits, hybrid selection with magnetic beads, and PCR amplification. The quality and the size of the fragments were assessed by high sensitivity DNA kit using Bioanalyzer 2100 (Agilent Technologies, CA, USA). Indexed samples were sequenced on Nextseq500 (Illumina, Inc., USA) with paired-end reads and target sequencing depth of 1,000× for tissue samples.

Bioinformatics Pipeline Information

Sequence data were mapped to the reference human genome (hg19) using Burrows–Wheeler Aligner v.0.7.10.[30] Local alignment optimization, duplication marking and variant calling were performed using Genome Analysis Tool Kit v.3.2,[31] and VarScan v.2.4.3.[32] Variants were filtered using the VarScan fpfilter pipeline, loci with depth less than 100 were filtered out. Base-calling in tissue samples required at least 8 supporting reads for single nucleotide variations (SNV) and five supporting reads for insertion-deletion variations (INDEL). Variants with population frequency over 0.1% in the ExAC, 1000 Genomes, dbSNP or ESP6500SI-V2 databases were grouped as single nucleotide polymorphisms (SNP) and excluded from further analysis. Remaining variants were annotated with ANNOVAR (February 01, 2016 release)[33] and SnpEff v.3.6.[34] Analysis of DNA translocation was performed using Factera v.1.4.3.[35] Copy number variations (CNVs) were analyzed based on the depth of coverage data of capture intervals. Coverage data were corrected against sequencing bias resulting from GC content and probe design. The average coverage of all captured regions was used to

normalize the coverage of different samples to comparable scales. Copy number was calculated based on the ratio between the depth of coverage in tumor samples and average coverage of an adequate number ($n > 50$) of samples without CNV as references per capture interval. CNV is called if the coverage data of the gene region was quantitatively and statistically significant from its reference control. The limit of detection for CNVs is 1.5 and 2.64 for deletions and amplifications, respectively.

Statistical Analysis

The Kruskal–Wallis test or Fisher's exact test was used to evaluate the differences in clinical covariables among the three groups (*WT/WT, TP53/WT*, and *MYC/TP53*). The PFS and OS of the patients were analyzed by the Kaplan–Meier method. The Cox proportional hazard regression model was used to evaluate the correlation between clinical covariables and PFS/OS, and the risk ratio and 95% confidence interval (CI) were obtained. All the reported *P* values were double-tailed, and *P* values less than 0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS25.0 for statistical analysis and GraphPadPrism version 7 to generate drawings.

Table 2. Baseline Patient and Tumor Characteristics by Mutation Subgroup.

Demographic	*WT/WT* ($n = 17$), *n* (%)	*TP53/WT* ($n = 36$), *n* (%)	*MYC/TP53* ($n = 12$), *n* (%)	*P* value
Median age	61(46–75)	59(44–75)	58(45–68)	.194
Range				
Gender				
Male	9(53)	17(47)	7(58)	.783
Female	8(47)	19(53)	5(42)	
Smoking status				
Smoker	7(41)	23(64)	8(67)	.242
Never smoker	10(59)	13(36)	4(33)	
Stage				
IIIA	4(23)	4(11)	1(8)	
IIIB	2(11)	3(8)	1(8)	.597
IV	11(66)	29(81)	10(84)	
ECOG PS				
0-1	14(82)	26(56)	6(50)	.044*
2	3(18)	10(44)	6(50)	
Histology				
Adenocarcinoma	17(100)	36(100)	10(83)	.029*
Nonadenocarcinoma	0(0)	0(0)	2(17)	
EGFR mutation				
19	12(70)	20(56)	9(75)	.358
21 L858R	5(30)	16(44)	3(25)	

Note: $*P < .05$.
Abbreviations: *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no *MYC* mutation; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

Results

Baseline Characteristics

We identified 65 patients with EGFR-positive NSCLC (Table 1). Among 65 patients, 17 had *TP53* and *MYC* wild-type mutations (*WT/WT*), 36 had *TP53* mutant and *MYC* wild-type mutations (*TP53/WT*), and 12 had coexisting *MYC/TP53* mutations (*MYC/TP53*) (Table 2). There was no significant difference in median age ($P = .194$), sex ($P = .783$), clinical stage ($P = .597$), smoking history ($P = .242$) or EGFR mutation type ($P = .358$) among the three groups. Compared with other groups, there were more patients with *MYC/TP53* wild-type tumors (*WT/WT*) with an ECOG score of 0-1 (82% *WT/WT* vs 56% *TP53/WT* vs 50% *MYC/TP53*; $P = .044$), with significant histological differences between groups ($P = .029$).

Univariate and Multivariate Analysis of the Relationship between Clinical Characteristics and Prognosis in Patients with EGFR Mutation NSCLC

We summarized the results of survival and response analysis of 65 patients with NSCLC who received EGFR-TKIs at any stage by Cox regression analysis. In univariate analysis (Table 3), Gene mutation status (*WT/WT*, *TP53/WT*, and *MYC/TP53*) was a significant predictor of PFS (HR: 0.596, 95% CI: 0.393-0.903, $P = .015$) and OS (HR: 0.427, 95% CI: 0.274-0.665, $P < .001$). In addition, clinical stage (IIIA, IIIB, and IV) and ECOG score (0-1, 2) were also predictors of PFS (HR: 0.131, 95% CI: 0.040-0.430, $P = .004$, HR 3.013, 95% CI: 1.876-4.839, $P < .001$) and OS (HR: 0.216, 95% CI: 0.067-0.703, $P = .010$, HR: 2.493, 95% CI: 1.638-3.794, $P < .001$). In the multivariate analysis adjusted for other variables (age, sex, smoking, and histology) (Table 4), we observed a significant correlation between PFS and clinical stage (HR: 0.126, 95% CI: 0.034-0.461, $P = .002$), ECOG score (HR: 2.556, 95% CI: 1.530-4.270, $P < .001$), and Gene mutation status (HR: 0.622, 95% CI: 0.389-0.996, $P = .048$). Similarly, OS was significantly correlated with ECOG score (HR:1.899, 95% CI: 1.221-2.954, $P = .004$) and Gene mutation status (HR: 0.427, 95% CI: 0.338-0.947, $P = .030$).

Analysis of the Survival status of the Three Groups of Patients

Because both univariate and multivariate analyses showed that the state of gene mutation (*WT/WT*, *TP53/WT*, and *MYC/TP53*) is a predictor of PFS and OS, we stratified the patients with EGFR-positive *WT/WT* (*MYC*, *TP53* wild-type mutation), *TP53/WT* (*MYC* wild-type, *TP53* mutation positive), and *MYC/TP53* comutations and compared the survival time of the three groups by Kaplan–Meier analysis (Figure 1). When 12 patients with *MYC/TP53* comutation were compared with the other two groups (*TP53/WT* and *WT/WT*), mPFS and mOS were significantly lower than those in the other two groups (mPFS: 4.1 months vs 6.0 months, 12.3 months, HR:

Table 3. Univariate Regression Analysis of PFS and OS in EGFR-Positive NSCLC.

	PFS			OS		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age	0.998	0.971-1.027	.912	0.983	0.955-1.011	.230
Gender						
men vs women	1.248	0.718-2.167	.432	1.334	0.767-2.323	.308
Histology						
ADK vs non-ADK	2.400	0.329-17.505	.388	0.368	0.049-2.788	.333
Stage						
IIIA vs IIIB vs IV	0.131	0.040-0.430	.004*	0.216	0.067-0.703	.010*
Smoking						
Ever vs never	0.688	0.391-1.211	.195	0.693	0.385-1.245	.219
ECOG PS						
0-1 vs 2	3.013	1.876-4.839	$<.001$*	2.493	1.638-3.794	$<.001$*
EGFR mutation						
19 vs 21 L858R	0.899	0.496-1.630	.726	0.748	0.410-1.363	.343
Genetic markers						
WT/WT vs *TP53/WT* versus *MYC/TP53*	0.596	0.393-0.903	.015*	0.427	0.274-0.665	$<.001$*

Note: *$P<.05$.
Abbreviations: ADK, adenocarcinoma; CI, confidence interval; HR, hazard ratio; *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no *MYC* mutation; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

0.769, 95% CI: 4.592–7.608, $P = .047$. mOS: 14.6 months vs 24.1 months, 31.5 months, HR: 3.170, 95% CI: 18.786–31.214, $P<.001$).

In addition, in patients whose PFS was less than 3 months, 33.3% (4/12) had *MYC/TP53* comutations, 22.2% (8/36) had *TP53/WT* mutations, and only 5.9% (1/17) had *WT/WT* mutations. Similarly, 50% of patients with OS less than 15 months were *MYC/TP53* comutants, 33.3% (12/36) had *TP53/WT* mutations, and 5.9% (1/17) had *WT/WT* mutations, which suggests that individuals with *MYC/TP53* comutations have poorer clinical outcomes and are more prevalent in patients with early resistance to EGFR-TKI treatment.

We also analyzed the secondary outcome ORR, DCR of 65 patients in these three groups (*WT/WT, TP53/WT*, and *MYC/TP53*) (Figure 2). The ORR, DCR of patients with *MYC/TP53* comutation was lower than that of the other two groups (ORR, 25% vs 44.4%, 70.6%, $P = .045$. DCR, 58.3% vs 72.2%, 82.4%, $P = .365$) (Table 5). There was no statistical significance in DCR among the three groups, but ORR showed statistical significance. Thus, patients with *MYC/TP53* comutations are more likely to develop drug resistance after early treatment with EGFR-TKIs and have a worse clinical outcome.

Evaluation of the Difference in PFS and OS between MYC/TP53 *and* TP53/WT

We found that there were differences in PFS and OS among the three groups of patients (*WT/WT*, *TP53/WT*, and *MYC/TP53*). To further analyze the nature of the differences, we made a pairwise comparison among the three groups of patients. (Figure 3A, B). There was no statistical significance in PFS between the patients with *MYC/TP53* and *TP53/WT* (4.1 months vs 6.0 months, HR: 0.775, 95% CI: 0.3858-1.557, $P = .25$), but there was statistical significance in OS (14.6 months vs 24.1 months, HR: 0.658, 95% CI: 0.3274-1.321, $P = .015$). OS was an independent predictor of prognosis in *MYC/TP53* and *TP53/WT*.

Evaluation of the Difference in PFS and OS Between MYC/TP53 *and* WT/WT

We then performed Kaplan–Meier analysis of PFS and OS in the *MYC/TP53* and *WT/WT* groups (Figure 3C, D). There were significant differences in PFS and OS between the patients with *MYC/TP53* and *WT/WT* (PFS: 4.1 months vs 12.3 months, HR: 0.378, 95% CI: 0.1668-0.8568, $P = .012$. OS: 14.6 months vs 31.5 months, HR: 0.5032, 95% CI: 0.222-1.14, $P < 0.001$). The results showed that both PFS and OS are independent predictors for the prognosis of *MYC/TP53* and *WT/WT*.

Effect of MYC *Mutation Site on Prognosis*

We then studied the effects of different *MYC* mutation sites on EGFR positive Chinese patients with advanced NSCLC, and Table 6 listed the *MYC* mutation sites as well as PFS and OS in 12 patients with *MYC/TP53* comutation. It can be seen that the PFS and OS of *MYC* short variant are longer than *MYC* amplification (mPFS: 7.0 months vs 3.4 months, HR: 2.059, 95% CI: 0.6027 to 7.033, $P = .21$. mOS: 24.8 months vs 11.3 months, HR: 2.195, 95% CI: 0.6425 to 7.497, $P = .031$) (Figure 4). The results showed that the patients with *MYC* amplification had a worse prognosis. Due to the small total sample size, a larger sample size needs to be collected in the future to confirm this conclusion.

Table 4. Multivariate Regression Analysis of PFS and OS in EGFR-Positive NSCLC.

	PFS			OS		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age	1.025	0.992-1.060	.143	1.001	0.968–1.035	.966
Gender						
Men vs women	0.873	0.417-1.828	.719	0.989	0.497–1.971	.976
Histology						
ADK s non-ADK	4.980	0.476-52.109	.180	0.421	0.041–4.275	.464
Stage						
IIIA versus IIIB versus IV	0.126	0.034-0.461	.002*	0.298	0.084-1.052	.059
Smoking						
Ever versus Never	0.665	0.311-1.425	.294	0.784	0.397-1.551	.485
ECOG PS						
0–1 versus 2	2.556	1.530-4.270	<.001*	1.899	1.221-2.954	.004*
EGFR mutation						
19 versus 21 L858R	0.694	0.351-1.372	.294	0.786	0.404-1.528	.478
Genetic markers						
WT/WT versus *TP53/WT* versus *MYC/TP53*	0.622	0.389-0.996	.048*	0.565	0.338-0.947	.030*

Note: *$P < .05$.

Abbreviations: ADK, adenocarcinoma; CI, confidence interval; HR, hazard ratio; *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no *MYC* mutation; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

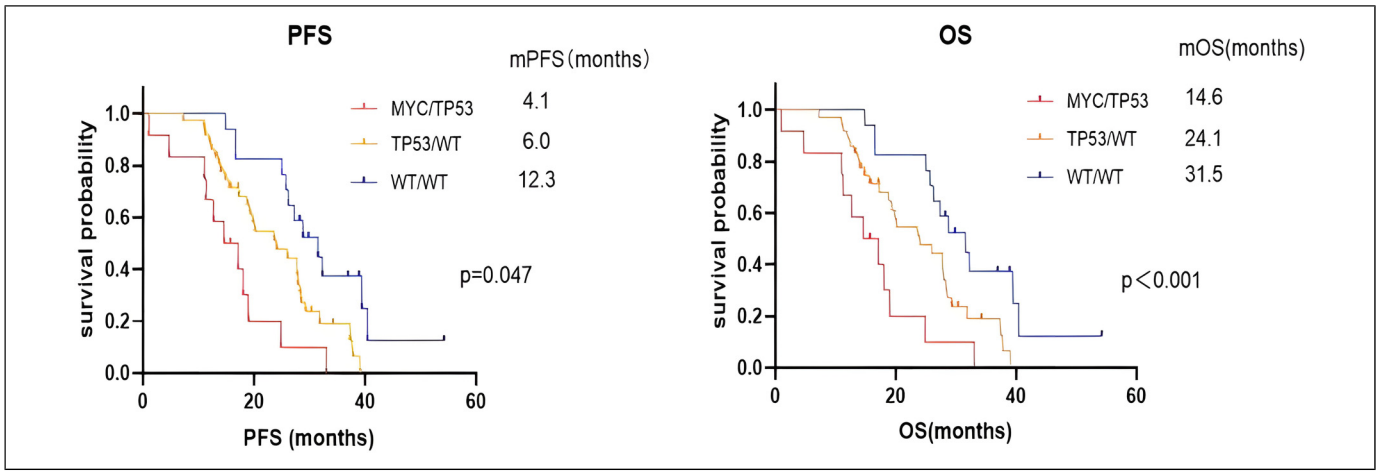

Figure 1. Analysis of the survival status of the three groups of patients. Abbreviations: PFS, progression-free survival; OS, overall survival; *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no MYC mutation; *MYC/TP503*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

Discussion

The *MYC* oncogene is a common transcription factor, and it is one of the most highly amplified oncogenes in a variety of human cancers.[12] In an earlier study, Yoo et al.[36] used IHC to report the overexpression of *MYC* in 147 cases of NSCLC, which showed that the overexpression of *MYC* accounted for 16% of NSCLC (24 of 147 cases). Other studies have shown that 14% of patients with *TP53* mutations in NSCLC have *MYC* gene amplification.[37] The conclusions of these studies are basically consistent with ours (18.5%, 12 of 65 cases). In our study, we compared the prognostic value of three groups of EGFR-positive NSCLC patients with *WT/WT* (*MYC* and *TP53* wild type mutation), *TP53/WT* (*MYC* wild type, *TP53* mutation positive) and *MYC/TP53* comutations and verified that the EGFR-positive *MYC/TP53* comutation was related to the significant shortening of PFS and OS and the decrease of DCR and ORR after EGFR-TKI treatment. This indicates that *MYC/TP53* comutation EGFR-positive NSCLC patients are more likely to develop drug resistance after early treatment with EGFR-TKI and have a poor clinical outcome. Subsequently, we further analyzed the prognosis of two subtypes of *MYC* mutations (*MYC* short variant and *MYC* amplification) in 12 patients with *MYC/TP53* comutation. We come to conclusion that the OS and PFS of *MYC* short variant are longer than *MYC* amplification, there was no significant difference in

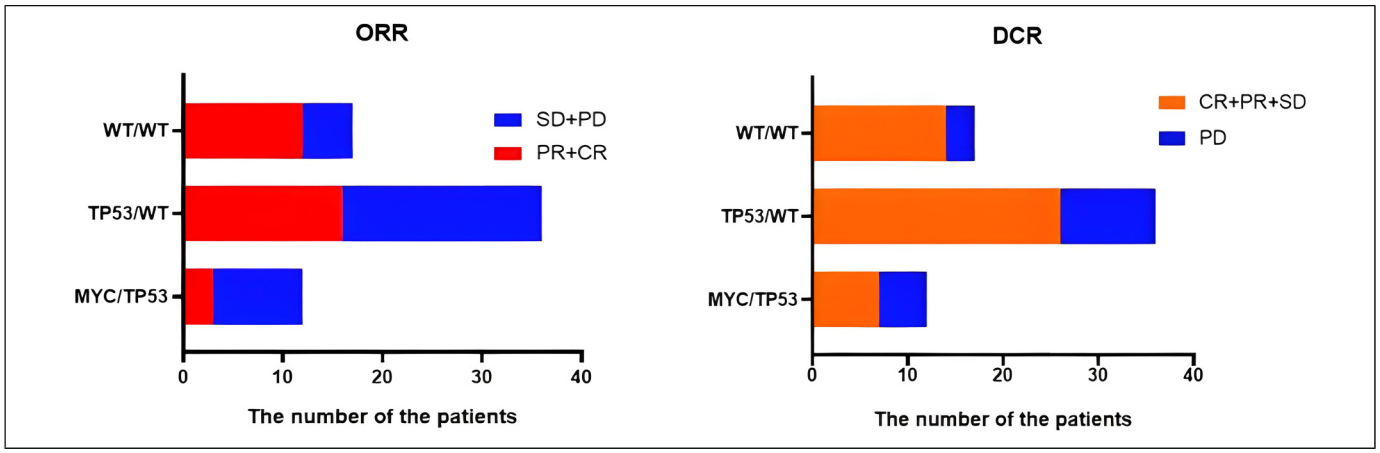

Figure 2. ORR and DCR of the three groups of patients. Abbreviations: ORR, objective response rate; DCR, disease control rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no MYC mutation; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

Table 5. Chart of Response After EGFR TKI Treatment in Three Subgroups of Patients(*WT/WT*, *TP53/WT*, *MYC/TP53*).

EGFR-TKIs	Response after EGFR TKI treatment			*P* value
	MYC/TP53	*TP53/WT*	*WT/WT*	
ORR	25% (3/12)	44.4% (16/36)	70.6% (12/17)	.045*
Median PFS	4.1	6.0	12.3	.047*
DCR	58.3% (7/12)	72.2% (26/36)	82.4% (14/17)	.365
Median OS	14.6	24.1	31.5	<.001*

Note: *$P < .05$.
Abbreviations: ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; OS, overall survival; *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no *MYC* mutation; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

PFS between the two groups ($P = .21$). However, the molecular mechanism of this phenomenon is still unclear.

The results of Hall et al[38] show that the activation of *MYC* leads to the accumulation of cholesterol ester (CEs) stored in lipid droplets, the subsequent inactivation of *MYC* leads to a further increase in CEs, and the accumulation of CEs leads to the further enhancement of the invasiveness of NSCLC. Seo et al.[16] used in situ fluorescence hybridization to determine that c-*MYC* gene amplification is an independent poor prognostic factor for DFS and OS in stage I lung cancer and may also be an independent poor prognostic factor for EGFR mutant adenocarcinoma. This study pointed out that the increase in the *c-MYC* 8 chromosome was often related to smoking history, male sex, and/or lymphatic invasion, but the significance of the correlation may be limited because the number of cases with chromosome eight increases is relatively small, with only five patients. However, this study only observed patients with IA-IIIB, and in our study, we extended these results to patients with IIIA-IV.

The *MYC* gene is widely studied in lymphoma, prostate cancer, colorectal cancer and small cell lung cancer,[39–42] and several studies have shown that overexpression of *MYC* is associated with drug resistance.[43,44] Brägelmann et al.[17] pointed out that as a carcinogenic driving factor, *MYC* may constitute a new therapeutic target in small cell lung cancer, and its core role in tumor maintenance provides a new opportunity for targeted therapy. In addition, Sabattini et al.[45] used the FISH detection method and identified *MYC* amplification as a diagnostic or prognostic marker for malignant lymphoma. However, there are few studies on *MYC* gene mutations in NSCLC, which may be related to the fact that the *MYC* gene promotes tumor progression and leads to the transformation of NSCLC into more malignant small cell lung cancer.[46]

TP53 is a common tumor suppressor gene, but because of its inoperability, *TP53* mutations are not always detected in the early NSCLC molecular spectrum.[47] Therefore, the incidence of comutations leading to *TP53* and other driving gene mutations may be underestimated. Similarly, the same is true of *MYC* mutations, and even when detecting *MYC* and *TP53* mutations, the frequency of comutations with *MYC* and *TP53* is not always reported, so there are few studies on the interaction of *MYC* and *TP53* comutations in NSCLC.

At present, the clinical data on the predictive effect of EGFR-positive *MYC/TP53* comutation NSCLC on the efficacy of EGFR-TKIs are still limited, and it is still controversial whether early drug resistance will be caused by EGFR-TKI treatment in EGFR-positive NSCLC patients with *MYC/TP53* comutation. In a recent case report, researchers found that a patient with EGFR-positive NSCLC developed drug resistance after treatment with EGFR-TKIs. After drug resistance, new *MYC* gene amplification, *RB1* mutation and *TP53* mutation were found in the patient's tissue.[48] It is well known that the *RB1* gene is considered as an important genetic marker for

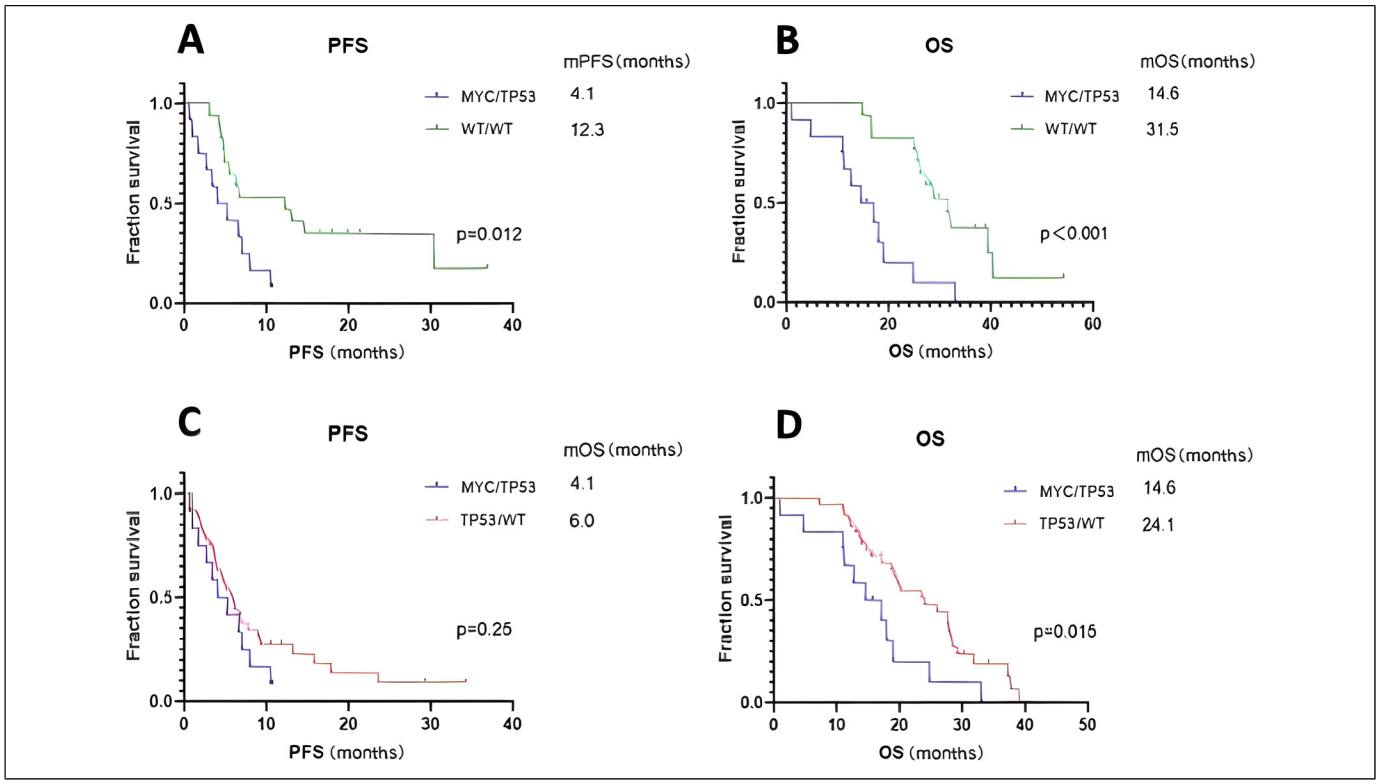

Figure 3. The survival curve of pairwise comparison of three subgroups (*WT/WT*, *TP53/WT*, and *MYC/TP53*). Abbreviations: PFS, progression-free survival; OS, overall survival; *TP53/WT*, group of patients with NSCLC harboring *TP53* mutation but no *MYC* mutation; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations; *WT/WT*, group of patients with NSCLC harboring no *TP53* or *MYC* mutation.

Table 6. *MYC* Mutation Site and Corresponding Survival Time.

Sample	Mutation site	PFS (months)	OS (months)
MYC/TP53–1	*MYC* short variant	10.7	15.7
MYC/TP53-2	*MYC* short variant	8.0	18.0
MYC/TP53-3	*MYC* short variant	7.0	33.0
MYC/TP53-4	*MYC* short variant	4.1	24.8
MYC/TP53-5	*MYC* short variant	2.7	12.7
MYC/TP53-6	*MYC* amplification	6.6	17.1
MYC/TP53-7	*MYC* amplification	10.5	11.0
MYC/TP53-8	*MYC* amplification	1.7	14.6
MYC/TP53-9	*MYC* amplification	3.4	11.3
MYC/TP53-10	*MYC* amplification	1.0	1.0
MYC/TP53-11	*MYC* amplification	5.2	19.0
MYC/TP53-12	*MYC* amplification	0.7	4.7

Abbreviations: PFS, progression-free survival; OS, overall survival; *MYC/TP53*, group of patients with NSCLC harboring *MYC* and *TP53* mutations

the transformation of NSCLC into small cell lung cancer, and researchers have shown that lung cancer with *EGFR/TP53/RB1* mutations has a unique risk of histological transformation, with 25% showing new SCLC or final small cell lung cancer transformation.[49] However, for NSCLC patients with EGFR-positive *MYC/TP53* comutation, the mechanism of this phenomenon is not clear. Studies have shown that proto-oncogenes in the *MYC* family, such as *MYC*, *MYCN*, and *MYCL*, are amplified and/or overexpressed in SCLC tumors, and they affect the tumor phenotype by controlling the dedifferentiation process from neuroendocrine cells to nonneuroendocrine cells[50] so that NSCLC can be transformed into small cell lung cancer. It is worth noting that in the data we studied, 3 of the 12 EGFR-positive patients with *MYC/TP53* comutation experienced the transformation of small cell lung cancer during EGFR-TKI treatment, which may provide a new research direction for the drug resistance mechanism of *MYC/TP53* comutation genes. Another study showed that osimertinib rapidly and sustainably reduced *c-MYC* levels mainly by enhancing protein degradation in EGFR-mutated NSCLC cell lines and xenotransplanted tumors, resulting in acquired drug resistance to osimertinib,[51] which is basically consistent with the conclusion of our study and can provide a scientific basis for the mechanism of drug resistance to EGFR-TKIs. Zhong et al.[52] studied the mechanism of primary drug resistance by NGS sequencing, analyzed the genetic changes of 11 patients with primary drug resistance to EGFR-TKIs and 11 patients sensitive to EGFR-TKIs after taking EGFR-TKIs, and found that *MYC* gene amplification was found in two sensitive patients and one patient with primary drug resistance. It was also found that the number of *MYC* copies in patients with primary drug resistance (13.6 times) was significantly higher than that in sensitive patients (4.8 times and 2.9 times). Thus, they concluded that the

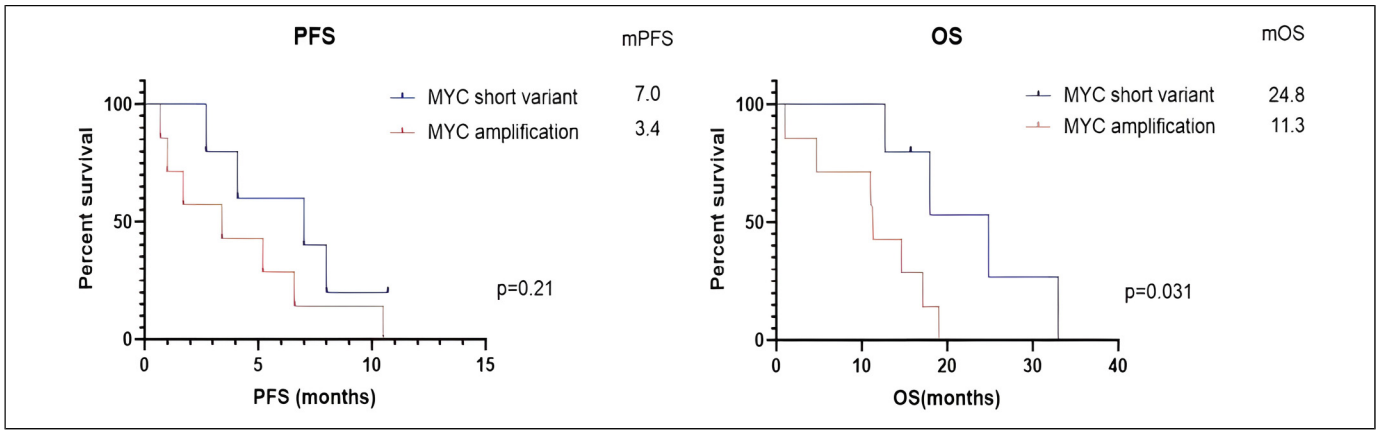

Figure 4. Survival curve of the effect of MYC mutation site on prognosis. Abbreviations: PFS, progression-free survival; OS, overall survival.

combination of *MYC* inhibitors and EGFR-TKIs may be a promising strategy to overcome the primary drug resistance of lung cancer to EGFR-TKIs. However, this observation needs to be confirmed because there were only 11 patients with EGFR-TKI primary drug resistance in this study, of whom only one patient had *MYC* gene amplification. Our study analyzed the overall prognostic levels of PFS and OS in 12 patients with *MYC/TP53* comutations and not only found that these patients were prone to early drug resistance when using EGFR-TKIs but also had a shorter survival time. Another study showed that EGFR-TKIs affect the expression of PD-L1 in NSCLC through the *c-MYC* pathway to promote drug resistance. Eun Young Kim et al introduced siRNA targeting *MYC* into H60 and H2009 cells with high expression of PD-L1 to study the relationship between *MYC* and PD-L1. They concluded that there was a significant positive correlation between PD-L1 and *MYC* expression ($\gamma = 0.210$). Compared with double-positive patients, patients with double-negative tumors had better PFS (31.1 months vs 7.1 months, $P = .011$) and OS (56.1 months vs 14.4 months, $P = .032$).[12] In addition, Alidousty et al.[37] by observing ChIP-Seq data, showed that the *MYC* binding site was located in the EML4 promoter region, and the overexpression of *MYC* in *TP53* mutant cells led to the upregulation of EML4-ALK, suggesting that there may be a mechanism of *MYC*-dependent drug resistance in patients with increased copies of yeast. These studies provide scientific ideas and methods for the mechanism of drug resistance in EGFR-positive *MYC/TP53* comutation patients and have been used to guide the follow-up use of drugs.

Our research has several limitations. First, the number of EGFR-positive patients with coexisting *MYC/TP53* mutations was relatively small, which limits the comparative analysis, so we have considered expanding the sample size for future research. Second, this study is a single-center retrospective study, and additional prospective studies are needed to validate the results. Finally, although this study compared the three groups (*WT/WT*, *TP53/WT*, and *MYC/TP53*) of patients' PFS, OS, ORR, and DCR, molecular experiments are still needed to explore the possible mechanisms in larger-scale experimental studies. It is worth noting that we are the first to study the prognostic and predictive value of *MYC/TP53* comutation in Chinese patients with advanced EGFR-positive NSCLC treated with oral EGFR-TKIs. We believe that our research contributes to the understanding of primary drug resistance and to the development of more personalized treatments. These findings must be confirmed in a larger patient population in the future.

Conclusions

Patients with *MYC/TP53* comutations with EGFR-positive advanced NSCLC are more likely to develop drug resistance after early treatment with EGFR-TKIs and have a worse clinical outcome.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The authors state that they have obtained approval from the Ethics Committee of Northern Jiangsu People's Hospital on January 4, 2017 (Approval: ID2017005). In addition, written informed consent has been obtained from the participants involved.

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ORCID iD

Jin Cao https://orcid.org/0000-0002-5433-2911

Supplemental Material

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References

1. Zhang ML, Zhao TT, Du WW, Yang ZF, Peng W, Cui ZJ. C-MYC-induced upregulation of LINC01503 promotes progression of non-small cell lung cancer. *Eur Rev Med Pharmacol Sci*. 2020;24(21):11120–11127.
2. Wang H, Zhang M, Tang W, et al. Mutation abundance affects the therapeutic efficacy of EGFR-TKI in patients with advanced lung adenocarcinoma: A retrospective analysis. *Cancer Biol Ther*. 2018;19(8):687–694.
3. Tomasini P, Mascaux C, Jao K, et al. Effect of coexisting KRAS and TP53 mutations in patients treated with chemotherapy for non-small-cell lung cancer. *Clin Lung Cancer*. 2019;20(3): e338–e345.
4. Lin C, Shi X, Yang S, et al. Comparison of ALK detection by FISH, IHC and NGS to predict benefit from crizotinib in advanced non-small-cell lung cancer. *Lung Cancer*. 2019;131:62–68.
5. VanderLaan PA, Rangachari D, Mockus SM, et al. Mutations in TP53, PIK3CA, PTEN and other genes in EGFR mutated lung cancers: Correlation with clinical outcomes. *Lung Cancer*. 2017;106:17–21.
6. Ma L, Li H, Wang D, et al. Dynamic cfDNA analysis by NGS in EGFR T790M-positive advanced NSCLC patients failed to the first-generation EGFR-TKIs. *Front Oncol*. 2021;11:643199.
7. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2012;13(3):239–246.
8. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. *Lancet Oncol*. 2010;11(2):121–128.
9. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009;361(10):947–957.
10. Little CD, Nau MM, Carney DN, et al. Amplification and expression of the c-myc oncogene in human lung cancer cell lines. *Nature*. 1983;306(5939):194–196.
11. Ulz P, Heitzer E, Speicher MR. Co-occurrence of MYC amplification and TP53 mutations in human cancer. *Nat Genet*. 2016;48(2):104–106.
12. Kim EY, Kim A, Kim SK, Chang YS. MYC Expression correlates with PD-L1 expression in non-small cell lung cancer. *Lung Cancer*. 2017;110:63–67.
13. Brik A, Weber DG, Casjens S, et al. Digital PCR for the analysis of MYC copy number variation in lung cancer. *Dis Markers*. 2020;2020:4176376.
14. Iwakawa R, Kohno T, Kato M, et al. MYC Amplification as a prognostic marker of early-stage lung adenocarcinoma identified by whole genome copy number analysis. *Clin Cancer Res*. 2011;17(6):1481–1489.
15. Alves Rde C, Meurer RT, Roehe AV. MYC Amplification is associated with poor survival in small cell lung cancer: A chromogenic in situ hybridization study. *J Cancer Res Clin Oncol*. 2014;140(12):2021–2025.
16. Seo AN, Yang JM, Kim H, et al. Clinicopathologic and prognostic significance of c-MYC copy number gain in lung adenocarcinomas. *Br J Cancer*. 2014;110(11):2688–2699.
17. Brägelmann J, Böhm S, Guthrie MR, Mollaoglu G, Oliver TG, Sos ML. Family matters: How MYC family oncogenes impact small cell lung cancer. *Cell Cycle*. 2017;16(16):1489–1498.
18. Gini B, Thomas N, Blakely CM. Impact of concurrent genomic alterations in epidermal growth factor receptor (EGFR)-mutated lung cancer. *J Thorac Dis*. 2020;12(5):2883–2895.
19. Roeper J, Falk M, Chalaris-Rißmann A, et al. TP53 co-mutations in EGFR mutated patients in NSCLC stage IV: A strong predictive factor of ORR, PFS and OS in EGFR mt + NSCLC. *Oncotarget*. 2020;11(3):250–264.
20. Hou H, Qin K, Liang Y, et al. Concurrent TP53 mutations predict poor outcomes of EGFR-TKI treatments in Chinese patients with advanced NSCLC. *Cancer Manag Res*. 2019;11:5665–5675.
21. Gazzeri S, Brambilla E, Caron de Fromentel C, et al. P53 genetic abnormalities and MYC activation in human lung carcinoma. *Int J Cancer*. 1994;58(1):24–32.
22. von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Ann Intern Med*. 2007;147(8):573–577.
23. Azam F, Latif MF, Farooq A, et al. Performance status assessment by using ECOG (Eastern cooperative oncology group) score for cancer patients by oncology healthcare professionals. *Case Rep Oncol*. 2019;12(3):728–736.
24. Jang GS, Kim MJ, Ha HI, et al. Comparison of RECIST version 1.0 and 1.1 in assessment of tumor response by computed tomography in advanced gastric cancer. *Chin J Cancer Res*. 2013;25(6):689–694.
25. Tang G, Cho M, Wang X. OncoDB: An interactive online database for analysis of gene expression and viral infection in cancer. *Nucleic Acids Res*. 2022;50(D1):D1334–D1339.
26. Ettinger DS, Wood DE, Aggarwal C, et al. NCCN Guidelines insights: Non-small cell lung cancer, version 1.2020. *J Natl Compr Canc Netw*. 2019;17(12):1464–1472.
27. Tavtigian SV, Greenblatt MS, Harrison SM, et al. Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. *Genet Med*. 2018;20(9):1054–1060.
28. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: A precision oncology knowledge base. *JCO Precis Oncol*. 2017;2017:PO.17.00011.
29. Wang B, Fan Y, Zhang L, et al. Evaluation of pembrolizumab monotherapy efficacy in advanced non-small-cell lung cancer by serial monitoring of circulating tumor DNA using next-generation sequencing. *Clin Med Insights Oncol*. 2022;16:11795549221075326.
30. Li H, Durbin R. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics (Oxford, England)*. 2009;25(14):1754–1760.
31. McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297–1303.

32. Koboldt DC, Zhang Q, Larson DE, et al. Varscan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res.* 2012;22(3):568–576.
33. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164.
34. Cingolani P, Platts A, Wang le L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly (Austin).* 2012;6(2):80–92.
35. Newman AM, Bratman SV, Stehr H, et al. FACTERA: A practical method for the discovery of genomic rearrangements at breakpoint resolution. *Bioinformatics (Oxford, England)*. 2014;30(23):3390–3393.
36. Yoo JY, Kim CH, Song SH, et al. Expression of caspase-3 and c-myc in non-small cell lung cancer. *Cancer Res Treat*. 2004;36(5):303–307.
37. Alidousty C, Baar T, Martelotto LG, et al. Genetic instability and recurrent MYC amplification in ALK-translocated NSCLC: A central role of TP53 mutations. *J Pathol*. 2018; 246(1):67–76.
38. Hall Z, Wilson CH, Burkhart DL, Ashmore T, Evan GI, Griffin JL. Myc linked to dysregulation of cholesterol transport and storage in nonsmall cell lung cancer. *J Lipid Res*. 2020;61(11):1390–1399.
39. Landsburg DJ. Management of patients with MYC-altered lymphomas. *Curr Hematol Malig Rep*. 2016;11(3):208–217.
40. Lee JK, Phillips JW, Smith BA, et al. N-Myc drives neuroendocrine prostate cancer initiated from human prostate epithelial cells. *Cancer Cell*. 2016;29(4):536–547.
41. Ren L, Zhou T, Wang Y, et al. RNF8 Induces β-catenin-mediated c-myc expression and promotes colon cancer proliferation. *Int J Biol Sci*. 2020;16(12):2051–2062.
42. Tokgun O, Tokgun PE, Inci K, Akca H. lncRNAs as potential targets in small cell lung cancer: MYC -dependent regulation. *Anticancer Agents Med Chem*. 2020;20(17):2074–2081.
43. Walker TL, White JD, Esdale WJ, Burton MA, DeCruz EE. Tumour cells surviving in vivo cisplatin chemotherapy display elevated c-myc expression. *Br J Cancer*. 1996;73(5):610–614.
44. McNeil CM, Sergio CM, Anderson LR, et al. . c-Myc overexpression and endocrine resistance in breast cancer. *J Steroid Biochem Mol Biol*. 2006;102(1-5):147–155.
45. Sabattini E, Bacci F, Sagramoso C, Pileri SA. WHO Classification of tumours of haematopoietic and lymphoid tissues in 2008: An overview. *Pathologica*. 2010;102(3):83–87.
46. Patel AS, Yoo S, Kong R, et al. Prototypical oncogene family Myc defines unappreciated distinct lineage states of small cell lung cancer. *Sci Adv*. 2021;7(5):eabc2578.
47. Barlesi F, Mazieres J, Merlio JP, et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: Results of a 1-year nationwide programme of the French cooperative thoracic intergroup (IFCT). *Lancet*. 2016;387(10026):1415–1426.
48. Yang Z, Lin Y, Wang H. Transformation of non-small cell lung cancer into small cell lung cancer in a patient with advanced lung cancer: A case report. *J Int Med Res*. 2021;49(8):3000605211035005.
49. Offin M, Chan JM, Tenet M, et al. Concurrent RB1 and TP53 alterations define a subset of EGFR-mutant lung cancers at risk for histologic transformation and Inferior clinical outcomes. *J Thorac Oncol*. 2019;14(10):1784–1793.
50. Ng J, Sutherland KD. NOTCH Your usual suspect: MYC charged with controlling neuroendocrine cell-fate in small cell lung cancer. *Cancer Cell*. 2020;38(1):17–20.
51. Zhu L, Chen Z, Zang H, et al. Targeting c-myc to overcome acquired resistance of EGFR mutant NSCLC cells to the third-generation EGFR tyrosine kinase inhibitor, osimertinib. *Cancer Res*. 2021;81(18):4822–4834.
52. Zhong J, Li L, Wang Z, et al. Potential resistance mechanisms revealed by targeted sequencing from lung adenocarcinoma patients with primary resistance to epidermal growth factor receptor (EGFR) Tyrosine Kinase Inhibitors (TKIs). *J Thorac Oncol*. 2017;12(12):1766–1778.