Research Article

The Value of Next-Generation Sequencing for Treatment in Non-Small Cell Lung Cancer Patients: The Observational, Real-World Evidence in China

Yan Zhang, Wen-Xiang Shen, Li-Na Zhou, Min Tang, Yue Tan, Chun-Xia Feng, Ping Li, Li-Qiang Wang, and Min-Bin Chen

Department of Oncology, Affiliated Kunshan Hospital of Jiangsu University, Jiangsu, China

Correspondence should be addressed to Min-Bin Chen; cmb1981@163.com

Received 25 April 2019; Accepted 9 August 2019; Published 25 January 2020

Academic Editor: Kwang Gi Kim

Copyright © 2020 Yan Zhang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Great success has been made in the targeting therapy of advanced non-small cell lung cancer (NSCLC). Nowadays, next generation sequencing (NGS) is acquirable and affordable in developed area of China. Using this feasible and accurate method of detecting therapeutic genes would help to select optimal treatments to extend patients survival. Here, we identified somatic mutations by NGS and analyzed the value for treatment of NSCLC in a real-world clinical setting.

Methods. NGS was carried out on biopsy samples obtained from 66 advanced unresectable NSCLC patients who had not received any treatment. 23 patients received liquid biopsy after failure of first-line targeted treatment. The mutation profiling as well as associations between mutations and clinicopathological characters was analyzed. The study also assessed the values of NGS for choosing treatment options and predicting prognosis in NSCLC patients. Results. 152 somatic mutations were identified in 45 (68.18%) tissue samples. The most frequently mutated genes were EGFR (42.42%), TP53 (31.82%) and KRAS (15.15%). Specifically, the most frequent EGFR mutation subtypes were exon 19 deletion (60.71%) and L858R in exon 21 (46.43%). 83.33% mutated patients received targeted therapy. Among the adenocarcinoma cases, patients with EGFR exon 19 deletion mutation have longer overall survival (OS) than the wide-type (36.0 months versus 19.0 months \( p = 0.046 \)). In addition, in the smoking group, patients with EGFR exon 19 deletion mutation tended to have longer OS (38.0 months versus 16.5 months \( p < 0.01 \)). After the failure of first-line targeted therapy, 23 EGFR mutated patients received liquid biopsy, and the positive rate of T790M mutation in EGFR exon 20 was 47.83%. T790M positive patients have longer progression-free survival (PFS) than the others (15 months versus 9.5 months \( p = 0.025 \)). Conclusions. The observational study from real-world demonstrated that using NGS in routine clinical detection may be useful in guiding the therapy decisions and benefit more Chinese NSCLC patients.

1. Introduction

Non-small cell lung cancer (NSCLC) contributes to over 80% of all lung cancer cases and it is one of the leading causes of cancer-related deaths worldwide [1]. Although the application of surgery, chemotherapy, radiation and targeted therapy was beneficial for some patients, most of patients died of relapse, metastasis or even adverse effects by treatment [2, 3]. Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKI) have been used to treat NSCLC since 2003 with great success in patients with EGFR mutations [4–6], which made physicians pay more attention to “individualized treatment”.

Unfortunately, acquired resistance to EGFR-TKI treatment occurs inevitably. It is well known that NSCLC patients with the EGFR exon 19 deletion or L858R mutation show initial responses to first-generation of TKI, such as gefitinib and erlotinib. After about 9–14 months treatment, more than half of the patients relapsed [7]. Possible mechanisms for the acquired resistance may be the appearance of second-site hot spot mutations [8]. The most common and famous alteration is EGFR T790M mutation. The efficiency of the third-generation EGFR-TKI osimertinib in treating EGFR T790M mutation has been demonstrated in several studies [9–11]. However, more targeted and rare mutations and biomarkers...
are needed to use in clinical practice for prolonging survival of NSCLC patients.

With the wide use of next-generation sequencing (NGS), the genetic basis of various diseases, especially human cancers, have been disclosed. NGS is a high-throughput method that can detect numerous genetic variations, such as single nucleotide variants, insertions and deletions, copy number variations, and gene fusions over larger genomic regions. It is also noted for its high sensitivity and specificity [12]. Consequently, NGS may be a good tool for guiding the treatment of NSCLC.

The aim of this study was to identify somatic mutations by NGS and analyze the value for treatment in NSCLC patients in a real-world clinical setting.

2. Materials and Methods

2.1. Study Population. This study included 66 histologically confirmed NSCLC cases diagnosed in Affiliated Kunshan Hospital of Jiangsu University between January 2010 and September 2017. They were all advanced unresectable NSCLC patients. Tissues were obtained by transbronchoscopic lung biopsy, lymph node biopsy, thoracocentesis or lumbar puncture. The personal data of each participant about clinical characteristics and survival information was collected from clinical record or family contact. The overall survival (OS) was defined as time from the data of diagnosis to the data of death or last visit. The progression-free survival (PFS) was calculated from the time of diagnosis to the time of progression, relapse, death, or the last follow-up. This prospective observational study was reviewed by our institutional review board and written informed consent was provided by each patient.

2.2. Tissue DNA and Plasma Cell-Free DNA Extraction. Tissues were stored at −80°C until DNA extraction. Genomic DNA was extracted using a QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA, USA) according to manufacturer’s instructions. Almost 20 ml fluid sample was collected with EDTA (0.5 mmol/L, pH 8.0) to reach a final concentration of 10 ng cf DNA under 500X reads and/or the frequency was <10% were considered noninformative. Mutations were detected using the Variant Caller plugin (version 3.6; Thermo Fisher Scientific, Inc.). Each mutation was verified using the Integrative Genome Viewer (IGV) from the Broad Institute (www.broadinstitute.org) [14]. The NGS testing process took about five to seven days.

2.3. NGS Library Preparation and Sequencing. NGS was carried out on all DNA samples. Sequencing libraries were prepared using a KAPA Hyper Prep kit (KAPA Biosystems, Boston, MA) with an optimized manufacturer’s protocol for different samples types. In brief, 250 ng−1 μg genomic DNA fragments or 10–250 ng cf DNA underwent end-repairing, A-tailing and ligation with indexed adapters sequentially, followed by size selection of genomic DNA using Agencourt AMPure XP beads (Beckman Coulter, Pasadena, CA). Finally, libraries were amplified by PCR and purified for target enrichment. Hybridization-based target enrichment was performed using GeneseqOne™ 416-gene panel (Nanjing Geneseq Technology Inc., Nanjing, China). Library fragment size was determined by an Agilent Technologies (Palo Alto, CA) 2100 Bioanalyzer. The target-enriched library was then sequenced on HiSeq4000 NGS platforms (Illumina) [13].

2.4. NGS Data Analysis. The raw data were aligned to Human Genome version 19 (hg19) using Torrent Suite software (version 3.6.2; Thermo Fisher Scientific, Inc.). The coverage analysis was performed using the Coverage Analysis plugin (version 3.6; Thermo Fisher Scientific, Inc.). Cases for which the quality was <20% and/or the average base coverage was <500X reads and/or the frequency was <10% were considered noninformative. Statistical analyses were performed using the Variant Caller plugin (version 3.6; Thermo Fisher Scientific, Inc.). Each mutation was verified using the Integrative Genome Viewer (IGV) from the Broad Institute (www.broadinstitute.org) [14]. The NGS testing process took about five to seven days.

2.5. Statistical Analysis. Comparisons between groups were performed using Chi-squared test. The Kaplan-Meier method and log-rank tests were used to compare survival curves. For all the analyses, a two-sided p value of <0.05 was defined as significance. Statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Patient Characteristics. As shown in Table 1, 37 (56.06%) enrolled patients were male, and 29 (43.94%) were female. The median age was 63 years old (range 33–87 years). Of all the 66 patients, 55 (83.33%) were diagnosed with lung adenocarcinoma, and 11 (16.67%) were squamous cell carcinoma. More than half (57.58%) of the patients had a history of smoking and 26 patients (39.39%) were never smokers. Another 2 cases lost the information of smoking history. There were 23 (34.85%) patients have the family history of cancer.

3.2. Mutation Profiling by NGS. A total of 66 biopsies, consisting of 60 tissue samples, three pleural effusion samples, one cerebrospinal fluid sample, and one peritoneal fluid sample were obtained from the 66 NSCLC patients. We identified 152 somatic mutations in 45 (68.18%) patients, while 21 (31.82%) patients had no somatic mutations observed. The most frequently mutated genes were EGRF (42.42%), TP53 (31.82%) and KRAS (15.15%). Other oncogenic driver mutations including PTEN (7.78%), BRAF (6.06%), PIK3CA (4.55%), ERBB2 (4.55%), and ALK (3.03%) were also detected among the 66 patients (Figure 1). Specifically, the most frequent EGRF mutation subtypes identified included exon 19 deletion (60.71%), L858R in exon 21 (46.43%). Almost all EGRF mutated patients carried either exon 19 or 21 mutation, and only one patient was detected to carry both mutations.
Stratified analyzed for adenocarcinoma, the most frequently mutated genes were \textit{EGFR} (49.09%), \textit{TP53} (34.55%) and \textit{KRAS} (16.36%), which were similar to the full analysis sets. The \textit{EGFR} exon 19 deletion mutation rate was higher than exon 21 L858R (30.91% versus 23.64%).

We further analyzed the information of 23 adenocarcinoma patients received the first generation of \textit{EGFR}-TKI treatment after they were told \textit{EGFR} mutated. These patients all received liquid biopsy after they relapsed or progressed. We found that 11 (47.83%) patients had mutation of \textit{EGFR} T790M in exon 20, which was known as the most common mutation associated with acquired drug resistance. Other rare mutations included \textit{EGFR} G719A (1.52%) and MET amplification (1.52%).

3.3. Clinicopathological Features and Genetic Mutations. We analyzed the association between genetic mutations and clinicopathologic variables of the enrolled patients. As shown in Table 1, \textit{EGFR} exon 19 deletion mutation was associated with sex ($p = 0.045$), smoking history ($p = 0.041$) and family history ($p = 0.016$) and pathological type ($p = 0.032$). In another word, \textit{EGFR} 19-del mutation had an increased frequency in female, nonsmokers, who had family history and adenocarcinoma patients. However, \textit{EGFR} L858R, \textit{PTEN}, \textit{ALK}, or \textit{KRAS} were not associated with any clinicopathologic variables.

3.4. Utilization of NGS for Treatment Option and Prognosis Prediction in NSCLC Patients. In this study, 55 (83.33%) patients received targeted therapy such as \textit{EGFR}-TKI or crizotinib after the detection of \textit{EGFR} mutations or \textit{ALK} gene rearrangements, and the others received chemotherapy. The median OS for target therapy patients were 24 months and the first-line PFS were 10.5 months. As shown in Figure 2, patients with \textit{EGFR} exon 19 deletion had longer OS than those with exon 21 L858R mutation (37.0 months versus 19.0 months, $p = 0.01$). Subsequently, we further conducted stratified analysis, finding that among the adenocarcinoma cases, patients with \textit{EGFR} 19 deletion mutation have longer OS than the wide-type (36.0 months versus 19.0 months, $p = 0.046$, Figure 3). In addition, in the smoking group, patients with \textit{EGFR} 19 deletion mutation tended to have longer OS (38.0 months versus 16.5 months, $p < 0.01$, Figure 4).

After the first step of NGS detection, most of the \textit{EGFR} mutated patients received first-generation \textit{EGFR}-TKIs. The media PFS of these patients was 11 months. After disease progression, 23 patients received liquid biopsy. 11 (47.83%) patients had mutation of T790M in \textit{EGFR} exon 20. All these 11 patients received osimertinib as the second-line targeted therapy. It was also shown that, T790M positive patients have longer OS than the negative ones in the second-line treatment (15 months versus 9.5 months, $p = 0.032$, Figure 5).

4. Discussion

In the present study, we demonstrated the real-world data of mutations detected by NGS in Chinese NSCLC patients. Most of the patients benefited from the targeted therapy. We identified that NGS can be applied to guide treatment and predict prognosis in NSCLC patients.
patients who carried driver mutations showed longer OS and PFS. Several studies have reported the use of NGS to detect the oncogenic driver mutations could guide therapy decisions and thus prolong the survival of NSCLC patients [16, 17]. We believe that our real-world data may add new evidence for it.

In our study, 23 EGFR mutated patients received second-line liquid biopsy after the failure of first-generation of its introduction in 2007, NGS technology has already made extraordinary advances, making the detection of genetic alterations to guide therapy to be more feasible. And as NGS becomes faster and less expensive, it’s sure to be used more frequently and with greater benefit for Chinese NSCLC patients [15]. In our real-world data, 83.33% patients chose NGS-guided targeted therapy in first-line and/or second-line therapy. These patients, especially adenocarcinoma patients who carried driver mutations showed longer OS and PFS. Several studies have reported the use of NGS to detect the oncogenic driver mutations could guide therapy decisions and thus prolong the survival of NSCLC patients [16, 17]. We believe that our real-world data may add new evidence for it.

In our study, 23 EGFR mutated patients received second-line liquid biopsy after the failure of first-generation of

| Characteristics            | All n = 66 (%) | EGFR 19 del | P value | EGFR L858R | P value |
|----------------------------|----------------|------------|---------|------------|---------|
|                           |                | Wide-type  | Mutant  | Wide-type  | Mutant  |
| Age (years)               |                | n = 49 (%) | n = 17 (%) | n = 53 (%) | n = 13 (%) |
| ≥65                       | 29 (43.94)     | 24 (48.98) | 5 (29.41) | 22 (41.51) | 7 (53.85) | 0.161 | 0.422 |
| <65                       | 37 (56.06)     | 25 (51.02) | 12 (70.59) | 31 (58.49) | 6 (46.15) |
| Sex                       |                |            |         |            |         | 0.045 | 0.858 |
| Male                      | 37 (56.06)     | 31 (63.27) | 6 (35.29) | 30 (56.60) | 7 (53.85) |
| Female                    | 29 (43.94)     | 18 (36.73) | 11 (64.71) | 23 (43.40) | 6 (46.15) |
| Smoking history           |                |            |         |            |         | 0.041 | 0.548 |
| No                        | 26 (39.39)     | 15 (30.61) | 11 (64.71) | 21 (39.62) | 5 (38.46) |
| Yes                       | 38 (57.58)     | 32 (65.31) | 6 (35.29) | 31 (58.49) | 7 (53.85) |
| Unknown                   | 2 (3.03)       | 2 (4.08)   | 0        | 1 (1.89)   | 1 (7.69)   |
| Family history            |                |            |         |            |         | 0.016 | 0.340 |
| No                        | 43 (65.15)     | 36 (73.47) | 7 (41.18) | 36 (22.03) | 7 (53.85) |
| Yes                       | 23 (34.85)     | 13 (26.53) | 10 (58.82) | 17 (77.97) | 6 (46.15) |
| Pathological type         |                |            |         |            |         | 0.032 | 0.333 |
| Adenocarcinoma            | 55 (83.33)     | 38 (77.55) | 17 (100) | 43 (81.13) | 12 (92.31) |
| Squamous cell carcinoma   | 11 (16.67)     | 11 (22.45) | 0        | 10 (18.87) | 1 (7.69)   |

EGFR 19-del mutation had an increased frequency in female (p = 0.045), non-smokers (p = 0.046), who had family history (p = 0.016) and adenocarcinoma patients (p = 0.032).

Figure 2: Kaplan-Meier survival curves of overall survival in NSCLC according to EGFR mutations.

Figure 3: Kaplan-Meier survival curves of overall survival in adenocarcinoma according to EGFR 19 deletion status.
We supposed that repeated NGS in relapse and metastasis NSCLC patients was essential and it also could guide the following treatment.

It was reported that, \( \text{EGFR} \) mutation rate varies in different countries, and the mutation rate was much higher in Asian people [18, 19]. Similar to the study conducted in southern China [20], we showed that \( \text{EGFR} \) was the most frequent mutation in NSCLC patients. The majority of \( \text{EGFR} \) mutations were exon 19 deletion (60.71%), L858R in exon 21 (46.43%). \( \text{EGFR} \) double mutation is not rare in Asia [21]. Analyzed for the 23 patients received second-line liquid biopsy, double mutation rate was 47.83% (11 cases), of which exon 19 deletion combined with T790M account for 81.82% (9/11). On the other hand, several studies conducted for Chinese patients reported L858R combined with T790M occurred more often [20, 22]. The different incidences of \( \text{EGFR} \) mutation pattern may cause by limited sample sizes and ethnic differences.

Besides \( \text{EGFR} \), the mutation rate of other oncogenic driver mutations in our study such as \( \text{PTEN}, \text{KRAS}, \text{ALK} \) and \( \text{BRAF} \) were consistent with previous studies [23, 24]. Although driver genes mutations were reported to be mutually exclusive in NSCLC [25], we found one case carried both \( \text{EGFR} \text{L858R} \) mutation and \( \text{ALK} \) rearrangement. The patient received first-line therapy of gefitinib, and the PFS was 8 months. With one cycle of crizotinib as second-line therapy, his tumor progressed again. The OS was 15 months which was shorter than the average. This result was similar to other study. Yang et al. showed the median PFS of patients with concurrent \( \text{EGFR}/\text{ALK} \) mutations treated with \( \text{EGFR-TKI} \) ranged from 5.0 to 11.2 months, relatively lower than patients harboring only \( \text{EGFR} \) mutation [26].

The use of NGS in this present study revealed that females, non-smokers, family history of cancer and adenocarcinoma patients had greater \( \text{EGFR} \) 19 deletion mutation rate, consistent with most previous reports [23, 27]. The difference of mutation rate between males and females may be caused by the higher smoking rate in males. It was also found from the stratified analysis that among the smokers, patients with \( \text{EGFR} \) exon 19 deletion mutation tended to have longer OS. We think it may be due to that the mutated patients were more likely to choose the targeted therapies. Patients with \( \text{EGFR} \) exon 19 deletion were reported to have longer survival than those with exon 21 mutation [28, 29], though the detailed mechanism remain unknown. Our results added new evidences for this conclusion.

5. Conclusions

In summary, we applied NGS in NSCLC tumor tissue at the moment of diagnosis and in liquid biopsy at the moment of progression in a subset of \( \text{EGFR} \) mutant patients. It was demonstrated that using NGS in routine clinical detection allows selecting a better treatment for patients and even improving PFS and OS. Further larger-scale studies focus on the prognostic value of NGS in NSCLC and other tumors are needed to confirm the advantages of this tool.
Data Availability

No data were used to support this study.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional (Institutional Review Board of Kunshan First People’s Hospital Affiliated to Jiangsu University-20180124) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Yan Zhang and Wen-Xiang Shen are contributed equally.

Acknowledgments

This work is supported by the National Natural Science Foundation (81773192); Natural Science Foundation of Jiangsu Province (BK20171248); Jiangsu Youth Medical Talents Project (QNRC2016527); Jiangsu Province “333 Project” Research Projects (2016-III-0367); The Foundation of tumor clinical and basic research team (KYC005).

References

[1] R. L. Siegel, K. D. Miller, and A. Jemal, “Cancer statistics, 2017,” CA: A Cancer Journal for Clinicians, vol. 67, no. 1, pp. 7–30, 2017.

[2] C. Mayo-de-Las-Casas, N. Jordana-Ariza, M. Garzon-Ibanez et al., “Large scale, prospective screening of EGFR mutations in the blood of advanced NSCLC patients to guide treatment decisions,” Annals of Oncology: Official Journal of the European Society for Medical Oncology, vol. 28, no. 9, pp. 2248–2255, 2017.

[3] N. Singh, A. N. Aggarwal, J. Kaur, and D. Behera, “Association of graded folic acid supplementation and total plasma homocysteine levels with hematological toxicity during first-line treatment of nonsquamous NSCLC patients with pemetrexed-based chemotherapy,” American Journal of Clinical Oncology, vol. 40, no. 1, pp. 75–82, 2017.

[4] T. S. Mok, Y. L. Wu, S. Thongprasert et al., “Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma,” The New England Journal of Medicine, vol. 361, no. 10, pp. 947–957, 2009.

[5] J. Y. Douillard, F. A. Shepherd, V. Hirsh et al., “Molecular predictors of outcome with gefitinib and docetaxel in previously treated non-small-cell lung cancer: data from the randomized phase III INTEREST trial,” Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, vol. 28, no. 5, pp. 744–752, 2010.

[6] R. Rosell, E. Carcereny, R. Gervais 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, The Lancet,” Oncology, vol. 13, pp. 239–246, 2012.

[7] T. Kaburagi, M. Kiyoshima, T. Nawa et al., “Acquired EGFR T790M mutation after relapse following EGFR-TKI therapy: a population-based multi-institutional study,” Anticancer Research, vol. 38, no. 5, pp. 3145–3150, 2018.

[8] G. P. Doss, B. Rajith, C. Chakraborty, N. NagaSundaram, S. K. Ali, and H. Zhu, “Structural signature of the G719S–T790M double mutation in the EGFR kinase domain and its response to inhibitors,” Scientific Reports, vol. 4, no. 1, p. 5868, 2014.

[9] S. Khozin, C. Weinstock, G. M. Blumenthal et al., “Osimertinib for the treatment of metastatic EGFR T790M mutation-positive non-small cell lung cancer,” Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, vol. 23, no. 9, pp. 2131–2135, 2017.

[10] J. C. Yang, M. J. Ahn, D. W. Kim et al., “Osimertinib in pretreated T790M-positive advanced non-small-cell lung cancer: AURA phase II extension component,” Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, vol. 35, no. 12, pp. 1288–1296, 2017.

[11] T. S. Mok, Y. L. Wu, M. J. Ahn et al., “Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer,” The New England Journal of Medicine, vol. 376, no. 7, pp. 629–640, 2017.

[12] N. Chennagiri, E. J. White, A. Frieden et al., “Orthogonal NGS for high throughput clinical diagnostics,” Scientific Reports, vol. 6, no. 1, p. 24650, 2016.

[13] Z. Wu, Z. Yang, C. S. Li et al., “Differences in the genomic profiles of cell-free DNA between plasma, sputum, urine, and tumor tissue in advanced NSCLC,” Cancer Medicine, vol. 8, no. 5, pp. 910–919, 2019.

[14] H. Thorvaldsdottir, J. T. Robinson, and J. P. Mesirov, “Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration,” Briefings in Bioinformatics, vol. 14, no. 2, pp. 178–192, 2013.

[15] M. Saito, T. Momma, and K. Kono, “Targeted therapy according to next generation sequencing-based panel sequencing,” Fukushima Journal of Medical Science, vol. 64, no. 1, pp. 9–14, 2018.

[16] M. Provenço, M. Torrente, V. Calvo et al., “Prognostic value of quantitative ctDNA levels in non small cell lung cancer patients,” Oncotarget, vol. 9, no. 1, pp. 488–494, 2018.

[17] L. Cao, L. Long, M. Li et al., “The utilization of next-generation sequencing to detect somatic mutations and predict clinical prognosis of Chinese non-small cell lung cancer patients,” OncoTargets and Therapy, vol. 11, pp. 2637–2646, 2018.

[18] Q. Zhou, X. C. Zhang, Z. H. Chen et al., “Relative abundance of EGFR mutations predicts benefit from gefitinib treatment for advanced non-small-cell lung cancer,” Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, vol. 29, no. 24, pp. 3316–3321, 2011.

[19] A. Tamiya, M. Tamiya, T. Nishihara et al., “Cerebrospinal fluid penetration rate and efficacy of afatinib in patients with EGFR mutation-positive non-small cell lung cancer with
Leptomeningeal Carcinomatosis: multicenter prospective study,” Anticancer Research, vol. 37, no. 8, pp. 4177–4182, 2017.

[20] J. Zhou, X. B. Song, H. He, Y. Zhou, X. J. Lu, and B. W. Ying, “Prevalence and clinical profile of EGFR mutation in non-small-cell lung carcinoma patients in Southwest China,” Asian Pacific Journal of Cancer Prevention: APJCP, vol. 17, no. 3, pp. 965–971, 2016.

[21] M. A. Lowder, A. E. Doerner, and A. Schepartz, “Structural differences between wild-type and double mutant EGFR modulated by third-generation kinase inhibitors,” Journal of the American Chemical Society, vol. 137, no. 20, pp. 6456–6459, 2015.

[22] X. Cai, J. Sheng, C. Tang et al., “Frequent mutations in EGFR, KRAS and TP53 genes in human lung cancer tumors detected by ion torrent DNA sequencing,” PLoS One, vol. 9, no. 4, p. e95228, 2014.

[23] S. Li, L. Li, Y. Zhu et al., “Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 Chinese cohorts,” British Journal of Cancer, vol. 110, no. 11, pp. 2812–2820, 2014.

[24] W. Hu, Y. Liu, and J. Chen, “Concurrent gene alterations with EGFR mutation and treatment efficacy of EGFR-TKIs in Chinese patients with non-small cell lung cancer,” Oncotarget, vol. 8, no. 15, pp. 25046–25054, 2017.

[25] J. F. Gainor, A. M. Varghese, S. H. Ou et al., “ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer,” Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, vol. 19, no. 15, pp. 4273–4281, 2013.

[26] J. J. Yang, X. C. Zhang, J. Su et al., “Lung cancers with concomitant EGFR mutations and ALK rearrangements: diverse responses to EGFR-TKI and crizotinib in relation to diverse receptors phosphorylation,” Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, vol. 20, no. 5, pp. 1383–1392, 2014.

[27] Y. L. Zhang, J. Q. Yuan, K. F. Wang et al., “The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis,” Oncotarget, vol. 7, pp. 78985–78993, 2016.

[28] Y. Wang, R. Q. Li, Y. Q. Ai et al., “Exon 19 deletion was associated with better survival outcomes in advanced lung adenocarcinoma with mutant EGFR treated with EGFR-TKIs as second-line therapy after first-line chemotherapy: a retrospective analysis of 128 patients,” Clinical and Translational Oncology: Official Publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico, vol. 17, pp. 727–736, 2015.

[29] Y. W. Won, J. Y. Han, G. K. Lee et al., “Comparison of clinical outcome of patients with non-small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutations,” Journal of Clinical Pathology, vol. 64, no. 11, pp. 947–952, 2011.