THERAPEUTIC STRATEGIES IN EGFR MUTANT LUNG CANCER

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THERAPEUTIC STRATEGIES IN EGFR MUTANT LUNG CANCER

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CASE REPORT
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Neoadjuvant Four-Drug Combination Therapy for NSCLC With EGFR Mutation Avoiding Total Pneumonectomy

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We report a case of successful neoadjuvant four-drug combination therapy to avoid total pneumonectomy. A 33-year-old male patient was diagnosed with locally advanced non-squamous NSCLC harboring EGFR mutation in the left lower lobe. The patient experienced significant clinical downstaging after two cycles of neoadjuvant therapy, including icotinib, carboplatin, pemetrexed, and bevacizumab. He underwent a successful lobectomy avoiding pneumonectomy. The patient showed no recurrence in the follow-up of a chest computed tomographic scan, which is 17 months after surgery. The promising results of this neoadjuvant combination therapy provided a novel therapeutic option for patients with locally advanced EGFR-mutated NSCLC facing total pneumonectomy.

Keywords: neoadjuvant, EGFR mutation, targeted (selective) treatment, chemothearpy, surgery

INTRODUCTION

Even with multidisciplinary treatment, the 5-year overall survival rates in stage III NSCLC patients are ∼25 to 35% (1). Molecularly targeted agents have been shown to improve the overall survival for patients with NSCLC harboring epidermal growth factor receptor (EGFR) mutation in the setting of advanced disease. Researchers have begun to seek the possibility of applying a single agent of EGFR- tyrosine kinase inhibitor (TKI) from advanced to earlier stage (2). However, despite achieving significant downstaging of tumors, local or distant disease relapsed in less than 1 year. Various combination therapy achieved the promising results in the setting of advanced stage, including chemotherapy/EGFR-TKI (3, 4), chemotherapy/Bevacizumab (5), EGFR-TKI/Bevacizumab (6, 7), and eventually chemotherapy/EGFR-TKI/Bevacizumab (8). Currently, some prospective trials (9–11) had investigated the critical role of EGFR-TKI (gefitinib or erlotinib) in neoadjuvant therapy, the objective response rate (ORR) was ranged from 42.1 to 54.5%, and the rate of major pathological regression (MPR) was from 9.7% up to 24.2%. Considering the neoadjuvant EGFR-TKI combined with chemotherapy or anti-angiogenesis was tolerable, and could improve the radical resection rate, so we proposed a four-agent combination therapy for locally advanced non-squamous non-small-cell lung cancer harboring EGFR mutations.

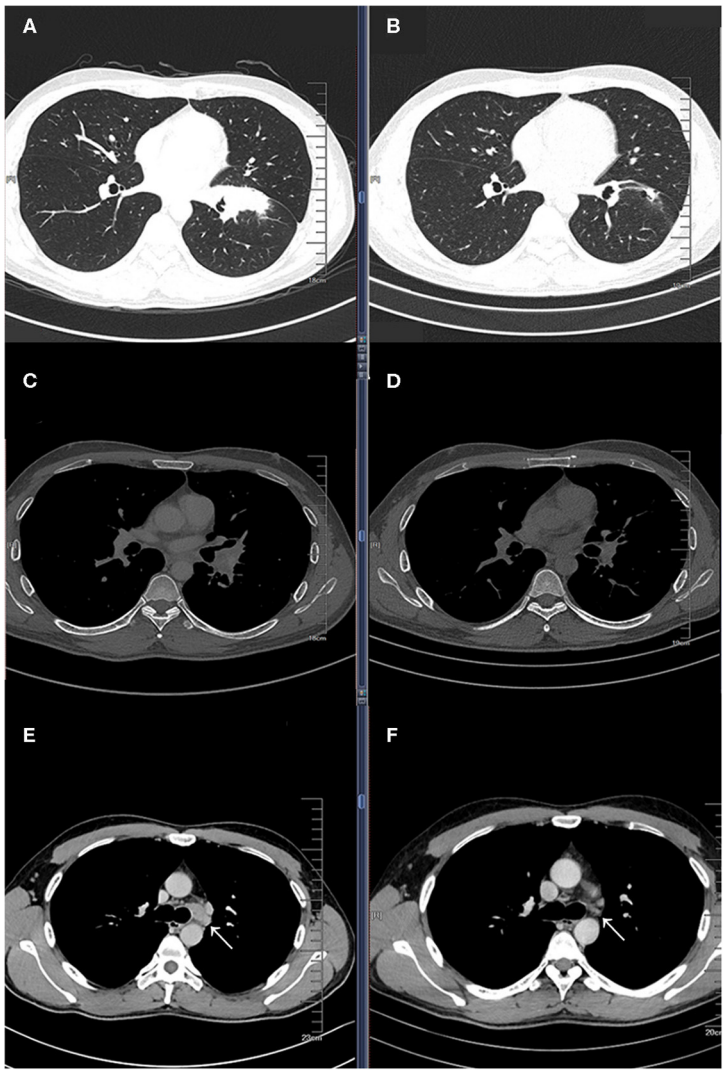

FIGURE 1 | Baseline computed tomography (CT) showing a lung mass in the left lower lobe pulmonary window **(A)** and mediastinal window **(C)**, and an enlarged left lower paratracheal (4L) lymph node **(E)**; CT images after two cycles of four drug combination therapy showing partial response in the pulmonary window **(B)** and mediastinal window **(D)**, and a significantly reduction in the 4L lymph node **(F)**.

CASE DESCRIPTION

A 33-year-old male smoker presented with cough and sputum was admitted to the department of thoracic surgery. Computed tomography (CT) revealed a lung mass in the left lower lobe (**Figures 1A,C**) and multiple hilar and mediastinal lymph nodes (**Figure 1E**). Cerebral magnetic resonance imaging and the bone scan did not show any lesion. The bronchoscopic biopsy confirmed the diagnosis of lung adenocarcinoma (cT2bN2M0, stage IIIA), and amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) showed an EGFR 19 exon deletion mutation. Multidisciplinary team discussion suggested neoadjuvant therapy over total pneumonectomy. Based on our previous experience of various combination therapies within our institute, four-drug combination therapy with fixed regimens was proposed, including icotinib 125 mg po, tid, and intravenous carboplatin 150 mg D1-D2, pemetrexed 800 mg D1, and bevacizumab 300 mg D1, given a young age and good general status. After two cycles of neoadjuvant therapy, the Positron emission tomography/Computed tomography (PET/CT) revealed that the tumor was reduced from 4.6 × 2.0 cm to 1.4 × 1.1 cm (**Figures 1B,D**) with a normal standard uptake value (SUV), and there were no enlarged mediastinal or hilar lymph nodes (**Figure 1F**). No grade 3/4 adverse event (AE), including hemoptysis, was experienced,while rash and gastrointestinal symptoms were the most frequent AEs based on the patient's self-report.

The re-evaluation workup suggested a significant downstaging of the tumor (cT1bN0M0, stage IA2). Then 6 weeks after the last cycle of combination regimens were given, a left lower lobectomy was proposed. However, an occult parietal

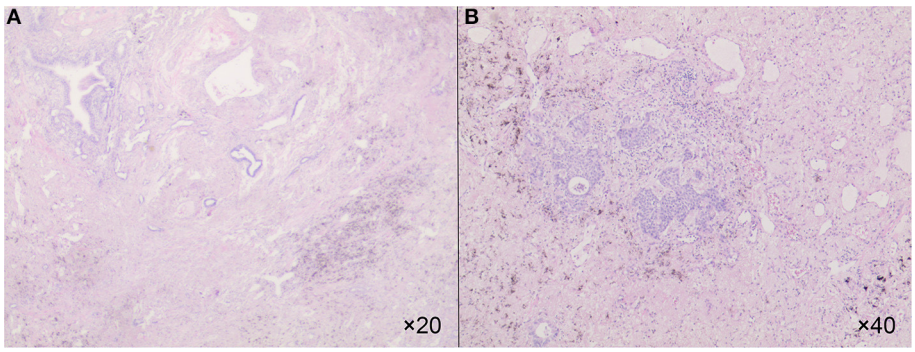

FIGURE 2 | The HE showed an increase in the number of residue cancer cells, but fibrosis still predominated in 20× **(A)** and in 40× **(B)**.

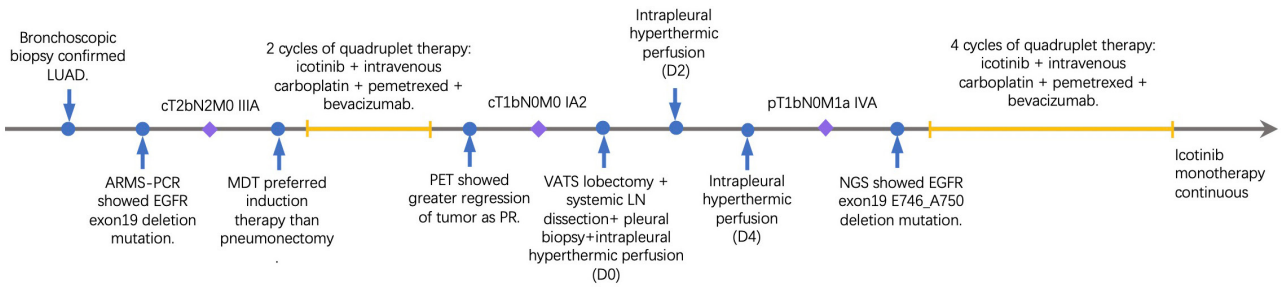

FIGURE 3 | The timeline showing the relevant data of treatment.

pleural metastasis was detected during surgery. In the operative fields, we observed tissue edema and hyperemia of the lung and moderate fibrosis of the external coat of vessels. The patient underwent a video-assisted thoracoscopic surgery (VATS) left lower lobectomy, systematic lymphadenectomy, pleural biopsy, and intrapleural hyperthermic perfusions, as well as two additional intrapleural hyperthermic perfusion performed on the next 2 days. The estimated intraoperative blood loss was nearly 200 ml due to mild tissue hyperemia of the lung. The perioperative recovery was uneventful. The patient was discharged on the seventh postoperative day. Pathology confirmed the diagnosis of lung adenocarcinoma with pleural metastasis (ypT1bN0M1a, stage IVA). The histologic findings indicated significant tumor regression. It was characterized by an increase in the number of residue cancer cells, but fibrosis still predominated (**Figures 2A,B**). No lymph nodes metastasis was found at the dissected nodes station 5 (0/1), 7 (0/2), and 10 (0/1); no vascular cancer thrombus and no residual tumor in the bronchial stumps were observed. The next-generation sequencing (NGS) of pathological specimens also confirmed EGFR 19 exon E746_A750 deletion mutation. Thus, after 1 month for recovery postoperatively, an additional of four-drug combination therapy was successfully conducted every 3 weeks ± 3 day for four cycles. Single-agent icotinib was continued, with a follow-up chest CT scan every 3 months and PET/CT every 6 months. The latest follow-up recorded no disease progression in a chest CT scan when it was 16.7 months after surgery and 19.4 months after initial treatment. The timeline of treatment with relevant data of care was shown in **Figure 3**.

DISCUSSION

The patient's course illustrates the successful use of four-drug combination therapy to treat a locally advanced non-squamous NSCLC harboring EGFR mutation. The patient experienced significant tumor downstaging and underwent lobectomy instead of total pneumonectomy.

Patients with locally advanced NSCLC, especially N2 disease, should be considered for neoadjuvant chemotherapy or chemoradiotherapy based on NCCN guidelines. Although EGFR-TKI monotherapy is considered the standard first-line treatment for patients with TKI-sensitive EGFR mutations, most patients develop recurrence within ∼1 year. The rationale for concurrent use of cytotoxic agents and bevacizumab is to reduce or even avoid potential *de novo* resistance (12). Sugawara (13) obtained the median progression-free survival (PFS) 18.3 and 15.3 months for the concurrent and sequential alternating regimens with gefitinib and carboplatin/pemetrexed in patients with EGFR-mutated NSCLC in a randomized phase II study, which is more promising in comparison to the PFS in previous studies with first-line gefitinib monotherapy (14). Two randomized clinical trials demonstrated erlotinib combined with bevacizumab harvest a median PFS of 16.0–16.9 months, which was significantly better than erlotinib monotherapy (7).

Under the expectation to provide high-level, customized treatment for patients with resectable NSCLC, several phase II clinical trials had revealed the efficacy and safety on respect of preoperative TKI therapy (10, 11). EMERGING-CTONG 1103 showed the improved ORR for neoadjuvant erlotinib with 54.1% compared to gemcitabine plus cisplatin chemotherapy with 34.3% and doubling PFS with erlotinib (21.5 months) vs. GC chemotherapy (11.4 months; $p < 0.001$). Zhang's study showed similar ORR to the previous study, and patients with MPR were associated with improved survival while no patients reported grade 3 or 4 AEs. These results suggest that biomarker-guided neoadjuvant EGFR-TKI treatment in resectable NSCLC is promising. Hence, we combined these four drugs to optimize the treatment effect by avoiding potential *de novo* resistance. This case did reflect a promising effect. It is possible that TKI played a key role in the significant response of preoperative therapy. However, the first generation of EGFR-TKI is reversible TKI, which leaves it a tumor inhibitor. Chemotherapy provides a curative potential, which is critical for significant response of preoperative therapy and long-term tumor control. Since no neoadjuvant therapy can guarantee a full transfer to complete resection. In the long-term, however, bevacizumab may reduce potential *de novo* resistance if complete resection is unachievable. So it was important to include bevacizumab in the combination therapy.

Apart from a slight increase in intraoperative blood loss, there were no significant adverse events reported in this case, which was mainly the result of careful case selection and a fixed lower dosage of the cytotoxic agents and is far from a standard requirement. In our institute, we have attempt to apply the EGFR-TKI, EGFR-TKI/Chemotherapy, and Chemotherapy/Bevacizumab in several locally advanced NSCLC patients. The regimens of Chemotherapy were all adjusted to this fixed lower dosage. No significant adverse event was observed in the treatment period. All patients underwent surgery successfully. Yet, during the surgery, different degrees of tissue edema and hyperemia of the lung and fibrosis of the external coat of vessels in the operative fields. In general, any therapy involving EGFR-TKI was expecting moderate to severe fibrosis of the external coat of vessels. There are a few reasons that accounted for the choice of icotinib in this patient. First, we did not choose erlotinib or gefitinib because there was a beneficence project in China so that the patient can acquire a free prescription after 10 months of using icotinib. So it was more economical to use this drug, given the non-inferior to gefitinib in efficacy with favorable safety in non-selected or EGFR-mutant NSCLC patients (15). Second, we did not choose osimertinib because it would be 10-fold higher than icotinib and nearly 20 times of cost if insurance was taken into consideration. Though median progression-free survival was significantly longer with osimertinib than SoC EGFR-TKI (16), the overall survival of the FLAURA study of osimertinib was not reported until recently (17).

In conclusion, treatment with this neoadjuvant combination therapy provided a novel therapeutic option for patients with locally advanced EGFR-mutated NSCLC facing total pneumonectomy.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JL and JH was involved in planning and supervised the study. All authors wrote the manuscript and designed the figures. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Bradley JD, Paulus R, Komaki R, Masters G, Blumenschein G, Schild S, et al. Standard-dose versus high-dose conformal radiotherapy with concurrent and consolidation carboplatin plus paclitaxel with or without cetuximab for patients with stage IIIA or IIIB non-small-cell lung cancer (RTOG 0617): a randomised, two-by-two factorial phase 3 study. *Lancet Oncol*. (2015) 16:187–99. doi: 10.1016/S1470-2045(14)71207-0
2. Mazzoni F, Petreni P, Perna M, Scotti V, Bongiolatti S, Livi L, et al. Afatinib with subsequent surgery in stage III NSCLC with EGFR mutation: lessons learned from two clinical experiences. *Tumor J*. (2018) 104:P5–9. doi: 10.1177/0300891618775204
3. Yang JC, Srimuninnimit V, Ahn MJ, Lin CC, Kim SW, Tsai CM, et al. First-line pemetrexed plus cisplatin followed by Gefitinib maintenance therapy versus Gefitinib monotherapy in East Asian never-smoker patients with locally advanced or metastatic nonsquamous non-small cell lung cancer: final overall survival results from a randomized phase 3 study. *J Thorac Oncol*. (2016) 11:370–9. doi: 10.1016/j.jtho.2015.11.008
4. Noronha V, Patil VM, Joshi A, Menon N, Chougule A, Mahajan A, et al. Gefitinib versus gefitinib plus pemetrexed and carboplatin chemotherapy in EGFR-mutated lung cancer. *J Clin Oncol*. (2020) 38:124–36. doi: 10.1200/JCO.19.01154
5. Yokoi T, Torii Y, Katashiba Y, Sugimoto H, Tanijiri T, Ogata M, et al. Phase II study of pemetrexed and carboplatin plus bevacizumab, followed by maintenance pemetrexed and bevacizumab in Japanese patients with

non-squamous non-small cell lung cancer. *Oncol Lett*. (2014) 8:2453–7. doi: 10.3892/ol.2014.2552
6. Nakagawa K, Garon EB, SetoT, Nishio M, Aix SP, Paz-Ares L, et al., Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. (2019) 20:1655–69. doi: 10.1016/S1470-2045(19)30634-5
7. Saito H, Fukuhara T, Furuya N, Watanabe K, Sugawara S, Iwasawa S, et al. Erlotinib plus bevacizumab versus erlotinib alone in patients with EGFR-positive advanced non-squamous non-small-cell lung cancer (NEJ026): interim analysis of an open-label, randomised, multicentre, phase 3 trial. *Lancet Oncol*. (2019) 20:625–35. doi: 10.1016/S1470-2045(19)30035-X
8. Kurata T, Nakaya A, Yokoi T, Niki M, Kibata K, Takeyasu Y, et al. Phase I/II study of erlotinib, carboplatin, pemetrexed, and bevacizumab in chemotherapy-naïve patients with advanced non-squamous non-small cell lung cancer harboring epidermal growth factor receptor mutation. *Genes Cancer*. (2017) 8:559–65. doi: 10.18632/genesandcancer.145
9. Xiong L, Li R, Sun J, Lou Y, Zhang W, Bai H, et al. Erlotinib as neoadjuvant therapy in stage IIIA (N2) mutation-positive non-small cell lung cancer: a prospective, single-arm, phase II study. *Oncologist*. (2019) 24:157–64. doi: 10.1634/theoncologist.2018-0120
10. Zhong WZ, Chen KN, Chen C, Gu CD, Wang J, Yang XN, et al. Erlotinib versus gemcitabine plus cisplatin as neoadjuvant treatment of stage IIIA-N2 -mutant non-small-cell lung cancer (EMERGING-CTONG 1103): a randomized phase II study. *J Clin Oncol*. (2019) 37:2235–45. doi: 10.1200/JCO.19.00075
11. Zhang Y, Fu F, Hu H, Wang S, Li Y, Hu H, et al. Gefitinib as neoadjuvant therapy for resectable stage II-IIIA non-small cell lung cancer: a phase II study. *J Thorac Cardiovasc Surg*. (2020). doi: 10.1016/j.jtcvs.2020.02.131. [Epub ahead of print].
12. Takahashi K, Saito H. Is epidermal growth factor receptor tyrosine kinase inhibitor in combination with cytotoxic chemotherapy a better treatment option for patients with EGFR-mutated non-small-cell lung cancer? *Transl Lung Cancer Res*. (2016) 5:98–101. doi: 10.3978/j.issn.2218-6751.2015.08.14
13. Sugawara S, Oizumi S, Minato K, Harada T, Inoue A, Fujita Y, et al. Randomized phase II study of concurrent versus sequential alternating gefitinib and chemotherapy in previously untreated non-small cell lung cancer with sensitive EGFR mutations: NEJ005/TCOG0902. *Ann Oncol*. (2015) 26:888–94. doi: 10.1093/annonc/mdv063
14. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. (2010) 362:2380–8. doi: 10.1056/NEJMoa0909530
15. Shi Y, Zhang L, Liu X, Zhou C, Zhang L, Zhang S, et al. Icotinib versus gefitinib in previously treated advanced non-small-cell lung cancer (ICOGEN): a randomised, double-blind phase 3 non-inferiority trial. *Lancet Oncol*. (2013) 14:953–61. doi: 10.1016/S1470-2045(13)70355-3
16. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. (2018) 378:113–25. doi: 10.1056/NEJMoa1713137
17. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, et al. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N Engl J Med*. (2020) 382:41–50. doi: 10.1056/NEJMoa1913662

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ERRATUM
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Erratum: Neoadjuvant Four-Drug Combination Therapy for NSCLC With EGFR Mutation Avoiding Total Pneumonectomy

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ORIGINAL RESEARCH
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Soluble PD-L1 as a Predictor of the Response to EGFR-TKIs in Non-small Cell Lung Cancer Patients With *EGFR* Mutations

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Programmed cell death ligand 1 (PD-L1) expressed on tumor tissues is a vital molecule for immune suppression and its impact on the response to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) has been reported. The significance of soluble PD-L1 (sPD-L1) for lung cancer patients remains unknown. This study investigated whether sPD-L1 could predict the response of *EGFR*-mutated non-small cell lung cancer (NSCLC) to EGFR-targeted therapy. We retrospectively evaluated patients who received first-line treatment with EGFR-TKIs for advanced NSCLC with *EGFR* mutations. Pre-treatment plasma concentrations of PD-L1 and on-treatment (1 month after treatment initiation) plasma concentrations of PD-L1 were measured using the R-plex Human PD-L1 assay. The association between the sPD-L1 level and the clinical outcome was analyzed. Among 66 patients who were eligible for the study, patients with high pre-treatment or on-treatment sPD-L1 levels had decreased objective response rate (ORR) compared with that of patients with low sPD-L1 levels (39.4 vs. 66.7%, $p = 0.026$ for pre-treatment sPD-L1 level, and 43.5 vs. 73.9%, $p = 0.025$ for on-treatment sPD-L1 level). A high baseline sPD-L1 level was associated with a shortened progression-free survival (PFS) rate (9.9 vs. 16.1 months, $p = 0.005$). Both univariate and multivariate analyses showed that a high baseline sPD-L1 level was an independent factor associated with the PFS (hazard ratio [HR] 2.56, $p = 0.011$). Our study revealed that the sPD-L1 level was strongly related to the outcome of EGFR-TKIs in NSCLC patients harboring *EGFR* mutations.

Keywords: soluble PD-L1, non-small cell lung cancer, EGFR-TKIs, efficacy, prediction

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INTRODUCTION

Lung cancer remains the leading cause of cancer-related death around the world. Despite significant improvements in the treatment of this malignancy, the prognosis remains poor (1). In recent decades, targeted therapies such as the EGFR-TKIs have markedly improved the management of NSCLC patients with *EGFR* mutations (2–4). Nevertheless, in most patients, the disease inevitably progresses despite an initial dramatic and rapid response to the EGFR-TKIs. Some patients demonstrate a primary resistance to EGFR-TKIs in spite of harboring *EGFR*-sensitive mutations

(5). Preclinical studies have suggested that the immune microenvironment can influence the effects of targeted therapy and may serve as one of the mechanisms of resistance to small molecule inhibitors (6–8), but the clinical significance of this interaction in *EGFR*-mutant NSCLC has not been well-verified.

The activation of the programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) pathway, which leads to exhausted T-cells and continuous cancer growth, has been identified as the most critical mechanism of tumor evasion (9). PD-1/PD-L1 antibodies have demonstrated impressive anti-tumor responses by releasing the PD-1/PD-L1-mediated control of the immune system, and this activity has therefore become a highly promising treatment strategy for NSCLC in recent years (10). However, NSCLC patients with *EGFR* mutations exhibited a rather low response to PD-1/PD-L1 checkpoint inhibitors (11). Recent studies have identified the association between upregulation of the PD-1/PD-L1 pathway and a resistance to EGFR-targeted therapy. Han et al. detected increased PD-L1 expression when patients acquired a resistance to EGFR-TKIs (12). High levels of PD-L1 expression were also reported to be correlated with a primary resistance and predicted a poor response to EGFR-TKIs (13, 14). These findings may provide implications for using PD-1/PD-L1 inhibitors in patients with *EGFR*-mutant NSCLC.

Evaluating PD-L1 expression in tumor tissue is challenging. First, it is not easy to obtain sufficient tumor samples for analysis from inoperable patients. Furthermore, the test results of PD-L1 expression may differ according to the anti-PD-L1 antibodies applied (15). And the results may vary due to the intra-tumor heterogeneity of PD-L1 expression (16). Soluble forms of PD-L1 (sPD-L1) have recently been identified in blood samples of patients with various malignancies (17–21). A previous study has shown that sPD-L1 may impair host immunity and contribute to systemic immunosuppression, subsequently leading to cancer progression and a poor clinical outcome (22). In lung cancer, it has been reported that high sPD-L1 levels in plasma were associated with a poor prognosis (18). The association between sPD-L1 level and clinical outcome of EGFR-TKIs have not been elaborated, however. Therefore, our study aimed to investigate the impact of sPD-L1 levels on the treatment response to EGFR-TKIs in treatment-naïve NSCLC patients with *EGFR* mutations.

MATERIALS AND METHODS

Study Population

For this retrospective study, we included patients with advanced NSCLC who had started EGFR-TKI treatment between 2014 and 2016 at the Shanghai Pulmonary Hospital. The inclusion criteria were a diagnosis of histologically or cytologically confirmed NSCLC, a sensitizing *EGFR* mutation (defined as *19DEL* or *L858R*), a treatment-naïve status regarding EGFR-TKIs and a thorough documentation of the response evaluation for patients. The treatment response was evaluated every 2–3 months using computerized tomography according to the Response Evaluation Criteria in Solid Tumors version 1.1. (23) Clinicopathological characteristics including gender, age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), histological type, presence of metastases, *EGFR* mutation status and smoking status, were obtained by a review of medical records. The study was approved by the Ethics Committee of the Shanghai Pulmonary Hospital and was conducted according to the Declaration of Helsinki.

Blood Samples

Blood samples were collected in EDTA tubes prior to the initiation of EGFR-TKI treatment and after 1 month of such treatment. Plasma samples were isolated by centrifugation and stored at −80°C until use. All experiments followed the standard biosecurity and safety procedures of Shanghai Pulmonary Hospital.

Determination of Soluble PD-L1 Levels

The plasma sPD-L1 level was measured using the R-plex Human PD-L1 kit from Meso Scale Discovery (Rockville, MD, USA) according to the manufacturer's instructions. All the samples were tested in duplicate.

Statistical Analysis

Continuous data were summarized as medians and ranges. When assessing changes in sPD-L1 levels, for each patient with available blood sample, we estimated the difference between levels at baseline and at 1 month after initiating an EGFR-TKI. Patients with a change in sPD-L1 level that was lower than the median difference for the entire population were considered to have a reduction in sPD-L1 levels, whereas others were considered to have no reduction in sPD-L1 levels. For pre-treatment and on-treatment sPD-L1 levels, values that were lower than the median concentration for the entire population were considered to be low, whereas those above or equal to the median concentration were considered to be high.

The relationship between categorical parameters was determined using a chi-square test or Fisher's exact test. The student's *t*-test or Mann-Whitney *U*-test was used for comparing continuous data according to the data distribution determined by the Kolmogorov-Smirnov test. Kaplan-Meier curves and the log-rank test were used to compare survival times across different patient groups. The Cox proportional hazards regression analysis was performed, and HRs and 95% confidence intervals (CIs) were calculated to determine the survival difference. Variables were included in the multivariate analysis if they were statistically significant ($p < 0.10$) in the univariate analysis. All statistical analyses were performed using GraphPad Prism software (version 8; GraphPad, Inc., LaJolla, CA) and SPSS statistical software (version 22.0; IBM Corporation, Armonk, NY). Results were considered statistically significant at a two-sided $p < 0.05$.

RESULTS

Distribution of Plasma sPD-L1 and Patient Characteristics

In total, 66 patients met the inclusion criteria and were enrolled in this study. On-treatment blood samples were collected for 46 of these patients. The distributions of pre-treatment and on-treatment plasma sPD-L1 concentrations are shown in **Table 1**.

TABLE 1 | Distribution of plasma soluble PD-L1 concentration.

Variable	Concentration (pg/ml)
Plasma sPD-L1 Level (Pre-treatment)	
Median (Range)	568.19 (344.96–1889.49)
Plasma sPD-L1 Level (On-treatment)	
Median (Range)	560.99 (305.13–2255.57)
Difference Among sPD-L1 Level	
Median (Range)	6.88 (−454.08–743.72)
% Change in sPD-L1 Level	
Median (Range) (%)	19.19 (0.5–116.61)

The median pre-treatment and on-treatment sPD-L1 levels were 568.19 pg/ml (range: 344.96–1889.49 pg/ml) and 560.99 pg/ml (range: 305.13–2255.57 pg/ml), respectively. The median difference among the pre-treatment and on-treatment sPD-L1 concentrations was 6.88 pg/ml (range: −454.08–743.72 pg/ml). The median % change in the pre-treatment and on-treatment sPD-L1 level was 19.19% (range: 0.5–116.61%).

The demographic and clinical characteristics are demonstrated in **Table 2**. Of the 66 patients, 30 (45.4%) were female and 36 (54.5%) were male. The median age was 61. Most patients were diagnosed with adenocarcinoma (93.9%, $n = 62$) and had an ECOG PS status of 0–1 (97.0%, $n = 64$). Fifty-six patients (84.8%) were at stage IIIB to IV at the time of diagnosis, and 10 patients (15.2%) were with recurred disease. A majority of patients were non-smokers (75.8%, $n = 50$), and 16 patients (24.2%) were current or former smokers. Regarding the baseline *EGFR* mutation status, 33 patients (50.0%) harbored the *exon 19 deletion* and 33 patients (50.0%) had the *exon 21 L858R* point mutation (of this latter group, one patient had a co-mutation of *L858R* and *L861Q*). A majority of patients were treated with first-line EGFR-TKIs (gefitinib: $n = 49$, icotinib: $n = 13$, erlotinib: $n = 2$) and two patients received afatinib treatment. As for the metastasis status, there were 22 patients (33.3%) with brain metastases, 24 patients (36.4%) with bone metastases, and four patients with liver metastases at diagnosis. Among all patients, 38 patients received the *EGFR T790M* test at progression; 22 of these patients harbored an *EGFR T790M* mutation when they became resistant to first-line EGFR-TKIs.

The patient cohort was divided into two groups based on the level of sPD-L1 before treatment had been initiated. There were no significant differences in gender, age, histological status, ECOG PS status, stage, smoking status, *EGFR* mutation status, type of EGFR-TKI treatment received, metastasis status or *T790M* mutation at progression between the low sPD-L1 expression group and high sPD-L1 expression group.

TABLE 2 | Patient characteristics.

Variable	All $N = 66$ (%)	Low sPD-L1 $N = 33$ (%)	High sPD-L1 $N = 33$ (%)	*P*-value
Gender				
Female	30 (45.4)	16 (48.5)	14 (42.4)	0.621
Male	36 (54.5)	17 (51.5)	19 (57.6)	
Age (years)				
Range	35–84	43–76	35–84	0.054
Median	61	55	63	
Histology				
Adenocarcinoma	62 (93.9)	31 (93.9)	31 (93.9)	1.000
NSCLC-NOS	4 (6.1)	2 (6.1)	2 (6.1)	
ECOG PS				
0–1	64 (97.0)	33 (100.0)	31 (93.9)	0.473
2	2 (3.0)	0 (0.0)	2 (6.1)	
Stage				
Recurrence	10 (15.2)	4 (12.1)	6 (18.2)	0.492
IIIB-IV	56 (84.8)	29 (87.9)	27 (81.8)	
Smoking				
Never	50 (75.8)	25 (75.8)	25 (75.8)	1.000
Current/former	16 (24.2)	8 (24.2)	8 (24.2)	
EGFR Status				
19DEL	33 (50.0)	20 (60.6)	13 (39.4)	0.085
L858R and others	33 (50.0)	13 (39.4)	20 (60.6)	
TKIs				
Gefitinib	49 (74.2)	24 (72.7)	25 (75.8)	0.207
Erlotinib	2 (3.0)	2 (6.1)	0 (0.0)	
Icotinib	13 (19.7)	5 (15.2)	8 (24.2)	
Afatinib	2 (3.0)	2 (6.1)	0 (0.0)	
Brain Metastasis				
Yes	22 (33.3)	10 (30.3)	12 (36.4)	0.602
No	44 (66.7)	23 (69.7)	21 (63.6)	
Bone Metastasis				
Yes	24 (36.4)	9 (27.3)	15 (45.5)	0.125
No	42 (63.6)	24 (72.7)	18 (54.5)	
Liver Metastasis				
Yes	4 (6.1)	2 (6.1)	2 (6.1)	1.000
No	62 (93.9)	31 (93.9)	31 (93.9)	
T790M Detected at Progression				
Yes	22 (33.3)	11 (33.3)	11 (33.3)	1.000
No	16 (24.2)	8 (24.2)	8 (24.2)	

NSCLC-NOS, non-small cell lung cancer-not otherwise specified; ECOG PS, Eastern Cooperative Oncology Group performance status.
L858R and others, one patient had a L858R and L861Q co-mutation.
P-values are calculated using Chi-square test or Fisher's exact test. Student' t-test was used for age. Bolded p-values indicate significance.

High sPD-L1 Expression Is Associated With a Poor Response to EGFR-TKIs

Clinical characteristics of patients and distributions of sPD-L1 concentrations according to the therapeutic response to EGFR-TKIs are listed in **Table 3**. The ORR among the whole cohort was 53.0%, with 35 patients achieving a partial response (PR) and no patient achieving a complete response. The plasma sPD-L1 levels were significantly correlated with the treatment response. Patients with a pre-treatment sPD-L1 level of <568.19

TABLE 3 | Clinical characteristics of patients and distributions of sPD-L1 concentrations according to the therapeutic response to EGFR-TKIs.

	Objective response rate (ORR)		*P*
	Yes *N* = 35	No *N* = 31	
Gender			
Female	46.7 (14/30)	53.3 (16/30)	0.344
Male	58.3 (21/36)	41.7 (15/36)	
Age			
<61	63.6 (21/33)	36.4 (12/33)	0.084
≥61	42.4 (14/33)	57.6 (19/33)	
ECOG PS			
0–1	54.7 (35/64)	45.3 (29/64)	0.217
2	0.0 (0/2)	100.0 (2/2)	
Stage			
Recurrence	40.0 (4/10)	60.0 (6/10)	0.581
IIIb/IV	55.4 (31/56)	44.6 (25/56)	
Smoking			
Never	50.0 (25/50)	50.0 (25/50)	0.383
Current/former	62.5 (10/16)	37.5 (6/16)	
EGFR Status			
19DEL	57.6 (19/33)	42.4 (14/33)	0.459
L858R and others	48.5 (16/33)	51.5 (17/33)	
TKIs			
Gefitinib	53.1 (26/49)	46.9 (23/49)	0.734
Erlotinib	50.0 (1/2)	50.0 (1/2)	
Icotinib	46.2 (6/13)	53.8 (7/13)	
Afatinib	100.0 (2/2)	0.0 (0/2)	
Brain Metastasis			
Yes	63.6 (14/22)	36.4 (8/22)	0.222
No	47.7 (21/44)	52.3 (23/44)	
Plasma sPD-L1 Levels (Pre-treatment)			
<568.19	66.7 (22/33)	33.3 (11/33)	**0.026**
≥568.19	39.4 (13/33)	60.6 (20/33)	
Plasma sPD-L1 Levels (On-treatment)			
<560.99	73.9 (17/23)	26.1 (6/23)	**0.025**
≥560.99	43.5 (10/23)	56.5 (13/23)	
Plasma sPD-L1 Reduction			
Yes	60.9 (14/23)	39.1 (9/23)	0.765
No	56.5 (13/23)	43.5 (10/23)	
T790M Detected at Progression			
Yes	68.2 (15/22)	31.8 (7/22)	0.132
No	43.8 (7/16)	56.2 (9/16)	

NSCLC-NOS, non-small cell lung cancer-not otherwise specified; ECOG PS, Eastern Cooperative Oncology Group performance status.
L858R and others, one patient had a L858R and L861Q co-mutation.
P-values are calculated using Chi-square test or Fisher's exact test. Bolded p-values indicate significance.

had an obviously higher ORR than those with a pre-treatment sPD-L1 level of more than or equal to 568.19 (66.7 vs. 39.4%, $p = 0.026$). Meanwhile, a higher on-treatment sPD-L1 level was also associated with a poor response to EGFR-TKIs. The ORR was 73.9% in patients with low on-treatment sPD-L1 levels, but the ORR was only 43.5% in patients with high on-treatment sPD-L1 levels. There were no differences in the treatment response between patients with or without a reduction of sPD-L1 levels. Other clinical characteristics, including gender, age, ECOG PS score, tumor stage, smoking status, *EGFR* status, and type of EGFR-TKI received were not associated with the therapeutic response.

We next compared both the pre-treatment and on-treatment sPD-L1 concentrations in patients who achieved a PR and patients who had a best response of stable disease (SD) or progressive disease (PD). The PR group demonstrated significantly lower levels of pre-treatment plasma sPD-L1 compared with the SD+PD group. As for the on-treatment plasma sPD-L1 levels, although the finding was marginally significant, the PR group also showed a lower level of sPD-L1. In whole patient group and subgroups divided by treatment response, the levels of sPD-L1 were not significantly changed by EGFR-TKIs treatment (**Figure 1**).

Lower Levels of sPD-L1 Before EGFR-TKI Initiation Are Associated With Improved Survival Rates

The median progression-free survival (PFS) in the whole patient group was 12.5 months (95% CI: 9.7–15.2 months). As demonstrated in **Figure 2**, patients with a lower level of pre-treatment sPD-L1 had a statistically superior PFS rate compared with patients with higher pre-treatment sPD-L1 levels. The median PFS was 16.1 months (95% CI: 13.0–19.2 months) vs. 9.9 months (95% CI: 8.6–11.2 months), and the log-rank *p*-value was 0.005. Although it was not statistically significant, a shorter PFS rate was also observed in patients with higher on-treatment sPD-L1 concentrations (median PFS, 11.1 vs. 16.4 months). The change in sPD-L1 levels was not correlated with the PFS rate of patients treated with EGFR-TKIs, however.

To further evaluate the potential impact of clinical variables on the therapeutic efficacy of treatment with first-line EGFR-TKIs, we performed both univariate and multivariate analyses on the whole patient cohort. Typical factors of age, sex, smoking history, stage, *EGFR* driver mutation type, brain metastasis status, sPD-L1 concentration, and *T790M* status at progression were included in the Cox regression analysis. A worse outcome for patients with high sPD-L1 levels before EGFR-TKI treatment was also found for the PFS rate in the Cox regression model with an HR of 2.56 (95% CI: 1.24–5.27, $p = 0.011$). No clinicopathological factors were associated with the PFS rate (**Table 4**), but the emergence of the *T790M* resistance mutation at progression was correlated with a better PFS rate (HR = 0.45, 95% CI: 0.22–0.94, $p = 0.033$).

DISCUSSION

A growing number of studies have demonstrated that sPD-L1 might play a crucial role in the prediction of the treatment response of PD-1/PD-L1 inhibitors and also the prognosis of cancer patients (17, 20, 21, 24). However, the significance of the sPD-L1 level in predicting the response to EGFR-TKIs in NSCLC patients remains unclear. The results of the present study

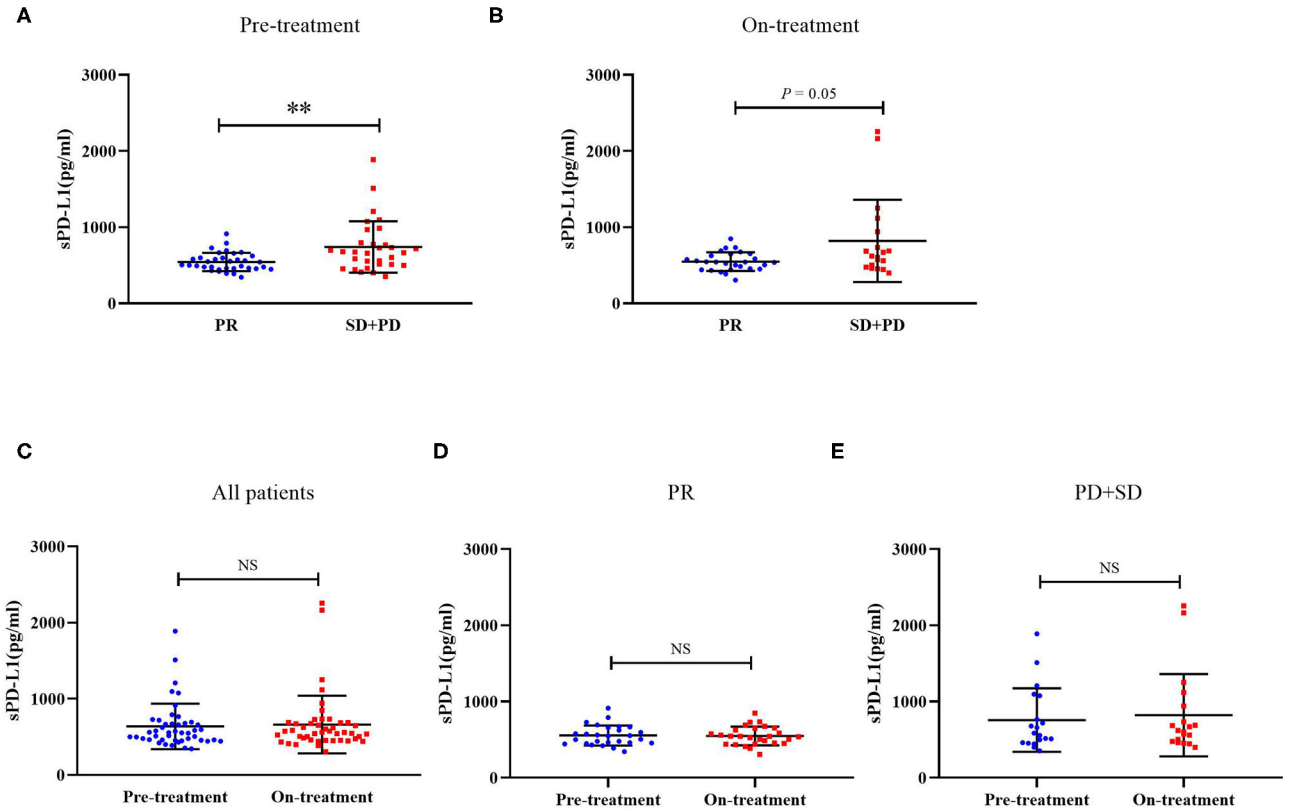

FIGURE 1 | (A) Distribution of sPD-L1 concentrations prior to the initiation of EGFR-TKI treatment according to therapeutic responses. **(B)** Distribution of sPD-L1 concentrations 1 month after initiating EGFR-TKI treatment according to therapeutic responses. **(C)** sPD-L1 concentrations at pre-treatment and on-treatment in whole patient group. **(D)** sPD-L1 concentrations at pre-treatment and on-treatment in patients with PR. **(E)** sPD-L1 concentrations at pre-treatment and on-treatment in patients with SD or PD (Results are presented as mean ± SD. $^{**}P < 0.01$; NS, Not Significant).

revealed that the ORR for first-line EGFR-TKI treatment was higher in *EGFR*-mutant NSCLC patients with low plasma sPD-L1 levels than in those with high sPD-L1 levels. Furthermore, a prolonged PFS rate was significantly associated with a lower pre-treatment sPD-L1 level. Our results suggested that the plasma PD-L1 concentration could be a promising marker for determining the efficacy of EGFR-TKIs for NSCLC patients harboring *EGFR* mutations.

The underlying mechanisms of generation and regulation of the soluble forms of PD-L1 are still unclear. One possible source is spliced variant. Zhou et al. showed that alternative splicing of PD-L1 occurred in all melanoma cell lines and splice variants could result in the secretion of sPD-L1 (21). Besides, it has been reported that tumor-derived extracellular vesicles including exosomes carried PD-L1 on their surfaces (25). Chen et al. demonstrated in their study that sPD-L1 could also be produced through proteolytic cleavage of membrane-bound proteins because the release of sPD-L1 was decreased after tumor cells were treated with the inhibitor of matrix metalloproteinase (26). Frigola et al. reported that the tumor stage and the presence of aggressive pathological features were associated with sPD-L1 levels in renal cell carcinoma, suggesting that circulating sPD-L1 might be derived from tumor tissue (22). Whether sPD-L1 concentrations are correlated with clinicopathological features such as tumor stage in lung cancer is controversial, however. Cheng et al. reported a positive association between sPD-L1 levels and stages of NSCLC (27). In advanced lung cancer, no obvious difference was identified in clinical stage between the low sPD-L1 and high sPD-L1 groups (18, 24). If most of the circulating PD-L1 is derived from membrane PD-L1 on tumor cells, the levels of sPD-L1 should be elevated with an increase in tumor burden. The fact that the patients involved in our study had advanced or recurrent lung cancer explains why we did not observe any correlation between the initial tumor stage and the sPD-L1 level. It has been reported that concentrations of sPD-L1 in blood samples from healthy donors increased as age grew (26). Interestingly, although it was only marginally significant, our results also revealed that sPD-L1 levels tended to be correlated with the age distribution in NSCLC patients. These data suggested that the level of circulating PD-L1 could be associated with the status of the entire immune system.

The impact of membrane form of PD-L1 on the treatment response and prognosis of NSCLC with EGFR mutations has been identified in recent studies (16, 28, 29). However, the conclusions remain controversial. In a study carried out by Lin

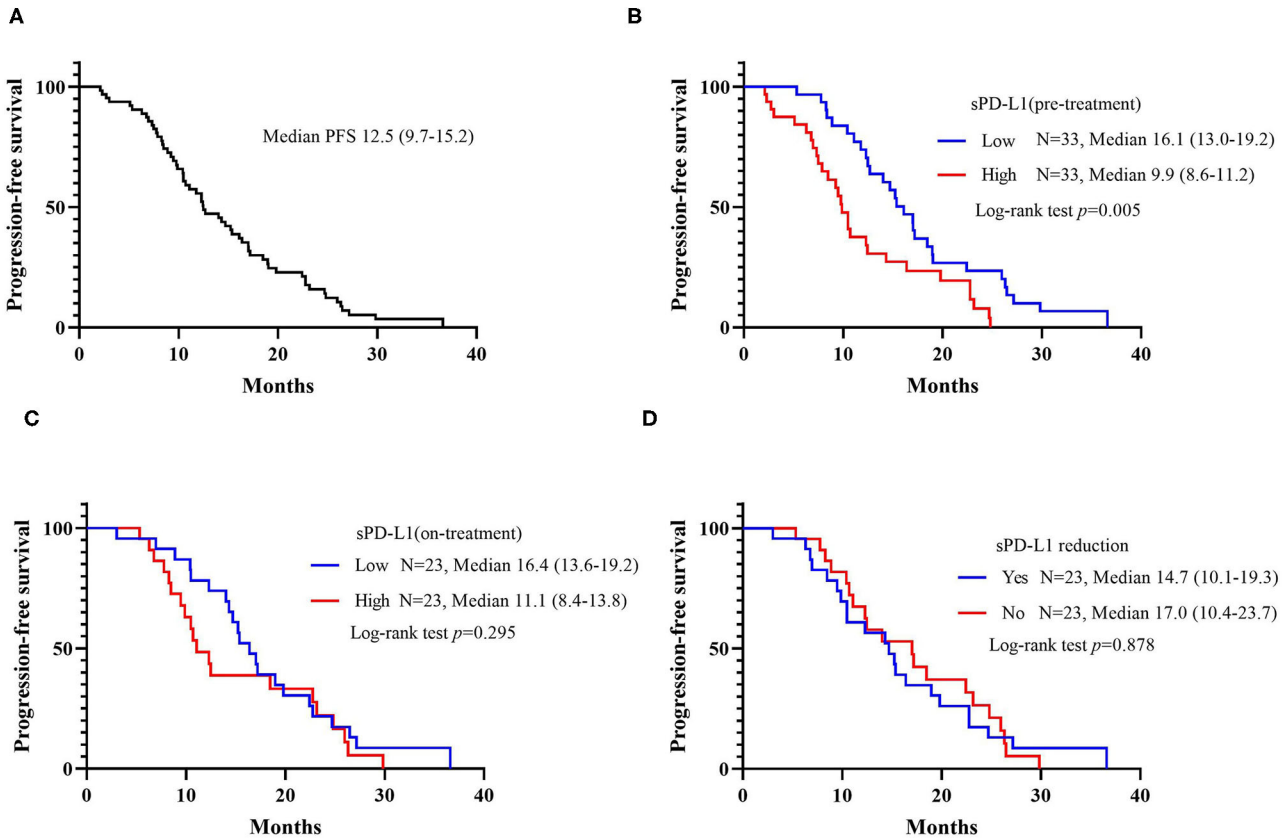

FIGURE 2 | Kaplan-Meier curves for progression-free survival (PFS). **(A)** PFS among all patients. **(B)** PFS according to the baseline level of sPD-L1. **(C)** PFS according to the sPD-L1 levels 1 month after initiating EGFR-TKI treatment. **(D)** PFS according to the changes in sPD-L1 during EGFR-TKI treatment.

et al. of *EGFR*-mutant lung adenocarcinoma patients, PD-L1 represented a favorable biomarker for the response to EGFR-TKIs and outcomes of these patients (28). There were also studies showed that high levels of PD-L1 expression were associated with a primary resistance and inferior response to EGFR-TKIs (13, 14). Because the soluble forms of PD-L1 are believed to be released from the PD-1/PD-L1 interaction site in tumor tissue, it is possible that the level of sPD-L1 may be correlated with membrane PD-L1 expression and also have a predictive or prognostic value. In our study, a higher level of sPD-L1 was significantly correlated with a lower ORR and a shorter PFS in *EGFR*-mutant NSCLC treated with EGFR-TKIs. In a recent study, Meyo et al. demonstrated that levels of sPD-L1 did not correlate with PFS in NSCLC patients with EGFR mutations (30). Several possible explanations of the conflicting results would be the differences in sPD-L1 testing assays, patients' characteristic and the definition of a low or high sPD-L1 level. Further studies should be done to validate the association between EGFR-TKI efficacy and sPD-L1 levels. The sPD-L1 level was not only revealing for targeted therapy; low sPD-L1 levels were also favorable markers for outcomes following chemotherapy and immunotherapy (20, 31, 32). In NSCLC, increasing evidences showed that sPD-L1 levels might represent a novel biomarker for the prediction of the efficacy of immune checkpoint therapy (24, 30, 32). These results supported the hypothesis that sPD-L1 binds to PD-1 on circulating T cells in peripheral blood before cytotoxic T cells reach the tumor site, thus impairing T cell-mediated antitumor immune activity and resulting in a poor treatment response for patients with high sPD-L1 levels.

Pre-clinical studies showed that the concentration of sPD-L1 was positively correlated with the expression of PD-L1 in various tumor cell lines and that sPD-L1 also played an important role in immunosuppression (26, 33). In studies carried out in lymphoma patients, serum sPD-L1 levels significantly correlated with the expression of PD-L1 in lymphoma cells and patients with low sPD-L1 levels demonstrated a favorable clinical outcome (33, 34). In gastric cancer, although serum sPD-L1 levels showed a trend of elevation in patients with high tissue PD-L1 expression, a statistically significance was not observed (20). A recent study performed in soft tissue sarcomas also revealed that there were no obvious differences in sPD-L1 levels between tissue PD-L1 positive group and PD-L1 negative group (35). One possible explanation is the mPD-L1 expression may vary within the same

TABLE 4 | Univariate and multivariate analysis of the clinical factors associated with progression-free survival.

Variable	Progression-free survival	
	Univariate analyses	Multivariate analyses
	HR (95% CI) *P*	HR (95% CI) *P*
Age:	1.15 (0.68–1.94)	
≥61 vs. <61	0.605	
Sex:	1.04 (0.62–1.76)	
male vs. female	0.881	
Smoking:	1.30 (0.71–2.39)	
current/former vs. never	0.391	
Stage:	1.35 (0.66–2.76)	
IIIB-IV vs. recurrence	0.412	
EGFR status:	1.36 (0.80–2.30)	
L858R and others vs. 19DEL	0.254	
Brain metastasis:	1.29 (0.74–2.23)	
Yes vs. No	0.371	
sPD-L1 level (pre-treatment):	2.15 (1.24–3.74)	2.56 (1.24–5.27)
≥568.19 vs. < 568.19	**0.007**	**0.011**
sPD-L1 level (on-treatment):	1.39 (0.75–2.57)	
≥560.99 vs. <560.99	0.299	
sPD-L1 reduction:	0.95 (0.51–1.77)	
Yes vs. No	0.879	
T790M detected at progression:	0.55 (0.28–1.10)	0.45 (0.22–0.94)
Yes vs. No	**0.089**	**0.033**

HR, hazard ratio; CI, confidence interval.
Bolded p-values indicate significance. Independent variables with $p < 0.10$ in the univariate analyses were included in the model.

tumor spatially and temporally. It is possible that assessment of PD-L1 expression from a single lesion or at a single time point may cause variability. The generation of sPD-L1 may also explain for the inconsistency of sPD-L1 and tissue PD-L1. Except for the main sources mentioned above, the circulating PD-L1 may also be produced by other sources like immune cells, cell injury, or cell death. The correlation between soluble forms and membrane PD-L1 in NSCLC has not been well-described. It is regrettable that the PD-L1 expression on tumor cells was not tested in our patients and that we could not, therefore, analyze the association between levels of membrane PD-L1 and sPD-L1. Costantini et al. revealed in their study that there was no association observed between IHC positivity of PD-L1 and sPD-L1 concentration at the time of diagnosis in NSCLC (32). Further study needs to be done to identify this correlation in NSCLC patients, especially in patients with *EGFR* mutations.

There have been studies supporting the theory that PD-L1 is a downstream molecule of EGFR signaling and EGFR-TKI could down-regulate PD-L1 expression on NSCLC cells by pathways like IL-6/JAK/STAT3, NKκB, or p-ERK1/2/p-c-Jun (36–38). However, the impact of EGFR-TKI treatment on sPD-L1 levels has not been well-elaborated in NSCLC patients with *EGFR* mutations. In our study, there was no significant change between the baseline and on-treatment sPD-L1 concentration. Similarly, Vecchiarelli et al. demonstrated in their study that sPD-L1 levels were elevated in NSCLC patients who received chemotherapy, but not in those who received treatments like TKIs or immunotherapy (39). There are evidences suggesting that EGFR-TKI may have an immunostimulatory effect by potentiating the induction of antigen presenting proteins in response to interferon-γ and enhancing T cells or NK cells mediated tumor killing (40–43). Considering the production of circulating PD-L1 was reported to be correlated with stimulation with interferon-γ (25), it is understandable that EGFR-TKI treatment did not decrease sPD-L1 levels like membrane PD-L1 on tumor cells do. Also, the sPD-L1 levels at the time when patients developed acquired resistance to EGFR-TKI treatment were not evaluated in this study. It has been reported that the expression of membrane PD-L1 was elevated when patients became resistant to first-line EGFR-TKIs (12). Further studies including a larger patient cohort should be done to verify this phenomenon with sPD-L1.

Emergence of the *T790M* resistance mutation accounts for 50–60% of cases with acquired resistance to first-generation EGFR-TKIs (44). Osimertinib, a third-generation EGFR-TKI that selectively inhibits the *EGFR T790M* mutation, has been a successful treatment for patients with *T790M*-positive NSCLC who have acquired resistance to prior-line EGFR-TKIs (45). However, the underlying mechanism is unknown in many patients with acquired resistance to EGFR-TKIs. Recently, the correlation between membrane PD-L1 expression and *T790M* status after disease progression during EGFR-TKI treatment was reported. It seemed that among *T790M*-negative patients, more demonstrated high levels of PD-L1 expression when they were resistant to first-line EGFR-TKIs (46), making us wonder if PD-L1 expression could represent a novel mechanism of resistance. In our study, although baseline sPD-L1 levels could predict the response to EGFR-TKIs, no significant association was observed between the plasma PD-L1 level and the *T790M* status. The small sample sizes in this study may have had an influence. Only 38 patients had a *T790M* mutation test when they progressed to prior-line EGFR-TKI treatment.

There are several limitations in this present study. First, as a retrospective study, the conclusions generated in our study still need further prospective studies to be confirmed. Second, our study mainly discussed the correlation between sPD-L1 level and response to EGFR-TKI treatment. The influence of sPD-L1 level on overall survival of NSCLC needs to be assessed in further studies. Third, as a study carried out in a single institution, the patient number is relatively small, especially when analyzing patients with secondary *T790M* mutation. Multi-centered study with a larger patient number is needed to verify our results.

In conclusion, this retrospective study revealed that high plasma sPD-L1 levels were associated with poor response to

EGFR-TKIs and that this finding could be a promising biomarker in patients with *EGFR*-mutant advanced NSCLC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Shanghai Pulmonary Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YJ, XL, and CZho designed this study and drafted the manuscript. YJ, XL, CZha, FZ, and JL reviewed the patient record, collected patient samples, and conducted the relevant experiments. YJ, CZha, GG, and WL performed the statistical analyses. SR and CS provided critical comments and revised the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* (2015) 65:87–108. doi: 10.3322/caac.21262
2. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol.* (2011) 12:735–42. doi: 10.1016/S1470-2045(11)70184-X
3. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol.* (2014) 15:213–22. doi: 10.1016/S1470-2045(13)70604-1
4. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med.* (2010) 362:2380–8. doi: 10.1056/NEJMoa0909530
5. Tan CS, Gilligan D, Pacey S. Treatment approaches for EGFR-inhibitor-resistant patients with non-small-cell lung cancer. *Lancet Oncol.* (2015) 16:e447–59. doi: 10.1016/S1470-2045(15)00246-6
6. Steinberg SM, Shabaneh TB, Zhang P, Martyanov V, Li Z, Malik BT, et al. Myeloid cells that impair immunotherapy are restored in melanomas with acquired resistance to BRAF inhibitors. *Cancer Res.* (2017) 77:1599–610. doi: 10.1158/0008-5472.CAN-16-1755
7. Kakavand H, Rawson RV, Pupo GM, Yang JYH, Menzies AM, Carlino MS, et al. PD-L1 expression and immune escape in melanoma resistance to MAPK inhibitors. *Clin Cancer Res.* (2017) 23:6054–61. doi: 10.1158/1078-0432.CCR-16-1688
8. Smith MP, Sanchez-Laorden B, O'Brien K, Brunton H, Ferguson J, Young H, et al. The immune microenvironment confers resistance to MAPK pathway inhibitors through macrophage-derived TNFα. *Cancer Discov.* (2014) 4:1214–29. doi: 10.1158/2159-8290.CD-13-1007
9. Balar AV, Weber JS. PD-1 and PD-L1 antibodies in cancer: current status and future directions. *Cancer Immunol Immunother.* (2017) 66:551–64. doi: 10.1007/s00262-017-1954-6
10. Meng X, Liu Y, Zhang J, Teng F, Xing L, Yu J. PD-1/PD-L1 checkpoint blockades in non-small cell lung cancer: new development and challenges. *Cancer Lett.* (2017) 405:29–37. doi: 10.1016/j.canlet.2017.06.033
11. Gainor JF, Shaw AT, Sequist LV, Fu X, Azzoli CG, Piotrowska Z, et al. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: a retrospective analysis. *Clin Cancer Res.* (2016) 22:4585–93. doi: 10.1158/1078-0432.CCR-15-3101
12. Han JJ, Kim DW, Koh J, Keam B, Kim TM, Jeon YK, et al. Change in PD-L1 expression after acquiring resistance to Gefitinib in EGFR-mutant non-small-cell lung cancer. *Clin Lung Cancer.* (2016) 17:263–70.e2. doi: 10.1016/j.cllc.2015.11.006
13. Hsu KH, Huang YH, Tseng JS, Chen KC, Ku WH, Su KY, et al. High PD-L1 expression correlates with primary resistance to EGFR-TKIs in treatment naive advanced EGFR-mutant lung adenocarcinoma patients. *Lung Cancer.* (2019) 127:37–43. doi: 10.1016/j.lungcan.2018.11.021
14. Su S, Dong Z-Y, Xie Z, Yan L-X, Li Y-F, Su J, et al. Strong programmed death ligand 1 expression predicts poor response and *de novo* resistance to EGFR tyrosine kinase inhibitors among NSCLC patients with EGFR mutation. *J Thorac Oncol.* (2018) 13:1668–75. doi: 10.1016/j.jtho.2018.07.016
15. Yu H, Boyle TA, Zhou C, Rimm DL, Hirsch FR. PD-L1 expression in lung cancer. *J Thorac Oncol.* (2016) 11:964–75. doi: 10.1016/j.jtho.2016.04.014
16. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res.* (2014) 20:5064–74. doi: 10.1158/1078-0432.CCR-13-3271
17. Finkelmeier F, Canli O, Tal A, Pleli T, Trojan J, Schmidt M, et al. High levels of the soluble programmed death-ligand (sPD-L1) identify hepatocellular carcinoma patients with a poor prognosis. *Eur J Cancer.* (2016) 59:152–9. doi: 10.1016/j.ejca.2016.03.002
18. Okuma Y, Hosomi Y, Nakahara Y, Watanabe K, Sagawa Y, Homma S. High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer. *Lung Cancer.* (2017) 104:1–6. doi: 10.1016/j.lungcan.2016.11.023
19. Chang B, Huang T, Wei H, Shen L, Zhu D, He W, et al. The correlation and prognostic value of serum levels of soluble programmed death protein 1 (sPD-1) and soluble programmed death-ligand 1 (sPD-L1) in patients with hepatocellular carcinoma. *Cancer Immunol Immunother.* (2019) 68:353–63. doi: 10.1007/s00262-018-2271-4
20. Shigemori T, Toiyama Y, Okugawa Y, Yamamoto A, Yin C, Narumi A, et al. Soluble PD-L1 expression in circulation as a predictive marker for recurrence and prognosis in gastric cancer: direct comparison of the clinical burden between tissue and serum PD-L1 expression. *Ann Surg Oncol.* (2019) 26:876–83. doi: 10.1245/s10434-018-07112-x
21. Zhou J, Mahoney KM, Giobbie-Hurder A, Zhao F, Lee S, Liao X, et al. Soluble PD-L1 as a biomarker in malignant melanoma treated with checkpoint blockade. *Cancer Immunol Res.* (2017) 5:480–92. doi: 10.1158/2326-6066.CIR-16-0329
22. Frigola X, Inman BA, Lohse CM, Krco CJ, Cheville JC, Thompson RH, et al. Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma. *Clin Cancer Res.* (2011) 17:1915–23. doi: 10.1158/1078-0432.CCR-10-0250

23. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* (2009) 45:228–47. doi: 10.1016/j.ejca.2008.10.026
24. Okuma Y, Wakui H, Utsumi H, Sagawa Y, Hosomi Y, Kuwano K, et al. Soluble programmed cell death ligand 1 as a novel biomarker for nivolumab therapy for non-small-cell lung cancer. *Clin Lung Cancer.* (2018) 19:410–7.e1. doi: 10.1016/j.cllc.2018.04.014
25. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature.* (2018) 560:382–6. doi: 10.1038/s41586-018-0392-8
26. Chen Y, Wang Q, Shi B, Xu P, Hu Z, Bai L, et al. Development of a sandwich ELISA for evaluating soluble PD-L1 (CD274) in human sera of different ages as well as supernatants of PD-L1+ cell lines. *Cytokine.* (2011) 56:231–8. doi: 10.1016/j.cyto.2011.06.004
27. Cheng S, Zheng J, Zhu J, Xie C, Zhang X, Han X, et al. PD-L1 gene polymorphism and high level of plasma soluble PD-L1 protein may be associated with non-small cell lung cancer. *Int J Biol Markers.* (2015) 30:e364–8. doi: 10.5301/jbm.5000170
28. Lin C, Chen X, Li M, Liu J, Qi X, Yang W, et al. Programmed death-ligand 1 expression predicts tyrosine kinase inhibitor response and better prognosis in a cohort of patients with epidermal growth factor receptor mutation-positive lung adenocarcinoma. *Clin Lung Cancer.* (2015) 16:e25–35. doi: 10.1016/j.cllc.2015.02.002
29. Takashima Y, Sakakibara-Konishi J, Hatanaka Y, Hatanaka KC, Ohhara Y, Oizumi S, et al. Clinicopathologic features and immune microenvironment of non-small-cell lung cancer with primary resistance to epidermal growth factor receptor tyrosine kinase inhibitors. *Clin Lung Cancer.* (2018) 19:352–9.e1. doi: 10.1016/j.cllc.2018.02.004
30. Tiako Meyo M, Jouinot A, Giroux-Leprieur E, Fabre E, Wislez M, Alifano M, et al. Predictive value of soluble PD-1, PD-L1, VEGFA, CD40 ligand and CD44 for nivolumab therapy in advanced non-small cell lung cancer: a case-control study. *Cancers.* (2020) 12:473. doi: 10.3390/cancers12020473
31. Buderath P, Schwich E, Jensen C, Horn PA, Kimmig R, Kasimir-Bauer S, et al. Soluble programmed death receptor ligands sPD-L1 and sPD-L2 as liquid biopsy markers for prognosis and platinum response in epithelial ovarian cancer. *Front Oncol.* (2019) 9:1015. doi: 10.3389/fonc.2019.01015
32. Costantini A, Julie C, Dumenil C, Helias-Rodzewicz Z, Tisserand J, Dumoulin J, et al. Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab. *Oncoimmunology.* (2018) 7:e1452581. doi: 10.1080/2162402X.2018.1452581
33. Nagato T, Ohkuri T, Ohara K, Hirata Y, Kishibe K, Komabayashi Y, et al. Programmed death-ligand 1 and its soluble form are highly expressed in nasal natural killer/T-cell lymphoma: a potential rationale for immunotherapy. *Cancer Immunol Immunother.* (2017) 66:877–90. doi: 10.1007/s00262-017-1987-x
34. Cho I, Lee H, Yoon SE, Ryu KJ, Ko YH, Kim WS, et al. Serum levels of soluble programmed death-ligand 1 (sPD-L1) in patients with primary central nervous system diffuse large B-cell lymphoma. *BMC Cancer.* (2020) 20:120. doi: 10.1186/s12885-020-6612-2
35. Asanuma K, Nakamura T, Hayashi A, Okamoto T, Iino T, Asanuma Y, et al. Soluble programmed death-ligand 1 rather than PD-L1 on tumor cells effectively predicts metastasis and prognosis in soft tissue sarcomas. *Sci Rep.* (2020) 10:9077. doi: 10.1038/s41598-020-65895-0
36. Chen N, Fang W, Zhan J, Hong S, Tang Y, Kang S, et al. Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. *J Thorac Oncol.* (2015) 10:910–23. doi: 10.1097/JTO.0000000000000500
37. Zhang N, Zeng Y, Du W, Zhu J, Shen D, Liu Z, et al. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. *Int J Oncol.* (2016) 49:1360–8. doi: 10.3892/ijo.2016.3632
38. Lin K, Cheng J, Yang T, Li Y, Zhu B. EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF-κB. *Biochem Biophys Res Commun.* (2015) 463:95–101. doi: 10.1016/j.bbrc.2015.05.030
39. Vecchiarelli S, Passiglia F, D'Incecco A, Gallo M, De Luca A, Rossi E, et al. Circulating programmed death ligand-1 (cPD-L1) in non-small-cell lung cancer (NSCLC). *Oncotarget.* (2018) 9:17554–63. doi: 10.18632/oncotarget.24785
40. Pollack BP, Sapkota B, Cartee TV. Epidermal growth factor receptor inhibition augments the expression of MHC class I and II genes. *Clin Cancer Res.* (2011) 17:4400–13. doi: 10.1158/1078-0432.CCR-10-3283
41. He S, Yin T, Li D, Gao X, Wan Y, Ma X, et al. Enhanced interaction between natural killer cells and lung cancer cells: involvement in gefitinib-mediated immunoregulation. *J Transl Med.* (2013) 11:186. doi: 10.1186/1479-5876-11-186
42. Kim H, Kim S-H, Kim M-J, Kim S-J, Park S-J, Chung J-S, et al. EGFR inhibitors enhanced the susceptibility to NK cell-mediated lysis of lung cancer cells. *J Immunother.* (2011) 34:372–81. doi: 10.1097/CJI.0b013e31821b724a
43. Okita R, Wolf D, Yasuda K, Maeda A, Yukawa T, Saisho S, et al. Contrasting effects of the cytotoxic anticancer drug gemcitabine and the EGFR tyrosine kinase inhibitor gefitinib on NK cell-mediated cytotoxicity via regulation of NKG2D ligand in non-small-cell lung cancer cells. *PLoS ONE.* (2015) 10:e0139809. doi: 10.1371/journal.pone.0139809
44. Lim SM, Syn NL, Cho BC, Soo RA. Acquired resistance to EGFR targeted therapy in non-small cell lung cancer: mechanisms and therapeutic strategies. *Cancer Treat Rev.* (2018) 65:1–10. doi: 10.1016/j.ctrv.2018.02.006
45. Cross DA, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov.* (2014) 4:1046–61. doi: 10.1158/2159-8290.CD-14-0337
46. Haratani K, Hayashi H, Tanaka T, Kaneda H, Togashi Y, Sakai K, et al. Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. *Ann Oncol.* (2017) 28:1532–9. doi: 10.1093/annonc/mdx183

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Effect of β-Blocker in Treatment-Naïve Patients With Advanced Lung Adenocarcinoma Receiving First-Generation EGFR-TKIs

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Background: Through activation of adrenergic receptors, chronic stress can trigger the secretion of neurotransmitters and hormones that enhance tumor growth, increase angiogenesis, and promote drug resistance. This study aimed to evaluate the effect of β-blockers in patients receiving first-line epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) for lung adenocarcinoma.

Methods: This retrospective cohort study enrolled patients with advanced lung adenocarcinoma under first-line EGFR-TKIs between 2011 and 2014 in the National Health Insurance Research Database of Taiwan. The effects of β-blockers use, defined as ≥60 defined daily doses within 180 days before initiation of EGFR-TKI therapy, on the 2-year time-to-discontinuation (TTD) of EGFR-TKIs and 4-year overall survival (OS) were investigated using Cox regression analyses with inverse propensity score weighting and sensitivity analysis in subgroup with either hypertension or ischemic heart diseases.

Results: Among 4988 enrolled patients, 552 (11.1%) were in the β-blocker group. Patients in the β-blocker group were more likely to be older than 75 and had diabetes mellitus and cardiovascular comorbidities. In Cox regression analysis, β-blocker usage was associated with a longer TTD (hazard ratio, HR: 0.91 [0.86–0.96]) and OS (HR: 0.68 [0.64–0.72]). The results also favored β-blocker group in sensitivity analysis.

Conclusions: In treatment-naïve patients with advanced lung adenocarcinoma under first-line EGFR-TKIs, prior use of β-blocker was associated with a better outcome. The findings encourage further prospective clinical study to validate the possibility of β-blockers as adjuvant anticancer therapy.

Keywords: lung cancer, epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), β-blocker, overall survival, time-to-discontinuation

INTRODUCTION

Epidermal growth factor receptor (EGFR) mutations account for 51.4% of advanced lung adenocarcinoma driver mutations in Asia and 15% to 22% in non-Asia area (1, 2). EGFR tyrosine kinase inhibitors (TKIs) are highly effective in patients with lung adenocarcinoma, harboring sensitive EGFR mutations (3, 4). Preventing emergence of acquired resistance is crucial in prolonging the overall survival (OS).

Chronic stress increases the production of stress hormones from the adrenal medulla and sympathetic neurons. The effects of stress hormones are mediated through binding to β-adrenergic receptors (ARs) on target cells, which contributes to tumor development and progression of multiple malignancies, such as non-small cell lung cancer (NSCLC) in animal models (5). Evidence from preclinical and epidemiological studies have implicated the strong association of stress hormones or behavioral changes with tumor cell growth, migration, invasion, and metastasis (6–8).

β-blockers are widely used in patients with hypertension (HTN), coronary artery disease, and arrhythmia. In preclinical studies, β-blockers were observed to inhibit cell growth, proliferation, and EGFR inhibitor acquired resistance in lung cancer cell lines (7, 9, 10). However, human studies on the therapeutic value of β-blockers in lung cancer are controversial. Certain studies have revealed no survival benefits with β-blockers (11, 12), whereas others have demonstrated prolonged survival (10). The possible mechanism is to decrease the stress stimulation cell growth and mutation by reducing growth hormone such as cyclic AMP (cAMP)-mediated pathways or insulin-like growth factor 2 (6, 13). Until now, no large-scale clinical data regarding the effect of β-blockers in patients with lung adenocarcinoma receiving EGFR-TKIs is available. Herein, a retrospective study was performed using the National Health Insurance Research Database (NHIRD) of Taiwan to investigate the effect of β-blockers on patients with lung adenocarcinoma receiving first-line EGFR-TKIs.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of National Taiwan University Hospital (NTUH REC: 201212001W). Given the retrospective design and use of an encrypted database in this study, the need for informed consent was waived.

Case Selection

Patients with lung cancer were identified using a compatible diagnosis (International Classification of Disease, 9th Revision, Clinical Modification [ICD-9-CM] code 162) from the Registry of Catastrophic Illness Patients Database, a subset of the NHIRD. Application to this registry obligated histological confirmation. The date on which patient applied to this registry for lung cancer was defined as the index date. The NHIRD was linked with the Taiwan Cancer Registry for histopathology and cancer stage and those with clinical stage IIIb or IV histology-confirmed adenocarcinoma were selected.

In this study, patients receiving gefitinib and erlotinib were enrolled because afatinib had not yet approved by the National Health Insurance (NHI) of Taiwan during study period. Both gefitinib and erlotinib required preaudit approval by the NHI administration and were of benefit to patients with lung adenocarcinoma harboring sensitive EGFR mutations during first-line therapy.

Information on key chemotherapeutic agents for NSCLC, as defined by the National Comprehensive Cancer Network guidelines, was retrieved from the NHIRD; the drugs included gemcitabine, vinorelbine, docetaxel, paclitaxel, etoposide, and pemetrexed (14). Patients who initiated EGFR-TKI therapy after the start date of key chemotherapeutic agent were excluded. The complete selection process is shown in **Figure 1**.

Outcome Measurement

Because of the FLAURA study, the median PFS and OS in Gefitinib/Erlotinib group were around 10.2 months and 31.8 months (15, 16). The endpoints of this study were time-to-discontinuation (TTD) of EGFR-TKIs within 2 years and 4-year overall survival (OS) (both starting from the first date of EGFR-TKIs). Discontinuation of EGFR-TKIs was based on the decision of primary care physicians as well as the expert panel of the NHI because approval of gefitinib and erlotinib was reaudited every 90 days, and the therapy was reissued only to patients without progression under to treatment with EGFR-TKIs, which was determined according to the response evaluation criteria in solid tumors (RECIST) group (i.e., stable disease, partial, or complete response) (17).

Exposure

The prescription duration of β-blockers was converted from the claims data according to the defined daily doses (DDDs) (18). β-blockers were identified by Anatomical Therapeutic Chemical codes C07AA, C07BA, C07CA, C07DA, C07FA, C07AB, C07BB, C07CB, C07DB, C07FB, C07AG, C07BG, and C07CG. Patients on β-blockers for ≥60 DDDs within 180 days before initiation of EGFR-TKI therapy were defined as β-blocker users, whereas others were classified as β-blocker nonusers. The definition was made because of the length of refillable prescriptions for patients with chronic illnesses in Taiwan.

Disease Severity

Disease severity of lung cancer was recorded according to the status between the index date and start date of EGFR-TKIs, including cachexia (19), intracranial metastasis (20), duration of hospitalization (days), and anemia (21). Patients were defined as having cachexia if they had received megestrol or medroxyprogesterone. Those exhibiting increased intracranial pressure (IICP) were considered as having intracranial metastasis, which was determined based on whether they had received glycerin or mannitol prescription. Patients who required transfusion of packed red blood cells (PRBCs) were defined as having anemia (22).

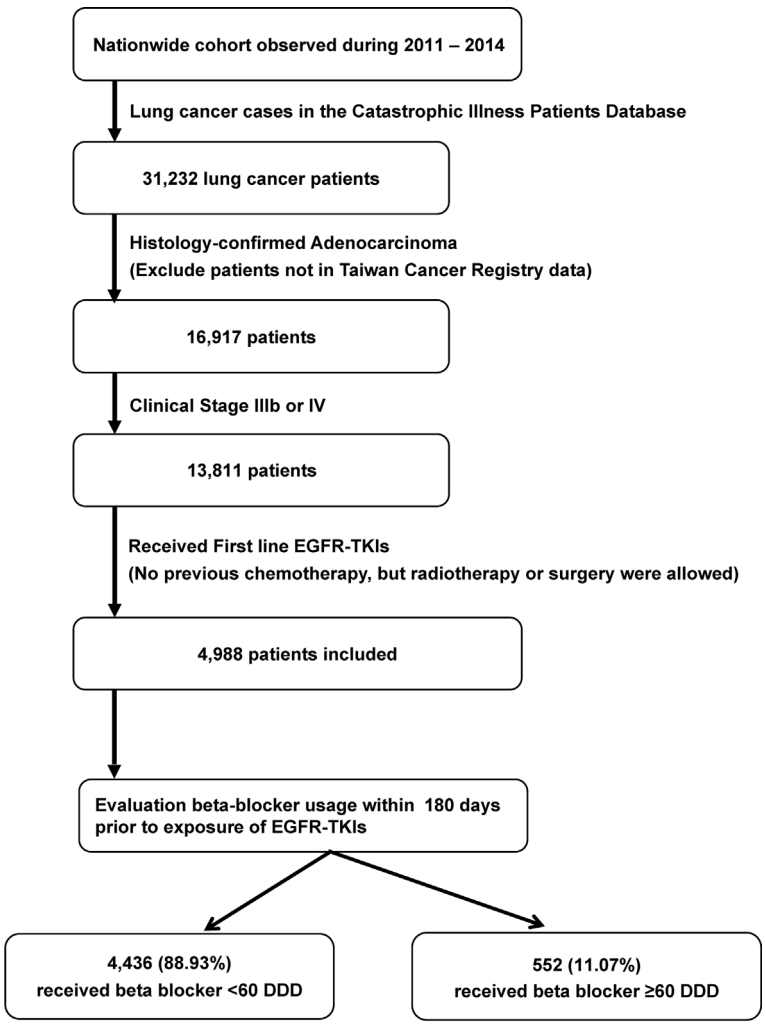

FIGURE 1 | Selection and disposition of the study subjects. EGFR-TKIs, epidermal growth factor receptor tyrosine kinase inhibitors; DDD, defined daily dose.

Comorbidities

Comorbidities such as chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), end-stage renal disease (ESRD), HTN, ischemic and other heart disease, cerebrovascular disease, peripheral artery disease, and other malignancies were identified by international classification of diseases, ninth revision, clinical modification (ICD-9-CM) code according to a previous study (23). Patients with vascular diseases were defined as those having at least one of the following comorbidities including HTN, heart disease, ischemic heart disease, cerebrovascular disease, and peripheral artery disease.

Statistical Analysis

Intergroup differences were compared using the *t* test or Mann–Whitney *U* test for continuous variables on the basis of their normality, and the chi-squared test or Fisher's exact test for categorical variables, as appropriate. For each variable, TTD within 2 years of EGFR-TKIs and 4-year OS were generated using the Kaplan–Meier method and compared using the log-rank test. Cox regression analysis was performed to identify the independent prognostic factors.

A propensity score was derived, which is the logit (probability) for receiving β-blockers or not calculated from a binomial logistic regression model by using crucial background covariates. Inverse propensity score weighting (IPSW) was used in the Cox model to adjust for potential confounders in selecting β-blocker users and nonusers (24).

In the multivariate analysis, potential interactions between variables were evaluated, and all variables were included. Statistical significance was set at $p < 0.05$. All analyses were conducted using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Sensitivity Analysis

To avoid confounding by the indications of β-blocker, mainly HTN and ischemic heart disease, a subgroup including patients

with either of the two comorbidities were formed to evaluate the effect of β-blocker use on TTD within 2 years of EGFR-TKIs and 4-year OS.

RESULTS

Patient Selection

For the 2011 to 2014 period, 31,232 patients with lung cancer were identified. After selection, a total of 4,988 patients were identified for further analysis (**Figure 1**). Among them, 552 (11.07%) received β-blocker ≥ 60 DDD and were classified into the β-blocker user group. The other 4,436 (88.93%) were classified into the β-blockers nonuser group.

Table 1 shows the demographic data of the enrolled cases. In the study cohort, 15.6% of the patients were aged 75 years or older, 41.8% of the patients were men, 86.2% of the patients were in stage IV, and 34.4% of the patients had distant metastases. Cachexia and IICP were noted in 28.8% and 22.9% of the patients, respectively. The mean duration of hospitalization was 2.9 days between the index date and start date of EGFR-TKI treatment, and the mean unit of PRBC transfusion was 1.6. The most common underlying comorbidities were vascular diseases (56.7%), HTN (40.8%), and ischemic heart disease (16.0%).

In compare with the β-blocker nonuser group, the user group had significantly more patients being >75 years old (22.3% vs. 14.8%, $p < 0.001$), having IICP (17.6% vs. 23.6%, $p = 0.001$) and other comorbidities, including DM (25.5% vs. 10.1%, $p < 0.001$), HTN (90.4% vs. 34.7%, $p < 0.001$), heart disease (30.8% vs. 13.9%, $p < 0.001$), ischemic heart disease (38.0% vs. 13.2%, $p < 0.001$), cerebrovascular disease (18.3% vs. 9.1%, $p < 0.001$), peripheral artery disease (6.7% vs. 3.3%, $p < 0.001$), vascular diseases (56.9% vs. 28.3%, $p < 0.001$), and malignancies other than lung cancer (7.8% vs. 4.6%, $p < 0.001$).

Propensity Score of β-Blocker Use

Factors in **Table 1** that were significantly associated with use of β-blockers were identified by logistic regression. All these significant factors were included in the propensity score calculation.

Prognostic Factors of 2-Year TTD of EGFR-TKIs

The Kaplan–Meier analysis with IPSW adjustment revealed that the β-blocker user group had a more favorable 2-year TTD than the nonuser group (HR: 0.85 [0.75–0.97]; **Figure 2A**). HTN was also a favorable prognostic factor. Poor prognostic factors included male sex, cachexia, IICP, longer duration of hospitalization, and PRBC transfusion (**Table 2**). In the multivariate Cox regression analysis with IPSW adjustment, β-blocker users were independently associated with a more favorable 2-year TTD than nonusers [HR: 0.91 (0.86–0.96)]. Other independent good prognostic factor was HTN [HR: 0.77 (0.72–0.82)]. Poor prognostic factors of the 2-year TTD included cachexia [HR: 1.37 (1.29–1.45)], IICP [HR: 1.16 (1.08–1.23)], PRBC transfusion [HR: 1.02 (1.02–1.03)], and DM [HR: 1.13 (1.02–1.24)] (**Table 2**).

Prognostic Factors of 4-Year Overall Survival

The Kaplan–Meier analysis with IPSW adjustment revealed that the β-blocker user group had more favorable 4-year OS than the nonuser group (HR: 0.85 [0.75–0.97]) (**Figure 2B**). Other poor prognostic factors of 4-year OS included male sex, age ≥ 75 years, cachexia, IICP, longer duration of hospitalization, PRBC transfusion, DM, and vascular disease (**Table 3**).

TABLE 1 | Patient characteristics, stratified by beta-blocker use.

Variables	All (*N* = 4988)	Beta-blocker <60 DDD (*n* = 4436)	Beta-blocker ≥60 DDD (*n* = 552)	*p* value
Male	2,087 (41.8%)	1,869 (42.1%)	218 (39.5%)	0.254
Age >75	779 (15.6%)	656 (14.8%)	123 (22.3%)	<0.001
Stage IV lung cancer	4,299 (86.2%)	3,833 (86.4%)	466 (84.4%)	0.226
Distant metastasis (M1b)	1,714 (34.4%)	1,537 (34.7%)	177 (32.1%)	0.247
Disease severity				
Megest use	1,437 (28.8%)	1,292 (29.1%)	145 (26.3%)	0.132
Mannitol/glycerol use	1,143 (22.9%)	1,046 (23.6%)	97 (17.6%)	0.001
Length of hospitalization (days)	2.9 ± 2.6	3.0 ± 2.6	2.8 ± 2.8	0.137
PRBC transfusion (unit)	1.6 ± 3.8	1.5 ± 3.8	1.6 ± 3.7	0.465
Comorbidity				
Diabetes mellitus	589 (11.8%)	448 (10.1%)	141 (25.5%)	<0.001
COPD	217 (4.4%)	197 (4.4%)	20 (3.6%)	0.437
Other malignancies	245 (4.9%)	202 (4.6%)	43 (7.8%)	<0.001
Hypertension	2,037 (40.8%)	1,538 (34.7%)	499 (90.4%)	<0.001
Heart disease	788 (15.8%)	618 (13.9%)	170 (30.8%)	<0.001
Ischemic heart disease	796 (16.0%)	586 (13.2%)	210 (38.0%)	<0.001
Cerebral vascular disease	504 (10.1%)	403 (9.1%)	101 (18.3%)	<0.001
Peripheral artery disease	185 (3.7%)	148 (3.3%)	37 (6.7%)	<0.001
End-stage renal disease	15 (0.3%)	11 (0.3%)	4 (0.7%)	0.129
Vascular diseases	2,828 (56.7%)	1,257 (28.3%)	1571 (56.9%)	<0.001

COPD, chronic obstructive pulmonary disease; DDD, defined daily dose; PRBC, packed red blood cell.

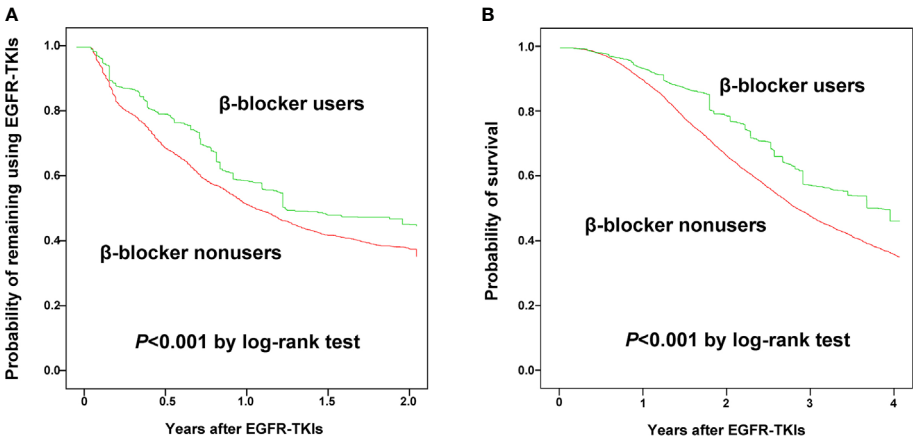

FIGURE 2 | Kaplan-Meier curves for time to discontinuation of epidermal growth factor receptor-tyrosine kinase inhibitors within 2 years **(A)** and 4-year overall survival **(B)** between β-blocker users and nonusers.

TABLE 2 | Multivariate Cox proportional hazards regression analysis for time to discontinuation of first-line epidermal growth factor receptor tyrosine kinase inhibitors in 2 years.

Variables	Kaplan–Meier Analysis			Multivariate Cox Regression		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Male	1.22	1.13–1.32	<0.001	0.92	0.87–0.97	0.002
Age >75	1.02	0.92–1.13	0.704	0.95	0.88–1.03	0.244
Beta–blocker ≥60 DDD	0.85	0.75–0.97	0.015	0.91	0.86–0.96	<0.001
Disease severity						
Megestrol use	1.70	1.57–1.84	<0.001	1.37	1.29–1.45	<0.001
Mannitol/Glycerol use	1.46	1.34–1.59	<0.001	1.16	1.08–1.23	<0.001
Length of hospitalization (days)	1.05	1.04–1.06	<0.001	1.01	0.99–1.01	0.706
PRBC transfusion (unit)	1.04	1.03–1.04	<0.001	1.02	1.02–1.03	<0.001
Comorbidity						
Diabetes mellitus	1.05	0.93–1.19	0.401	1.13	1.02–1.24	0.038
COPD	1.03	0.86–1.24	0.755	0.90	0.76–1.06	0.253
Hypertension	0.88	0.81–0.95	0.002	0.88	0.82–0.94	<0.001
Vascular disease	0.99	0.91–1.08	0.793	1.01	0.94–1.08	0.784

Multivariate Cox regression adjusted for sex, age, disease severity, and comorbidities, including COPD, diabetes mellitus, end-stage renal disease, hypertension, heart disease, ischemic heart disease, cerebral vascular disease, and peripheral artery disease.
COPD, chronic obstructive pulmonary disease; DDD, defined daily dose.

In the multivariate Cox regression analysis with IPSW adjustment, the β-blocker user group was independently associated with a more favorable 4-year OS than the nonuser group [HR: 0.68 (0.64–0.72)]. Other independent factors of a poor prognosis included age ≥ 75 years (HR: 1.22 (1.12–1.33)], cachexia [HR: 1.42 (1.33–1.51)], IICP [HR: 1.47 (1.37–1.58)], longer duration of hospitalization [HR: 1.02 (1.01–1.03)], PRBC transfusion [HR: 1.02 (1.01–1.03)], DM [HR: 1.12 (1.02–1.24)], HTN [HR: 1.12 [1.04–1.20)], and vascular disease [HR: 1.22 (1.13–1.31)] (**Table 3**).

Subgroup Analysis

The benefit of β-blockers on both 2-year TTD and 4-year OS was observed in male patients, patients aged ≥50 years, those with stage IV diseases, those with cachexia, those with or without IICP, those without DM, those without HTN, those without vascular disease, and those without COPD. In contrary, β-blockers was not beneficial in patients with DM and those with vascular disease. β-blockers was a poor prognostic factor in patients aged <50 years (**Figures 3A, B**).

In patients with either HTN or COPD, the benefit was only observed in 2-year TTD but not 4-year OS. On the other hand, in patients with stage IIIb disease or non-cachexic patients, the benefit of β-blocker was only seen in 4-year OS. Female patients were associated with shorter 2-year TTD but not 4-year OS (**Figures 3A, B**).

Sensitivity Analysis

A total of 2,507 patients with either HTN or ischemic heart disease were included in the sensitivity analysis. Among them, 511 (20.38%) received β-blocker ≥ 60 DDD and 1,996 (79.62%) received β-blocker < 60 DDD. The results of multivariate Cox

TABLE 3 | Multivariate Cox proportional hazards regression analysis for 4-year overall survival after using first-line epidermal growth factor receptor tyrosine kinase inhibitors.

Variable	Kaplan–Meier Analysis			Multivariate Cox Regression		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Male	1.17	1.09–1.27	<0.001	0.82	0.77–0.87	<0.001
Age >75	1.28	1.16–1.43	<0.001	1.22	1.12–1.33	<0.001
Beta-blocker ≥60 DDD	0.91	0.80–1.03	0.145	0.68	0.64–0.72	<0.001
Disease severity						
Megest use	1.57	1.45–1.70	<0.001	1.42	1.33–1.51	<0.001
Mannitol/glycerol use	1.48	1.36–1.61	<0.001	1.47	1.37–1.58	<0.001
Length of hospitalization (days)	1.05	1.04–1.07	<0.001	1.02	1.01–1.03	<0.001
PRBC transfusion (unit)	1.03	1.03–1.04	<0.001	1.02	1.01–1.03	<0.001
Comorbidity						
Diabetes mellitus	1.15	1.02–1.30	0.019	1.12	1.02–1.24	0.025
COPD	1.13	0.94–1.36	0.191	0.99	0.83–1.17	0.884
Hypertension	1.00	0.92–1.08	0.871	1.12	1.04–1.20	0.002
Vascular disease	1.15	1.06–1.25	0.001	1.22	1.13–1.31	<0.001

Multivariate Cox regression adjusted for sex, age, disease severity, and comorbidities, including COPD, diabetes mellitus, end-stage renal disease, hypertension, heart disease, ischemic heart disease, cerebral vascular disease, and peripheral artery disease.
COPD, chronic obstructive pulmonary disease; DDD, defined daily dose.

analysis with IPSW adjustment are presented in **Tables S1** and **S2**. Use of β-blockers remained an independent prognostic factor for 2-year TTD of EGFR-TKIs [HR: 0.89 (0.82–0.97)] with a trend of better 4-year OS [HR: 0.93 (0.85–1.01)].

DISCUSSION

This nationwide cohort study investigating the effect of β-blocker usage on stage IIIb/IV lung adenocarcinoma has two major findings. First, in patients with lung adenocarcinoma harboring sensitive EGFR mutation receiving first-generation EGFR-TKIs, prior use of β-blockers was independently associated with a better 2-year TTD and 4-year OS compared with nonusers. The benefit from prior use of β-blockers may also exist in lung cancer patients with either HTN or ischemic heart diseases. Second, the survival benefit of β-blockers was even greater for men and those with age ≥ 50 years, stage IV disease, cachexia, IICP, and absence of comorbidities.

The eight hallmarks of cancer development and progression are revised in 2011 (25). The stress hormones are highly associated with each of these hallmarks (26), which makes β-blockers a potential adjuvant therapy in malignancies (27–29).

A previous study has demonstrated that stress neurotransmitters activate stem cell-like cells in NSCLC through multiple cAMP-mediated pathways, and the growth of NSCLC xenografts in a mouse model was significantly decreased after stress reduction (13). Another study found that mice expressing lung-specific insulin-like growth factor type-1 receptor exhibited accelerated lung tumor development in response to chronic stress *via* exocytosis of insulin-like growth factor 2 (6). Moreover, a cell-line study suggested that nicotine facilitates growth and progression of NSCLC and pharmacological intervention using β-blockers may lower the risk of NSCLC development in smokers (10).

This is the first human study showing the benefit of β-blockers in patients with lung adenocarcinoma harboring EGFR mutation receiving first-line EGFR-TKIs. Previously, several observational studies have discussed the therapeutic value of β-blockers in lung cancer, but the results are controversial. In one retrospective study that included patients with stage I to IIIa NSCLC, the administration of β-blockers during the perioperative period did not improve recurrence-free or overall survival (12). Another study retrospectively reviewed 722 patients with NSCLC who received definitive radiotherapy or concurrent chemoradiotherapy, administration of β-blockers was associated with significantly more favorable distant metastasis-free survival [HR: 0.67 (0.50–0.91)], disease-free survival [HR: 0.74 (0.58-0.95)], and OS [HR: 0.78 (0.63–0.97)] (30). Recently, a population-based cohort study including patients with all stage lung cancer in Germany demonstrated β-blocker use before and after diagnosis was not associated with a more favorable OS (11).

It is already known that EGFR-TKIs delay tumor progression greater than chemotherapy. However, only a small advantage in OS was noted in previous studies probably due to drug resistance (3, 4, 31). It usually occurs within 1–2 years of starting therapy. EGFR target alteration accounts for approximately 60% acquired resistance, and T790M is the most common mutation (32). Recently, a mouse and cell-line model study demonstrated β2-activation of adrenergic receptors (β2-ARs) on NSCLC cells due to stress hormones, which cooperatively signal with mutant EGFR, resulting in the inactivation of the tumor suppressor liver kinase B1 and subsequent induction of interleukin-6 expression (5). This preclinical concept was used in LUX-lung 3 study and confirmed in patients receiving afatinib. Afatinib improved PFS, with a median PFS of 13.6 and 11.1 months in the β-blockers group and non-β-blockers group, respectively. The likelihood of progression reduction in the afatinib group was 75% and 40% in the β-blockers group and non-ß-blockers group, respectively (7).

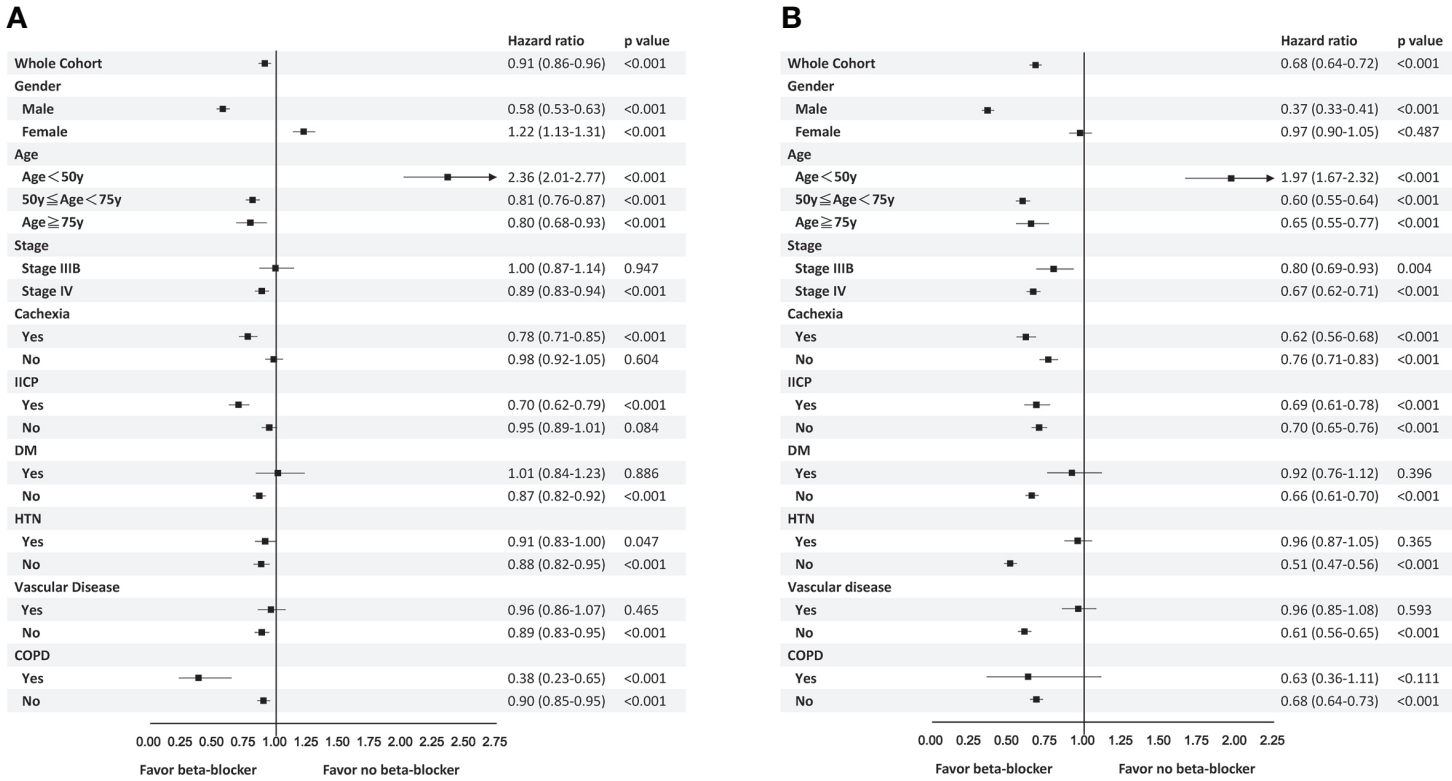

FIGURE 3 | Subgroup analyses for time to discontinuation of epidermal growth factor receptor-tyrosine kinase inhibitors within 2 years **(A)** and 4-year overall survival **(B)**.

In our study, we observed that former β-blocker use prolonged survival in patients with lung adenocarcinoma harboring sensitive EGFR mutation receiving first-line EGFR-TKIs. The result was consistent with a previous study that chronic stress hormones promote EGFR-TKI resistance and combinations of β-blockers and EGFR-TKIs may delay drug resistance (7). In sensitivity analysis, beta-blocker remains protective in TTD of EGFR-TKIs but not OS. The inconsistence may due to that the cause of death may result from underlying comorbidities but not lung cancer.

The present study has some limitations. First, though we have included cachexia, IICP, red blood cell transfusion, and duration of hospitalization as surrogates for disease severity, the performance status of each patient was unavailable and likely to bias the results. Second, beta-blocker use during EGFR-TKI treatment was not considered in the study. This could also introduce bias. Third, NHIRD does not contain information on smoking status, an important prognostic factor in previous studies (33, 34). However, we use COPD as a surrogate of smoking status for adjustment.

CONCLUSION

The results of this study suggest that in treatment-naïve patients with advanced lung adenocarcinoma receiving first-line EGFR-TKIs, prior β-blocker use was associated with a longer TTD and OS. The benefit remains present after considering the confounders. The findings encourage further prospective clinical study to test the possibility of using β-blockers as adjuvant anticancer therapy not only in lung cancer patient with hypertension or cardiovascular disease, but also normotensive patients. Second, β-blocker user during EGFR-TKI treatment was not considered in the study and the definition of β-blocker user was somewhat arbitrary. Both could introduce bias either toward or against null hypothesis. A prospective observational study using time-dependent analysis in patients with hypertension or those for whom β-blocker is indicated may be necessary to confirm the findings and provide more solid evidence.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by This study was approved by the Institutional Review Board of National Taiwan University Hospital (NTUH REC: 201601007W). Given the retrospective design and use of an encrypted database in this study, the need for informed consent was waived. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

C-HC, J-YW, and J-YS: conceptualization, methodology, and software. C-HC, C-HL: validation and formal analysis. C-HL, J-FZ, and J-YW: statistical analysis. C-HC, J-YW: investigation and resources. C-HC, C-HL, and J-CK: data curation and writing. C-HC, C-HL, J-CK, J-FZ, L-YC, M-CL, J-YW, J-YS, and C-JY: manuscript review and visualization. J-YW, J-YS, and C-JY: Supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.583529/full#supplementary-material

REFERENCES

1. Shi Y, Au JS-K, Thongprasert S, Srinivasan S, Tsai C-M, Khoa MT, et al. A Prospective, Molecular Epidemiology Study of EGFR Mutations in Asian Patients with Advanced Non–Small-Cell Lung Cancer of Adenocarcinoma Histology (PIONEER). *J Thoracic Oncol* (2014) 9(2):154–62. doi: 10.1097/JTO.0000000000000033
2. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* (2014) 511(7511):543–50. doi: 10.1038/nature13385
3. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *New Engl J Med* (2009) 361(10):947–57. doi: 10.1056/NEJMoa0810699
4. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, 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):239–46. doi: 10.1016/s1470-2045(11)70393-x

5. Al-Wadei HA, Plummer HK,3, Ullah MF, Unger B, Brody JR, Schuller HM. Social stress promotes and gamma-aminobutyric acid inhibits tumor growth in mouse models of non-small cell lung cancer. *Cancer Prev Res (Philadelphia Pa)* (2012) 5(2):189–96. doi: 10.1158/1940-6207.capr-11-0177
6. Jang HJ, Boo HJ, Lee HJ, Min HY, Lee HY. Chronic Stress Facilitates Lung Tumorigenesis by Promoting Exocytosis of IGF2 in Lung Epithelial Cells. *Cancer Res* (2016) 76(22):6607–19. doi: 10.1158/0008-5472.can-16-0990
7. Nilsson MB, Sun H, Diao L, Tong P, Liu D, Li L, et al. Stress hormones promote EGFR inhibitor resistance in NSCLC: Implications for combinations with beta-blockers. *Sci Trans Med* (2017) 9(415):eaao4307. doi: 10.1126/scitranslmed.aao4307
8. Saxena A, Becker D, Preeshagul I, Lee K, Katz E, Levy B. Therapeutic Effects of Repurposed Therapies in Non-Small Cell Lung Cancer: What Is Old Is New Again. *Oncol* (2015) 20(8):934–45. doi: 10.1634/theoncologist.2015-0064
9. Chaudhary KR, Yan SX, Heilbroner SP, Sonett JR, Stoopler MB, Shu C, et al. Effects of β-Adrenergic Antagonists on Chemoradiation Therapy for Locally Advanced Non-Small Cell Lung Cancer. *J Clin Med* (2019) 8(5):575. doi: 10.3390/jcm8050575
10. Al-Wadei HA, Al-Wadei MH, Schuller HM. Cooperative regulation of non-small cell lung carcinoma by nicotinic and beta-adrenergic receptors: a novel target for intervention. *PloS One* (2012) 7(1):e29915. doi: 10.1371/journal.pone.0029915
11. Weberpals J, Jansen L, Haefeli WE, Hoffmeister M, Wolkewitz M, Herk-Sukel M, et al. Pre- and post-diagnostic beta-blocker use and lung cancer survival: A population-based cohort study. *Sci Rep* (2017) 7(1):2911. doi: 10.1038/s41598-017-02913-8
12. Cata JP, Villarreal J, Keerty D, Thakar DR, Liu DD, Sood AK, et al. Perioperative beta-blocker use and survival in lung cancer patients. *J Clin Anesthesia* (2014) 26(2):106–17. doi: 10.1016/j.jclinane.2013.10.004
13. Banerjee J, Papu John AM, Schuller HM. Regulation of nonsmall-cell lung cancer stem cell like cells by neurotransmitters and opioid peptides. *Int J Cancer* (2015) 137(12):2815–24. doi: 10.1002/ijc.29646
14. Network NCC. *National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer (Version 4.2016).* (2016) Fort Washington (PA): National Comprehensive Cancer Network. Available at: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf, [cited 2016 July 6].
15. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *New Engl J Med* (2018) 378(2):113–25. doi: 10.1056/NEJMoa1713137
16. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *New Engl J Med* (2019) 382(1):41–50. doi: 10.1056/NEJMoa1913662
17. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Institute* (2000) 92(3):205–16. doi: 10.1093/jnci/92.3.205
18. WHO International Working Group for Drug Statistics Methodology. *Guidelines for ATC classification and DDD assignment*. Oslo, Norway: WHO Collaborating Centre for Drug Statistics Methodology (2011).
19. Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *J Clin Oncol Off J Am Soc Clin Oncol* (2013) 31(12):1539–47. doi: 10.1200/jco.2012.45.2722
20. Sperduto PW, Kased N, Roberge D, Xu Z, Shanley R, Luo X, et al. Summary report on the graded prognostic assessment: an accurate and facile diagnosis-specific tool to estimate survival for patients with brain metastases. *J Clin Oncol Off J Am Soc Clin Oncol* (2012) 30(4):419–25. doi: 10.1200/jco.2011.38.0527
21. Caro JJ, Salas M, Ward A, Goss G. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer* (2001) 91(12):2214–21. doi: 10.1002/1097-0142(20010615)91:12<2214::AID-CNCR1251>3.0.CO;2-P
22. Chang CH, Lee CH, Ko JC, Chang LY, Lee MC, Wang JY, et al. Gefitinib or erlotinib in previously treated non-small-cell lung cancer patients: a cohort study in Taiwan. *Cancer Med* (2017) 6(7):1563–72. doi: 10.1002/cam4.1121
23. Lee CH, Lee MC, Lin HH, Shu CC, Wang JY, Lee LN, et al. Pulmonary tuberculosis and delay in anti-tuberculous treatment are important risk factors for chronic obstructive pulmonary disease. *PloS One* (2012) 7(5):e37978. doi: 10.1371/journal.pone.0037978
24. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behav Res* (2011) 46(3):399–424. doi: 10.1080/00273171.2011.568786
25. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144(5):646–74. doi: 10.1016/j.cell.2011.02.013
26. Tang J, Li Z, Lu L, Cho CH. beta-Adrenergic system, a backstage manipulator regulating tumour progression and drug target in cancer therapy. *Semin Cancer Biol* (2013) 23(6 Pt B):533–42. doi: 10.1016/j.semcancer.2013.08.009
27. Childers WK, Hollenbeak CS, Cheriyath P. beta-Blockers Reduce Breast Cancer Recurrence and Breast Cancer Death: A Meta-Analysis. *Clin Breast Cancer* (2015) 15(6):426–31. doi: 10.1016/j.clbc.2015.07.001
28. Colucci R, Moretti S. The role of stress and beta-adrenergic system in melanoma: current knowledge and possible therapeutic options. *J Cancer Res Clin Oncol* (2016) 142(5):1021–9. doi: 10.1007/s00432-015-2078-z
29. Braadland PR, Ramberg H, Grytli HH, Tasken KA. beta-Adrenergic Receptor Signaling in Prostate Cancer. *Front Oncol* (2014) 4:375. doi: 10.3389/fonc.2014.00375
30. Wang HM, Liao ZX, Komaki R, Welsh JW, O'Reilly MS, Chang JY, et al. Improved survival outcomes with the incidental use of beta-blockers among patients with non-small-cell lung cancer treated with definitive radiation therapy. *Ann Oncol Off J Eur Soc Med Oncol* (2013) 24(5):1312–9. doi: 10.1093/annonc/mds616
31. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol* (2014) 15(2):213–22. doi: 10.1016/s1470-2045(13)70604-1
32. Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: learning from lung cancer. *Nat Rev Clin Oncol* (2014) 11(8):473–81. doi: 10.1038/nrclinonc.2014.104
33. Tammemagi CM, Neslund-Dudas C, Simoff M, Kvale P. Smoking and lung cancer survival: the role of comorbidity and treatment. *Chest* (2004) 125(1):27–37. doi: 10.1378/chest.125.1.27
34. Tsao AS, Liu D, Lee JJ, Spitz M, Hong WK. Smoking affects treatment outcome in patients with advanced nonsmall cell lung cancer. *Cancer* (2006) 106(11):2428–36. doi: 10.1002/cncr.21884

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Role of *ARL4C* in Erlotinib Resistance: Activation of the *Jak2/Stat 5/β-Catenin* Signaling Pathway

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Cancer patients who initially benefit from Erlotinib, a drug targeting *EGFR* path, eventually develop resistance to the drug. The underlying mechanism is largely unknown. This study investigated the role of *ARL4C* in Erlotinib resistance development of NSCLC. qRT-PCR and Western blotting were performed to analyze the expression of mRNA and protein of *ARL4C* in two NSCLC cell lines (HCC827 and PC-9). Several assays (MTS, colony formation, transwell migration, luciferase reporter, and chromatin-immunoprecipitation) were used to explore the role of *ARL4C* in biofunctional changes of Erlotinib-resistant cells and their associations with *Jak2/Stat 5/β-catenin* signaling. Results demonstrated that (1) long-term use of Erlotinib resulted in downregulation of *ARL4C*; (2) overexpression of ARL4C could regain the sensitivity to Erlotinib in the drug-resistant HCC827/ER cells, while downregulation of *ARL4C* increased HCC827, and PC-9 cells' resistance to the drug; (3) Erlotinib-induced downregulation of *ARL4C* resulted in phosphorylation of *Jak2/Stat5* and upregulation of *β-catenin* and their related molecules *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7*, which promoted the malignant behaviors of Erlotinib-resistant cells; (4) chromatin immunoprecipitation and luciferase reporter assay revealed that Stat5 could bind to *β-catenin* promoter to upregulate molecules to maintain the malignant behaviors, which might count for how Erlotinib-resistant cell survived while *EGFR* path was blocked; (5) the expression of *ARL4C* was not associated with known EGFR gene mutations in both Erlotinib-resistant cells and NSCLC tissues. Our data suggest that Erlotinib resistance of NSCLCs is associated with downregulation of *ARL4C* via affecting *Jak/Stat/β-catenin* signaling. *ARL4C* could serve as a biomarker to predict the effectiveness of TKI targeting therapy and a potential therapeutic target for overcoming Erlotinib resistance in NSCLC.

Keywords: non-small cell lung cancer, *ARL4C*, TKI resistance, *β-catenin*, *Jak*, *Stat*

INTRODUCTION

Lung cancer is the most commonly diagnosed malignancy and a leading cause of cancer death worldwide (1). Non-small cell lung cancer (NSCLC) accounts for ∼80% of all lung cancer cases (2). *EGFR* (Epidermal Growth Factor Receptor) tyrosine kinase inhibitors (TKIs) are a group of important targeting drugs for the treatment of NSCLCs with *EGFR* mutations, including exon 19 deletions or L858R substitutions. However, the acquired drug resistance occurs within 1 year after the treatment with the first generation of EGFR-TKI, which is related partially with the T790M secondary gatekeeper mutation (3), or activation of other alternative pathways, such as *HGF/Met* (hepatocyte growth factor/mesenchymal-epithelial transition factor), *HER2* (Erb-B2 Receptor Tyrosine Kinase 2), and *PIK3CA* (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) (4). However, mechanisms of TKI resistance in about 30% cases remain unclear (5). Seeking for the unknown mechanisms is a hot research topic in lung cancer research (6).

With selected methods, we found that downregulation of *ARL4C* (ADP-ribosylation factor-like 4C) might be associated with TKI resistance. *ARL4C*, also known as arl7, is a 192-amino-acid protein belonging to a small GTP enzyme. It is one of the subfamily members of ADP ribosylation factor and plays an important role in vesicle transport and signal transduction (7). The biofunction of *ARL4C* is not very clear yet. A study conducted by Su and his colleagues showed that high-level expression of *ARL4C* could inhibit the migration of ovarian cancer cells. Patients with high level of *ARL4C* mRNA had a good prognosis (8). Another study with gastric cancer showed that the expression of *ARL4C* was abnormal and the molecule was involved in tumor cell growth and cell migration (9). Using immunohistochemical analysis, Fujii et al. showed that *ARL4C* was abnormally expressed in lung cancer tissues. It was involved in the proliferation and invasion of lung cancer cells *in vitro* and *in vivo* (10). However, the role of *ARL4C* in TKI resistance is unexplored.

In this study, we investigated the role of *ARL4C* in TKI resistance of NSCLC cells by analyzing its functions with various assays. Our data demonstrated for the first time that *ARL4C* contributed to TKI resistance by activation of the *JAK/STAT* (Janus Kinase/signal transducer and activator of transcription) signaling pathway for survival when *EGFR* path blocked. The results suggest that *ARL4C* could be a promising biomarker for patients who likely benefit from TKI-based targeting therapy.

METHODS

NSCLC Tissue Samples

NSCLC tissues from 42 patients (22 men and 20 women with a median age of 60.5 years old) who have undergone lung cancer resection in Fujian Cancer Hospital between January 2008 and June 2009 were collected. None of the patients received chemotherapy before surgery, and cancer tissues were obtained immediately after surgical resection. One part of every cancer specimen was frozen at −80°C for measuring *ARL4C* mRNA level. The remaining part of the tissues was fixed in formalin and embedded in paraffin (FFPE) for detecting EGFR mutations.

Selection of TKI Erlotinib-Resistant Cell Line

HCC827 and PC-9 (*EGFR* 19del) cell lines purchased from ATCC were cultured in H1640 medium, containing 10% FBS (GIBCO BRL, Rockville, MD, U.S.A. Cat No. 10099233), 100 U/ml penicillin, and 100 μg/ml streptomycin in an incubator containing 5% CO_2 at 37°C. The cells were exposed to gradually increased concentration of Erlotinib (Selleck, Houston, TX, USA. Cat No. S1023) from 0, 100, 200, 400, 800, 1,600, and 3,200 nM in their culture medium. After passing 17 generations in 6 months of the selection, Erlotinib was removed from the medium. The cells growing in 3,200 nM of Erlotinib were labeled as TKI Erlotinib-resistant (ER) cell lines HCC827/ER and PC-9/ER, respectively.

ARL4C Overexpression or Knockdown Cell Lines

Several virus vectors were purchased from Hanheng Biotechnology Co Ltd (Beijing, China). To create cell lines with *ARL4C* overexpression, *ARL4C* expression vector HBLV-ARL4C (pHBLV-CMV-mcs-3flag-EF1-ZsGReen-T2A-PURO inserted with *ARL4C* gene) was used. The original vector was used as vector alone control. For *ARL4C* knockdown in cells, HBLV-ARL4C-shrna1, HBLV-ARL4C-shrna2, and HBLV-ARL4C-shrna3 were used and their parental vector *pHBLV-U6-MCS-CMV-Zsgreen* was used as vector alone control. HCC827, HCC827/ER, PC-9, and PC-9/ER cells cultured in six-well-plates (5×10^5/well) were infected with 10 MOI of respective viral vectors in the presence of 6 μg/ml of polyamine. After 48 h, the cells were selected with 2 μg/ml of Puromycin for 2 weeks to obtain stable infected cells. Newly established cells were HCC827/ER/vector, HCC827/ER/ARL4C-OE, HCC827/vector, HCC827/ARL4C-SH, PC-9/ER/vector, PC-9/ER/ARL4C-OE, PC-9/vector, and PC-9/ARL4C-SH. The symbols -OE and -SH means overexpression and knockdown, respectively.

Detection of mRNA Levels of *ARL4C*, *β-Catenin*, *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP-7*

The level of ARL4C mRNA expression was determined in 42 lung cancer tissues by real-time PCR. ARL4C and *β*-atenin, *Axin2* (Axis Inhibition Protein 2), *CD44*, *Ccnd1* (Cyclin D1), *Lgr-5* (Leucine Rich Repeat Containing G Protein-Coupled Receptor 5), and *MMP-7* (Matrix Metallopeptidase 7) were assessed in NSCLC cell lines (HCC827, PC-9, HCC827/ER, and PC-9/ER) also by real-time PCR. Total RNA was extracted from tissues and cell lines using Trizol reagent (Invitrogen, Grand Island, NY, USA) following the manufacturer's instruction. cDNA was synthesized from 1 μg of total RNA using M-MuLV reverse transcriptase (Promega, Madison, WI, USA). Real-time PCR was performed using SYBR1 Green Dye detection systems (Roche, Switzerland). The primers for amplifying *ARL4C*, *β-catenin*, *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP-7* are shown in

Supplementary Table 1. Real-time PCR parameters were 95°C for 10 min, followed by 40 cycles of 95°C for 10 s, 55°C for 10 s and 72°C for 20 s, and 40°C for 30 s at the end of the 40 cycles. Relative quantity of mRNA expression was calculated by using the $2^{-\Delta\Delta Ct}$ method. All measurements were repeated in triplicate.

Relative mRNA expression levels of *ARL4C* were quantified in all lung cancer samples using the comparative $2^{-\Delta\Delta Ct}$ method and lung cancer sample T27 as reference reported previously (11, 12). The housekeeping gene glyceraldehyde phosphate dehydrogenase (*GAPDH*) was used to normalize expression levels of *ARL4C*.

Protein Levels of ARL4C, β-Catenin, and JAK/STAT Signaling

Cells were harvested and lysed using RIPA buffer (50 mM Tris–Cl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.1% SDS, and 1% NP-40) supplemented with protease inhibitor cocktail (Abcam, Cambridge, MA, USA. Cat No. ab65621). The cell lysates were centrifuged at 12,000 rpm for 30 min at 4°C. Supernatants were collected and protein concentrations were determined by BCA protein assay (Thermo Scientific, Rockford, Illinois, USA). The supernatants (each with 25 μg proteins) were electrophoresed on 10–12% polyacrylamide gel with sodium dodecyl sulfate (SDS) and then transferred onto nitrocellulose membranes (Millipore, Burlington, MA, USA) at 100 V for 1.5 h. After blocking with 3% BSA in TBST (TBS−1% Tween 20) for 1 h, the membranes were incubated with primary antibodies of ARL4C (1:500. Abcam, Cambridge, UK, Cat No. ab122025), and 1:1,000 diluted antibodies against β-catenin (CST, Danvers, MA, USA. Cat No. 8480), p-JAK 2 (CST, Danvers, MA, USA. Cat No. 4406), and p-STAT5 (Cell Signaling Technology, Danvers, MA, USA. Cat No. 4322) overnight at 4°C, respectively. After wash, the membranes were further incubated with horseradish peroxidase-conjugated anti-rabbit antibody. Finally, protein bands were developed with the enhanced chemiluminescence Western blot detection kit Immobilon ECL Ultra Western HRP Substrate (Millipore, Bedford, MA, USA. Cat No. WBULS0500), and images were captured on image station 4,000 mm pro (Carestream, Canada). Image J program was used to quantify the protein band intensity relative to loading control of β-actin.

Effects of Erlotinib on Cell Proliferation and IC50

Cells were plated into 96-well-plates (5,000 cells/well) and grew overnight. Different concentrations of Erlotinib (0, 100, 200, 400, 800, 1,600, and 3,200 nM) were added into the cell cultures in triplicate. After culturing for 72 h, 20 μl of MTS (Promega, Madison, WI, USA. Cat No. g3582) and 100 μl of serum containing medium were added to each well-followed by incubation at 37°C for 2 h. H1640 only with serum was used as background control. The absorption of the palates at 490 nm was read on a Bio-Rad (Hercules, CA, USA) plate reader (model 680). The proliferation of Erlotinib-treated cells was normalized with the control cells (no Erlotinib). IC50 was calculated with SPSS17.0. The experiment was repeated three times.

Colony Formation Assay

For colony-formation assay, cells (about 500 cells/well) were seeded and grew in six-well-plates for 48 h before adding Erlotinib. After 14 days, the cells were fixed in methanol and stained with 0.2% crystal violet. Cell colonies (>50 cells/colony) were pictured with Image Scanner (GE, Piscataway, NJ, USA). The number of colonies in each well was counted using *Image J*.

Transwell Invasion Experiment

Cell concentration was adjusted to 7×10^5/ml, and 100 μl of the cell suspension was placed onto the upper chamber of each well on 24-well-Transwell plates coated with 1 mg/ml fibronectin (Millipore). The lower chamber contained medium with 20% FBS. After incubation at 37°C for 48 h, the cells in the upper chamber were wiped off with cotton swabs, and the cells in the other side of chamber membrane were fixed with methanol for 15 min, dried, stained with 0.1% crystal violet, and randomly pictured with a magnification of ×200 under inverted microscope (Olympus, Japan). The cells were counted with *Image J* program and the average number of the cells from 15 fields was used. The experiment was repeated three times.

Luciferase Assays

Using the online transcription factor binding sites (TFBS) software (http://alggen.lsi.upc.es), we predicted the *CTNNB1* promoter containing a *STAT5A* binding site. *CTNNB1* (*β-catenin*) promoter (−200 to 0 regions) was inserted into *pGL3-basic vector* (Promega, Madison, WI, USA) as *pGL3-CTNNB1* luciferase reporter plasmid (wild-pGL3-CTNNB1). The STAT5 site of the *CTNNB1* promoter was mutated (from 5′-atttttctgtcag-3′ to 5′-taaaaagacagtc-3′) and was cloned into *pGL3-basic vector* to generate *CTNNB1* promoter mutated reporter plasmid mut-pGL3-CTNNB1. All constructs were verified by sequencing. For the luciferase reporter assay, HEK293T cells, HEK293T/ARL4C-SH, HCC827, and HCC827/ARL4C-SH cells were transfected with *pGL3-CTNNB1-Luc* or *mut-pGL3-CTNNB1* using X-treme GENE HP DNA Transfection Reagent (Roche, Basel, Switzerland. Cat No. 6366236001). Renilla luciferase was used as internal control. Forty-eight hours later, the transfected cells were harvested and the luciferase activity was measured by Dual-luciferase Reporter Assay System (Promega Corporation, Madison, WI, USA). The relative firefly luciferase activity was calculated by normalizing transfection efficiency using the Renilla luciferase activity.

Chromatin-Immunoprecipitation (ChIP)

ChIP assay was carried out using a SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology, Beverly, MA, USA, Cat No. 9002) following the manufacturer's instruction. Briefly, 5×10^6 cells were fixed with 1% formaldehyde and quenched in 0.125 M glycine. Cells were sonicated by Bioruptor Sonication System UCD-300. DNA was immunoprecipitated by either control IgG or phospho-stat5 antibody. Precipitated DNA samples and inputs were amplified by PCR. The primers used for the amplification of stat5 binding site in β-catenin promoter are

5′-cctcttccccgttgtttcca-3′ (sense) and 5′-ggggtgattctttgctaatttca-3′ (antisense).

Detection of *EGFR* Mutations in Both NSCLC Samples and Cell Lines

Two methods were used to detect *EGFR* mutations. For 42 NSCLC paraffin specimens, DNA was obtained using a paraffin tissue DNA Extraction kit (Qiagen, Hilden, Germany. Cat No. 56404). The concentration of DNA was adjusted to 1 ng/ml, and EGFR mutations were detected using the amplification refractory mutation system (ARMS) with human EGFR Mutations Detection kit (Amoy Diagnostics, Xiamen, China. Cat No. ADx-EG01) according to the manufacturer's protocol as previously described (13). Briefly, ARMS-PCR assay was performed in a 50-μl volume containing 5 μl of PCR buffer, 10 pM forward and reverse primers, 20 pM probe, and 12.5 mM dNTPs. The thermocycling conditions were as follows: 95°C for 5 min, then 15 cycles of 95°C for 25 s, 64°C for 20 s, and 72°C for 20 s, followed by 31 cycles of 93°C for 25 s, 60°C for 35 s, and 72°C for 20 s.

To determine if there is any association between *ARL4C* expression and *EGFR* mutations, eight cell lines with different levels of *ARL4C* were examined with the next-generation sequencing (NGS). DNA was extracted using the GONOROAD kit (Qiagen, Hilden, Germany) and 200 ng of DNA was used to build the library using NEBNext Ultra II DNA library Prep Kit for Illumina (NEB, Ipswich, MA, USA). Integrated DNA technologies (IDT, Skokie, IL, USA) customized probes were used for hybridization capture. All libraries were performed on an MGISEQ2000 instrument according to the manufacturer's instructions (BGI, Shenzhen, Guangdong, China) with 200 cycles, standing for paired-end 100 bp. Then, IDT 10-hotspot gene panels from all eight libraries were used, which included *ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *NRAS*, *PIK3CA*, *RET*, and *ROS1*, and were quantitated using Library Quantification Kit-Illumina/Universal (Kapa Biosystems, Wilmington, MA, USA) on an ABI 7500 Real Time PCR system (Applied Biosystems, Waltham, MA, USA). The mutations were detected by the following methods: the Trimmomatic (version 0.39, parameter: PE -threads 4 -phred33 ILLUMINACLIP: adapter.fa:2:30:10 MINLEN:15), and the adapter sequences were AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA and AAGTCGGATCGTAGCCATGTCGTTCTGTGAGCCAAGGAGTTG and were used to narrow down the raw sequencing data (Fastq), filtering out the adapter contamination reads and low-quality reads to get clean data. Bwa Aln (Version: 0.7.12-r1039) algorithm was used to align the clean data of the human reference genome (hg19) and to get the Sequence Alignment/Map format (sam) file. For the Binary Alignment/Map format (bam) file, the sam file was sorted and the artificial duplication reads were removed by samtools (Version: 0.1.19-44428cd). According to the bed interval file of the 10-hotspot gene panels, Freebayes (version: v1.0.2-6-g3ce827d, parameter: -j -m 10 -q 30 -F 0.001 -C 1 -t bed.file –f hg19.fa) was used to determine the single-nucleotide polymorphisms (SNPs) and insertions or deletions (indels) and then ANNOVAR was used for the annotation.

Statistical Analysis

All data were presented as mean ± SD. Student's *t*-test was used for analysis. ANOVA was used to determine the statistical difference between or among different experimental groups. The value $P < 0.05$ was considered as statistically significant difference.

RESULTS

TKI Resistant NSCLC Cell Lines Expressed a Low Level of *ARL4C*

Two NSCLC cell lines, HCC827 and PC-9, were subjected to Erlotinib selection in culture. After 6 months, IC50 of Erlotinib was significantly increased from 289 to 1,843 nM for HCC827 and from 71.08 to 5232.12 nM for PC-9. These Erlotinib-resistant (ER) cell lines were named HCC827/ER and PC-9/ER, which were 5.37 and 73.60 times more resistant to Erlotinib than their parental cells, respectively (**Figures 1A–C**). However, results of qPCR and Western blotting demonstrated that the levels of *ARL4C* were significantly lower in HCC827/ER and PC-9/ER cells than in their parental cells (**Figures 1D–G**, $P < 0.0001$). Furthermore, *β-Catenin* expression significantly increased in HCC827/ER and PC-9/ER, compared with their parental cells (**Figures 1D–G**, both $P < 0.0001$). Collectively, these results showed that the expression of *ARL4C* was reduced in TKI-resistant cells (HCC827/ER, PC-9/ER), while the expression of *β-catenin* was elevated in TKI-resistant cells.

Knocking Down *ARL4C* Resulted in Enhancing Erlotinib Resistance and Increasing Cell Migration

To confirm that the downregulation of *ARL4C* was associated with the increased Erlotinib resistance, sh-ARL4C-knockout lentivirus was used to infect HCC827 and PC-9 cells. Western blotting showed that the expression level of *ARL4C* in the infected cells HCC827/ARL4C-sh and PC-9/ARL4C-sh were significantly lower than those in control cells infected with lentivirus vector alone (HCC827/Vector and PC-9/Vector) (**Figures 2A–C**, both $P < 0.0001$).

Effect of low *ARL4C* on Erlotinib IC50 was examined with MTS assay. Results showed that knockdown of *ARL4C* significantly increased Erlotinib resistance as compared to their parental cells. IC50 values of Erlotinib for HCC827, HCC827/vector, and HCC827/ARL4C-sh were 222.8, 393.2, and 1236.2 nM, respectively. The values for PC-9, PC-9/vector, and PC-9/ARL4C-sh were 71.2, 68.23, and 877.97 nM, respectively (**Figures 2D–F**, $P < 0.01$).

The cells with knockdown of *ARL4C* (HCC827/ARL4C-sh and PC-9/ARL4C-sh) had a marked enhancement of proliferation and colony formation, compared to their parental HCC827 and PC-9 cells when treated with Erlotinib (**Figures 2G–J**, both $P < 0.001$). These *ARL4C* low-expressing cell lines HCC827/ARL4C-sh and PC-9/ARL4C-sh cells also

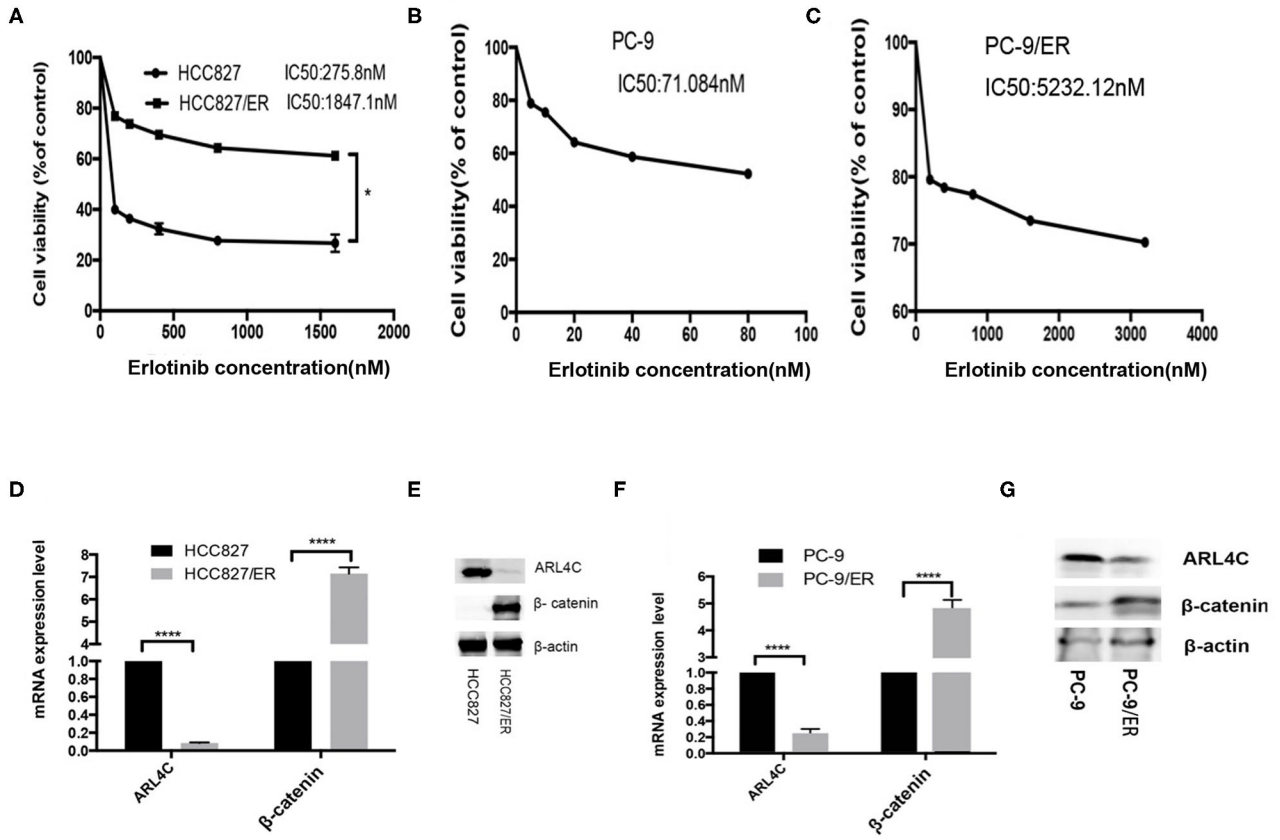

FIGURE 1 | Expression of *ARL4C* and *β-Catenin* in TKI-resistant NSCLC cell lines. **(A)** MTS assay of the proliferation of in Erlotinib-resistant HCC827/ER and Erlotinib-sensitive HCC827 cells in different concentrations of Erlotinib. **(B,C)** MTS assay of the dose response of Erlotinib-sensitive PC-9 cells **(B)** and Erlotinib-resistant PC-9/ER **(C)** to different concentrations of Erlotinib. **(D,E)** qPCR and Western blotting analyzed *ARL4C* and β-catenin expression in HCC827/ER and HCC827 cells. **(F,G)** qPCR and Western blotting detected *ARL4C* and *β-catenin* expression in PC-9/ER and PC-9 cells. *$P < 0.05$; ****$P < 0.0001$.

had a significantly increased capability of the migration, passing through the polyester membrane, compared with control cells HCC827/vector and PC-9/vector (**Figures 2K,L**, both $P < 0.001$).

These results suggest that knockdown *ARL4C* enhances TKI Erlotinib tolerance, colony formation, and migration of NSCLC cells.

Overexpression of *ARL4C* in Erlotinib-Resistant HCC827/ER and PC-9/ER Cells Inhibited Their Tolerance to Erlotinib and Cell Migration

To further confirm the relationship between Erlotinib resistance and *ARL4C* expression, both Erlotinib-resistant HCC827/ER and PC-9/ER cells were infected with *ARL4C* overexpression lentivirus to regain the *ARL4C* expression. Results of qPCR and Western blot showed that the expression levels of *ARL4C* in HCC827/ER/ARL4C-OE and PC-9/ER/ARL4C-OE were significantly higher than those of vector alone control cells, HCC827/ER/Vector, and PC-9/ER/Vector (**Figures 3A–C**, $P < 0.001$).

To examine the effect of high level of *ARL4C* on cell susceptibility to Erlotinib, the IC50 was measured with MTS assay. Results showed that the overexpression of *ARL4C* significantly decreased Erlotinib resistance of both HCC827/ER and PC-9/ER cells. IC50 values of Erlotinib for HCC827/ER, HCC827/ER/Vector, and HCC827/ER/ARL4C-OE cells were 1.85, 1.71, and 0.47 μM, respectively. The values for PC-9/ER, PC-9/ER/Vector, and PC-9/ER/ARL4C-OE cells were 5232.1, 5146.1, and 928.1 nM, respectively (**Figures 3D,E**, both $P < 0.05$).

Similarly, the effect of high level of *ARL4C* on cell colony formation and migration in the presence of Erlotinib was tested. Results showed that the overexpression of *ARL4C* in HCC827/ER/ARL4C-OE and PC-9/ER/ARL4C-OE cells resulted in an reduced sensitivity to Erlotinib as compared to HCC827/ER and PC-9/ER cells (**Figures 3F–I**, $P < 0.01$, $P < 0.001$, and $P < 0.0001$). The migration of HCC827/ER/ARL4C-OE and PC-9/ER/ARL4C-OE was significantly decreased. The number of cells passing through the polyester membrane decreased,

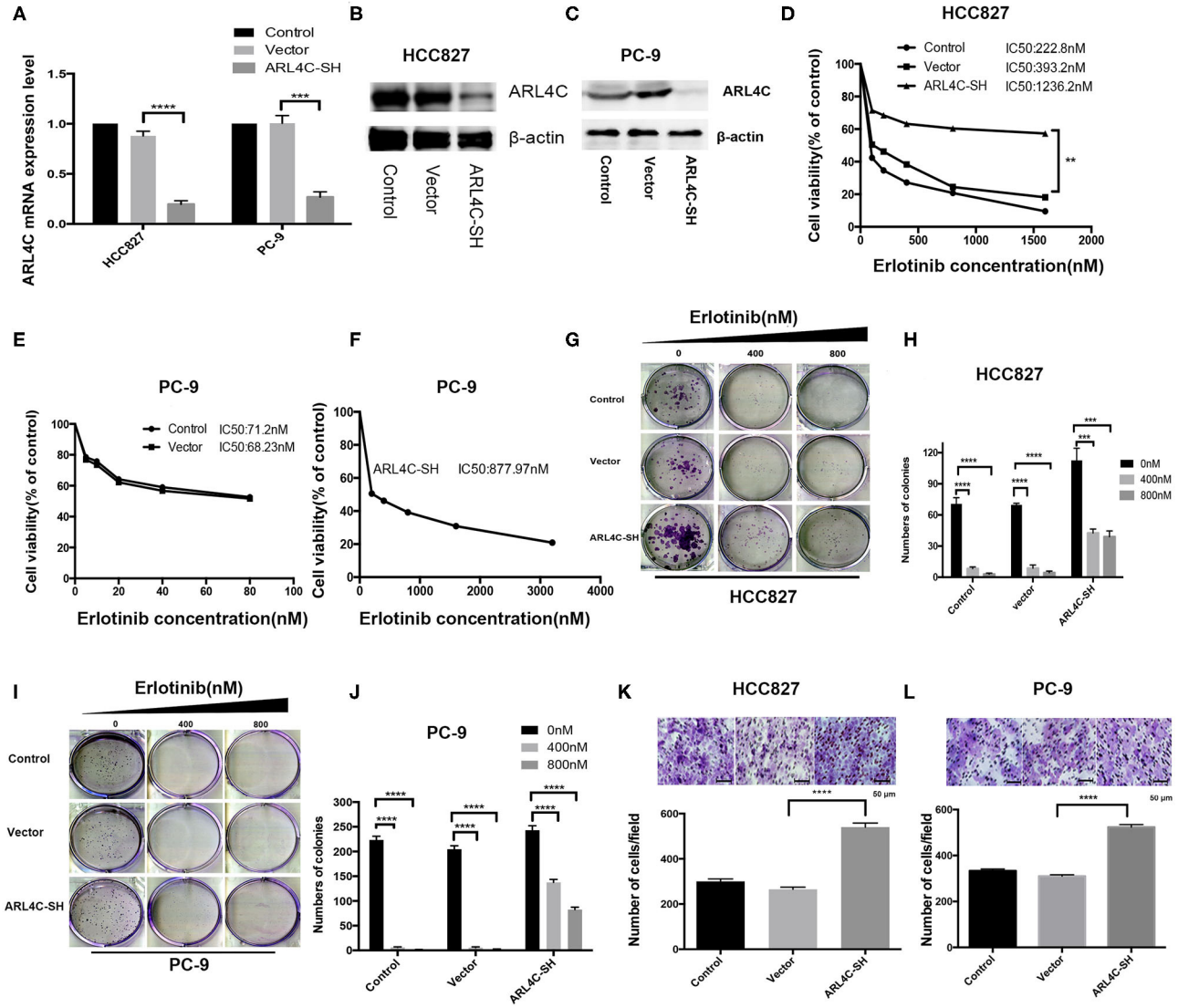

FIGURE 2 | *ARL4C* knockdown enhances Erlotinib resistance of lung cancer cells. **(A)** qPCR quantification of *ARL4C* mRNA levels in *ARL4C* knockdown cells HCC827 (HCC827-ARL4C-SH) and PC-9 (PC-9-ARL4C-SH), control vector-infected HCC827 (HCC827-Vector) and PC-9 (PC-9-Vector), and their parental HCC827 and PC-9 control cells. **(B)** Western blotting detection of *ARL4C* expression in HCC827-ARL4C-SH, HCC827-Vector, and HCC827 (control) cells, and in **(C)** in PC-9-ARL4C-SH, PC-9-Vector, and PC-9 (control) cells. **(D)** IC50 of HCC827-ARL4C-SH, HCC827-Vector, and HCC827 (control) cells measured by MTS. **(E,F)** Erlotinib IC50 of PC-9-ARL4C-SH, PC-9-Vector, and PC-9 (control) cells measured by MTS. **(G,H)** Colony formation of HCC827-ARL4C-SH, HCC827-Vector, and HCC827 (control) cells. **(I,J)** Colony formation of PC-9-ARL4C-SH, PC-9-Vector, and PC-9 (control) cells. **(K)** Transwell assays of the migration of HCC827-ARL4C-SH, HCC827-Vector, and HCC827 (control) cells. **(L)** Transwell assays of the migration of PC-9-ARL4C-SH, PC-9-Vector, and PC-9 (control) cells. $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

compared to control cells HCC827/ER-vector and PC-9/ER-vector (**Figures 3J,K**, both $P < 0.0001$).

These data suggest that overexpression of *ARL4C* in Erlotinib-resistant lung cancer cells increases the sensitivity of the cells to TKI Erlotinib and inhibits cell migration.

Enhance of *β-Catenin* Is Critical for ARL4C-Associated Erlotinib Resistance

It has been shown that cells resistant to Erlotinib expressed a high level of β-catenin (12). Our results also showed that *ARL4C* was expressed at a significantly low level in Erlotinib-resistant HCC827/ER and PC-9/ER cells, compared to Erlotinib-sensitive HCC827 and PC-9 cells (**Figures 1D–G**, $P < 0.0001$), while *β-Catenin* expression significantly increased in Erlotinib-resistant cells (**Figures 1D–G**, $P < 0.0001$). Therefore, we speculate that *β-catenin* might contribute to *ARL4C*-associated Erlotinib resistance.

To test our hypothesis, the effect of *ARL4C* on the *β-catenin* expression in HCC827 and HCC827/ER cells was examined. As expected, knockdown of *ARL4C* with lentivirus vectors

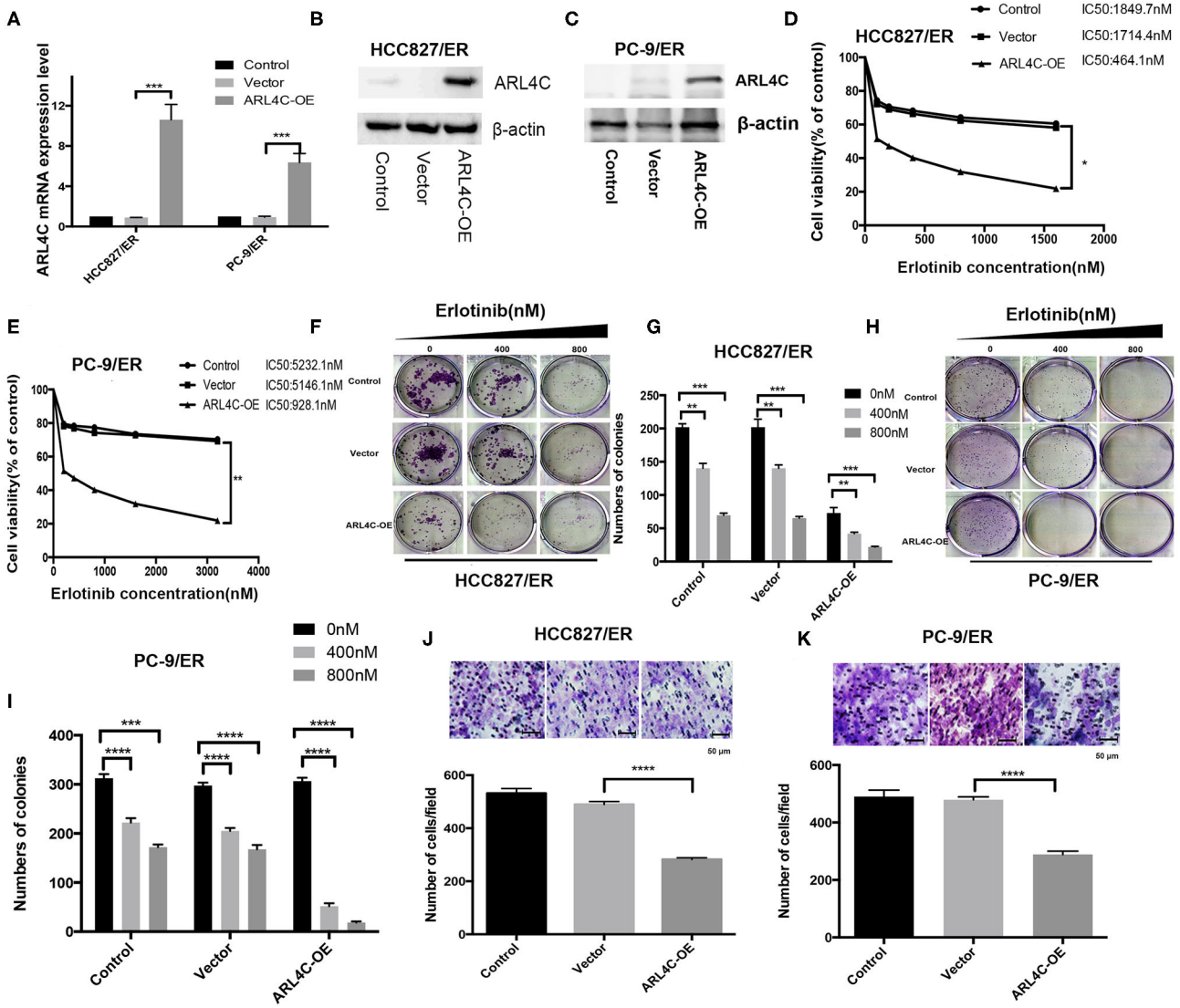

FIGURE 3 | *ARL4C* overexpression decreases Erlotinib resistance of lung cancer cells. **(A)** Left: qPCR quantification of *ARL4C* expression in HCC827/ER ARL4C-overexpressing (HCC827/ER-ARL4C-OE), HCC827/ER-Vector (HCC827/ER-Vector), and HCC827/ER (control) cells. Right: qPCR quantification of *ARL4C* expression in PC-9/ER ARL4C-overexpressing (PC-9/ER-ARL4C-SH), PC-9/ER-ARL4C-control (PC-9/ER-Vector), and PC-9/ER (control) cells. **(B)** Western blotting detection of *ARL4C* expression in HCC827/ER-ARL4C-OE, HCC827/ER-Vector, and HCC827/ER (control) cells. **(C)** Western blotting detection of *ARL4C* expression in PC-9/ER-ARL4C-OE, PC-9/ER vector, and PC-9/ER (control) cells. **(D,E)** Erlotinib IC50 for HCC827/ER and PC-9 /ER cells ARL4C overexpressing with HBLV-ARL4C-SH3 and Vector measured by MTS. **(F,G)** Colony formation of HCC827/ER-ARL4C-OE, HCC827/ER-Vector, and HCC827/ER (control) cell. **(H,I)** Colony formation of PC-9/ER-ARL4C-OE, PC-9/ER-vector, and PC-9/ER (control) cells. **(J)** Transwell assay of the migration of HCC827/ER-ARL4C-OE, HCC827/ER-Vector, and HCC827/ER (control) cells. **(K)** Transwell assay of the migration of PC-9/ER-ARL4C-OE, PC-9/ER-vector, and PC-9/ER (control) cells. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

HBLV-ARL4C-shrna1s significantly increased the expression of *β-catenin* in HCC827 cells. Furthermore, the expression of *β-catenin*-regulated target genes, *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7*, was also significantly increased in *ARL4C* shRNA transfected cells (**Figures 4A,B**, $P < 0.05$). In contrast, overexpression of *ARL4C* significantly suppressed the expression of β-catenin as well as β-catenin-regulated target genes, *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7*, in HCC827/ER cells (**Figures 4C,D**, $P < 0.01$; $P < 0.001$).

To further confirm the relationship between *ARL4C* and *β-catenin*, a rescue assay was performed by overexpressing *β-catenin* in HCC827/ER/ARL4C-OE. qPCR and Western blotting showed that the expression level of β-catenin in HCC827/ER/ARL4C-OE/CTNNB1-OE was significantly higher than that in HCC827/ER/ARL4C-OE (**Figures 4E,F**, $P < 0.05$ and $P < 0.0001$). Moreover, the expression of ARL4C-inhibited β-catenin-target genes *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7* was increased (**Figure 4G**, $P < 0.01$, $P < 0.001$, and

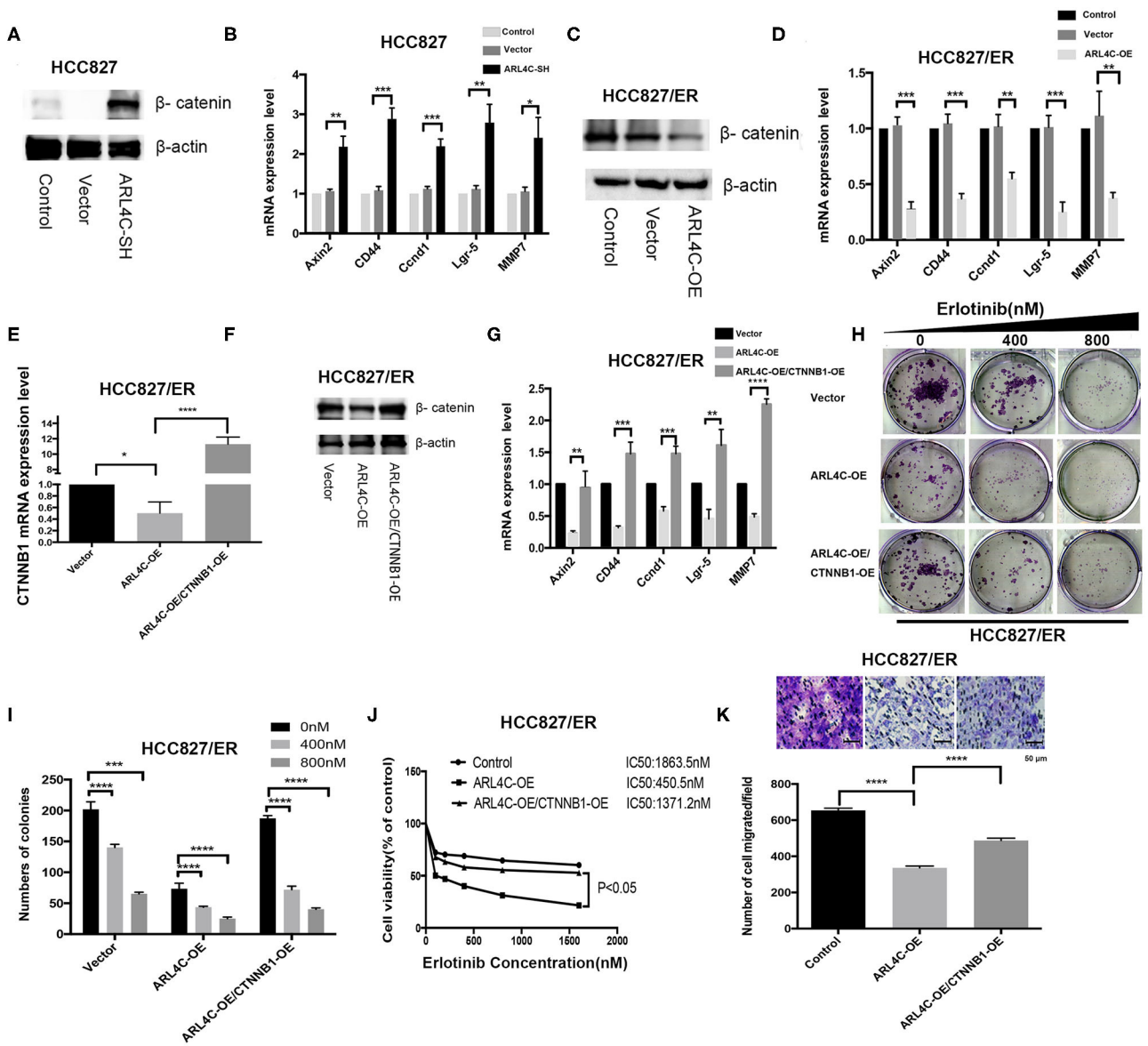

FIGURE 4 | Enhancement of *β-catenin* is critical for *ARL4C* to reduce TKI Erlotinib resistance. **(A)** Western blot showed β-catenin protein expression in HCC827-ARL4C-SH, HCC827-Vector, and HCC827 (control) cells. **(B)** The expression of *β-catenin*-target genes *Axin*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7* in HCC827-ARL4C-SH, HCC827-Vector, and HCC827 (control) cells detected by real-time PCR. **(C)** Detection of β-catenin protein expression in HCC827/ER-ARL4C-OE, HCC827/ER-Vector, and HCC827/ER (control) cells by Western blot. **(D)** The expression of *β-catenin*-target genes *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7* in HCC827/ER-ARL4C-OE, HCC827/ER-Vector, and HCC827/ER (control) cells measured by real-time PCR. **(E,F)** *β-catenin* (*CTNNB1*) expression in HCC827/ER/ARL4C-OE, HCC827/ER/ARL4C-OE–vector, and HCC827/ER/ARL4C-OE-CTNNB1-OE in the rescue assay analyzed by real-time PCR and Western blot. **(G)** Real-time PCR quantified the expression of *β-catenin*-target genes, *Axin2, CD44, Ccnd1, Lgr-5*, and *MMP7* in HCC827/ER/ARL4C-OE, HCC827/ER/ARL4C-OE–vector, and HCC827/ER/ARL4C-OE-CTNNB1-OE. **(H,I)** Colony formation of HCC827/ER/ARL4C-OE, HCC827/ER/ARL4C-OE–vector, and HCC827/ER/ARL4C-OE-CTNNB1-OE. **(J)** Erlotinib IC50 for HCC82/ER/ARL4C-OE, HCC827/ER/ARL4C-OE–vector, and HCC827/ER/ARL4C-OE-CTNNB1-OE measured by MTS assay. **(K)** Transwell assays on the migration of HCC827/ER/ARL4C-OE, HCC827/ER/ARL4C-OE–vector, and HCC827/ER/ARL4C-OE-CTNNB1-OE. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

$P < 0.0001$), which was positively correlated to the expression of *β-catenin*.

In clonogenic assays, it was found that the overexpression of *β-catenin* significantly increased the proliferation of ARL4C-overexpressed HCC827/ER cells when treated with Erlotinib (**Figures 4H,I**, $P < 0.001$ and $P < 0.0001$). More importantly, the overexpression of *β-catenin* by lentivirus significantly decreased ARL4C-induced Erlotinib sensitivity in HCC827/ER cells. IC50 values of Erlotinib for HCC827/Vector, HCC827/ER/ARL4C-OE, and HCC827/ER/ARL4C-OE/CTNNB1-OE were 1863.5,

450.5, and 1371.2 nM, respectively (**Figure 4J**, $P < 0.05$). Transwell migration assay also showed that the overexpression of *β-catenin* increased the migration of *ARL4C*-overexpressed HCC827/ER cells (**Figure 4K**, $P < 0.0001$). These results suggest that *β-catenin* has an opposite effect to *ARL4C* on the development of TKI Erlotinib resistance of NSCLC cells.

ARL4C Regulates *β-Catenin* Expression Through *JAK2/STAT5A* Signal Activation

Previous studies showed that several relevant downstream signals, such as *ERK*, *JAK*, and *AKT*, might be involved in tumor-promoting effects of *ARL4C* (14). To further explore potential mechanisms related to the acquired TKI resistance, phosphorylation levels of *JAK* were examined in *ARL4C* knockdown-HCC827 cells. Results showed that the phosphorylation level of *JAK2* was significantly increased, compared to that in control cells (**Figure 5A**). Consistently, the overexpression of *ARL4C* significantly decreased the phosphorylation of *JAK2* in HCC827/ER cells, compared to that in control cells (**Figure 5B**). The *JAK-STAT* signaling pathway not only regulates tumor development but also is closely related to TKI resistance (14). These results indicated that *ARL4C* abnormal expression affected the phosphorylation of *JAK2*.

We further studied if *ARL4C* regulated *STAT5A*. In HCC827 cells, the knockdown of *ARL4C* upregulated the phosphorylation of *STAT5A* as detected by Western blot analysis (**Figure 5C**). In contrast, the overexpression of *ARL4C* in HCC827/ER cells downregulated the phosphorylation of *STAT5A* (**Figure 5D**).

Chromatin immunoprecipitation showed that the overexpression of *ARL4C* resulted in the loss of the promoter of *STAT5* binding to *CTNNB1* (**Figure 5E**). We found that luciferase expression directed by a 200-bp fragment of the *β-catenin* promoter containing this *STAT5A* site was increased in *ARL4C* downregulated HEK293T cell (**Figure 5F**). Similarly, downregulation of *ARL4C* resulted in a significant enhancement of *β-catenin* promoter activity in HCC827 cells (**Figure 5G**). These results indicated that the *β-catenin* promoter contained a conserved *STAT5A* binding site. *STAT5A* acts as a transcription factor in regulating the mRNA expression of *β-catenin* by binding and activating the β-catenin promoter.

These data suggest that the regulation of *β-catenin* expression by *ARL4C* might be through the *JAK2/STAT5A* signal pathway in the regulating TKI resistance of NSCLC.

Detection of *EGFR* Mutations

To explore the association between *EGFR* mutation and *ARL4C*, *EGFR* mutations in 42 NSCLC paraffin samples were detected with amplification refractory mutation system (ARMS) with human *EGFR* Mutations Detection kit. Among 42, 20 were found with *EGFR* mutations and only 1 had a T790M mutation, while the other 21 had no *EGFR* mutation (in **Supplementary Table 2**). In these 42 tissues, mRNA expression level of ARL4C in the EGFR mutation group was -0.064 ± 0.091 (lgRQ, $RQ = 2^{-\Delta\Delta Ct}$), while the level in the group without EGFR mutation was -0.023 ± 0.099, indicating that the expression of *ARL4C* was not associated with *EGFR* gene mutations (**Figure 6C**, $P = 0.7668$).

NGS was used to detect the *EGFR* mutation in eight cell lines (HCC827, HCC827/ER, HCC827/ER/ARL4C-OE, HCC827/ER/ARL4C-OE/CTNNB1-OE, PC-9, PC-9/ER, PC-9/ER/ARL4C-OE, and PC-9/ER/ARL4C-OE/CTNNB1-OE) with different levels of *ARL4C* expression. Results (**Figures 6A,B**, **Supplementary Table 3**) showed that there was a high frequency of sensitive mutation (exon19:c.2236_2250del:p.746_750del) and no T790M and C797S mutations associated with Erlotinib-induced drug-resistant cells of HCC827/ER and PC-9/ER was found. There was a high frequency of synonymous mutation (exon20:c.G2361A:p.Q787Q) in HCC827, and no new mutation was caused by the overexpression of *ARL4C* in HCC827/ER and PC-9/ER or by the overexpression of *CTNNB1* in HCC827/ER/ARL4C-OE and PC-9/ER/ARL4C-OE.

DISCUSSION

In this study, the underlying mechanism by which the resistance of Erlotinib, an *EGFR*-TKI, in NSCLC was explored. The study revealed for the first time that *ARL4C* was downregulated and *β-catenin* was upregulated in Erlotinib-resistant cells via *JAK2/STAT5A* signaling pathway. By manipulating the expression of these two genes (overexpression or knocking down expression), the study provided evidences that the inhibition of *ARL4C* selectively enhanced the resistance of HCC827 and PC-9 cells to Erlotinib, while the overexpression of *ARL4C* enhanced the sensitivity of the cancer cells to the drug. The regulation of the drug resistance of lung cancer cells by *ARL4C* was through activating the *β-catenin/JAK2/STAT5A* signaling pathway. These data indicate for the first time that ARL4C plays an important role in the resistance of NSCLC cells to Erlotinib.

Several mechanisms that mediate TKI resistance have been identified: (A) *EGFR* exon 20 T790M mutation (14); (B) *Met* gene amplification (15); (C) *HER2* gene amplification (16); and (D) *PIK3CA* mutation (4), which counts for the majority of gene mutations responsible for the TKI resistance. However, one third of causes of the TKI resistance remains unknown. Our data (**Figure 6**) showed that Erlotinib-induced *ARL4C* reduction did not associate with any currently known mutations of *EGFR*, indicating that *ARL4C* is an independent factor for the development of Erlotinib resistance.

EGFR path is one of the major oncogenic pathways via *Ras/Raf/MEK/ERK* signaling to promote the cancer cell proliferation, migration, and invasion. When the *EGFR* path is blocked by Erlotinib, the cancer cells have to develop another oncogenic path for their continuous survival. Our data supported that the downregulation of *ARL4C* upregulated *β-catenin* via activation of *JAK2/STAT5*, which could help the *EGFR*-path-blocked cancer cells to regain their malignant behaviors (**Figure 6D**).

ARL4C is a member of the ADP-ribosylation factor family of GTP-binding proteins. The abnormal expression of *ARL4C* possesses carcinogenic effect on many types of tumors (17). Recent studies have indicated that *ARL4C* could be induced by

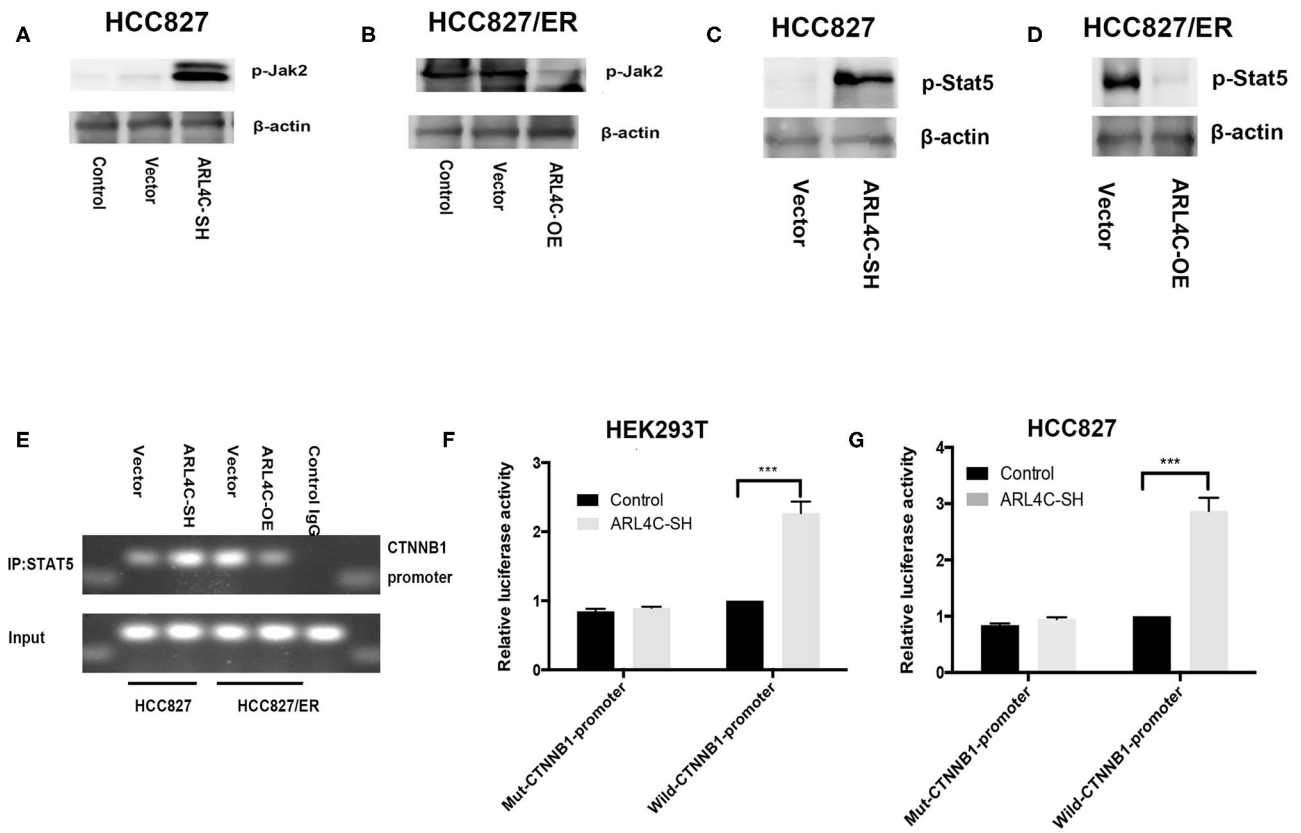

FIGURE 5 | *ARL4C* regulates β-catenin expression through the *JAK2/STAT5A* signal pathway. **(A–D)** The expression of p-JAK2 in HCC827-ARL4C-SH, HCC827-Vector, and HCC827 (control) cells **(A)** and in HCC827/ER-ARL4C-OE, HCC827/ER-Vector, and HCC827/ER (control) cells **(B)** detected by Western blot. *STAT5* protein expression in HCC827-ARL4C-SH, HCC827-Vector cells **(C)** and in HCC827/ER-ARL4C-OE, HCC827/ER-Vector cells **(D)** also detected by Western blot. **(E)** The enrichment of the *β-catenin* (*CTNNB1*) promoter in immunoprecipitated (IP) *STAT5* and the input of *STAT5* in HCC827/ER-ARL4C-OE, HCC827/ER-Vector, HCC827-ARL4C-SH, and HCC827-Vector and followed by Western blot. Unrelavant IgG was used as a negative control staining. **(F,G)** Luciferase activity in HEK293T cells **(F)** and HCC827 cells **(G)** co-transfected with pGL3-CTNNB1-Luc or mut-pGL3-CTNNB1-Luc and HBLV-ARL4C-SH3 by the Dual-Luciferase Reporter Assay System, $^{***}P < 0.001$.

EGF and promotes the motility of cancer cells by remodeling actin cytoskeleton through *Arf6* activation (18), which might be involved in the migration, invasion, and proliferation of cancer cells (18). Consistent with this, our data showed that when *EGF* path was blocked by Erlotinib, the *ARL4C* was reduced, which triggered the upregulation of the *JAK2/STAT5/β-catenin* path to replace the *EGFR* oncogenic signal path. Similarly, our data supported the idea that the inhibition of *ARL4C* in wild-type HCC827 and PC-9 cells via sh-RNA could selectively enhance the Erlotinib tolerance, while the overexpression of *ARL4C* could enhance the Erlotinib sensitivity. The results indicate that *ARL4C* could be a "switch" between the *EGFR* path and the *JAK2/STAT5/β-catenin* path to maintain the proliferation, migration, and invasion of cancer cells when cells are exposed to Erlotinib and *EGFR* path is blocked.

It is well-known that *Wnt/β-catenin* signaling is a classic pathway with a crucial role in NSCLC progression. *β-catenin* is a key component in the *Wnt* signaling cascade and is involved in cancer cell proliferation and tumor progression (19). In the present study, a significant upregulation of *β-catenin* was observed in Erlotinib-resistant HCC827/ER and PC-9/ER cells compared with their parental cells HCC827 and PC-9. The result is in accordance with a previous observation that Wnt/β-catenin not only participates in the proliferation, invasion, and metastasis of tumor cells but also induces drug resistance (20). Nuclear accumulation of *β-catenin* was associated with *EGFR* mutations (21) and *β-catenin* overexpression was associated with NSCLC cell resistance to gefitinib (20). *DDX17* nucleocytoplasmic shuttling promotes acquired gefitinib resistance in NSCLC cells via activation of *β-catenin* (22). *Rab25* promotes Erlotinib resistance by activating the *β1 integrin/AKT/β-catenin* pathway in NSCLC (23). Alone this line, our finding added another path, *ARL4C/JAK2/STAT5/β-catenin*, as a new means for cancer cells to escape the survival inhibition by Erlotinib. Our data showed that in the presence of Erlotinib, the overexpression of *β-catenin* significantly increased the proliferation and decreased Erlotinib sensitivity in HCC827/ER-OE and PC-9/ER-OE (**Figures 4H–K**). Thus, *ARL4C*-induced Erlotinib resistance was via *β-catenin* signaling.

It is well-known that the *JAK-STAT* signaling pathway serves a crucial role in cell immunity, division, death, and tumor formation (24). The two families involved in this pathway are

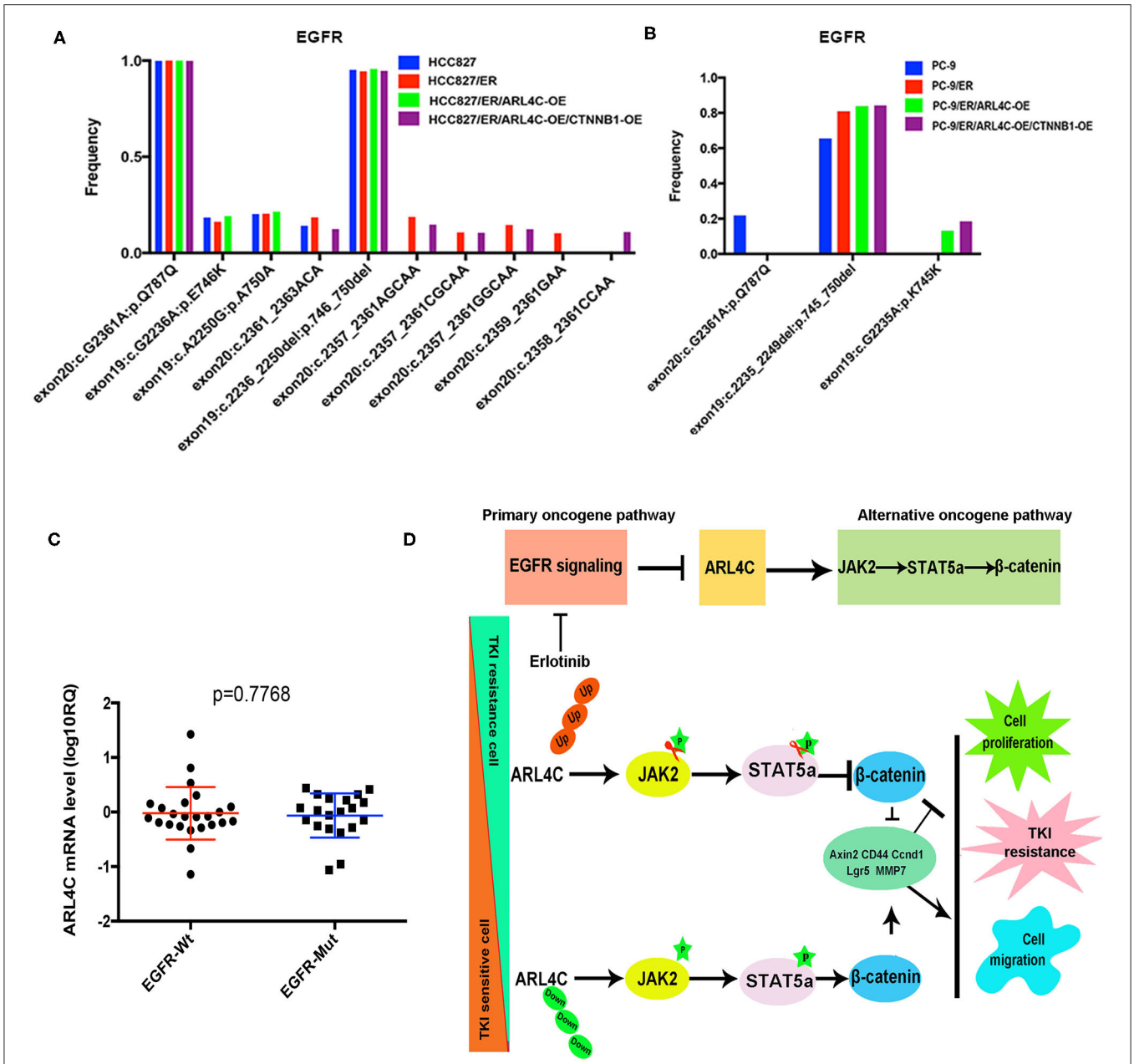

FIGURE 6 | *ARL4C* alteration is not associated with known-mutated *EGFR* genes but affects *JAK/STAT/β-Catenin* path to stimulate the malignant behaviors of NSCLC cells: EGFR mutations were detected by ARMS with human *EGFR* Mutations Detection kit in 42 NSCLC samples. Sensitive *EGFR* mutations: Exon19:c.2236_2250del: p.746_750del; Synonymous mutation: Exon20:c.G2361A:p.Q787Q. **(A)** No differences of *EGFR* mutations among HCC827, HCC827/ER, HCC827/ER/ARL4C-OE, and HCC827/ER/ARL4C-OE /CTNNB1-OE cell lines. **(B)** No differences of EGFR mutations among PC-9, PC-9/ER, PC-9/ER/ARL4C-OE, and PC-9/ER/ARL4C-OE/CTNNB1-OE cell lines. **(C)** Distribution of ARL4C mRNA levels in NSCLC patients with EGFR mutations and without EGFR mutation(s). Bold lines represent the mean value for each patient cohort, RQ = $2^{-\Delta\Delta Ct}$. Similar expression level of *ARL4C* was found in 42 cases of NSCLC tissues, regardless of their *EGFR* mutation status. **(D)** A graphic illustration of the role of *ARL4C* in Erlotinib resistance. In TKI-resistant *ARL4C*-reduced NSCLC cells, the overexpression of *ARL4C* inhibited the expression of *β-catenin* by phosphorylation of *JAK/STAT*, resulting in an inhibitory effect on TKI resistance, cell proliferation, and migration, which was related to *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7*. Likewise, in the downregulation of *ARL4C* in sensitive cell lines, the results were vice versa.

Janus kinases (*JAK*s) and signal transducer and activator of transcription proteins (*STAT*s), encoded by the genes *JAK* (*JAK1*, *JAK2*, *JAK3*, and *TYK2*) and *STAT* (*STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5A*, *STAT5B*, and *STAT6*), respectively (25). The continuous activation of *JAK/STAT* could promote malignant transformation of cells, leading to the development of cancers including NSCLC (21). The inappropriate activation of *Stat3* or *IL-7*-mediated *IL-7R-JAK3/STAT5* pathway is associated with an unfavorable prognosis in NSCLC patients and correlated with chemoresistance and radioresistance (24). Inhibition of the

JAK/STAT3 signaling pathway has therefore been recognized as a promising therapeutic strategy for NSCLC (25). In addition, it was reported that (1) the inhibition of *gp130-Jak-Stat3* signaling could partially inhibit *Wnt–β-catenin*–mediated intestinal tumor growth and regeneration (26); (2) *Stat3* and *β-catenin* were involved in tumorigenesis (27). By using chromatin immunoprecipitation and luciferase reporter assays, this study revealed for the first time that the promoter region of *β-catenin* contained a conserved *STAT5A* binding site. *STAT5A* acted as a transcription factor regulating the mRNA expression of *β-Catenin* by binding to and activating the *β-catenin* promoter, forming *ARL4C/JAK2/STAT5/β-catenin* axis for the survival of Erlotinib-resistant cells.

Our data also supported that the biofunctions of *ARL4C/JAK2/STAT5/β-catenin* axis were carried out by a set of molecules, such as *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7*. *Axin2* is responsible for the stability of *β-catenin* (28); *CD44* is a famous stem cell marker and adhesion molecule to enhance the proliferation, migration, and invasion (29); *Ccnd1* enhances the proliferation of cancer cells via promoting the cell's G1/S transition (30); *Lgr-5* is a biomarker for stem cells, involved in tumor development (31). *MMP7* promotes the migration and invasion of cancer cells (32). All these functional molecules were upregulated in Erlotinib-resistant cells when *ARL4C* was suppressed and *JAK2/STAT5/β-catenin* axis was activated (**Figure 6D**), serving as executors for the malignant behaviors when the *EGFR* path was blocked in NSCLC cells.

The Erlotinib stress-related *ARL4C/JAK2/STAT5/β-catenin* axis and the downstream *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7* could be therapeutic targets when considering how to reverse Erlotinib resistance and how to enhance the Erlotinib killing effects (**Figure 6D**).

CONCLUSION

This study demonstrated for the first time that the *ARL4C/JAK2/STAT5/β-catenin* axis and their downstream molecules *Axin2*, *CD44*, *Ccnd1*, *Lgr-5*, and *MMP7* could help to bypass *EGFR* oncogenic path and serve as an alternative new signal/function path to maintain the survival and malignant behaviors for HCC827 and PC9 NSCLC cells under the stress of Erlotinib.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: NCBI BioProject (PRJNA648039) (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA648039).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of the Fujian Provincial Tumor Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL, LZ, ZY, and YS designed the study, analyzed data, and wrote the manuscript. ZY, DH, and ZH collected and analyzed the NSCLC lung tissues for *ARL4C* expression level. JL, ZC, and CZ performed all the experiments, collected data, and created the figures. TH carried out the bioinformatics and statistical analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.585292/full#supplementary-material

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* (2020) 70:7–30. doi: 10.3322/caac.21590
2. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature.* (2018) 553:446–54. doi: 10.1038/nature25183
3. Wu SG, Shih JY. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non-small cell lung cancer. *Mol Cancer.* (2018) 17:38. doi: 10.1186/s12943-018-0777-1
4. Andrews Wright NM, Goss GD. Third-generation epidermal growth factor receptor tyrosine kinase inhibitors for the treatment of non-small cell lung cancer. *Transl Lung Cancer Res.* (2019) 8(Suppl. 3):S247–64. doi: 10.21037/tlcr.2019.06.01

5. Majem M, Remon J. Tumor heterogeneity.evolution through space and time in EGFR mutant non small cell lung cancer patients. *Transl Lung Cancer Res.* (2013) 2:226–37. doi: 10.3978/j.issn.2218-6751.2013.03.09
6. Berger AH, Brooks AN, Wu X, Shrestha Y, Chouinard C, Piccioni F, et al. High-throughput phenotyping of lung cancer somatic mutations. *Cancer Cell.* (2016) 30:214–28. doi: 10.1016/j.ccell.2016.06.022
7. Matsumoto S, Fujii S, Kikuchi A. Arl4c is a key regulator of tubulogenesis and tumourigenesis as a target gene of Wnt-beta-catenin and growth factor-Ras signalling. *J Biochem.* (2017) 161:27–35. doi: 10.1093/jb/mvw069
8. Su D, Katsaros D, Xu S, Xu H, Gao Y, Biglia N, et al. ADP-ribosylation factor-like 4C (ARL4C), a novel ovarian cancer metastasis suppressor, identified by integrated genomics. *Am J Transl Res.* (2015) 7:242–56.
9. Chen X, Su Z, Wang S, Xu H. Clinical and prognostic significance of Arl4c expression in colorectal cancer. *Cancer Biomark.* (2016) 16:253–7. doi: 10.3233/CBM-150562
10. Kimura K, Matsumoto S, Harada T, Morii E, Nagatomo I, Shintani Y, et al. ARL4C is associated with initiation and progression of lung adenocarcinoma and represents a therapeutic target. *Cancer Sci.* (2020) 111:951–61. doi: 10.1111/cas.14303
11. Yuan JS, Reed A, Chen F, Stewart CN Jr. Statistical analysis of real-time PCR data. *BMC Bioinform.* (2006) 7:85. doi: 10.1186/1471-2105-7-85
12. Lin XD, Chen SQ, Qi YL, Zhu JW, Tang Y, Lin JY. Overexpression of thrombospondin-1 in stromal myofibroblasts is associated with tumor growth and nodal metastasis in gastric carcinoma. *J Surg Oncol.* (2012) 106:94–100. doi: 10.1002/jso.23037
13. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med.* (2009) 361:947–57. doi: 10.1056/NEJMoa0810699
14. Lu X, Yu L, Zhang Z, Ren X, Smaill JB, Ding K. Targeting EGFR(L858R/T790M) and EGFR(L858R/T790M/C797S) resistance mutations in NSCLC: current developments in medicinal chemistry. *Med Res Rev.* (2018) 38:1550–81. doi: 10.1002/med.21488
15. Wang Q, Yang S, Wang K, Sun SY. MET inhibitors for targeted therapy of EGFR TKI-resistant lung cancer. *J Hematol Oncol.* (2019) 12:63. doi: 10.1186/s13045-019-0759-9
16. de Langen AJ, Jebbink M, Hashemi SMS, Kuiper JL, de Bruin-Visser J, Monkhorst K, et al. Trastuzumab and paclitaxel in patients with EGFR mutated NSCLC that express HER2 after progression on EGFR TKI treatment. *Br J Cancer.* (2018) 119:558–64. doi: 10.1038/s41416-018-0194-7
17. Fujii S, Matsumoto S, Nojima S, Morii E, Kikuchi A. Arl4c expression in colorectal and lung cancers promotes tumorigenesis and may represent a novel therapeutic target. *Oncogene.* (2015) 34:4834–44. doi: 10.1038/onc.2014.402
18. Harada T, Matsumoto S, Hirota S, Kimura H, Fujii S, Kasahara Y, et al. Chemically modified antisense oligonucleotide against ARL4C inhibits primary and metastatic liver tumor growth. *Mol Cancer Ther.* (2019) 18:602–12. doi: 10.1158/1535-7163.MCT-18-0824
19. Stewart DJ. Wnt signaling pathway in non-small cell lung cancer. *J Natl Cancer Inst.* (2014) 106:djt356. doi: 10.1093/jnci/djt356
20. Cao S, Wang Z, Gao X, He W, Cai Y, Chen H, et al. FOXC1 induces cancer stem cell-like properties through upregulation of beta-catenin in NSCLC. *J Exp Clin Cancer Res.* (2018) 37:220. doi: 10.1186/s13046-018-0894-0
21. Suzuki M, Shigematsu H, Nakajima T, Kubo R, Motohashi S, Sekine Y, et al. Synchronous alterations of Wnt and epidermal growth factor receptor signaling pathways through aberrant methylation and mutation in non small cell lung cancer. *Clin Cancer Res.* (2007) 13:6087–92. doi: 10.1158/1078-0432.CCR-07-0591
22. Li K, Mo C, Gong D, Chen Y, Huang Z, Li Y, et al. DDX17 nucleocytoplasmic shuttling promotes acquired gefitinib resistance in non-small cell lung cancer cells via activation of beta-catenin. *Cancer Lett.* (2017) 400:194–202. doi: 10.1016/j.canlet.2017.02.029
23. Wang J, Zhou P, Wang X, Yu Y, Zhu G, Zheng L, et al. Rab25 promotes erlotinib resistance by activating the beta1 integrin/AKT/beta-catenin pathway in NSCLC. *Cell Prolif.* (2019) 52:e12592. doi: 10.1111/cpr.12592
24. Groner B, von Manstein V. Jak Stat signaling and cancer: opportunities, benefits and side effects of targeted inhibition. *Mol Cell Endocrinol.* (2017) 451:1–14. doi: 10.1016/j.mce.2017.05.033
25. Chai EZ, Shanmugam MK, Arfuso F, Dharmarajan A, Wang C, Kumar AP, et al. Targeting transcription factor STAT3 for cancer prevention and therapy. *Pharmacol Ther.* (2016) 162:86–97. doi: 10.1016/j.pharmthera.2015.10.004
26. Phesse TJ, Buchert M, Stuart E, Flanagan DJ, Faux M, Afshar-Sterle S, et al. Partial inhibition of gp130-Jak-Stat3 signaling prevents Wnt-beta-catenin-mediated intestinal tumor growth and regeneration. *Sci Signal.* (2014) 7:ra92. doi: 10.1126/scisignal.2005411
27. Wu J, Keng VW, Patmore DM, Kendall JJ, Patel AV, Jousma E, et al. Insertional Mutagenesis Identifies a STAT3/Arid1b/beta-catenin Pathway Driving Neurofibroma Initiation. *Cell Rep.* (2016) 14:1979–90. doi: 10.1016/j.celrep.2016.01.074
28. Song X, Wang S, Li L. New insights into the regulation of Axin function in canonical Wnt signaling pathway. *Protein Cell.* (2014) 5:186–93. doi: 10.1007/s13238-014-0019-2
29. Bhattacharya R, Mitra T, Ray Chaudhuri S, Roy SS. Mesenchymal splice isoform of CD44 (CD44s) promotes EMT/invasion and imparts stem-like properties to ovarian cancer cells. *J Cell Biochem.* (2018) 119:3373–83. doi: 10.1002/jcb.26504
30. Gennaro VJ, Stanek TJ, Peck AR, Sun Y, Wang F, Qie S, et al. Control of CCND1 ubiquitylation by the catalytic SAGA subunit USP22 is essential for cell cycle progression through G1 in cancer cells. *Proc Natl Acad Sci USA.* (2018) 115:E9298–307. doi: 10.1073/pnas.1807704115
31. Choi YJ, Kim N, Lee HS, Park SM, Park JH, Yoon H, et al. Expression of leucine-rich repeat-containing G-protein coupled receptor 5 and CD44: potential implications for gastric cancer stem cell marker. *J Cancer Prevent.* (2016) 21:279–87. doi: 10.15430/JCP.2016.21.4.279
32. Han B, Zhou B, Qu Y, Gao B, Xu Y, Chung S, et al. FOXC1-induced non-canonical WNT5A-MMP7 signaling regulates invasiveness in triple-negative breast cancer. *Oncogene.* (2018) 37:1399–408. doi: 10.1038/s41388-017-0021-2

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Efficacy and Tolerability of Erlotinib 100 mg/d vs. Gefitinib 250 mg/d in EGFR-Mutated Advanced Non-small Cell Lung Cancer (E100VG250): An Open-Label, Randomized, Phase 2 Study

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Background: Erlotinib-based combination therapy leads to increased efficacy but also toxicity for EGFR-mutated NSCLC. Reducing the dose of erlotinib could improve treatment tolerability, but few evidences are available regarding its efficacy at reduced dose. This randomized phase-2 study intends to compare the efficacy and tolerability between lower dose erlotinib (100 mg/d) and standard dose gefitinib (250 mg/d) in EGFR-mutated NSCLC.

Methods: Patients with EGFR-mutated advanced NSCLC were randomized at 1:1 ratio to receive erlotinib 100 mg/d or gefitinib 250 mg/d until disease progression or unacceptable toxicity. The primary endpoint was disease control rate (DCR).

Results: Between April 2013 and September 2018, 171 patients were randomized to receive erlotinib (n = 85) and gefitinib (n = 86); 74 in the erlotinib group and 83 in the gefitinib group were include in analysis. DCR with erlotinib and gefitinib were 91% [95% CI 81.7–95.3] and 93% [85.1–96.6], respectively (P = 0.613). Response rate was 62% [50.8–72.4] in the erlotinib group and 53% [42.4–63.4] in the gefitinib group (P = 0.247). No significant difference was observed between erlotinib and gefitinib in median progression-free survival [10.1 vs. 11.3 months, HR = 1.295 [0.893–1.879], P = 0.171] and median overall survival [26.6 vs. 28.7 months, HR = 0.999 [0.637–1.569], P = 0.998]. Subgroup analyses by line of treatment, EGFR subtypes and status of central nervous system (CNS) metastasis found similar results. More toxicity [any-grade, 80 [96%] vs. 66 [89]; grade 3–4, 11 [13%] vs. 4 [5%]] and toxicity-related discontinuation [10 [12%] vs. 3 [4%]] occurred with gefitinib compared with erlotinib. But no significant difference was observed.

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Conclusion: Lower dose erlotinib (100 mg/d) achieved comparable efficacy compared with standard dose gefitinib (250 mg/d) in EGFR-mutated NSCLC.

Clinical Trial Registration: https://clinicaltrials.gov, identifier: NCT01955421.

Keywords: non-small cell lung cancer, randomized controlled trial, lower dose, erlotinib, gefitinib, EGFR mutation

INTRODUCTION

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are standard first-line treatment for EGFR mutation-driven non-small cell lung cancer (NSCLC) (1). Median progression-free survival (PFS) was 10–13 months with first-generation TKIs (gefitinib and erlotinib), 14.7 months with second-generation TKI (dacomitinib), and 18.9 months with third-generation agent (osimertinib) (2–7). Given that most EGFR-driven NSCLC patients fail to benefit from the recent advance in immunotherapy, treatment options after the exhaustion of targeted therapy are highly limited (8). Therefore, it remains a crucial need to develop EGFR TKI-based combination therapies that can optimize tumor control and delay disease progression (9–11). In the recent RELAY trial, the combination of erlotinib and ramucirumab yielded an unprecedented median PFS of 19.4 months, accompanied by a 72% incidence of grade 3–4 treatment-related adverse events (10).

Reducing the dose of erlotinib, which is now approved at its maximal tolerated dose (MTD) 150 mg/d (12), may improve the tolerability of combination therapy. However, data regarding the efficacy of lower dose erlotinib are limited and mutually contradictory. Preclinical models and phase-1 pharmacokinetic data suggested that erlotinib 25 mg/d led to similar antitumor effect compared with gefitinib 250 mg/d (12, 13). Retrospective studies also supported this notion by showing similar PFS between patients treated with reduced-dose erlotinib ($\leq$ 100 mg/d) and those with standard dose (14). *Post-hoc* analyses that might be subjected to survival bias found a correlation between dose reduction of EGFR TKIs and better treatment outcomes (15, 16). A single-arm phase-2 trial showed 50 mg/d erlotinib achieved an objective response rate of 60% in elderly or frail patients (17). Nevertheless, another single-arm, prospective study reported contradictory findings, where no objective response was observed in patients treated with erlotinib 50 mg/d (18).

There has been no prospective, randomized controlled trial (RCT) directly comparing lower dose erlotinib with standard dose erlotinib or gefitinib in EGFR-mutated advanced NSCLC. Therefore, to properly addressed this problem, we designed this randomized, phase-2 study comparing the efficacy and tolerability of erlotinib 100 mg/d vs. gefitinib 250 mg/d in patients with EGFR-mutated advanced NSCLC.

PATIENTS AND METHODS

Study Design and Patients

This is an open-label, randomized, phase-2 study to compare the efficacy and tolerability of erlotinib 100 mg/d vs. gefitinib 250 mg/d in patients with EGFR-mutated, advanced NSCLC.

Eligibility criteria were aged at least 18 years; histologically or cytologically confirmed stage IIIB/IV NSCLC defined by American Joint Committee on Cancer (AJCC) staging criteria (version 7); stage IIIB had no indication for curative treatment; harbored EGFR exon 19 or 21 sensitizing mutations detected by direct sequencing or Amplification Refractory Mutation System (ARMS); measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (19); an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; adequate bone marrow, liver and kidney function; no prior exposure to EGFR TKIs, able to swallow tablets and resolution to grade 1 or less adverse events due to any previous anticancer treatment. Patients with EGFR T790M mutations, clinically unstable CNS metastasis (symptomatic, or needed treatment within 4 weeks, or pia mater disease), clinically relevant cardiovascular diseases, history of interstitial lung diseases, other active malignancies or active infectious diseases were excluded.

Study protocol was approved by the ethics committee of Sun Yat-sen University Cancer Center. All patients had provided written informed consent before the study entry. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization guidelines for good clinical practice.

Procedures

Eligible patients were randomly assigned to received erlotinib 100 mg/d or gefitinib 250 mg/d at a 1:1 ratio using an interactive web-response system with a computer-generated random sequence. Patients and investigators were all unmasked to treatment allocation. Treatment could be delayed for up to 2 weeks for recovery from toxicities, and was reintroduced at the same dosage when recover to grade 1 or baseline. Dose modification of was not allowed. Treatment continued until radiographic progression according to RECIST version 1.1, or intolerable toxicity or withdrawal of consent.

Baseline CT scans and brain MRI were mandated for every patient. Tumor assessment by CT scans were performed 4 weeks after randomization, and every 8 weeks after the first assessment. For patients with baseline CNS metastasis, CT scans, and brain MRI were both performed for every assessment. Tumor responses were evaluated by investigators according to RECIST version 1.1. Patients were evaluated for adverse events at every visit. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Treatment adherence was monitored by monthly telephone follow-up.

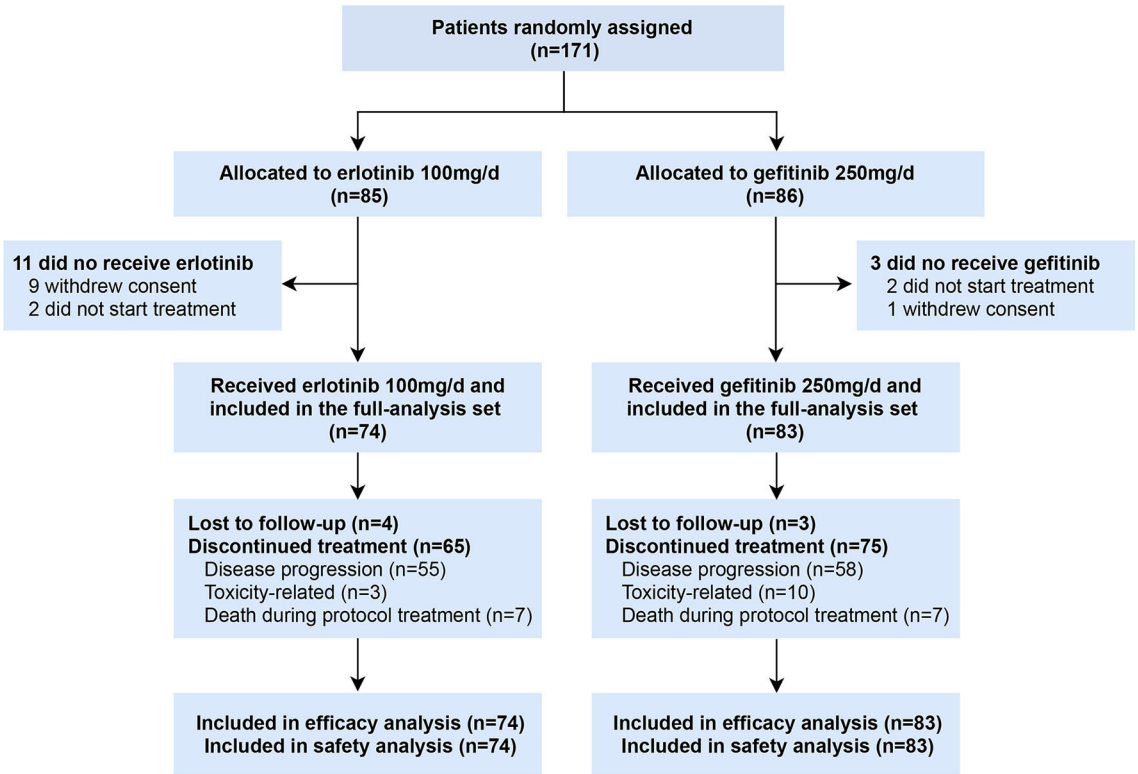

FIGURE 1 | CONSORT flow diagram (Data cutoff date: Sept 30, 2019).

Outcomes

The primary endpoint was disease control rate (DCR) in the full-analysis set, defined as the sum proportion of patients achieving complete responses, or partial responses or stable diseases according to RECIST version 1.1. Secondary endpoints included objective response rate (ORR, the sum proportion of patients achieving complete responses or partial responses), PFS (the time from randomization to disease progression or death from any cause), and overall survival (OS, the time from randomization to date of death from any cause). Prespecified subgroup analyses were planned to evaluate efficacy of erlotinib 100 mg/d in treatment-naïve patients, patients with different EGFR mutations (exon 19 deletions, L858R mutations), and patients with or without baseline CNS metastasis.

Statistical Analysis

This randomized phase-2 trial was designed to investigate the efficacy and tolerability of erlotinib at 100 mg/d compared with gefitinib at 250 mg/d and determine whether it will be useful to proceed to a phase-3 non-inferiority trial. The criteria for proceeding to a phase-3 non-inferiority trial should be that the lower limit of the 95% CI on difference in DCR (i.e., the lower 95% CI for the DCR of erlotinib group minus DCR of gefitinib group) was not more than 12%.We estimated a DCR of 91% for gefitinib 250 mg/d based on the data from WJTOG3405 and ICOGEN (3, 20). Therefore, comparable efficacy could be concluded and a phase-3 non-inferiority trial was warranted if the lower limit of the 95% CI of DCR with erlotinib 100 mg/d was > 79%. At least 71 patients are required in each group to draw a useful conclusion with an 80% statistical power at a two-sided significance level of 5%. Assuming a 12% dropout rate, the estimated sample size was set at 160 patients with 80 patients for each group.

Patient characteristics, tumor responses, and adverse events were compared between the two groups using the χ^2 or Fisher's exact test. Survival was estimated using the Kaplan-Meier method. A two-sided log-rank test was used to compare survival between two treatment groups. Estimates of the treatment effect on survival were summarized as a hazard ratio (HR) for erlotinib vs. gefitinib with a two-sided 95% confidence interval (CI). HR and the corresponding 95% CI were calculated with the Cox proportional hazards regression model. All *P*-values were two-sided. Analyses were conducted using SAS, version 9.3 (SAS Institute, Cary, NC). This study is registered at ClinicalTrials.gov as NCT01955421.

RESULTS

Patients Characteristics

Between April 2013 and September 2018, 171 patients were enrolled, of whom 85 were randomly assigned to receive erlotinib 100 mg/d and 86 to gefitinib 250 mg/d. Ten patients withdrew before initiation of treatment and four patients received other EGFR inhibitors (2 afatinib, 2 icotinib)

TABLE 1 | Baseline patient characteristics of the study.

Patient characteristics	No. of patients (%)			*P*
	Erlotinib (*n* = 74)	Gefitinib (*n* = 83)	All patients (*n* = 157)	
Age, years				0.934
Median (range)	57 (27–77)	56 (32–82)	56 (27–82)	
< 65	61 (82)	68 (82)	129 (82)	
≥ 65	13 (18)	15 (18)	28 (18)	
Sex				0.256
Male	37 (50)	34 (41)	71 (45)	
Female	37 (50)	49 (59)	86 (55)	
ECOG PS				0.422
0–1	70 (95)	81 (98)	151 (96)	
2	4 (5)	2 (2)	6 (4)	
Histology				0.851
Adeno	69 (93)	78 (94)	147 (94)	
Non-adeno[a]	5 (7)	5 (6)	10 (6)	
Disease stage				0.602
IV	72 (97)	82 (99)	154 (98)	
IIIB	2 (3)	1 (1)	3 (2)	
Line of EGFR-TKI				0.774
1st line	55 (74)	60 (72)	115 (73)	
2nd line or beyond[b]	19 (26)	23 (28)	42 (27)	
Prior Surgery				0.828
Yes	15 (20)	18 (22)	33 (21)	
No	59 (80)	65 (78)	124 (79)	
Prior Radiotherapy				0.736
Yes	5 (7)	4 (5)	9 (6)	
No	69 (93)	79 (95)	148 (94)	
Prior Chemotherapy[c]				0.774
Yes	19 (26)	23 (28)	42 (27)	
No	55 (74)	60 (72)	115 (73)	
Baseline CNS metastasis				0.694
Yes	29 (39)	30 (36)	59 (38)	
No	45 (61)	53 (64)	98 (62)	
Baseline Liver metastasis				0.316
Yes	13 (18)	20 (24)	33 (21)	
No	61 (82)	63 (76)	124 (79)	
EGFR mutation				0.593
Exon19 deletion	46 (62)	45 (54)	91 (58)	
L858R mutation	27 (36)	36 (43)	63 (40)	
Others[d]	1 (1)	2 (2)	3 (2)	

ECOG PS, Eastern Cooperative Oncology Group performance status; Adeno, adenocarcinoma; EGFR TKI, epidermal growth factor receptors tyrosine kinase inhibitors; EGFR, epidermal growth factor receptors.

[a] *non-adenocarcinoma included squamous-cell carcinoma (n = 6), large-cell carcinoma (n = 3), and bronchoalveolar carcinoma (n = 1).*

[b] *included four patients in the third-line settings.*

[c] *patients who had received chemotherapy were all treated with at least one cycle of platinum-based doublet chemotherapy.*

[d] *included two patients with L861Q mutations, one patient with G719A mutation.*

TABLE 2 | Response to treatment by RECIST 1.1.

Best Response	No. of Patients (%)											
	Full-analysis set		Treatment-naïve		Exon19 deletion		L858R mutation		With baseline CNS metastasis		No. baseline CNS metastasis	
	Erlotinib (*n* = 74)	Gefitinib (*n* = 83)	Erlotinib (*n* = 55)	Gefitinib (*n* = 60)	Erlotinib (*n* = 46)	Gefitinib (*n* = 45)	Erlotinib (*n* = 27)	Gefitinib (*n* = 36)	Erlotinib (*n* = 29)	Gefitinib (*n* = 30)	Erlotinib (*n* = 45)	Gefitinib (*n* = 53)
Partial response	46 (62)	44 (53)	38 (69)	33 (55)	31 (67)	26 (58)	15 (56)	17 (47)	16 (55)	13 (43)	30 (67)	31 (58)
Stable disease	21 (28)	33 (40)	14 (25)	23 (38)	13 (28)	18 (40)	8 (30)	15 (42)	10 (34)	14 (47)	11 (24)	19 (36)
Progressive disease	7 (9)	6 (7)	3 (5)	4 (7)	2 (4)	1 (2)	4 (15)	4 (11)	3 (10)	3 (10)	4 (9)	3 (6)
ORR	46 (62)	44 (53)	38 (69)	33 (55)	31 (67)	26 (58)	15 (56)	17 (47)	16 (55)	13 (43)	30 (67)	31 (58)
95% CI	(50.8–72.4)	(42.4–63.4)	(56.0–79.7)	(42.5–66.9)	(53.0–79.1)	(43.3–71.0)	(37.3–72.4)	(32.0–63.0)	(37.5–71.6)	(27.3–60.8)	(52.1–78.6)	(45.1–70.7)
P-value	0.247		0.120		0.343		0.513		0.363		0.405	
DCR	67 (91)	77 (93)	52 (95)	56 (93)	44 (96)	44 (98)	23 (85)	32 (89)	26 (90)	27 (90)	41 (91)	50 (94)
95% CI	(81.7–95.3)	(85.1–96.6)	(85.1–98.1)	(84.1–97.4)	(85.5–98.8)	(88.4–99.6)	(67.5–94.1)	(74.7–95.6)	(73.6–96.4)	(74.4–96.5)	(79.3–96.5)	(84.6–98.1)
P-value	0.613		1.000		1.000		0.715		1.000		0.670	

95% CI, 95% confidence interval; ORR, objective response rate; DCR, disease control rate.

(**Figure 1**). A total of 157 patients who received at least one dose of investigated drugs were included in the analysis population (full-analysis set: 74 erlotinib, 83 gefitinib). Baseline demographic and clinicopathological characteristics of patients were balanced between two groups (**Table 1**). Most patients with baseline brain metastasis were asymptomatic and untreated (20 erlotinib, 25 gefitinib). Thirteen patients received brain radiotherapy (8 erlotinib, 5 gefitinib) and one patient in the erlotinib group received surgical resection of brain metastasis before enrollment.

Response and Survival

The data cutoff date was September 30, 2019, when 113 progression events had occurred. Median follow-up was 21.4 months (Interquartile range: 12.7–28.6).

Treatment responses of the full-analysis set and subgroup population are presented in **Table 2**. Best percentage changes in the target lesion for two groups are shown in **Figure 2**. The proportion of patients achieved disease control with erlotinib 100 mg/d was similar to those with standard dose gefitinib [91% [95%CI 81.7–95.3] vs. 93% [95%CI 85.1–96.6], $P = 0.613$, **Table 2**]. The difference in DCR between erlotinib and gefitinib group was 2% and the lower 95% CI for the difference in DCR was 11.3%. Therefore, the primary endpoint of this study was met. Forty six patients [62% [95%CI 50.8–72.4]] in the erlotinib group and 44 patients [53% [95%CI 42.4–63.4]] in the gefitinib group had an objective response, respectively ($P = 0.247$). Median time to response was also similar between erlotinib and gefitinib [29 days [95% CI 26–63] vs. 32 days [95% CI 28–85], $P = 0.142$]. However, median duration of response with erlotinib 100 mg/d was significantly shorter than with standard dose gefitinib [7.7 months [95% CI 6.1–10.1] vs. 10.6 months [95% CI 6.3–12.9], $P = 0.020$]. Subgroup analyses were performed by the line of treatment, mutation subtypes and status of CNS metastasis. In terms of DCR and ORR, no significant difference was observed between lower dose erlotinib and standard dose gefitinib in subgroup populations (**Table 2**).

PFS was similar between erlotinib and gefitinib [10.1 months [95% CI 9.1–11.2] vs. 11.3 months [95% CI 10.4–12.1], HR = 1.295 [95% CI 0.893–1.879], $P = 0.171$, **Figure 3A**]. Subgroup analyses by line of treatment, mutation subtypes, and status of CNS metastasis detected no significant difference in PFS between the two groups (**Figure 3B**). With regard to the patterns of disease progression, 39 patients (39/55, 71%) with lower dose erlotinib and 36 patients (36/58, 62%) with standard dose gefitinib experienced disease progression at all sites, respectively ($P = 0.320$). Twenty-one patients (21/55, 38%) with erlotinib and 31 patients (31/58, 53%) with gefitinib had disease progression in the CNS ($P = 0.104$). Among them, six patients (6/55, 11%) with lower dose erlotinib developed newly onset brain metastasis, while 14 patients (14/58, 24%) with standard dose gefitinib had newly onset brain metastasis ($P = 0.066$).

Median OS with lower dose erlotinib was numerically shorter than standard dose gefitinib, but the difference was not significant [26.6 months [95% CI 22.4–30.8] vs. 28.7 months [95% CI 24.2–33.1], HR = 0.999 [95% CI 0.637–1.569], $P = 0.998$, **Figure 3C**]. Subgroup analyses by line of treatment, mutation subtypes, and status of CNS metastasis showed no significant difference in OS between the two groups either (**Figure 3D**).

Treatment-Related Toxicity

Toxicity was evaluable in 157 patients (**Table 3**). The most common treatment-related toxicity was skin and mucosa disorder, including rash, pruritus, stomatitis, and paronychia. Grade 1–2 liver dysfunction and diarrhea were also common in both groups.

No significant difference was observed in the incidence of adverse events of any grade or adverse events of grade 3–4 between erlotinib and gefitinib. Numerically, higher incidence of alanine transaminase (ALT) and aspartate transaminase (AST) elevation was observed in the gefitinib group, but the difference was not significant [ALT: 22 [27%] vs. 15 [20%], $P = 0.358$; AST: 21 [25%] vs. 11 [15%], $P = 0.105$]. Numbers of patients with adverse events of any grade and adverse events of grade 3–4 were also higher with standard dose gefitinib compared with lower dose erlotinib [gefitinib vs. erlotinib: any-grade, 80 [96%] vs. 66 [89]; grade 3–4, 11 [13%] vs. 4 [5%]]. In the erlotinib group, three patient discontinued treatment because of serious skin toxicities. In the gefitinib group, 10 patients discontinued treatment because of grade-3 liver dysfunction ($n = 7$), grade-3 rash ($n = 2$), or grade-2 stomatitis ($n = 1$). No significant difference in toxicity-related treatment discontinuation between the two groups ($P = 0.085$). No treatment-related death occurred.

DISCUSSION

This is the first randomized controlled trial to directly compare lower dose erlotinib with standard dose gefitinib in EGFR-mutated NSCLC. The study objective was to evaluate whether erlotinib administered at 100 mg/d, two-thirds of its approved dose, could deliver similar efficacy compared with gefitinib 250 mg/d. According to our results, the lower 95% CI difference in DCR was < 12%, indicating the need in proceeding to a phase-3 non-inferiority trial. Erlotinib 100 mg/d was comparable to gefitinib 250 mg/d in terms of disease control, tumor response, PFS, OS, and toxicity, supporting the use of 100 mg/d erlotinib in patients with EGFR-mutated, advanced NSCLC.

Erlotinib and gefitinib are both first-generation EGFR TKI. Gefitinib was administered at 250 mg/d, almost one-third of its MTD, while erlotinib was administered exactly at its MTD, 150 mg/d (12, 21, 22). Several retrospective studies have reported that dose reduction of erlotinib correlated with better response and longer survival (13, 15, 16). However, restricted by the inherent limitations of retrospective analysis, no study could provide conclusive evidence on the efficacy of reduced dose erlotinib. Additionally, given the 3–6% cerebrospinal fluid penetration rates of erlotinib and its active metabolite (23, 24), the concern that dose reduction may result in higher rate of CNS failure further discourage the use of lower dose erlotinib. In the full-analysis set of the present study, efficacy with erlotinib 100 mg/d

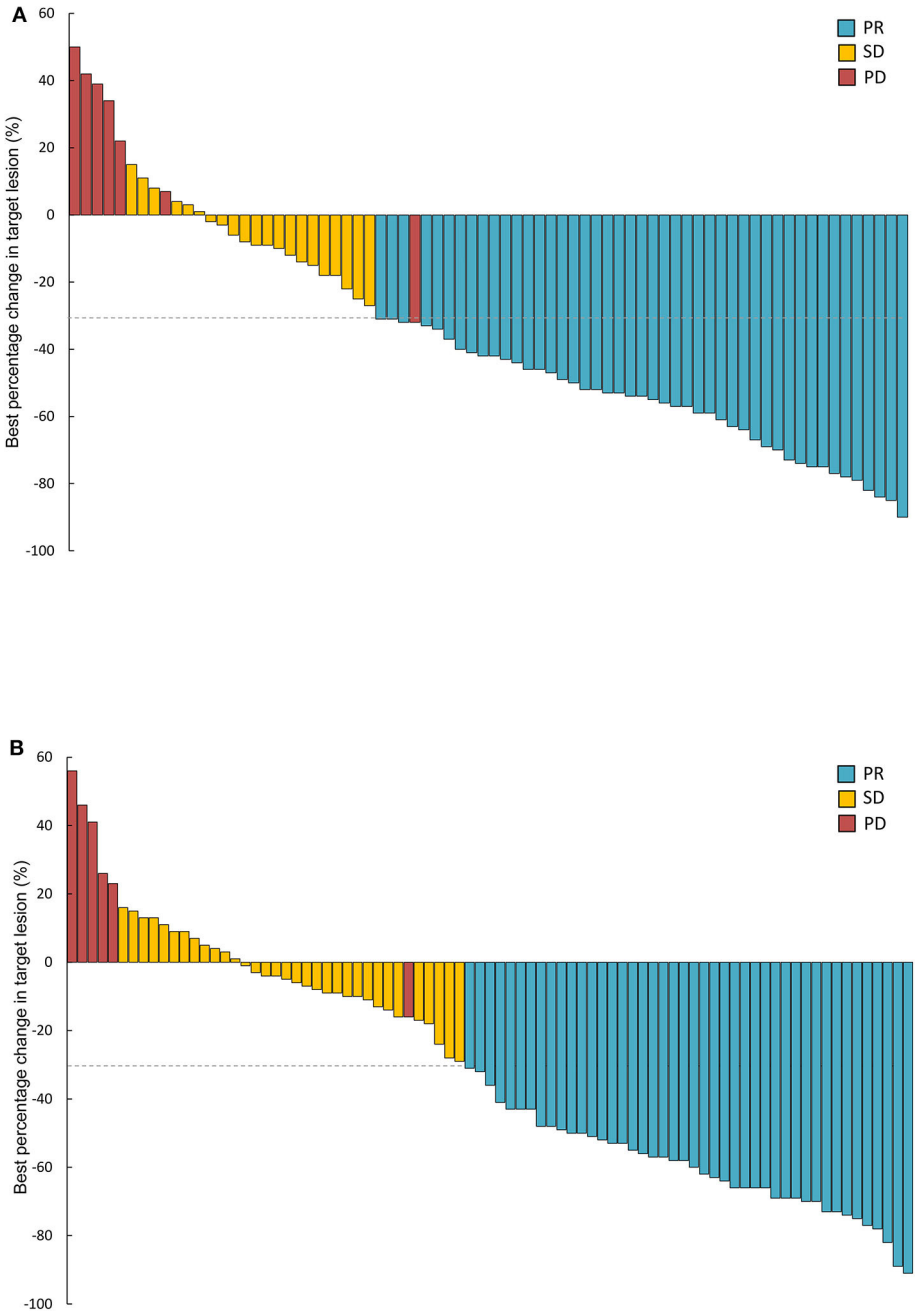

FIGURE 2 | Waterfall plots of best percentage changes in the target lesions at baseline in two groups. **(A)** Erlotinib 100 mg/d group ($n = 74$). **(B)** Gefitinib 250 mg/d group ($n = 83$). PR, partial response; SD, stable disease; PD, progressive disease.

was comparable to those with gefitinib 250 mg/d. Subgroup analysis in patients with baseline CNS metastasis [29 [39%] in erlotinib group, 30 [36%] in gefitinib group] also found that disease control, tumor response, PFS, and OS with erlotinib 100 mg/d were similar to those with gefitinib 250 mg/d. These results suggest that pharmacokinetic factor may not be the main reason for CNS failure in these patients. Erlotinib 100 mg/d is of sufficient efficacy for EGFR-mutated NSCLC patients who carried clinically stable CNS metastasis.

Interestingly, although no significant difference in PFS was observed between lower dose erlotinib and standard dose gefitinib, duration of response (DOR) with gefitinib 250 mg/d was significantly longer than with erlotinib 100 mg/d. Consistent results were observed in another study. Yamada et al. (18) treated

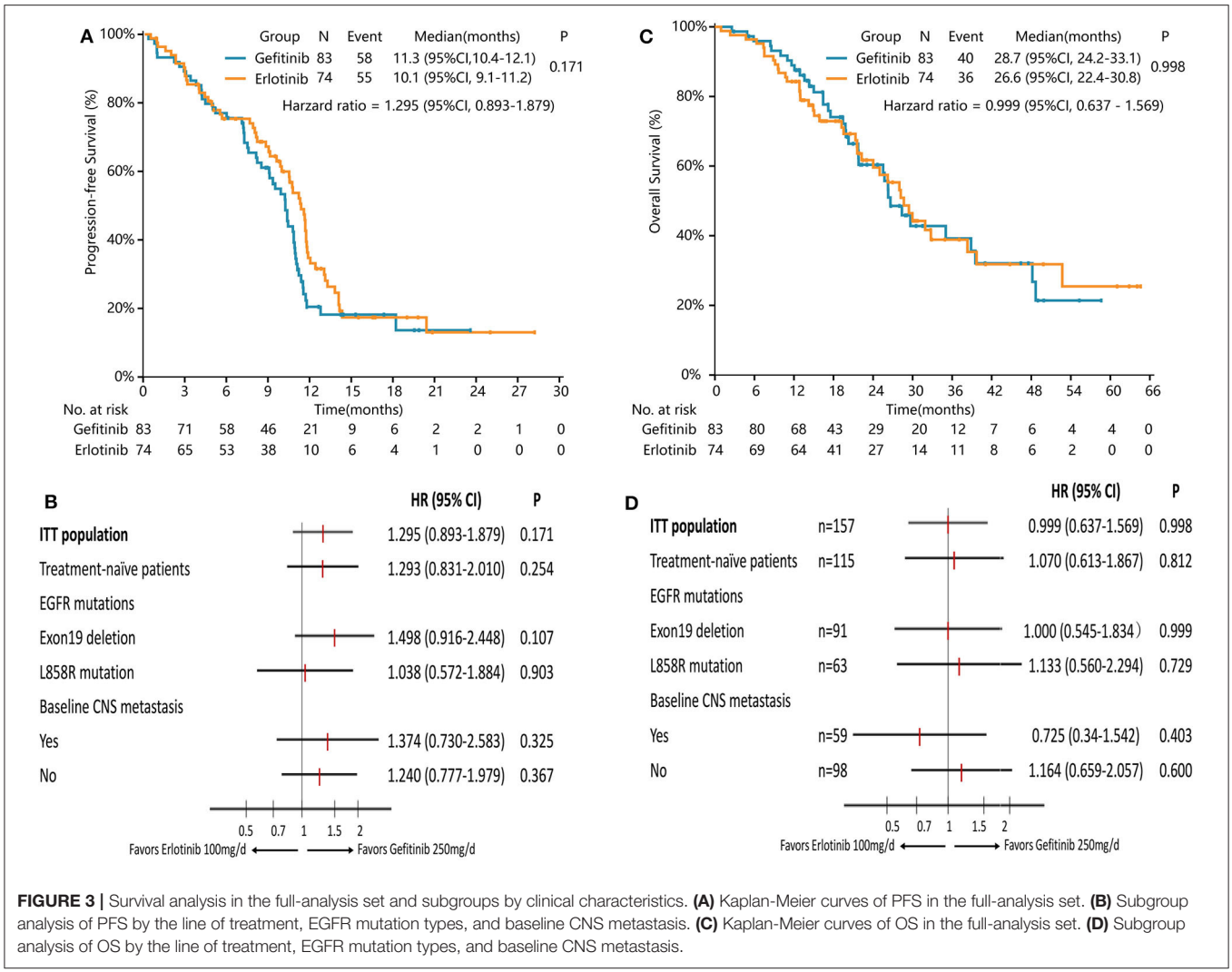

FIGURE 3 | Survival analysis in the full-analysis set and subgroups by clinical characteristics. **(A)** Kaplan-Meier curves of PFS in the full-analysis set. **(B)** Subgroup analysis of PFS by the line of treatment, EGFR mutation types, and baseline CNS metastasis. **(C)** Kaplan-Meier curves of OS in the full-analysis set. **(D)** Subgroup analysis of OS by the line of treatment, EGFR mutation types, and baseline CNS metastasis.

patients with erlotinib 50 mg/d, and then escalated the dose to 150 mg/d in patients with no response. They found that patients having progressive disease at 50 mg/d did not obtain any response when the dose was increased to 150 mg/d. While four patients who had shown tumor shrinkage at 50 mg/d erlotinib achieved partial response with increased dose. These findings indicate that pharmacokinetic factors caused by dose modification may play a greater role in treatment-sensitive clones, but little in resistant clones. Consistently, Foo et al. (22) reported that erlotinib at 150 mg/d failed to substantially inhibit tumors with preexisting T790M clones. Therefore, as long as the administered dose is sufficiently potent in suppressing sensitive clones, disease control and PFS of treatment would not be significantly influenced by dose reduction, as demonstrated in the present study. For patients with responsive tumors, erlotinib 100 mg/d is of ample efficacy, while increasing the dose to 150 mg/d only led to increased toxicity but few incremental efficacies.

By demonstrating the comparable efficacy between lower dose erlotinib and standard dose gefitinib in EGFR-mutated NSCLC, our results could facilitate the development of EGFR TKI-based combination therapies. For example, c-Met amplification has been established as a resistant mechanism to EGFR-TKIs. The combination of erlotinib and crizotinib led to a marked tumor shrinkage (> 50%) in a patient with EGFR-mutant and c-Met-amplified lung adenocarcinoma (25). However, the combination also caused intolerable toxicity that forced a dose reduction to erlotinib 75 mg/d and crizotinib 250 mg/d. The combination of erlotinib 150 mg/d with bevacizumab, ramucirumab, nivolumab, or cabozantinib were also investigated in other studies, where increased efficacy and toxicity were reported for the combination therapy (9–11, 26, 27). Results of the present study indicate the alternative role of lower dose erlotinib in combination therapies, which could lead to comparable efficacy and improved tolerability.

There are some limitations of the current study. First, the recruitment took 5 years to complete because of several competitive trials were initiated during this time. The approval of osimertinib in China further affected the enrollment of this

TABLE 3 | Treatment-related adverse events.

Adverse event	No. of Patients (%)							
	Erlotinib ($n = 74$)				Gefitinib ($n = 83$)			
	All grade	Grade 1–2	Grade 3	Grade 4	All grade	Grade 1–2	Grade 3	Grade 4
Rash	35 (47)	32 (43)	2 (3)	1 (1)	33 (40)	31 (37)	2 (2)	0
Diarrhea	12 (16)	12 (16)	0	0	16 (19)	15 (18)	1 (1)	0
Pruritus	9 (12)	9 (12)	0	0	15 (18)	15 (18)	0	0
Stomatitis	6 (8)	6 (8)	0	0	8 (10)	8 (10)	0	0
Increased ALT	15 (20)	14 (19)	1 (1)	0	22 (27)	16 (19)	5 (6)	1 (1)
Increased AST	11 (15)	11 (15)	0	0	21 (25)	17 (20)	3 (4)	1 (1)
Neutropenia	3 (4)	3 (4)	0	0	1 (1)	1 (1)	0	0
Increase bilirubin	3 (4)	3 (4)	0	0	8 (10)	8 (10)	0	0
Paronychia	2 (3)	2 (3)	0	0	3 (4)	3 (4)	0	0
Fatigue	1 (1)	1 (1)	0	0	2 (2)	2 (2)	0	0
Nausea/vomiting	1 (1)	1 (1)	0	0	4 (5)	4 (5)	0	0
Infection	1 (1)	1 (1)	0	0	1 (1)	1 (1)	0	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

study, because many patients preferred osimertinib over first-generation TKIs. Second, we were unable to evaluate serum concentrations of erlotinib administered at 100 mg/d in this study due to the lack pharmacokinetic data. Finally, study sample size was calculated with DCR as the primary endpoint and the number of participants enrolled in the erlotinib arm was < prespecified 80 participants. A total of 157 patients may not be large enough to tell the mild difference in PFS between the two groups. Only a non-significant trend toward improved tolerability was observed with lower dose erlotinib, which could also be attributed to the small sample size. Future studies with larger sample size are warranted to expand on our findings.

In conclusion, this study provided the first RCT-based evidence on efficacy and tolerability of 100 mg erlotinib in EGFR-mutated, advanced NSCLC. Compared with gefitinib at 250 mg/d, erlotinib at 100 mg/d yielded comparable efficacy in terms of disease control, tumor response, median PFS, and median OS. Similar results were also observed in patients in the first-line setting, patients with different EGFR mutations and patients with or without baseline CNS metastasis. Therefore, in Stage IV EGFR mutated NSCLC, this study showed that erlotinib 100 mg/d had similar DCR compared with gefitinib 250 mg/d. A randomized phase-3 non-inferiority trial with PFS as a primary endpoint is required to confirm the non-inferiority of erlotinib 100 mg/d when compared with gefitinib 250 mg/d.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Sun Yat-sen University Cancer Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LZ, SZ, ZheZ, and WF contributed to study design, data collection, data interpretation, and drafting of the manuscript. LZ supervised the study. SZ, ZheZ, WF, YZ, ZhoZ, and SH contributed to data collection and management. All authors were involved in the provision of study materials and patients, data interpretation, contributed to the writing, and critical review of the manuscript.

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REFERENCES

1. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. (2004) 350:2129–39. doi: 10.1056/NEJMoa040938
2. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H. Gefitinib or chemotherapy for non–smallcell lung cancer with mutated EGFR. *N Engl J Med*. (2010) 362:2380–8. doi: 10.1056/NEJMoa0909530
3. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, 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) 11:121–8. doi: 10.1016/S1470-2045(09)70364-X
4. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, 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) 13:239–46. doi: 10.1016/S1470-2045(11)70393-X
5. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. (2018) 378:113–25. doi: 10.1056/NEJMoa1713137
6. Wu YL, Cheng Y, Zhou X, Lee KH, Nakagawa K, Niho S, et al. Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomised, open-label, phase 3 trial. *Lancet Oncol*. (2017) 18:1454–66. doi: 10.1016/S1470-2045(17)30608-3
7. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol*. (2011) 12:735–42. doi: 10.1016/S1470-2045(11)70184-X
8. Lee CK, Man J, Lord S, Links M, Gebski V, Mok T, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small cell lung cancer-A meta-analysis. *J Thorac Oncol*. (2017) 12:403–7. doi: 10.1016/j.jtho.2016.10.007
9. Gettinger S, Hellmann MD, Chow LQM, Borghaei H, Antonia S, Brahmer JR, et al. Nivolumab plus erlotinib in patients with EGFR-mutant advanced NSCLC. *J Thorac Oncol*. (2018) 13:1363–72. doi: 10.1016/j.jtho.2018.05.015
10. Nakagawa K, Garon EB, Seto T, Nishio M, Ponce Aix S, Paz-Ares L, et al. Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. (2019) 20:1655–69. doi: 10.1016/S1470-2045(19)30634-5
11. Saito H, Fukuhara T, Furuya N, Watanabe K, Sugawara S, Iwasawa S, et al. Erlotinib plus bevacizumab versus erlotinib alone in patients with EGFR-positive advanced non-squamous non-small-cell lung cancer (NEJ026): interim analysis of an open-label, randomised, multicentre, phase 3 trial. *Lancet Oncol*. (2019) 20:625–35. doi: 10.1016/S1470-2045(19)30035-X
12. Hidalgo M, Siu LL, Nemunaitis JJ, Rizzo J, Hammond LA, Takimoto C. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J Clin Oncol*. (2001) 19:3267–79. doi: 10.1200/JCO.2001.19.13.3267
13. Yeo WL, Riely GJ, Yeap BY, Lau M, Warner J, Bodio K. Erlotinib at a dose of 25 mg daily for non-small cell lung cancers with EGFR mutations. *J Thorac Oncol*. (2010) 5:1048–53. doi: 10.1097/JTO.0b013e3181dd1386
14. Lampson BL, Nishino M, Dahlberg SE, Paul D, Santos AA, Janne PA, et al. Activity of erlotinib when dosed below the maximum tolerated dose for EGFR-mutant lung cancer: implications for targeted therapy development. *Cancer*. (2016) 122:3456–63. doi: 10.1002/cncr.30270
15. Binder D, Buckendahl AC, Hubner RH, Schlattmann P, Temmesfeld-Wollbruck B, Beinert T, et al. Erlotinib in patients with advanced non-small-cell lung cancer: impact of dose reductions and a novel surrogate marker. *Med Oncol*. (2012) 29:193–8. doi: 10.1007/s12032-010-9767-x
16. Satoh H, Inoue A, Kobayashi K, Maemondo M, Oizumi S. Low-Dose gefitinib treatment for patients with advanced non-small cell lung cancer harboring sensitive epidermal growth factor receptor mutations. *J Thorac Oncol*. (2011) 6:1413–17. doi: 10.1097/JTO.0b013e31821d43a8
17. Miyamoto S, Azuma K, Ishii H, Bessho A, Hosokawa S, Fukamatsu N, et al. Low-dose erlotinib treatment in elderly or frail patients with egfr mutation-positive non-small cell lung cancer: a multicenter phase 2 trial. *JAMA Oncol*. (2020) 6:e201250. doi: 10.1001/jamaoncol.2020.1250
18. Yamada K, Aono H, Hosomi Y, Okamoto H, Kato T, Komase Y, et al. A prospective, multicentre phase II trial of low-dose erlotinib in non-small cell lung cancer patients with EGFR mutations pretreated with chemotherapy: Thoracic Oncology Research Group 0911. *Eur J Cancer*. (2015) 51:1904–10. doi: 10.1016/j.ejca.2015.06.120
19. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. (2009) 45:228–47. doi: 10.1016/j.ejca.2008.10.026
20. Shi Y, Zhang L, Liu X, Zhou C, Zhang L, Zhang S, et al. Icotinib versus gefitinib in previously treated advanced non-small-cell lung cancer (ICOGEN): a randomised, double-blind phase 3 non-inferiority trial. *Lancet Oncol*. (2013) 14:953–61. doi: 10.1016/S1470-2045(13)70355-3
21. Baselga J, Rischin D, Ranson M, Calvert H, Raymond E, Kieback DG, et al. Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. *J Clin Oncol*. (2002) 20:4292–302. doi: 10.1200/JCO.2002.03.100
22. Foo J, Chmielecki J, Pao W, Michor F. Effects of pharmacokinetic processes and varied dosing schedules on the dynamics of acquired resistance to erlotinib in EGFR-mutant lung cancer. *J Thorac Oncol*. (2012) 7:1583–93. doi: 10.1097/JTO.0b013e31826146ee
23. Togashi Y, Masago K, Fukudo M, Terada T, Fujita S, Irisa K. Cerebrospinal fluid concentration of erlotinib and its active metabolite OSI-420 in patients with central nervous system metastases of non-small cell lung cancer. *J Thorac Oncol*. (2010) 5:950–5. doi: 10.1097/JTO.0b013e3181e2138b
24. Togashi Y, Masago K, Fukudo M, Tsuchido Y, Okuda C, Kim YH, et al. Efficacy of increased-dose erlotinib for central nervous system metastases in non-small cell lung cancer patients with epidermal growth factor receptor mutation. *Cancer Chemother Pharmacol*. (2011) 68:1089–92. doi: 10.1007/s00280-011-1691-z
25. Dietrich MF, Yan SX, Schiller JH. Response to Crizotinib/Erlotinib combination in a patient with a primary EGFR-mutant adenocarcinoma and a primary c-met-amplified adenocarcinoma of the lung. *J Thorac Oncol*. (2015) 10:e23–5. doi: 10.1097/JTO.0000000000000448
26. Neal JW, Dahlberg SE, Wakelee HA, Aisner SC, Bowden M, Huang Y, et al. Erlotinib, cabozantinib, or erlotinib plus cabozantinib as second-line or third-line treatment of patients with EGFR wild-type advanced non-small-cell lung cancer (ECOG-ACRIN 1512): a randomised, controlled, open-label, multicentre, phase 2 trial. *Lancet Oncol*. (2016) 17:1661–71. doi: 10.1016/S1470-2045(16)30561-7
27. Seto T, Kato T, Nishio M, Goto K, Atagi S, Hosomi Y, et al. Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring EGFR mutations (JO25567): an open-label, randomised, multicentre, phase 2 study. *Lancet Oncol*. (2014) 15:1236–44. doi: 10.1016/S1470-2045(14)70381-X

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Integrating Liquid Biopsy and Radiomics to Monitor Clonal Heterogeneity of EGFR-Positive Non-Small Cell Lung Cancer

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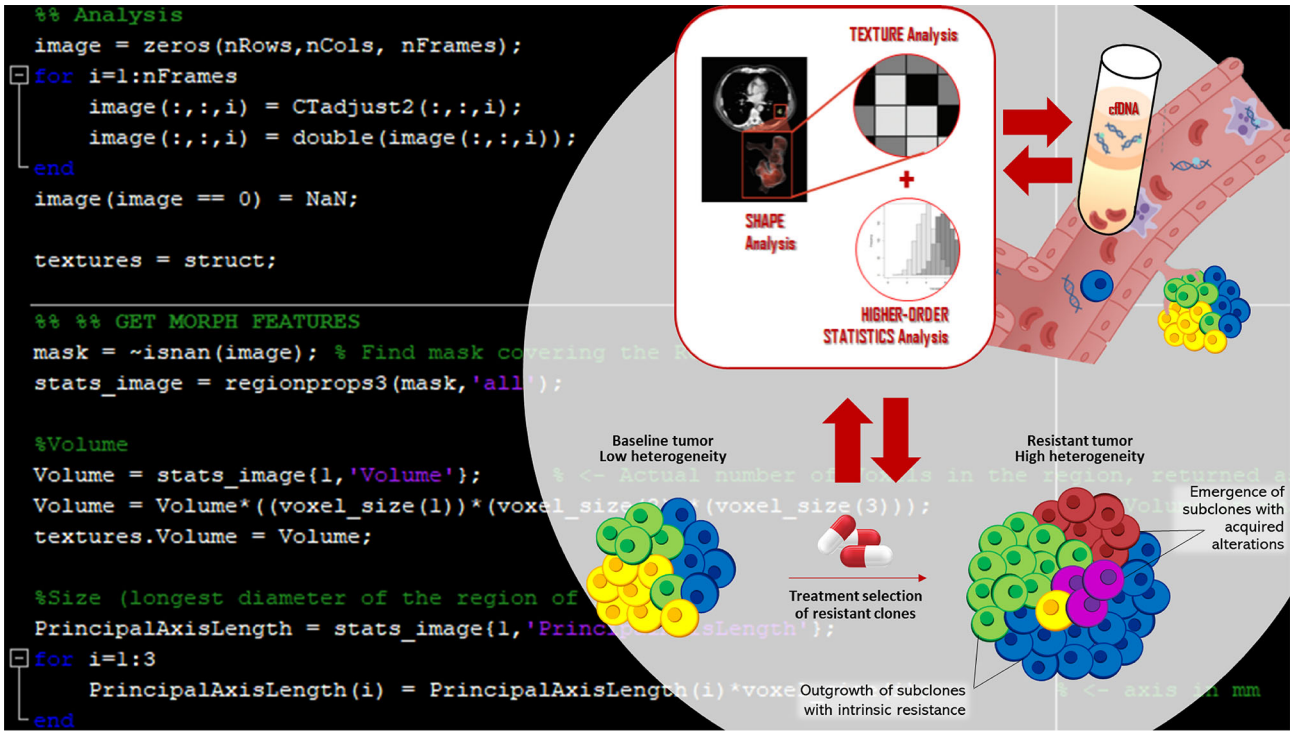
Background: EGFR-positive Non-small Cell Lung Cancer (NSCLC) is a dynamic entity and tumor progression and resistance to tyrosine kinase inhibitors (TKIs) arise from the accumulation, over time and across different disease sites, of subclonal genetic mutations. For instance, the occurrence of EGFR T790M is associated with resistance to gefitinib, erlotinib, and afatinib, while EGFR C797S causes osimertinib to lose activity. Sensitive technologies as radiomics and liquid biopsy have great potential to monitor tumor heterogeneity since they are both minimally invasive, easy to perform, and can be repeated over patient's follow-up, enabling the extraction of valuable information. Yet, to date, there are no reported cases associating liquid biopsy and radiomics during treatment.

Case presentation: In this case series, seven patients with metastatic EGFR-positive NSCLC have been monitored during target therapy. Plasma-derived cell free DNA (cfDNA) was analyzed by a digital droplet PCR (ddPCR), while radiomic analyses were performed using the validated LifeX® software on computed tomography (CT)-images. The dynamics of EGFR mutations in cfDNA was compared with that of radiomic features. Then, for each EGFR mutation, a radiomic signature was defines as the sum of the most predictive features, weighted by their corresponding regression coefficients for the least absolute shrinkage and selection operator (LASSO) model. The receiver operating characteristic (ROC) curves were computed to estimate their diagnostic performance. The signatures achieved promising performance on predicting the presence of EGFR mutations (R^2 = 0.447, p <0.001 EGFR activating mutations R^2 = 0.301, p = 0.003 for T790M; and R^2 = 0.354, p = 0.001 for activating plus resistance mutations), confirmed by ROC analysis.

Conclusion: To our knowledge, these are the first cases to highlight a potentially promising strategy to detect clonal heterogeneity and ultimately identify patients at risk of progression during treatment. Together, radiomics and liquid biopsy could detect the appearance of new mutations and therefore suggest new therapeutic management.

Keywords: non-small cell lung cancer, EGFR, liquid biopsy, cell free DNA, radiomics, tyrosine kinase inhibitors, precision medicine



GRAPHICAL ABSTRACT |

INTRODUCTION

Tyrosine kinases inhibitors (TKIs), such as gefitinib, erlotinib, afatinib or osimertinib, are the first-line treatments in patients with advanced NSCLC and activating EGFR mutation (1–4) since they improved progression-free survival (PFS) compared with conventional chemotherapy (5). Nevertheless, NSCLC is a dynamic entity, and tumor progression and resistance to treatment arise from the accumulation of independent genetic mutations in subclones, over time and across different disease sites, thereby resulting in temporal and spatial heterogeneity (6). Moreover, treatment exerts selective pressure on cancer cells, and only those bearing either primary or secondary resistance mutations will survive (7–9).

The concept of a single-site biopsy to monitor disease dynamics is practically unfeasible since it is invasive and may result in underestimation of heterogeneity (10). Instead, liquid biopsy—allowing the analysis of cell-free DNA (cfDNA) (11)—better reflects the mutational status from the overall sites of disease (12), being able to identify emerging sub-clones responsible for treatment resistance (13).

Besides, radiomics has emerged as a novel field of research (14), dealing with the extraction and analysis of specific features from diagnostic images (15), and potentially reflecting the pathophysiological processes and the heterogeneity of tumors genetics (16). Recent data has shown that also texture analysis of radiological images can identify NSCLCs bearing EGFR mutations (17, 18).

The combined approach of radiomics and liquid biopsy has the potential to understand the dynamics of molecular lesions, supporting clinical decision-making.

To date, no reports correlate the dynamics of EGFR mutations in cfDNA with that of radiomic features. The present study aimed to assess such correlation in a case series

Abbreviations: AIC, Akaike's Information Criteria; AUC, area under the curve of the ROC; CT, computed tomography; cfDNA, cell-free DNA; ddPCR, digital droplet polymerase chain reaction; DNA, deoxyribonucleic acid; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; ex19del, exon 19 deletion; GLCM, gray level co-occurrence matrix; GLNUr, gray-level non-uniformity for run; GLNUz, gray-level non-uniformity for zone; GLRLM, gray-level run length matrix; GLZLM, gray-level zone length matrix; HGRE, high gray-level run emphasis; HGZE, high gray-level zone emphasis; KV, kilovolt; LASSO, least absolute shrinkage and selection operator; LGRE, low gray-level run emphasis; LGZE, low gray-level zone emphasis; LRE, long-run emphasis; LRHGE, long-run high gray-level emphasis; LRLGE, long-run low gray-level emphasis; LZE, long-zone emphasis; LZHGE, long-zone high gray-level emphasis; LZLGE, long-zone low gray-level emphasis; mAs, milliampere-seconds; min, minutes; ml, milliliter; mm, millimeter; n, number; NGLDM, neighborhood gray-level different matrix; NSCLC, non-small cell lung cancer; PD, progressive disease; PFS, progression-free survival; PR, partial response; rpm, revolutions per minute; RLNU, run length non-uniformity; ROC, receiver operating characteristic; RP, run percentage; RTKs, receptor tyrosine kinases; SCC, squamous cell cancer; SCLC, small cell lung cancer; SD, stable disease; SRE, short-run emphasis; SRHGE, short-run high gray-level emphasis; SRLGE, short-run low gray-level emphasis; STD, standard deviation; SZE, short-zone emphasis; SZHGE, short-zone high gray-level emphasis; SZLGE, short-zone low gray-level emphasis; TKI, tyrosine kinase inhibitor; ZLNU, zone length non-uniformity; ZP, zone percentage.

of seven patients with EGFR mutant NSCLC, and to build a multi-parametric signature of clonal heterogeneity.

PATIENTS

The study retrospectively matched clinical, molecular and imaging databases of seven patients with histologically proven EGFR-positive NSCLC (exon 19 deletion [ex19del], exon 21 [L858R], or other mutations [i.e. L861Q]), and candidate to a first/second or third-generation EGFR-TKIs. Enrolled patients underwent blood sampling 1) before the first dose of TKI (baseline), 2) every two months, 3) and at each of the instrumental (i.e. imaging) disease re-evaluation throughout the follow-up. Complete (CR) and partial response (PR), disease stabilization (SD) and disease progression (PD) were defined following RECIST (v. 1.1) criteria. CT scans were collected at baseline and every 3–6 months as per clinical practice (19) and then used for radiomic analysis. The interval between follow-up medical visits (3 vs. 6 months) was based on clinical decision-making on an individual basis (19). Clinical data were collected from medical records. A written consent form was obtained from all patients. The study was approved by the institutional ethics committee of University Hospital, Pisa, Italy (protocol 5625/2015), and performed in accordance with the provisions established by the Helsinki Declaration.

METHODS

cfDNA Extraction and Analysis

cfDNA was extracted from 3 ml of plasma using the QIAmp Circulating Nucleic Acid kit (Qiagen®, Hilden, Germany) and then eluted in 100 µl of the buffer, as previously described (20). EGFR mutations (ex19del, L858R, T790M, and C797S) were investigated by digital droplet polymerase chain reaction (ddPCR) using the ddPCR Mutation Assay (BioRad®, Hercules, CA). A fluorescence intensity threshold of 3,000 was set as a cut-off point; the sample was considered as mutant positive when at least one droplet was above the threshold level. The number of mutant alleles was reported as copies/ml.

CT Segmentation and Extraction of Radiomic Features

Images were extracted from multiple non-contrast material-enhanced thoraco-abdominal computed tomography (CT)-scans (SIEMENS CT Sensation 64®; kilovoltage = 120 KV and exposure = 165 mAs; CT slice = 1.5 mm) (21). All CT examinations were reconstructed using B30f kernel (22). The radiomic analysis was performed by one author, using the validated LifeX® software (LifeX®, IMIV, CEA, Inserm, CNRS, Orsay, France) (23–25), after appropriate manual segmentation of the volumes of interest (VOIs; i.e. the lesions). Thirty-six radiomic features including three shapes, two gray-level histogram, six gray-level co-occurrence matrix (GLCM), 11 gray-level run lengths matrix (GLRLM), three Neighborhood Grey-Level Different Matrix (NGLDM) and 11 Grey-Level Zone-Length Matrix (GLZLM) features, were computed.

Selection of Radiomic Features and Data Analysis

Each patient had a longitudinal dataset of several scans to match with respective temporally linked liquid biopsy data. To calculate to which extent the variation between the radiomic features is correlated to EGFR mutation status, a logistic least absolute shrinkage and selection operator (LASSO) regression model adopting a 27-fold Monte Carlo cross-validation was applied and executed in Matlab R2019a (MatLab® software, The Math Works Inc., Natick, MA) (26, 27). The LASSO logistic model was used to reduce the number of radiomic features and estimate the maximum-likelihood fitted regression coefficients for the remaining ones. The LASSO computation was performed to assess the radiomic features about the copies/ml of EGFR activating mutations (ex19del/L858R), as well as about the emergence of resistance mutations (T790M and C797S) and the total copies/ml (ex19del/L858R together with T790M and C797S) occurring in EGFR in patients progressed to TKI treatments. Selected radiomic features are reported in **Supplemental Table 1** in the online version. Then, radiomic signatures were calculated as the sum of the selected features weighted by their corresponding regression coefficients for the LASSO models. The receiver operating characteristic (ROC) curve analysis was computed to estimate the diagnostic performance of such signatures and select the optimal thresholds.

Moreover, Kendall's correlation coefficient (tau-b, τb) was calculated to determine the strength of the association between changes in radiomic features and changes in matched liquid biopsy-derived data, over time (28).

Differences were considered significant at $p < 0.05$. Statistical analysis was performed using the open-source statistical language R (R Foundation for Statistical Computing, Vienna, Austria) through the free and open statistical software program JAMOVI® (Version 1.1.9; retrieved from https://www.jamovi.org).

RESULTS

Five patients presented the ex19del activating mutation at diagnosis and two were carriers of the L858R. Three patients were treated with afatinib, two with erlotinib, and two with gefitinib as first-line TKI. Clinical characteristics are summarized in **Supplemental Table 2** in the online version, while plasma monitoring for each of them is reported in **Figure 1**. Overall, at baseline, the median activating EGFR copies/ml was higher than T790M and was often related to disease control, whereas the T790M amount was not. At disease progression, T790M was detected in plasma and/or tissue in all patients; therefore, osimertinib treatment was started, except in one patient, since the drug was not yet available (**Figure 1G**). In two patients disease progression occurred due to C797S mutation, in addition to ex19del and T790M.

The dynamics of EGFR mutations were significantly associated to specific changes in radiomic features over time

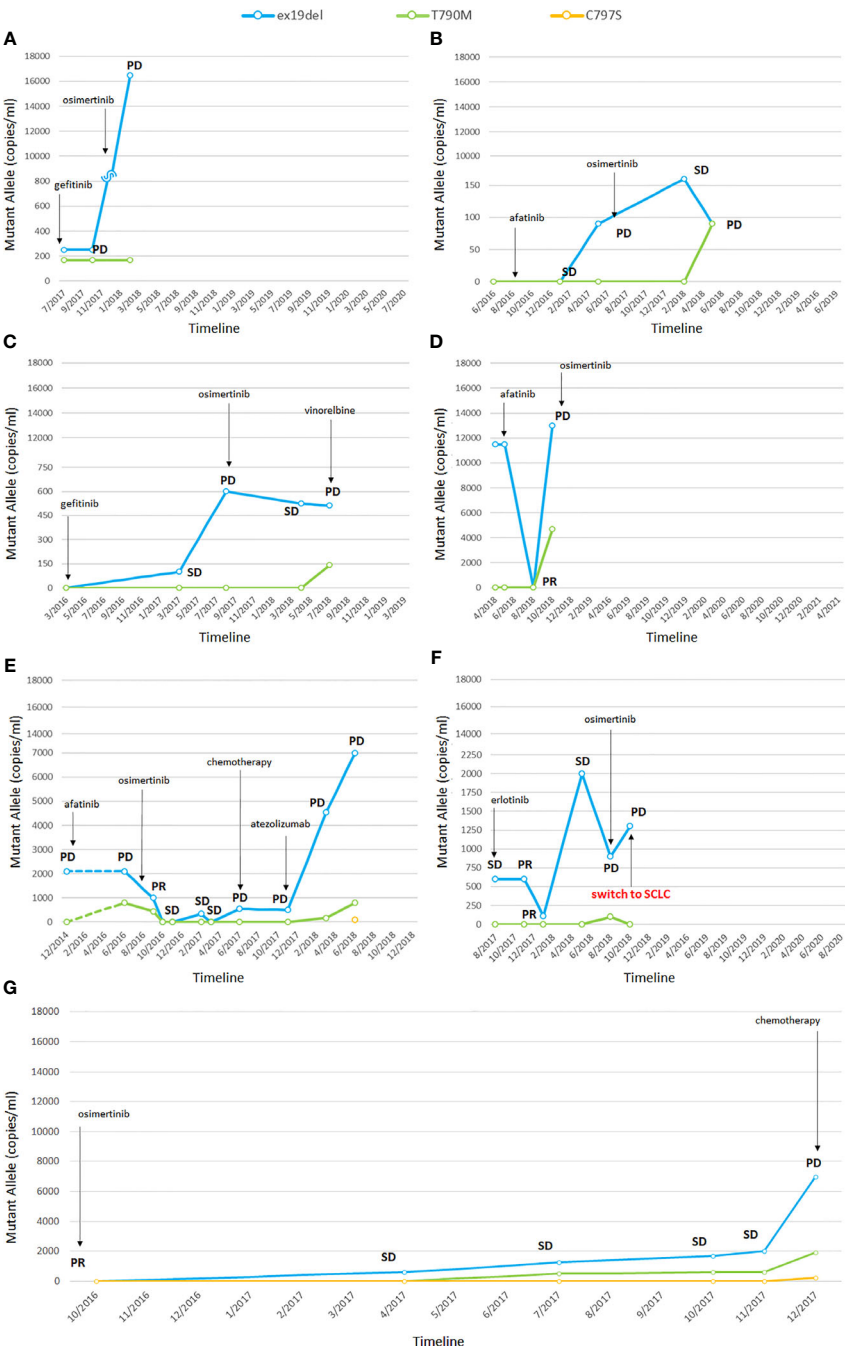


FIGURE 1 | Changes in EGFR mutations detected by liquid biopsy **(A–G)**. In one patient the tumor transformed into small cell lung cancer (SCLC) **(F)**. PR, partial response; SD, stable disease; PD, progressive disease; SCLC, small cell lung cancer. The numbers before the year indicate the months while the letters **(A–G)** refer to single patients.

($p < 0.05$; **Supplemental Table 3** in the online version). Radiomic features selected at least once in the LASSO models with respect to the number of copies/ml of mutant EGFR L858R/ex19del were combined in a signature (R).

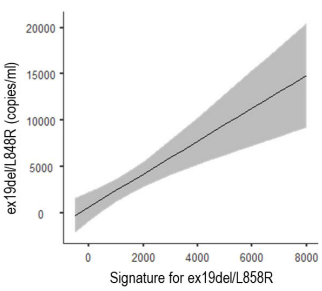
$$R = \sum radiomic \;\; signature * regression \;\; coefficients$$

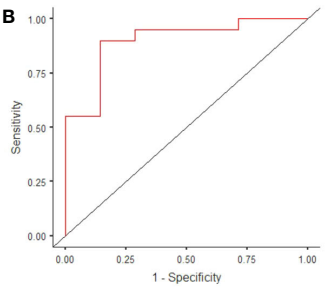
The signature evidenced good capability—with acceptable representativeness—in predicting the number of copies/ml of the activating EGFR ($R^2 = 0.447$, Akaike's Information Criteria (AIC) = 515, $p < 0.001$) (**Figure 2A**), and the optimal cut-point estimated from the ROC curve showed 88.9% accuracy, 90% sensitivity, and 85.7% specificity, with the area under the curve (AUC) of 0.90

A

Estimated Marginal Means – Signature for ex19del/L858R

	Signature	Marginal Mean	SE	95% Confidence Interval Lower	95% Confidence Interval Upper
⁻	-341	53.9	865	-1727	1835
μ	1221	2828.1	606	1581	4075
⁺	2784	5602.4	865	3822	7383

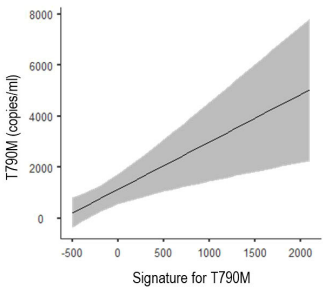


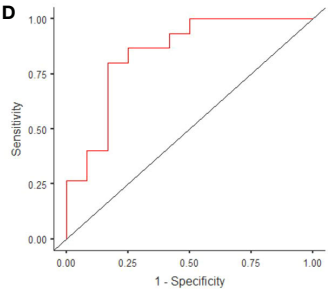


C

Estimated Marginal Means – Signature for T790M

	Signature	Marginal Mean	SE	95% Confidence Interval Lower	95% Confidence Interval Upper
⁻	-718	-189	362	-935	557
μ	-260	660	254	137	1182
⁺	198	1509	362	763	2255

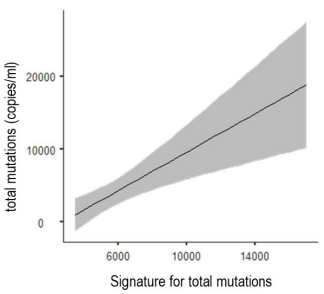




E

Estimated Marginal Means – Signature for total mutations

	Signature	Marginal Mean	SE	95% Confidence Interval Lower	95% Confidence Interval Upper
⁻	3009	379	1220	-2134	2892
μ	5448	3603	855	1843	5363
⁺	7887	6828	1220	4314	9341



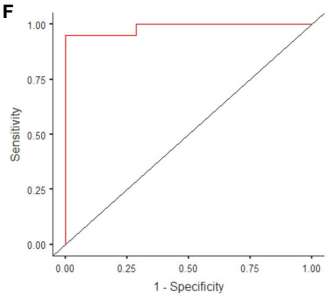


FIGURE 2 | Estimated Marginal Means and ROC analysis for radiomic signature concerning ex19del/L858R **(A, B)**, T790M **(C, D)**, and total copies/ml (i.e. ex19del/L858R together with T790M and C797S) **(E, F)**. ⁻, mean-1SD; μ, mean; ⁺, mean+1STD.

(**Figure 2B**). Signatures and their predicting representativeness were also evaluated with respect to the copies/ml of T790M mutation and the total copies/ml of mutations in patients progressed to TKIs. The model predicting the T790M copies/ml showed $R^2 = 0.301$ and AIC = 468 ($p = 0.003$), with 81.5% accuracy, 80% sensitivity and 83.3% specificity of the optimal threshold, and AUC of 0.84 (**Figures 2C, D**). The model predicting the total copies/ml of all mutations displayed $R^2 = 0.354$ and AIC = 534 ($p = 0.001$), with 96.3% accuracy, 95% sensitivity, and 100% specificity of the optimal threshold, with AUC of 0.98 (**Figures 2E, F**); no correlation emerged for C797S.

DISCUSSION

To date, several works studied the potential of radiomics in the non-invasive prediction of EGFR mutational status and showed promising results (18, 24, 29–32). However, none of them addressed the subclonal heterogeneity that occurs asynchronously. In this study, we endeavored to highlight the great potential of integrating radiomics and liquid biopsy, as both are minimally invasive, easy to perform, and can be repeated over patients' follow-up visits.

We did it by incorporating multiple radiomic functions into a signature (R) that could reliably predict the EGFR mutation status during treatment, and demonstrating significant correlations between radiomics and liquid biopsy data.

Of note, sphericity of lung lesions decreased with the increase of T790M copies/ml and, more generally, with the total copies/ml of mutant alleles, highlighting the association between spherical disproportion and neoplastic progression, aggressiveness and resistance to therapy (33). Besides, copies/ml of the T790M mutation were directly correlated with GLCM dissimilarity (a measure of local intensity variation of the voxel gray levels), and direct relationships also emerged between ex19del/L858R copies/ml, GLCM energy and contrast (**Supplemental Table 3**). The GLCM energy is a characteristic that describes the order status of the system and refers to the uniformity of the gray level between voxel pairs. The GLCM contrast highlights how many nearby sub-areas of heterogeneity differ within each lesion.

Interestingly, we found no significant correspondence between tumor volume (ml) and mutational status (**Supplemental Table 3**).

Our results are consistent with those from Park et al. (34) and Lee et al. (35), testing the stability and reliability of radiomic features to evaluate tumor heterogeneity. Notably, Lee and collaborators (35), by studying the variability of radiomics features and their relationship with tumor size and shape upon 260 lung nodules, found that only a few features—including spherical disproportion and dissimilarity—showed high reproducibility in correlation with nodule status. This is probably because radiomics in lung cancer is different from in other oncology fields. Lung cancer resides in an environment rich with air, while other cancers primarily consist of soft tissue and reside in the interstitium (36). More than the usual volume changes, tumor progression is associated with shape and density changes from ground-glass opacity (GGO) to solid component (37–39). Thus, radiomics in the lung should jointly consider the tumor core geometry along with textural changes to properly model lung cancers. Nevertheless, reproducibility studies are lacking, and more evidences are needed to provide suggestions for future lung radiomics investigations.

There was also no significant correlation between changes in radiomic characteristics and C797S dynamics, probably due to the low sample size. However, the landscape of mechanisms of resistance dramatically changes considering osimertinib, and future studies to better investigate radiomic changes correlating with C797S dynamics will be needed (40). The appearance of the EGFR C797S mutation accounts for 6–10% after osimertinib as first line and 10–26% as second line (41). Furthermore, co-occurring with T790M has potential implications for treatment: when C797S and T790M occur on the same allele (*cis*), no response to EGFR TKIs alone or in combination can be expected, while the C797S in trans with the T790M mutation confers sensitivity to a combination of first/third-generation drugs (42–44).

Our results took advantage of consistently examining a few patients over a period, and of correlating changes in their radiomic features with the respective dynamics of EGFR mutations in cfDNA. The resulting signatures showed a good capability—with acceptable representativeness—in predicting the tumor mutational status. Unfortunately, given the low number of subjects, we found no radiomic signature that can be reliably associated with clinical outcomes, but we plan to look for it in the future. Future larger prospective clinical trials will also need to validate these findings and give us the chance to look for new resistance signatures, such as the one related to SCLC transformation, which is an important potential mechanism of resistance for to first/second and third-generation EGFR-TKIs (8), but to date, only a new tissue biopsy could allow to find it. Clinicians may consider using the signature as a new supporting tool, in accordance with their experience and judgment.

Liquid biopsy and radiomics have both advantages and drawbacks making them complementary methods. Although they are appealing options at progression, to track mechanisms of resistance (40, 45–48), there are still too few laboratory applications for liquid biopsy, and molecular protocols need to be standardized. Furthermore, there are difficulties in detection, and extremely sensitive and specific analytical methods are required to deal with small quantities of easily degradable materials. Lastly, it is still unclear whether liquid biopsy provides a representative sampling of all genetic clones or whether there is a propensity for specific subpopulations within the intra-tumor heterogeneity (49). Similarly, radiation, dearth of standardization for image acquisition, computational approaches and feature selection, as well as the black-box problem (i.e. non-interpretable advanced machine-learning algorithms that work like black boxes, hindering clinical translation), limit the use of radiomics, which should be considered as an indirect and non-detailed quantification of the underlying biological processes. Therefore, to strengthen the trustworthiness of the results, radiomics-based genotype predictions could be compared with information from liquid biopsy (16), over time. A combination of these two minimally invasive strategies, together with cutting-edge data analysis strategies, could be more valuable and reliable than their independent use and may help decode tumor information regarding the type, aggressiveness, progression, and response to treatment (29, 50). A study from the University of Oklahoma reported that while radiomics and genomics models were capable of predicting survival, accuracy significantly improved when both data were combined (51). Besides, while it is possible to avoid unnecessary radiation by using liquid biopsy, on the other hand, we can use radiomics to refine liquid biopsy results and provide a full-field analysis of patient's lesions in virtually real-time response. Both techniques, providing a new instrumental and therefore objective diagnostic support, are able to reduce the need for invasive (and often difficult to perform) biopsies and favor an approach that promptly suggests a change in treatment strategy over the follow-up.

To the best of our knowledge, this is the first study investigating the longitudinal trajectory of NSCLC from both the radiomic and liquid biopsy points of view. As far as we know, the parallelism between the dynamics of EGFR mutation status and radiomic features is potentially dependent on the progressive enrichment of tumor tissue by treatment-resistant clones.

CONCLUSION

Radiomic signatures may represent a clinically relevant readout of EGFR mutational status and provide a non-invasive biomarker to monitor targeted drug therapies in NSCLC. Indeed, with the availability of big data and cutting-edge analysis strategies (such as machine learning), the information coming from tumor genotype and phenotype decoded via imaging (29), may predict treatment failures suggesting a change in treatment strategy earlier than with conventional methods. Nevertheless, it should be noted that such techniques will not substitute tissue biopsy in the near future, since they will require the aid of other parameters to be correctly interpreted and acted upon (52).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional ethics committee of University Hospital, Pisa, Italy (protocol 5625/2015). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Conception and design: RD, MDR, FC. Provision of study material or patients: IP, CR, SV, ML, AC, SC, ER, FC, AD, AR, RD. Collection and assembly of data: MDR, FC, SV, CR, IP, ML, SC, ER. Data analysis and interpretation: MDR, FC, CR, SC. Manuscript writing and final approval of manuscript: all authors. Financial support: RD. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, 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) 13:239–46. doi: 10.1016/S1470-2045(11)70393-X
2. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* (2010) 362:2380–8. doi: 10.1056/NEJMoa0909530
3. Ricciuti B, Baglivo S, De Giglio A, Chiari R. Afatinib in the first-line treatment of patients with non-small cell lung cancer: clinical evidence and experience. *Ther Adv Respir Dis* (2018) 12:1–13. doi: 10.1177/1753466618808659
4. Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* (2018) 378:113–25. doi: 10.1056/NEJMoa1713137
5. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman J, Chirieac LR, et al. Non-Small Cell Lung Cancer, Version 5.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* (2017) 15:504–35. doi: 10.6004/jnccn.2017.0050
6. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* (2018) 15:81–94. doi: 10.1038/nrclinonc.2017.166
7. Yu HA, Arcila ME, Hellmann MD, Kris MG, Ladanyi M, Riely GJ. Poor response to erlotinib in patients with tumors containing baseline EGFR T790M mutations found by routine clinical molecular testing. *Ann Oncol Off J Eur Soc Med Oncol* (2014) 25:423–8. doi: 10.1093/annonc/mdt573
8. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* (2011) 3:75ra26. doi: 10.1126/scitranslmed.3002003
9. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* (2013) 19:2240–7. doi: 10.1158/1078-0432.CCR-12-2246
10. Rolfo C, Mack PC, Scagliotti GV, Baas P, Barlesi F, Bivona TG, et al. Liquid Biopsy for Advanced Non-Small Cell Lung Cancer (NSCLC): A Statement Paper from the IASLC. *J Thorac Oncol* (2018) 13:1248–68. doi: 10.1016/j.jtho.2018.05.030
11. Alix-Panabières C, Pantel K. Clinical prospects of liquid biopsies. *Nat BioMed Eng* (2017) 1:65. doi: 10.1038/s41551-017-0065
12. Esposito A, Criscitiello C, Locatelli M, Milano M, Curigliano G. Liquid biopsies for solid tumors: Understanding tumor heterogeneity and real time monitoring of early resistance to targeted therapies. *Pharmacol Ther* (2016) 157:120–4. doi: 10.1016/j.pharmthera.2015.11.007
13. Del Re M, Addeo A, Passaro A, Petrini I, van Schaik RH, Danesi R. Circulating tumor DNA and the future of EGFR-mutant lung cancer treatment. *Pharmacogenomics* (2019) 20:1255–7. doi: 10.2217/pgs-2019-0150
14. Lambin P, Rios-Velazquez E, Leijenaar R, Carvalho S, van Stiphout RGPM, Granton P, et al. Radiomics: extracting more information from medical images using advanced feature analysis. *Eur J Cancer* (2012) 48:441–6. doi: 10.1016/j.ejca.2011.11.036
15. Gillies RJ, Kinahan PE, Hricak H. Radiomics: Images Are More than Pictures, They Are Data. *Radiology* (2016) 278:563–77. doi: 10.1148/radiol.2015151169
16. Neri E, Del Re M, Paiar F, Erba P, Cocuzza P, Regge D, et al. Radiomics and liquid biopsy in oncology: the holons of systems medicine. *Insights Imaging* (2018) 9:915–24. doi: 10.1007/s13244-018-0657-7
17. Nair JKR, Saeed UA, McDougall CC, Sabri A, Kovacina B, Raidu BVS, et al. Radiogenomic Models Using Machine Learning Techniques to Predict EGFR Mutations in Non-Small Cell Lung Cancer. *Can Assoc Radiol J* (2020) 17:1–11. doi: 10.1177/0846537119899526
18. Zhao W, Wu Y, Xu Y, Sun Y, Gao P, Tan M, et al. The Potential of Radiomics Nomogram in Non-invasively Prediction of Epidermal Growth Factor Receptor Mutation Status and Subtypes in Lung Adenocarcinoma. *Front Oncol* (2019) 9:1485. doi: 10.3389/fonc.2019.01485
19. Ng C, Pircher A, Augustin F, Kocher F. Evidence-based follow-up in lung cancer? *memo - Mag Eur Med Oncol* (2020) 13:73–7. doi: 10.1007/s12254-020-00575-3
20. Del Re M, Tiseo M, Bordi P, D'Incecco A, Camerini A, Petrini I, et al. Contribution of KRAS mutations and c.2369C > T (p.T790M) EGFR to acquired resistance to EGFR-TKIs in EGFR mutant NSCLC: a study on circulating tumor DNA. *Oncotarget* (2017) 8:13611–9. doi: 10.18632/oncotarget.6957
21. Berenguer R, Pastor-Juan MDR, Canales-Vazquez J, Castro-Garcia M, Villas MV, Mansilla Legorburo F, et al. Radiomics of CT Features May Be Nonreproducible and Redundant: Influence of CT Acquisition Parameters. *Radiology* (2018) 288:407–15. doi: 10.1148/radiol.2018172361
22. Choe J, Lee SM, Do K-H, Lee G, Lee J-G, Lee SM, et al. Deep Learning-based Image Conversion of CT Reconstruction Kernels Improves Radiomics Reproducibility for Pulmonary Nodules or Masses. *Radiology* (2019) 292:365–73. doi: 10.1148/radiol.2019181960
23. Nioche C, Orlhac F, Boughdad S, Reuzé S, Goya-Outi J, Robert C, et al. LIFEx: a freeware for radiomic feature calculation in multimodality imaging to accelerate advances in the characterization of tumor heterogeneity. *Cancer Res* (2018) 78(16):4786–9.
24. Jia T-Y, Xiong J-F, Li X-Y, Yu W, Xu Z-Y, Cai X-W, et al. Identifying EGFR mutations in lung adenocarcinoma by noninvasive imaging using radiomics

features and random forest modeling. *Eur Radiol* (2019) 29:4742–50. doi: 10.1007/s00330-019-06024-y
25. Li S, Ding C, Zhang H, Song J, Wu L. Radiomics for the prediction of EGFR mutation subtypes in non-small cell lung cancer. *Med Phys* (2019) 46:4545–52. doi: 10.1002/mp.13747
26. de Jong EEC, Sanders KJC, Deist TM, van Elmpt W, Jochems A, van Timmeren JE, et al. Can radiomics help to predict skeletal muscle response to chemotherapy in stage IV non-small cell lung cancer? *Eur J Cancer* (2019) 120:107–13. doi: 10.1016/j.ejca.2019.07.023
27. Xu T, Sun J, Bi J. Longitudinal LASSO: Jointly Learning Features and Temporal Contingency for Outcome Prediction. KDD '15: Proceedings of the 21th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining. (2015) 1345–54. doi: 10.1145/2783258.2783403
28. Ma Y. On Inference for Kendall's τ within a Longitudinal Data Setting. *J Appl Stat* (2012) 39:2441–52. doi: 10.1080/02664763.2012.712954
29. Aerts HJWL, Grossmann P, Tan Y, Oxnard GR, Rizvi N, Schwartz LH, et al. Defining a Radiomic Response Phenotype: A Pilot Study using targeted therapy in NSCLC. *Sci Rep* (2016) 6:33860. doi: 10.1038/srep33860
30. Yang X, Dong X, Wang J, Li W, Gu Z, Gao D, et al. Computed Tomography-Based Radiomics Signature: A Potential Indicator of Epidermal Growth Factor Receptor Mutation in Pulmonary Adenocarcinoma Appearing as a Subsolid Nodule. *Oncologist* (2019) 24:e1156–64. doi: 10.1634/theoncologist.2018-0706
31. Liu Y, Kim J, Balagurunathan Y, Li Q, Garcia AL, Stringfield O, et al. Radiomic Features Are Associated With EGFR Mutation Status in Lung Adenocarcinomas. *Clin Lung Cancer* (2016) 17:441–8.e6. doi: 10.1016/j.cllc.2016.02.001
32. Digumarthy SR, Padole AM, Lo Gullo R, Sequist LV, Kalra MK. Can CT radiomic analysis in NSCLC predict histology and EGFR mutation status? *Med (Baltimore)* (2019) 98:e13963. doi: 10.1097/MD.0000000000013963
33. Limkin EJ, Reuzé S, Carré A, Sun R, Schernberg A, Alexis A, et al. The complexity of tumor shape, spiculatedness, correlates with tumor radiomic shape features. *Sci Rep* (2019) 9:4329. doi: 10.1038/s41598-019-40437-5
34. Park BW, Kim JK, Heo C, Park KJ. Reliability of CT radiomic features reflecting tumour heterogeneity according to image quality and image processing parameters. *Sci Rep* (2020) 10:3852. doi: 10.1038/s41598-020-60868-9
35. Lee S-H, Cho H-H, Lee HY, Park H. Clinical impact of variability on CT radiomics and suggestions for suitable feature selection: a focus on lung cancer. *Cancer Imaging Off Publ Int Cancer Imaging Soc* (2019) 19:54. doi: 10.1186/s40644-019-0239-z
36. Kang H, Lee HY, Lee KS, Kim J-H. Imaging-Based Tumor Treatment Response Evaluation: Review of Conventional, New, and Emerging Concepts. *Korean J Radiol* (2012) 13:371–90. doi: 10.3348/kjr.2012.13.4.371
37. Chong Y, Kim J-H, Lee HY, Ahn YC, Lee KS, Ahn M-J, et al. Quantitative CT variables enabling response prediction in neoadjuvant therapy with EGFR-TKIs: are they different from those in neoadjuvant concurrent chemoradiotherapy? *PloS One* (2014) 9:e88598. doi: 10.1371/journal.pone.0088598
38. Kobayashi Y, Fukui T, Ito S, Usami N, Hatooka S, Yatabe Y, et al. How long should small lung lesions of ground-glass opacity be followed? *J Thorac Oncol* (2013) 8:309–14. doi: 10.1097/JTO.0b013e31827e2435
39. Staring M, Pluim JPW, de Hoop B, Klein S, van Ginneken B, Gietema H, et al. Image Subtraction Facilitates Assessment of Volume and Density Change in Ground-Glass Opacities in Chest CT. *Invest Radiol* (2009) 44(2):61–6. doi: 10.1097/RLI.0b013e318197fcb7
40. Del Re M, Cucchiara F, Petrini I, Fogli S, Passaro A, Crucitta S, et al. erbB in NSCLC as a molecular target: current evidences and future directions. *ESMO Open* (2020) 5:e000724. doi: 10.1136/esmoopen-2020-000724
41. Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. *N Engl J Med* (2017) 376:629–40. doi: 10.1056/NEJMoa1612674
42. Wang Z, Yang J-J, Huang J, Ye J-Y, Zhang X-C, Tu H-Y, et al. Lung Adenocarcinoma Harboring EGFR T790M and In Trans C797S Responds to Combination Therapy of First- and Third-Generation EGFR TKIs and Shifts Allelic Configuration at Resistance. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer* (2017) 12:1723–7. doi: 10.1016/j.jtho.2017.06.017
43. Arulananda S, Do H, Musafer A, Mitchell P, Dobrovic A, John T. Combination Osimertinib and Gefitinib in C797S and T790M EGFR-Mutated Non-Small Cell Lung Cancer. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer* (2017) 12:1728–32. doi: 10.1016/j.jtho.2017.08.006
44. Niederst MJ, Hu H, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z, et al. The Allelic Context of the C797S Mutation Acquired upon Treatment with Third-Generation EGFR Inhibitors Impacts Sensitivity to Subsequent Treatment Strategies. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2015) 21:3924–33. doi: 10.1158/1078-0432.CCR-15-0560
45. Kuang Y, Rogers A, Yeap BY, Wang L, Makrigiorgos M, Vetrand K, et al. Noninvasive detection of EGFR T790M in gefitinib or erlotinib resistant non-small cell lung cancer. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2009) 15:2630–6. doi: 10.1158/1078-0432.CCR-08-2592
46. Sundaresan TK, Sequist LV, Heymach JV, Riely GJ, Jänne PA, Koch WH, et al. Detection of T790M, the Acquired Resistance EGFR Mutation, by Tumor Biopsy versus Noninvasive Blood-Based Analyses. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2016) 22:1103–10. doi: 10.1158/1078-0432.CCR-15-1031
47. Oxnard GR, Thress KS, Alden RS, Lawrance R, Paweletz CP, Cantarini M, et al. Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non–Small-Cell Lung Cancer. *J Clin Oncol* (2016) 34:3375–82. doi: 10.1200/JCO.2016.66.7162
48. Kim H, Park CM, Keam B, Park SJ, Kim M, Kim TM, et al. The prognostic value of CT radiomic features for patients with pulmonary adenocarcinoma treated with EGFR tyrosine kinase inhibitors. *PloS One* (2017) 12:e0187500. doi: 10.1371/journal.pone.0187500
49. Sisson BA, Uvalic J, Kelly K, Selvam P, Hesse AN, Ananda G, et al. Technical and Regulatory Considerations for Taking Liquid Biopsy to the Clinic: Validation of the JAX PlasmaMonitor(TM) Assay. *Biomark Insights* (2019) 14:1–11. doi: 10.1177/1177271919826545
50. Grossmann P, Stringfield O, El-Hachem N, Bui MM, Rios Velazquez E, Parmar C, et al. Defining the biological basis of radiomic phenotypes in lung cancer. *Elife* (2017) 6:e23421. doi: 10.7554/eLife.23421
51. Emaminejad N, Qian W, Guan Y, Tan M, Qiu Y, Liu H, et al. Fusion of Quantitative Image and Genomic Biomarkers to Improve Prognosis Assessment of Early Stage Lung Cancer Patients. *IEEE Trans BioMed Eng* (2016) 63:1034–43. doi: 10.1109/TBME.2015.2477688
52. Pesapane F, Suter MB, Rotili A, Penco S, Nigro O, Cremonesi M, et al. Will traditional biopsy be substituted by radiomics and liquid biopsy for breast cancer diagnosis and characterisation? *Med Oncol* (2020) 37:29. doi: 10.1007/s12032-020-01353-1

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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Lung Adenocarcinoma Harboring *EGFR* Kinase Domain Duplication (*EGFR*-KDD) Confers Sensitivity to Osimertinib and Nivolumab: A Case Report

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Background: Kinase domain duplication of EGFR (*EGFR*-KDD) is a rare oncogenic driver alteration and serves as a potential therapeutic target. Its effect on EGFR–tyrosine kinase inhibitors (TKIs), especially the third-generation drug Osimertinib, and immune checkpoint inhibitors (ICIs) remains inconclusive.

Case Presentation: A 45-year old male with lung adenocarcinoma progressed with liver metastasis after receiving pemetrexed and cisplatin as adjuvant chemotherapy. Targeted next-generation sequencing (NGS) identified an *EGFR*-KDD in the resected left upper lung. Icotinib was used in the following treatment and the liver metastasis was found to shrink but the progression-free survival (PFS) only lasted for 4 months with the appearance of right hepatic metastasis. Meantime, the same *EGFR*-KDD was identified in the left hepatic re-biopsy. Afterward, the patient benefited from the third-line therapy of Osimertinib with a PFS as long as 21 months. Then he progressed with enlarged mediastinal lymph nodes, and targeted NGS consistently identified *EGFR*-KDD, as well as a new *RELN* p.G1774E mutation. Given the continually increasing tumor mutation burden (TMB, 3.4 mutation/Mb) and PD-L1 expression-based tumor proportion score (TPS, 1%), Nivolumab was used as the fourth-line salvage therapy, which lead to considerable efficacy, with decreased blood carcinoembryonic antigen (CEA), regressed mediastinal lymph nodes, and reduced liver metastases.

Conclusions: Our case provided direct evidence to support the role of Osimertinib in the treatment of *EGFR*-KDD, as well as added valuable insights into application of immune-based therapeutics in the specific subgroups bearing *EGFR* alteration(s).

Keywords: lung adenocarcinoma, targeted next-generation sequencing, *EGFR*-KDD, Osimertinib, Nivolumab

BACKGROUND

The discovery of oncogenic aberrations in epidermal growth factor receptor (*EGFR*), which commonly occur as 19 exon deletion or L858R mutation, boosts the treatment of targeted therapy in non-small cell lung cancer (NSCLC). As a rare *EGFR* alteration, kinase domain duplication (KDD), firstly identified as a driver aberration and therapeutic target in 2015, is an in-frame duplication in exons that encode the EGFR tyrosine kinase domain (1). The current reported prevalence of *EGFR*-KDD in NSCLCs is 0.04% (2) in European and American and 0.07% (3)~0.12% (4) in East Asian patients, respectively. When with this rare aberration, the response to EGFR-tyrosine kinase inhibitor (TKI) and immune checkpoint inhibitors (ICIs) remains inconclusive. Here we described a case with advanced lung adenocarcinoma harboring *EGFR*-KDD who achieved differentiate response to first and third generation EGFR-TKIs as well as programmed death receptor-1 (PD-1) inhibitor Nivolumab.

CASE PRESENTATION

A 45-year-old male underwent a left upper lobectomy and postoperative pathology revealed invasive stage IIIA lung adenocarcinoma (**Figure 1**). Targeted next- generation sequencing (NGS) with a customized panel (Geneseeq Prime panel) designed to target 425 cancer-specific genes was performed, and four somatic mutations and copy number alterations (CNAs) were identified, including *EGFR*-KDD of exon 18-25 [mutant allele frequency (MAF): 13.5%], *EGFR* amplification (4.5-fold), TP53 p.Y220C (MAF: 37.0%), and *RB1* single copy loss (**Figure 1**). The tumor mutation burden (TMB) was estimated to be 1.1 mutation/Mb. The patient received pemetrexed and cisplatin as adjuvant chemotherapy. Four months later, he progressed with liver metastasis in left lobe (**Figure 1**).

Then, the patient was treated with Icotinib and the metastasis shrunk. Unfortunately, the drug resistance was observed only after 4 months, as evidenced by the fact that previously responsive liver lesion progressed. Left hepatic re-biopsy confirmed metastatic adenocarcinoma and target sequencing (Geneseeq Prime panel) detected the same *EGFR*-KDD (MAF: 4.9%) as well as mutation of *TP53* p.Y220C (MAF: 0.5%) (**Figure 1**). The TMB was calculated as 2.2 mutation/Mb.

Docetaxel and cisplatin were initiated as the second-line therapy. However, the left hepatic metastasis enlarged rapidly after 2 cycles of chemotherapy. The blood carcinoembryonic antigen (CEA) level increased from 9.5 mg/ml (before chemotherapy) to 22.7 mg/ml. Even worse, a right hepatic metastasis appeared soon afterward. Radiofrequency ablation (RFA) of liver was conducted on both of the left and right hepatic metastases, but no reduction in liver lesions was observed, and the CEA level showed a slight increase from 7.3 to 10.3 mg/ml.

Afterward, the patient started taking Osimertinib (80 mg once daily). Encouragingly, both liver lesions showed significant regression (**Figure 1**). One month after initiation of Osimertinib, the CEA level decreased to 5.4 mg/ml, and remained at normal level for 18 months. Moreover, the progression-free survival (PFS) reached 21 months. However, the CEA level increased to 23.1 mg/ml at the 19th month after the initiation of Osimertinib treatment, and 2 months later, the patient progressed with enlarged mediastinal lymph nodes (**Figure 1**) with the CEA level of 73.9 mg/ml. Resampling and targeted sequencing (Geneseeq Prime panel) consistently identified *EGFR*-KDD (MAF: 33.9%), as well as *EGFR* amplification (6.6-fold), *TP53* p.Y220C (MAF: 53.3%), and a new mutation of *RELN* p.G1774E (MAF: 45.4%) (**Figure 1**). The estimated TMB increased to 3.4 mutation/Mb. In addition, the assessment of PD-L1 expression using antibody 28-8 (pharm Dx, Dako's Platform) showed tumor proportion score (TPS) of 1%.

On these bases, the fourth-line salvage therapy using Nivolumab was prescribed and the therapeutic efficacy was

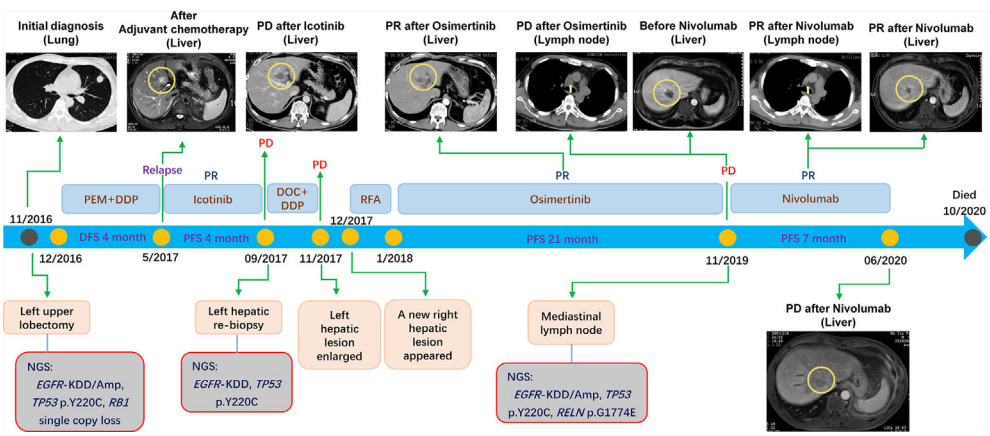

FIGURE 1 | The timeline showing the history of treatment and examinations for the patient under current study.

considerable, as evidenced by the decreased CEA, regressed mediastinal lymph nodes, reduced metastases in both left and right liver (**Figure 1**). Specifically, the CEA level decreased from 143.6 to 41.8 mg/ml one month later. The PFS reached 7 months and no obvious adverse effects were observed. The quality of life was in good status during the Nivolumab treatment. After that, the patient progressed with enlarged liver metastasis. Unfortunately, the patient was also infected with tuberculosis, and his condition took a sharp turn for the worse due to both tumor progression and tuberculosis. The families gave up further treatment and the patient died 4 months later.

DISCUSSION

Classical *EGFR* alterations confer continual activation of protein kinase function and sensitivity to EGFR TKI (5). As a rare oncogenic variant, *EGFR*-KDD is able to form asymmetric homo-dimer and thus activate *EGFR* signaling pathway (1). Several pilot studies confirmed the effectiveness of EGFR-TKIs in NSCLCs harboring *EGFR*-KDD (1, 3, 4, 6–9) (**Table 1**). In our case, the patient bearing *EGFR*-KDD was sensitive to Icotinib and Osimertinib with PFS of 4 and 21 months, respectively. According to previous reports, there are greatly varying efficacies across the first-generation TKIs against *EGFR*-KDD, among which the longest PFS up to 6 years was achieved by Gefitinib (6). In our case, a PFS of only 4 months was observed on Icotinib treatment. In comparison, the third-generation TKI Osimertinib presented an encouraging PFS as long as 21 months. The mechanism underlying such difference in the clinical outcomes is worth investigation. Most recently, our group conducted a molecular dynamics simulation-guided study of *EGFR*-KDD effect on different TKIs (10). It was shown that Gefitinib, as the first-generation EGFR-TKI, suffered from more disturbances in the *EGFR*-KDD binding event than the third-generation EGFR-TKI, Osimertinib. Moreover, Osimertinib was found with higher binding affinity toward *EGFR*-KDD than Gefitinib. These results provide the structural basis of evidence that Osimertinib, compared to the first generation TKI, is able to bind and thus inhibit *EGFR*-KDD with more potency.

ICIs serve as a new standard of care for advanced NSCLCs with no *EGFR* mutation. However, the study concerning the therapeutic effect of ICIs on *EGFR* mutant lung cancer is sparse and the outcome seems not optimistic. Previous evidence showed that compared with chemotherapy, there was no superiority in terms of overall survival (OS) when ICIs were used as the second line treatment among *EGFR*-mutant subgroup (11). The Atlantic trial demonstrated the overall response rate (ORR) of ICIs was 9.8% with averaged PFS of only 1.9 months among *EGFR*+/*ALK*+ individuals (12). Cho et al. also suggested *EGFR* mutant NSCLC patients benefited less from ICIs treatment (13). Similar results were found in Italian Nivolumab expanded access program, which showed ORR of 8.8% among *EGFR* mutant subgroup (14). Consistently, a retrospective study by Hastings et al., concluded with an ORR of 9.9% for ICIs treatment in *EGFR*-mutant NSCLCs (15). Despite these, it is worth to mention that adding atezolizumab to standard-of-care Bevacizumab and chemotherapy increased PFS and OS benefit among the *EGFR*-mutant patients (16).

Of note, *EGFR* aberrations were found to be corelated with significantly increased rate of tumor growth after ICIs monotherapy (17). Pilot study suggested that *EGFR* pathway activation resulted in a signature of immuno-suppression, driving immune escape (18). Furthermore, certain *EGFR* aberrations, including *EGFR* 19Del and T790M, are considered to be related to ICIs-induced hyper-progressive disease (HPD). Recently, our group reported a patient with *EGFR* 20 exon insertion and MYC amplification who suffered from HPD after treatment of Nivolumab, resulting in rapid death in 2 months (19). *Ex vivo* study exhibited that PDX model carrying *EGFR* 21 exon L858R mutation also mirrored the clinical observation of HPD following Nivolumab treatment (20). Here, our patient significantly benefited from ICIs treatment in the presence of *EGFR*-KDD. Emerging evidence showed *EGFR* 20 exon insertion mutation tended to present higher PD-L1 expression than classic *EGFR* mutation, and in turn, was related with improved outcome in response to ICIs (21). Case series showed patients harboring *EGFR* G719X mutations along with high PD-L1 expression conferred sensitivity to ICIs-based treatment (22). The aforementioned Hastings' study (15) further investigated the

TABLE 1 | Summary of response to EGFR-TKIs in NSCLCs harboring EGFR-KDDs.

Study	Population	Best response to TKIs	TKIs, response and PFS
Our case	East Asian	PR	Icotinib, PR, 4m; Osimertinib, PR, 21m
Gallant et al. (1)	American	PR	Afatinib, PR, 10m
Baik et al. (6)	American	PR	Gefitinib, PR, 6y; Erlotinib, PR, 3y
Wiest et al. (7)	Germany	PR	Afatinib, PR, NA
Zhu et al. (8)	East Asian	SD	Icotinib, SD, 11m (Not reach)
Xu et al. (9)	East Asian	PR	Afatinib, PR, NA
Wang et al. (3)	East Asian	SD	Icotinib, SD, NA
Wang et al. (4)	East Asian	PD	Erlotinib, PD, 2m; Osimertinib, PD, 2m
	East Asian	PR	Gefitinib, PR, 5m; Afatinib, PD, 2m Osimertinib, PR, 4m (Not reach)
	East Asian	SD	Gefitinib, SD, 11m
	East Asian	PR	Icotinib+apatinib, PR, 4m (Not reach)
	East Asian	PD	Gefitinib, PD, 3m Erlotinib, PD, 5m

efficacy differences between various *EGFR* subtypes. Therapeutic efficacy was best for *EGFR* G719 but worst for *EGFR* L861Q. For common mutant subgroups, *EGFR* 19Del showed worse response than *EGFR* L858R. On contrary, negative association between *EGFR* alteration and HPD was observed from two independent cohorts (23, 24). These data suggest the responsiveness to ICIs in patients with *EGFR* aberrations may differ in terms of specific aberrant type. To overcome the low response rates to PD-1 pathway blockade, highly specific patient(s) with *EGFR*-driven tumor should be screened out for ICIs monotherapy and combinations.

There are several limitations in the present study. Owing to the coverage of currently used sequencing panel, it was not available to explore the molecular basis of mechanism underlying the drug resistance observed in the clinic, e.g., Icotinib and Osimertinib. According to previous studies, there exist varying conclusions as to the efficacies of the first-generation EGFR-TKIs in the treatment of *EGFR*-KDD, as well as the uncertain response to ICIs among *EGFR* mutant tumors. In this context, our current case report only provided an example but not guidance for the clinical intervention, which clearly demands more extensive investigations.

Collectively, our case provides direct evidence to support the role of Osimertinib in the treatment of *EGFR*-KDD, as well as added valuable insights into application of immune-based therapeutics in the specific subgroups bearing *EGFR* alteration(s).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Hwa Mei Hospital, University of Chinese Academy of Sciences. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

GZ designed the entire study. JL carried out patient clinical management and sample collection. JY, RC, and GD analyzed the data. JL and JY wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Gallant JN, Sheehan JH, Shaver TM, Bailey M, Lipson D, Chandramohan R, et al. EGFR Kinase Domain Duplication (EGFR-KDD) Is a Novel Oncogenic Driver in Lung Cancer That Is Clinically Responsive to Afatinib. *Cancer Discov* (2015) 5(11):1155–63. doi: 10.1158/2159-8290.CD-15-0654
2. Konduri K, Gallant JN, Chae YK, Giles FJ, Gitlitz BJ, Gowen K, et al. EGFR Fusions as Novel Therapeutic Targets in Lung Cancer. *Cancer Discov* (2016) 6 (6):601–11. doi: 10.1158/2159-8290.CD-16-0075
3. Wang W, Xu C, Zhu Y, Fang M, Zhuang W, Chen Y, et al. An EGFR KDD Data in the Chinese NSCLC Population and the Response to EGFR-TKIs: A Multicenter Study. *J Thoracic Oncol* (2018) 13(12):S1081–1. doi: 10.1016/j.jtho.2018.10.102
4. Wang J, Li X, Xue X, Ou Q, Wu X, Liang Y, et al. Clinical outcomes of EGFR kinase domain duplication to targeted therapies in NSCLC. *Int J Cancer* (2019) 144(11):2677–82. doi: 10.1002/ijc.31895
5. Carey KD, Garton AJ, Romero MS, Kahler J, Thomson S, Ross S, et al. Kinetic analysis of epidermal growth factor receptor somatic mutant proteins shows increased sensitivity to the epidermal growth factor receptor tyrosine kinase inhibitor, erlotinib. *Cancer Res* (2006) 66(16):8163–71. doi: 10.1158/0008-5472.CAN-06-0453
6. Baik CS, Wu D, Smith C, Martins RG, Pritchard CC. Durable Response to Tyrosine Kinase Inhibitor Therapy in a Lung Cancer Patient Harboring Epidermal Growth Factor Receptor Tandem Kinase Domain Duplication. *J Thorac Oncol* (2015) 10(10):e97–9. doi: 10.1097/JTO.0000000000000586
7. Wiest G, Kohlhäufel M, Müller J, Lakis S, Wesseler C, Mariotti E, et al. Detection of an EGFR kinase domain duplication in a lung adenocarcinoma patient by liquid biopsy using hybrid capture based next generation sequencing. *New Oncol* (2016) Abstract #291. Available at:https://www.newoncology.com/fileadmin/content/Download_pdfs/Wiest_et_al_DGHO-2016-Abstract_final.pdf.
8. Zhu YC, Wang WX, Xu CW, Tan QH, Li JY, Zhuang W, et al. Lung adenocarcinoma patient with an EGFR kinase domain duplication (KDD) and the response to Icotinib. *J Thorac Dis* (2018) 10(5):E359–63. doi: 10.21037/jtd.2018.04.162
9. Xu C, Wang W, Zhang Q, Wu B, Zhuang W, Zhu Y, et al. A Novel Oncogenic Driver in a Lung Adenocarcinoma Patient Harboring an EGFR-KDD and Response to Afatinib. *J Thoracic Oncol* (2018) 13(10):S1032–3. doi: 10.1016/j.jtho.2018.08.1991
10. Jin R, Li J, Jin Z, Lu Y, Shao YW, Li W, et al. Osimertinib confers potent binding affinity to EGFR Kinase Domain Duplication. *Int J Cancer* (2019) 145 (10):2884–5. doi: 10.1002/ijc.32617
11. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* (2015) 373(17):1627–39. doi: 10.1056/NEJMoa1507643
12. Garassino MC, Cho BC, Kim JH, Mazieres J, Vansteenkiste J, Lena H, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. *Lancet Oncol* (2018) 19(4):521–36. doi: 10.1016/S1470-2045(18)30144-X
13. Cho JH, Jung HA, Lee SH, Ahn JS, Ahn MJ, Park K, et al. Impact of EGFR mutation on the clinical efficacy of PD-1 inhibitors in patients with pulmonary adenocarcinoma. *J Cancer Res Clin* (2019) 145(5):1341–9. doi: 10.1007/s00432-019-02889-0
14. Garassino MC, Gelibter AJ, Grossi F, Chiari R, Soto Parra H, Cascinu S, et al. Italian Nivolumab Expanded Access Program in Nonsquamous Non-Small Cell Lung Cancer Patients: Results in Never-Smokers and EGFR-Mutant Patients. *J Thorac Oncol* (2018) 13(8):1146–55. doi: 10.1016/j.jtho.2018.04.025

15. Hastings K, Yu H, Wei W, Sanchez-Vega F, DeVeaux M, Choi J, et al. EGFR mutation subtypes and response to immune checkpoint blockade treatment in non-small cell lung cancer. *Ann Oncol* (2019) 30(8):1311–20. doi: 10.1093/annonc/mdz141
16. Mok TSK, Socinski MA, Reck M, Jotte RM, Lim DWT, Cappuzzo F, et al. IMpower150: An exploratory analysis of efficacy outcomes in patients with EGFR mutations. *Ann Oncol* (2018) 29(suppl_9):ix173–8. doi: 10.1093/annonc/mdy483.008
17. Kato S, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after Immunotherapy: Analysis of Genomic Alterations Associated with Accelerated Growth Rate. *Clin Cancer Res* (2017) 23 (15):4242–50. doi: 10.1158/1078-0432.CCR-16-3133
18. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov* (2013) 3(12):1355–63. doi: 10.1158/1535-7163.TARG-13-B290
19. Huang X, Xia L, Lan F, Shao YW, Li W, Xia Y. Treatment of Nivolumab Results in Hyperprogressive Disease in a Patient Harboring EGFR Exon 20 Insertion and MYC Amplification. *J Thorac Oncol* (2019) 14(9):e189–91. doi: 10.1016/j.jtho.2019.04.009
20. Lo Russo G, Moro M, Sommariva M, Cancila V, Boeri M, Centonze G, et al. Antibody-Fc/FcR Interaction on Macrophages as a Mechanism for Hyperprogressive Disease in Non-small Cell Lung Cancer Subsequent to PD-1/PD-L1 Blockade. *Clin Cancer Res* (2019) 25(3):989–99. doi: 10.1158/1078-0432.CCR-18-1390
21. Cardona AF, Rojas L, Zatarain-Barron ZL, Freitas HC, Granados ST, Castillo O, et al. CLICaP, EGFR exon 20 insertion in lung adenocarcinomas among Hispanics (geno1.2-CLICaP). *Lung Cancer* (2018) 125:265–72. doi: 10.1016/j.lungcan.2018.10.007
22. Taniguchi Y, Tamiya A, Ishii S, Atagi S. Effect of pembrolizumab on patients harboring uncommon epidermal growth factor receptor mutations. *Ann Oncol* (2018) 29(5):1331–3. doi: 10.1093/annonc/mdy087
23. Kim CG, Kim KH, Pyo KH, Xin CF, Hong MH, Ahn BC, et al. Hyperprogressive disease during PD-1/PD-L1 blockade in patients with non-small-cell lung cancer. *Ann Oncol* (2019) 30(7):1104–13. doi: 10.1093/annonc/mdz123
24. Ferrara R, Mezquita L, Texier M, Lahmar J, Audigier-Valette C, Tessonnier L, et al. Hyperprogressive Disease in Patients With Advanced Non-Small Cell Lung Cancer Treated With PD-1/PD-L1 Inhibitors or With Single-Agent Chemotherapy. *JAMA Oncol* (2018) 4(11):1543–52. doi: 10.1001/jamaoncol.2018.3676

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Changes of Brain Structure in Patients With Metastatic Non-Small Cell Lung Cancer After Long-Term Target Therapy With EGFR-TKI

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Purpose: Epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) therapy is the routine treatment for patients with metastatic non-small cell lung cancer (NSCLC) harboring positive EGFR mutations. Patients who undergo such treatment have reported cognitive decline during follow-up. This study, therefore, aimed to evaluate brain structural changes in patients receiving EGFR-TKI to increase understanding of this potential symptom.

Method: The medical records of 75 patients with metastatic NSCLC (without brain metastasis or other co-morbidities) who received EGFR-TKI therapy from 2010 to 2017 were reviewed. The modified Scheltens Visual Scale and voxel-based morphometry were used to evaluate changes in white matter lesions (WML) and gray matter volume (GMV), respectively.

Results: The WML scores were higher at the 12-month [8.65 ± 3.86; 95% confidence interval (CI), 1.60–2.35; $p < 0.001$] and 24-month follow-ups (10.11 ± 3.85; 95% CI, 2.98–3.87; $p < 0.001$) compared to baseline (6.68 ± 3.64). At the 24-month follow-up, the visual scores were also significantly higher in younger patients (3.89 ± 2.04) than in older patients (3.00 ± 1.78; $p = 0.047$) and higher in female patients (3.80 ± 2.04) than in male patients (2.73 ± 1.56; $p = 0.023$). Additionally, significant GMV loss was observed in sub-regions of the right occipital lobe (76.71 voxels; 95% CI, 40.740–112.69 voxels), left occipital lobe (93.48 voxels; 95% CI, 37.48–149.47 voxels), and left basal ganglia (37.57 voxels; 95% CI, 21.58–53.57 voxels) (all $p < 0.005$; cluster-level false discovery rate < 0.05).

Conclusions: An increase in WMLs and loss of GMV were observed in patients with metastatic NSCLC undergoing long-term EGFR-TKI treatment. This might reflect an unknown side-effect of EGFR-TKI treatment. Further prospective studies are necessary to confirm our findings.

Keywords: non-small cell lung cancer, epidermal growth factor receptor-tyrosine kinase inhibitor, white matter lesion, gray matter, MRI

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INTRODUCTION

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide. About 40–50% of Asian patients with NSCLC harbor epidermal growth factor receptor (EGFR) mutations, and distant metastases are observed in nearly 40% of these patients at initial diagnosis (1). The approval of gefitinib, the first-generation EGFR tyrosine kinase inhibitor (TKI), led to the development of molecular targeted therapy for lung cancer (2). Prospective phase III trials have established that EGFR-TKIs are superior to chemotherapy for patients harboring an EGFR mutation (3–6). Therefore, EGFR-TKIs have been recommended as first-line treatment for such patients in clinical guidelines (7, 8). Consequently, EGFR-TKI therapy has been routinely prescribed for patients with EGFR mutations worldwide.

The known side effects of targeted therapy with EGFR-TKIs include rashes, diarrhea, hepatic impairment, mucositis, and interstitial pneumonia (9). However, during routine follow-ups, patients with NSCLC undergoing EGFR-TKI treatment in our department, the Oncology Department of West-China Hospital, have reported cognitive decline after starting EGFR-TKI treatment, a side effect that has not previously been reported. Meanwhile, a recent study evaluated the neuropsychological performance of patients with NSCLC who underwent targeted therapy and reported that depression and/or anxiety were correlated with the treatment, but details regarding drug utilization in this study were not clear (10). Potential reasons for these neuropsychiatric symptoms remain unknown.

Many studies have reported that epidermal growth factor (EGF) is involved in the main biological pathway of neurodevelopment and repair of nerve injury by promoting the proliferation, regeneration, and development of neurons (11–13). Thus, the inhibition of the EGF pathway caused by EGFR-TKIs could negatively influence the differentiation, maturation, and rehabilitation of neural cells, which may lead to chronic cognitive function impairments. In this context, exploring structural changes in patients' brains before and after EGFR-TKI treatment could help in determining the causes of cognitive decline.

It has been reported that white matter lesions (WMLs) are an indication of cognitive impairment, especially in the elderly and patients with specific comorbidities (14, 15). The loss of gray matter volume (GMV) has also been associated with mild cognitive impairment, including memory loss and attention and language dysfunction (16, 17). MRI-based studies have confirmed that WMLs and gray matter atrophy (GMA) could be the primary imaging correlates of early dementia and mild cognitive impairment in several chronic diseases, including Parkinson's disease, diabetes mellitus, and Alzheimer's disease, among others (18–20).

Patients with metastatic NSCLC who undergo EGFR-TKI treatment at our hospital receive routine MRIs. This allowed us to investigate the potential impact of long-term EGFR-TKI therapy on patients' brains, which has not yet been evaluated. Thus, for the first time, we collected brain MRI images of patients with NSCLC to investigate changes in WMLs and GMV.

Abbreviations: EGFR-TKI, Epidermal growth factor receptor-tyrosine kinase inhibitor; NSCLC, Non-small cell lung cancer; WML, white matter lesion; GMV, gray matter volume; MRI, magnetic resonance imaging; GMA, gray matter atrophy; VBM, voxel-based morphometry; FDR, false discovery rate; FLAIR, fluid attenuated inversion recovery.

MATERIALS AND METHODS

This retrospective study was approved by the ethics committee at West China Hospital, Sichuan University and was in full accordance with the International Conference of Harmonization Good Clinical Practice Guidelines. Informed consent was obtained during follow-up, and for those who were lost to follow-up (e.g., death, emigration), we were granted permission by the ethics committee at West China Hospital, Sichuan University for an informed consent waiver.

Patients with pathologically-confirmed metastatic NSCLC between 2010 and 2017 in West China Hospital, Sichuan University were included. The inclusion criteria were as follows: a) positive for EGFR mutation (19 exon deletion or 21 exon L858R mutation detected by amplification refractory mutation system, differential display-polymerase chain reaction, or next generation sequencing), b) received first-generation EGFR-TKIs (gefitinib, erlotinib, or icotinib, according to the Chinese Food and Drug Administration's approval); and c) brain MRI data available during follow-up at our hospital. Exclusion criteria were as follows: a) suffering from a neurological or psychiatric disease; b) presence of comorbidities that might influence the patient's brain structure (e.g., type 2 diabetes, hypertension, chronic kidney disease); c) identification of brain metastases during MRI at baseline or anytime during the evaluation period; d) substantial abuse including alcohol or narcotics; and e) any other concurrent systematic therapy (e.g., chemotherapy, anti-angiogenetic therapy, or immunotherapy).

Treatment and Follow-Up

After EGFR-TKI treatment initiation, objective assessments of all the eligible patients were recorded every 3 months according to the RESIST criteria (21). The patients underwent a brain MRI every 6 months when no neural symptoms or physical signs were observed; otherwise, a brain MRI was performed immediately to rule-out brain metastasis. The EGFR-TKI dose was modified according to the instructions specific to each drug. The duration of the follow-up period was ≥ 24 months.

Image Acquisition

Image data were retrospectively collected from our hospital's PACS (Picture Archiving and Communication System). Since there are multiple MRI scanners at our hospital, T2-FLAIR (fluid attenuated inversion recovery) images for WML assessments were acquired using different scanners from two different manufacturers (GE and Siemens) and with varying magnetic field intensities (1.5-T, n = 154 person-time; and 3.0-T, n = 82 person-time). Since the WML diagnostic features were high signal spots in the T2-FLAIR sequence, the evaluation of visual scores were not affected by the different scanners. A high resolution T1WI (1.0 mm/slice) MRI was not routinely used for all the patients due to its extra charge;

however, we screened the image data for available high resolution T1WI MRIs before and after about 1-year treatment, which were obtained by the same 3.0 T MRI system. The images of 21 patients (13 by Siemens scanners; 8 by GE scanners) were determined to be suitable for GMV analysis.

Definitions and Acquisition of White Matter Lesion and Gray Matter Volume

WMLs are regions of white matter that have an abnormal white matter fiber tract, which present as hyperintense regions on MRI T2-FLAIR sequence images with different shapes categorized as: periventricular caps, rims, or halos; subcortical multiple punctuates or patchy lesions; and partially or completely confluent lesions. They are often divided into two broad categories, namely, periventricular WMLs (attached to the ventricular system) and deep WMLs (located at the subcortical white matter area) (22, 23).

WMLs from axial T2-FLAIR images were evaluated using the modified Scheltens Visual Scale (SVS; **Supplementary Material, Section A**), with which periventricular and white matter hyperintensities are semi-quantitively rated. The modified SVS is used to rate WMLs in the periventricular region on a 7-point scale (0–6) and those in the subcortical region on a 25-point scale (0–24) according to the size and number of lesions (24, 25). The modified SVS was used to evaluate the WMLs seen on T2-FLAIR images at baseline and after 12 months and 24 months of EGFR-TKI therapy (one representative patient is shown in **Supplementary Material, Section B**).

Gray matter is a major component of brain parenchyma and consists of neuronal cell bodies, neuropils (dendrites and axons), glial cells, and capillaries. It is distinguished from white matter in that it contains numerous neuronal cell bodies and relatively few myelinated axons. GMV is determined using optimized voxel-based morphometry (VBM) (26), a computational neuroanatomy method that measures the number of voxels of gray matter after separating them from white matter using T1WI.

In this study, the GMV analysis was performed using the Statistical Parametric Mapping Package (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/), with the VBM-based diffeomorphic anatomical registration using the exponentiated lie algebra (VBM-DARTEL) toolbox (27). First, the high-resolution images of all the patients before and after treatment were segmented into gray matter, white matter, and cerebrospinal fluid. Second, the segmented gray matter was smoothed to create the primary DARTAL template. After 18 iterative operations with raw segmented gray matter images, six templates were created and the sixth template, which is considered to have maximum accuracy and sensitivity (28), was registered to the Montreal Neurological Institute space. All the patients' GMVs before and after treatment were obtained for further statistical analysis.

Statistical Analysis

The normality of the WML SVS scores was tested using the Shapiro-Wilk test. Paired sample t-tests were used to test differences in the WML scores before and after treatment. Independent sample t-tests were used to evaluate variations in WMLs from baseline according to sex, age, type of mutation, and type of TKI therapy.

Changes in GMV before and after EGFR-TKI therapy were tested using paired sample t-tests (uncorrected p value < 0.001), and corrected using a false discovery rate (FDR) of $p < 0.05$ at cluster level and peak level. A cluster level test takes into account the size of the cluster that consists of adjacent voxels as test objects, and a cluster size above the voxel's threshold has a statistical significance suitable for small samples. For the peak level test, each voxel is regarded as an independent test subject, meaning a much stricter FDR is required for viability.

RESULTS

Patient Characteristics

The median age of all 75 patents with NSCLC was 60 years (range, 38–71 years) and the majority were women (49/75, 65.3%). Forty-one (54.7%) and 34 (45.3%) patients were positive for EGFR 19 exon deletion and 21 exon L858R transformation, respectively. The median duration of intracranial progression-free survival was 32.0 months (range, 23.0–89.0 months). For the 21 patients included in the GMV analysis, the median age was 59 years (range, 43–70 years) and the majority were female (12/21, 57.1%) (**Table 1**).

Changes in White Matter Lesions

The SVS scores of the WMLs at baseline varied between 0 and 17.00, and increased at the 12-month and 24-month follow-ups (**Figure 1A**). Compared to baseline (6.68 ± 3.64), the scores were significantly higher at the 12-month [8.65 ± 3.86; 95% confidence interval (CI) 1.56–2.35, $p < 0.001$] and 24-month (10.11 ± 3.85; 95% CI 2.98–3.87, $p < 0.001$) follow-ups (**Figure 1B**).

Sub-group analyses showed that the SVS scores at baseline were significantly higher in older patients (> 60 years) than in younger patients (≤ 60 years) (7.62 ± 3.56 vs. 5.67 ± 3.49, respectively; $p = 0.019$). Compared to older patients, the younger patients also showed significantly higher SVS scores at the 24-month follow-

TABLE 1 | Baseline characteristics of patients in present study.

Baseline characteristics	Patients for WML analysis, number	Patients for GMV analysis, number
Age (years)	60 (range of 38–71)	59 (range of 43–70)
Sex (%)		
Male	26 (34.7)	9 (42.9)
Female	49 (65.3)	12 (57.1)
ECOG performance status (%)		
0	38 (50.7)	10 (47.6)
1	37 (49.3)	11 (52.4)
EGFR mutation (%)		
19 del	41 (54.7)	12 (57.1)
21L858R	34 (45.3)	9 (42.9)
EGFR-TKI (%)		
Gefitinib	30 (40)	13 (61.9)
non-Gefitinib	45 (60)	8 (38.1)
Progression-free survival (months)	32 (range of 23-89)	

Data are median (IQR) or number (%); ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

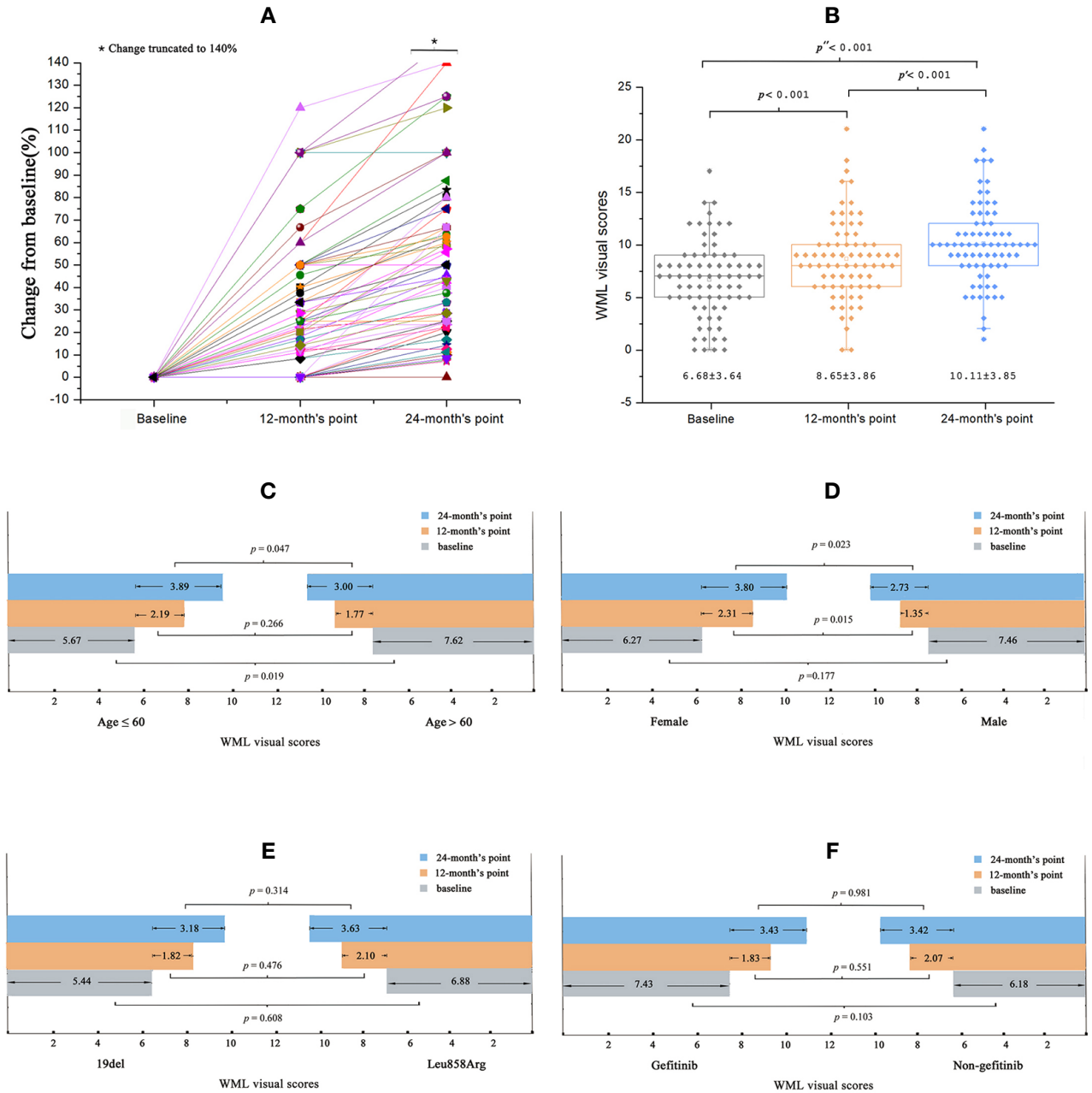

FIGURE 1 | Baseline and changes of WML visual scores in all patients. **(A)** During the treatment of EGFR-TKI, the patient's WML visual scores increased progressively. **(B)** Comparing to the baseline scores, the scores were significantly changed at the 12-months' point and changed more obviously at the 24-months' point. **(C)** Sub-group analysis: the baseline WML visual score was significantly higher in elder patients. The WML visual scores increased more significantly at the 24-month point in younger patients than elder patients. **(D)** There was no difference between the baseline WML visual scores among female and male patients, while the visual scores increased more significantly in female patients at the 12-month's point and 24-month's point than that in the male patients. **(E, F)** No significant differences of the WML visual scores was observed between EGFR mutation types or EGFR-TKIs the patients receiving.

up (3.00 ± 1.78 vs. 3.89 ± 2.04, respectively; p = 0.047) but not at the 12-month (2.19 ± 1.69 vs. 1.77 ± 1.56, respectively; p = 0.266) follow-up (**Figure 1C**). For the SVS scores at baseline, no significant differences were found between female and male patients (6.27 ± 3.37 vs. 7.46 ± 4.05, respectively; p = 0.177). However, SVS scores were significantly higher for female patients than male patients at the 12-month (2.31 ± 1.66 vs. 1.35 ± 1.44, respectively; p = 0.015) and 24-month (3.80 ± 2.04 vs. 2.73 ± 1.56, respectively; p = 0.023) follow-ups (**Figure 1D**). No significant differences in SVS scores were observed according to the different EGFR mutations or EGFR-TKI treatments at baseline or at the 12-month or 24-month follow-ups (all p > 0.05; **Figures 1E, F**).

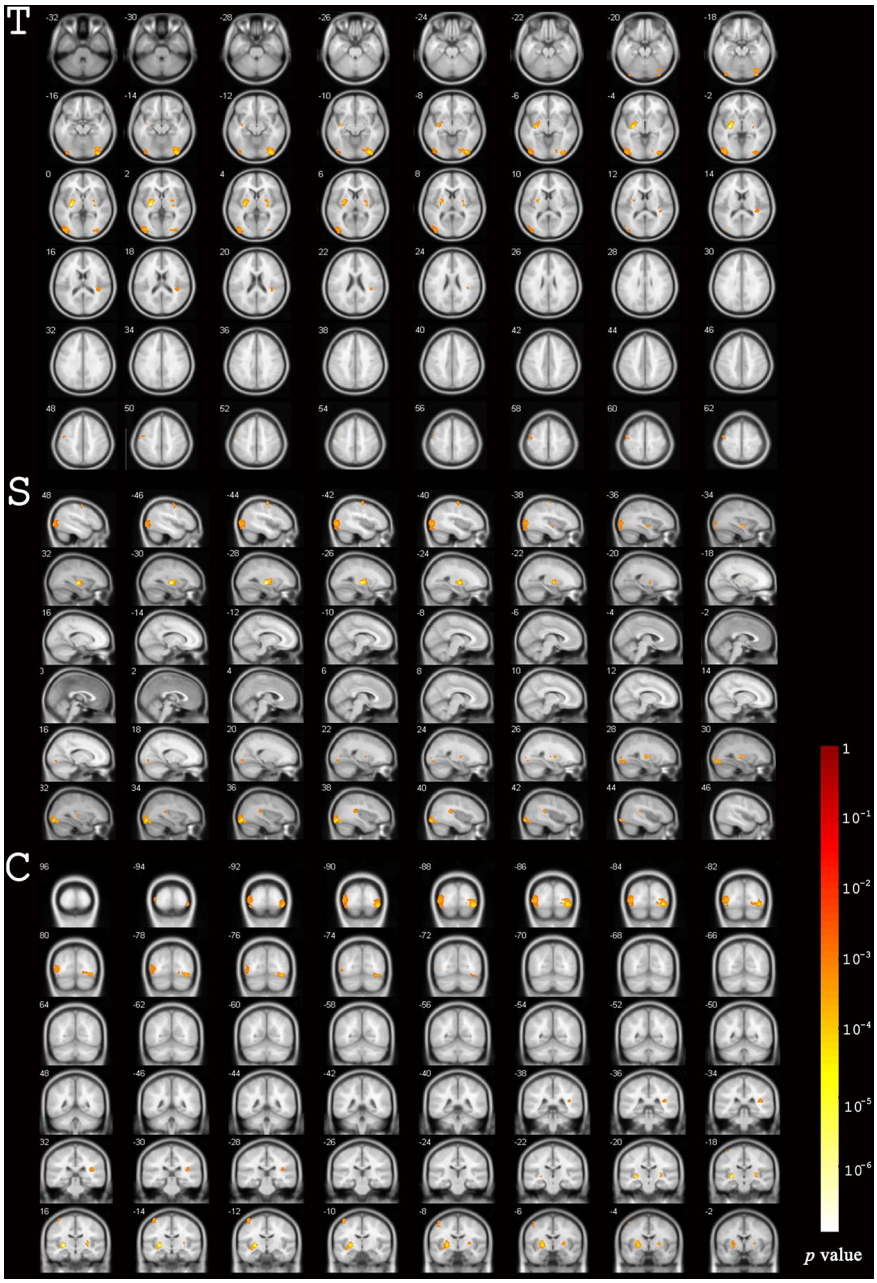

FIGURE 2 | Differences in gray matter volume before and after EGFR-TKI treatment. Significant differences were identified using voxel-based paired sample T-test.

Changes in Gray Matter Volume

The total GMV of patients was 673.8 ± 58.5 cm^3 and 667.6 ± 60.3 cm^3 at baseline and the 12-month follow-up, respectively. Uncorrected GMV loss ($p < 0.001$) was identified in brain MRIs (**Figure 2**). The total voxel values were clearly lower after EGFR-TKI treatment than at baseline in three main clusters: the sub-regions of the middle and inferior occipital cortex (1,697 voxels); the right middle and inferior occipital cortex extending to the lingual gyrus and entorhinal cortex (1,660 voxels); and the left lentiform nucleus, which included the putamen and pallidum (1,145 voxels) (**Table 2**). Mild GMV loss was observed in the left precentral gyrus, which included part of Brodmann area 6 (321 voxels); two independent clusters at the right lentiform nucleus (142 and 125 voxels), both of which included parts of the putamen and pallidum; and the right insula extending to the superior temporal gyrus (269 voxels).

After the cluster-level FDR was corrected to $p < 0.05$, significant GMV loss remained in three main clusters: the right middle and inferior occipital cortex extending to the lingual and entorhinal gyrus, the left lentiform nucleus, and the middle and

TABLE 2 | Sub-regions with GMV atrophy of patients treated with EGFR-TKIs.

Cluster	Total voxels	Main brain sub-regions					
		Region1	Voxels	Region2	Voxels	Region3	Voxels
1	1697	Occipital_Mid_L	1153	Occipital_Inf_L	377	–	–
2	1660	Occipital_Inf_R	705	Occipital_Mid_R	573	Fusiform_R	247
3	1145	Lentiform Nucleus_L	782	Putamen_L	604	Pallidum_L	174
4	321	Precentral Gyrus_L	211	Brodmann area 6	96	–	–
5	269	Insula_R	109	Temporal_Sup_R	84	–	–
6	142	Lentiform Nucleus_R	115	Putamen_R	106	Pallidum_R	9
7	125	Lentiform Nucleus_R	123	Putamen_R	79	Pallidum_R	45

GMV, gray mater volume.

inferior occipital cortex (p = 0.012, p = 0.003, and p = 0.003, respectively) (**Table 3**, **Figure 3**). The peak-level FDR-corrected analysis showed no significant difference between GMV atrophies at baseline and the 12-month follow-up in these three main clusters (p = 0.054, p = 0.653, and p = 0.885, respectively). The other four clusters were not significant after cluster-level or peak-level FDR correction (all p > 0.05).

DISCUSSION

Previous studies have revealed that the most common adverse effects of EGFR-TKI therapy are skin rashes (31.4%), diarrhea (14.2%), pruritus (6.7%), and hepatic toxicity (3.8%) (9). For the first time, using a series of brain MRIs, significant worsening of WMLs and GMA were observed among patients with advanced-stage NSCLC receiving long-term EGFR-TKI treatment.

The existing literature suggests that EGF is expressed in the cortical plate during neural development, promoting the neurite outgrowth of cortical neurons (11), and the EGFR pathway is linked to multiple nerve cell events, such as proliferation, differentiation, and apoptosis (12). Recently, an EGFR pathway-regulating compound (yhhu-3792) was reported to induce cognitive impairment in mice by inhibiting neural pathways in the hippocampus (29). This microvascular anomaly is believed to be one the principle causes of WMLs, as EGFR and vascular EGF pathways are closely related and share common downstream signaling pathways (30). Therefore, long-term EGFR-TKI therapy could potentially induce WMLs and GMA.

GMA and WMLs are reportedly associated with a rapid or excessive decline in global cognitive performance, executive function, and processing efficiency (17, 31, 32). Furthermore, structural changes in the brain are strongly correlated with a patient's cognitive status (17, 33, 34). In the present study, the reduction in gray matter was nearly 0.92% after 12 months of EGFR-TKI treatment, while a large cross-sectional study (479 healthy participants) using SPM8 to measure age-related changes in GMV reported a global loss of 0.57% per year (35). Even though directly comparing these two studies is not sufficient evidence, it may be used to some extent to demonstrate the difference between patients receiving EGFR-TKI therapy and healthy people, especially given the difficulty in collecting longitudinal image data of healthy people along aging. Physical frailty-related GMV loss has been observed in the bilateral frontal and occipital cortices, while cognitive impairment-related GMV loss has been observed in the bilateral frontal, occipital, and temporal cortices (17). Similarly, we observed significant GMV loss mainly in sub-regions of the bilateral occipital lobes and the left basal ganglia. Unfortunately, no cognitive function tests (e.g., Wechsler adult intelligence scale-III, mini-mental state examination, color trails test, etc.) were performed on the patients who received EGFR-TKI treatment at baseline or during follow-up. We could not, therefore, analyze possible cognitive impairments caused by the changes in brain structure in the patients.

Previous studies on healthy elderly populations have reported either no significant progression of WMLs associated with age (36), or a mild increase of 0.2 to 0.4% per year (37), In this study, however, we observed significant deterioration of WMLs in patients with NSCLC who received EGFR-TKI therapy. The baseline WML score was significantly higher in older patients than in younger patients (p = 0.019), which is in accordance with recent reported findings (14, 22). At the 24-month follow-up, however, the WML scores

TABLE 3 | Statistic information of sub-regions with GMV reduction in patients treated with EGFR-TKIs.

Cluster	Cluster-level			Peak-level			X (mm)	Y (mm)	Z (mm)
	Equivk	*p* (un-corr)	*p* (FDR-corr)	T	*p* (uncorr)	*p* (FDR-corr)			
1	1145	0.001	0.012	6.88	0.000	0.054	–29	–17	–2
2	1660	0.000	0.003	4.84	0.000	0.653	35	–86	–11
3	1697	0.000	0.003	3.78	0.001	0.885	–39	–83	0
4	321	0.06	0.387	3.83	0.001	0.885	–44	–12	65
5	142	0.194	0.825	4.09	0.000	0.885	29	–18	5
6	125	0.222	0.825	3.69	0.001	0.885	26	–5	2
7	269	0.082	0.424	3.50	0.001	0.885	41	–33	17

GMV, gray matter volume; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; Equivk, equivalent voxels k; Corr, corrected; FDR, false discovery rate; X, Y, Z, x,y and z axis. respectively.

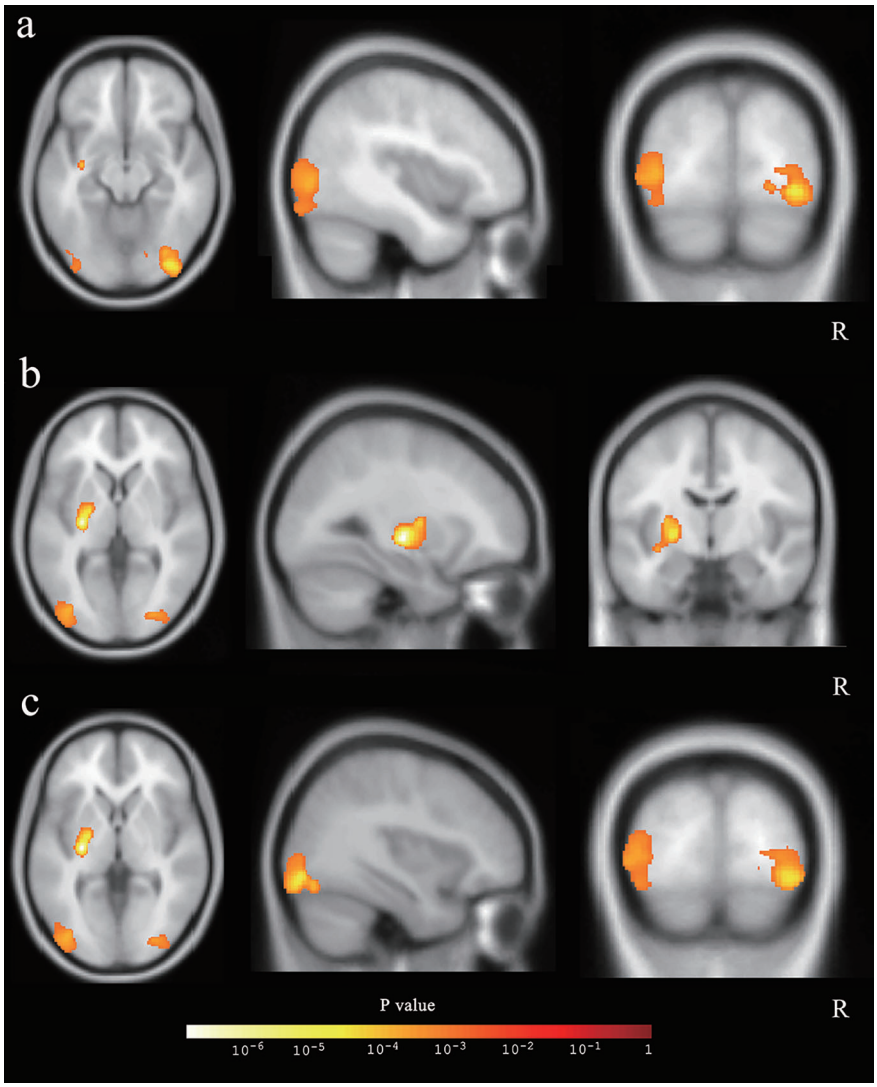

FIGURE 3 | The three main clusters with reduced gray matter volume. The three clusters were showed in axial, sagittal, and coronal positions: **(A)** the right middle and inferior occipital cortex, **(B)** the left lentiform nucleus, and **(C)** the middle and inferior occipital cortex.

were significantly higher in younger patients than older ones ($p = 0.047$), indicating that the younger patients were more sensitive to therapy. This could be explained by the higher proliferation rate of neural stem and progenitor cells in younger compared to older people, leading to a higher chance they would be affected by EGFR-TKI therapy. Women reportedly have significantly more WMLs relative to white matter volume than men (2.8 vs. 2.4%, respectively; $p < 0.001$) (38), and a greater marked progression of subcortical WML and incident lacunar infarcts than men (39). Similarly, the changes in WML scores in this study were more significant in female patients than in male patients at the 12-month and 24-month follow-ups. However, there were no significant differences in WML scores based on the type of EGFR mutation or EGFR-TKI therapy received.

For dementia research, visual rating scores from routine brain MRIs, which are recognized as a practical and inexpensive way to improve diagnostic accuracy, are recommended for assessing cognitive impairment. Prior to the application of more advanced image analysis techniques in clinical practice, visual rating scales were widely used and recommended for evaluating patients clinically with suspected dementia and was considered a diagnostic criterion for numerous types of dementia (22, 23, 40). Compared to several other visual rating scales, including the Fazekas scale (41), Rotterdam Scan Study (RSS) scale (37), modified SVS (24), Koedam posterior atrophy (PA) scale (42), and Prins scale (25), which were developed specifically to rate the vulnerability of brain regions to atrophy in different types of dementias, the SVS has been recommended for observing longitudinal changes in WMLs for chronic diseases and their relationship to clinical variables (43–45). GMV changes have been evaluated using the VBM-toolbox on SPM8, and the reliability of extracting quantitative brain metrics, such as GMV,

in clinical-quality MRIs has been justified (46). Uncorrected voxel-based statistics increase the sensitivity as FDR increases (28). In this study, the FDR-corrected analysis was performed to minimize bias. Thus, the theoretical foundation and MRI analysis performed in the present study were relatively robust and have been validated by a large number of studies worldwide.

However, this study also had some limitations. First, the retrospective nature and relatively limited sample size of this study restricts its value in routine practice. Additionally, since a cognitive analysis was not conducted, interpreting the potential relationship between changes in the brain structure and cognition could not be assessed, even though significant worsening of the brain structure was observed. Second, data on mental status (depression or anxiety) before and during follow-up were not available, and subtle mental symptoms are difficult for patients to detect themselves. However, concomitant neuropsychiatric symptoms, such as depression or anxiety, in patients with NSCLC who undergo target therapy may exist, as reported previously (10). Third, systemic chemotherapy might have affected patients' cognitive function (47, 48). Previous evaluations of cognition and brain structure changes in patients with lung cancer have demonstrated cognitive impairments after chemotherapy (49). Consequently, patients with lung cancer who undergo chemotherapy could not be used as controls. Additionally, no healthy volunteers were analyzed as controls in this retrospective setting. Therefore, the brain alterations observed in the present study should be interpreted cautiously unless they are validated by prospective data sets.

CONCLUSION

This retrospective structural analysis of a series of brain MRIs showed significant worsening of WMLs and GMA in patients with advanced-stage NSCLC undergoing chronic EGFR-TKI treatment, which may indicate that this could be an unknown side-effect of EGFR-TKI treatment. Further prospective studies are being designed to more definitively determine the effects of long-term EGFR-TKI treatment on cognitive ability.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of West China Hospital, Sichuan University. The patients/participants provided their written informed consent to participate in this study. For patients were lost to follow-up (e.g., death, emigration), the informed consent waiver was permitted by the ethics committee at West China Hospital, Sichuan University.

AUTHOR CONTRIBUTIONS

YG conceived and designed the study. BY, CL, and YG collected, analyzed, and interpreted the data and drafted the article. BoT and BiT contributed to the evaluation of WML. MY, LZ, YiZ, JZ, MH, FP, YoL, YX, YaZ, XZ, JX, YaL, YW, ZL, YouL, and SL interpreted the data and revised the paper critically. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.573512/full#supplementary-material

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA Cancer J Clin* (2018) 68:7–30. doi: 10.3322/caac.21442
2. Ciardiello F, Tortora G. A Novel Approach in the Treatment of Cancer: Targeting the Epidermal Growth Factor Receptor. *Clin Cancer Res* (2001) 7:2958.
3. Holleman MS, van Tinteren H, Groen HJ, Al MJ, Uyl-de Groot CA. First-line tyrosine kinase inhibitors in EGFR mutation-positive non-small-cell lung cancer: a network meta-analysis. *Onco Targets Ther* (2019) 12:1413–21. doi: 10.2147/OTT.S189438
4. Loong HH, Kwan SS, Mok TS, Lau YM. Therapeutic Strategies in EGFR Mutant Non-Small Cell Lung Cancer. *Curr Treat Pptions Oncol* (2018) 19:58. doi: 10.1007/s11864-018-0570-9
5. Tan DS, Yom SS, Tsao MS, Pass HI, Kelly K, Peled N, et al. The International Association for the Study of Lung Cancer Consensus Statement on Optimizing Management of EGFR Mutation-Positive Non-Small Cell Lung Cancer: Status in 2016. *J Thorac Oncol* (2016) 11:946–63. doi: 10.1016/j.jtho.2016.05.008
6. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, 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) 13:239–46. doi: 10.1016/S1470-2045(11)70393-X
7. David S, Ettinger DEW, Aggarwal C. *NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer NCCN guidelines Version 4*. National Comprehensive Cancer Network (2019).
8. Wu YL, Planchard D, Lu S, Sun H, Yamamoto N, Kim DW, et al. Pan-Asian adapted Clinical Practice Guidelines for the management of patients with metastatic non-small-cell lung cancer: a CSCO-ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS. *Ann Oncol* (2019) 30:171–210. doi: 10.1093/annonc/mdy554
9. Shah RR, Shah DR. Safety and Tolerability of Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors in Oncology. *Drug Saf* (2019) 42:181–98. doi: 10.1007/s40264-018-0772-x

10. Kang HL, Chen VC, Hung WL, Hsiao HP, Wang WH. Preliminary comparison of neuropsychological performance in patients with non-small-cell lung cancer treated with chemotherapy or targeted therapy. *Neuropsychiatr Dis Treat* (2019) 15:753–61. doi: 10.2147/NDT.S194642
11. Goldshmit Y, Greenhalgh CJ, Turnley AM. Suppressor of cytokine signalling-2 and epidermal growth factor regulate neurite outgrowth of cortical neurons. *Eur J Neurosci* (2004) 20:2260–6. doi: 10.1111/j.1460-9568.2004.03698.x
12. Kim IS, Koppula S, Park SY, Choi DK. Analysis of Epidermal Growth Factor Receptor Related Gene Expression Changes in a Cellular and Animal Model of Parkinson's Disease. *Int J Mol Sci* (2017) 18(12):430. doi: 10.3390/ijms 18020430
13. Furnari FB, Cloughesy TF, Cavenee WK, Mischel PS. Heterogeneity of epidermal growth factor receptor signalling networks in glioblastoma. *Nat Rev Cancer* (2015) 15:302–10. doi: 10.1038/nrc3918
14. Bolandzadeh N, Davis JC, Tam R, Handy TC, Liu-Ambrose T. The association between cognitive function and white matter lesion location in older adults: a systematic review. *BMC Neurol* (2012) 12:126. doi: 10.1186/1471-2377-12-126
15. Kloppenborg RP, Nederkoorn PJ, Geerlings MI, van den Berg E. Presence and progression of white matter hyperintensities and cognition: a meta-analysis. *Neurology* (2014) 82:2127–38. doi: 10.1212/WNL.0000000000000505
16. Li M, Meng Y, Wang M, Yang S, Wu H, Zhao B, et al. Cerebral gray matter volume reduction in subcortical vascular mild cognitive impairment patients and subcortical vascular dementia patients, and its relation with cognitive deficits. *Brain Behav* (2017) 7:e00745. doi: 10.1002/brb3.745
17. Chen YS, Chen HL, Lu CH, Chen MH, Chou KH, Tsai NW, et al. Reduced lateral occipital gray matter volume is associated with physical frailty and cognitive impairment in Parkinson's disease. *Eur Radiol* (2019) 29:2659–68. doi: 10.1007/s00330-018-5855-7
18. Prange S, Metereau E, Thobois S. Structural Imaging in Parkinson's Disease: New Developments. *Curr Neurol Neurosci Rep* (2019) 19:50. doi: 10.1007/s11910-019-0964-5
19. Dore V, Villemagne VL, Bourgeat P, Fripp J, Acosta O, Chetelat G, et al. Cross-sectional and longitudinal analysis of the relationship between Abeta deposition, cortical thickness, and memory in cognitively unimpaired individuals and in Alzheimer disease. *JAMA Neurol* (2013) 70:903–11. doi: 10.1001/jamaneurol.2013.1062
20. Goldstein FC, Mao H, Wang L, Ni C, Lah JJ, Levey AI. White Matter Integrity and Episodic Memory Performance in Mild Cognitive Impairment: A Diffusion Tensor Imaging Study. *Brain Imaging Behav* (2009) 3:132–41. doi: 10.1007/s11682-008-9055-y
21. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* (2000) 92:205–16. doi: 10.1093/jnci/92.3.205
22. Kim KW, MacFall JR, Payne ME. Classification of white matter lesions on magnetic resonance imaging in elderly persons. *Biol Psychiatry* (2008) 64:273–80. doi: 10.1016/j.biopsych.2008.03.024
23. Valdes Hernandez MDC, Chappell FM, Munoz Maniega S, Dickie DA, Royle NA, Morris Z, et al. Metric to quantify white matter damage on brain magnetic resonance images. *Neuroradiology* (2017) 59:951–62. doi: 10.1007/s00234-017-1892-1
24. Scheltens P, Barkhof F, Leys D, Pruvo JP, Nauta JJP, Vermersch P, et al. A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. *J Neurol Sci* (1993) 114:7–12. doi: 10.1016/0022-510X(93)90041-V
25. Prins ND, van Straaten EC, van Dijk EJ, Simoni M, van Schijndel RA, Vrooman HA, et al. Measuring progression of cerebral white matter lesions on MRI: visual rating and volumetrics. *Neurology* (2004) 62:1533–9. doi: 10.1212/01.WNL.0000123264.40498.B6
26. Davies RR, Scahill VL, Graham A, Williams GB, Graham KS, Hodges JR. Development of an MRI rating scale for multiple brain regions: comparison with volumetrics and with voxel-based morphometry. *Neuroradiology* (2009) 51:491–503. doi: 10.1007/s00234-009-0521-z
27. Ashburner J, Friston KJ. Voxel-based morphometry–the methods. *NeuroImage* (2000) 11:805–21. doi: 10.1006/nimg.2000.0582
28. Radua J, Canales-Rodriguez EJ, Pomarol-Clotet E, Salvador R. Validity of modulation and optimal settings for advanced voxel-based morphometry. *NeuroImage* (2014) 86:81–90. doi: 10.1016/j.neuroimage.2013.07.084
29. Lu H, Cheng G, Hong F, Zhang L, Hu Y, Feng L. A Novel 2-Phenylamino-Quinazoline-Based Compound Expands the Neural Stem Cell Pool and Promotes the Hippocampal Neurogenesis and the Cognitive Ability of Adult Mice. *Stem Cells (Dayton Ohio)* (2018) 36:1273–85. doi: 10.1002/stem.2843
30. Lichtenberger BM, Tan PK, Niederleithner H, Ferrara N, Petzelbauer P, Sibilia M. Autocrine VEGF signaling synergizes with EGFR in tumor cells to promote epithelial cancer development. *Cell* (2010) 140:268–79. doi: 10.1016/j.cell.2009.12.046
31. Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Jolles J, Koudstaal PJ, et al. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain J Neurol* (2005) 128:2034–41. doi: 10.1093/brain/awh553
32. Fujishima M, Maikusa N, Nakamura K, Nakatsuka M, Matsuda H, Meguro K. Mild cognitive impairment, poor episodic memory, and late-life depression are associated with cerebral cortical thinning and increased white matter hyperintensities. *Front Aging Neurosci* (2014) 6:306. doi: 10.3389/fnagi.2014.00306
33. Garcia-Garcia R, Cruz-Gomez AJ, Mangas-Losada A, Urios A, Forn C, Escudero-Garcia D, et al. Reduced resting state connectivity and gray matter volume correlate with cognitive impairment in minimal hepatic encephalopathy. *PloS One* (2017) 12:e0186463. doi: 10.1371/journal.pone.0186463
34. Brickman AM, Zahodne LB, Guzman VA, Narkhede A, Meier IB, Griffith EY, et al. Reconsidering harbingers of dementia: progression of parietal lobe white matter hyperintensities predicts Alzheimer's disease incidence. *Neurobiol Aging* (2015) 36:27–32. doi: 10.1016/j.neurobiolaging.2014.07.019
35. Yu T, Korgaonkar MS, Grieve SM. Gray Matter Atrophy in the Cerebellum-Evidence of Increased Vulnerability of the Crus and Vermis with Advancing Age. *Cerebellum (London England)* (2017) 16:388–97. doi: 10.1007/s12311-016-0813-x
36. Matsusue E, Sugihara S, Fujii S, Ohama E, Kinoshita T, Ogawa T. White matter changes in elderly people: MR-pathologic correlations. *Magn Reson Med Sci* (2006) 5:99–104. doi: 10.2463/mrms.5.99
37. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry* (2001) 70:9–14. doi: 10.1136/jnnp.70.1.9
38. Fatemi F, Kantarci K, Graff-Radford J, Preboske GM, Weigand SD, Przybelski SA, et al. Sex differences in cerebrovascular pathologies on FLAIR in cognitively unimpaired elderly. *Neurology* (2018) 90:e466–73. doi: 10.1212/WNL.0000000000004913
39. van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. *Stroke* (2008) 39:2712–9. doi: 10.1161/STROKEAHA.107.513176
40. Kipps CM, Davies RR, Mitchell J, Kril JJ, Halliday GM, Hodges JR. Clinical significance of lobar atrophy in frontotemporal dementia: application of an MRI visual rating scale. *Dement Geriatr Cogn Disord* (2007) 23:334–42. doi: 10.1159/000100973
41. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol* (1987) 149:351–6. doi: 10.2214/ajr.149.2.351
42. Koedam EL, Lehmann M, van der Flier WM, Scheltens P, Pijnenburg YA, Fox N, et al. Visual assessment of posterior atrophy development of a MRI rating scale. *Eur Radiol* (2011) 21:2618–25. doi: 10.1007/s00330-011-2205-4
43. Gouw AA, van der Flier WM, van Straaten EC, Pantoni L, Bastos-Leite AJ, Inzitari D, et al. Reliability and sensitivity of visual scales versus volumetry for evaluating white matter hyperintensity progression. *Cerebrovasc Dis (Basel Switzerland)* (2008) 25:247–53. doi: 10.1159/000113863
44. Harper L, Fumagalli GG, Barkhof F, Scheltens P, O'Brien JT, Bouwman F, et al. MRI visual rating scales in the diagnosis of dementia: evaluation in 184 post-mortem confirmed cases. *Brain J Neurol* (2016) 139:1211–25. doi: 10.1093/brain/aww005
45. Robinson-Papp J, Navis A, Dhamoon MS, Clark US, Gutierrez-Contreras J, Morgello S. The Use of Visual Rating Scales to Quantify Brain MRI Lesions in Patients with HIV Infection. *J Neuroimaging* (2018) 28:217–24. doi: 10.1111/jon.12466

46. Adduru VR, Michael AM, Helguera M, Baum SA, Moore GJ. Leveraging Clinical Imaging Archives for Radiomics: Reliability of Automated Methods for Brain Volume Measurement. *Radiology* (2017) 284:862–9. doi: 10.1148/radiol.2017161928
47. Matsos A, Johnston IN. Chemotherapy-induced cognitive impairments: A systematic review of the animal literature. *Neurosci Biobehav Rev* (2019) 102:382–99. doi: 10.1016/j.neubiorev.2019.05.001
48. Hermelink K, Buhner M, Sckopke P, Neufeld F, Kaste J, Voigt V, et al. Chemotherapy and Post-traumatic Stress in the Causation of Cognitive Dysfunction in Breast Cancer Patients. *J Natl Cancer Inst* (2017) 109(10): djx057. doi: 10.1093/jnci/djx057
49. Simó M, Root JC, Vaquero L, Ripollés P, Jové J, Ahles T, et al. Cognitive and brain structural changes in a lung cancer population. *J Thorac Oncol* (2015) 10:38–45. doi: 10.1097/JTO.0000000000000345

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Treatment Options of First-Line Tyrosine Kinase Inhibitors and Subsequent Systemic Chemotherapy Agents for Advanced EGFR Mutant Lung Adenocarcinoma Patients: Implications From Taiwan Cancer Registry Cohort

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Objectives: Large-scale, population-based real-world studies on the treatment outcomes of first-line tyrosine kinase inhibitors (TKIs) and subsequent systemic chemotherapy agents for lung adenocarcinoma (with activating epidermal growth factor receptor [EGFR] mutations) remain limited.

Materials and Methods: From March 2014 to December 2016, patients with advanced lung adenocarcinoma, identified from the Taiwan Cancer Registry were included in this study if they received any of the three TKIs as first-line treatment. The primary outcome was overall survival (OS). The secondary outcome was time-to-treatment discontinuation (TTD).

Results: A total of 4,889 patients (median age: 67 years and two-thirds with distant metastasis) were recruited (1,778 gefitinib, 1,599 erlotinib, and 1,512 afatinib users). A 1:1 propensity score (PS)-matched cohorts of 1,228 afatinib/erlotinib and 1054 afatinib/gefitinib was created. After PS matching, it was found that afatinib was not associated with better OS (afatinib vs. erlotinib, HR: 0.96, 95% CI: 0.86–1.07; afatinib vs. gefitinib, HR: 0.91, 95% CI: 0.81–1.02). In the subgroup analysis, afatinib demonstrated a survival benefit in patients with active smoking (afatinib vs. erlotinib, HR: 0.69, 95% CI: 0.51–0.93; afatinib vs. gefitinib, HR: 0.67, 95% CI: 0.48–0.94) and ECOG > 1 (afatinib vs. erlotinib, HR: 0.79, 95% CI: 0.63–0.99; afatinib vs. gefitinib, HR: 0.78, 95% CI: 0.62–0.98). A total of 41.1% (n = 1992) of first-line TKI users received subsequent chemotherapy. Among the three TKI groups, pemetrexed usage was associated with better OS compared with other chemotherapy agents, with the exception of gemcitabine in the afatinib and gefitinib groups. Pemetrexed and gemcitabine had the longest TTD of 3–4 months.

Conclusions: Among patients with *EGFR* mutant lung adenocarcinoma, afatinib use may not provide longer OS compared with first-generation TKIs. Afatinib may be preferably considered among patients with active smoking and should not be withheld among those with worse performance status. With 40% of patients receiving subsequent chemotherapy, pemetrexed may be the preferred agent, while gemcitabine can be a reasonable alternative.

Keywords: lung adenocarcinoma, gefitinib, erlotinib, afatinib, epidermal growth factor receptor mutation, subsequent therapy

INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths in the 21st century (1, 2). Adenocarcinoma is the major histological type of non-small cell lung cancer (NSCLC), but the standard care for patients with metastatic NSCLC has shifted from traditional platinum-based doublets to precision targeted therapy to the driver genes with mutations, such as the epidermal growth factor receptor (*EGFR)*, anaplastic lymphoma kinase (*ALK*), *ROS-1*, and *BRAF* (3, 4). Targeting lung adenocarcinoma with *EGFR* mutations among Asians is important because they have a significantly higher prevalence of the *EGFR* mutation compared with the Caucasians (5–7). Multiple generations of EGFR tyrosine kinase inhibitors (TKIs) have been effective as first-line therapy for advanced *EGFR*-mutant lung adenocarcinoma patients (8–12).

Gefitinib, erlotinib, and afatinib are widely prescribed first-line TKIs worldwide. All of them provide robust and similar effects in advanced *EGFR*-mutant lung adenocarcinoma patients (11, 13, 14). Although afatinib has minimal clinical significance in progression-free survival (PFS) compared with gefitinib (median PFS 11.0 vs. 10.9 months, respectively) in the first-line setting (15), it did not show improved overall survival (OS) compared with gefitinib (13, 14). Recently, several real-world studies have investigated the characteristics and clinical effectiveness of these three EGFR TKIs administered in advanced lung adenocarcinoma patients (16–20). However, the conclusions from these studies may not provide convincing evidence for clinical practice because of their limited case numbers, discrepant recruitment time, disproportional populations in which TKIs were used, and lack of information on subsequent therapy after first-line EGFR TKI failure (16–20).

Prolonging cancer patients' OS is a major goal of all cancer treatments, and understanding the optimal treatment sequences is a key factor that allows patients to live longer. p.T790M in the *EGFR* gene is the most common acquired resistance mechanism following first-line TKI treatment (21), and osimertinib proved to be effective in patients with the *EGFR* p.T790M mutation as a standard second-line treatment (22). Owing to the unavailability of osimertinib in some situations, for cases without acquired p.T790M or accessible tumor tissues for re-biopsy, chemotherapy remains an important subsequent therapy after first-line TKI (23, 24). Furthermore, only, few studies have investigated the optimal regimen of chemotherapy as second-line treatment in patients who are p.T790M negative or have an unknown acquired resistance mechanism after first-line TKI failure (25, 26).

This study, therefore, aimed to investigate the treatment sequences and clinical outcomes of treatment-naïve, EGFR-mutant advanced lung adenocarcinoma patients receiving TKIs in a real-world, population-based setting. Additionally, we explored the prognostic factors of TKI users and treatment durations of individuals after they underwent subsequent chemotherapy. Our results were informative with respect to clinical decision-making.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the Institutional Review Board (IRB) committee of the National Taiwan University Hospital Hsinchu Branch (NTUH-HC REC: 105-040-E). The IRB waived the requirement of informed consent because the utilized data were de-identified in this study.

Study Design and Population

This study used the Taiwan Cancer Registry (TCR), which is a population-based registry system that includes 90% of all cancer patients in Taiwan (27, 28). We identified patients with advanced lung adenocarcinoma, including those in stages IIIb and IV (M1a and M1b) from the TCR during March 2014 and December 2016. Patients were included if they received gefitinib, erlotinib, or afatinib as first-line treatment within 60 days after diagnosis. Patients were excluded if they received chemotherapy prior to first-line TKI therapy. In Taiwan, gefitinib, erlotinib, and afatinib have been sequentially reimbursed by the Taiwan National Health Insurance (NHI) as first-line therapy for advanced EGFR-mutant lung adenocarcinoma since June 2011, November 2013, and May 2014, respectively (29). Considering that the study period could be an important confounding variable, which could strongly influence the outcome by improving lung cancer treatment, we truncated our dataset to the date of afatinib approval for use (May 2014) in Taiwan.

Abbreviations: BMI, body mass index; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; HR, hazard ratio; NHI, National Health Insurance; OS, overall survival; PFS, progression-free survival; SD, standard deviation; TKI, tyrosine kinase inhibitor; TTD, time-to-treatment discontinuation.

During the study period, physicians applied for TKI use prior to TKI initiation, and the application was reviewed by the experts of the NHI committee. *EGFR* mutation results, clinical images, pathology, and clinical information were provided along with the application for TKIs. For every 3 months, physicians provided the imaging evidence of partial remission or stable disease to allow further TKI use (https://www.nhi.gov.tw/).

After TKI use, the recruited cohort was then followed, and mortality was confirmed using mortality data from the Department of Statistics, Taiwan. Underlying diseases, TKI use, and duration were ascertained from the Taiwan NHI database (28, 30, 31). Using the linkage between the above-mentioned databases, we longitudinally followed our cohort patients till December 31, 2017.

Data Collection and Definition

TNM staging data at diagnosis available in the TCR were made according to the International Association for the Study of Lung Cancer 7th edition lung cancer staging system (32). Accordingly, the metastasis (M) category of stage IV lung cancer was subdivided into M1a for cases with intra-thoracic metastases (including pleural seeding, malignant pleural/pericardial effusion, and contralateral pulmonary nodules) and M1b for cases with distant metastases (32). The patients' performance status was represented as the Eastern Cooperative Oncology Group (ECOG) scores (33). Meanwhile, the Charlson comorbidity index (CCI) was used to assess the patients' comorbidities using the NHI claims data (34), but malignancy-related score was excluded (cancer-free CCI) as it was previously reported (27). Hospital levels were classified hierarchically into medical centers, regional hospitals, and local hospitals (35). The defining codes for lung adenocarcinoma in the TCR in the NHI are summarized in **Supplementary Table S1**. We also categorized second-line chemotherapy agents into pemetrexed, gemcitabine, paclitaxel, docetaxel, vinorelbine, and others.

Statistical Analyses

We used proportions or means to describe the demographics and clinical characteristics of the patients. Categorical variables were analyzed using chi-square tests. One-way ANOVA or Student's t-test was applied for continuous variables. The cohort entry date was that of diagnosis. OS, the primary outcome, was defined as the period from the date of diagnosis to death. Participants were censored if they were still alive at the end of the study period (December 31, 2017). The secondary outcome was time-to-treatment discontinuation (TTD), which was defined as the interval between the date of TKI treatment or chemotherapy initiation and discontinuation. The BMIs were missing for 8% of the patients, but we still considered it important to include BMIs in the final analysis. We imputed the missing values of BMI by age and sex with linear regression methods.

The propensity score (PS) for the probability of TKI administration was derived using a logistic regression model, which included potential confounders such as, age, sex, ECOG, BMI, cancer staging, smoking, alcoholism, CCI, year of TKI use, and hospital level. A 1:1 matched cohort group of afatinib/erlotinib and afatinib/gefitinib was created. Variables that remained significantly different after PS matching were further adjusted in the final model. In this study, only the categorical BMI groups were imbalanced among the different TKI groups, while the absolute values of BMIs were not different between the matched groups.

Subgroup analysis was performed among the different age groups, BMI groups, ECOG groups, sexes, smoking habit, and stages (IIIb, M1a, and M1b). We also compared the OS of five common chemotherapy agents, including pemetrexed, gemcitabine, vinorelbine, paclitaxel, and docetaxel, among the three TKI groups using multivariate Cox regression.

We used SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) for data analyses. A p value of < 0.05 on a two-sided test was considered statistically significant.

RESULTS

Demographics and Clinical Variables of the Study

Between May 2014 and December 2016, a total of 4,889 advanced lung adenocarcinoma patients with the *EGFR* mutation receiving TKIs (including 1,778 gefitinib, 1,599 erlotinib, and 1,512 afatinib) as first-line therapy were included in our study (**Figure 1**). The baseline characteristics of the enrolled patients are summarized in **Table 1**. The median age of all patients was 67 years, and the majority was female (n = 3,083, 63.1%). Meanwhile, 4,669 (95.5%) patients had stage IV disease. Most eligible patients had relatively good performance status (ECOG $\leqq$ 1: n = 3,780, 77.3%) and had never smoked (n = 3,684, 75.3%).

Regarding the comparison among afatinib, gefitinib, and erlotinib users' characteristics, afatinib users were significantly younger (64.4 ± 11.4 vs. 70.9 ± 12.0 vs. 66.6 ± 11.7 years, $p < 0.0001$), with higher BMIs (23.6 ± 3.54 vs. 23.0 ± 3.71 vs. 23.4 ± 4.10 kg/m^2, $p = 0.0028$) and better ECOG performance status (ECOG $\leqq$ 1, 83.9% vs. 71.9% vs. 77.1%, $p < 0.0001$), were more active smokers (13.2% vs. 9.6% vs. 12.1%, $p = 0.0004$), and had lower CCIs (0.60 ± 1.67 vs. 0.93 ± 2.11 vs. 0.82 ± 2.12. $p < 0.0001$) (**Table 1**).

OS and TTD of Advanced Lung Adenocarcinoma Patients Harboring *EGFR* Mutations and Receiving TKIs as First-Line Therapy

Among all patients, mortality was 48.5% (n = 734) of afatinib, 57.0% (n = 912) of erlotinib, and 62.5% (n = 1112) of gefitinib users. The Kaplan–Meier curve of the three TKIs and OS is illustrated in **Supplementary Figure 1A**. While less than 50% of afatinib users had mortality events, we calculated the OS of users recruited during 2014 and 2015. The OS (mean [median] ± standard deviation, SD) of the gefitinib, erlotinib, and afatinib users recruited during 2014 and 2015 was 20.2 (20) ± 11.7, 20.7 (22) ± 11.7, and 21.8 (24) ± 11.0 months, respectively. The TTD (mean [median] ± SD) of the gefitinib, erlotinib, and afatinib groups was 12.8 (11) ± 9.6, 12.2 (11) ± 9.0, and 13.6 (13) ± 8.9 months, respectively (**Supplementary Figure 1B**).

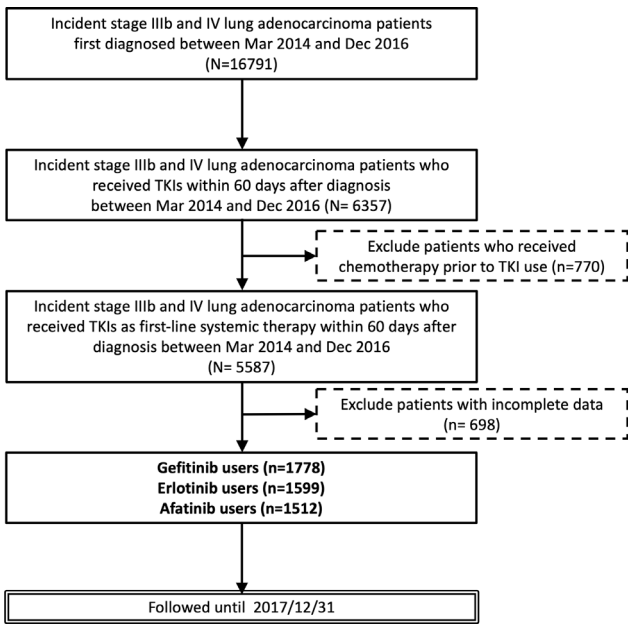

FIGURE 1 | Flow chart of patient recruitment.

Comparing OS and TTD of Matched Afatinib/Erlotinib and Afatinib/Gefitinib Users

In the PS 1:1 matched cohort, two cohorts of 1,228/1,228, afatinib/erlotinib users and 1,054/1,054, afatinib/gefitinib users were assembled. The variables were balanced between the matched groups, while the categorical BMI group remained unbalanced within the groups. The average of BMI, however, remained balanced between the groups (erlotinib vs. afatinib, 23.5 ± 4.3 vs. 23.6 ± 3.6, p = 0.6102; gefitinib vs. afatinib, 23.4 ± 3.5 vs. 23.3 ± 3.9, p = 0.6242) (**Table 1**).

In the Cox regression analysis, afatinib was not associated with better OS compared with erlotinib (HR: 0.96, 95% CI: 0.86–1.07, p = 0.4673, **Figure 2A**) or gefitinib (HR: 0.91, 95% CI: 0.81–1.02, p = 0.0971, **Figure 2B**). In contrast, patients with afatinib still had longer TTD compared with erlotinib (HR: 0.89, 95% CI: 0.81–0.98, p = 0.0176) (**Supplementary Figure 2A**) and gefitinib (HR: 0.84, 95% CI: 0.76–0.92, p = 0.0004) (**Supplementary Figure 2B**).

Matched Subgroups Analysis of Afatinib Versus Erlotinib/Gefitinib

A forest plot of the matched subgroups analysis comparing OS between afatinib and first-generation TKIs users is illustrated in **Figure 3**. Interestingly, we found that afatinib use reached statistical significance among the subgroups of active smokers (afatinib vs. erlotinib, HR: 0.69, 95% CI: 0.51–0.93, p = 0.0151; afatinib vs. gefitinib, HR: 0.67, 95% CI: 0.48–0.94, p = 0.022) and ECOG > 1 (afatinib vs. erlotinib, HR: 0.79, 95% CI: 0.63–0.99, p = 0.0375; afatinib vs. gefitinib, HR: 0.78, 95% CI: 0.62–0.98, p = 0.0319). When comparing afatinib and gefitinib, afatinib was also associated with better OS among those with normal BMI (HR: 0.84, 95% CI: 0.73–0.97, p = 0.0149) and M1b staging (HR: 0.83, 95% CI: 0.72–0.95, p = 0.0085).

Meanwhile, TTD between afatinib and first-generation TKI users was also compared in the matched subgroups analysis (**Supplementary Figure 3**). Afatinib use could provide longer TTD among the subgroups of patients aged 45–65 years, with normal BMIs (18–24), M1b staging, female sex, any performance status, and active and non-smokers.

Subsequent Therapies After First-Line EGFR TKI Treatment

Forty-five patients, including 16 afatinib, 18 erlotinib, and 11 gefitinib users were still receiving TKI at the end of the follow-up. In total, 1,992 of 4,844 patients (41.1%) received subsequent treatment as second-line therapy. A total of 729 patients (41.3%) in the gefitinib group, 661 patients (41.8%) in the erlotinib group, and 602 (40.2%) in the afatinib group received subsequent chemotherapy after receiving EGFR TKIs **(Figure 4)**. Of the 1,992 patients receiving subsequent chemotherapy, 1,120 patients (56.2%) received platinum-based doublets as treatment, i.e., 359 patients (49.2% of 729) in the gefitinib group, 372 patients (56.3% of 661) in the erlotinib group, and 389 (64.6% of 602) in the afatinib group (**Supplementary Table S2**). Pemetrexed (1,088 of 1,992 patients, 54.6%) constituted the majority of second-line regimens, followed by vinorelbine (n = 433, 21.7%), gemcitabine (n = 160, 8.0%), docetaxel (n = 123, 6.2%), and paclitaxel (n = 64, 3.2%). Pemetrexed (76.9%) and gemcitabine (50.6%) were the most common partners for platinum drugs, and only 18.5% of patients with vinorelbine simultaneously received platinum drugs (**Supplementary Table S2)**. For subsequent therapy in subgroup analyses, patients who received erlotinib and afatinib as first-line treatment had a significantly higher proportion of pemetrexed usage (n = 379, 57.3%, p = 0.0025 and n = 350, 58.1%, p = 0.0012, respectively) as second-line therapy compared to the gefitinib group (n = 359, 49.2%). Among gefitinib users, a higher proportion of patients received vinorelbine (n = 189, 25.9%) than that in the erlotinib and afatinib groups (n = 132, 20.0%, p = 0.0085 and n = 112, 18.6%, p = 0.0015, respectively) **(Figure 4)**.

OS Among Different Second-Line Systemic Chemotherapy After First-Line EGFR TKI Treatment

After first-line TKI therapy, the TTD of subsequent systemic chemotherapy was around 2.7–3.6 months (pemetrexed: 3.36 ± 3.53 months; vinorelbine: 2.82 ± 4.23 months; gemcitabine: 3.60 ± 5.56 months; docetaxel: 2.74 ± 3.08 months; paclitaxel 2.73 ± 2.96 months) (**Supplementary Table S3)**. As second-line therapy, pemetrexed and gemcitabine both have longer TTD compared with other chemotherapy agents, regardless of first-line TKI agents (**Figure 4**).

Comparing the OS of different second-line chemotherapy regimens, pemetrexed was associated with better OS than was vinorelbine in gefitinib users with advanced *EGFR* mutant lung adenocarcinoma (Ref: pemetrexed, HR: 1.65, 95% CI: 1.28–2.13,

TABLE 1 | Characteristics of advanced lung adenocarcinoma patients receiving epidermal growth factor receptor TKIs as first-line systemic therapy.

	Overall patients (n = 4889)	Gefitinib (n = 1778)	Erlotinib (n = 1599)	Afatinib (n = 1512)	*p** value(gefitinib/erlotinib/afatinib)	Erlotinib/Afatinib matched cohort			Gefitinib/Afatinib matched cohort		
						Erlotinib (n = 1228)	Afatinib (n = 1228)	*p* value	Gefitinib (n = 1054)	Afatinib (n = 1054)	*p* value
Age (mean ± SD)	67.3 ± 11.9	70.9 ± 12.0	66.6 ± 11.7	64.4 ± 11.4	<0.0001	65.5 ± 11.6	65.3 ± 11.2	0.6058	66.8 ± 11.9	66.9 ± 10.88	0.8721
<45 years	157 (3.2)	41 (2.3)	50 (3.1)	66 (4.4)	<0.0001	43 (3.5)	35 (2.9)	0.2747	38 (3.6)	22 (2.1)	0.1044
45–65 years	1989 (40.7)	560 (31.5)	677 (42.3)	752 (49.7)		559 (45.5)	595 (48.5)		440 (41.8)	455 (43.2)	
>65 years	2743 (56.1)	1177 (66.2)	872 (54.5)	694 (45.9)		626 (51.0)	598 (48.7)		576 (54.7)	577 (54.7)	
Male, *n* (%)	1806 (36.9)	519 (29.2)	650 (40.7)	637 (42.1)	<0.0001	523 (42.6)	514 (41.8)	0.7131	379 (36.0)	375 (35.6)	0.8558
BMI (mean ± SD)	23.3 ± 3.79	23.0 ± 3.71	23.40 ± 4.10	23.6 ± 3.5	0.0028	23.5 ± 4.3	23.6 ± 3.6	0.6102	23.4 ± 3.5	23.3 ± 3.9	0.6242
<18	262 (5.4)	127 (7.1)	87 (5.4)	48 (3.2)	<0.0001	65 (5.3)	39 (3.2)	0.0265	73 (6.9)	38 (3.6)	0.0023
18–24	3330 (68.1)	1204 (67.7)	1091 (68.2)	1035 (68.5)		824 (67.1)	827 (67.4)		687 (65.2)	726 (68.9)	
>24	1297 (26.5)	447 (25.1)	421 (26.3)	429 (28.4)		339 (27.6)	362 (29.5)		294 (27.9)	290 (27.5)	
Staging, *n* (%)											
IIIb	220 (4.5)	93 (5.2)	58 (3.6)	69 (4.6)	<0.0001	53 (4.3)	52 (4.2)	0.8676	51 (4.8)	49 (4.6)	0.8623
M1a	1415 (28.9)	610 (34.3)	366 (22.9)	439 (29.0)		323 (26.3)	312 (25.4)		342 (32.4)	332 (31.5)	
M1b	3254 (66.6)	1075 (60.5)	1175 (73.5)	1004 (66.4)		852 (69.4)	864 (70.4)		661 (62.7)	673 (63.9)	
ECOG, *n* (%)											
ECOG ≦ 1	3780 (77.3)	1279 (71.9)	1233 (77.1)	1268 (83.9)	<0.0001	1006 (81.9)	996 (81.1)	0.6032	837 (79.4)	844 (80.1)	0.7044
ECOG > 1	1109 (22.7)	499 (28.1)	366 (22.9)	244 (16.1)		222 (18.1)	232 (18.9)		217 (20.6)	210 (19.9)	
Smoking, *n* (%)											
Active smoker	565 (11.6)	171 (9.6)	194 (12.1)	200 (13.2)	0.0031	152 (12.4)	145 (11.81)	0.7434	118 (11.2)	120 (11.4)	0.9587
Ever smoker	640 (13.1)	181 (10.2)	234 (14.6)	225 (14.9)		193 (15.7)	183 (14.9)		125 (11.9)	121 (11.5)	
Never smoker	3684 (75.3)	1426 (80.2)	1171 (73.2)	1087 (71.9)		883 (71.9)	900 (73.3)		811 (76.9)	813 (77.1)	
Alcohol Drinking, *n* (%)											
Active drinker	531 (10.9)	198 (11.1)	165 (10.3)	168 (11.1)	<0.0001	145 (11.8)	142 (11.6)	0.8515	117 (11.1)	108 (10.3)	0.7127
Quitted	203 (4.1)	48 (2.7)	77 (4.8)	78 (5.2)		60 (4.9)	66 (5.9)		38 (3.6)	43 (4.1)	
Never drinker	4155 (85.0)	1532 (86.2)	1357 (84.9)	1266 (83.7)		1023 (83.3)	1020 (83.1)		899 (85.3)	903 (85.7)	
CCI (mean ± SD)	0.79 ± 1.99	0.93 ± 2.11	0.82 ± 2.12	0.60 ± 1.67	<0.0001	0.73 ± 1.83	0.70 ± 1.79	0.9455	0.66 ± 1.79	0.65 ± 1.75	0.6568
Year of use, *n (%)*											
2014	1215 (24.9)	518 (29.1)	450 (28.1)	247 (16.3)	<0.0001	257 (20.9)	228 (18.6)	0.1063	225 (21.4)	215 (20.4)	0.801
2015	1820 (37.2)	708 (39.8)	566 (35.4)	546 (36.1)		428 (34.9)	475 (38.7)		404 (38.3)	417 (39.6)	
2016	1854 (37.9)	552 (31.1)	583 (36.5)	719 (47.6)		543 (44.2)	525 (42.8)		425 (40.3)	422 (40.0)	
Hospital level, *n (%)*											
Medical center	3012 (61.6)	1014 (57.0)	1030 (64.4)	968 (64.0)	<0.0001	795 (64.7)	785 (63.9)	0.9136	643 (61.0)	636 (60.3)	0.9241
Regional hospital	1828 (37.4)	747 (42.0)	552 (34.5)	529 (35.0)		421 (34.3)	431 (35.1)		398 (37.8)	406 (38.5)	
Local hospital	49 (1.0)	17 (1.0)	17 (1.1)	15 (1.0)		12 (1.0)	12 (1.0)		13 (1.2)	12 (1.1)	

BMI, body mass index; CCI, Charlson comorbidity index; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation; TKI, tyrosine kinase inhibitor.
**comparison among three groups (gefitinib, erlotinib, afatinib).*

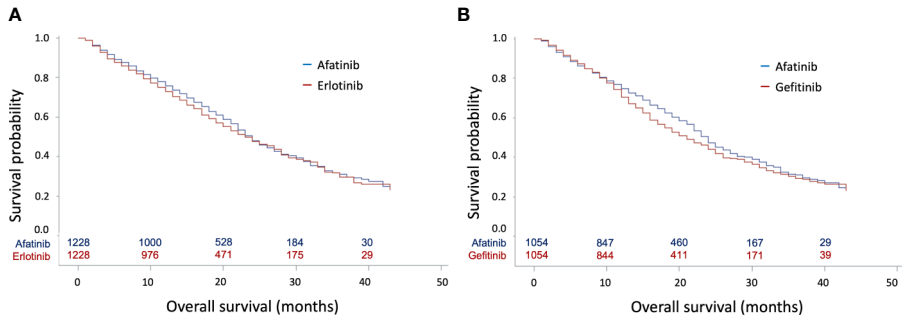

FIGURE 2 | Kaplan–Meier curves for overall survival (OS) according to tyrosine kinase inhibitors (TKIs) in matched cohorts. **(A)** Kaplan–Meier curves for OS between matched afatinib and erlotinib users; **(B)** Kaplan–Meier curves for OS between matched afatinib and gefitinib users.

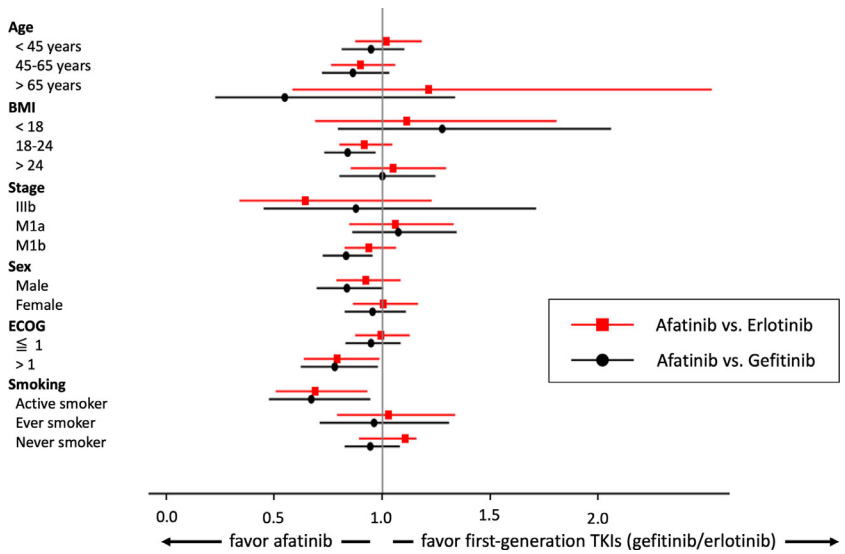

FIGURE 3 | Forest plot for the matched subgroup analysis on overall survival.

p = 0.0001) (**Table 2**). In erlotinib users, pemetrexed showed superiority in the longest TTD of all regimens as second-line treatment. Pemetrexed and gemcitabine had similar OS, which was longer than that of vinorelbine, docetaxel, or paclitaxel among afatinib users.

DISCUSSION

Our study showed that afatinib did not provide the evidence of a survival advantage over gefitinib and erlotinib. In the subgroup analysis, afatinib was associated with better OS among patients with active smoking and poor performance status. While 40% of patients were able to receive second-line chemotherapy agents, pemetrexed was associated with better OS across the three TKI groups. An alternative choice may be gemcitabine.

To the best of our knowledge, this study is the single largest cohort study to investigate the effectiveness of three EGFR TKIs (16, 18). We found that compared with first-generation TKIs, patients receiving afatinib were younger, were more likely to be male, had higher BMIs, and had better performance status. Previous studies have shown that afatinib provided longer PFS than first-generation TKIs (15, 18, 20), but adverse effects in patients receiving afatinib were also more frequently observed (15). In real-world practice, our research showed that the baseline characteristics could significantly influence the clinicians' judgments and preferences while choosing one of the TKIs (gefitinib, erlotinib, or afatinib) as first-line treatment. Afatinib may be preferred among those who are younger, are male, have higher BMIs, are active smokers, and have better performance status. Interestingly, we found that the proportion of afatinib users among all TKI users had increased in the recent years. In 2016, the number of afatinib users surpassed either

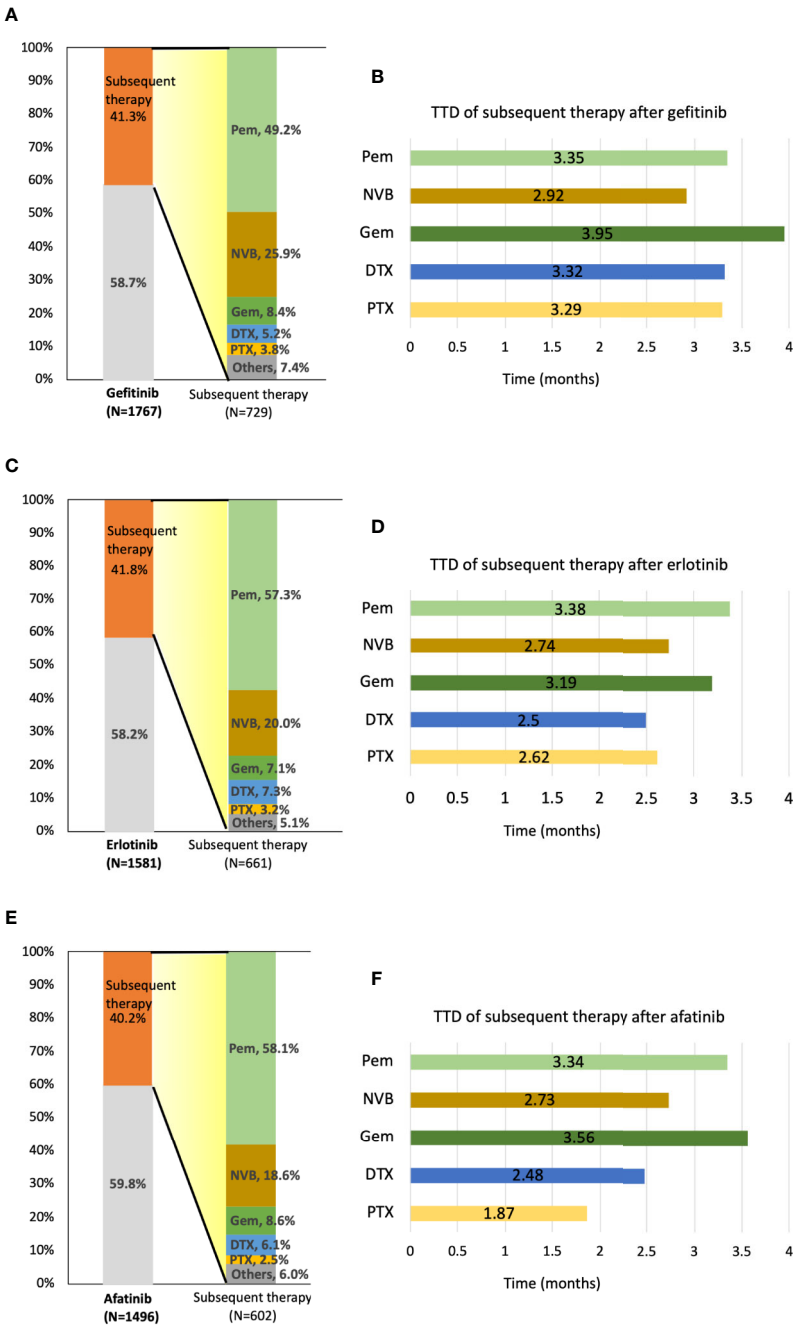

FIGURE 4 | Distribution and time-to-treatment discontinuation (TTD) of second-line chemotherapy agents by different TKIs. **(A)** Percentage of patients who received subsequent therapy and the distribution of second-line treatment agents after gefitinib administration. **(B)** TTD of five second-line treatment agents after gefitinib administration. **(C)** Percentage of patients who received subsequent therapy and the distribution of second-line treatment agents after erlotinib administration. **(D)** TTD of five second-line treatment agents after erlotinib administration. **(E)** Percentage of patients who received subsequent therapy and the distribution of second-line treatment agents after afatinib administration. **(F)** TTD of five second-line treatment agents after afatinib administration. DTX, docetaxel; GEM, gemcitabine; NVB, vinorelbine; Pem, pemetrexed; PTX, paclitaxel.

erlotinib or gefitinib users. As physicians became experienced in managing patients with afatinib (especially the toxicity profiles), they selected afatinib over erlotinib or gefitinib.

In PS matching analysis, afatinib use was not associated with better OS compared with erlotinib or gefitinib use. In the subgroup analysis, afatinib use was associated with survival benefit among patients with ECOG > 1 and active smoking. While one may argue that failure to demonstrate the clinical evidence of survival benefit may be due to the relatively small sample size in previous randomized control trials and other

TABLE 2 | Comparison of the overall survival of five common chemotherapy regimens as a subsequent therapy of the three EGFR TKI groups.

	Gefitinib			Erlotinib			Afatinib		
	Hazard ratio	**95% CI**	***p* value**	**Hazard ratio**	**95% CI**	***p* value**	**Hazard ratio**	**95% CI**	***p* value**
Pemetrexed	Ref			Ref			Ref		
Gemcitabine	1.26	0.86–1.85	0.239	1.87	1.20–2.90	0.0053	1.37	0.90–2.09	0.146
Vinorelbine	1.65	1.28–2.13	0.0001	1.59	1.19–2.12	0.0019	1.67	1.21–2.32	0.0021
Docetaxel	1.50	0.96–2.36	0.0767	1.61	1.08–2.41	0.0191	2.08	1.37–3.16	0.0006
Paclitaxel	1.44	0.86–2.41	0.1627	1.81	1.01–3.25	0.0463	2.24	1.15–4.33	0.0173

CI, confidence interval.

observational studies, our study may have the current largest cohort, including more than 1,000 participants in each TKI group (14, 16, 17, 36, 37). More importantly, we used PS matching, which is a more robust way of controlling confounders in observational studies, and this analysis strategy was not performed in previous observational studies (38).

Clinically, afatinib could be an effective treatment for lung adenocarcinoma patients with the *EGFR* mutation and brain metastasis (39). Subgroup analysis showed that afatinib provided better OS in patients with distant metastases (stage M1b) compared with gefitinib, but this benefit was not observed when compared with the erlotinib group. Active/current smokers usually have lower *EGFR* mutation rates (especially exon 19 deletion and p.L858R) than never-smoking female patients (8, 40). Notably, our study (through subgroup analysis) showed that afatinib could provide significantly longer OS in active smokers than could gefitinib/erlotinib. The above-mentioned benefits may be because afatinib is a member of the pan-ErbB family of inhibitors. It could covalently and irreversibly bind to the intracellular tyrosine kinase domain of the EGFR and effectively treat common (exon 19 deletion and p.L858R) and uncommon *EGFR* mutations (41–43). Furthermore, this real-world study provided additional information on the minor population with a poor performance status (ECOG >1), which is often excluded by randomized controlled trials to evaluate the efficacy and safety of the study drugs. None of the patients (among the 310 patients) with ECOG > 1 in the phase IIb LUX-Lung 7 trial (afatinib vs. gefitinib) were enrolled (19), and only 6 (2.3%) patients with ECOG = 2 (among 256 patients) in the phase III CTONG 0901 trial (gefitinib vs. erlotinib) were included (13). In contrast, there were 1,109 patients (up to 22.7% of total 4,889 patients) with ECOG > 1 in real-world practice, and afatinib surprisingly demonstrated superior TTD and OS benefits compared with first-generation TKIs in patients with worse performance status.

Approximately 40%–60% of acquired *EGFR* p.T790M develops after the patients receive first-line TKIs (44, 45), and osimertinib was approved as second-line treatment by the US Food and Drug Administration (FDA) and Taiwan Food and Drug Administration in November 2015 and November 2016, respectively. In the FLAURA trial, 14.1% (39 of 277) of patients received chemotherapy and 30.7% (85 of 277) of patients in the gefitinib/erlotinib group received osimertinib as second-line treatment (46). Osimertinib, however, was not reimbursed by the NHI during the study period. Shifting to osimertinib treatment after first-line TKI failure, therefore, was not widely used during our study period in Taiwan, and platinum-based doublets remained the standard for second-line treatment. Our real-world study indicated that 41.1% of all TKI patients in Taiwan could have subsequent cytotoxic chemotherapy as an effective treatment. While chemotherapy may be the most important and preferred systemic therapy after the failure of first-line osimertinib treatment, only 32.3% (90 of 279) of patients in the osimertinib group of the FLAURA trial received chemotherapy as a subsequent systemic therapy (46). In our study, pemetrexed (54.6%) and vinorelbine (21.7%) were the most common subsequent chemotherapy agents. Pemetrexed was the most preferred subsequent therapy in clinical practice owing to its efficacy, tolerability, and convenience in administration (47), and vinorelbine was also frequently prescribed because of the oral route of administration and less toxicity in elderly patients (48). In this real-world study, only 56.2% of patients used platinum-based doublets as subsequent chemotherapy agents after TKI failure. Interestingly, pemetrexed and gemcitabine were found to be the most common partners for platinum drugs. Meanwhile, pemetrexed as a subsequent therapy could provide the best TTD benefit among all agents in erlotinib users. Furthermore, pemetrexed and gemcitabine demonstrated similar effectiveness in TTD among gefitinib and afatinib users. These findings from our claims database epidemiological studies could provide personalized guidance in clinical practice, complementary to biomarker and genetic risk factor studies for oncological patients.

There were some limitations in our study. First, detailed information on *EGFR* mutation sites was not available in the TCR database. Therefore, the effectiveness of different generation TKIs could not be compared with common or uncommon mutations. Meanwhile, the causes of TTD and TKI-related toxicity profiles could not be readily clarified. In the subsequent treatment analysis, osimertinib was not reimbursed by the Taiwan NHI. Therefore, self-financed or clinical trial osimertinib users could not be identified in this study. Finally, the FLAURA trial demonstrated the superior efficacy and safety of osimertinib compared with gefitinib and erlotinib as first-line TKIs in *EGFR* mutant NSCLC patients, and osimertinib is therefore currently considered the standard for first-line therapy (46). However, data regarding the activity of osimertinib in patients harboring rare *EGFR* mutations are limited. Economic issues, such as high cost and the lack of insurance reimbursement may preclude osimertinib use in real-world. Meanwhile, the optimal therapeutic strategy for disease progression after osimertinib administration may still be

ambiguous for physicians because of the lack of large-scale real-world data. Gefitinib, erlotinib, and afatinib are, therefore, still used as first-line treatment in many *EGFR* mutant NSCLC patients.

Our study indicates that despite the increasing use of afatinib as first-line TKI for *EGFR* mutant, late-stage adenocarcinoma patients, afatinib use was not associated with longer OS than were first-generation TKIs. Afatinib administration, however, may be considered among active smokers. Additionally, for patients with poor performance status, afatinib administration may also lead to survival benefits and should not be withheld due to the fear of toxicity. For second-line chemotherapy, pemetrexed may be the preferred agent, and gemcitabine can also be considered as a reasonable alternative.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) committee of National Taiwan University Hospital Hsinchu Branch. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

M-RL and S-KL designed all experiments. M-RL, S-KL, C-HC, Y-FW, and L-TK conducted the experiments and analyzed and interpreted the results. C-YY, J-YS, J-YW, J-CK, C-YY, and C-JY supervised the project. M-RL, C-HC, and S-KL wrote the draft manuscript. C-YY, J-YS, J-YW, J-CK, C-YY, and C-JY reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.590356/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | Kaplan–Meier curves for overall survival (OS) and time-to-treatment discontinuation (TTD) for 3 tyrosine kinase inhibitors (TKIs). **(A)** Kaplan–Meier curves of OS for afatinib, erlotinib, and gefitinib administration; **(B)** Kaplan–Meier curves of TTD for afatinib, erlotinib, and gefitinib administration.

SUPPLEMENTARY FIGURE 2 | (A) Kaplan–Meier curves of time-to-treatment discontinuation (TTD) between matched afatinib and erlotinib groups; **(B)** Kaplan–Meier curves for TTD between matched afatinib and gefitinib groups

SUPPLEMENTARY FIGURE 3 | Forest plot for the matched subgroup analysis on time-to-treatment discontinuation.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* (2019) 69(1):7–34. doi: 10.3322/caac.21551
2. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WE, et al. The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer. *J Thorac Oncol* (2016) 11(1):39–51. doi: 10.1016/j.jtho.2015.09.009
3. Ettinger DS, Wood DE, Aggarwal C, Aisner DL, Akerley W, Bauman JR, et al. NCCN Guidelines Insights: Non-Small Cell Lung Cancer, Version 1.2020. *J Natl Compr Canc Netw* (2019) 17(12):1464–72. doi: 10.6004/jnccn.2019.0059
4. Mu Y, Yang K, Hao X, Wang Y, Wang L, Liu Y, et al. Clinical Characteristics and Treatment Outcomes of 65 Patients With BRAF-Mutated Non-small Cell Lung Cancer. *Front Oncol* (2020) 10:603. doi: 10.3389/fonc.2020.00603
5. Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol* (2014) 9(2):154–62. doi: 10.1097/JTO.0000000000000033
6. Kawaguchi T, Koh Y, Ando M, Ito N, Takeo S, Adachi H, et al. Prospective Analysis of Oncogenic Driver Mutations and Environmental Factors: Japan Molecular Epidemiology for Lung Cancer Study. *J Clin Oncol* (2016) 34 (19):2247–57. doi: 10.1200/JCO.2015.64.2322
7. Zhang YL, Yuan JQ, Wang KF, Fu XH, Han XR, Threapleton D, et al. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget* (2016) 7(48):78985–93. doi: 10.18632/oncotarget.12587
8. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) 361(10):947–57. doi: 10.1056/NEJMoa0810699
9. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* (2011) 12 (8):735–42. doi: 10.1016/S1470-2045(11)70393-X

10. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) 31(27):3327–34. doi: 10.1200/JCO.2012.44.2806
11. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* (2018) 378(2):113–25. doi: 10.1056/NEJMoa1713137
12. Chen F, Chen N, Yu Y, Cui J. Efficacy and Safety of Epidermal Growth Factor Receptor (EGFR) Inhibitors Plus Antiangiogenic Agents as First-Line Treatments for Patients With Advanced EGFR-Mutated Non-small Cell Lung Cancer: A Meta-Analysis. *Front Oncol* (2020) 10:904. doi: 10.3389/fonc.2020.00904
13. Yang JJ, Zhou Q, Yan HH, Zhang XC, Chen HJ, Tu HY, et al. A phase III randomised controlled trial of erlotinib vs gefitinib in advanced non-small cell lung cancer with EGFR mutations. *Br J Cancer* (2017) 116(5):568–74. doi: 10.1038/bjc.2016.456
14. Paz-Ares L, Tan EH, O'Byrne K, Zhang L, Hirsh V, Boyer M, et al. Afatinib versus gefitinib in patients with EGFR mutation-positive advanced non-small-cell lung cancer: overall survival data from the phase IIb LUX-Lung 7 trial. *Ann Oncol* (2017) 28(2):270–7. doi: 10.1093/annonc/mdw611
15. Park K, Tan EH, O'Byrne K, Zhang L, Boyer M, Mok T, et al. Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): a phase 2B, open-label, randomised controlled trial. *Lancet Oncol* (2016) 17(5):577–89. doi: 10.1016/S1470-2045(16)30033-X
16. Lau SC, Chooback N, Ho C, Melosky B. Outcome Differences Between First- and Second-generation EGFR Inhibitors in Advanced EGFR Mutated NSCLC in a Large Population-based Cohort. *Clin Lung Cancer* (2019) 20(5):e576–e83. doi: 10.1016/j.cllc.2019.05.003
17. Lin YT, Chen JS, Liao WY, Ho CC, Hsu CL, Yang CY, et al. Clinical outcomes and secondary epidermal growth factor receptor (EGFR) T790M mutation among first-line gefitinib, erlotinib and afatinib-treated non-small cell lung cancer patients with activating EGFR mutations. *Int J Cancer* (2019) 144(11):2887–96. doi: 10.1002/ijc.32025
18. Tu CY, Chen CM, Liao WC, Wu BR, Chen CY, Chen WC, et al. Comparison of the effects of the three major tyrosine kinase inhibitors as first-line therapy for non-small-cell lung cancer harboring epidermal growth factor receptor mutations. *Oncotarget* (2018) 9(36):24237–47. doi: 10.18632/oncotarget.24386
19. Krawczyk P, Kowalski DM, Ramlau R, Kalinka-Warzocha E, Winiarczyk K, Stencel K, et al. Comparison of the effectiveness of erlotinib, gefitinib, and afatinib for treatment of non-small cell lung cancer in patients with common and rare EGFR gene mutations. *Oncol Lett* (2017) 13(6):4433–44. doi: 10.3892/ol.2017.5980
20. Kuan FC, Li SH, Wang CL, Lin MH, Tsai YH, Yang CT. Analysis of progression-free survival of first-line tyrosine kinase inhibitors in patients with non-small cell lung cancer harboring leu858Arg or exon 19 deletions. *Oncotarget* (2017) 8(1):1343–53. doi: 10.18632/oncotarget.13815
21. Wu SG, Shih JY. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non-small cell lung cancer. *Mol Cancer* (2018) 17(1):38. doi: 10.1186/s12943-018-0777-1
22. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. *N Engl J Med* (2017) 376(7):629–40. doi: 10.1056/NEJMoa1612674
23. Wu YL, Planchard D, Lu S, Sun H, Yamamoto N, Kim DW, et al. Pan-Asian adapted Clinical Practice Guidelines for the management of patients with metastatic non-small-cell lung cancer: a CSCO-ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS. *Ann Oncol* (2019) 30(2):171–210. doi: 10.1093/annonc/mdy554
24. Hanna N, Johnson D, Temin S, Baker SJr., Brahmer J, Ellis PM, et al. Systemic Therapy for Stage IV Non-Small-Cell Lung Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. *J Clin Oncol* (2017) 35(30):3484–515. doi: 10.1200/JCO.2017.74.6065
25. Reck M, Mok TSK, Nishio M, Jotte RM, Cappuzzo F, Orlandi F, et al. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. *Lancet Respir Med* (2019) 7(5):387–401. doi: 10.1016/S2213-2600(19)30084-0
26. Mok TSK, Kim SW, Wu YL, Nakagawa K, Yang JJ, Ahn MJ, et al. Gefitinib Plus Chemotherapy Versus Chemotherapy in Epidermal Growth Factor Receptor Mutation-Positive Non-Small-Cell Lung Cancer Resistant to First-Line Gefitinib (IMPRESS): Overall Survival and Biomarker Analyses. *J Clin Oncol* (2017) 35(36):4027–34. doi: 10.1200/JCO.2017.73.9250
27. Lee MR, Yu KL, Kuo HY, Liu TH, Ko JC, Tsai JS, et al. Outcome of stage IV cancer patients receiving in-hospital cardiopulmonary resuscitation: a population-based cohort study. *Sci Rep* (2019) 9(1):9478. doi: 10.1038/s41598-019-45977-4
28. Lee MR, Lee CH, Wang JY, Lee SW, Ko JC, Lee LN. Clinical impact of using fluoroquinolone with low antimycobacterial activity on treatment delay in tuberculosis: Hospital-based and population-based cohort study. *J Formos Med Assoc* (2020) 119(1 Pt 2):367–76. doi: 10.1016/j.jfma.2019.06.008
29. Yao ZH, Liao WY, Ho CC, Chen KY, Shih JY, Chen JS, et al. Real-World Data on Prognostic Factors for Overall Survival in EGFR Mutation-Positive Advanced Non-Small Cell Lung Cancer Patients Treated with First-Line Gefitinib. *Oncologist* (2017) 22(9):1075–83. doi: 10.1634/theoncologist.2016-0331
30. Lee MR, Lee MC, Chang CH, Liu CJ, Chang LY, Zhang JF, et al. Use of Antiplatelet Agents and Survival of Tuberculosis Patients: A Population-Based Cohort Study. *J Clin Med* (2019) 8(7):1–13. doi: 10.3390/jcm8070923
31. Lee MR, Ho CM, Lee CH, Lee MC, Chang LY, Yu KL, et al. Tuberculosis contact investigation in an intermediate burden setting: implications from a large tuberculosis contact cohort in Taiwan. *Eur Respir J* (2017) 50(2):1–4. doi: 10.1183/13993003.00851-2017
32. Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol* (2007) 2(8):706–14. doi: 10.1097/JTO.0b013e31812f3c1a
33. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* (1982) 5(6):649–55. doi: 10.1097/00000421-198212000-00014
34. Quan H, Sundararajan V, Halfon P, Fong A, Burnand B, Luthi JC, et al. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. *Med Care* (2005) 43(11):1130–9. doi: 10.1097/01.mlr.0000182534.19832.83
35. Chen JH, Chung CH, Wang YC, Hsu SN, Huang WY, Chien WC. Prevalence and Mortality-Related Factors of Multiple Myeloma in Taiwan. *PloS One* (2016) 11(12):e0167227. doi: 10.1371/journal.pone.0167227
36. Holleman MS, van Tinteren H, Groen HJ, Al MJ, Uyl-de Groot CA. First-line tyrosine kinase inhibitors in EGFR mutation-positive non-small-cell lung cancer: a network meta-analysis. *Onco Targets Ther* (2019) 12:1413–21. doi: 10.2147/OTT.S189438
37. Chen YH, Chen YF, Chen CY, Shih JY, Yu CJ. Clinical factors associated with treatment outcomes in EGFR mutant non-small cell lung cancer patients with brain metastases: a case-control observational study. *BMC Cancer* (2019) 19(1):1006. doi: 10.1186/s12885-019-6140-0
38. Kuss O, Blettner M, Borgermann J. Propensity Score: an Alternative Method of Analyzing Treatment Effects. *Dtsch Arztebl Int* (2016) 113(35-36):597–603. doi: 10.3238/arztebl.2016.0597
39. Hoffknecht P, Tufman A, Wehler T, Pelzer T, Wiewrodt R, Schutz M, et al. Efficacy of the irreversible ErbB family blocker afatinib in epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI)-pretreated non-small-cell lung cancer patients with brain metastases or leptomeningeal disease. *J Thorac Oncol* (2015) 10(1):156–63. doi: 10.1097/JTO.0000000000000380
40. D'Angelo SP, Pietanza MC, Johnson ML, Riely GJ, Miller VA, Sima CS, et al. Incidence of EGFR exon 19 deletions and L858R in tumor specimens from men and cigarette smokers with lung adenocarcinomas. *J Clin Oncol* (2011) 29(15):2066–70. doi: 10.1200/JCO.2010.32.6181
41. Yang JC, Sequist LV, Geater SL, Tsai CM, Mok TS, Schuler M, et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. *Lancet Oncol* (2015) 16(7):830–8. doi: 10.1016/S1470-2045(15)00026-1

42. Kim Y, Lee SH, Ahn JS, Ahn MJ, Park K, Sun JM. Efficacy and Safety of Afatinib for EGFR-mutant Non-small Cell Lung Cancer, Compared with Gefitinib or Erlotinib. *Cancer Res Treat* (2019) 51(2):502–9. doi: 10.4143/crt.2018.117
43. Liang SK, Hsieh MS, Lee MR, Keng LT, Ko JC, Shih JY. Real-world experience of afatinib as a first-line therapy for advanced EGFR mutation-positive lung adenocarcinoma. *Oncotarget* (2017) 8(52):90430–43. doi: 10.18632/oncotarget.19563
44. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* (2013) 19(8):2240–7. doi: 10.1158/1078-0432.CCR-12-2246
45. Wu SG, Liu YN, Tsai MF, Chang YL, Yu CJ, Yang PC, et al. The mechanism of acquired resistance to irreversible EGFR tyrosine kinase inhibitor-afatinib in lung adenocarcinoma patients. *Oncotarget* (2016) 7(11):12404–13. doi: 10.18632/oncotarget.7189
46. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *N Engl J Med* (2020) 382(1):41–50. doi: 10.1056/NEJMoa1913662
47. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2008) 26(21):3543–51. doi: 10.1200/JCO.2007.15.0375
48. Cesare G, Francesco P, Ciro G, Antonio R, Francesco S, Silvio M, et al. Effects of vinorelbine on quality of life and survival of elderly patients with advanced non-small-cell lung cancer. The Elderly Lung Cancer Vinorelbine Italian Study Group. *J Natl Cancer Inst* (1999) 91(1):66–72. doi: 10.1093/jnci/91.1.66

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SYSTEMATIC REVIEW
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Neoadjuvant EGFR-TKI Therapy for EGFR-Mutant NSCLC: A Systematic Review and Pooled Analysis of Five Prospective Clinical Trials

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Purpose: The role of neoadjuvant epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) targeted therapy for patients with EGFR-mutant non-small cell lung cancer (NSCLC) has not been clarified. A pooled analysis of prospective clinical trials was conducted to evaluate the efficacy and safety of neoadjuvant EGFR-TKI therapy.

Methods: The PubMed, Embase, Web of Science, and Cochrane Library databases, as well as meeting abstracts were searched for prospective clinical trials evaluating the efficacy and safety of neoadjuvant EGFR-TKI for treatment of EGFR-mutant NSCLC. The main outcomes included the objective response rate (ORR), downstaging rate, surgical resection rate (SRR), pathologic complete response (pCR) rate, progression-free survival (PFS), and adverse events.

Results: A total of five, phase II, prospective, clinical trials involving 124 patients with resectable or potentially resectable EGFR-mutant NSCLC treated with neoadjuvant erlotinib or gefitinib treatment were included in this pooled analysis. The median neoadjuvant medication time was 42 (range, 21–56) days and the median time of response evaluation was 45 (range, 42–56) days. The pooled ORR was 58.5% [95% confidence interval (CI), 45.5%–71.8%] and the surgical resection and complete resection (R0) rates were 79.9% (95% CI, 65.3%–94.5%) and 64.3% (95% CI, 43.8%–84.8%), respectively. In the stage IIIA subgroup (n = 68), the pooled ORR, SRR, and R0 rate were 51.4%, 72.9%, and 57.0%, respectively, while the downstaging and pCR rates were 14.0% and 0.0%, respectively. The pooled median PFS and overall survival were 13.2 and 41.9 months, respectively. Of the most common grade 3/4 adverse events in the overall group, the incidences of hepatotoxicity and skin rash were 5.3% and 14.7%, respectively. The most commonly reported postoperative complications were lung infection, arrhythmia, and pneumothorax.

Conclusion: Neoadjuvant EGFR-TKI therapy provides a feasible treatment modality for patients with resectable or potentially resectable EGFR-mutant NSCLC, with satisfactory surgical outcomes and low toxicity. Although further phase III clinical trials are needed to confirm these findings, it is necessary to explore the feasibility of a more effective EGFR-

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TKI combination neoadjuvant therapy given the modest downgrade and pCR rates for EGFR-TKI alone.

Keywords: neoadjuvant, non-small cell lung cancer, efficacy, safety, epidermal growth factor receptor-tyrosine kinase inhibitors

INTRODUCTION

Lung cancer is the most common malignancy and the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for 80%–85% of all lung cancers (1). For patients with early resectable NSCLC, surgery remains the cornerstone of treatment. Although resection can achieve good local control, the rates of regional recurrence and distant metastasis remain very high. As preoperative systemic therapy has the potential to reduce disease stage and facilitate surgical resection, in addition to the value of drug sensitivity tests to guide postoperative treatment, a series of studies of neoadjuvant systematic therapies, including chemotherapy, targeted therapy, and immunotherapy, have been conducted to explore the possibility of improving the cure rate and survival rate (2–5). Multiple meta-analyses based on large-scale prospective randomized controlled trials (RCTs) confirmed a modest survival benefit of preoperative chemotherapy for NSCLC (6, 7).

For patients with oncogenic driver (e.g., epidermal growth factor receptor [EGFR], anaplastic lymphoma kinase, and proto-oncogene ROS1)-positive advanced NSCLC, targeted therapy with small molecule tyrosine kinase inhibitors (TKIs) has greatly improved the therapeutic outcomes and has become the first-line treatment standard. As compared to chemotherapy, EGFR-TKIs significantly improve the objective response rate (ORR) and progression-free survival (PFS) for patients with EGFR-mutant advanced NSCLC (8–11). Beyond that, for EGFR-mutant stage II or III NSCLC patients, as compared with chemotherapy/placebo, postoperative adjuvant EGFR-TKI therapy significantly prolongs disease-free survival (DFS), with a 3-year DFS rate of 34%–80% in the EGFR-TKI group versus 20%–28% in the chemotherapy/placebo group (12–14).

In view of the robust anti-tumor activity and tumor remission rate of EGFR-TKI against EGFR-mutant advanced diseases, many recent studies have explored the feasibility of neoadjuvant EGFR-TKI therapy for the treatment of NSCLC. However, most of these studies were single arm prospective clinical trials. A prospective phase II RCT launched by the Chinese Thoracic Oncology Group (CTONG) 1103 reported at the 2018 European Society for Medical Oncology (ESMO) meeting that, as compared with neoadjuvant chemotherapy, neoadjuvant EGFR-TKI therapy for patients with EGFR-mutant stage IIIA NSCLC had a significant advantage in PFS (21.5 vs. 11.4 months; hazard ratio = 0.39; $p < 0.001$) (5). Therefore, the aim of this pooled analysis based on prospective clinical trials was to evaluate the efficacy and safety of neoadjuvant EGFR-TKI therapy in patients with resectable or potentially resectable EGFR-mutant NSCLC, and to provide a basis for decision-making on neoadjuvant EGFR-TKI therapy.

Abbreviation: AEs, adverse events; CI, confidence interval; CTONG, Chinese Thoracic Oncology Group; DFS, disease-free survival; EGFR, epidermal growth factor receptor; ESMO, European Society for Medical Oncology; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; pCR, pathologic complete response; RCTs, randomized controlled trials; SRR, surgical resection rate; TKI, tyrosine kinase inhibitor; TRAEs, treatment-related adverse events.

MATERIALS AND METHODS

Search Strategy

This pooled analysis was conducted in accordance with the guidelines of the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) (15). The PubMed, Embase, Web of Science, and Cochrane Library databases, as well as meeting abstracts from the American Society of Clinical Oncology, ESMO, European Lung Cancer Conference, and World Conference on Lung Cancer were searched for relevant trials using the following search terms: "lung cancer" AND "EGFR" AND "neoadjuvant" OR "induction" OR "preoperative". The reference lists of the enrolled studies were carefully scanned to ensure that all relevant literature was retrieved. The final literature search was performed on March 20, 2020.

Inclusion Criteria

The inclusion criteria were as follows: 1) prospective studies that evaluated the efficacy or safety of preoperative EGFR-TKI for resectable or potentially resectable NSCLC with an EGFR-sensitive mutation; 2) outcomes that included at least one of these endpoints: ORR, PFS, DFS, overall survival (OS), surgery resection rate (SRR), complete (R0) resection rate, downstaging rate, pathologic complete response (pCR) rate, or adverse events (AEs); and 3) the inclusion of ≥ 10 cases.

Data Extraction

Two authors screened the authorship and titles to extract preliminary eligible studies and exclude duplicate studies. Then, the titles, abstracts, and full text of the retrieved articles were further screened to identify studies that met the inclusion criteria. Two authors independently extracted data from all eligible studies, which included 1) the name of the first author and the publication year; 2) study characteristics, including patient characteristics, disease stage, EGFR mutant type, preoperative and postoperative therapies, medication time, and timing of surgery; 3) ORR, SRR (defined as the percentage of patients who underwent surgery after neoadjuvant therapy), downstage rate, R0 resection rate (defined as the percentage of patients who underwent radical resection after neoadjuvant therapy), pCR rate (defined as the proportion of patients with

no tumor cells in all pathologic samples surgically resected after neoadjuvant therapy); 4) DFS (defined as the time from surgery to tumor recurrence or death from any cause), PFS (defined as the time from the neoadjuvant treatment to disease progression or death from any cause), and OS (defined as the time from neoadjuvant treatment to the date of death or the last follow-up); and 5) AEs during neoadjuvant treatment and the perioperative period.

Statistical Analysis

Statistical analyses were performed with Stata 15.0 software (StataCorp LLC, College Station, TX, USA). The data of the main outcomes of each study were pooled, which included the ORR, SRR, downstage rate, R0 resection rate, pCR rate, median PFS, median OS, and incidence rate of AEs. Statistical heterogeneity among the studies was detected with the I^2 statistic. If the probability (p) value was ≤ 0.05 or I^2 > 50% indicated significant heterogeneity, a random-effects model (DerSimonian-Laird method) was used. Otherwise, a fixed-effects model (inverse-variance method) was used.

Sensitivity Analysis and Publication Bias

Sensitivity analyses were performed for the ORR results based on the leave-one-out approach. The potential for publication bias in the reported ORR values was assessed using funnel plots, with the appropriate accuracy intervals.

RESULTS

Study Population and Patient Characteristics

A PRISMA flow diagram of the literature search process is shown in **Figure 1**. A total of five, phase II, prospective, clinical trials involving 124 patients with resectable or potentially resectable EGFR-mutant NSCLC were included in this pooled analysis. Among the five studies, three were single arm trials and two were RCTs. Three studies included patients with only stage IIIA disease (5, 15, 16), while the other two included patients with stages IA–IIB or II–IIIA disease without further stratification (**Table 1**) (18, 19). The data of 68 patients with stage IIIA disease from three studies were extracted as a subgroup for independent analysis (5, 16, 17).

The characteristics of patients in the included studies are summarized in **Table 2**. All patients had an ECOG performance status score of 0–1 point, while 68 (54.8%) were treated with neoadjuvant erlotinib and 56 (45.2%) with neoadjuvant gefitinib.

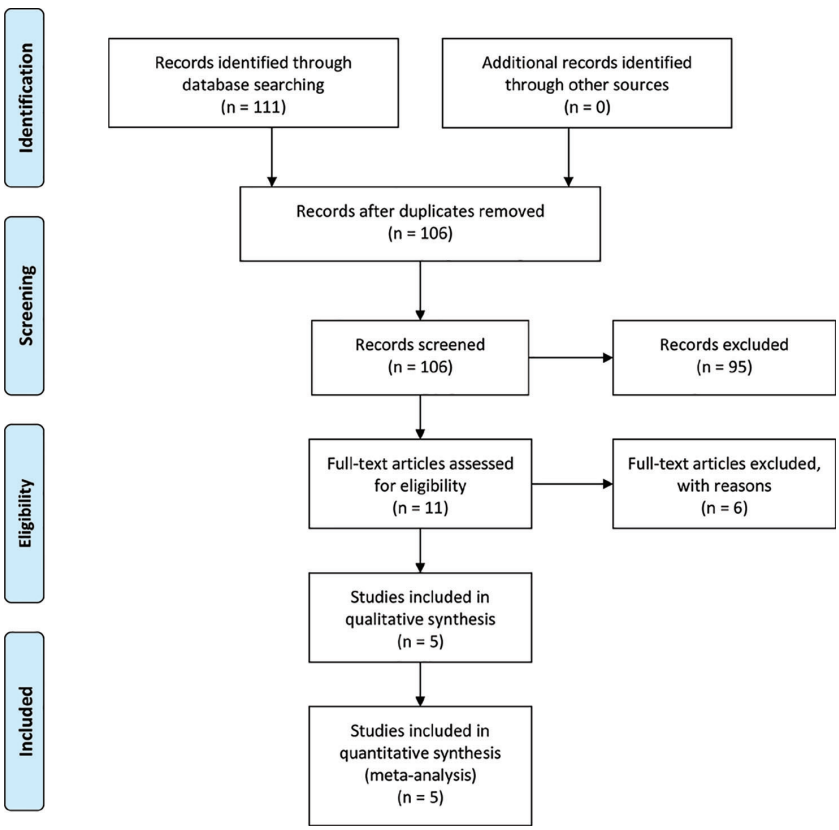

FIGURE 1 | A flow diagram of the literature search and selection process.

TABLE 1 | Characteristics of included studies.

Study	Zhong (5)	Xiong (16)	Zhong (17)	Rizvi (18)	Zhang (19)
Enrollment years	2011–2017	2011–2014	2008–2011	2004–2008	2013–2015
Case number	37	19	12	21	35
Clinical stage	IIIA	IIIA	IIIA	IA–IIB	II–IIIA
Preoperative Tx	Erlotinib	Erlotinib	Erlotinib	Gefitinib	Gefitinib
Tx duration (day)	42	56	42	21	42
Postoperative Tx	Erlotinib (1 year)	chemotherapy	NR	Gefitinib (2 years)	chemotherapy
ORR	54.1%	42.0%	58.3%	81.0%	54.5%
Operation time[#]	One week	NR	One week	Two days	NR
Downstage rate	10.8%	21.1%	16.7%	NR	20.0%
Surgery rate	83.8%	73.7%	50.0%	100%	94.3%
R0 rate	73.0%	68.4%	25.0%	NR	82.8%
pCR rate	0	0	NR	NR	12.1%
DFS (mo)	NR	10.3	8.6	NR	33.5
PFS (mo)	21.5	11.2	6.9	NR	NR
OS (mo)	45.8	51.6	14.5	NR	NR
SAEs	0	10.5%	16.7%	NA	0

Tx, treatment; [#]the time from drug discontinuance to surgery; SA, single arm; RCT, randomized controlled trial; ORR, objective response rate; pCR, pathologic complete response; DFS, disease-free survival; PFS, progression-free survival; OS, overall survival; NR, not report; NA, not available; SAEs: grade3/4 adverse events during preoperative therapy.

The median medication time was 42 (range, 21–56) days. The median time of response evaluation was 45 (range, 42–56) days.

ORR, SRR, and Postoperative Outcomes

The pooled overall ORR was 58.5% [95% confidence interval (CI), 45.5%–71.8%] (**Figure 2A**). The surgical resection and R0 rates were 79.9% (95% CI, 65.3%–94.5%) and 64.3% (95% CI, 43.8%–84.8%), respectively (**Figures 2B–C**). In the stage IIIA subgroup, the pooled ORR was 51.4% (95% CI, 39.7%–63.2%) (**Figure 3A**), while the surgical resection and R0 rates were 72.9% (95% CI, 55.7%–90.1%) and 56.8% (95%CI, 29.8%–83.8%), respectively (**Figures 3B–C**). The downstaging rate was 14.0% (95% CI, 5.6%–21.8%) (**Figure 3D**), the pCR rate extracted from two studies was 0.0%, the pooled median PFS was 13.2 months (95% CI, 2.7–23.7) (**Figure 4**), and the pooled median OS was 41.9 months, which was calculated using a weighted average of single study medians because of insufficient data of the 95% CI values (20).

TABLE 2 | Characteristics of included patients (n=124).

Characteristics	Case number (%)
ECOG 0–1	124 (100%)
Age median (range)	60 (57–67)
Sex	
Male	35 (28.2%)
Female	68 (54.4%)
Unknown	21 (17.4%)
Smoke status	
Ever	25 (20.2%)
Never	66 (53.2%)
Unknown	33 (26.6%)
Histology	
Adenocarcinoma	62 (51.7%)
Non-adenocarcinoma	6 (5.0%)
Unknown	52 (43.3%)
Clinical stage	
IA–IIB (17, 18)	29 (23.4%)
IIIA	95 (76.6%)
Mutation status	
Exon 19 deletion	68 (54.8%)
Exon 21 L858R	56 (45.2%)
Preoperative Tx	
Erlotinib	68 (54.8%)
Gefitinib	56 (45.2%)

Safety

The most common AEs observed during neoadjuvant treatment are listed in **Table 3**. The most common AEs were rash and diarrhea. The pooled incidence rates of any grade and grade ≥ 3 rash were 54.9% and 14.7%, respectively. The pooled incidence rate of any grade diarrhea was 14.7%. No grade ≥ 3 diarrhea was reported. The pooled incidence rates of any grade and grade ≥ 3 hepatotoxicity were 7.7% and 5.3%, respectively. Other AEs, including paronychia, stomatitis, and leukopenia, etc., were reported by limited studies (**Table 3**).

The postoperative complications reported by four studies are listed in **Table 4** (5, 16, 17, 19). The postoperative complications reported by two or more studies included lung infection, arrhythmia, and pneumothorax, but there were no actual concrete data. Other postoperative complications included poor incision healing, chest tube drainage for > 7 days, postoperative bleeding, chylothorax, and pulmonary artery injury, but without concrete data. There was no report of increased operative difficulty or perioperative death.

Sensitivity Analysis and Publication Bias

The results of the leave-one-out sensitivity analyses for the ORR are summarized in **Figure 5A**. The estimated ORR of each study was similar to the pooled ORR value and 95% CI. Potential publication bias was assessed using funnel plots with ORR. The funnel plots were symmetrical, indicating no publication bias (**Figure 5B**).

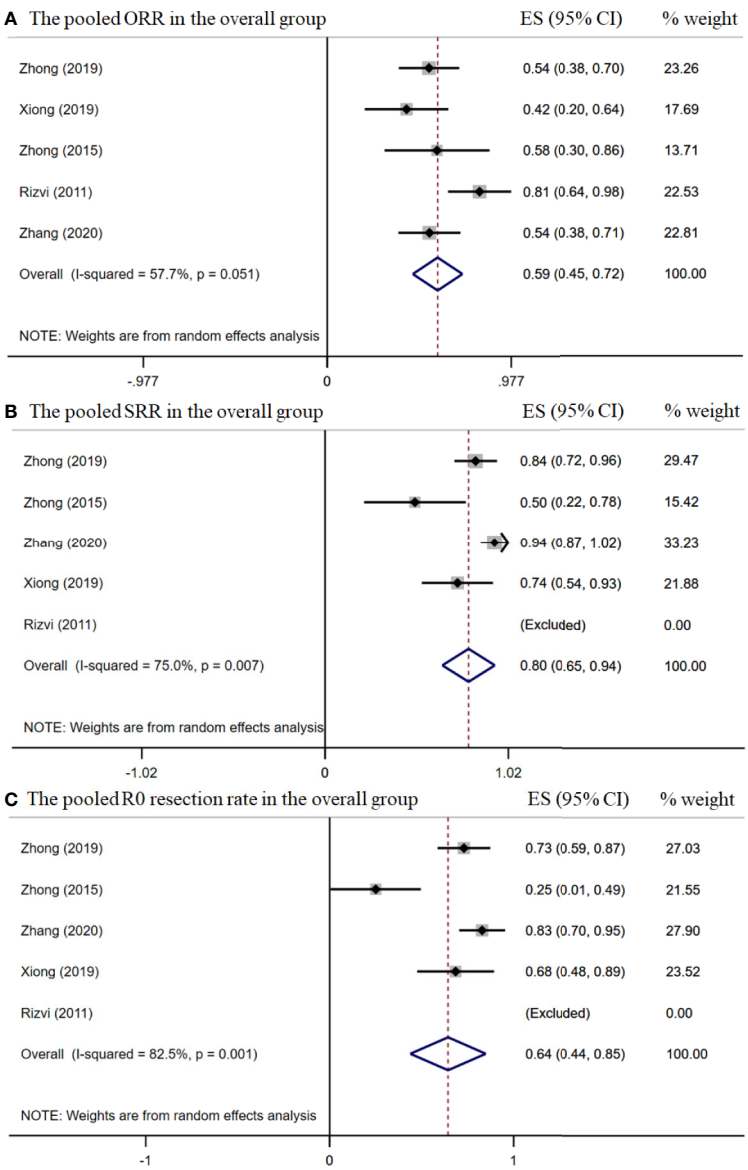

FIGURE 2 | The pooled efficacy rates in the overall group. The ORR **(A)**; SRR **(B)**; and R0 resection rate **(C)**.

DISCUSSION

Neoadjuvant chemotherapy is now an acceptable treatment approach for resectable or potentially resectable NSCLC (21). However, the role of neoadjuvant targeted therapy remains unclear due to the lack of prospective phase III RCTs. Our pooled analysis indicated that neoadjuvant EGFR-TKI therapy may provide a feasible treatment modality for patients with resectable or potentially resectable EGFR-mutant NSCLC, with satisfactory surgical outcomes and low toxicity. Although further phase III clinical trials are needed to confirm these findings, especially whether neoadjuvant EGFR-TKI treatment can improve survival of such patients, several controversial questions were addressed.

The first question is whether neoadjuvant EGFR-TKI was more effective than neoadjuvant chemotherapy for EGFR-mutant NSCLC patients. If the group of patients being treated had advanced unresectable or metastatic NSCLC, this question was not difficult to answer. As for patients with advanced NSCLC with EGFR-sensitive mutations, more than a dozen phase III RCTs studies have reached a consistent conclusion that, as compared to platinum-based doublet chemotherapy, EGFR-TKIs significantly improved the median PFS (9–20 months) and ORR (60%–80%) (8–11). In the phase II EMERGING (CTONG 1103) study, which included a total of 72 patients with stage IIIA-N2 EGFR-mutated NSCLC and compared neoadjuvant erlotinib with neoadjuvant chemotherapy of gemcitabine plus cisplatin, the primary endpoint of ORR was 54.1% (95% CI, 37.2%–70.9%)

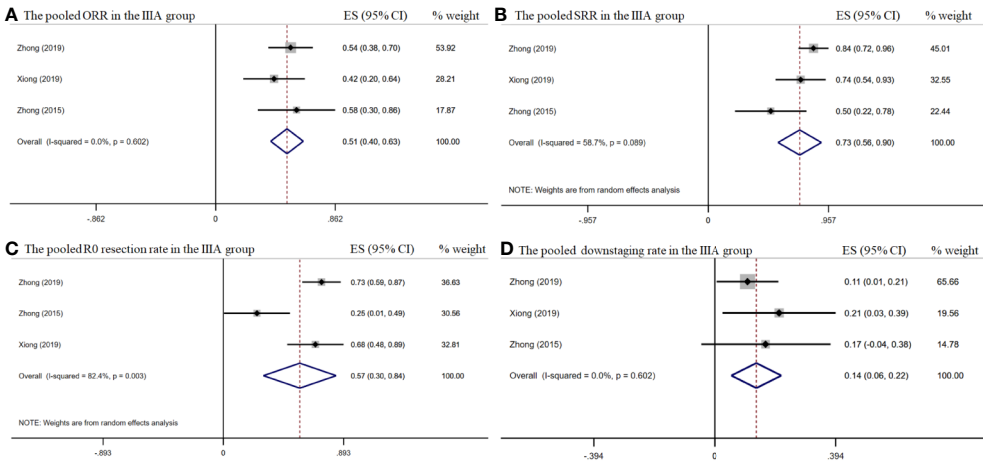

FIGURE 3 | The pooled efficacy rates in the stage IIIA subgroup. The ORR **(A)**; SRR **(B)**; R0 resection rate **(C)**; and downstaging rate **(D)**.

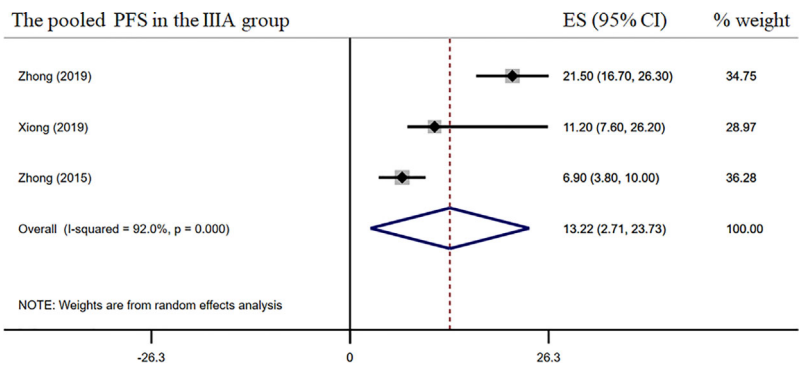

FIGURE 4 | Median PFS of the stage IIIA subgroup.

TABLE 3 | The main toxicity of neoadjuvant EGFR-TKI therapy.

	subcutaneous tissue disorders				Hematologic	Gastrointestinal	Hepatorenal	
	Rash		Paronychia	Stomatitis	Leukopenia	Diarrhea	Abnormal liver function	
	All grade	≥3 grade	All grade	All grade	≥3 grade	All grade	All grade	≥3 grade
Studies*	4	4	2	1	1	4	4	4
Patients*	103	103	72	37	19	103	103	103
Events	65	2	3	4	1	26	2	1
pooled incidence rates (%)	54.9	14.7	3.8	10.8	5.3	14.7	7.7	5.3
Range (%)	30.3–79.6	2.7–26.8	0–8.2	NA	NA	2.7–26.8	0–16.6	NA

**Number of studies reporting this toxicity and number of patients included in these studies. NA, Not Applicable.*

versus 34.3% (95% CI, 17.7%–50.8%), respectively, with an odds ratio of 2.26 (95% CI, 0.87–5.84; p = 0.092) (5). Although the difference was not statistically significant, the ORR tended to be higher for neoadjuvant EGFR-TKI treatment than neoadjuvant chemotherapy. In the present study, the pooled ORRs of overall stage I–IIIA patients and stage IIIA subgroup were 58.5% and 51.4%, respectively, both of which were numerically superior to those in previous studies of neoadjuvant chemotherapy (28%–49%) (22–24). Among these neoadjuvant chemotherapy studies, most patients had stage IB–IIIA NSCLC with predominant squamous cell carcinomas or adenocarcinomas treated with three cycles of platinum-based chemotherapy followed by surgical resection. Despite the insufficient sample size of CTONG 1103, the secondary endpoint (PFS) was significantly

TABLE 4 | Postoperative complications.

Study*	Lung infection	Sinus tachycardia or arrhythmia	Chylothorax	Poor incision healing	Lung infection or left-sided pneumothorax	Chest tube drainage for >7 days	Postoperative bleeding	Pulmonary artery injury
Zhong (5)	2 (6.5%)	2 (6.5%)		2 (6.5%)	1 (3.2%)	1 (3.2%)		
Zhong (17)							1 (16.7%)	
Zhang (19)			4 (12.1%)					
Rizvi (18)	Y	Y			Y			Y

**Studies reported the result of postoperative complications; Y, Study just reported the events without concrete data.*

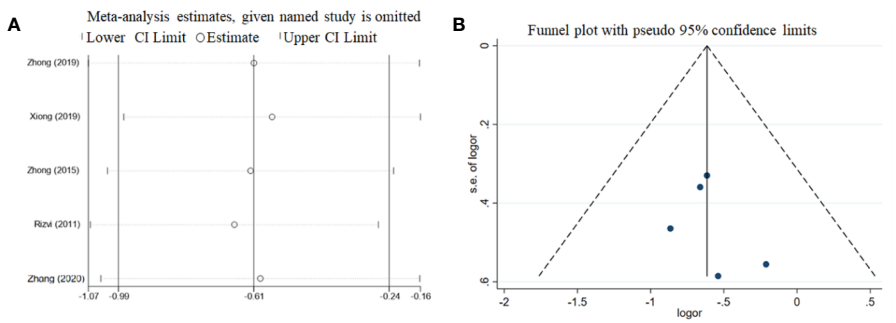

FIGURE 5 | Sensitivity analyses **(A)** and funnel plot **(B)** of the ORR among the included studies.

improved. The median PFS was 21.5 months with erlotinib vs. 11.4 months with chemotherapy (HR, 0.39; 95% CI, 0.23 to 0.67; $p < 0.001$). However, there was no significant difference in OS between the two groups (45.8 vs. 39.2 months; HR, 0.77; 95% CI, 0.41–1.45; $p = 0.417$). The limited number of patients and differences in follow-up treatment may be the main reasons for the lack of differences in OS. In the present study, the pooled median OS for neoadjuvant EGFR-TKIs in the stage IIIA group was 41.9 months, which is comparable to the OS results of previous studies of neoadjuvant chemotherapy (median range, 16–55 months) (22–24). Due to the lack of more RCTs, it was unclear whether neoadjuvant EGFR-TKI therapy could improve OS as compared to neoadjuvant chemotherapy. Ongoing large phase III RCTs (e.g., NCT03203590) will further clarify the difference in OS between neoadjuvant chemotherapy and neoadjuvant EGFR-TKI.

Surprisingly, the higher ORR for neoadjuvant EGFR-TKI treatment did not appear to be associated with a remarkable improvement in surgical outcomes. In the CTONG 1103 study, the surgical resection and R0 rates were 83.8% and 73%, respectively, in the preoperative EGFR-TKI group, and 68.6% and 62.9%, respectively, in the preoperative chemotherapy group, while the downstaging and pCR rates were only 10.8% and 0%, and 2.9% and 0%, respectively in the two groups. In a study by Zhang et al., neoadjuvant therapy with gefitinib for 35 patients with operable stage II-IIIA NSCLC with EGFR-sensitive mutations led to a pCR of 12.1% (4/33), major pathological response rate of 24.2% (8/33), and an ORR of 54% (18/33) (19). In the present review, data of 68 patients with stage IIIA-N2 NSCLC were extracted from three studies for independent analysis. In this subgroup, the surgical resection and R0 rates were 79.7% and 56.8%, respectively. However, the downstaging and pCR rates were merely 14.0% and 0%, respectively. Numerically, a portion of surgical outcomes in the present study, especially the downstaging and pCR rates, was inferior to those in previous studies of neoadjuvant chemotherapy (22–24). In a phase III RCT comparing induction chemoradiation with induction chemotherapy, which included 232 patients with stage IIIA-N2 NSCLC, the surgical resection and R0 rates in the induction chemotherapy group were 82% and 81%, respectively, and the downstaging and pCR rates were 53.0% and 16%, respectively (n = 117) (25). In another large RCT involving 354 patients with stage IB–IIIA NSCLC (excluding N2 disease), the surgical resection and R0 rates in the neoadjuvant chemotherapy group were 89.9% and 93%, respectively (22). Consistently, the EGFR mutation status was not elucidated in these previous studies.

In brief, neoadjuvant EGFR-TKI therapy could significantly shrink tumor volume and improve radiological responses, while increasing the curative resection rate. However, this impressive tumor shrinkage effect has not been translated into changes in disease stage or pCR rate. We believe that the spatial heterogeneity within and between tumors may be the main reason for this unexpected result.

The second question is the timing of EGFR-TKI medication for patients with operable NSCLC with EGFR-sensitive mutations, as it remains unclear whether preoperative or postoperative administration of EGFR-TKI, or both is more beneficial for these patients. Indeed, if the patient population is diagnosed with EGFR-mutant NSCLC after surgery, particularly stage IIIa-pN2, the question has been positively answered with adjuvant EGFR-TKI treatment. Several RCTs reported that adjuvant EGFR-TKI therapy significantly improved DFS (28.7 vs. 18.0 months for the ADJUVANT study; 42.4 vs. 21.0 months for the EVAN study, and not reached vs. 20.4 months for the

ADAURA study, respectively) as compared with adjuvant chemotherapy or placebo for patients with postoperative stage II-III NSCLC with EGFR-sensitive mutations (12–14), thus providing strong evidence for adjuvant EGFR-TKI therapy. In the present study, the pooled median PFS and OS for neoadjuvant EGFR-TKIs in the stage IIIA group were 13.2 and 41.9 months, respectively, but median PFS values varied between 6.9 and 21.5 months. In the CTONG 1103 study, the erlotinib-treatment group of patients who were intended to receive neoadjuvant erlotinib therapy for 42 days and adjuvant erlotinib therapy for 1 year obtained a median PFS of 21.5 months (5). In a study by Xiong et al., patients who received neoadjuvant erlotinib therapy for 56 days and three cycles of adjuvant chemotherapy achieved a median PFS of 11.2 months (16). In the CSLC0702 study, the median PFS was only 6.9 months (17). This difference might be attributed to inconsistencies in subsequent adjuvant therapies (postoperative chemotherapy vs. adjuvant EGFR-TKIs vs. postoperative radiotherapy etc.). Clinically, for patients with operable EGFR-mutant NSCLC, adjuvant chemotherapy, EGFR-TKI, or a combination of both are currently acceptable treatment options, although the most efficacious remains controversial. For potentially resectable NSCLC, neoadjuvant EGFR-TKI should be considered given the better ORR, SRR, and safety as compared to chemotherapy.

The third question concerns the duration of neoadjuvant EGFR-TKI therapy. In our pooled analysis, the median medication duration was 42 (range, 21–56) days and the efficacy evaluation time was 45 (range, 42–56) days. Of note, for advanced NSCLC, the ORR for neoadjuvant EGFR-TKI was slightly lower than that for first-line EGFR-TKI (58% vs. 62%–70%, respectively). For advanced disease, the efficacy evaluation time commonly ranged between 42 and 56 days (8–11). Different durations of drug exposure might influence efficacy. In the five included studies, the ORR varied from 42% to 81%. Paradoxically, Rizvi et al. reported a medication time of 21 days and ORR of 81% (18), while Xiong et al. reported a medication time of 56 days and ORR of only 42% (16). Obviously, patient characteristics and the neoadjuvant drugs of EGFR-TKIs differed among these studies. The study by Xiong et al. was limited to patients with stage IIIA NSCLC treated with erlotinib therapy, while the study by Rizvi et al. was limited to patients with IA–IIB early-stage NSCLC treated with gefitinib. According to the ORR results and postoperative outcomes, 42 days is a rational medication time for clinical treatment because the effect would not be evaluated prematurely, the delay in surgical intervention would not too long, and toxicities would not obviously increase.

The last question addresses the safety of neoadjuvant EGFR-TKI. Neoadjuvant TKI therapy appears to be generally well tolerated. Similar to the AEs as the first-line treatment for patients with advanced disease, the common side effects were skin rash, diarrhea, and other skin and subcutaneous tissue disorders, as well as hepatotoxicity. The incidence of grade 3/4 adverse events was 5.3% for hepatotoxicity and 14.7% for skin rash. Surgery was no delayed for any patient due to treatment-related AEs (TRAEs). In contrast, TRAEs, including perioperative death and treatment-induced surgery delay, limit the application of preoperative chemotherapy (22, 26). In total, 48%–60% of AEs were grade 3/4 and 6% of TRAEs led to permanent discontinuation of chemotherapy (25, 26).

We were more concerned with surgical difficulties and risks, and intra- and postoperative complications. In the CTONG 1103 study, the types of resection in the erlotinib and chemotherapy groups were lobectomy (64.9% vs. 54.3%), bi-lobectomy (13.5% vs. 14.3%), and pneumonectomy (5.4% vs. 0.0%, respectively). Zhang et al. reported lobectomy in 93.9% of patients and bi-lobectomy in 6.1% (19). The most common postoperative complications were lung infection, arrhythmia, and pneumothorax. No perioperative death, increase in surgical difficulty, or postoperative complications caused by neoadjuvant EGFR-TKI was observed.

There were some limitations to this pooled analysis. Although there was no publication bias, the included studies were all phase II clinical trials with small sample sizes. Furthermore, differences in patient characteristics may have influenced the results. In addition, different medications for different EGFR-TKI types were not stratified, so it remains to be determined whether efficacy differed among the different EGFR-TKIs.

CONCLUSION AND FUTURE PERSPECTIVES

Although data from prospective phase III RCTs evaluating the role of neoadjuvant targeted therapy for patients with EGFR-mutant NSCLC are lacking, the results of this pooled analysis indicated that short-term (median, 42 days; range, 21–56 days) neoadjuvant EGFR-TKI therapy provided a feasible treatment modality for patients with resectable or potentially resectable EGFR-mutant NSCLC, with satisfactory surgical resection and R0 rates (80% and 64.3%, respectively), but modest downstaging and pathological complete response rates (14% and 0%, respectively). The incidence of grade 3/4 toxicity was low. Because the studies included in this pooled analysis were all phase II clinical trials with small sample sizes, further studies with well-designed phase III clinical trials are warranted to confirm the efficacy and safety of neoadjuvant EGFR-TKIs. An ongoing clinical trial (NCT03203590) is investigating the efficacy and safety of gefitinib neoadjuvant targeted therapy and vinorelbine/carboplatin neoadjuvant chemotherapy for resectable stage II-IIIA NSCLC patients with EGFR mutation.

There is an urgent need to explore a more effective neoadjuvant targeted therapy regimen given the modest downgrade and pCR rates for EGFR-TKI alone. Two RCTs showed that first-line treatment with gefitinib plus chemotherapy achieved a significantly higher ORR (84% vs. 67% for the NEJ009 study; 75% vs. 63% for the study by Noronha et al.), longer PFS (median, 20.9 vs. 11.9 months for the NEJ009 study; 16 vs. 8 months for the study by Noronha et al.) and longer OS than gefitinib alone for patients with EGFR-mutant advanced NSCLC (27, 28). Given the strong ORRs and PFS, it is very worthwhile to design clinical trials to validate the feasibility of chemotherapy combined with EGFR-TKI as a neoadjuvant therapy for EGFR-mutant NSCLC.

Because the results of adjuvant osimertinib in the phase III ADAURA study were impressive, a single arm phase II trial (NCT03433469) is ongoing to evaluate the efficacy of osimertinib as a neoadjuvant therapy for patients with surgically resectable (stage

I-IIIA) EGFR-mutant NSCLC, and a phase III trial neoADAURA (NCT04351555) is planned to compare neoadjuvant osimertinib, with or without chemotherapy, and chemotherapy alone for resectable NSCLC (29). These prospective clinical studies will confirm whether and what type of EGFR-TKI neoadjuvant treatment can improve survival of patients with EGFR mutations.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

J-TM: conceptualization, methodology, manuscript review, and revision. LS: writing of the original draft. Y-JG: data extraction and collection. JS: data extraction and collection. WJ: software. Y-RW: manuscript review and revision. S-LZ: Software. L-TH: formal analysis. J-ZZ: table editing. C-BH: conceptualization, methodology, and supervision. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* (2020) 70(1):7–30. doi: 10.3322/caac.21590
2. Früh M, Betticher DC, Stupp R, Xyrafas A, Peters S, Ris HB, et al. Multimodal treatment in operable stage III NSCLC: A pooled analysis on long-term results of three SAKK trials (SAKK 16/96, 16/00, and 16/01). *J Thorac Oncol* (2019) 14(1):115–23. doi: 10.1016/j.jtho.2018.09.011
3. Kwiatkowski DJ, Rusch VW, Chaft JE, Johnson BE, Nicholas A, Wistubaet II, et al. Neoadjuvant atezolizumab in resectable non-small cell lung cancer (NSCLC): Interim analysis and biomarker data from a multicenter study (LCMC3). *J Clin Oncol* (2019) 37(suppl; abstr 8503):8503-8503. doi: 10.1200/JCO.2019.37.15_suppl.8503
4. Cascone T, William WN, Weissferdt A, Lin HY, Leung CH, Carter BW, et al. Neoadjuvant nivolumab (N) or nivolumab plus ipilimumab (NI) for resectable non-small cell lung cancer (NSCLC): Clinical and correlative results from the NEOSTAR study. *J Clin Oncol* (2019) 37(suppl; abstr 8504):8504-8504. doi: 10.1200/JCO.2019.37.15_suppl.8504
5. Zhong WZ, Chen KN, Chen C, Gu CD, Wang J, Yang XN, et al. Erlotinib versus gemcitabine plus cisplatin as neoadjuvant treatment of stage IIIA-N2 EGFR-mutant non-small-cell lung cancer (EMERGING-CTONG 1103): A randomized phase II study. *J Clin Oncol* (2019) 37(25):2235–45. doi: 10.1200/JCO.19.00075
6. Song WA, Zhou NK, Wang W, Chu XY, Liang CY, Tian XD, et al. Survival benefit of neoadjuvant chemotherapy in non-small cell lung cancer an updated meta-analysis of 13 randomized control trials. *J Thorac Oncol* (2010) 5(4):510–6. doi: 10.1097/JTO.0b013e3181cd3345
7. NSCLC Meta-analysis Collaborative Group. Preoperative chemotherapy for non-small cell lung cancer: a systematic review and meta-analysis of individual participant data. *Lancet* (2014) 383(9928):1561–71. doi: 10.1016/S0140-6736(13)62159-5
8. Inoue A, Kobayashi K, Maemondo M, Sugawara S, Oizumi S, Isobe H, et al. Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naive non-small cell lung cancer with sensitive EGFR gene mutations (NEJ002). *Ann Oncol* (2013) 24:54–9. doi: 10.1093/annonc/mds214
9. Zhou C, Wu YL, Chen G, Feng JF, Liu XQ, Wang CL, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* (2011) 12:735–42. doi: 10.1016/S1470-2045(11)70184-X
10. Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J Clin Oncol* (2011) 29:2866–74. doi: 10.1093/annonc/mds214
11. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, 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):121–8. doi: 10.1016/S1470-2045(09)70364-X
12. Zhong WZ, Wang Q, Mao WM, Xu ST, Wu L, Shen Y, et al. Gefitinib versus vinorelbine plus cisplatin as adjuvant treatment for stage II-IIIA (N1-N2) EGFR-mutant NSCLC (ADJUVANT/ CTONG1104): a randomised, open-label, phase 3 study. *Lancet Oncol* (2018) 19(1):139–48. doi: 10.1016/S1470-2045(17)30729-5
13. Yue D, Xu S, Wang Q, Li X, Shen Y, Zhao H, et al. Erlotinib versus vinorelbine plus cisplatin as adjuvant therapy in Chinese patients with stage IIIA EGFR mutation-positive non-small-cell lung cancer (EVAN): a randomised, open-label, phase 2 trial. *Lancet Respir Med* (2018) 6(11):863–73. doi: 10.1016/S2213-2600(18)30277-7
14. Wu YL, Tsuboi M, He J, John T, Grohe C, Majem M, et al. ADAURA Investigators. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. *N Engl J Med* (2020) 383(18):1711–23. doi: 10.1056/NEJMoa2027071
15. Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* (2009) 339:b2535. doi: 10.1136/bmj.b2535
16. Xiong L, Li R, Sun J, Lou Y, Zhang W, Bai H, et al. Erlotinib as neoadjuvant therapy in stage IIIA (N2) EGFR mutation-positive non-small cell lung cancer: A prospective, single-arm, phase II study. *Oncologist* (2019) 24(2):157–e64. doi: 10.1634/theoncologist.2018-0120
17. Zhong W, Yang X, Yan H, Zhang X, Su J, Chen Z, et al. Phase II study of biomarker-guided neoadjuvant treatment strategy for IIIA-N2 non-small cell lung cancer based on epidermal growth factor receptor mutation status. *J Hematol Oncol* (2015) 8:54. doi: 10.1186/s13045-015-0151-3
18. Rizvi NA, Rusch V, Pao W, Chaft JE, Ladanyi M, Miller VA, et al. Molecular characteristics predict clinical outcomes: prospective trial correlating response to the EGFR tyrosine kinase inhibitor gefitinib with the presence of sensitizing mutations in the tyrosine binding domain of the EGFR gene. *Clin Cancer Res* (2011) 17(10):3500–6. doi: 10.1158/1078-0432.CCR-10-2102
19. Zhang Y, Fu F, Hu H, Li Y, Hu H, Chen H. Gefitinib as neoadjuvant therapy for resectable stage II-IIIA non-small cell lung cancer: A phase II study [published online ahead of print, 2020 Mar 19]. *J Thorac Cardiovasc Surg* (2020) S0022-5223(20)30625-5. doi: 10.1016/j.jtcvs.2020.02.131
20. Ma JT, Sun J, Sun L, Zhang SL, Huang LT, Han CB. Efficacy and safety of apatinib in patients with advanced nonsmall cell lung cancer that failed prior chemotherapy or EGFR-TKIs: A pooled analysis. *Med (Baltimore)* (2018) 97(35):e12083. doi: 10.1097/MD.0000000000012083
21. NCCN *Clinical Practice Guidelines in Oncology for Non-Small Cell Lung Cancer, Version 6* (2018). Available at: www.nccn.org/patients (Accessed August 17, 2018).

22. Pisters KM, Vallières E, Crowley JJ, Franklin WAC.OMMAJ.R.X.X.X PA, Ginsberg RJ, et al. Surgery with or without preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer: Southwest Oncology Group Trial S9900, an intergroup, randomized, phase III trial. *J Clin Oncol* (2010) 28(11):1843–9. doi: 10.1200/JCO.2009.26.1685
23. Gilligan D, Nicolson M, Smith I, Groen H, Dalesio O, Goldstraw P, et al. Preoperative chemotherapy in patients with resectable non-small cell lung cancer: results of the MRC LU22/NVALT 2/EORTC 08012 multicentre randomised trial and update of systematic review. *Lancet* (2007) 369 (9577):1929–37. doi: 10.1016/S0140-6736(07)60714-4
24. Nagai K, Tsuchiya R, Mori T, Tada H, Ichinose Y, Koike T, et al. A randomized trial comparing induction chemotherapy followed by surgery with surgery alone for patients with stage IIIA N2 non-small cell lung cancer (JCOG 9209). *J Thorac Cardiovasc Surg* (2003) 125(2):254–60. doi: 10.1067/mtc.2003.15
25. Pless M, Stupp R, Ris HB, Stahel RA, Weder W, Thierstein S, et al. Induction chemoradiation in stage IIIA/N2 non-small-cell lung cancer: a phase 3 randomised trial. *Lancet* (2015) 386(9998):1049–56. doi: 10.1016/S0140-6736(15)60294-X
26. Depierre A, Milleron B, Moro-Sibilot D, Chevret S, Quoix E, Lebeau B, et al. Preoperative chemotherapy followed by surgery compared with primary surgery in resectable stage I (except T1N0), II, and IIIa non-small-cell lung cancer. *J Clin Oncol* (2002) 20(1):247–53. doi: 10.1200/JCO.2002.20.1.247
27. Hosomi Y, Morita S, Sugawara S, Kato T, Fukuhara T, Gemma A, et al. Gefitinib alone versus gefitinib plus chemotherapy for non-small-cell lung cancer with mutated epidermal growth factor receptor: NEJ009 Study. *J Clin Oncol* (2020) 38(2):115–23. doi: 10.1200/JCO.19.01488
28. Noronha V, Patil VM, Joshi A, Menon N, Chougule A, Mahajan A, et al. Gefitinib versus gefitinib plus pemetrexed and carboplatin chemotherapy in EGFR-mutated lung cancer. *J Clin Oncol* (2020) 38(2):124–36. doi: 10.1200/JCO.19.01154
29. Friedlaender A, Addeo A, Russo A, Gregorc V, Cortinovis D, Rolfo CD. Targeted therapies in early stage NSCLC: Hype or hope? *Int J Mol Sci* (2020) 21(17):6329. doi: 10.3390/ijms21176329

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CASE REPORT
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Case Report: Afatinib Treatment in a Patient With NSCLC Harboring a Rare *EGFR* Exon 20 Mutation

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Unlike most other primary epidermal growth factor receptor (*EGFR*) mutations in non-small cell lung cancer (NSCLC), exon 20 insertions, comprising approximately 4% to 10% of all *EGFR* mutations, are generally considered to be resistant to EGFR tyrosine kinase inhibitors (TKIs). However, *EGFR* exon 20 insertions are structurally and pharmacologically heterogeneous, with variability in their position and size having implications for response to different EGFR TKIs. The second-generation ErbB family blocker, afatinib, is approved for the first-line treatment of *EGFR* mutation-positive NSCLC and has been shown to have a broad inhibitory profile against common and uncommon *EGFR* mutations. Here, we describe a patient with bilateral multifocal lung adenocarcinoma harboring a very rare *EGFR* exon 20 insertion (c.2317_2319dup3; p.H773dup), who has been receiving treatment with afatinib for 4.5 years. To our knowledge, this is the first report describing long-term benefit for a patient treated with afatinib with this rare exon 20 insertion. We are aware of two further cases with this rare *EGFR* mutation. One patient, also reported here, has early-stage lung adenocarcinoma and has not yet received systemic therapy for NSCLC. The other patient received afatinib in the context of a global compassionate use program and had progressive disease. Our findings may be of clinical relevance for patients carrying tumors with this rare mutation as epidemiological evidence suggests that p.H773dup may function as a driver mutation in NSCLC. Together with previous preclinical and clinical evidence for the activity of afatinib against certain *EGFR* exon 20 insertions, these findings warrant further investigation.

Keywords: afatinib, *EGFR* mutation, exon 20 insertion, H773dup, long-term response, NSCLC, uncommon mutation

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INTRODUCTION

In non-small cell lung cancer (NSCLC), activating mutations in the epidermal growth factor receptor (*EGFR*) gene are reported in approximately 10% to 15% of Caucasian and 50% of Asian patients (1). *EGFR* mutation-positive tumors tend to be dependent on EGFR signaling for their growth and survival and, consequently, several EGFR-targeted therapeutics have been developed and approved.

Two types of *EGFR* mutation, exon 19 deletions (Del19) and the exon 21 substitution L858R, represent approximately 45% and 40% of all *EGFR* mutations, respectively (2). Del19 and L858R are therefore classed as common *EGFR* mutations and are the best characterized in terms of their association with response to EGFR TKIs (3). For these mutations, drugs approved by the United States (US) Food & Drug Administration (FDA) for *EGFR* mutation-positive NSCLC comprise three generations of EGFR tyrosine kinase inhibitors (TKIs) — the first-generation reversible EGFR TKIs, erlotinib and gefitinib, the second-generation irreversible ErbB family blockers, afatinib and dacomitinib, and the third-generation irreversible, EGFR wild-type sparing TKI, osimertinib (4).

Many of the less common mutations are also sensitive to EGFR inhibitors. Afatinib, in particular, shows a broad preclinical activity across uncommon *EGFR* mutations (5), and has demonstrated clinical efficacy against uncommon mutations such as G719X, S768I, and L861Q (in exons 18, 20, and 21, respectively) (6). Based on these findings, the US indication for afatinib was extended to include S768I, L861Q and G719X mutations (7), while all activating *EGFR* mutations were already included in the labels in Europe (8).

Treatment options for the most prevalent uncommon mutations, i.e. *EGFR* exon 20 insertions (~4–10% of all *EGFR* mutations), are not clear and represent an area of unmet need. A recent Phase 2 study, ZENITH20, assessed poziotinib, a covalent inhibitor of EGFR and human epidermal growth factor receptor 2 (HER2), in pretreated NSCLC patients with exon 20 insertions. Although the study did not meet its primary endpoint, there was some evidence of clinical activity, with an objective response rate (ORR) of 15%, disease control rate of 69% and median progression-free survival (PFS) of 4.2 months. Treatment-naïve cohorts using alternative dosing regimens to improve tolerability are ongoing (9). Other investigational agents, including the EGFR/HER2 inhibitor, TAK-788 (10) and the bispecific EGFR/cMET antibody, JNJ-372 (11), are in clinical development. While, in principle, most *EGFR* exon 20 insertions could be considered as oncogenic driver mutations, since they promote interleukin 3- and EGF-independent growth of Ba/F3 cells (5), structural and pharmacological differences between specific mutations mean that their sensitivity to targeted treatment differs depending on the inhibitor used and the mutational context. Some exon 20 insertions exist as compound mutations, which could also contribute to EGFR-TKI resistance (5). With the exception of A763_Y764insFQEA (12), most *EGFR* exon 20 insertion mutations are resistant to first-generation EGFR TKIs, while the efficacy of second-/third-generation TKIs against these mutations is less clear (13). Indeed, a post-hoc analysis of the LUX-Lung 2, 3, and 6 trials suggested limited activity of afatinib treatment in patients with *EGFR* exon 20 insertions (6). In 23 such patients the ORR was 8.7% and median PFS was 2.7 months (6). In contrast, data from the afatinib uncommon *EGFR* mutations database indicate that afatinib has modest but apparent clinical activity. The response rate in 70 TKI-naïve patients with exon 20 insertions was 24% and median duration of response was 11.9 months (14). In addition to the mutations known to be responsive to EGFR TKIs, a number of rare exon 20 insertions showed sensitivity to afatinib including A767delinsASVD (13) and A767_S768insSVA (15).

Previously published preclinical evidence shows that afatinib displays inhibitory activity against some *EGFR* exon 20 insertion mutations (5, 16–18). Using ectopically expressed *EGFR*-mutants in NIH-3T3 cell lines, we found that, consistent with previous findings, afatinib was at least 100-fold more potent against G719S (Exon 18) and L861Q (Exon 21) mutations (**Figure 1**) compared to erlotinib. For most exon 20 mutations, EGFR phosphorylation was inhibited by afatinib at concentrations exceeding the clinically relevant C_{max} of 100 nM (except D770_N771insNPG), while erlotinib was ineffective in reducing constitutive phosphorylation in the exon 20 mutations tested at concentrations up to 10000 nM (**Figure 1**). The observed biomarker modulation data is in-line with previously reported proliferation data in the BA/F3 system (IC_{50} of afatinib for Y764_V765insHH, A767_V769dupASV, and D770_N771insNPG were 134, 158, and 43 nM, respectively) (10, 16). Taken together, these data show differential sensitivity of EGFR exon 20 mutations to different EGFR-TKIs in preclinical and clinical contexts. Careful evaluation of the EGFR mutational context, including potency and therapeutic window, will be essential to select appropriate treatments for patients harboring tumors with EGFR exon 20 mutations.

Here, we report on a case of a patient with a very rare *EGFR* exon 20 insertion (c.2317_2319dup3; p.H773dup) who has been receiving treatment with afatinib for 4.5 years. In addition, we describe a patient in the early stages of lung cancer treatment, who underwent surgery at the same institution and had an identical p.H773dup mutation.

CASE REPORT 1

Patient 1 was a 62-year-old, female, ex-smoker (20 pack-years) with no major comorbidities. She was incidentally diagnosed in November 2014 with a 6.4 cm mass in the left upper lobe and multiple ground glass nodules in both lungs, the largest of which was located in the right middle lobe, with a maximum diameter of 1.8 cm. Histopathology following resection of the left upper lobe confirmed lung adenocarcinoma (Grade 2; pT2b pN1 [2/17 lymph nodes positive]; V0 R0), with partially papillary and partially tubular morphology. Molecular pathology based on Sanger sequencing of *EGFR* exons 18 to 21 indicated no *EGFR* mutation, and the tumor was *ALK* fluorescence *in situ* hybridization negative and ROS1 immunohistochemistry-negative (**Figure 2**).

The patient received 4 cycles of adjuvant chemotherapy with carboplatin and vinorelbine from January 2015 until April 2015, but a computed tomography (CT) scan in May 2015 showed disease progression. The scan revealed several pulmonary focal ground-glass opacities (GGOs) in both lungs, some of which had increased in size compared with preoperative CT findings (**Figure 3**). Biopsy of a GGO lesion of the right upper lobe in

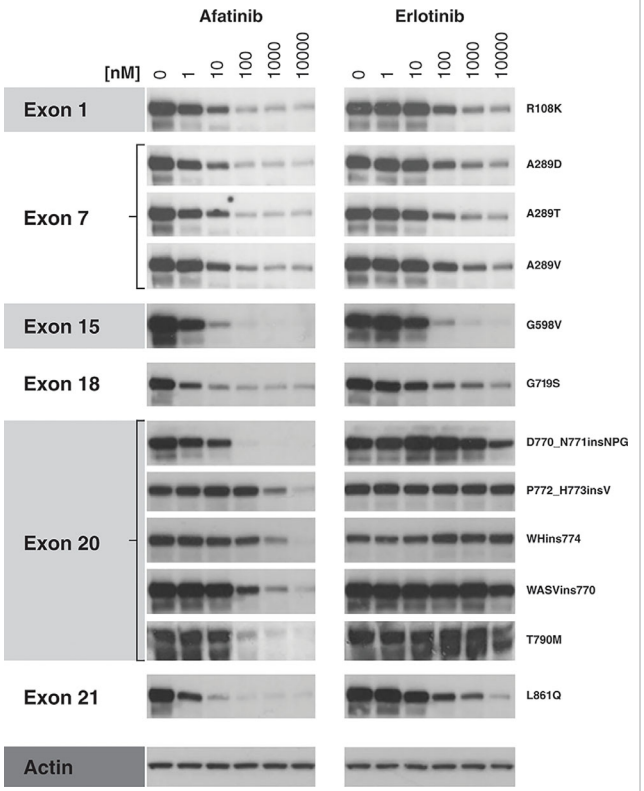

FIGURE 1 | Inhibition of *EGFR* mutant protein autophosphorylation by afatinib and erlotinib in cellular assays. NIH-3T3 cells (ATCC; #CRL-1658) were cultured in supplemented Dulbecco's Modified Eagle Medium and cultivated at 37°C/5% CO_2 in a humidified atmosphere to maintain <80% confluence. Cells were then transfected with one of 12 *EGFR* mutant plasmid constructs, using 4 µg of DNA for EGFR variants G598V, D770_N771insNPG, P772_H773insV, WHins774, and T790M, and 2.5 µg of constructs R108K, A289D, A289T, A289V, G719S, WASVins770, and L861Q, diluted in 250 µl serum-free culture medium. A diluted Lipofectamine-DNA mix was then added drop-wise to the cells. 48 h post-transfection, cells were treated for 2 h with afatinib or erlotinib (1–10,000 nM) or were left untreated. At 2 h post-treatment, protein lysates were prepared using lysis buffer and the effect of TKI treatment on EGFR tyrosine-1068 phosphorylation was analyzed by Western blot (primary antibody: phospho-specific anti-EGFR [Y1068] antibody; Abcam, #ab40815, 1:1,000 dilution; secondary antibody: goat anti-rabbit; Dako, #P0448, 1:1,000 dilution). Actin was used as a loading control. *EGFR*, epidermal growth factor receptor.

June 2015 indicated Grade 2 adenocarcinoma of the lung that was partially tubular and partially lepidic.

Further Sanger sequencing detected an *EGFR* exon 20 insertion mutation (NM_005228.3 [EGFR]: codon 2317_2319dupCAC; p.H773dup; **Figures 4A, B**). From July 2015, the patient received afatinib 40 mg/day, which was reduced to 30 mg/day in August 2015 and further reduced to 20 mg/day in October 2015 due to diarrhea. The patient achieved stable disease until May 2016, followed by progression, which was treated with stereotactic irradiation to two lesions (40.5 Grays in 3 fractions to each lesion) in the left lower lobe and one lesion in the right upper lobe. She remained on treatment with afatinib (20 mg/day) and had stable disease again from July 2016 until July 2017 (**Figure 3**), when progression ensued and she received stereotactic irradiation to one lesion in the left lower lobe.

Two liquid biopsies were performed, in January and August 2017. No *EGFR* or any other mutation was detected in either sample, using next-generation sequencing (NGS) with a colon/lung 22-gene (including *EGFR*) panel from Thermo Fisher Scientific. The sample from January was additionally analyzed with digital PCR for Del19 and T790M mutations, with none detected. In December 2018, a third liquid biopsy was performed, this time employing an NGS liquid biopsy lung circulating free DNA (11-gene) panel from Thermo Fisher Scientific. Again, no mutations were detected.

Ileocecal resection was performed in April 2018 because of ischemic necrosis of the cecum, and no carcinoma infiltration was detected in the resection specimen. Because of slightly enlarged retrocaval lymph nodes in a CT scan from December 2018, lymph node and lung biopsies were performed in January 2019. Endobronchial ultrasound-guided biopsy of the mediastinal lymph nodes revealed no carcinoma infiltration, and bronchoscopy with biopsy lower right lobe (B10) identified no carcinoma in the lung parenchyma. However, *Escherichia coli* and *Klebsiella pneumonia* were detected in the bronchoalveolar lavage, and the patient received antibiotic treatment with ciprofloxacin. A CT scan in February 2019 showed a decrease in size of the previously enlarged lymph nodes. Treatment with afatinib is ongoing as of October 2020, and the patient continues to have stable disease (**Figure 3**), with an Eastern Cooperative Oncology Group performance status of 0.

CASE REPORT 2

Patient 2 was referred to our clinic for surgery in September 2019. The patient had previously undergone resection of breast carcinoma, and received radiotherapy and hormone ablation therapy. A lung tumor was detected with position emission tomography-CT scan, and resection of the left lower lobe with lymphadenectomy was performed in September 2019, due to suspicion of lung metastasis of the breast carcinoma. Histology revealed invasive adenocarcinoma, and NGS with the Oncomine Focus Assay (Thermo Fisher Scientific) identified an *EGFR* exon 20 insertion (NM_005228.3 [EGFR]: codon 2317_2319dupCAC; p.H773dup; **Figures 4C, D**) that was identical to the mutation identified in Patient 1.

After tumor resection, the patient was re-transferred to their previous hospital.

DISCUSSION AND CONCLUDING REMARKS

Exon 20 insertion mutations are the third most common type of *EGFR* mutation, after Del19 and L858R (3). Similar to Del19 and L858R, *EGFR* exon 20 insertions can result in sustained EGFR signaling and function as oncogenic drivers. However,

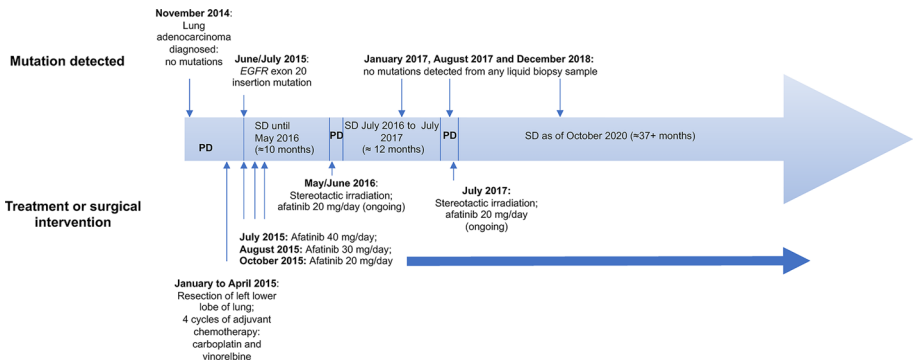

FIGURE 2 | Patient 1 case history and time line of key events. PD, progressive disease; SD, stable disease.

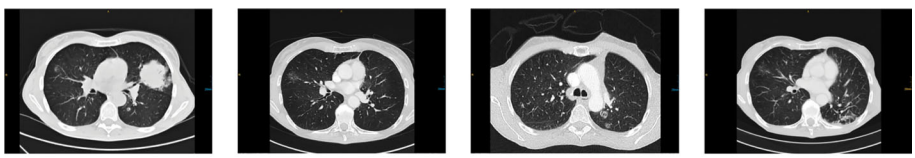

FIGURE 3 | Patient 1 clinical course, including treatment history and CT scans. CT, computed tomography.

despite their importance as potentially targetable mutations, the clinicopathologic characteristics and molecular spectrum of exon 20-mutant tumors have not been explored in most patient populations, and the biology underlying the heterogeneous responses of different genomic variants to targeted therapies is not well understood (3).

The first case presented herein describes a patient with NSCLC harboring an *EGFR* exon 20 insertion mutation who achieved durable stable disease with afatinib and remains on treatment after 4.5 years. To the best of our knowledge, this is the first report describing long-term benefit for a patient treated with afatinib with this rare exon 20 insertion. We are aware of two further cases with this same, rare *EGFR* mutation. One patient, also reported here, has early-stage lung adenocarcinoma and has not yet received systemic therapy for NSCLC. The other patient received afatinib in the context of a global compassionate use program and had progressive disease (19, 20). It is not uncommon for patients to have concurrent mutations alongside exon 20 insertions (21); therefore, we speculate that the difference in afatinib response could be owing to the presence of an additional mutation. For example, TP53 mutations are one of the most common concurrent mutations alongside exon 20 insertion (21) and is possibly associated with a lower likelihood of response to EGFR TKIs (22). Overall, these findings are particularly interesting and suggest that afatinib may provide a new therapeutic option for the particular type of mutation discussed here.

The exon 20 H773dup insertion, annotated as H773_V774insH, has been reported previously, although such reports have not provided evidence for a potential driver role for this particular mutation (3, 23). To determine its prevalence in current databases, we searched for the occurrence of H773dup within the American Association for Cancer Research (AACR) Genomics Evidence Neoplasia Information Exchange (GENIE) database (The AACR Project GENIE Consortium, release 5.0), which comprises almost 60,000 samples across 81 major cancer types (24). We found 15 cases of H773dup in NSCLC, glioma, and endometrial cancer, showing prevalences of 0.12%, 0.06%, and 0.12%, respectively (**Table 1A**). In line with Qin et al. (21), we also find co-mutations in cancer-related genes like TP53 (4/15), PIK3CA (1/15), or PTEN (1/15) which might contribute to different responses upon treatment with TKIs. As a second source, we queried FoundationCore (version MI20190726), a proprietary database provided by Foundation Medicine. FoundationCore contains almost 300,000 clinical specimens and represents, to our knowledge, the biggest available database of its kind, allowing us to exhaustively describe the H773dup mutation landscape. In line with the GENIE results, we found H773dup mutations in NSCLC (adenocarcinoma and squamous cell carcinoma) and glioma (glioblastoma and anaplastic astrocytoma) at a prevalence of 0.1%, as well as in other (niche) indications. **Table 1B** lists all instances of H773dup, highlighting its widespread occurrence. In addition to mutation information, FoundationCore provides

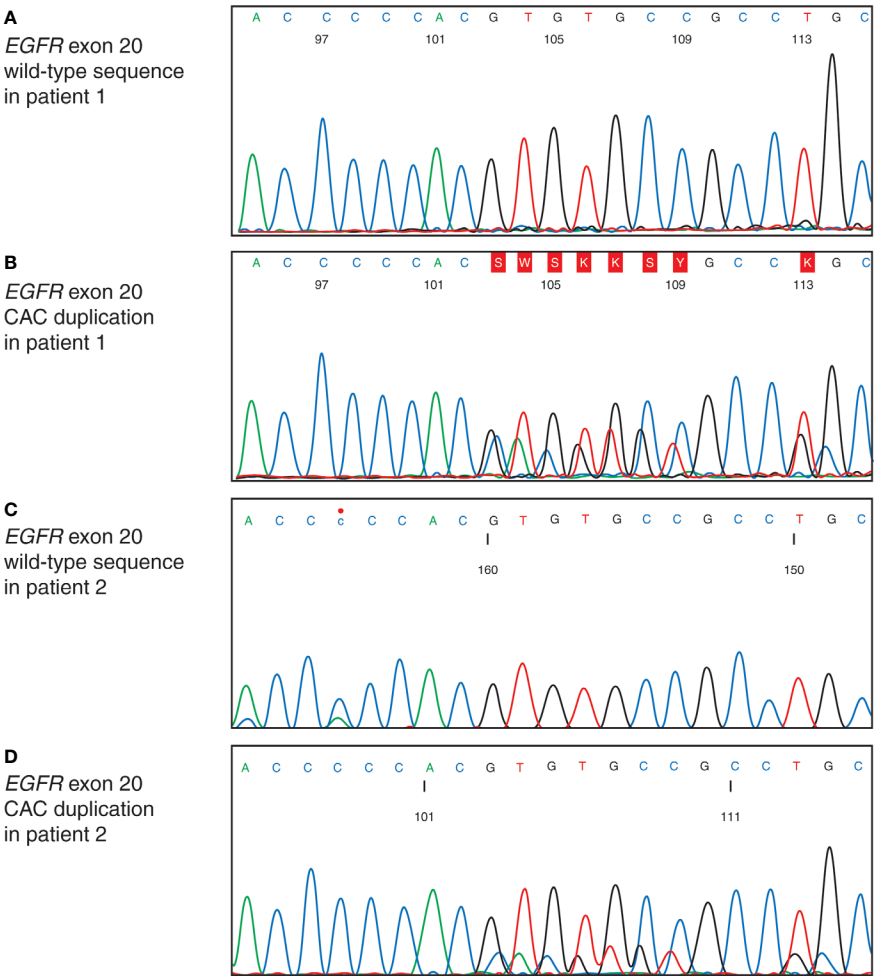

FIGURE 4 | Insertion site of *EGFR* exon 20 insertion mutation. Sequencing electropherogram of *EGFR* exon 20 showing: **(A)** wild-type sequence of the adenocarcinoma from patient 1 resected in 2014; **(B)** duplication of a CAC base triplet in the adenocarcinoma from patient 1 biopsied in 2015. *EGFR*, epidermal growth factor receptor; CAC, cytosine adenine cytosine; **(C)** wild-type sequence of the adenocarcinoma from patient 2 resected in 2019; **(D)** duplication of a CAC base triplet in the adenocarcinoma from patient 2 biopsied in 2019.

copy-number information for all samples. Interestingly, 33% and 43% of lung adenocarcinomas of glioblastomas, respectively, show co-occurring *EGFR* amplifications, which are indicative of H773dup having a functional role. This corroborates an earlier claim that *EGFR* exon 20 insertions may function as driver mutations that are potentially susceptible to effective targeted therapy, based on exon 20 insertions being enriched in never-smokers and Asian patients (23).

The discrepancy in the results of *EGFR* mutation testing in 2014 and 2015 may at least partly reflect the fact that Patient 1 had a multifocal adenocarcinoma, as high rates of discordance in *EGFR* mutation status and subtype have been observed between GGO lesions from the same patient (25, 26). Alternatively, this could be attributable to emergence of a secondary *EGFR* mutation after chemotherapy. The clinical implications of such spatial and temporal heterogeneity are that the use of multiple biopsies on multiple lesions and repeated biopsies upon progression, together with the use of sensitive techniques such as NGS, may be needed to fully characterize the molecular pathology of multifocal adenocarcinomas before and after treatment.

Afatinib was generally well tolerated, and diarrhea adverse events (AEs) were managed effectively with dose reductions to 20 mg/day, enabling Patient 1 to remain on afatinib treatment while still experiencing clinical benefit. This finding is consistent with previous reports that tolerability-guided dose reductions of afatinib are effective in mitigating drug-related AEs, without compromising efficacy (27).

EGFR exon 20 insertions are highly variable in position and size, with structural heterogeneity having implications for response to EGFR TKIs (3, 28). An *in-silico* modelling study predicted that insertions between codons 769 and 775 may be resistant to currently available EGFR TKIs, while insertions proximal to codon 769 are predicted to retain sensitivity (3). Additionally, it appears that different genomic variants confer heterogeneity in response to different EGFR-targeted therapies. Hirano et al. performed MTS

TABLE 1 | Occurrence of H773dup mutations in the AACR Project GENIE and FoundationCore databases.

A) AACR Project GENIE

Cancer Type	Total number of samples	Number of H773dup mutations	Prevalence (%)	95% CI
NSCLC	9090	11	0.121	0.060–0.216
Glioma	3214	2	0.062	0.008–0.225
Endometrial cancer	1668	2	0.120	0.015–0.432

B) FoundationCore

Cancer Type	Total number of samples	Number of H773dup mutations	Prevalence (%)	Co-occurring *EFGR* amplification (%)
Lung adenocarcinoma	33096	24	0.1	33.33
Brain glioblastoma	6162	7	0.1	42.86
Lung NSCLC (Nos)	7197	5	0.1	20.00
Bladder urothelial (transitional cell) carcinoma	3789	4	0.1	0
Kidney urothelial carcinoma	603	2	0.3	100.00
Breast phyllodes tumor	88	2	2.3	0
Lung squamous cell carcinoma	8117	2	0.02	0
Ureter urothelial carcinoma	293	1	0.3	0
Kidney renal papillary carcinoma	342	1	0.3	0
Ovary serous carcinoma	7216	1	0.01	100.00
Lung typical carcinoid	72	1	1.4	0
Brain anaplastic astrocytoma	887	1	0.1	0
Uterus endometrial adenocarcinoma endometrioid	1854	1	0.1	0

AACR, American Association for Cancer Research; EGFR, epidermal growth factor receptor; GENIE, Genomics Evidence Neoplasia Information Exchange; NSCLC, non-small cell lung cancer; Nos, not otherwise specified.

assays using cells harboring four different types of *EGFR* exon 20 insertions. Afatinib potently inhibited the growth of cells harboring *EGFR* A763_Y764insFQEA (IC_{50} 3 nM vs 44 nM for osimertinib), and afatinib and osimertinib showed similar efficacy against Y764_V765insHH (134 vs 237 nM), A767_V769dupASV (158 vs 333 nM), and 770_N771insNPG (43 vs 42 nM) (16). In contrast, erlotinib and rociletinib were relatively ineffective against these mutations (16). Our own preclinical findings also demonstrated heterogeneous responses, with exon 20 mutations at different amino acid positions showing a range of sensitivities to afatinib, while being largely insensitive to erlotinib (example in **Figure 1**). A retrospective analysis of Chinese patients, in which 85 unique *EGFR* exon 20 insertion variants were identified in 547 cases (of 24,468 patients screened) indicated heterogeneous response to EGFR TKIs in the clinic. PFS differed significantly among six representative *EGFR* exon 20 insertion variants (p=0.017) with p.A763_Y764insFQEA associated with better PFS than other insertions. Afatinib and osimertinib were associated with higher disease control rate than first-generation TKIs (21).

Emerging clinical evidence further supports the hypothesis that some exon 20 insertion mutations may be sensitive to afatinib. Among patients treated with afatinib as part of the global Named Patient Use (NPU) program, 100/723 patients with any *EGFR* mutations had uncommon mutations, including 20 patients with exon 20 insertions (20). The ORR among these patients was 35% (vs 23.4% in the overall NPU population). Cases have also been reported of patients with *EGFR* exon 20 insertions responding to afatinib. In one report, two patients with *de novo EGFR* exon 20 insertions (D770_N771insSVD and Ser768_Asp770dup) showed rapid clinical improvement after afatinib treatment, but disease progression quickly ensued in one patient (29). Although the authors concluded that responses to afatinib could be short lived in some patients (29), another case study of a patient with an A767_S768insSVA tandem duplication demonstrated a durable response to afatinib, with the patient surviving for over 3 years from the start of treatment (15). In a separate report, one patient with exon 20 insertion (initially H773_V774insH, D770_N771insG, V769_D770insASV, D770_N771insSVD) was treated with osimertinib, but mutation testing following progression suggested that the mutation site had changed to A767delinsASVD only. The patient subsequently received afatinib treatment, during which the primary tumor regressed and pleural effusion was significantly reduced, with a PFS of 7.4 months (13).

An alternative strategy that has been tested against tumors with *EGFR* exon 20 insertions is to combine EGFR TKIs with anti-EGFR monoclonal antibody treatment. Preclinical evaluation of afatinib or osimertinib plus cetuximab demonstrated a mild but statistically significant additive antitumor effect of these combinations against several *EGFR* exon 20 insertion mutations *in vitro*. Afatinib plus cetuximab also significantly inhibited the growth of tumors harboring *EGFR* A767_V769dupASV and *EGFR* Y764_V765insHH, *in vivo*, while single-agent treatments did not (30). With regard to clinical data, among four patients with *EGFR* exon 20 insertions treated with afatinib plus cetuximab in the Netherlands, three patients had a partial response (PR), and the median PFS was 5.4 months (31).

Unlike *EGFR* exon 20 insertions, the spectrum of *HER2* exon 20 mutations in NSCLC is narrower, with A775_G776insYVMA accounting for most cases (18). Nevertheless, as with *EGFR* exon 20 insertions, similar heterogeneity in responses of different *HER2* exon 20 insertions to afatinib has been reported. One study

investigating specific *HER2* exon 20 insertions in a Chinese cohort found that patients with tumors harboring G778_P780dup achieved numerically longer median PFS (10 vs 3.3 months, p=0.32) and overall survival (19.7 vs 7 months, p=0.16) with afatinib versus non-G778 patients, which is consistent with *in vitro* results suggesting that Glycine778 may facilitate inhibitor binding to HER2 (32). Among patients who received afatinib in a global NPU program, 12 patients with information available on the type of *HER2* mutation had an exon 20 mutation, among whom 10 patients (83%) had A775_G776insYVMA (33). Four of these patients remained on afatinib for more than 1 year, and this subgroup demonstrated a median time to treatment failure of 9.6 months, compared with just 1.9 months in the other two patients, both with M774 duplications.

Preclinical studies also suggest that irreversible EGFR TKIs such as afatinib and dacomitinib are active against *HER2* exon 20 insertions, but at ~100-fold higher concentrations than are necessary to inhibit Del19 or L858R models (34, 35). Consequently, Costa et al. tested intermittent pulsatile doses of afatinib in preclinical models of NSCLC with *HER2* exon 20 insertions, with the aim of achieving intermittent plasma concentrations that would exceed the threshold for efficacy, while improving tolerability versus daily dosing. Pulse afatinib induced anti-tumor activity in these models, and evidence of clinical activity (one PR and one stable disease) was observed among three patients with advanced *HER2* exon 20 insertion-mutated NSCLC treated with off-label pulse afatinib (36). Overall, there is accumulating evidence that afatinib may provide a viable therapeutic option for patients with at least some types of *EGFR* and *HER2* exon 20 insertion, with different approaches having been evaluated in this difficult-to-treat population.

Regarding other treatment options, a Phase 2 trial of poziotinib did not meet its primary endpoint, however the trial is ongoing in other cohorts: treatment-naïve NSCLC patients with exon 20 insertions and alternative dosing regimens to improve tolerability (9). However, despite preliminary activity, a request for breakthrough therapy designation for poziotinib for the treatment of *EGFR* exon 20 insertion mutation-positive NSCLC was rejected by the FDA. Currently, there are no treatments approved in this particular indication, although other TKIs designed to target exon 20 insertions, such as TAK-788, which has received breakthrough therapy designation from the FDA, are in early clinical development (37).

At the time that afatinib treatment was initiated in our patient, there was a lack of investigational treatments for patients with *EGFR* exon 20 insertions, and chemotherapy was and still is the standard treatment choice for these patients. In our case, the patient relapsed shortly after adjuvant chemotherapy, and afatinib treatment was chosen after detection of an exon 20 insertion mutation due to its broad inhibitory profile against uncommon *EGFR* mutations.

In conclusion, our report describes two rare cases of patients with H773dup, one of whom was treated with afatinib for 4.5 years (still on treatment at the time of this report). Our findings are in line with epidemiological evidence that this very rare (~0.1% prevalence in NSCLC), albeit widespread (across tumor types), mutation has a functional role as a driver mutation in NSCLC and can be treated with appropriate EGFR-targeted therapy. Finally, the long time on treatment and durable stable disease observed in Patient 1 is testament to afatinib's manageable safety profile, and suggests that afatinib may be a viable therapeutic option for patients with tumors harboring this exon 20 insertion mutation, particularly those for whom chemotherapy is unsuccessful. Together with previous preclinical and clinical evidence supporting afatinib's activity against certain *EGFR* exon 20 insertions, these findings warrant further investigation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from both participants for the publication of this case report and any potentially identifying information/images.

AUTHOR CONTRIBUTIONS

SZM, BK, MB, and LM collected and assembled the data, analyzed and interpreted the data, and drafted the manuscript. HP analyzed and interpreted the data, and drafted the manuscript. AC conceived and designed the study, analyzed and interpreted the data, and drafted the manuscript. FS conceived and designed the study, collected, assembled, analyzed and interpreted the data, and drafted the manuscript. All authors provided final approval of the manuscript and agreed to be accountable for all aspects of the work, which includes ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Chan BA, Hughes BG. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. *Transl Lung Cancer Res* (2015) 4:36–54. doi: 10.3978/j.issn.2218-6751.2014.05.01
2. Kim EY, Cho EN, Park HS, Hong JY, Lim S, Youn JP, et al. Compound EGFR mutation is frequently detected with co-mutations of actionable genes and associated with poor clinical outcome in lung adenocarcinoma. *Cancer Biol Ther* (2016) 17:237–45. doi: 10.1080/15384047.2016.1139235
3. Arcila ME, Nafa K, Chaft JE, Rekhtman N, Lau C, Reva BA, et al. EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. *Mol Cancer Ther* (2013) 12:220–9. doi: 10.1158/1535-7163.Mct-12-0620
4. Girard N. Optimizing outcomes in EGFR mutation-positive NSCLC: which tyrosine kinase inhibitor and when? *Future Oncol* (2018) 14:1117–32. doi: 10.2217/fon-2017-0636
5. Kohsaka S, Nagano M, Ueno T, Suehara Y, Hayashi T, Shimada N, et al. A method of high-throughput functional evaluation of *EGFR* gene variants of unknown significance in cancer. *Sci Transl Med* (2017) 9:eaan6566. doi: 10.1126/scitranslmed.aan6566
6. Yang JC, Sequist LV, Geater SL, Tsai CM, Mok TS, Schuler M, et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. *Lancet Oncol* (2015) 16:830–8. doi: 10.1016/s1470-2045(15)00026-1
7. FDA. *Gilotrif® (afatinib) Highlights of Prescribing Information* (2018). Available at: https://docs.boehringer-ingelheim.com/Prescribing%20Information/PIs/Gilotrif/Gilotrif.pdf (Accessed July 4, 2019).
8. EMA. *Giotrif (EPAR) Summary of Product Characteristics* (2018). Available at: https://www.ema.europa.eu/en/documents/product-information/giotrif-epar-product-information_en.pdf (Accessed July 4, 2019).
9. Le X, Goldman JW, Clarke JM, Tchekmedyian N, Piotrowska Z, Chu D, et al. Poziotinib shows activity and durability of responses in subgroups of previously treated EGFR exon 20 NSCLC patients. *J Clin Oncol* (2020) 38: Abstract9514. doi: 10.1200/JCO.2020.38.15_suppl.9514
10. Janne PA, Neal JW, Camidge DR, Spira AI, Piotrowska Z, Horn L, et al. Antitumor activity of TAK-788 in NSCLC with EGFR exon 20 insertions. *J Clin Oncol* (2019) 37(Suppl 15):Abstract9007. doi: 10.1200/JCO.2019.37.15_suppl.9007
11. Haura EB, Cho BC, Lee JS, Han J-Y, Lee KH, Sanborn RE, et al. JNJ-61186372 (JNJ-372), an EGFR-cMet bispecific antibody, in EGFR-driven advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* (2019) 37(Suppl 15): Abstract9009. doi: 10.1200/JCO.2019.37.15_suppl.9009
12. Yasuda H, Park E, Yun C-H, Sng NJ, Lucena-Araujo AR, Yeo W-L, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. *J Sci Transl Med* (2013) 5:216ra177. doi: 10.1126/scitranslmed.3007205
13. Cai Y, Wang X, Guo Y, Sun C, Xu Y, Qiu S, et al. Successful treatment of a lung adenocarcinoma patient with a novel EGFR exon 20-ins mutation with afatinib: a case report. *Medicine (Baltimore)* (2019) 98:e13890. doi: 10.1097/md.0000000000013890
14. Yang JC, Schuler M, Popat S, Miura S, Heeke S, Park K, et al. Afatinib for the treatment of NSCLC harboring uncommon EGFR mutations: a database of 693 cases. *J Thorac Oncol* (2020) 15:803–15. doi: 10.1016/j.jtho.2019.12.126
15. Chan RT. Afatinib for an EGFR exon 20 insertion mutation: a case report of progressive stage IV metastatic lung adenocarcinoma with 54 months' survival. *Asia Pac J Clin Oncol* (2018) 14(Suppl 1):7–9. doi: 10.1111/ajco.12853
16. Hirano T, Yasuda H, Tani T, Hamamoto J, Oashi A, Ishioka K, et al. In vitro modeling to determine mutation specificity of EGFR tyrosine kinase inhibitors against clinically relevant EGFR mutants in non-small-cell lung cancer. *Oncotarget* (2015) 6:38789–803. doi: 10.18632/oncotarget.5887
17. Kobayashi Y, Mitsudomi T. Not all epidermal growth factor receptor mutations in lung cancer are created equal: Perspectives for individualized treatment strategy. *Cancer Sci* (2016) 107:1179–86. doi: 10.1111/cas.12996
18. Kosaka T, Tanizaki J, Paranal RM, Endoh H, Lydon C, Capelletti M, et al. Response heterogeneity of EGFR and HER2 exon 20 insertions to covalent EGFR and HER2 inhibitors. *J Cancer Res* (2017) 77:2712–21. doi: 10.1158/0008-5472.CAN-16-3404
19. *Uncommon EGFR Mutations*. Available at: https://www.uncommonegfrmutations.com/Results/Search# (Accessed April 30, 2020).
20. Cappuzzo F, Soo R, Hochmair M, Schuler M, Lam KC, Stehle G, et al. Global named patient use program of afatinib in advanced non-small-cell lung carcinoma patients who progressed following prior therapies. *Future Oncol* (2018) 14:1477–86. doi: 10.2217/fon-2017-0666
21. Qin Y, Jian H, Tong X, Wu X, Wang F, Shao YW, et al. Variability of EGFR exon 20 insertions in 24 468 Chinese lung cancer patients and their divergent responses to EGFR inhibitors. *Mol Oncol* (2020) 14:1695–704. doi: 10.1002/1878-0261.12710
22. Canale M, Petracci E, Delmonte A, Bronte G, Chiadini E, Ludovini V, et al. Concomitant TP53 mutation confers worse prognosis in EGFR-mutated non-small cell lung cancer patients treated with TKIs. *J Clin Med* (2020) 9:1047. doi: 10.3390/jcm9041047
23. Oxnard GR, Lo PC, Nishino M, Dahlberg SE, Lindeman NI, Butaney M, et al. Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. *J Thorac Oncol* (2013) 8:179–84. doi: 10.1097/JTO.0b013e3182779d18
24. The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *J Cancer Discov* (2017) 7:818–31. doi: 10.1158/2159-8290.CD-17-0151
25. Liu M, He W-X, Song N, Yang Y, Zhang P, Jiang G-N. Discrepancy of epidermal growth factor receptor mutation in lung adenocarcinoma presenting as multiple ground-glass opacities. *Eur J CardioThorac Surg* (2016) 50:909–13. doi: 10.1093/ejcts/ezw113
26. Tanvetyanon T, Boyle TA. Clinical implications of genetic heterogeneity in multifocal pulmonary adenocarcinomas. *J Thorac Dis* (2016) 8:E1734–8. doi: 10.21037/jtd.2016.12.06
27. Yang JC-H, Sequist LV, Zhou C, Schuler M, Geater SL, Mok T, et al. Effect of dose adjustment on the safety and efficacy of afatinib for EGFR mutation-positive lung adenocarcinoma: post hoc analyses of the randomized LUX-Lung 3 and 6 trials. *Ann Oncol* (2016) 27:2103–10. doi: 10.1093/annonc/mdw322
28. O'Kane GM, Bradbury PA, Feld R, Leighl NB, Liu G, Pisters KM, et al. Uncommon EGFR mutations in advanced non-small cell lung cancer. *Lung Cancer* (2017) 109:137–44. doi: 10.1016/j.lungcan.2017.04.016
29. Costa M, Lewgoy J. P3.13-13 Afatinib in lung adenocarcinoma harboring de novo EGFR exon 20 insertions. *J Thorac Oncol* (2018) 13:S981. doi: 10.1016/j.jtho.2018.08.1853
30. Hasegawa H, Yasuda H, Hamamoto J, Masuzawa K, Tani T, Nukaga S, et al. Efficacy of afatinib or osimertinib plus cetuximab combination therapy for non-small-cell lung cancer with EGFR exon 20 insertion mutations. *Lung Cancer* (2019) 127:146–52. doi: 10.1016/j.lungcan.2018.11.039
31. van Veggel B, de Langen AJ, Hashemi SMS, Monkhorst K, Heideman DAM, Thunnissen E, et al. Afatinib and cetuximab in four patients with EGFR exon 20 insertion–positive advanced NSCLC. *J Thorac Oncol* (2018) 13:1222–6. doi: 10.1016/j.jtho.2018.04.012
32. Liu Z, Wu L, Cao J, Yang Z, Zhou C, Cao L, et al. Clinical characterization of ERBB2 exon 20 insertions and heterogeneity of outcomes responding to afatinib in Chinese lung cancer patients. *Onco Targets Ther* (2018) 11:7323–31. doi: 10.2147/ott.S173391
33. Peters S, Curioni-Fontecedro A, Nechushtan H, Shih J-Y, Liao W-Y, Gautschi O, et al. Activity of afatinib in heavily pretreated patients with ERBB2 mutation–positive advanced NSCLC: findings from a global named patient use program. *J Thorac Oncol* (2018) 13:1897–905. doi: 10.1016/j.jtho.2018.07.093
34. Engelman JA, Zejnullahu K, Gale CM, Lifshits E, Gonzales AJ, Shimamura T, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. *Cancer Res* (2007) 67:11924–32. doi: 10.1158/0008-5472.Can-07-1885
35. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. *Oncogene* (2008) 27:4702–11. doi: 10.1038/onc.2008.109
36. Costa DB, Jorge SE, Moran JP, Freed JA, Zerillo JA, Huberman MS, et al. Pulse afatinib for ERBB2 exon 20 insertion–mutated lung adenocarcinomas. *J Thorac Oncol* (2016) 11:918–23. doi: 10.1016/j.jtho.2016.02.016
37. Neal J, Doebele R, Riely G, Spira A, Horn L, Piotrowska Z, et al. P1.13-44 Safety, PK, and preliminary antitumor activity of the oral EGFR/HER2 exon

20 inhibitor TAK-788 in NSCLC. *J Thorac Oncol* (2018) 13:S599. doi: 10.1016/j.jtho.2018.08.901

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Detection of EGFR Mutations in Cerebrospinal Fluid of EGFR-Mutant Lung Adenocarcinoma With Brain Metastases

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Background: We aimed to investigate the feasibility of detecting epidermal growth factor receptor (EGFR) mutations in cell-free DNA (cfDNA) from cerebrospinal fluid (CSF) and plasma of advanced lung adenocarcinoma (LADC) with brain metastases (BMs) by droplet digital polymerase chain reaction (ddPCR).

Methods: Thirty advanced LADC patients with BMs were enrolled, and their matched CSF and plasma samples were collected. Droplet digital PCR was used to test cfDNA in CSF and plasma for EGFR mutation status. The clinical response and prognosis were evaluated.

Results: Out of 30 patients, there were 21 females and 9 males, aged 34-75 years. In all of the cases, CSF cytology were negative. In ddPCR assays, 10 patients (33.3%) had EGFR mutation in CSF, including 3 cases of EGFR T790M mutation, and 16 patients (53.3%) had EGFR mutation in plasma, including 6 cases of EGFR T790M mutation. Five patients with activating EGFR mutations in CSF achieved an intracranial partial response (iPR) after combination treatment with the first-generation EGFR-tyrosine kinase inhibitors. Three patients with EGFR T790M mutations in CSF achieved iPR after second-line osimertinib treatment. The median overall survival and intracranial progression-free survival were 17.0 months and 11.0 months, respectively.

Conclusion: It was feasible to test EGFR mutation in cerebrospinal fluid and plasma. In LADC patients with brain metastasis, cerebrospinal fluid can be used as a liquid biopsy specimen to guide treatment strategy by monitoring EGFR mutation status.

Keywords: lung adenocarcinoma, brain metastases, cerebrospinal fluid, EGFR mutation, droplet digital PCR

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INTRODUCTION

Brain metastases (BMs) occurred in 25-50% of non-small-cell lung cancer (NSCLC) patients (1, 2), 30-60% of those had epidermal growth factor receptor (EGFR) activating mutation lung adenocarcinoma (LADC) (3, 4). Median overall survival of NSCLC patients with BMs ranged from 3 to 15 months in an unselected population without EGFR-tyrosine kinase inhibitors (EGFR-

TKIs) targeted therapy (5, 6), and 18 to 58 months in EGFR-mutant patients with EGFR-TKIs treatment (7, 8). EGFR-TKIs have been established as the standard therapy for EGFR-sensitizing mutant (EGFRm, mainly refer to L858R or 19del) advanced NSCLC. In EGFRm patients, first-line EGFR-TKIs treatment has a good response rate of 50 to 80%. However, patients who respond to EGFR-TKIs eventually develop resistance to these drugs, with a median progression-free survival around 9 to 13 months (9).

There are various mechanisms for the development of resistance to EGFR-TKIs. Approximately 50% of the patients who initially respond well to EGFR-TKIs develop resistance due to the occurrence of secondary mutation T790M, an amino acid substitution at position 790 in EGFR from a threonine to a methionine (10, 11). This is the most common mechanism of acquired resistance to EGFR-TKIs. In China, the third-generation EGFR-TKI, osimertinib, is standard treatment for patients with advanced EGFR T790M-mutated NSCLC who have been pre-treated with early-generation EGFR-TKIs (gefitinib, erlotinib, icotinib, or afatinib) (12).

Intracranial progress is the main cause of EGFR-TKIs treatment failure (4, 13). In clinical practice, biopsy of BMs lesions is rarely performed, which results in poor understanding of the resistance mechanisms of EGFR-TKIs therapy in NSCLC with BMs. Liquid biopsy of CSF cfDNA may provide potential information about intracranial lesions. Recent studies have demonstrated that driver and resistance mutations can be identified by droplet digital polymerase chain reaction (ddPCR) or next-generation sequencing (NGS) in CSF circulating cell-free DNA (cfDNA) in patients with central nervous system (CNS) metastases (14–16). Herein, to explore the alternative detection of EGFR mutation status, ddPCR was used to examine the mutation status in CSF and plasma. And the clinical efficacy of EGFR-mutant LADC with BMs was studied based on real clinical practice, including treatment with EGFR-TKIs alone or combined with chemotherapy and radiotherapy.

MATERIALS AND METHODS

Patient Population

Between July 2014 and June 2017 in Beijing Chest Hospital, Capital Medical University (Beijing, China), 30 pathologically confirmed LADC patients with BMs harboring the activating EGFR mutation in their primary tumors were enrolled. The inclusion criteria were as follows: 1) activating EGFR mutation (19del or L858R) in original tissues determined by amplification refractory mutation system polymerase chain reaction (ARMS-PCR); 2) radiological computed tomography or magnetic resonance imaging (MRI) confirmed brain metastases without leptomeningeal metastases; and 3) received lumbar puncture and CSF cytology was negative.

All patients provided written informed consent before specimen collection. This study was reviewed and approved by the institutional review board (IRB)/ethics committee of Beijing Chest Hospital, Capital Medical University.

Specimen Collection and Processing

CSF samples were obtained by lumbar puncture. Peripheral blood samples were obtained from venous blood. Tumor tissue samples were collected from primary and/or metastatic sites *via* surgical resection or biopsy. The CSF and matched peripheral blood were collected into ethylene diamine tetraacetic acid (EDTA) anti-coagulated tubes from all included subjects. Within 2 h of CSF or peripheral blood sample collection, the sample was placed on ice and centrifuged at 1,000×g at 4°C for 10 min. The CSF supernatant or plasma was transferred to sterilized prelabeled cryotubes, the tubes were stored at -80°C for further exploration.

Extraction and Quantification of Cell-Free DNA

The 2 mL of CSF or plasma was used for the extraction of cell-free DNA. Stored samples were thawed at room temperature and then centrifuged at 10,000×g at 4 °C for 30 min to remove residual precipitated cellular components and various particles. Circulating cell-free DNA was extracted according to the procedure of the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Hilden, Germany). The concentration of cfDNA was measured with the Qubit dsDNA HS Assay kit (Invitrogen, Life Technologies, CA, USA) on a Qubit 3.0 Fluorometer (Invitrogen, Life Technologies, CA, USA) following manufacturer's instructions.

EGFR Mutation Analysis

ARMS-PCR for Tissue EGFR Mutations

The initial tissue EGFR mutations were detected by ARMS-PCR with the AmoyDx Human EGFR Gene Mutation Fluorescence PCR Diagnostic Kit (Amoy Diagnostics, Xiamen, China), which had been approved by the National Medical Products Administration for *in vitro* diagnostics use. This kit can cover the 29 most common types of EGFR mutations in exons 18 to 21 of lung cancers, including T790M, L858R, L861Q, S768I, and G719X point mutation; three insertions in exon 20; and 19 deletions in exon 19 (19del). All the experiments were carried out according to the manufacturer's protocols.

Droplet Digital PCR for cfDNA EGFR Mutations

We only detected EGFR 19del, L858R, and T790M mutations for each specimen by ddPCR, and the experiments were carried out at Amoy Diagnostics Co., Ltd (Xiamen, China). Droplet digital PCR was performed using the QX200 AutoDG Droplet Digital PCR System (BioRad, Hercules, CA, USA) according to the manufacturer's protocol. The method of ddPCR assays has been reported previously and the established sensitivity was 0.04% (17). In short, the ddPCR detection platform can produce about 20,000 droplets of mutant and wild-type DNA emulsion, and the PCR reaction can be carried out in individual droplets. After PCR reaction, positive or negative fluorescence signals were produced in each droplet, indicating whether an EGFR mutant existed or not. In EGFR 19del detection, a 15-base pair peptide nucleic acid (PNA) was introduced to block the amplification of wild-type alleles by targeting the common 19del

region, E746 to A750. The FAM-labeled probes were targeted at wild-type and mutant allele amplicons of EGFR exon 19 to reflect deletion mutants in the PNA targeting region. A VIC-labeled probe was designed to target EGFR exon 2 for total EGFR gene input control. The 19 common types of EGFR 19del in the ARMS-PCR kit were all detected by ddPCR analysis. EGFR L858R and T790M were detected by a FAM-labeled probe targeting the mutant region and a VIC-labeled probe targeting the wild-type region, respectively. Human genomic DNA was used as negative control to determine the cutoff of allele calling. We used the QuantaSoft software (version 1.6.6.0320; BioRad, Hercules, CA USA) for ddPCR data analysis of the allele calls. In the test of non-template control reaction, random events occurred occasionally in a single droplet. Therefore, samples of at least two droplets in the FAM signal positive region were regarded as mutation positive. Mutations values were reported as mutant allele frequency (MAF), defined as the proportion of mutant to wild-type PCR products in the ddPCR readout.

Statistical Analysis

All statistical analyses were performed with SPSS version 24.0 (SPSS Inc., Chicago, IL, USA), or GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, CA, USA). Frequency tabulation and summary statistics provided the characteristics of data distribution. The intracranial objective responses were evaluated for all patients based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (18), and the therapeutic response was evaluated as complete response (CR), partial response (PR), stable disease (SD), or progression disease (PD). Fisher's exact method was used to compare intracranial objective responses (CR+PR versus SD+PD) between EGFR status in different kinds of liquid samples. Kaplan-Meier estimation was used to designate progress-free survival (PFS) and overall survival (OS), and the significant difference was determined by the log-rank test. OS was calculated from the day of diagnosis of brain metastasis to the day of death. Intracranial PFS was calculated from the date of diagnosis of brain metastasis until the date of progression of previous lesions or the appearance of a new lesion. A two-sided p value less than 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

All of the 30 included patients were Chinese with histologically confirmed lung adenocarcinoma, and brain metastases (BMs) were diagnosed by imaging. At the time of diagnosis with BMs, in all of cases, CSF cytology were negative and examinations of cranial imaging showed no leptomeningeal metastases (LMs). The median age was 58 years (range, 34 to 75 years); nine patients were male and 21 patients were female. The majority (n = 24; 80.0%) were given a good Eastern Cooperative Oncology Group performance status (ECOG PS < 2). Most patients had four or more brain lesions (n = 23; 76.7%). At the time of initial diagnosis with BMs, 19 patients had received no prior treatment, 10 had received first-generation EGFR-TKIs (gefitinib, erlotinib, or icotinib), and one patient had received chemotherapy alone. After diagnosis with BMs, all the patients received systemic treatments, including 20 patients with EGFR-TKIs alone (five cases with second-line EGFR-TKI of osimertinib), six patients with chemotherapy followed by EGFR-TKIs, and four patients with chemotherapy alone. Twenty-one patients had whole brain radiotherapy (WBRT) for local treatment of BMs, one of them also underwent stereotactic radiosurgery (SRS). The baseline clinical characteristics including age, gender, smoking status, ECOG PS, BMs status, and systemic and local treatments are summarized in **Table 1** and detailed case by case in **Supplementary Table 1**.

EGFR Mutation Status in Tumor Tissue and Liquid Samples

EGFR mutations were detected in primary tumor tissues by ARMS-PCR assays, tissue EGFR 19del mutations were identified in 18 cases (60.0%) and EGFR L858R mutations were detected in 12 patients (40.0%).

Droplet digital PCR assays were performed for paired liquid samples, CSF and plasma samples. In the CSF samples, EGFR mutations were present in 10 patients (33.3%), the more

TABLE 1 | Clinicopathologic characteristics of 30 patients.

Characteristic	Value or no. of patients	%
Patients	30	
Age, years		
Median	58	
Range	34-75	
Sex		
Male	9	30.0
Female	21	70.0
ECOG PS		
0	3	10.0
1	21	70.0
2	3	10.0
3	3	10.0
Smoking status		
Never	23	76.7
Current	7	23.3
Tumor histology		
Adenocarcinoma	30	100.0
Primary tissue EGFR status		
19del	18	60.0
L858R	12	40.0
No. of brain metastases		
≤3	7	23.3
>3	23	76.7
BMs at the time of diagnosis		
Yes	19	63.3
No	11	36.7
First-generation TKI treatment		
Prior BMs	10	33.3
Post BMs	20	66.7
BMs local treatment		
WBRT ± SRS	21	70.0
None	9	30.0

No., number; ECOG PS, Eastern Cooperative Oncology, Group performance score; EGFR, epidermal growth factor receptor; BMs, brain metastasis; TKI, tyrosine kinase inhibitor; WBRT, whole brain radiotherapy; SRS, stereotactic radiosurgery.

common mutation was 19del (six patients), followed by L858R mutation (four patients). To our surprise, there were three cases (patients 15, 17, and 29) with EGFR T790M mutation in CSF (two accompanied with 19del, one accompanied with L858R). In plasma samples, EGFR mutations were identified in 16 patients (53.3%), including six cases with EGFR T790M mutation (three patients with L858R mutation, two patients with 19del mutation, and one patient with T790M mutation alone). In total, 19 patients found EGFR mutation in CSF or in plasma, no EGFR mutation was found in cerebrospinal fluid or plasma in 11 patients (**Table 2**). All EGFR T790M mutations (nine samples in seven patients) were found during or after EGFR-TKIs treatments.

Correlation Between EGFR Mutation in Liquid Samples and Clinical Responses of BMs

In 30 patients, the best intracranial response rates were 3.3% CR (n = 1), 60.0% PR (n = 18), 30.0% stable disease (n = 9), and 6.7% PD (n = 2).

EGFR mutations were found in the CSF samples of 10 patients, the five patients with activating EGFR mutations (19del or L858R) achieved intracranial partial response (iPR) after treatment with a combination of WBRT and first-generation EGFR-TKIs, and three patients (patient 15, 17, and 29) with the EGFR T790M mutation were identified after first-generation EGFR-TKIs treatments, then achieved iPR after treatment with second-line osimertinib alone (**Figure 1**). One patient (patient 22) with an EGFR 19del mutation in CSF received first-line gefitinib, and intracranial lesions were stable, the other patient (patient 3) with an EGFR L858R mutation in CSF received two lines of chemotherapy before BMs, and was then treated with gefitinib, but the intracranial lesion progressed.

In liquid samples (CSF or plasma), the RECIST rates of CR, PR, SD, and PD were 5.3%, 73.7%, 15.8%, and 5.3% for patients with EGFRm (in CSF or plasma) and 0%, 36.4%, 54.5%, and 9.1% for patients with wild-type EGFR (EGFRw, in CSF and plasma). The best intracranial response rate (CR+PR) was 78.9% for patients with EGFRm versus 36.4% for patients with EGFRw. There was a significant difference when the numbers of the two groups were compared (CR+PR versus SD+PD, $p = 0.047$).

TABLE 2 | EGFR testing result.

Patient	Initial primary tissue EGFR mutation*	CSF EGFR mutation		Plasma EGFR mutation	
		Status	MAF	Status	MAF
Liquid biopsy at the time of diagnosis with brain metastasis					
1	19del	WT		T790M	0.3%
2	19del	WT		19del	5.3%
3	L858R	L858R	37.9%	L858R	2.0%
4	L858R	WT		WT	
5	19del	WT		WT	
7	L858R	WT		WT	
8	19del	19del	69.7%	19del/T790M	11.0%/7.0%
11	19del	WT		WT	
13	L858R	L858R	32.8%	WT	
15	19del	19del/T790M	13.2%/0.5%	19del/T790M	14.4%/3.5%
16	L858R	WT		WT	
18	L858R	WT		WT	
19	19del	19del	43.3%	19del	14.9%
21	19del	WT		19del	3.7%
22	19del	19del	21.8%	19del	7.4%
23	L858R	WT		L858R	20.5%
24	19del	WT		19del	11.4%
25	19del	WT		WT	
26	19del	WT		19del	0.8%
27	L858R	L858R	7.2%	L858R	5.2%
28	19del	19del	6.9%	WT	
29	19del	19del/T790M	35.7%/12.1%	WT	
30	19del	WT		19del	15.3%
Liquid biopsy after brain metastasis progression					
6	L858R	WT		L858R/T790M	10.8%/26.5%
9	L858R	WT		WT	
10	19del	WT		WT	
12	L858R	WT		L858R/T790M	2.4%/0.2%
14	19del	WT		WT	
17	L858R	L858R/T790M	16.2%/2.0%	L858R/T790M	8.1%/2.2%
20	19del	WT		WT	

CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; MAF, mutant allele frequency; WT wild-type.

**EGFR mutations were detected in primary tumor tissues at the time of initial diagnosis with lung cancer by amplification refractory mutation system polymerase chain reaction (ARMS-PCR) assays.*

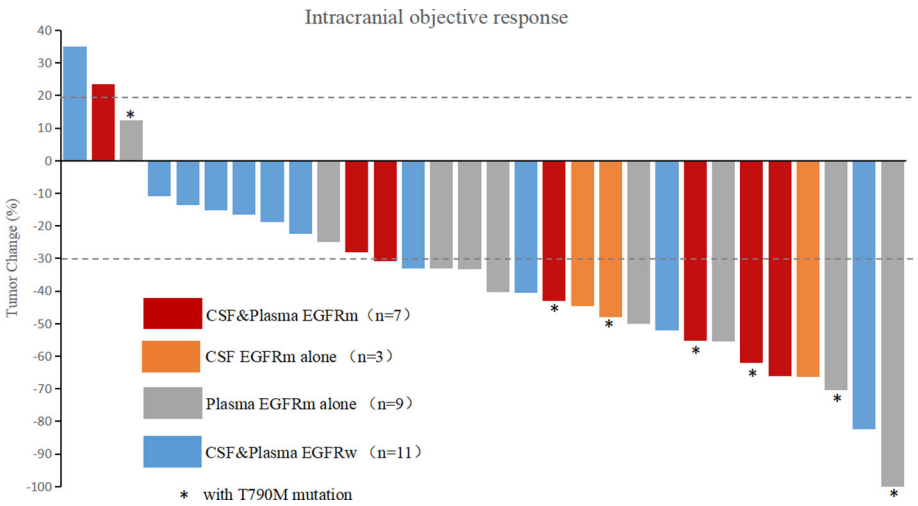

FIGURE 1 | Waterfall plot of intracranial best response. CSF, cerebrospinal fluid; EGFRm, mutant epidermal growth factor receptor; EGFRw, wild-type epidermal growth factor receptor; T790M, an amino acid substitution at position 790 in EGFR from a threonine to a methionine.

The waterfall plot of intracranial objective response is shown in **Figure 1**, and the RECIST rates of CR, PR, SD, and PD according to EGFR mutation status of CSF and plasma samples are listed in **Table 3** and detailed in **Supplementary Table 2**.

Correlation Between EGFR Mutation in Liquid Samples and Prognosis of BMs

Fourteen patients were alive at the time of this analysis. The median iPFS and OS from the time of diagnosis with BMs were 11.0 months and 17.0 months, respectively (**Figures 2A, B**).

To emphasize the clinical significance of different EGFR mutation status in liquid samples (EGFRm vs EGFRw), prognosis survival was also evaluated. In CSF samples, the median iPFS of EGFRm and EGFRw were 12.0 months and 8.0 months, respectively (p = 0.337), and the median OS of EGFRm and EGFRw were not reached and 17.0 months, respectively (p = 0.404; **Figures 2C, D**). In plasma samples, the median iPFS of EGFRm and EGFRw were 12.0 months and 8.0 months, respectively (p = 0.059), and the median OS of EGFRm and EGFRw were 31.0 months and 11.0 months, respectively (p = 0.003; **Figures 2E, F**). In liquid samples, the median iPFS of EGFRm (CSF or plasma) and EGFRw (CSF and plasma) were 12.0 months and 6.0 months, respectively (p = 0.014), and the median OS of EGFRm (CSF or plasma) and EGFRw (CSF and plasma) were 31.0 months and 11.0 months, respectively (p = 0.002; **Figures 2G, H**).

A Case Presentation

A 66-year-old woman (patient 29) was diagnosed with stage Ia lung adenocarcinoma at disease baseline and underwent a radical right upper lobectomy with video-assisted thoracoscopic surgery (VATS) in February 2010, EGFR 19del was discovered in the primary lung lesion by ARMS-PCR. In January 2011, the tumor recurred and one of the left ribs was involved. After regional radiotherapy of the involved rib, she responded to erlotinib for 58 months before developing brain metastasis (**Figure 3A**) with a central nervous system (CNS) symptom of intermittent headaches. A brain MRI did not shown any evidence of LMs and her cerebrospinal fluid pressure was in the normal range. Tumor cells were not identified in CSF. EGFR 19del and T790M mutations were identified by ddPCR in the CSF sample (**Table 2**, **Figure 3A**),

TABLE 3 | Summary of intracranial objective response in different EGFR mutation status.

Intracranial Response	n (%)	CSF EGFR		P*	Plasma EGFR		P*	CSF/plasma EGFR		P*
		Mut, n (%)	Wt, n (%)		Mut, n (%)	Wt, n (%)		Mut, n (%)	Wt, n (%)	
ALL	30 (100.0)	10 (33.3)	20 (66.7)		16 (53.3)	14 (46.7)		19 (63.3)	11 (36.7)	
CR+PR	19 (63.3)	8 (80.0)	11 (55.0)	0.247	12 (75.0)	7 (50.0)	0.257	15 (78.9)	4 (36.4)	0.047
CR	1 (3.3)	0 (0)	1 (5.0)		1 (6.3)	0 (0)		1 (5.3)	0 (0)	
PR	18 (60.0)	8 (80.0)	10 (50.0)		11 (68.8)	7 (50.0)		14 (73.7)	4 (36.4)	
SD+PD	11 (36.7)	2 (20.0)	9 (45.0)		4 (25.0)	7 (50.0)		4 (21.1)	7 (63.6)	
SD	9 (30.0)	1 (10.0)	8 (40.0)		3 (18.8)	6 (42.9)		3 (15.8)	6 (54.5)	
PD	2 (6.7)	1 (10.0)	1 (5.0)		1(6.3)	1 (7.1)		1 (5.3)	1 (9.1)	

EGFR, epidermal growth factor receptor; CSF, cerebrospinal fluid; Mut, mutant; Wt, wild-type; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease.
**P value was assessed by using Fisher's exact test to compare the number of two groups (CR+PR versus SD+PD).*

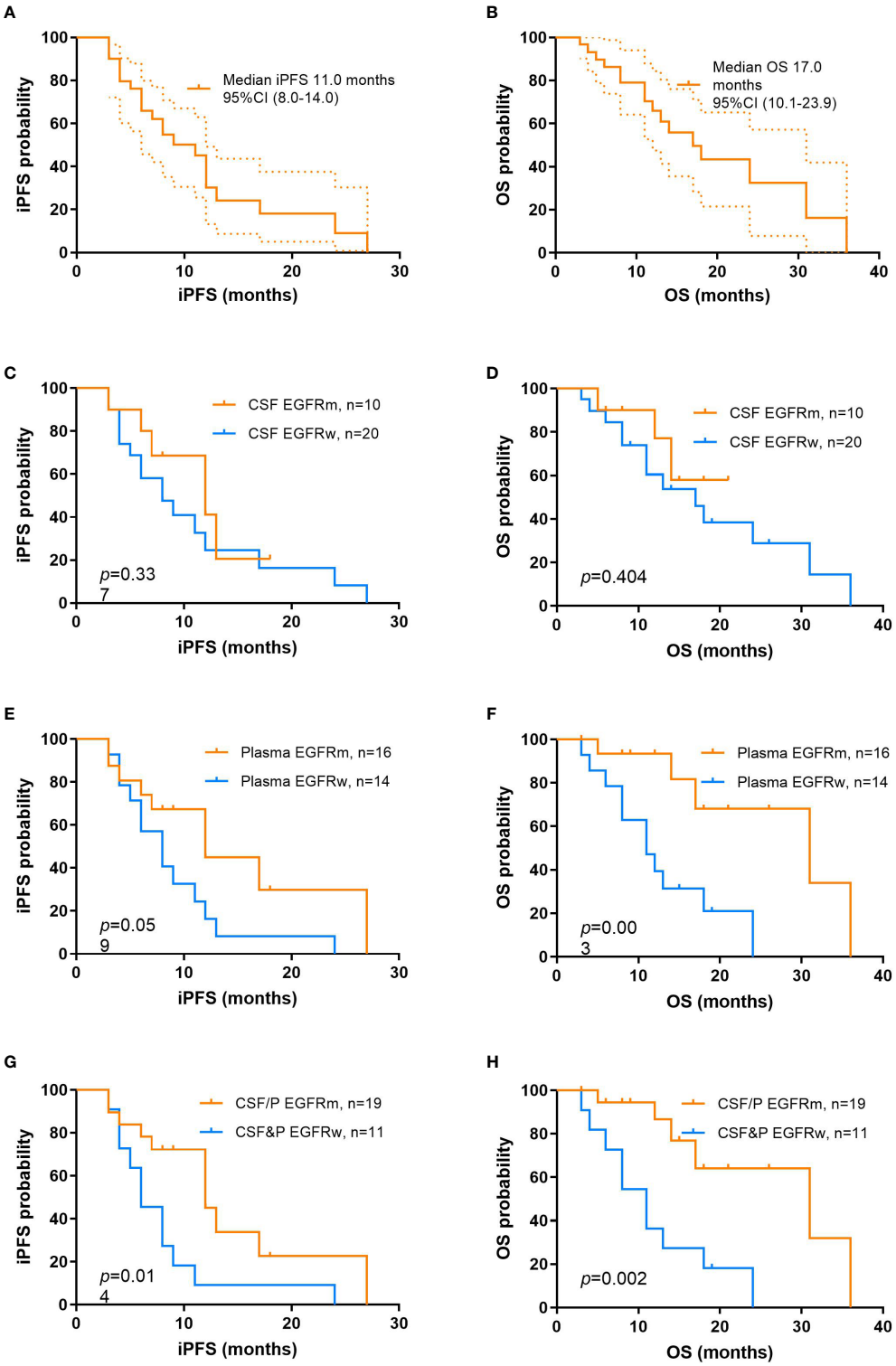

FIGURE 2 | Kaplan-Meier curves of iPFS and OS from the time of diagnosis of brain metastasis in different groups of study. **(A)** iPFS for the overall population; **(B)** OS for the overall population; iPFS **(C)** and OS **(D)** between patients with EGFRm and EGFRw in CSF; iPFS **(E)** and OS **(F)** between patients with EGFRm and EGFRw in plasma; iPFS **(G)** and OS **(H)** between patients with EGFRm and EGFRw in CSF and plasma. iPFS, intracranial progression-free survival; OS, overall survival; EGFRm, mutant epidermal growth factor receptor; EGFRw, wild-type epidermal growth factor receptor; CSF, cerebrospinal fluid.

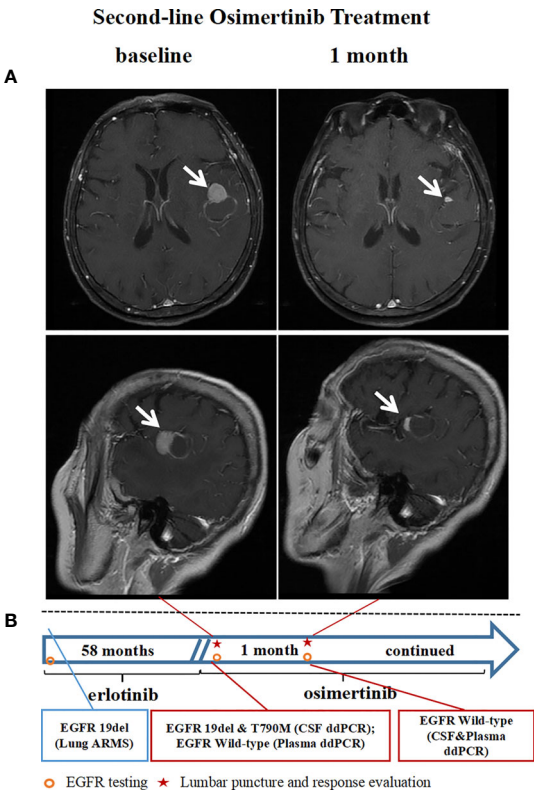

FIGURE 3 | Case presentation: A case received second-line osimertinib treatment after a CSF EGFR T790M mutation was identified by ddPCR. **(A)** Brain MRI imaging tests before and after osimertinib treatment. **(B)** The timeline and results of EGFR mutation status identified by ddPCR. CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; T790M, an amino acid substitution at position 790 in EGFR from a threonine to a methionine; ddPCR, droplet digital PCR; MRI, magnetic resonance imaging.

while the mutations were not found in a blood sample at the same time. The patient received second-line osimertinib and achieved intracranial PR after one month of treatment (**Figure 3**). At this time point, the EGFR mutations were not found either in the CSF or in plasma sample.

DISCUSSION

It is feasible to detect EGFR mutations of CSF cfDNA by ddPCR in advanced NSCLC patients with brain metastasis. As one of the PCR methods, ddPCR is more sensitive than NGS methods and ARMS-PCR for a low abundance of DNA (19). Our results show that the ddPCR method may be suitable for detecting low abundance mutation DNA in CSF. In our study, with the use of ddPCR, we found that CSF EGFR mutations were identified in one third of 30 included cases, and EGFR T790M mutations were found in three patients. To emphasize an important point, all the patients in this study had been confirmed to have brain metastases without leptomeningeal metastases by radiological magnetic resonance imaging or computed tomography, and CSF cytology of all cases were negative. Previous studies on the detection of EGFR mutations in cerebrospinal fluid mainly included patients with leptomeningeal metastases. In 2016, Zhao et al. studied seven patients with leptomeningeal metastases to test EGFR mutations in paired CSF and plasma samples (20). In a recent study, regardless of CSF cytology results, EGFR mutations were detected by NGS in 100% of CSF cfDNA in 26 cases with leptomeningeal metastases of EGFR-mutant NSCLC. However, high-confidence somatic alterations by NGS were found in all 16 (100%) patients with positive CSF cytology and 4 of the 16 (25%) with negative CSF cytology with radiographic evidence for CNS metastases (21). In a similar study on EGFR status in patients with neoplastic meningitis by DNA sequencing, EGFR mutations were reported in 45% of the patients with positive CSF cytology and 30% of the patients with negative CSF cytology (22). Our findings indicate that analysis of CSF cfDNA can be useful for monitoring relevant molecular pathological information of patients with negative tumor cytology in CSF.

Due to the existence of the blood-brain barrier, CSF cfDNA can not be fully circulated in the blood system, thus, plasma can not fully represent the 'real world' of intracranial lesions (23). In 10 EGFR mutant cases of CSF, 70% of samples had a concordant EGFR status in their paired plasma samples. Importantly, in one case, T790M mutations were identified in a CSF sample, while the mutations were not found in a blood sample at the same time.

We were unable to compare the EGFR status of cerebrospinal fluid samples and intracranial tumors due to the absence of intracranial metastatic tumor tissue. In our study, EGFR mutation detection rate was lower in CSF (33.3%) than that in plasma (53.3%). This is partly due to lower levels of cfDNA in cerebrospinal fluid than in blood. Though recent reports have demonstrated that circulating tumor DNA (ctDNA) was more abundant in CSF than that in plasma of breast cancer (24). In 2 ml of the liquid samples of this study, lower overall cfDNA yields were obtained from CSF (mean ± SD, plasma: 64.59 ± 41.25 ng versus CSF: 23.70 ± 9.52 ng. **Supplementary Figure 1**).

Initial detection of EGFR mutations is necessary to guide TKI treatment, and EGFR mutation status of cfDNA in plasma correlates to TKI response, PFS, as well as OS (25, 26). In this study, 71.4% (5/7) of patients with activating EGFRm (19del or L858R) in CSF samples achieved iPR after treatment with a combination of WBRT and first-generation EGFR-TKIs, and 100% (3/3) of patients with an EGFR T790M mutation achieved iPR after treatment with second-line osimertinib alone. The median iPFS and OS of EGFRm were 12.0 months and 8.0 months, respectively, which were numerically superior to that of EGFRw, however no statistical difference was reached. When we combined the EGFR mutation results of CSF and that of plasma for analysis, the best intracranial response rate (CR+PR) was 78.9% for patients with EGFRm (CSF or plasma) versus 36.4% for patients with EGFRw (CSF and plasma). There was a significant difference. The median iPFS of EGFRm (CSF or plasma) and EGFRw (CSF and plasma) were 12.0 months and 6.0 months, respectively (p = 0.014), and the median OS of EGFRm (CSF or plasma) and EGFRw (CSF and plasma) were 31.0 months and 11.0 months, respectively (p = 0.002). The accurate identification of tumors with sensitized EGFR mutations, the most common targetable molecular alteration in lung adenocarcinoma, and acquired drug resistance mutations during treatment is a clinical priority. In a recent study, Huang et al. enrolled 35 patients with central nervous system metastases, (including 20 brain metastases and 15 leptomeningeal metastases) to investigate EGFR mutational status in cfDNA from paired CSF and plasma samples. In brain metastases patients, sensitizing EGFR mutations in the CSF or plasma were detected in 5/10 (50%) and 6/11 (54.5%) cases, and EGFR T790M mutations in the CSF or plasma were found in 0/10 (0%) and 4/11 (36.4%) cases (27). The EGFR T790M mutation is the most common mechanism of acquired resistance to first- and second-generation EGFR-TKIs, being present in 50%-60% of the cases (10, 11, 28). The EGFR T790M mutation can be detected accurately by liquid biopsy, and the presence of any detectable T790M ctDNA may be clinically relevant (29, 30). T790M status by liquid biopsy is well correlated with the response of third-generation TKIs (12, 31, 32). Dynamic repeat testing may provide more information about the mechanism of resistance. In this study, we found three cases with a T790M mutation from CSF (including one with wild-type EGFR in a paired blood sample), and all three patients achieved iPR after treatment with second-line osimertinib alone.

There were some limitations to this study. Firstly, this study was a single center retrospective study with a relatively small sample size, resulting in a low statistical power to detect associations. Secondly, as BM lesions biopsies were invasive and difficult to access, we were unable to compare the EGFR genetic profiles between intracranial tissue and CSF. Thirdly, cerebrospinal fluid sampling was 2 ml, and cfDNA yields were relatively few, which may affect the EGFR information of cfDNA. Finally, given the limitations of ddPCR, we only studied the T790M mutation in CSF, which was the most common drug resistance mechanism of first- and second-generation EGFR-TKIs, the other resistance mechanisms were not detected. NGS of cfDNA from CSF may be a better choice for comprehensive genetic profiles to explore the mechanisms of resistance beyond the T790M mutation.

In conclusion, our study demonstrates that it is feasible to test EGFR mutation in CSF and plasma. In LADC patients with brain metastasis, cerebrospinal fluid can be used as a liquid biopsy specimen to guide the treatment strategy by monitoring EGFR mutation status. For advanced LADC patients with BMs harboring EGFR mutation, dynamically monitoring the EGFR mutation status of CSF will be an appropriate choice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Beijing Chest Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ZL, LS, and NC conceived and designed the research. LS, JT, HT, LG, WHW, HW, ZCL, LT, WW, HL, QM, LX, and ZL performed the measurements or analyzed the results. LS and ZL wrote the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.622142/full#supplementary-material

REFERENCES

1. Khalifa J, Amini A, Popat S, Gaspar LE, Faivre-Finn C. Brain Metastases from NSCLC: Radiation Therapy in the Era of Targeted Therapies. *J Thorac Oncol* (2016) 11(10):1627–43. doi: 10.1016/j.jtho.2016.06.002
2. Sørensen JB, Hansen HH, Hansen M, Dombernowsky P. Brain metastases in adenocarcinoma of the lung: frequency, risk groups, and prognosis. *J Clin Oncol* (1988) 6(9):1474–80. doi: 10.1200/JCO.1988.6.9.1474
3. Heon S, Yeap BY, Britt GJ, Costa DB, Rabin MS, Jackman DM, et al. Development of Central Nervous System Metastases in Patients with Advanced Non–Small Cell Lung Cancer and Somatic EGFR Mutations Treated with Gefitinib or Erlotinib. *Clin Cancer Res* (2010) 16(23):5873–82. doi: 10.1158/1078-0432.CCR-10-1588
4. Lee YJ, Choi HJ, Kim SK, Chang J, Moon JW, Park IK, et al. Frequent central nervous system failure after clinical benefit with epidermal growth factor receptor tyrosine kinase inhibitors in Korean patients with nonsmall-cell lung cancer. *Cancer-Am Cancer Soc* (2010) 116(5):1336–43. doi: 10.1002/cncr.24877
5. Patchell RA. The management of brain metastases. *Cancer Treat Rev* (2003) 29(6):533–40. doi: 10.1016/S0305-7372(03)00105-1
6. Sperduto PW, Kased N, Roberge D, Xu Z, Shanley R, Luo X, et al. Summary Report on the Graded Prognostic Assessment: An Accurate and Facile Diagnosis-Specific Tool to Estimate Survival for Patients With Brain Metastases. *J Clin Oncol* (2012) 30(4):419–25. doi: 10.1200/JCO.2011.38.0527
7. Magnuson WJ, Yeung JT, Guillod PD, Gettinger SN, Yu JB, Chiang VL. Impact of Deferring Radiation Therapy in Patients With Epidermal Growth Factor Receptor–Mutant Non-Small Cell Lung Cancer Who Develop Brain Metastases. *Int J Radiat Oncol Biol Phys* (2016) 95(2):673–9. doi: 10.1016/j.ijrobp.2016.01.037
8. Yang J, Zhou C, Huang Y, Feng J, Lu S, Song Y, et al. Icotinib versus whole-brain irradiation in patients with EGFR -mutant non-small-cell lung cancer and multiple brain metastases (BRAIN): a multicentre, phase 3, open-label, parallel, randomised controlled trial. *Lancet Respir Med* (2017) 5(9):707–16. doi: 10.1016/S2213-2600(17)30262-X
9. Mok T, Yang J, Lam K. Treating Patients With EGFR-Sensitizing Mutations: First Line or Second Line—Is There a Difference? *J Clin Oncol* (2013) 31(8):1081–8. doi: 10.1200/JCO.2012.43.0652
10. Oxnard GR, Arcila ME, Sima CS, Riely GJ, Chmielecki J, Kris MG, et al. Acquired Resistance to EGFR Tyrosine Kinase Inhibitors in EGFR-Mutant Lung Cancer: Distinct Natural History of Patients with Tumors Harboring the T790M Mutation. *Clin Cancer Res* (2011) 17(6):1616–22. doi: 10.1158/1078-0432.CCR-10-2692
11. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of Tumor Specimens at the Time of Acquired Resistance to EGFR-TKI Therapy in 155 Patients with EGFR-Mutant Lung Cancers. *Clin Cancer Res* (2013) 19(8):2240–7. doi: 10.1158/1078-0432.CCR-12-2246
12. Mok TS, Wu Y, Ahn M, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or Platinum–Pemetrexed in EGFR T790M–Positive Lung Cancer. *N Engl J Med* (2017) 376(7):629–40. doi: 10.1056/NEJMoa1612674
13. Park SJ, Kim HT, Lee DH, Kim KP, Kim SW, Suh C, et al. Efficacy of epidermal growth factor receptor tyrosine kinase inhibitors for brain metastasis in non-small cell lung cancer patients harboring either exon 19 or 21 mutation. *Lung Cancer* (2012) 77(3):556–60. doi: 10.1016/j.lungcan.2012.05.092
14. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol* (2017) 14(9):531–48. doi: 10.1038/nrclinonc.2017.14
15. Wang Y, Springer S, Zhang M, McMahon KW, Kinde I, Dobbyn L, et al. Detection of tumor-derived DNA in cerebrospinal fluid of patients with primary tumors of the brain and spinal cord. *Proc Natl Acad Sci* (2015) 112(31):9704–9. doi: 10.1073/pnas.1511694112
16. Pentsova EI, Shah RH, Tang J, Boire A, You D, Briggs S, et al. Evaluating Cancer of the Central Nervous System Through Next-Generation Sequencing of Cerebrospinal Fluid. *J Clin Oncol* (2016) 34(20):2404–15. doi: 10.1200/JCO.2016.66.6487
17. Zhu G, Ye X, Dong Z, Lu YC, Sun Y, Liu Y, et al. Highly Sensitive Droplet Digital PCR Method for Detection of EGFR-Activating Mutations in Plasma Cell–Free DNA from Patients with Advanced Non–Small Cell Lung Cancer. *J Mol Diagn* (2015) 17(3):265–72. doi: 10.1016/j.jmoldx.2015.01.004
18. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* (2009) 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026
19. Goldman JW, Noor ZS, Remon J, Besse B, Rosenfeld N. Are liquid biopsies a surrogate for tissue EGFR testing? *Ann Oncol* (2018) 29(suppl_1):i38–46. doi: 10.1093/annonc/mdx706
20. Zhao J, Ye X, Xu Y, Chen M, Zhong W, Sun Y, et al. EGFR mutation status of paired cerebrospinal fluid and plasma samples in EGFR mutant non-small cell lung cancer with leptomeningeal metastases. *Cancer Chemother Pharmacol* (2016) 78(6):1305–10. doi: 10.1007/s00280-016-3155-y
21. Li YS, Jiang BY, Yang JJ, Zhang XC, Zhang Z, Ye JY, et al. Unique genetic profiles from cerebrospinal fluid cell-free DNA in leptomeningeal metastases of EGFR-mutant non-small-cell lung cancer: a new medium of liquid biopsy. *Ann Oncol* (2018) 29(4):945–52. doi: 10.1093/annonc/mdy009
22. Shingyoji M, Kageyama H, Sakaida T, Nakajima T, Matsui Y, Itakura M, et al. Detection of Epithelial Growth Factor Receptor Mutations in Cerebrospinal Fluid from Patients with Lung Adenocarcinoma Suspected of Neoplastic Meningitis. *J Thorac Oncol* (2011) 6(7):1215–20. doi: 10.1097/JTO.0b013e318219aaae
23. De Mattos-Arruda L, Mayor R, Ng C, Weigelt B, Martinez-Ricarte F, Torrejon D, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. *Nat Commun* (2015) 6(1):8839. doi: 10.1038/ncomms9839
24. Murtaza M, Dawson S, Pogrebniak K, Rueda OM, Provenzano E, Grant J, et al. Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. *Nat Commun* (2015) 6(1):8760. doi: 10.1038/ncomms9760
25. Mok T, Wu YL, Lee JS, Yu CJ, Sriuranpong V, Sandoval-Tan J, et al. Detection and Dynamic Changes of EGFR Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy. *Clin Cancer Res* (2015) 21(14):3196–203. doi: 10.1158/1078-0432.CCR-14-2594
26. Wang Z, Cheng Y, An T, Gao H, Wang K, Zhou Q, et al. Detection of EGFR mutations in plasma circulating tumour DNA as a selection criterion for first-line gefitinib treatment in patients with advanced lung adenocarcinoma (BENEFIT): a phase 2, single-arm, multicentre clinical trial. *Lancet Respir Med* (2018) 6(9):681–90. doi: 10.1016/S2213-2600(18)30264-9

27. Huang R, Xu X, Li D, Chen K, Zhan Q, Ge M, et al. Digital PCR-Based Detection of EGFR Mutations in Paired Plasma and CSF Samples of Lung Adenocarcinoma Patients with Central Nervous System Metastases. *Target Oncol* (2019) 14(3):343–50. doi: 10.1007/s11523-019-00645-5
28. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, et al. EGFR Mutation and Resistance of Non–Small-Cell Lung Cancer to Gefitinib. *N Engl J Med* (2005) 352(8):786–92. doi: 10.1056/NEJMoa044238
29. Oxnard GR, Thress KS, Alden RS, Lawrance R, Paweletz CP, Cantarini M, et al. Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non–Small-Cell Lung Cancer. *J Clin Oncol* (2016) 34(28):3375–82. doi: 10.1200/JCO.2016.66.7162
30. Jenkins S, Yang JC, Ramalingam SS, Yu K, Patel S, Weston S, et al. Plasma ctDNA Analysis for Detection of the EGFR T790M Mutation in Patients with Advanced Non–Small Cell Lung Cancer. *J Thorac Oncol* (2017) 12(7):1061–70. doi: 10.1016/j.jtho.2017.04.003
31. Hochmair MJ, Buder A, Schwab S, Burghuber OC, Prosch H, Hilbe W, et al. Liquid-Biopsy-Based Identification of EGFR T790M Mutation-Mediated Resistance to Afatinib Treatment in Patients with Advanced EGFR Mutation-Positive NSCLC, and Subsequent Response to Osimertinib. *Target Oncol* (2019) 14(1):75–83. doi: 10.1007/s11523-018-0612-z
32. Su PL, Yang SC, Chen YL, Wu YL, Lin CY, Chang WY, et al. Real-world outcomes of NSCLC patients receiving tissue or circulating tumor DNA-guided osimertinib treatment. *Cancer Med-Us* (2019) 8(13):5939–47. doi: 10.1002/cam4.2485

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Comprehensive Comparison Between Adjuvant Targeted Therapy and Chemotherapy for EGFR-Mutant NSCLC Patients: A Cost-Effectiveness Analysis

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Background: Chemotherapy has been the current standard adjuvant treatment for early-stage non-small-cell lung cancer (NSCLC) patients, while recent studies showed benefits of epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI). We conducted a cost-effectiveness analysis to comprehensively evaluate the benefit of EGFR-TKI compared with chemotherapy for early-stage EGFR-mutant NSCLC patients after resection from the perspective of the Chinese health care system.

Method: A Markov model was established. Clinical data were based on the phase 3, ADJUVANT trial, where stage II-IIIA, EGFR-mutant NSCLC patients were randomized into gefitinib group or chemotherapy group after resection. Cost parameters mainly included costs of drugs, examinations, and adverse events (AEs). Effect parameters were evaluated by quality-adjusted life year (QALY). Outcomes contained incremental cost-effective ratio (ICER), average cost-effective ratio (ACER), and net benefit. The willingness-to-pay threshold was set as 3 times per capita gross domestic product ($30,828/QALY). Sensitivity analyses were also conducted to verify the stability of the model.

Results: Patients who received gefitinib had both a higher cost ($12,057.98 vs. $11,883.73) and a higher QALY (1.55 vs. 1.42) than patients who received chemotherapy. With an ICER of $1,345.62/QALY, adjuvant gefitinib was of economic benefit compared with chemotherapy. The ACER and net benefit were also consistent (gefitinib vs. chemotherapy, ACER: $7,802.30/QALY vs. $8,392.77/QALY; net benefit: $35,584.85 vs. $31,767.17). Sensitivity analyses indicated the stability of the model and the impact of utility.

Conclusion: Adjuvant EGFR-TKI application for early-stage EGFR-mutant NSCLC patients was cost-effective compared with chemotherapy, which might provide a reference for clinical decision-making and medical insurance policy formulation in China.

Keywords: non-small-cell lung cancer, epidermal growth factor receptor-tyrosine kinase inhibitor, cost-effectiveness analysis, adjuvant therapy, chemotherapy

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BACKGROUND

Non-small-cell lung cancer (NSCLC) is the major type of lung cancer, among which, approximately 25% are diagnosed with early-stage NSCLC and are supposed to undergo surgical resection (1, 2). However, the high postoperative recurrence rate has a negative impact on prognosis, with approximately 30% for stage I patients and up to 75% for stage III patients (3–5). Common relapse after surgery for NSCLC patients highlights the importance of optimizing adjuvant treatment regions to eliminate residual tumors (6). Previous studies have shown that postoperative cisplatin-based chemotherapy could bring survival benefits to NSCLC patients with a 5–10% improvement in 5-year overall survival (OS) rate, furthermore, the combination of vinorelbine and cisplatin is currently the standard adjuvant treatment regimen for resected stage II–III NSCLC patients (7–10). However, the toxicity of chemotherapy reduces the compliance of patients and therapeutic efficacy (4).

Epidermal growth factor receptor (EGFR) mutation is the most common type of genomic alteration in NSCLC, with an incidence range of 10–20% in Caucasians to 50% in Asian populations (11). The efficacy of EGFR-tyrosine kinase inhibitor (EGFR-TKI) for advanced EGFR-mutant NSCLC patients is well accepted, and current studies are exploring the application of EGFR-TKI in the adjuvant setting (12). A meta-analysis confirmed the disease-free survival (DFS) benefit of adjuvant EGFR-TKI compared with both placebo (hazard ratio [HR]: 0.59, 95% confidence interval [CI]: 0.40–0.88, P = 0.009) and chemotherapy (HR: 0.42, 95%CI: 0.19–0.93, P = 0.03) for EGFR-mutant patients, however, OS analysis only showed superior tendency without significant benefit, besides patients administrated with EGFR-TKI had fewer adverse events (AEs) than patients receiving chemotherapy (risk ratio [RR]: 0.26, 95% CI: 0.18–0.38, P <0.00001) (13).

In clinical practice, despite survival benefits, cost and quality of life are also important considerations for treatment decisions. Patient's quality of life reflects both the physical and psychological status of patients, besides, the impact of AEs is also included. Multi-dimensional assessments are not only conducive to a comprehensive evaluation and decision making but could also improve the compliance of patients. Thus, a cost-effectiveness analysis was conducted to evaluate the benefit of EGFR-TKI compared with chemotherapy as adjuvant therapy for EGFR-mutant NSCLC patients after resection, in order to select the optimal adjuvant therapy comprehensively and provide guidance for both clinical decision making and health insurance policy formulation.

METHOD

Clinical Data

The clinical data was based on a phase 3, randomized, open-label ADJUVANT trial (CTONG1104, NCT01405079), patients who underwent complete resection (R0) and diagnosed with stage II–IIIA (N1–N2), EGFR mutation-positive (exon 19 deletion or exon 21 Leu858Arg) NSCLC were eligible from multi-centers in China (14, 15). After complete resection and randomization, patients were allotted into targeted therapy group (receiving gefitinib 250 mg once daily for 24 months, oral administration) or chemotherapy group (receiving vinorelbine 25 mg/m² on days 1 and 8 plus cisplatin 75 mg/m² on day 1, every 3 weeks for four cycles, intravenous administration). After a median follow-up of 80 months, results showed patients receiving gefitinib achieved a superior DFS (median DFS: 30.8 months vs. 19.8 months, HR: 0.56, 95%CI: 0.40–0.79, P = 0.001) than those administrated with chemotherapy, while OS analysis did not show a significant difference between gefitinib and chemotherapy group (median OS: 75.5 months vs. 62.8 months, HR: 0.92, 95%CI: 0.62–1.36, P = 0.674). Besides, in terms of AEs, patients receiving gefitinib suffered from fewer AEs than patients in the chemotherapy group (AEs: 58% vs. 80%, grades 3–4: 12% vs. 48%). Detailed information was listed in **Table 1**.

Cost-Effectiveness Parameters

A Markov model was established using Treeage Pro with a 21-day cycle length, a 10-year horizon, a 3% annual discount rate, and three mutually independent Markov states: DFS, progressive disease (PD), and die. All patients were in DFS state initially and transferred into other states according to progressive and survival probabilities. The structure of the Markov model was presented in **Figure 1**. Progression and survival probabilities were extracted and calculated from DFS and OS Kaplan–Meier curves respectively in the ADJUVANT trial by GetData Graph Digitizer and R software (14, 15). Fitting to the Weibull model, the following formulas were used to calculate progressive or survival probability P and transition probability at time t: $P = 1 - \text{Exp}(-r \times t)$; $Pt = 1 - \text{Exp}[\lambda(t-u)^{\gamma} - \lambda t^{\gamma}]$, where r represented for the progressive or survival rate, u was the cycle length, λ and γ were the scale and shape parameter separately (16).

Costs were extracted from local hospitals and published literature. For specific calculation of drug doses and costs, we

TABLE 1 | Clinical data.

	Gefitinib	Chemotherapy
Administration	Gefitinib (250 mg once daily) for 24 months	Vinorelbine (25 mg/m² on days 1 and 8) plus cisplatin (75 mg/m² on day 1) every 3 weeks for four cycles
Median DFS (95%CI)	30.8 (26.7–36.6)	19.8 (15.4–23.0)
HR (95%CI)	0.56 (0.40–0.79)	
P	0.001	
Median OS (95%CI)	75.5 (46.6–NC)	62.8 (45.8–NC)
HR (95%CI)	0.92 (0.62–1.36)	
P	0.674	
AE	58%	80%
Grades 3–4 AE	12%	48%

DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; AE, adverse event.

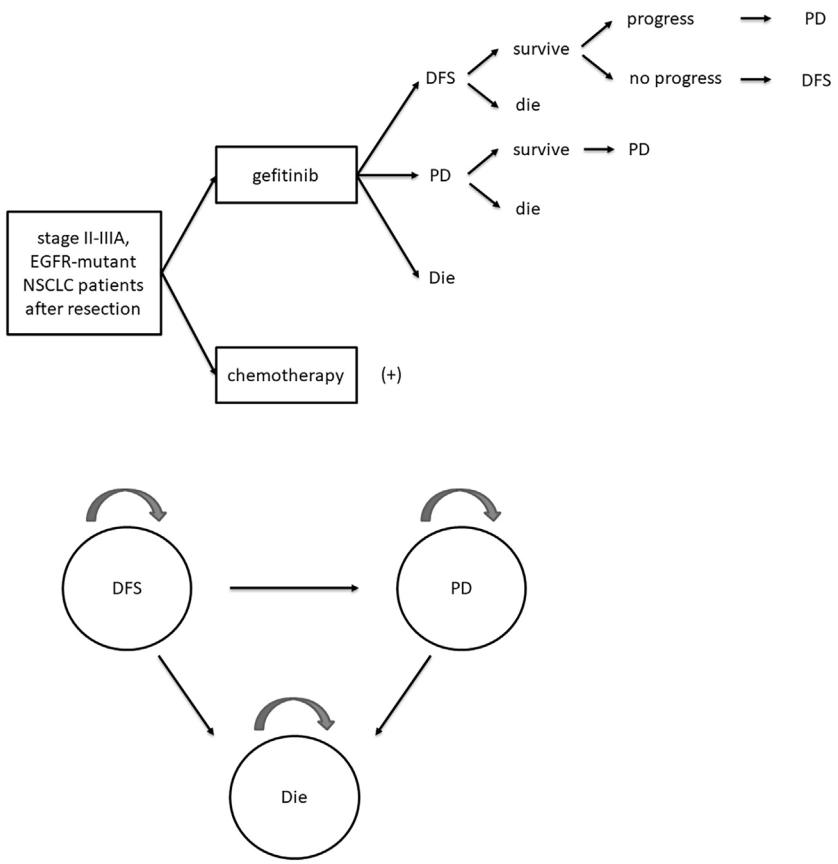

FIGURE 1 | Schematic diagram of Markov model. DFS, disease-free survival; PD, progressive disease; EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer.

assumed a typical patient with a 1.64 m height, a 65 kg weight, and a body surface area (BSA) of 1.72 m^2 according to previous cost-effectiveness analysis evaluating chemotherapy (17). Direct medical costs of drugs (gefitinib, vinorelbine, and cisplatin), imaging examinations, laboratory tests, follow-up, supportive care, grade ≥3 AEs, and PD state were calculated as US dollars (exchange rate: 6.8409) (17, 18). Common expenses for both groups such as costs of surgery and EGFR tests were not calculated since they did not affect the cost-effectiveness results.

Effects of treatments were representative by quality-adjusted life year (QALY), which is a comprehensive evaluation index of patients' survival period and quality of life. Health state utility parameters were extracted from published literature and were ranged from 0 to 1 with 1 representing the best physical and psychological conditions. Extracted utilities contained utilities for DFS state, including oral therapy (0.8) and intravenous therapy (0.76) respectively; PD state (0.7); death (0); and AEs of grades 3–4 (−0.0731), thereinto considering the unavailability of accurate utilities of various AEs, the average value was obtained for substitution based on published studies (19, 20). Specific utilities were adjusted based on clinical reports in the ADJUVANT trial. Cost and utility parameters were listed in **Table 2**.

Cost-Effectiveness and Sensitivity Analyses

The primary outcome of the study was the incremental cost-effective ratio (ICER), which is the ratio of incremental cost and incremental effect between the two groups. Secondary outcomes were the average cost-effective ratio (ACER, the ratio of average cost and average effect) and net benefit (willingness-to-pay [WTP] × effect − cost). The cost-effectiveness analysis was conducted in the perspective of the Chinese health care system and the WTP threshold was set as three times per capita gross domestic product (GDP, $30,828/QALY).

Both one-way sensitivity analysis and probabilistic sensitivity analysis were conducted by Treeage Pro. One-way sensitivity analysis was displayed as a Tornado diagram to explore the most influential factor on the Markov model. Cost parameters were evaluated with a range of 30% based on the baseline value, while a 20% range was set for both utilities and survival probabilities. Detailed information was shown in **Supplemental**

TABLE 2 | Baseline parameters.

Baseline parameters	Value	Specification
Cost		
Gefitinib	23.33	0.25 g * 1
Vinorelbine	8.16	1 ml/10 mg * 1
Cisplatin	2.80	6 ml/30 mg * 1
CT Scan-Lung	54.84	Once
CT Scan-Abdomen	52.34	Once
MRI Scan-brain	91.38	Once
Electrocardiograph	3.80	Once
Echocardiography	48.60	Once
Enhanced CT Scan-Lung	134.44	Once
Enhanced CT Scan-Abdomen	248.29	Once
Bone scan	183.41	Once
PET-CT	1,154.98	Once
Abdominal ultrasonography	26.38	Once
Routine blood test	3.22	Once
Blood biochemical examination	25.29	Once
Routine urine test	4.39	Once
Coagulation test	9.36	Once
Artery blood gas	21.93	Once
Follow-up	55.60	Per cycle
Supportive care	337.50	Per cycle
AE	507.40	Per cycle
PD	1,877.25	First cycle
Utility		
DFS, oral therapy	0.80	
DFS, intravenous therapy	0.76	
PD	0.70	
Death	0.00	
AE	−0.07	

CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; AE, adverse event; PD, progressive disease; DFS, disease-free survival.

Table 1. Probabilistic sensitivity analysis was conducted through Monte Carlo Simulation with 1,000 iterations, cost parameters were hypothesized to fit gamma distribution, while utilities and survival probabilities were assumed to be beta distributed (21). Results were displayed as cost-effectiveness acceptability curve and net monetary benefit acceptability curve in order to represent the cost-effective iterations with various WTP thresholds.

Statement

Clinical data in the current manuscript were extracted from a published clinical trial (ADJUVANT trial/CTONG1104/NCT01405079) and therefore ethics approval or specific consent procedures were not required for this study.

RESULTS

Patients receiving gefitinib achieved a better QALY than patients receiving chemotherapy (1.55 vs. 1.42) with an incremental QALY of 0.13, however, the gefitinib group also had a higher cost than the chemotherapy group ($12,057.98 vs. $11,883.73) with an incremental cost of $174.24. The ICER was $1,345.62/QALY, which indicated the administration of gefitinib was cost-effective compared to chemotherapy in the perspective of the Chinese health care system. The cost-effectiveness analysis curve was shown in **Figure 2**. As for secondary outcomes, the gefitinib group showed a lower ACER and a higher net benefit than the chemotherapy group (ACER: $7,802.30/QALY vs. $8,392.77/QALY; net benefit: $35,584.85 vs. $31,767.17). Specific results were listed in **Table 3**.

As for sensitivity analyses, one-way sensitivity analysis showed that the utility of patients receiving gefitinib in DFS state was the most dominant influence index, followed by the utility of DFS patients receiving chemotherapy and utility of PD patients receiving gefitinib. The tornado diagram was shown in **Supplemental Figure 1**. While in terms of probabilistic sensitivity analysis, the cost-effectiveness acceptability curve showed even at a WTP of $1,500, the gefitinib group was of more economic benefit than the chemotherapy group, which was displayed in **Figure 3**. Net monetary benefit acceptability curve showed advantages gradually expanded with the increase of

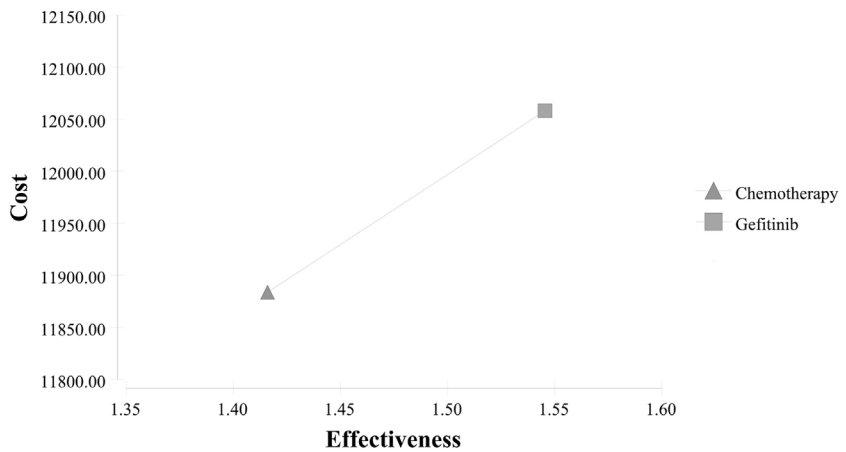

FIGURE 2 | Results of cost-effectiveness analysis.

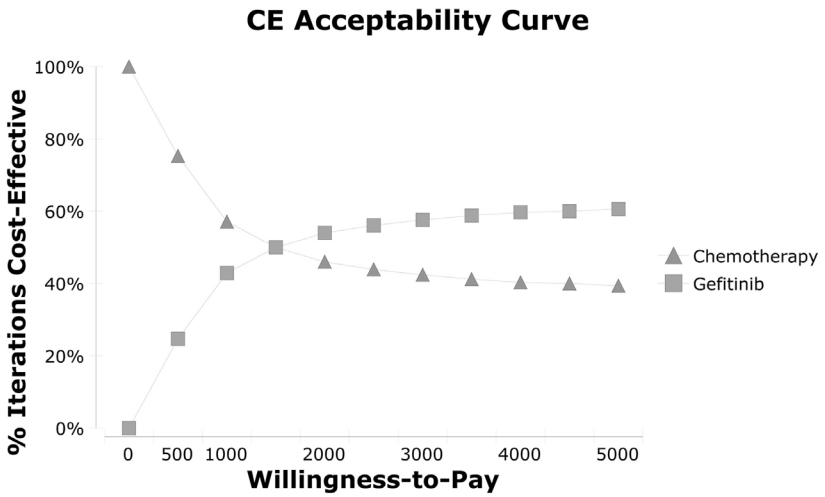

FIGURE 3 | Cost-effectiveness acceptability curve. CE, cost-effectiveness.

TABLE 3 | Results.

	QALY	IE	Cost	IC	ICER	ACER	Net benefit
Gefitinib	1.55		12,057.98			7,802.30	35,584.85
Chemotherapy	1.42	0.13	11,883.73	174.24	1,345.62	8,392.77	31,767.17

QALY, quality-adjusted life year; IE, incremental effect; IC, incremental cost; ICER, incremental cost-effective ratio; ACER, average cost-effective ratio.

WTP (**Supplemental Figure 2**). Probability distributions were listed in **Supplemental Table 2**.

DISCUSSION

Although several clinical trials confirmed the superior DFS benefit of adjuvant EGFR-TKI over both chemotherapy and placebo, none of them showed a long-term survival benefit, in addition, it was also suggested that two years of treatment course was not conducive to the adherence of patients due to chronic AEs, hence the adjuvant application of EGFR-TKI was still controversial (22). Thus, we conducted a comprehensive evaluation of adjuvant EGFR-TKI benefits from multi-dimensions including clinical survival benefit, quality of life, and costs. According to the outcomes, administration of EGFR-TKI brought a higher cost of $174.24 and a higher QALY of 0.13, the ICER was $1,345.62/QALY, which showed prominent advantages.

Previous cost-effectiveness analyses demonstrated that the application of adjuvant chemotherapy had superior benefits compared with the observation group for early-stage NSCLC patients in the Canadian health care system perspective (23). While other cost-effectiveness studies showed economic benefits of prognostic tests in guiding adjuvant chemotherapy, which was also from the perspective of the United States and Canada health care systems (24–26). However, there was still a lack of economic evaluations on EGFR-TKI in the adjuvant setting, making this study the first cost-effectiveness analysis to comprehensively evaluate the benefit of adjuvant EGFR-TKI therapy for early-stage EGFR-mutant NSCLC patients.

Currently, only advanced EGFR mutation-positive NSCLC patients receiving first-generation EGFR-TKI are included in the Chinese medical insurance policy. Considering the consistent DFS, safety, and cost-effectiveness benefits of first-generation EGFR-TKI application for early-stage EGFR mutation-positive NSCLC patients, it is suggested that the reimbursement policy could be further expanded. In addition, the cost of gefitinib was based on the ADJUVANT trial, while the cost of domestic gefitinib was cheaper, which could further expand the benefits.

In spite of the positive outcomes, further explorations and developments are still required in this field. Firstly, since the survival benefit of the third-generation EGFR-TKI, osimertinib for advanced EGFR-mutant NSCLC patients has been verified, studies are also exploring the efficacy of osimertinib in the adjuvant setting for EGFR-mutant NSCLC patients after complete resection (27). The phase 3 ADAURA (NCT02511106) trial demonstrated that patients receiving osimertinib achieved a significant superior DFS compared with those receiving placebo (stage II–IIIA patients: HR, 0.17; 99.06%CI, 0.11–0.26; P <0.001; stage IB–IIIA patients: HR, 0.20; 99.12%CI, 0.14–0.30; P <0.001) (28). We did not include osimertinib in the cost-effectiveness analysis due to the immature survival data, further exploration could be conducted with mature data. Secondly, although EGFR-TKI monotherapy could reduce AEs, considering tumor heterogeneity and the efficacy of other treatment regimens, including chemotherapy and radiotherapy, different combination therapies should also be further investigated for assessing the optimal adjuvant therapy (22). Thirdly, there is still a lack of relevant studies for treatments after resistance to EGFR-TKI adjuvant therapy, which should be further explored as well (5).

The main limitation of the study was that the outcomes were restricted to geographical regions and populations. Despite the phase 2, single-arm, SELECT trial also showed efficacy of adjuvant EGFR-TKI therapy based on major non-Asian population (5-year DFS rate: 56%, 5-year OS rate: 86%), studies showed that both EGFR mutation rate and therapeutic efficacy of EGFR-TKI are related to ethnicity with distinctive clinicopathologic characteristics (3, 29). Both the clinical data and cost parameters of this cost-effectiveness analysis were based on Chinese populations, thus it is not suitable to generalize the outcomes to Caucasians or other populations.

CONCLUSION

In the cost-effectiveness analysis, we comprehensively evaluated the benefit of adjuvant EGFR-TKI application for early-stage EGFR mutation-positive NSCLC patients by synthesizing clinical survival data, quality of life and cost parameters. The ICER was $1,345.62/QALY and demonstrated economic benefits from the perspective of the Chinese health care system. Our results could further propel the development of precision treatment, and provide a reference for clinical decision-making and medical insurance policy formulation in China.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Clinical data in the current manuscript were extracted from published clinical trial (ADJUVANT trial/CTONG1104/NCT01405079) and therefore ethics approval or specific consent procedures were not required for this study.

AUTHOR CONTRIBUTIONS

Guarantor of integrity of the entire study: JC. Study concepts and design: JC and WL. Literature research: WL and HG. Data analysis: WL and LL. Manuscript preparation: WL and JC. Manuscript editing: JC. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. He Q, Xin P, Zhang M, Jiang S, Zhang J, Zhong S, et al. The impact of epidermal growth factor receptor mutations on the prognosis of resected non-small cell lung cancer: a meta-analysis of literatures. *Transl Lung Cancer Res* (2019) 8(2):124–34. doi: 10.21037/tlcr.2019.03.14
2. Friedlaender A, Addeo A, Russo A, Gregorc V, Cortinovis D , Rolfo CD. Targeted Therapies in Early Stage NSCLC: Hype or Hope? *Int J Mol Sci* (2020) 21(17):6329. doi: 10.3390/ijms21176329
3. Pennell NA, Neal JW, Chaft JE, Azzoli CG, Jänne PA, Govindan R, et al. SELECT: A Phase II Trial of Adjuvant Erlotinib in Patients With Resected Epidermal Growth Factor Receptor-Mutant Non-Small-Cell Lung Cancer. *J Clin Oncol* (2019) 37(2):97–104. doi: 10.1200/JCO.18.00131
4. Xu ST, Xi JJ, Zhong WZ, Mao WM, Wu L, Shen Y, et al. The Unique Spatial-Temporal Treatment Failure Patterns of Adjuvant Gefitinib Therapy: A Post Hoc Analysis of the ADJUVANT Trial (CTONG 1104). *J Thorac Oncol* (2019) 14(3):503–12. doi: 10.1016/j.jtho.2018.11.020
5. Kauffmann-Guerrero D. Adjuvant TKI treatment of EGFR-mutant lung cancer-already ripe for decision? *Transl Lung Cancer Res* (2020) 9(4):964–6. doi: 10.21037/tlcr.2020.04.13
6. Romero D. Adjuvant TKIs - a long-term matter. *Nat Rev Clin Oncol* (2019) 16 (2):67. doi: 10.1038/s41571-018-0144-6
7. Kris MG, Gaspar LE, Chaft JE, Kennedy EB, Azzoli CG, Ellis PM, et al. Adjuvant Systemic Therapy and Adjuvant Radiation Therapy for Stage I to IIIA Completely Resected Non-Small-Cell Lung Cancers: American Society of Clinical Oncology/Cancer Care Ontario Clinical Practice Guideline Update. *J Clin Oncol* (2017) 35(25):2960–74. doi: 10.1200/JCO.2017.72.4401
8. 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(9):719–27. doi: 10.1016/S1470-2045(06)70804-X
9. Pignon JP, Tribodet H, Scagliotti GV, Douillard JY, Shepherd FA, Stephens RJ, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol* (2008) 26(21):3552–9. doi: 10.1200/JCO.2007.13.9030
10. Douillard JY, Tribodet H, Aubert D, Shepherd FA, Rosell R, Ding K, et al. Adjuvant cisplatin and vinorelbine for completely resected non-small cell lung cancer: subgroup analysis of the Lung Adjuvant Cisplatin Evaluation. *J Thorac Oncol* (2010) 5(2):220–8. doi: 10.1097/JTO.0b013e3181c814e7
11. Li WQ, Cui JW. Non-small cell lung cancer patients with ex19del or exon 21 L858R mutation: distinct mechanisms, different efficacies to treatments. *J Cancer Res Clin Oncol* (2020) 146(9):2329–38. doi: 10.1007/s00432-020-03296-6
12. Wu YL, Zhou C, Liam CK, Wu G, Liu X, Zhong Z, et al. First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. *Ann Oncol* (2015) 26(9):1883–9. doi: 10.1093/annonc/mdv270
13. Cheng H, Li XJ, Wang XJ, Chen ZW, Wang RQ, Zhong HC, et al. A meta-analysis of adjuvant EGFR-TKIs for patients with resected non-small cell lung cancer. *Lung Cancer* (2019) 137:7–13. doi: 10.1016/j.lungcan.2019.08.002
14. Zhong WZ, Wang Q, Mao WM, Xu ST, Wu L, Shen Y, et al. Gefitinib versus vinorelbine plus cisplatin as adjuvant treatment for stage II-IIIA (N1-N2) EGFR-mutant NSCLC (ADJUVANT/CTONG1104): a randomised, open-label, phase 3 study. *Lancet Oncol* (2018) 19(1):139–48. doi: 10.1016/S1470-2045(17)30729-5

15. Wu YL, Zhong W, Wang Q, Mao W, Xu ST, Wu L, et al. CTONG1104: Adjuvant gefitinib versus chemotherapy for resected N1-N2 NSCLC with EGFR mutation—Final overall survival analysis of the randomized phase III trial 1 analysis of the randomized phase III trial. *J Clin Oncol* (2020) 38:9005. doi: 10.1200/JCO.2020.38.15_suppl.9005
16. Li W, Bai R, Qian L, Chen N, Zhao Y, Han F, et al. Cost-effectiveness of icotinib versus whole-brain irradiation with or without chemotherapy in EGFR-mutant NSCLC patients with brain metastases. *Asia Pac J Clin Oncol* (2020). doi: 10.1111/ajco.13291
17. Lu S, Ye M, Ding L, Tan F, Fu J, Wu B. Cost-effectiveness of gefitinib, icotinib, and pemetrexed-based chemotherapy as first-line treatments for advanced non-small cell lung cancer in China. *Oncotarget* (2017) 8(6):9996–10006. doi: 10.18632/oncotarget.14310
18. Zeng X, Karnon J, Wang S, Wu B, Wan X, Peng L. The cost of treating advanced non-small cell lung cancer: estimates from the chinese experience. *PloS One* (2012) 7(10):e48323. doi: 10.1371/journal.pone.0048323
19. Labbé C, Leung Y, Silva Lemes JG, Stewart E, Brown C, Cosio AP, et al. Real-World EQ5D Health Utility Scores for Patients With Metastatic Lung Cancer by Molecular Alteration and Response to Therapy. *Clin Lung Cancer* (2017) 18(4):388–95.e4. doi: 10.1016/j.cllc.2016.12.015
20. Brown J, Cook K, Adamski K, Lau J, Bargo D, Breen S, et al. Utility values associated with advanced or metastatic non-small cell lung cancer: data needs for economic modeling. *Expert Rev Pharmacoecon Outcomes Res* (2017) 17 (2):153–64. doi: 10.1080/14737167.2017.1311210
21. Handorf EA, McElligott S, Vachani A, Langer CJ, Bristol Demeter M, Armstrong K, et al. Cost effectiveness of personalized therapy for first-line treatment of stage IV and recurrent incurable adenocarcinoma of the lung. *J Oncol Pract* (2012) 8(5):267–74. doi: 10.1200/JOP.2011.000502
22. Jassem J. Adjuvant EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer: still an investigational approach. *Transl Lung Cancer Res* (2019) 8(Suppl 4):S387–s90. doi: 10.21037/tlcr.2019.09.02
23. Ng R, Hasan B, Mittmann N, Florescu M, Shepherd FA, Ding K, et al. Economic analysis of NCIC CTG JBR.10: a randomized trial of adjuvant vinorelbine plus cisplatin compared with observation in early stage non-small-cell lung cancer–a report of the Working Group on Economic Analysis, and the Lung Disease Site Group, National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* (2007) 25(16):2256–61. doi: 10.1200/JCO.2006.09.4342
24. Roth JA, Billings P, Ramsey SD, Dumanois R, Carlson JJ. Cost-effectiveness of a 14-gene risk score assay to target adjuvant chemotherapy in early stage non-squamous non-small cell lung cancer. *Oncologist* (2014) 19(5):466–76. doi: 10.1634/theoncologist.2013-0357
25. Wong KM, Ding K, Li S, Bradbury P, Tsao MS, Der SD, et al. A Cost-Effectiveness Analysis of Using the JBR.10-Based 15-Gene Expression Signature to Guide Adjuvant Chemotherapy in Early Stage Non-Small-Cell Lung Cancer. *Clin Lung Cancer* (2017) 18(1):e41–e7. doi: 10.1016/j.cllc.2016.06.009
26. Stenehjem DD, Bellows BK, Yager KM, Jones J, Kaldate R, Siebert U, et al. Cost-Utility of a Prognostic Test Guiding Adjuvant Chemotherapy Decisions in Early-Stage Non-Small Cell Lung Cancer. *Oncologist* (2016) 21(2):196–204. doi: 10.1634/theoncologist.2015-0162
27. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* (2018) 378(2):113–25. doi: 10.1056/NEJMoa1713137
28. Wu YL, Tsuboi M, He J, John T, Grohe C, Majem M, et al. Osimertinib in Resected EGFR-Mutated Non-Small-Cell Lung Cancer. *N Engl J Med* (2020) 383(18):1711–23. doi: 10.1056/NEJMoa2027071
29. Tanaka S, Uchino J. Erlotinib as standard adjuvant therapy for resectable EGFR mutation-positive non-small cell lung cancer. *Transl Lung Cancer Res* (2019) 8(Suppl 4):S369–s72. doi: 10.21037/tlcr.2019.06.05

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The Impact of Variant Allele Frequency in EGFR Mutated NSCLC Patients on Targeted Therapy

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EGFR mutations represent the most common currently targetable oncogenic driver in non-small cell lung cancer. There has been tremendous progress in targeting this alteration over the course of the last decade, and third generation tyrosine kinase inhibitors offer previously unseen survival rates among these patients. Nonetheless, a better understanding is still needed, as roughly a third of patients do not respond to targeted therapy and there is an important heterogeneity among responders. Allelic frequency, or the variant EGFR allele frequency, corresponds to the fraction of sequencing reads harboring the mutation. The allelic fraction is influenced by the proportion of tumor cells in the sample, the presence of copy number alterations but also, most importantly, by the proportion of cells within the tumor that carry the mutation. Mutations that occur early in tumor evolution, often called clonal or truncal, have a higher allelic frequency than late, subclonal mutations, and are more often drivers of cancer evolution and attractive therapeutic targets. Most, but not all, EGFR mutations are clonal. Although an exact estimate of clonal proportion is hard to derive computationally, the allelic frequency is readily available to clinicians and could be a useful surrogate. We hypothesized that tumors with low allelic frequency of the EGFR mutation will respond less favorably to targeted treatment.

Keywords: TKI, allele frequency, EGFR, NSCLC, VAF, allelic frequency

HIGHLIGHTS

We present a retrospective analysis of the impact of allelic frequency on survival in patients with EGFR mutant non-small cell lung cancer. We then combine allelic frequency with the presence of co-mutations, a known negative predictive factor for targeted therapy in this setting.

INTRODUCTION

Lung cancer is the most commonly diagnosed malignancy worldwide and the leading cause of cancer-related mortality (1). It comprises NSCLC, accounting for 85% of all diagnoses and SCLC. In the last decade, there has been a dramatic surge in the use of targeted therapy, which consists of identifying tumor driving alterations and using small tyrosine kinase inhibitors to block the oncogenic signals (2).

The most common current therapeutic target in lung adenocarcinoma (ADC) consists of epidermal growth factor receptor (EGFR) mutations (3).

EGFR is a monomeric transmembrane receptor tyrosine kinase controlling major molecular pathways of cellular proliferation (4). Upon activation, EGFR phosphorylates tyrosine residues in the intracellular domain, dimerizing and activating downstream signaling including RAS-RAF-MAPK-MEK, STAT, and PI3K-AKT-mTOR pathways, leading to cellular division and proliferation (4).

Activating EGFR mutations in ADC are most common among non-smokers, and younger, female, Asian lung cancer patients (5). The prevalence of EGFR mutations has a significant ethnic variation. They occur in roughly 15% of Caucasian any-stage ADC patients, according to the TCGA, but 22-62% of East Asians with stage III/IV ADC (6). Among East Asian never smokers, EGFR mutations can be found in approximately 80% of advanced lung ADC patients. Furthermore, among a cohort of metastatic, multi-treated, predominantly Caucasian, ADC cases, EGFR was detected in 27% of patients, suggesting that mutations are more common in advanced disease (7).

There are many subtypes of EGFR mutations in ADC, though exon 19 microdeletions and exon 21 point-substitutions comprise 90% of these (8). These common pathogenic variants are highly responsive to small molecule tyrosine kinase inhibitors (TKIs).

Nearly a decade ago, TKIs became standard first-line therapy for EGFR mutant NSCLC. First-generation (erlotinib, gefitinib) and second-generation (afatinib, dacomitinib) TKIs yielded superior outcomes and lower toxicity compared to chemotherapy doublets (9). The appearance of on-target resistance mechanisms, namely exon 20 T790M mutations, prompted the development of osimertinib, a third-generation EGFR TKI. Using the latter upfront was subsequently proven to be superior to prior generation TKIs, both in in terms of progression free survival (PFS) and overall survival (OS) (10). Yet, not all patients derive a similar benefit from TKIs, regardless of the generation of the therapy.

Today, the gold standard for detecting EGFR alterations is through next generation sequencing (NGS), allowing for the detection of a wide panel of oncogenic drivers, including numerous EGFR variants, as well as quantifying the alterations (11). Tumors with oncogenic drivers, such as EGFR in NSCLC, usually depend on a single activated oncogene (12). It yields a survival advantage in this isolated cell line, explaining the low tumor mutation burden (TMB) commonly associated with these diseases (13). The lack of acquired neoantigens through mutations provides a less inflammatory microenvironment and poor in tumor-infiltrating CD8+ lymphocytes (14). This likely, in part, explains why EGFR mutant NSCLC is less sensitive to immune check-point inhibition, which is not part of the front-line therapeutic algorithm for these patients.

In a previous paper, we established that co-occurring genomic mutations may explain the lack of efficacy among a subset of these patients (15). When performing NGS we also calculate the allelic frequency, or mutant allele frequency, corresponding to the fraction of sequencing reads harboring the mutation. The allelic fraction is influenced by the proportion of tumor cells in the sample, the presence of copy number alterations but also, most importantly, by the proportion of cells within the tumor that carry the mutation. Mutations that occur early in tumor evolution, often called clonal or truncal, have a higher allelic frequency than late, subclonal mutations, and are more often drivers of cancer evolution and attractive therapeutic targets (16). Most, but not all, *EGFR* mutations are clonal (17). Although an exact estimate of clonal proportion is hard to derive computationally, the allelic frequency is readily available to clinicians and could be a useful surrogate. We hypothesized that tumors with low allelic frequency of the *EGFR* mutation will respond less favorably to targeted treatment.

METHODS

We identified all patients treated with front-line TKIs (gefitinib, erlotinib, afatinib, osimertinib, dacomitinib) in our centre for advanced EGFR mutated NSCLC between January 2016 and January 2020. We identified 42 patients. Eleven were excluded due to the unavailability of variant allelic frequency data. We reviewed patient records, radiologic and pathology reports to extract clinical and pathological and radiological outcomes. All biopsies were performed at baseline, before the introduction of any therapy. We recorded date of death, if applicable, or the date of the last follow-up visit. All living patients enrolled signed a standardized general research consent form, providing access to their medical records. The study was approved by the regional Ethics Committee (CCER 2020-01628).

We assessed two clinical outcomes, the OS (primary) and PFS (secondary). PFS was calculated from the date of TKI initiation to that of radiological progression or death. OS was calculated from TKI initiation to the date of death, based on the vital status in February, 2020. Patient characteristics included sex, age at diagnosis, smoking status, performance status (PS) and presence of brain metastases at diagnosis. The patient population has been described previously (15).

Next-Generation Sequencing

We extracted the data from the clinical sequencing reports that were found in the patient files.

The sequencing of tumor DNA was performed for clinical purposes using the IonAmpliseq Hotspot Panel V2 (ThermoFisher scientific) on an IonTorrent Proton sequencer. The tumor cellularity was estimated on hematoxylin and eosin slides and the mutant allele frequency of EGFR were recorded. Co-occurring mutations present on the Ion Ampliseq Hotspot Panel V2 were also recorded. The

pathogenicity of mutations, namely their influence on protein function, was assessed based on international databases: ClinVar, Catalog of Somatic Mutations in Cancer (COSMIC) and oncoKB, as well as their described impact on treatment resistance in current medical literature (15).

Copy number variation analysis using the Oncoscan CNV assay (ThermoFisher) was available for some samples, and allowed us to estimate *EGFR* copy number as normal (2 copies) or gain (more than 2 copies).

Statistical Analysis

The data were analysed in the R language and environment for statistics (version 4.0.2, https://www.r-project.org). We used Kaplan-Meier survival estimates for visual representation, plotted with the Survminer package (version 0.4.8). Cox proportional-hazards univariable and multivariable models were used to test the association of key variables with progression-free and overall survival by calculating hazard ratios and their corresponding 95% confidence intervals. The Wald test was used to assess the statistical significance of Cox models at the usual $\alpha < 0.05$. Pearson's test was used to correlate the visually estimated tumor cellularity with the allelic frequency. Fisher's exact test was used to compare differences in the distribution of allelic frequency (low or high) between clinically relevant groups.

RESULTS

The median allelic frequency of the *EGFR* mutation was 0.47 (interquartile range: 0.24-0.65), in accordance with the assumption that it is often clonal. Nevertheless, there was considerable variation between patients, with two tumors having an allelic frequency of less than 0.1 and seven tumors an allelic frequency less than 0.2. The visually estimated median tumor cellularity was 0.63 (interquartile range: 0.5-0.9), which is sufficient for molecular analyses. Interestingly, tumor cellularity was weakly correlated with mutation allelic frequency (Pearson's rho=0.23, P=0.21).

It should be noted that the *EGFR* allelic frequency was not normally distributed (**Supplementary Figure 1** – density plot) and would therefore not be optimal for use as a continuous variable in Cox survival models. Based on the observed tumor cellularity, we would expect clonal *EGFR* mutations to present an allelic frequency of at least 0.31 on average (average cellularity divided by half), even in the absence of copy number gains, which are common in NSCLC and increase the observed allelic frequency. We therefore used a simple cut-off of 0.30 to separate low from high allelic frequency, as a surrogate marker of early, clonal mutations versus late, subclonal mutations. This cut-off also corresponds to the visual plateau between the two main modes of the allelic frequency distribution (**Supplementary Figure 1** – density plot). As expected in this context, most mutations (22 of 31, 71%) were classified as having a high allelic frequency.

Although this was not a prospective randomized trial, the clinical characteristics of the patients were balanced between the high and low groups. The general characteristics of the cohort from which these patients were drawn has also been previously described (15). Specifically, there was no statistically significant difference between *EGFR* mutation allelic frequency and age (Fisher's test P=0.456), sex (Fisher's test P=1.0), PS (Fisher's test P=0.689), smoking status (Fisher's test P=0.286) or first line treatment with osimertinib (Fisher's test P=0.704).

A high *EGFR* mutation allelic frequency was associated with longer PFS (HR 0.27, 95% 0.09-0.79, P=0.017) (**Figure 1**). This association was robust and persisted even after adjustment for age over 65, sex, smoking and use of osimertinib up-front (13 patients), with a hazard ratio estimate that remained statistically significant in bivariable models (**Table 1A**). It should be noted that only age over 65 was associated with longer PFS in bivariable models with allelic frequency. The statistical significance of the *EGFR* allelic frequency increased further in a multivariable model including the above clinical variables (*EGFR* HR=0.112, 95% 0.023-0.547, P=0.007, **Table 1B**).

Of the other clinical variables, only male sex was associated with shorter PFS in univariable models. Based on our previous publication, we knew that patients with resistance co-mutation had shorter PFS. For the remaining patients (N=25), without a resistance co-mutation, a high allelic frequency still predicted longer PFS (HR 0.20, 95% 0.04-0.91, P=0.038).

High allelic frequency was not associated with significant difference in overall survival (HR 0.47, 95% 0.17-1.30, P=0.14), despite a visually obvious separation of the Kaplan-Meier curves (**Figure 2**). This result did not change significantly after adjustment for clinical variables of interest in bivariable models (**Table 1C**). Even though the *EGFR* hazard ratio improved in a multivariable model with clinical variables, it did not attain significance, remaining a statistical trend (*EGFR* HR=0.319, 95% 0.09-1.11, P=0.073 **Table 1D**).

As noted above, the allelic frequency was not normally distributed. Even after log transformation, the martingale residuals showed a nonlinear fit against PFS in a Cox model (**Supplementary Figure 2** – martingale pfs). Nevertheless, when the log-transformed *EGFR* allelic frequency was used as a continuous variable, the association with PFS remained consistent (HR=0.589, 95% 0.35-0.99, P=0.0452). Again, there was no association with OS (HR=0.638, 95% 0.37-1.1, P=0.114). Both of these results should be considered exploratory.

The *EGFR* copy number can influence the allelic frequency of the mutation. Indeed, in our data, most tumors with high allelic frequency also had copy number gains (15/20), while tumors with low allelic frequency typically did not have such gains (2/9). This difference was significant (Fisher's test OR=9.51, P=0.014). Despite this observation, the presence of copy number gains in *EGFR* did not predict PFS (HR=0.578, 95% 0.24-1.41, P=0.229) or OS (HR=0.537, 95% 0.22-1.34, P=0.182).

By combining the presence of resistance co-mutations in other cancer-related genes with the allelic frequency of the *EGFR* mutation, we hypothesized that we would more accurately capture the driver status of *EGFR* and predict treatment response. Even though resistance co-mutations in other genes were more often associated with low *EGFR* mutation allelic frequency (3 of 5), the very small numbers preclude a statistically significant conclusion (Fisher's test OR

TABLE 1A | Bivariable PFS.

Clinical variable	Clinical variable coefficients			EGFR coefficients		
	HR	95% CI	P-value	HR	95% CI	P-value
Age over 65	0.291	0.102-0.830	0.021	0.134	0.037-0.488	0.002
Male sex	2.206	0.898-5.420	0.085	0.337	0.115-0.991	0.048
PS 1 (vs PS0)	0.594	0.195-1.802	0.357	0.300	0.102-0.885	0.029
Current smoker	1.119	0.354-3.533	0.848	0.263	0.087-0.798	0.018
Osimertinib (vs other)	0.402	0.146-1.107	0.078	0.257	0.090-0.738	0.012

TABLE 1B | Multivariable PFS.

Clinical variable	Clinical variable coefficients		
	HR	95 % CI	P-value
Age over 65	0.268	0.071-1.009	0.052
Male sex	1.732	0.657-4.566	0.267
PS 1 (vs PS0)	0.975	0.243-3.917	0.972
Current smoker	2.750	0.568-13.318	0.209
Osimertinib (vs other)	0.502	0.149-1.698	0.268
EGFR high AF	0.112	0.023-0.547	0.007

TABLE 1C | Bivariable OS.

Clinical variable	Clinical variable coefficients			EGFR coefficients		
	HR	95% CI	P-value	HR	95% CI	P-value
Age over 65	0.859	0.350-2.106	0.740	0.485	0.740-0.485	0.157
Male sex	2.695	1.069-6.795	0.036	0.482	0.036-0.482	0.154
PS 1 (vs PS0)	0.317	0.090-1.124	0.075	0.382	0.075-0.382	0.078
Current smoker	1.199	0.364-3.945	0.765	0.450	0.765-0.450	0.141
Osimertinib (vs other)	0.764	0.240-2.429	0.648	0.483	0.648-0.483	0.153

TABLE 1D | Multivariable OS.

Clinical variable	Clinical variable coefficients		
	HR	95% CI	P-value
Age over 65	0.664	0.212-2.084	0.483
Male sex	2.438	0.919-6.467	0.073
PS 1 (vs PS0)	0.295	0.074-1.185	0.085
Current smoker	2.426	0.519-11.348	0.260
Osimertinib (vs other)	0.993	0.275-3.584	0.992
EGFR high AF	0.319	0.092-1.110	0.073

A. A summary of bivariable Cox models of PFS including clinical variables (one for each row) and the EGFR allelic frequency as a binary variable (high versus low). B. A multivariable model of PFS including clinical variables and the EGFR allelic frequency as a binary variable (high versus low). C. A summary of bivariable COX models of OS including clinical variables (one in each row) and the EGFR allelic frequency as a binary variable (high versus low). D. A multivariable model of OS including clinical variables and the EGFR allelic frequency as a binary variable (high versus low).

4.7, P=0.131). In that sense, the information obtained from co-mutations and allelic frequency appears to be complementary. We therefore defined a "sensitive" tumor as one that did not harbor resistance co-mutations and in which the *EGFR* mutation had a high allelic frequency. Under that definition, 20 of 31 tumors were classified as sensitive (64%), compared with 88% when only the co-mutation was considered.

Sensitive tumors were associated with significantly longer PFS (HR 0.22, 95% 0.07-0.61, P=0.004) and OS (HR 0.35, 95% 0.13-0.90, P=0.029) (**Figures 3** and **4**). The association with both PFS and OS remained significant after adjustment for clinical variables in a series of bivariable models with age over 65, sex, performance status, smoking and osimertinib use upfront (**Table 2**). Furthermore, EGFR sensitivity remained significantly associated with PFS (HR=0.137, 95% 0.04-0.51, P=0.003) and OS (HR=0.196, 95% 0.06-0.69, P=0.011) in multivariable models including all the above variables. Of the other clinical variables, only male sex was predictive of shorter OS in the multivariable model (HR=2.707, 95% 1.00-7.31, P=0.049). Twelve-month PFS was 0% in the insensitive group, compared with 41% in the sensitive group. At one year, OS was 10% in the insensitive group, compared with 79% in the sensitive

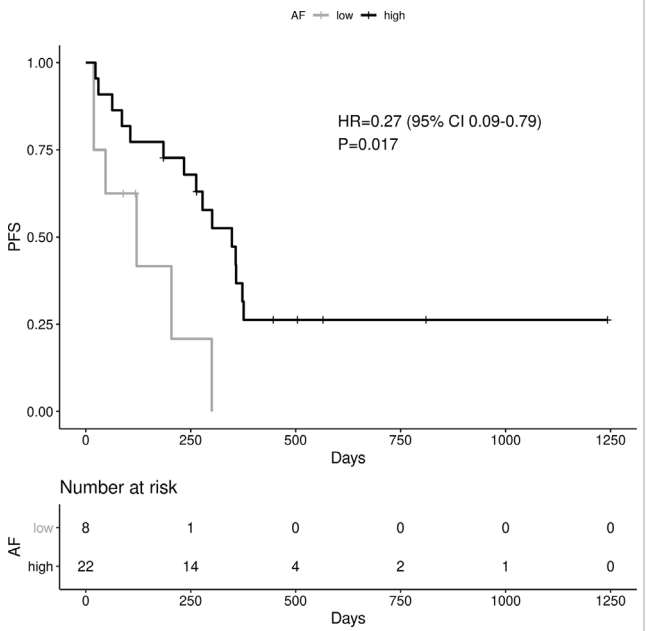

FIGURE 1 | Impact of EGFR allele frequency on PFS.

group. Patients with insensitive tumors were almost 10 times more likely to die before 12 months than patients with sensitive tumors (Fisher's test OR 9.7, P=0.007).

DISCUSSION

Biologically, it could be surmised that increased allelic frequency would be correlated with an increased probability of the variant being an oncogenic driver. There is not much literature assessing the impact of EGFR allelic frequency in NSCLC, and further complicating matters, available data are discordant. In addition, several studies are based on circulating tumor DNA and comparisons with tissue biopsies may not be always appropriate.

A retrospective analysis from the Shizuoka Lung Cancer Mutation Study found that among 705 enrolled patients, 102 lung adenocarcinoma patients carried the typical EGFR L858R exon 21 mutation (18). Forty-eight patients were assessed both by pyrosequencing, a non-electrophoretic real-time sequencing approach, and by outsourced polymerase chain reaction (PCR) laboratory tests. Among these patients, the median mutant allelic frequency was 18.5% (8% to 82%). Receiver operating characteristic curves found that a mutant allelic frequency of 9% resulted in 100% sensitivity and 99% specificity. The authors then used this cut-off to ass the impact of allelic frequency on survival in patients receiving TKI. The PFS among patients with a mutant allelic frequency ≤9% was 92 days, compared to 284 days for those with a frequency greater than 9% (p=0.0027), suggesting a predictive role of this variable. It should be noted that this study did not analyze allelic frequency among patients with EGFR exon 19 deletions, as this mutation was only detected qualitatively using fragment analysis in this initiative.

A further retrospective trial using digital droplet PCR based performed analyses on archived tissue from 233 lung cancer patients treated with first-generation EGFR TKIs (19). The results supported previous finding, as the authors found a correlation between mean allele frequency and clinical outcomes. Among patients with a partial response, the mean allele frequency was 48.6%, while it was 27.4% in patients with stable disease and 9.5% among those with progressive disease (p for partial response versus disease stability: 0.0078, partial response versus progressive disease: 0.000001, stable disease versus progressive disease 0.029).

The largest available dataset based on a prospective study is an unplanned retrospective analysis from the phase III CTONG 0901 trial, which compared the efficacy of erlotinib to gefitinib in advanced NSCLC harboring EGFR exon 19 or 21 mutations, measured by NGS. Among 194 patients with EGFR mutant NSCLC, the median mutant allelic frequency was 25.8%, with a range of 1.4% to 86.2%. Patients were divided into two groups, high mutant allelic frequency (25.8 to 86.2%) and low allelic frequency (1.4 to 25.8%). The authors evaluated ORR, PFS and OS. The first ORR did not differ between groups, at 56.2% and 57.5% in the high and low groups, respectively. Similarly, there was no difference in PFS, at 11.2 and 12.4 months ($P = 0.509$) nor OS at 20.5 and 23.1 months ($P = 0.500$), in the high and low allelic frequency groups, respectively (20).

Among patients receiving osimertinib in second-line with a T790M resistance mutation after failure of an earlier generation EGFR TKI, the maximum EGFR somatic allele frequency of EGFR variants measured in circulating tumor DNA does not appear to predict response rate or survival. However, the ratio of T790M allele frequency to maximum EGFR somatic allele frequency is highly predictive of ORR (p=0.002) and PFS (p=0.006) (21). A retrospective analysis on 54 patients mirrored these results (21). Both of these retrospective analyses are supported by a recent prospective trial on 34 patients who progressed on first or second-generation TKIs and developed T790M resistance mutations (22). After enrolment, there was a baseline plasma sample and patients started osimertinib. Cell-free DNA was analyzed by digital droplet PCR to calculate the mutant allele frequency. Patients with higher non-T790M mutant EGFR allele frequencies fared less well than those with lower frequencies, while higher T790M ratios provided superior PFS (6 months versus not reached, p=0.01). These studies highlight the potential predictive value of mutant allelic frequency in the second-line setting.

In our cohort of patients with EGFR-mutant NSCLC treated with targeted therapy in the front-line setting, the variant allelic frequency is associated with survival outcomes, whether they are receiving first or third generation TKIs. While our cohort is small in size, there is a clear PFS improvement and trend toward OS benefit among patients whose disease harbors a mutant allelic frequency greater than 0.3, or 30%. This appears to be an independent predictive factor for outcomes in our bivariable model.

TABLE 2A | Bivariable PFS.

Clinical Variable	Clinical variable coefficients			EGFR sensitive		
	HR	95% CI	P-value	EGFR HR	95% CI	P-value
Age over 65	0.415	0.171-1.005	0.051	0.174	0.059-0.509	0.001
Male sex	2.208	0.906-5.385	0.081	0.251	0.088-0.718	0.010
PS 1 (vs PS0)	0.671	0.217-2.076	0.488	0.237	0.081-0.698	0.009
Current smoker	1.298	0.398-4.237	0.666	0.200	0.066-0.606	0.004
Osimertinib (vs other)	0.453	0.164-1.251	0.127	0.229	0.081-0.646	0.005

TABLE 2B | Multivariable PFS.

Clinical variable	HR	95% CI	P-value
Age over 65	0.369	0.123-1.109	0.076
Male sex	2.404	0.923-6.264	0.073
PS 1 (vs PS0)	1.036	0.248-4.335	0.961
Current smoker	3.099	0.610-15.750	0.173
Osimertinib (vs other)	0.562	0.160-1.975	0.369
EGFR sensitive	0.137	0.037-0.507	0.003

TABLE 2C | Bivariable OS.

Clinical Variable	Clinical variable coefficients			EGFR sensitive		
	HR	95% CI	P-value	EGFR HR	95% CI	P-value
Age over 65	0.955	0.382-2.386	0.922	0.350	0.132-0.930	0.035
Male sex	2.806	1.105-7.123	0.030	0.335	0.128-0.879	0.026
PS 1 (vs PS0)	0.311	0.087-1.112	0.072	0.283	0.099-0.808	0.018
Current smoker	1.483	0.427-5.145	0.535	0.305	0.106-0.877	0.028
Osimertinib (vs other)	0.843	0.262-2.711	0.775	0.354	0.135-0.926	0.034

TABLE 2D | Multivariable OS.

Clinical variable	HR	95% CI	P-value
Age over 65	0.596	0.187-1.903	0.382
Male sex	2.707	1.003-7.305	0.049
PS 1 (vs PS0)	0.268	0.066-1.084	0.065
Current smoker	3.536	0.667-18.731	0.138
Osimertinib (vs other)	1.189	0.316-4.471	0.798
EGFR sensitive	0.196	0.055-0.693	0.011

(A) A summary of bivariable Cox models of PFS including clinical variables (one for each row) and the EGFR sensitivity as a binary variable (sensitive vs insensitive). (B) A multivariable Cox model of PFS including clinical variables (one for each row) and the EGFR sensitivity as a binary variable (sensitive vs insensitive). (C) A summary of bivariable COX models of OS including clinical variables (one in each row) and the EGFR sensitivity as a binary variable (sensitive vs insensitive). (D) A multivariable Cox model of OS including clinical variables (one for each row) and the EGFR sensitivity as a binary variable (sensitive vs insensitive).

Furthermore, after correcting for the presence of co-occurring pathogenic mutations, known to predict inferior outcomes in this population, the difference remains significant. Combining both predictive factors differentiates patients into very distinct prognostic groups. One could question the role of allelic frequency given the stronger predictive impact of resistance co-mutations; however, it is important to note that co-mutations are rare. By combining the two factors, we classify 64% of tumors as likely to be sensitive to EGFR TKIs, revealing 36% prone to respond less favorably to therapy. When we consider co-mutations alone, only 12% are classified as insensitive. The latter have a greater association with overall prognosis, but the former may have a more meaningful clinical impact, due to its wider applicability and role in predicting the efficacy of front-line TKIs. The combination of these two was consistently associated with both PFS and OS in univariable, bivariable and multivariable analyses and the magnitude of the effect was clinically very significant.

EGFR variant allelic frequency was driven by copy number status but did not correlate with the visually estimated tumor cellularity in our data. We believe that the allelic frequency also captures the proportion of cells carrying the mutation and is therefore able to separate tumors with a truncal mutation, which are more likely to respond favorably, from tumors with subclonal *EGFR* alterations, which are more likely resistant. In that sense, we feel that the *EGFR* variant allelic frequency can be a useful biomarker in the clinic.

The small sample size is a limitation of the interpretation of our results. Despite the sample size and the lack of

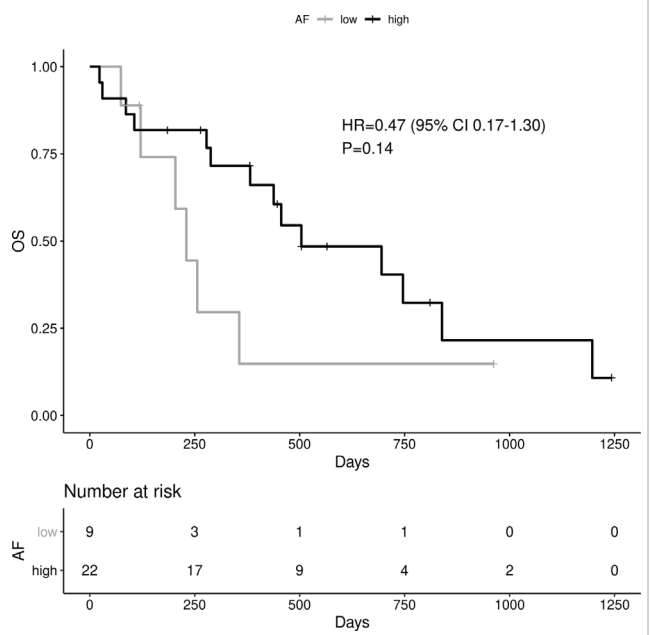

FIGURE 2 | Impact of EGFR allele frequency on OS.

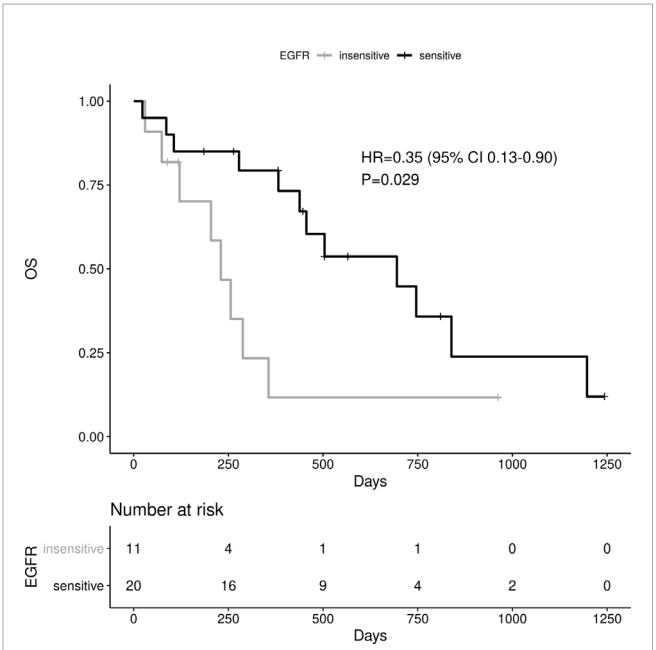

FIGURE 4 | Impact of tumor sensitivity on OS.

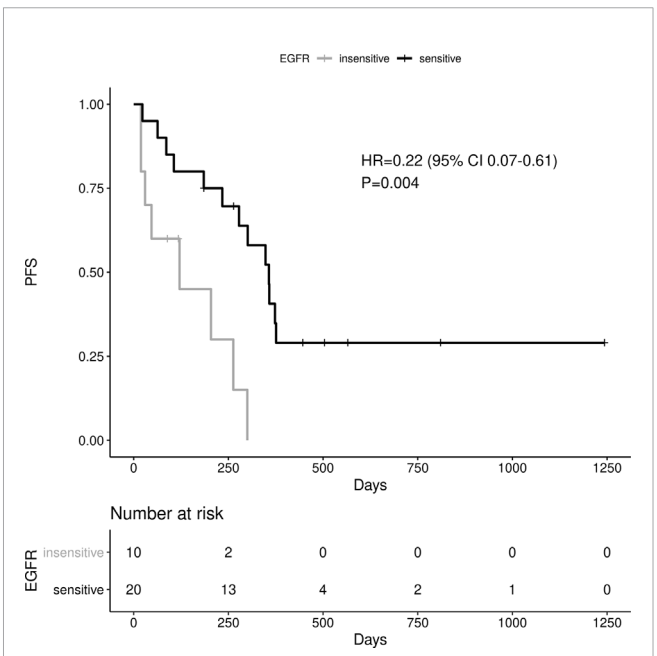

FIGURE 3 | Impact of tumor sensitivity on PFS.

randomization, the data appear balanced between groups, especially with respect to the use of osimertinib as a first line, which could be a potential confounder.

There is no well-established allelic frequency cut-off to classify EGFR mutations and no clear method for deriving the cut off. Our choice of cut-off is based on the visual estimation of tumor cellularity by an experienced molecular pathologist and the expectation that a homogeneous population of EGFR mutant cells would produce an allelic frequency of at least half the tumor cellularity, corresponding to one mutant allele out of two alleles (the other being normal). There are situations where this may not occur for other reasons, such as the amplification of the normal allele, but there is no selection pressure in favor of the normal allele. It is unclear whether this cut-off will translate to other cohorts, but our assumptions are general and not specific to our institution or the period of data collection.

Finally, we do not have TMB estimates for most of our patients. This could be relevant as high TMB is known to be associated with poor prognosis in patients whose cancer harbors an EGFR mutation (23).

CONCLUSION

The mutant allelic frequency of EGFR in NSCLC appears to be associated with clinical outcomes among patients treated with TKIs. In spite of our small cohort size, we note a clear PFS improvement in patients with a high EGFR allelic frequency compared to those with a low frequency. There is a clear trend toward improved OS, though it is not significant. This predictive biomarker is independent of the generation of TKIs used and of the presence of resistance co-mutations. Interestingly, by combining the latter with variant allelic frequency, we identify two clear prognostic groups, resistant and sensitive patients to TKIs. The complementary nature of these analyses and clinical implications of our results require further validation.

DATA AVAILABILITY STATEMENT

The data were obtained from the patient record of the university hospital of Geneva and are not public. We are willing to share the extracted, de-identified data upon reasonable request. The primary sources (documents from the digital patient record) cannot be released, due to the presence of identifying information.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by EC Geneva. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.644472/full#supplementary-material

Supplementary Figure 1 | *EGFR* allelic frequency distribution density plot.

Supplementary Figure 2 | Martingale PFS.

REFERENCES

1. https://www.who.int/news-room/fact-sheets/detail/cancer.
2. Bhullar KS, Lagarón NO, McGowan EM, Parmar I, Hubbard A, et al. Kinase-targeted cancer therapies: progress, challenges and future directions. *Mol Cancer* (2018) 17:48–8. doi: 10.1186/s12943-018-0804-2
3. Chan BA, Hughes BGM. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. *Trans Lung Cancer Res* (2015) 4:36–54. doi: 10.3978/j.issn.2218-6751.2014.05.01
4. Wee P, Wang Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. *Cancers* (2017) 9:52. doi: 10.3390/cancers9050052
5. Cheng YI, Gan YC, Liu D, Davies M, Li WM, Field JK, et al. Potential genetic modifiers for somatic EGFR mutation in lung cancer: a meta-analysis and literature review. *BMC Cancer* (2019) 19:1068–8. doi: 10.1186/s12885-019-6317-6
6. Shi Y, Au JS-K, Thongprasert S, Srinivasan S, Tsai C-M, Khoa MT, et al. A Prospective, Molecular Epidemiology Study of EGFR Mutations in Asian Patients with Advanced Non–Small-Cell Lung Cancer of Adenocarcinoma Histology (PIONEER). *J Thoracic Oncol* (2014) 9:154–62. doi: 10.1097/JTO.0000000000000033
7. Jordan EJ, Kim HR, Arcila ME, Barron D, Chakravarty D, Gao J, et al. Prospective Comprehensive Molecular Characterization of Lung Adenocarcinomas for Efficient Patient Matching to Approved and Emerging Therapies. *Cancer Discovery* (2017) 7:596–609. doi: 10.1158/2159-8290.CD-16-1337
8. Tian Y, Zhao J, Ren P, Wang B, Zhao C, Shi C, et al. Different subtypes of EGFR exon19 mutation can affect prognosis of patients with non-small cell lung adenocarcinoma. *PloS One* (2018) 13:e0201682–e0201682. doi: 10.1371/journal.pone.0201682
9. Wu Y-L, Planchard D, Lu S, Sun H, Yamamoto N, Kim D-W, et al. Pan-Asian adapted Clinical Practice Guidelines for the management of patients with metastatic non-small-cell lung cancer: a CSCO–ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS. *Ann Oncol* (2019) 30:171–210. doi: 10.1093/annonc/mdy554
10. Ramalingam S, Gray J, Ohe Y, Cho BC, Vansteenkiste J, Zhou C, et al. Osimertinib vs comparator EGFR-TKI as first-line treatment for EGFRm advanced NSCLC (FLAURA): Final overall survival analysis. *Ann Oncol* (2019) 30:v914–5. doi: 10.1093/annonc/mdz394.076
11. Legras A, Barritault M, Tallet A, Fabre E, Guyard A, Rance B, et al. Validity of Targeted Next-Generation Sequencing in Routine Care for Identifying Clinically Relevant Molecular Profiles in Non–Small-Cell Lung Cancer: Results of a 2-Year Experience on 1343 Samples. *J Mol Diagnostics* (2018) 20:550–64. doi: 10.1016/j.jmoldx.2018.04.002
12. Weinstein IB. Addiction to oncogenes–the Achilles heal of cancer. *Science* (2002) 297:63–4. doi: 10.1126/science.1073096
13. Nagahashi M, Sato S, Yuza K, Shimada Y, Ichikawa H, Watanabe S, et al. Common driver mutations and smoking history affect tumor mutation burden in lung adenocarcinoma. *J Surg Res* (2018) 230:181–5. doi: 10.1016/j.jss.2018.07.007
14. Alex F, Alfredo A. Promising predictors of checkpoint inhibitor response in NSCLC. *Expert Rev Anticancer Ther* (2020) 20:931–7. doi: 10.1080/14737140.2020.1816173
15. Chevallier M, Tsantoulis P, Addeo A, Friedlaender A. Influence of Concurrent Mutations on Overall Survival in EGFR-mutated Non-small Cell Lung Cancer. *Cancer Genomics Proteomics* (2020) 17:597–603. doi: 10.21873/cgp.20216
16. Spurr L, Li M, Alomran N, Zhang Q, Restrepo P, Movassagh M, et al. Systematic pan-cancer analysis of somatic allele frequency. *Sci Rep* (2018) 8:7735. doi: 10.1038/s41598-018-25462-0
17. Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, et al. Tracking the Evolution of Non-Small-Cell Lung Cancer. *N Engl J Med* (2017) 376:2109–21. doi: 10.1056/NEJMoa1616288
18. Ono A, Kenmotsu H, Watanabe M, Serizawa M, Mori K, Imai H, et al. Mutant allele frequency predicts the efficacy of EGFR-TKIs in lung adenocarcinoma harboring the L858R mutation. *Ann Oncol* (2014) 25:1948–53. doi: 10.1093/annonc/mdu251
19. Kim JH, Kim HS, Kim BJ. Prognostic value of KRAS mutation in advanced non-small-cell lung cancer treated with immune checkpoint inhibitors: A meta-analysis and review. *Oncotarget* (2017) 8:48248–52. doi: 10.18632/oncotarget.17594
20. Li X-M, Yang J-J, Wu Y-L. Association of allele frequency of EGFR mutation with efficacy of EGFR TKIs in advanced non-small cell lung cancer. *J Clin Oncol* (2019) 37:e20678–8. doi: 10.1200/JCO.2019.37.15_suppl.e20678
21. Zheng Q, Hong S, Huang Y, Zhao H, Yang Y, Hou X, et al. EGFR T790M relative mutation purity predicts osimertinib treatment efficacy in non-small cell lung cancer patients. *Clin Transl Med* (2020) 9:17. doi: 10.1186/s40169-020-0269-y
22. Del Re M, Bordi P, Rofi E, Restante G, Valleggi S, Minari R, et al. The amount of activating EGFR mutations in circulating cell-free DNA is a marker to monitor osimertinib response. *Br J Cancer* (2018) 119:1252–8. doi: 10.1038/s41416-018-0238-z
23. Offin M, Guo R, Wu SL, Sabari J, Land JD, Ni A, et al. Immunophenotype and Response to Immunotherapy of RET-Rearranged Lung Cancers. *JCO Precis Oncol* (2019) 3:1–8. doi: 10.1200/PO.18.00386

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CASE REPORT
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Lung Adenocarcinoma Patient Harboring *EGFR*-KDD Achieve Durable Response to Afatinib: A Case Report and Literature Review

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The rapid development of epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) has revolutionized the treatment of patients with advanced or metastatic non-small cell lung cancer (NSCLC) harboring *EGFR* mutations including but not limited to exon 19 deletions (19 del) and point mutation L858R in exon 21. However, the efficacy of EGFR-TKIs in patients with rare mutations, such as *EGFR*-kinase domain duplication (KDD), remains elusive. *EGFR*-KDD often results from in-frame tandem duplication of *EGFR* exons 18–25, causing subsequent constitutive activation of EGFR signaling. Several case reports have revealed the efficacies of EGFR-TKIs in advanced lung adenocarcinoma (LUAD) with *EGFR*-KDD but yielded variable antitumor responses. In the present study, we report a 61-year-old male patient diagnosed with T1N3M0 (stage IIIB) LUAD harboring *EGFR*-KDD involving exons 18–25. He was treated with afatinib and achieved partial response (PR) with progression-free survival (PFS) of 12 months and counting. Our work, confirming *EGFR*-KDD as an oncogenic driver and therapeutic target, provides clinical evidence to administer EGFR-TKIs in patients with advanced LUAD harboring *EGFR*-KDD.

Keywords: afatinib, EGFR-KDD, lung adenocarcinoma, next-generation sequencing, tyrosine kinase inhibitor

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INTRODUCTION

Lung cancer as the leading cause of cancer-related mortality worldwide accounts for almost one-quarter of all cancers (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases, including two major histological subtypes, adenocarcinoma and squamous cell carcinoma (2). With the advancements of sequencing technologies, it has been well-known that the initiation and development of some NSCLCs, especially lung adenocarcinoma (LUAD), are commonly driven by specific genetic alterations in oncogenes leading to abnormal proteins that can be targeted (2). The discovery of actionable mutations in NSCLC has changed the treatment paradigm from cytotoxic chemotherapy to molecular-targeted therapy.

Epidermal growth factor receptor (*EGFR*) is the most common driver oncogene in NSCLC especially in Asian patients (3, 4). Both exon 19 deletions (19 del) and the point mutation L858R at exon 21 are common mutations, accounting for more than 85% of *EGFR*-mutant NSCLC,

which predict sensitivity to EGFR-tyrosine kinase inhibitors (TKIs) (5). Uncommon mutations such as *EGFR* T790M mutation and exon 20 insertions have been documented to predict resistance to EGFR-TKI. However, whether patients harboring other uncommon mutations accounting for about 10% of all *EGFR* mutations obtain clinical benefit from EGFR-TKI is seldom investigated because in the majority of clinical trials investigating the efficacy of EGFR-TKIs, only patients with sensitizing *EGFR* mutations, 19 del, and L858R, are included (6, 7). Here, we presented a patient with metastatic NSCLC harboring *EGFR*-kinase domain duplication (KDD) who derived durable response to first-line treatment of second-generation EGFR-TKI afatinib with a progression-free survival (PFS) of 12 months and counting.

CASE PRESENTATION

A 61-year-old man with a smoking index of 600 (15 cigarettes/day for 40 years) presented with a cough for 2 weeks. He had no family history of cancer. Chest CT scans revealed a mass located in the lower lobe of the left lung, and a CT-guided percutaneous lung biopsy revealed LUAD. In addition, the ultrasound revealed the enlargement of the left and right supraclavicular lymph nodes (SCLNs). Subsequently, an ultrasound-guided percutaneous biopsy of the right SCLNs was performed and the patient was diagnosed with T1N3M0 (IIIB) LUAD in August 2019. The patient had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 1. Immunohistochemistry testing (IHC 22C3) for programmed death ligand-1 (PD-L1) was

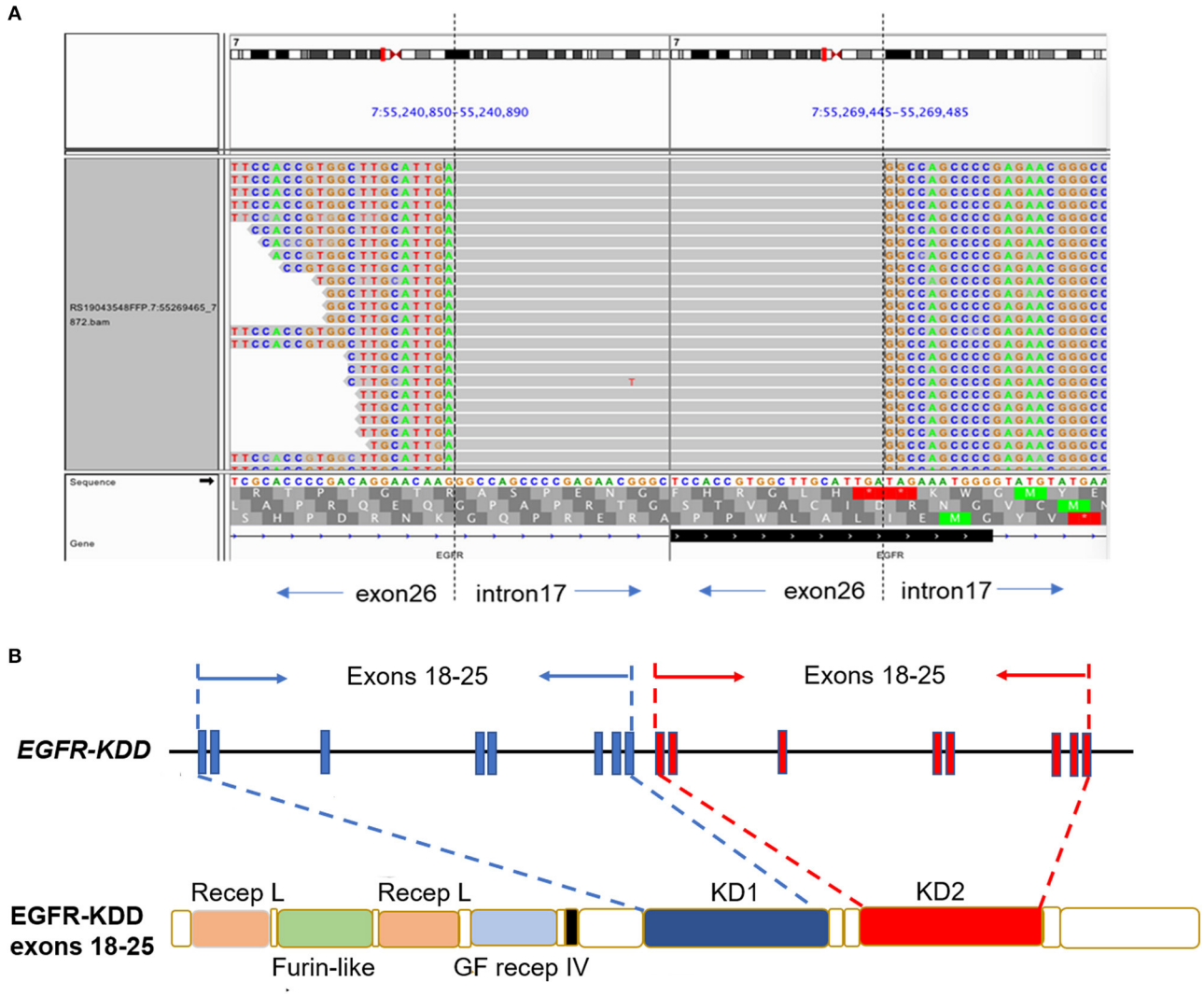

FIGURE 1 | The LUAD patient harboring canonical *EGFR*-KDD involving exons 18–25. **(A)** Visualization of canonical *EGFR*-KDD using the Integrative Genomics Viewer (IGV) browser. The dashed lines indicate the genomic breakpoints. **(B)** The genetic and protein domain structures of *EGFR*-KDD. *EGFR*/EGFR, epidermal growth factor receptor; KDD, kinase domain duplication; LUAD, lung adenocarcinoma; Recep L, Receptor L domain; Furin-like, Furin-like cysteine rich region; GF recep IV, Growth factor receptor domain IV; KD, tyrosine kinase domain.

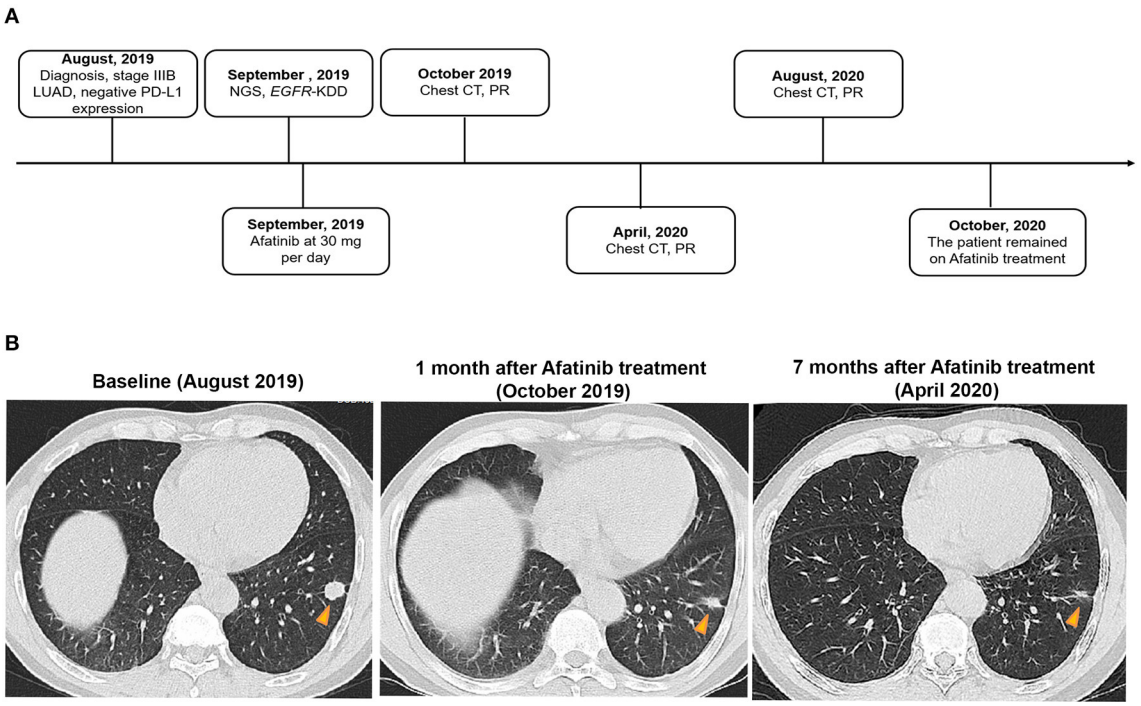

FIGURE 2 | A summary of the treatment procedure of the patient. **(A)** The entire treatment procedure. LUAD, lung adenocarcinoma; PR, partial response; NGS, next-generation sequencing; *EGFR*, human epidermal growth factor receptor 2; KDD, kinase domain duplication; CT, computed tomography; PD-L1, programmed death ligand-1. **(B)** Chest CT imaging of the LUAD patient prior to afatinib treatment, 1 month after the treatment, and 7 months after the treatment. The yellow arrows indicate the lesions. LUAD, lung adenocarcinoma; CT, computed tomography.

performed on the primary tumor biopsy. IHC analyses revealed the patient was negative for PD-L1 expression with membranous expression of PD-L1 on <1% of tumor cells. Capture-based targeted sequencing (Burning Rock Biotech, Guangzhou, China) was performed on the primary tumor sample, which revealed canonical *EGFR*-KDD involving exons 18–25 (**Figure 1**) and negative for well-known actionable alterations occurring in *EGFR*. He refused to receive chemotherapy and was administered with afatinib (30 mg per day, orally) in September 2019. **Figure 2A** illustrated the treatment procedure of the patient. The patient achieved partial response (PR) with significant shrinkage of the tumor, from a diameter of 17 mm to 11 mm 1 month after afatinib treatment and it further reduced to 6 mm at 7 months (**Figure 2B**). The ECOG PS of the patient decreased to 0 after 3 months of afatinib treatment. In August 2020, chest CT still showed a PR after 11 months of afatinib treatment. Afatinib was well-tolerated, with grade 1 rash that did not require medical treatment for control and grade 2 diarrhea (3–4 times per day) that disappeared after symptomatic treatment for 2 months. There were no treatment-related adverse events leading to discontinuation. As of the submission of this manuscript, the patient still remained on the treatment, with a PFS of 12 months and counting. The patient was satisfied with the effect of EGFR-TKI afatinib treatment.

We also reviewed the previously reported six cases harboring *EGFR*-KDD, of which three cases showed PR to first- or second-generation EGFR-TKI treatment. The clinical characteristics and outcomes of patients with NSCLC harboring *EGFR*-KDD before TKI treatment in our and previous studies are summarized in **Table 1**.

DISCUSSION

This study presents the clinical evidence of a patient with *EGFR*-KDD driven metastatic LUAD benefiting from afatinib as the first-line treatment with a PFS of 12 months and counting. To the best of our knowledge, this is the longest PFS among all reported studies.

Epidermal growth factor receptor- kinase domain duplication as the rare alteration is identified only in 0.12% (12/10,759) of all NSCLCs and 0.24% of all *EGFR*-mutant patients in East Asian population (10). KDD is a special type of large genomic rearrangements occurring in the kinase domain of protein kinase genes, which results in a novel mechanism for protein kinase activation in tumor cells. *EGFR*-KDD as the most well-studied KDD often results from in-frame tandem duplication of *EGFR* exon 18–25, causing subsequent constitutive activation of EGFR signaling (8). Preclinical data demonstrate that *EGFR*-KDD confers constitutive activity to the EGFR tyrosine kinase and sensitivity to EGFR-TKIs including erlotinib, afatinib, and osimertinib (8). Several instances of clinical evidence have revealed the efficacies of EGFR-TKIs in advanced LUAD but yielded variable antitumor responses (8–11). The first case of

TABLE 1 | Clinical characteristics and outcomes of patients with NSCLC harboring *EGFR*-KDD in our and previous studies.

Pt No.	Publication	Year of publication	Age	Gender/ Ethnicity	Diagnosis/ Stage	EGFR-TKI Treatment/line No.	Concurrent mutations	Response to TKI	PFS
1	Gallant et al. (8)	2015	33	Male/American	LUAD/IV	Afatinib/2nd line	None	PR	7 cycles of therapy
2	Zhu et al. (9)	2018	63	Female/Chinese	LUAD/IV	Icotinib/1st line	NA	SD	11 months, NR
3	Wang et al. (10)	2019	61	Male/Chinese	LUAD/IV	Erlotinib/2nd line Osimertinib 3rd line	*TP53* R280G	PD PD	2 months 2 months
4	Wang et al. (10)	2019	63	Male/Chinese	LUAD/IV	Gefitinib/1st line Afatinib/2nd line Osimertinib/3rd line	*ERBB2* amp	PR PD PR	5 months 2 months 4 months, NR
5	Wang et al. (10)	2019	67	Male/Chinese	LUAD/IV	Icotinib/2nd line	*TP53* Y220C *PIK3CA* E81G	PR	4 months, NR
6	Chen et al. (11)	2020	59	Male/Chinese	LUAD/IV	Afatinib/1st line	*TP53* R282W *CTNNB1* S37Y	SD	10 months, NR
7	Our study		61	Male/Chinese	LUAD/IIIB	Afatinib/1st line	None	PR	12 months, NR

EGFR, epidermal growth factor receptor; KDD, kinase domain duplication; NSCLC, non-small cell lung cancer; PD, progressive disease; PR, partial response; SD, stable disease; NA, not available; NR, not reached; Pt, patient; No., number; LUAD, lung adenocarcinoma; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

EGFR-KDD in LUAD is reported by Gallant et al. where a 33-year-old male smoker diagnosed with stage IV LUAD did not carry any common *EGFR* mutations but achieved PR after being treated with afatinib (8). Stable disease (SD) response to afatinib was observed in a 59-year-old patient with LUAD (11). Furthermore, studies have also documented that patients with *EGFR*-KDD-positive LUAD showed response to first-generation EGFR-TKI icotinib and third-generation EGFR-TKI osimertinib (9, 10). Baik et al. reported a female patient with bronchoalveolar carcinoma achieved a durable response to gefitinib and erlotinib, and *EGFR*-KDD was detected in the advanced stage (12), we cannot determine whether this *EGFR*-KDD is a primary or a secondary alteration after TKI treatment. However, in contrast, an *EGFR*-KDD-positive LUAD patient refractory to EGFR-TKIs has also been reported (10), a 61-year-old male patient harboring *EGFR*-KDD of exon 18–25 concurrent with *TP53* R280G mutation progressed shortly after undergoing therapies of erlotinib and osimertinib for only 2 months. Among seven cases harboring *EGFR*-KDD (including the current case), four cases achieved PR response to first- or second-TKIs treatment, and reached an objective response rate (ORR) of 57%. More evidence or clinical trials are needed to evaluate the efficacy of EGFR-TKIs in patients with LUAD harboring *EGFR*-KDD.

Previous studies have revealed that afatinib-related adverse events occur in more than 97% of patients with NSCLC (6, 7). Diarrhea (88.3%) and rash (80.8%) are the most common adverse events (7). In the present study, grade 1 rash and grade 2 diarrhea were reported by the patient. This patient had good adherence to the afatinib treatment. The rash did not require medical treatment for control. The diarrhea disappeared after symptomatic treatment for 2 months. Afatinib was well-tolerated and effective in the patient with advanced LUAD harboring *EGFR*-KDD.

A few limitations are associated with our study. Due to the nature of research, only one patient was incorporated into the work. Large cohort studies or clinical trials should be launched to verify the efficacy and safety of afatinib as the first-line treatment for patients with advanced LUAD harboring *EGFR*-KDD.

Here, we reported a patient who was *EGFR*-KDD-positive and showed durable response to EGFR-TKI therapy, thereby confirming *EGFR*-KDD is an oncogenic driver and a therapeutic target. Our work provided clinical evidence to administer EGFR-TKI in advanced NSCLC patients harboring *EGFR*-KDD and paving the way for its potential clinical utilization.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethic Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

PW designed the study and wrote the manuscript. LZ and ZW involved in diagnosing, treating, providing follow-up for the patient, and collecting data for this report. HD and SC conducted the genetic test and analysis. All authors read and approved the final version of the manuscript for submission.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* (2020) 70:7–30. doi: 10.3322/caac.21590
2. Gridelli C, Rossi A, Carbone DP, Guarize J, Karachaliou N, Mok T, et al. Non-small-cell lung cancer. *Nat Rev Dis Primers.* (2015) 1:15009. doi: 10.1038/nrdp.2015.9
3. Douillard JY, Ostoros G, Cobo M, Ciuleanu T, McCormack R, Webster A, et al. First-line gefitinib in Caucasian EGFR mutation-positive NSCLC patients: a phase-IV, open-label, single-arm study. *Br J Cancer.* (2014) 110:55–62. doi: 10.1038/bjc.2013.721
4. Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol.* (2014) 9:154–62. doi: 10.1097/JTO.0000000000000033
5. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med.* (2009) 361:958–67. doi: 10.1056/NEJMoa0904554
6. Sequist LV, Yang JCH, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol.* (2013) 31:3327–34. doi: 10.1200/JCO.2012.44.2806
7. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Onco.* (2014) 15:213–22. doi: 10.1016/S1470-2045(13)70604-1
8. Gallant JN, Sheehan JH, Shaver TM, Bailey M, Lipson D, Chandramohan R, et al. EGFR kinase domain duplication (EGFR-KDD) is a novel oncogenic driver in lung cancer that is clinically responsive to afatinib. *Cancer Discov.* (2015) 5:1155–63. doi: 10.1158/2159-8290.CD-15-0654
9. Zhu YC, Wang WX, Xu CW, Tan QH, Li JY, Zhuang W, et al. Lung adenocarcinoma patient with an EGFR kinase domain duplication (KDD) and the response to icotinib. *J Thorac Dis.* (2018) 10:E359–63. doi: 10.21037/jtd.2018.04.162
10. Wang J, Li X, Xue X, Ou Q, Wu X, Liang Y, et al. Clinical outcomes of EGFR kinase domain duplication to targeted therapies in NSCLC. *Int J Cancer.* (2019) 144:2677–82. doi: 10.1002/ijc.31895
11. Chen D, Li XL, Wu B, Zheng XB, Wang WX, Chen HF, et al. A novel oncogenic driver in a lung adenocarcinoma patient harboring an EGFR-KDD and response to afatinib. *Front Oncol.* (2020) 10:867. doi: 10.3389/fonc.2020.00867
12. Baik CS, Wu D, Smith C, Martins RG, Pritchard CC. Durable response to tyrosine kinase inhibitor therapy in a lung cancer patient harboring epidermal growth factor receptor tandem kinase domain duplication. *J Thorac Oncol.* (2015) 10:e97–9. doi: 10.1097/JTO.0000000000000586

Conflict of Interest: HD and SC were employed by Burning Rock Biotech.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
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First-Generation EGFR-TKI Plus Chemotherapy Versus EGFR-TKI Alone as First-Line Treatment in Advanced NSCLC With EGFR Activating Mutation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Objective: The aim of this meta-analysis was to evaluate efficacy and toxicity of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) in combination with chemotherapy (CT) compared to EGFR-TKI monotherapy as first-line treatment in advanced non-small cell lung cancer (NSCLC) harboring activating EGFR mutation.

Methods: A systematic literature search of randomized controlled trials using Cochrane Library, PubMed, Embase, and Web of Science, was performed up to Jan. 7th, 2020. Hazard ratios (HRs) with 95% confidence intervals (CI) were calculated as effect values for progress-free survival (PFS) and overall survival (OS). Risk ratio (RR) and Odds ratio (OR) were calculated as effect values for objective response rate (ORR) and toxicity, respectively.

Results: A total of eight randomized trials involving 1,349 advanced NSCLC patients with sensitive EGFR mutation were included in the meta-analysis. All patients in both groups received first-generation TKI as first-line treatment. The pooled HR of PFS and OS was 0.56 (95% CI = 0.50–0.64; P <0.00001) and 0.70 (95% CI = 0.54–0.90; P = 0.005), respectively. Subgroup analysis showed significantly higher OS advantages in patients receiving doublet CT (P = 0.02) and concurrent therapy (P = 0.002). The ORR in the EGFR-TKI plus CT group was significantly higher than in the EGFR-TKI monotherapy group (RR = 1.18, 95% CI = 1.10–1.26). The combination regimen showed a higher incidence of chemotherapy-induced toxicities. Subgroup analysis indicated that doublet chemotherapy rather than single-agent chemotherapy significantly increased incidence of grade 3 or higher leukopenia, neutropenia and anemia.

Conclusions: Compared with EGFR-TKI monotherapy, the combination of first-generation EGFR-TKI and CT, especially when applying concurrent delivery of platinum-based doublet chemotherapeutic drugs, significantly improve ORR and prolong PFS and

OS in first-line treatment for advanced EGFR-mutated NSCLC. Although increasing incidence of chemotherapy-induced toxicities occurs in the combination group, it is well tolerated and clinically manageable.

Keywords: EGFR-TKI, chemotherapy, first-line, advanced, NSCLC, mutation

INTRODUCTION

Lung cancer is the leading cause of cancer morbidity and mortality worldwide, with 2.1 million new cases and 1.8 million deaths estimated in 2018 (1). Non-small cell lung cancer (NSCLC) accounts for nearly 85% of all cases of lung cancer. Due to ineffective screening method and insidious symptom, lung cancer is usually diagnosed at an advanced stage in a majority of patients. Systematic therapy, therefore, remains the pivotal treatment approach for NSCLC in clinical practice.

Epidermal growth factor receptor (EGFR) is one of the most significant driver genes in lung cancer and its mutated form tempts constitutive activation of the EGFR tyrosine kinase, leading to uncontrolled growth and proliferation of tumor. Approximately, 10–15% of NSCLC patients in Europe and 30–35% of NSCLC patients in Asia harbor activating EGFR mutation (2, 3). An Individual Patient Data Meta-Analysis of six large randomized controlled trials (RCTs) suggested that compared with chemotherapy, first-line EGFR tyrosine kinase inhibitor (TKI) significantly prolonged progression-free survival (PFS) (median PFS = 11.0 vs. 5.6 months; Hazard Ratio (HR) = 0.37, 95% confidence intervals (CI) = 0.32–0.42, P <0.001) in EGFR-mutated NSCLC patients (4). Thus, first-line EGFR-TKI monotherapy, including representative gefitinib and erlotinib, is currently the mainstay treatment for naive advanced EGFR mutation positive NSCLC patients (5).

Inevitably, most patients who initially respond to an EGFR-TKI over 8–12 months, eventually develop resistance to first- or second-generation drugs (6). In order to prolong the survival \outcome, combination therapy of EGFR-TKI with other therapeutic drugs is an emerging promising approach. As one of promising combined strategy, EGFR-TKI plus chemotherapy has long been evaluated to overcome or delay resistance in advanced NSCLC since the early 2000s (7). Due to lack of EGFR-mutation status selection, however, preliminary studies failed to demonstrate the survival benefit of EGFR-TKI in combination with chemotherapy (8, 9). Recently, many phase II-III RCTs have investigated the EGFR-TKI plus chemotherapy combination in selected NSCLC patient with activating EGFR mutation (10). These studies with EGFR sensitive mutation had mixed overall survival (OS) results. Meta-analysis assessing the efficacy and toxicity of EGFR-TKI in combination with chemotherapy as first-line treatment in advanced NSCLC with EGFR activating mutation, has not yet been reported to our best knowledge. Therefore, we synthesized the results of different studies in this meta-analysis, to provide more objective data for the optimal clinical use of EGFR-TKI combined with chemotherapy.

MATERIAL AND METHODS

Search Strategy

Our study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (11). A comprehensive search of PubMed, Embase, Web of Science, and Cochrane databases was conducted to identify all relevant full-length literatures on the comparison of EGFR-TKI plus chemotherapy to EGFR-TKI alone as first-line treatment in advanced non-small cell lung cancer with activating EGFR mutation, up till Jan. 7th, 2020. Keywords including non-small-cell lung cancer, EGFR, TKI, and chemotherapy were used for initial search of eligible literatures. For instance, the following retrieval strategy was used on PubMed: (lung cancer OR lung carcinoma OR lung neoplasm) AND (epidermal growth factor receptor OR EGFR) AND (tyrosine kinase inhibitor OR TKI OR gefitinib OR erlotinib OR icotinib OR afatinib OR dacomitinib OR osimertinib) AND (chemotherapy OR pemetrexed OR gemcitabine OR paclitaxel OR vinorelbine) AND (first line OR untreat* OR naive). To obtain additional related articles, references cited in the eligible studies were also searched manually.

Selection Criteria

Inclusion criteria were as follows: (1) the patients were histologically diagnosed with advanced NSCLC; (2) the randomized trials were performed to evaluate the efficacy and safety of compared the EGFR-TKI plus chemotherapy to EGFR-TKI alone as first-line treatment in advanced non-small cell lung cancer with activating EGFR mutation; (3) the studies with affluent data for pooling the survival results, response rate, and toxicity. Exclusion criteria were as follows: (a) nonoriginal research articles with limited data, such as letters, case reports, reviews, comments, and conference abstracts; (b) duplicates of previous publications; and (c) studies with a sample size of less than 30 analyzable lesions.

Data Extraction and Quality Assessment

Basic information of each individual study was extracted by two reviewers (QW and WXL) independently. Any discrepancies were resolved by discussion and consensus during the process of research selection and data extraction or by consulting the third investigator (FX) when necessary. The following information was extracted: name of first author, trial name, publication year, trial phase, treatment arms, participants' characteristics, number of patients evaluable for analysis and other clinical characteristics. The primary data for calculation were the HR with 95% CI for PFS and OS, the number of patients who experienced a partial response or complete response, the number of patients that developed all grade toxicities.

A specific tool recommended by the Cochrane Collaboration was applied to assess the risk of bias for each identified study. Biases were categorized as selection bias, performance bias, detection bias, attrition bias, and reporting bias (12).

Statistical Analysis

All statistical analysis was performed using the Review Manager 5.3 software (Cochrane Library, Oxford, UK) and STATA 12.0 software (Stata Corp., College Station, TX). Cochrane's Q statistic and I^2 (I^2 >50% was considered substantially heterogeneous) statistic test were used to evaluate the heterogeneity between the eligible studies (13). The random effect model was used when there was significant heterogeneity between studies; otherwise, the fixed effect model was used. Publication bias was assessed *via* funnel plot with Begg's rank correlation. A two-sided p-value of <0.05 was considered to be statistically significant.

RESULTS

Study Selection

A comprehensive literature search yielded 1,732 non-duplicate papers. Of these, 21 full-text articles were screened for assessment of eligibility in the review. Finally, eight studies comparing EGFR-TKI plus chemotherapy with EGFR-TKI alone as first-line treatment in advanced non-small cell lung cancer with activating EGFR mutation were included in this meta-analysis (8, 14–20). The detail excluded studies from the 13 potential literatures was summarized in the supplementary selection of study. The flow diagram of studies selection was summarized in **Figure 1**.

Characteristics of Eligible Studies

From eight clinical trials, a total of 1,349 advanced NSCLC patients with sensitive EGFR mutation (704 in EGFR-TKI combination group and 645 in EGFR-TKI monotherapy group), were available for the meta-analysis. The great majority of histological type was adenocarcinoma. Of these EGFR-mutated patients, exon 19 deletion and L858R point mutation accounted for 55.7% (751/1,349) and 40.9% (552/1,349), respectively. As for first-line EGFR-TKI treatment, patients in all trials received first-generation drugs, including gefitinib (six studies), erlotinib (one study) and icotinib (one study). Most of trials involved platinum-based doublet chemotherapy, apart from two trials (8, 16). In addition, concurrent drug delivery of TKI and chemotherapy were engaged in four of these studies, three studies were intercalated, and one study was sequential. The characteristics of the included studies are listed in **Table 1**.

Progression-Free Survival

The median PFS as the primary end point of the studies ranged from 7.2 to 20.9 months in the EGFR-TKI combination arm and ranged from 4.7 to 16.6 months in EGFR-TKI monotherapy arm. The heterogeneity was not significant (I^2 = 11%; P = 0.34), and hence a fixed-effects model was used to pool the data (**Figure 2A**). The pooled HR of PFS in total population with activating EGFR mutation was 0.56 (95% CI = 0.50–0.64; P <0.00001; **Figure 2A**), which indicated that EGFR-TKI combination therapy significantly reduced the risk of disease progression compared with EGFR-TKIs alone. Furthermore, the pooled HR of PFS in patients with Exon 19 deletion or L858R point mutation was 0.54 (95% CI = 0.45–0.65; P <0.00001; **Figure 2B**) and 0.52 (95% CI = 0.42–0.65; P <0.00001; **Figure 2C**), respectively, retrieved from five included studies.

Subgroup analysis of chemotherapy drugs revealed that double-agents mighty induced longer PFS (double-agents, HR = 0.54, 95% CI = 0.47–0.62; single-agent, HR = 0.66, 95% CI = 0.50–0.87; **Figure 3A**). Moreover, subgroup analysis of combination timing indicated statistically significant PFS in concurrent and intercalated therapy (HR = 0.55, 95% CI = 0.47–0.64 and HR = 0.57, 95% CI = 0.45–0.73, respectively; **Figure 3A**), but was not statistically significant in sequential therapy (HR = 0.83; 95% CI = 0.42–1.63; **Figure 3A**).

Overall Survival

The median OS in the included studies ranged from 18.5 to 50.9 months in the EGFR-TKI combination arm and ranged from 14.2 to 45.7 months in EGFR-TKI monotherapy arm. The pooled HR of OS in total EGFR sensitive mutation sites between two arms was 0.70 (95% CI = 0.54–0.90; P = 0.005; **Figure 4A**), which indicated that combination therapy significantly improved the OS compared with EGFR-TKIs alone. Furthermore, the pooled HR of OS in patients with exon 19 deletion or L858R point mutation was 0.60 (95% CI = 0.42–0.86; P = 0.005; **Figure 4B**) and 0.82 (95% CI = 0.57–1.18; P = 0.28), respectively, retrieved from two trials. It revealed that overall survival benefit from EGFR-TKI in combination with chemotherapy might occur in patients with positive 19 deletion mutation other than in L858R mutation.

Subgroup analysis of chemotherapy drugs revealed that double-agents significantly improved PFS (double-agents, HR = 0.67, 95% CI = 0.48–0.94; single-agent, HR = 0.80, 95% CI = 0.56–1.14; **Figure 3B**). In addition, subgroup analysis of combination timing indicated statistically significant OS in concurrent therapy (HR = 0.65, 95% CI = 0.49–0.86; **Figure 3B**), but was not statistically significant in intercalated and sequential therapy (HR = 0.64, 95% CI = 0.39–1.07 and HR = 1.57, 95% CI = 0.72–3.41, respectively; **Figure 3B**).

Objective Response Rate

All of eight studies reported the data of objective response rate (ORR). The heterogeneity was not significant (I^2 = 0%; P = 0.66), and hence a fixed-effects model was used to pool the data (**Figure 5**). The meta-analysis demonstrated that pooled ORR in the EGFR-TKI plus chemotherapy group was significantly higher than in the EGFR-TKI monotherapy group (RR = 1.18, 95% CI = 1.10–1.26; p <0.00001; **Figure 5**).

Toxicities

Compared with the EGFR-TKI alone, the addition of chemotherapy to TKI was associated with a higher incidence

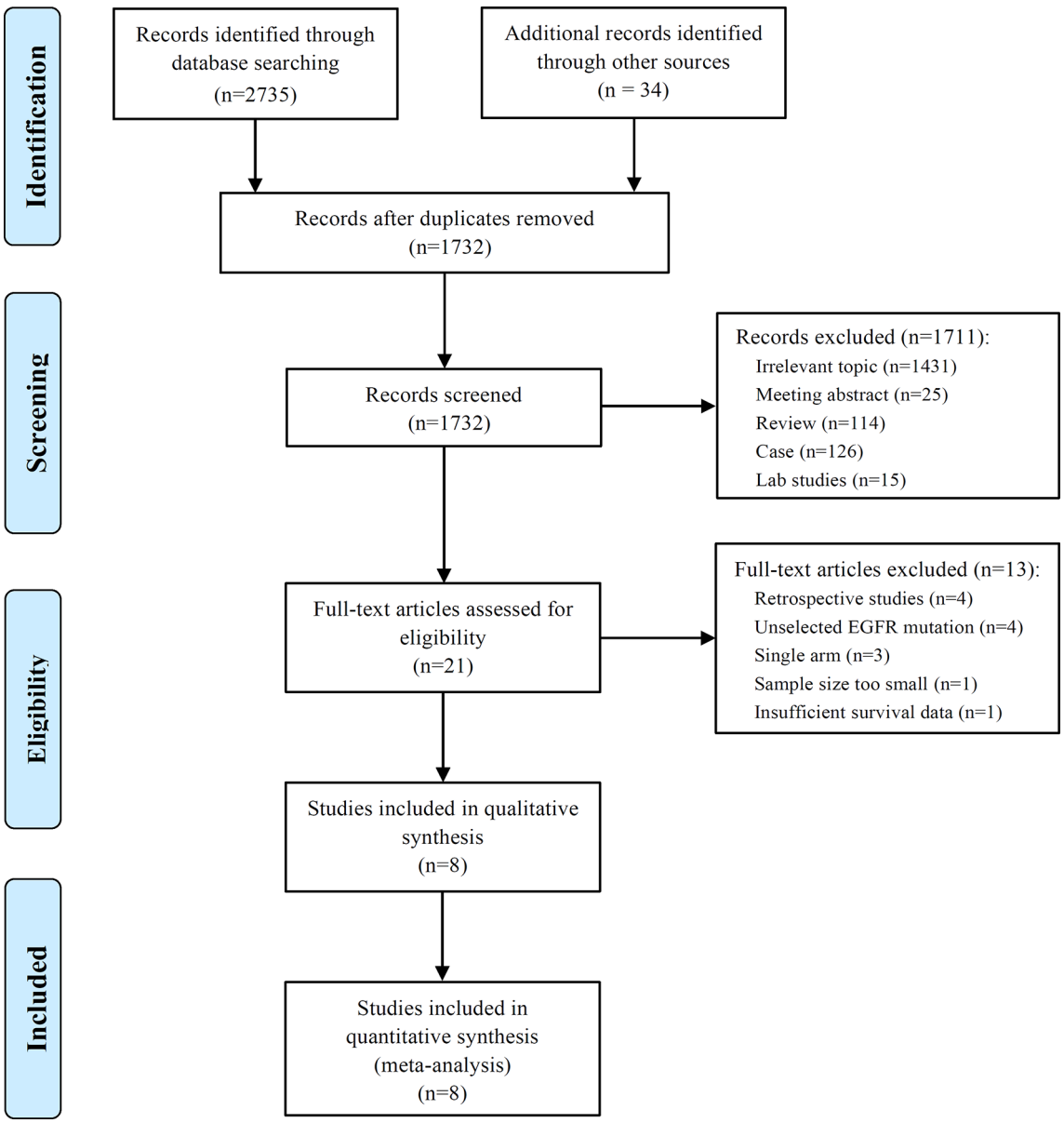

FIGURE 1 | The flow diagram of studies selection.

of any grade hematologic toxicities, such as neutropenia (grades 1–2, OR = 16.84, 95% CI = 9.04–31.36; grade 3 or higher, OR = 10.03, 95% CI = 1.04–96.69) and thrombocytopenia (grades 1–2, OR = 7.04, 95% CI = 4.73–10.48; grade 3 or higher, OR = 43.41, 95% CI = 6.01–313.71). Similarly, the combination therapy significantly increased the incidence of chemotherapy-induced toxicities, including any grade fatigue, anorexia, nausea and vomiting, and grade 3 or higher diarrhea. Subgroup analysis indicated that doublet chemotherapy significantly increased incidence of grade 3 or higher leukopenia (OR = 37.30, 95% CI = 7.26–191.63, **Figure 6A**), neutropenia (OR = 54.79, 95% CI = 13.21–227.24, **Figure 6B**) and anemia (OR = 14.28, 95% CI = 6.10–33.43, **Figure 6C**), while those differences were not significant in single-agent chemotherapy subgroup.

Nevertheless, no significant differences were founded in terms of any grade rash and grade 3 or higher liver dysfunction when applying combined treatment. The detail results are illustrated in **Table 2**.

Risk of Bias and Publication Bias

As defined by the Cochrane's manual for systematic reviews, all of included studies had a low risk of bias (**Figure S1**). In addition, no publication bias for PFS and OS was found based on Begg's test (P = 0.05 and P = 0.39, respectively; **Figure S2**).

TABLE 1 | Characteristics of the included randomized trials in the meta-analysis.

Study	Year	Country	Phase	Group	Type of combination	No. of evaluable patients	Medianage (years)	No. of EGFR mutation		Adenocarcinoma (%)	Efficacy		
								19 del	L858R		ORR	PFS	OS
CALGB 30406	2012	USA	II	Paclitaxel plus carboplatin+ E	Concurrent	33	60	16	17	84	73%	17.2 m	38.1 m
				E		33	58	23	10	88	70%	14.1 m	31.3 m
Yang et al. (15)	2014	East Asia	III	Pemetrexed plus cisplatin +G	Sequential	26	59	14	10	97	65.4%	12.9 m	32.4 m
				G		24	59	11	13	97	70.8%	16.6 m	45.7 m
An et al. (8)	2016	China	II	Pemetrexed +G	Intercalated	45	65.7	16	29	100	80.0%	18.0 m	34.0 m
				G		45	66.9	17	28	100	73.3%	14.0 m	32.0 m
Cheng et al. (16)	2016	East Asia	II	Pemetrexed +G	Concurrent	126	62	65	52	NA	80.2%	15.8 m	43.4 m
				G		65	62	40	23	NA	73.8%	10.9 m	36.8 m
Han et al. (17)	2017	China	II	Pemetrexed plus carboplatin +G	Intercalated	40	NA	21	19	100	82.5%	17.5 m	32.6 m
				G		41	NA	21	20	100	65.9%	11.9 m	25.8 m
NEJ009	2019	Japan	III	Pemetrexed plus carboplatin +G	Concurrent	170	64.8	93	69	98.8	84%	20.9 m	50.9 m
				G		172	64.0	95	67	98.8	67%	11.9 m	38.8 m
Noronha	2019	India	III	Pemetrexed plus carboplatin +G	Concurrent	174	54	107	60	98	75.3%	16.0 m	NR
				G		176	56	109	60	97	62.5%	8.0 m	17.0 m
Xu et al. (18)	2019	China	II	Pemetrexed plus carboplatin +I	Intercalated	90	58.6	51	38	100	77.8%	16.0 m	36.0 m
				I		89	61.0	52	37	100	64.0%	10.0 m	34.0 m

E, erlotinib; G, Gefitinib; I, icotinib; ORR, objective response rate; PFS, progression free survival; OS, over survival; NA, not available; NR, not reach; EGFR, epidermal growth factor receptor; m, months.

DISCUSSION

As the most common driver gene of lung adenocarcinoma, the status of EGFR mutation, has been gradually founded to be the most useful predictor of efficacy for EGFR-TKI over the past decade (21). The addition of chemotherapy to EGFR-TKI as first-line treatment for EGFR-mutated NSCLC has been reevaluated to overcome or delay resistance and prolong survival time (10). To comprehensively assess the effectiveness and toxicity of EGFR-TKI combined with chemotherapy, we systematically reviewed published randomized trials and performed a meta-analysis. The meta-analysis included eight RCTs with a combined total of 1,349 participants with EGFR-mutated NSCLC. Our results demonstrated that compared with EGFR-TKI monotherapy, the combination of first-generation EGFR-TKI and CT, especially when applying concurrent delivery of double-agents CT, significantly improve ORR (RR = 1.18, 95% CI = 1.10–1.26; p <0.00001) and prolong PFS (HR= 0.56 (95% CI = 0.50–0.64; P <0.00001) and OS (HR = 0.70 (95% CI = 0.54–0.90; P = 0.005), in first-line treatment for advanced NSCLC harboring activating EGFR mutation.

Growing evidence suggests that exon 19 deletions and L8585R point mutation are two different disease entities in the matter of response to TKIs and prognosis (22–25). Kuan et al. conducted a meta-analysis of eight trials comparing EGFR-TKI with chemotherapy as first-line treatment in EGFR-mutated NSCLC (24). Their results showed that TKI monotherapy demonstrated PFS benefit in patients with exon 19 deletions (HR = 0.27, 95% CI = 0.21–0.35) more than L858R (HR = 0.45, 95% CI = 0.35–0.58). How about the results when TKI combined with chemotherapy? In our study, the pooled HR of PFS in exon 19 deletion and L858R point mutation from five trials was 0.54 (95% CI = 0.45–0.65) and 0.52 (95% CI = 0.42–0.65), respectively, which were consistent. It indicated that compared with 19 deletion,

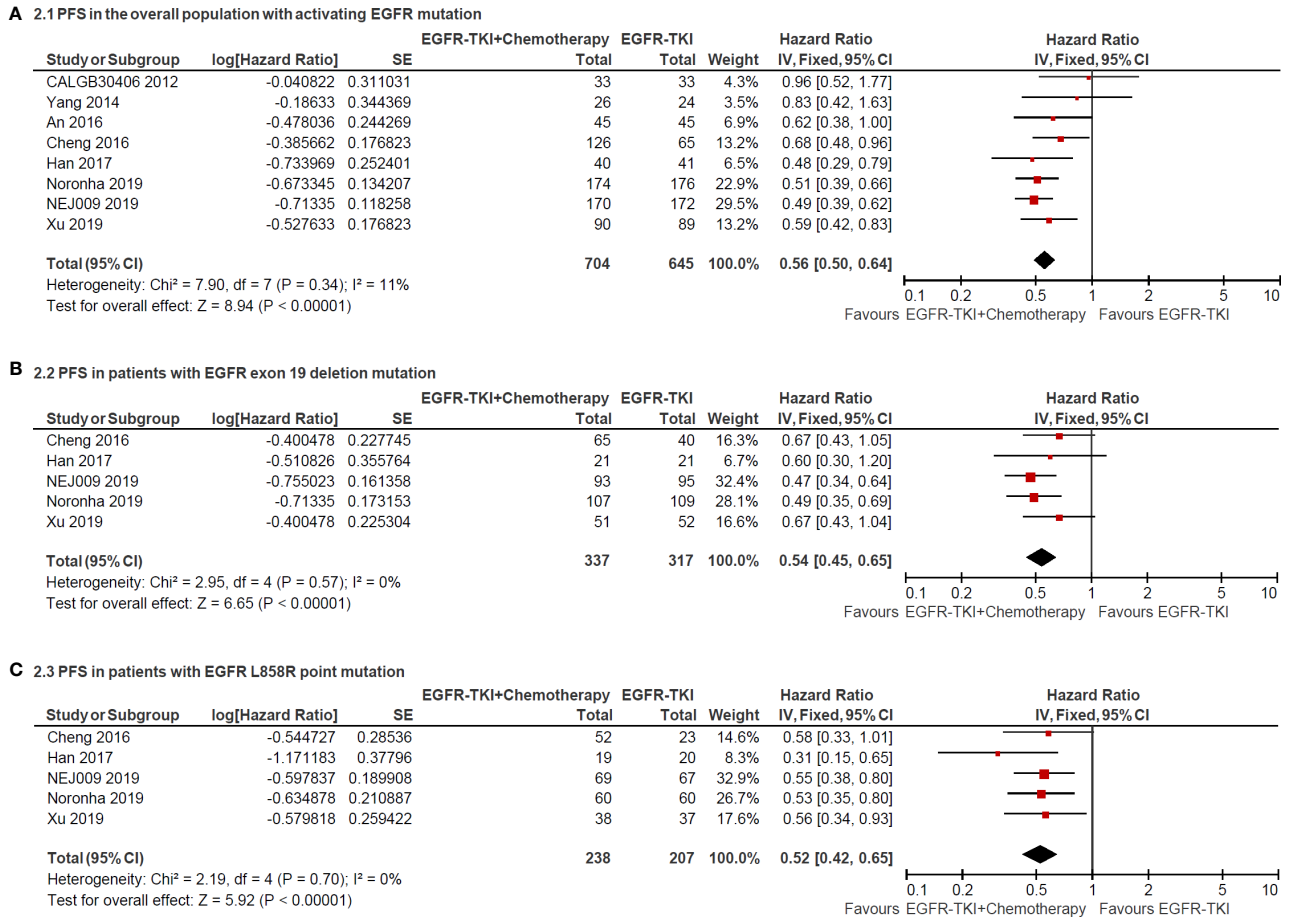

FIGURE 2 | Forest plot of hazard ratio of progress-free survival in overall patients with all sites of positive activating EGFR mutation **(A)**; in patients with positive exon 19 deletion mutation **(B)** and positive L858R point mutation **(C)**.

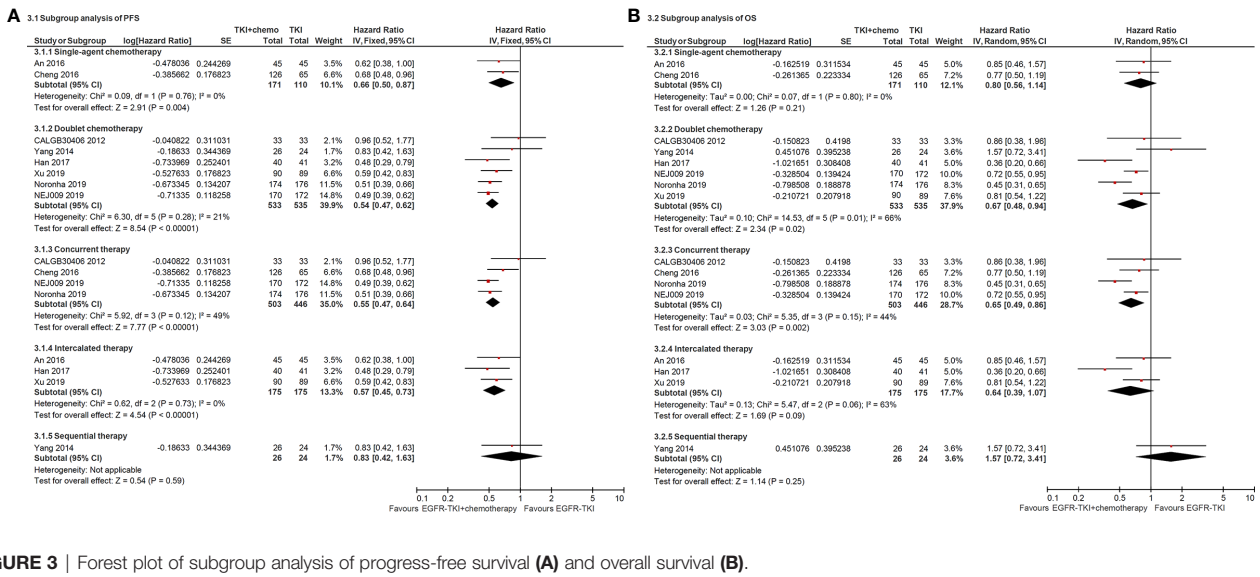

FIGURE 3 | Forest plot of subgroup analysis of progress-free survival **(A)** and overall survival **(B)**.

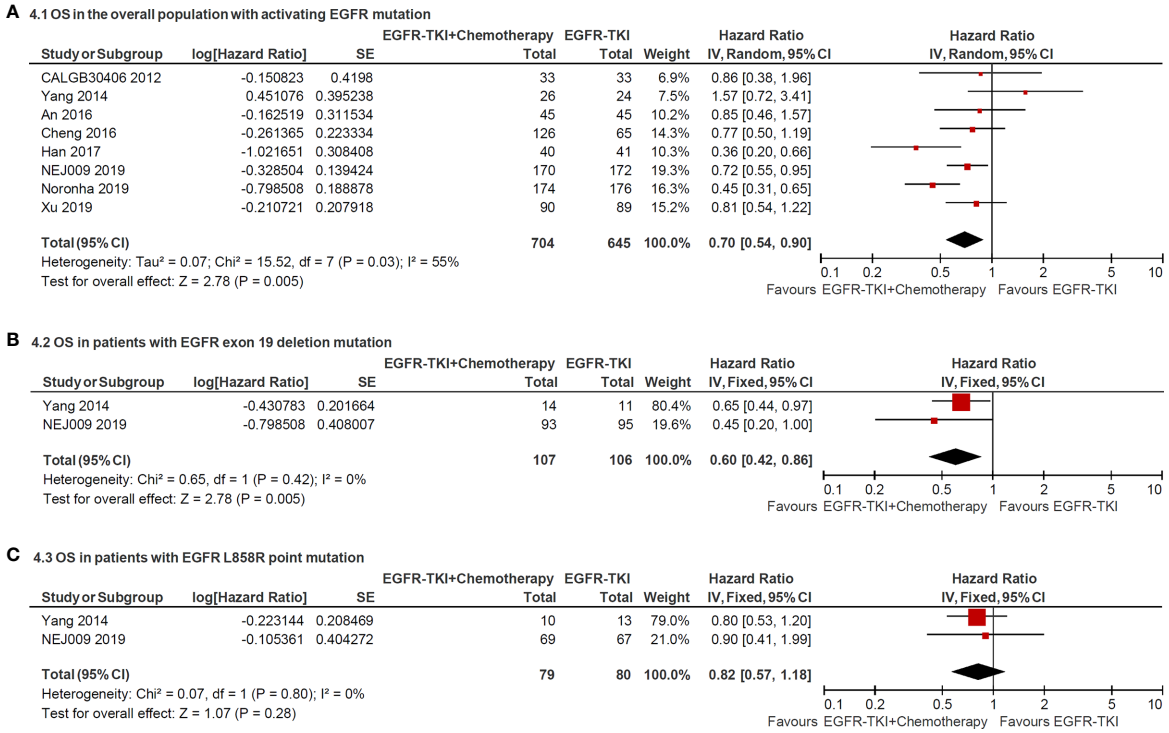

FIGURE 4 | Forest plot of the hazard ratio of overall survival in patients with: **(A)** all sites of positive activating EGFR mutation; **(B)** positive exon 19 deletion mutation and **(C)** positive L858R point mutation.

the PFS of patient with L858R could be more prolonged after TKI combined with chemotherapy. This might be related to increase in ORR of L858R patient after combined chemotherapy.

Currently, first-generation EGFR-TKI monotherapy is still the mainstay of first-line treatment for EGFR-mutated NSCLC, despite the third generation TKI is preferred recommended for first-line therapy. Although the PFS can be substantially prolonged by first-generation TKI compared with platinum-based doublet chemotherapy, none of the first-generation TKIs provide an overall survival benefit revealed by several meta-analyses (4, 26–28). Development of new-generation TKIs or combined therapy is promising strategy to improve OS. Recent ARCHER 1050 (29) and FLAURA (30) trials have shown that second-generation dacomitinib and third-generation osimertinib, significantly prolong the OS and then both of them have been approved for first-line treatment in EGFR-mutated NSCLC (31). As for combined strategy, adding chemotherapy to EGFR-TKI is main approach. Our meta-analysis indicated that first-generation

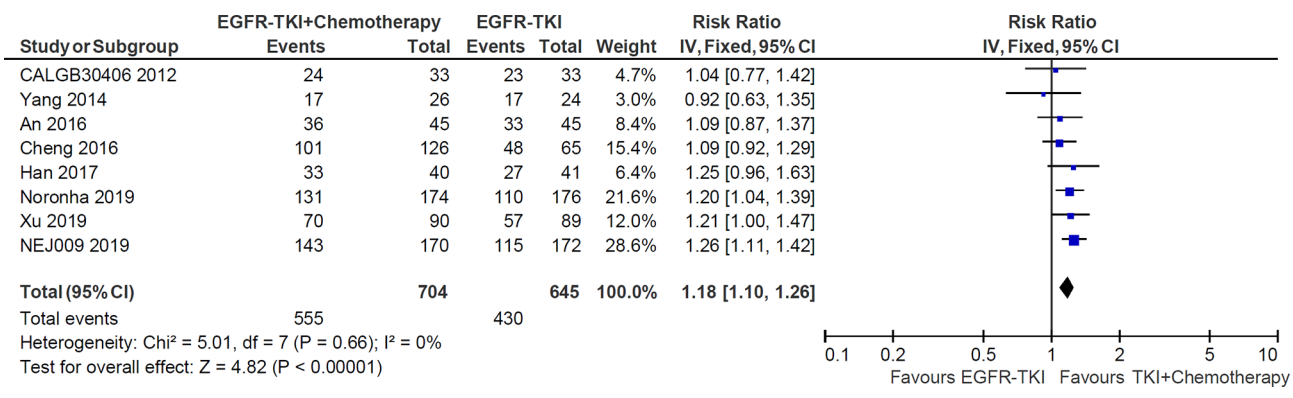

FIGURE 5 | Forest plot of Risk ratio of objective response rate in EGFR-TKI plus chemotherapy group and EGFR-TKI monotherapy group.

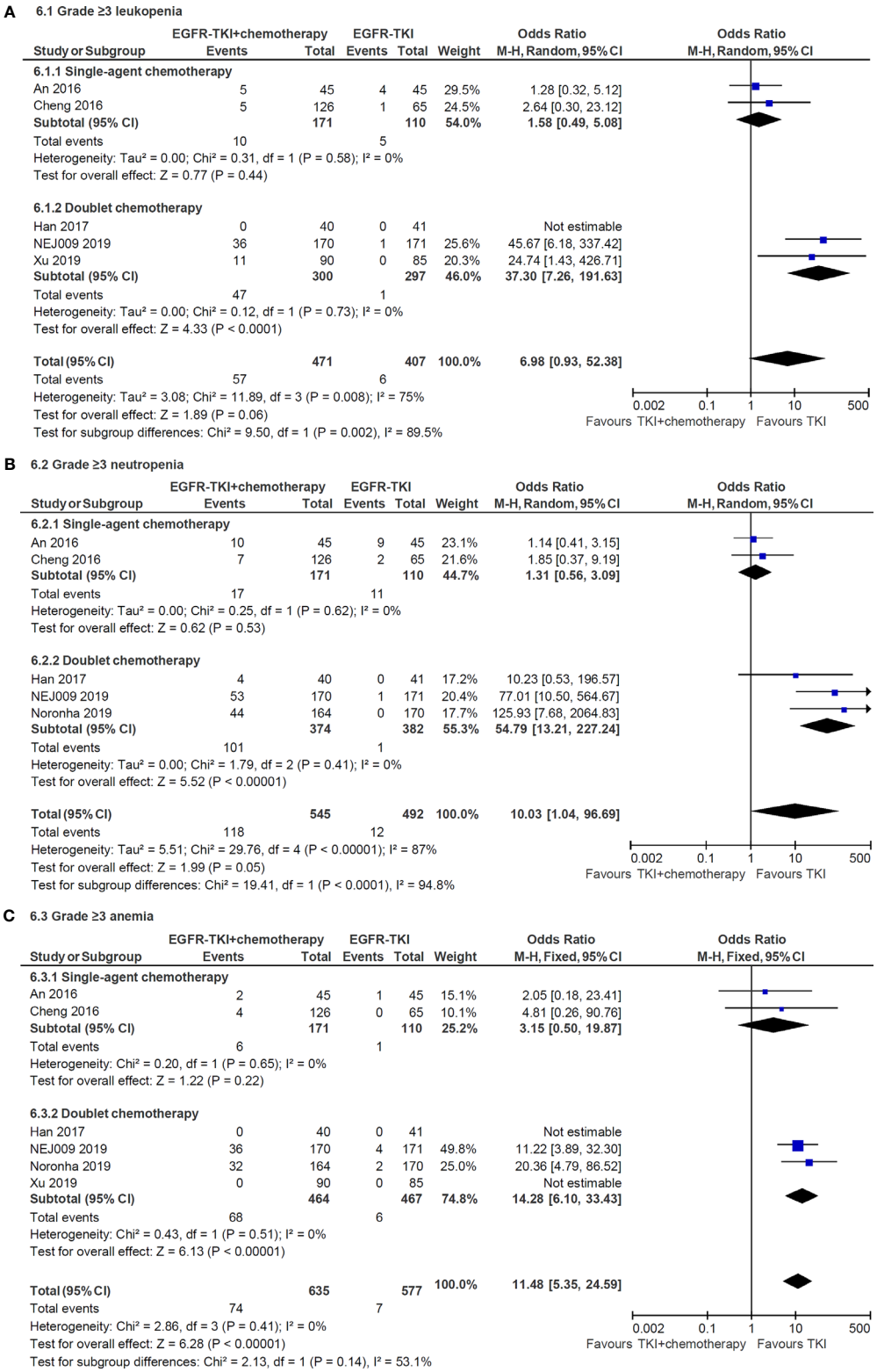

FIGURE 6 | Subgroup analysis of grade 3 or higher hematologic toxicities for leukopenia **(A)**, neutropenia **(B)** and anemia **(C)** in single-agent and doublet chemotherapy.

TABLE 2 | Pooled odds ratio of toxicities in included randomized trials.

Toxicities	No. of trials	Events in EGFR-TKI + Chemotherapy group	Events in EGFR-TKI monotherapy group	Odds Ratio(95% CI)	Pvalue	Heterogeneity (I^2) (%)
Leukopenia						
Grades 1–2	4	117/426	24/362	5.72 (3.59–9.12)	<0.001	45
Grade ≥3	5	57/471	6/407	6.98 (0.93–52.38)	0.06	75
Neutropenia						
Grades 1–2	4	143/500	11/447	16.84 (9.04–31.36)	<0.001	48
Grade ≥3	5	118/545	12/492	10.03 (1.04–96.69)	0.05	87
Thrombocytopenia						
Grades 1–2	4	164/464	39/467	7.04 (4.73–10.48)	<0.001	0
Grade ≥3	5	37/509	0/512	43.41 (6.01–313.71)	<0.001	0
Anemia						
Grades 1–2	5	261/590	158/532	3.01 (1.72–5.28)	<0.001	58
Grade ≥3	6	74/635	7/577	11.48 (5.35–24.59)	<0.001	0
Liver dysfunction						
Grades 1–2	5	308/590	206/532	1.71 (1.34–2.19)	<0.001	0
Grade ≥3	6	82/635	63/577	1.56 (0.74–3.31)	0.25	68
Rash						
Grades 1–2	5	342/590	312/532	0.96 (0.55–1.68)	0.90	78
Grade ≥3	6	27/635	23/577	1.14 (0.64–2.01)	0.66	0
Diarrhea						
Grades 1–2	5	226/590	182/532	1.15 (0.90–1.47)	0.26	47
Grade ≥3	6	35/635	19/577	1.94 (1.08–3.47)	0.03	0
Fatigue						
Grades 1–2	5	272/590	126/532	3.69 (1.99–6.85)	<0.001	71
Grade ≥3	6	25/635	7/577	2.80 (1.29–6.07)	0.009	13
Anorexia						
Grades 1–2	5	310/590	132/532	4.00 (3.06–5.25)	<0.001	10
Grade ≥3	6	19/635	2/577	6.12 (1.79–21.00)	0.004	0
Nausea and vomiting						
Grades 1–2	5	227/590	61/532	6.01 (2.94–12.26)	<0.001	74
Grade ≥3	6	20/635	4/577	4.10 (1.53–11.01)	0.005	0

TKI in combination with chemotherapy significantly improved the OS compared with EGFR-TKI alone (HR = 0.70, 95% CI = 0.54–0.90, P = 0.005). Despite head-to-head RCTs are lacking in directly comparing the efficacy, first-generation EGFR-TKI combined with chemotherapy might seem to prolong OS more than dacomitinib (HR = 0.76, 95% CI = 0.58–0.99, P = 0.044) and osimertinib (HR = 0.80, 95% CI = 0.64–1.00, P = 0.046), according to the results of HR. Based on those inspiring results, third-generation osimertinib combined with chemotherapy is speculated as a treatment strategy that could maximize the length of OS in EGFR-mutated NSCLC patients. Studies on the combination of osimertinib with chemotherapy in EGFR-mutated NSCLC, including TAKUMI and FLAURA2 trials, are currently ongoing and eagerly awaited (10).

Preclinical data indicate that the intercalated or sequential combination of EGFR-TKIs with cytotoxic agents has shown more efficacy than in the concurrent way. A possible explanation is that TKI drugs could induce the G1-phase arrest of tumor cells, which conferred a protection against the cytotoxic activity of pemetrexed (32–34). In our subgroup analysis, however, we founded that the benefit of PFS in concurrent administration (HR = 0.55, 95% CI = 0.47–0.64) were consistent with in intercalated administration (HR = 0.57, 95% CI = 0.45–0.73), and that only concurrent administration did confer an OS benefit to patients with EGFR-mutated NSCLC (HR = 0.65, 95% CI = 0.49–0.86). Our indirectly compared results could be proved by the results of NEJ005 trial, which compared concurrent versus sequential alternating gefitinib and chemotherapy in previously untreated NSCLC with sensitive EGFR mutations (35). In addition, our subgroup analysis revealed an OS benefit in doublet chemotherapy combination group (HR: 0.67, 95% CI: 0.48–0.94), not in single-agent chemotherapy combination group (HR: 0.80, 95% CI: 0.56–1.14). But this conclusion should be applied with caution in clinical practice because only two studies adopted single-agent chemotherapy.

Adding chemotherapy to TKI also increased toxicity, notably, while increasing efficacy. Our meta-analysis indicated that most of the increased toxicities were a result of chemotherapy-induced myelosuppression and gastrointestinal toxicity, as may be expected. The incidences of serious (grade 3 or higher) hematologic toxicities from chemotherapy combination group in the meta-analysis, including leukopenia (12.1%), neutropenia (21.7%), thrombocytopenia (7.3%) and anemia (11.7%), were similar with those landmark trials in which platinum-based doublet chemotherapy were used as first-line treatment approach for the control group (36–39). Otherwise, no significant differences were founded in terms of TKI-induced toxicities, such as any grade rash and grade 3 or higher liver dysfunction, when applying combined treatment in our meta-analysis. Meaningfully, TKI combined with chemotherapy did not significantly increase each other's serious side effects. Therefore, the toxicities of combination therapy are manageable. Our subgroup analysis of toxicity found

that compared with doublet chemotherapy, single-agent approach did not significantly increase grade 3 or higher hematologic toxicities, it may because chemotherapeutic drug in the single-agent group both adopted pemetrexed, which is generally considered to have mild toxicity (40, 41).

Admittedly, our meta-analysis has several limitations. First of all, some of results, especially in subgroup analysis, did not cover all enrolled patients due to the deficiency of detailed data, which might have an impact on the conclusion. Moreover, the outcome of OS would be confounded by the low proportion of patients in the controlled group receiving chemotherapy after experiencing progression on first-line TKI monotherapy and our study was underpowered for assessment of such effect. Meantime, the proportion of the third-generation EGFR-TKI osimertinib usage was relatively low, in that osimertinib was used for only 11–15% and 22% of patients after the first TKI treatment in the NEJ009 trial and the study by Noronha et al., respectively. Therefore, the conclusion of overall survival benefit might not be overvalued. Thirdly, the subgroup analyses of different first-generation EGFR-TKI drugs, including gefitinib, erlotinib and icotinib, were not performed due to in lack of sufficient included studies. Thus, it is not clear whether the efficacies of different first-generation drugs will have differences when combined with chemotherapy. What's more, the fact that this meta-analysis is not based on individual patient data represents a limitation to the interpretation of results, since this approach could tend to overestimate treatment effects. Finally, all included literatures in the meta-analysis were English language publications, which may omit other languages' studies so as to increase the publication bias.

In conclusion, our results demonstrate that compared with first-generation EGFR-TKI monotherapy, the combination of EGFR-TKI and chemotherapy, especially when applying concurrent delivery of platinum-based doublet chemotherapeutic drugs, significantly improve ORR and prolong PFS and OS of first-line treatment in advanced NSCLC patients harboring activating EGFR mutation. Although increasing incidence of chemotherapy-induced toxicities occurs in the combination group, it is well tolerated and clinically manageable. Thus, the combination of first-generation EGFR-TKI and chemotherapy may represent a new option for first-line treatment in EGFR-mutated NSCLC. Greatly inspired by the promising results of first-generation TKI combination therapy, the results of ongoing randomized trials regarding third-generation EGFR-TKI, such as osimertinib, in combination with chemotherapy, are eagerly awaited.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

QW, WXL and FX conceived the study. QW, WXL, WL and TW performed the systematic review of the literature and FX was consulted for a final decision in case of controversy. LH performed the statistical analyses. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.598265/full#supplementary-material

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(6):394–424. doi: 10.3322/caac.21492
2. Zhang YL, Yuan JQ, Wang KF, Fu XH, Han XR, Threapleton D, et al. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget* (2016) 7(48):78985–93. doi: 10.18632/oncotarget.12587
3. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* (2009) 361(10):958–67. doi: 10.1056/NEJMoa0904554
4. Lee CK, Davies L, Wu YL, Mitsudomi T, Inoue A, Rosell R, et al. Gefitinib or Erlotinib vs Chemotherapy for EGFR Mutation-Positive Lung Cancer: Individual Patient Data Meta-Analysis of Overall Survival. *J Natl Cancer Inst* (2017) 109(6):djw279. doi: 10.1093/jnci/djw279
5. Wu YL, Planchard D, Lu S, Sun H, Yamamoto N, Kim DW, et al. Pan-Asian adapted Clinical Practice Guidelines for the management of patients with metastatic non-small-cell lung cancer: a CSCO-ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS. *Ann Oncol* (2019) 30(2):171–210. doi: 10.1093/annonc/mdy554
6. Yi L, Fan J, Qian R, Luo P, Zhang J. Efficacy and safety of osimertinib in treating EGFR-mutated advanced NSCLC: A meta-analysis. *Int J Cancer* (2019) 145(1):284–94. doi: 10.1002/ijc.32097
7. Yang JC, Mok T, Han B, Orlando M, Puri T, Park K. A Review of Regimens Combining Pemetrexed With an Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor in the Treatment of Advanced Nonsquamous Non-Small-Cell Lung Cancer. *Clin Lung Cancer* (2018) 19(1):27–34. doi: 10.1016/j.cllc.2017.06.013
8. An C, Zhang J, Chu H, Gu C, Xiao F, Zhu F, et al. Study of Gefitinib and Pemetrexed as First-Line Treatment in Patients with Advanced Non-Small Cell Lung Cancer Harboring EGFR Mutation. *Pathol Oncol Res* (2016) 22 (4):763–8. doi: 10.1007/s12253-016-0067-4

9. Zhang M, Guo H, Zhao S, Wang Y, Yang M, Yu J, et al. Efficacy of epidermal growth factor receptor inhibitors in combination with chemotherapy in advanced non-small cell lung cancer: a meta-analysis of randomized controlled trials. *Oncotarget* (2016) 7(26):39823–33. doi: 10.18632/oncotarget.9503
10. Rebuzzi SE, Alfieri R, La Monica S, Minari R, Petronini PG, Tiseo M. Combination of EGFR-TKIs and chemotherapy in advanced EGFR mutated NSCLC: Review of the literature and future perspectives. *Crit Rev Oncol Hematol* (2019) 146:102820. doi: 10.1016/j.critrevonc.2019.102820
11. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PloS Med* (2009) 6(7):e1000097. doi: 10.1371/journal.pmed.1000097
12. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj* (2011) 343:d5928. doi: 10.1136/bmj.d5928
13. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *Bmj* (2003) 327(7414):557–60. doi: 10.1136/bmj.327.7414.557
14. Jänne PA, Wang X, Socinski MA, Crawford J, Stinchcombe TE, Gu L, et al. Randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 trial. *J Clin Oncol* (2012) 30(17):2063–9. doi: 10.1200/JCO.2011.40.1315
15. Yang JC-H, Kang JH, Mok T, Ahn M-J, Srimuninnimit V, Lin C-C, et al. First-line pemetrexed plus cisplatin followed by gefitinib maintenance therapy versus gefitinib monotherapy in East Asian patients with locally advanced or metastatic non-squamous non-small cell lung cancer: a randomised, phase 3 trial. *Eur J Cancer (Oxford Engl 1990)* (2014) 50(13):2219–30. doi: 10.1016/j.ejca.2014.05.011
16. Cheng Y, Murakami H, Yang PC, He J, Nakagawa K, Kang JH, et al. Randomized Phase II Trial of Gefitinib With and Without Pemetrexed as First-Line Therapy in Patients With Advanced Nonsquamous Non-Small-Cell Lung Cancer With Activating Epidermal Growth Factor Receptor Mutations. *J Clin Oncol* (2016) 34(27):3258–66. doi: 10.1200/JCO.2016.66.9218
17. Han B, Jin B, Chu T, Niu Y, Dong Y, Xu J, et al. Combination of chemotherapy and gefitinib as first-line treatment for patients with advanced lung adenocarcinoma and sensitive EGFR mutations: A randomized controlled trial. *Int J Cancer* (2017) 141(6):1249–56. doi: 10.1002/ijc.30806
18. Xu L, Qi Q, Zhang Y, Cui J, Liu R, Li Y. Combination of icotinib and chemotherapy as first-line treatment for advanced lung adenocarcinoma in patients with sensitive EGFR mutations: A randomized controlled study. *Lung Cancer* (2019) 133:23–31. doi: 10.1016/j.lungcan.2019.05.008
19. Hosomi Y, Morita S, Sugawara S, Kato T, Fukuhara T, Gemma A, et al. Gefitinib Alone Versus Gefitinib Plus Chemotherapy for Non-Small-Cell Lung Cancer With Mutated Epidermal Growth Factor Receptor: NEJ009 Study. *J Clin Oncol* (2020) 38(2):115–23. doi: 10.1200/JCO.19.01488
20. Noronha V, Patil VM, Joshi A, Menon N, Chougule A, Mahajan A, et al. Gefitinib Versus Gefitinib Plus Pemetrexed and Carboplatin Chemotherapy in EGFR-Mutated Lung Cancer. *J Clin Oncol* (2020) 38(2):124–36. doi: 10.1200/JCO.19.01154
21. Roengvoraphoj M, Tsongalis GJ, Dragnev KH, Rigas JR. Epidermal growth factor receptor tyrosine kinase inhibitors as initial therapy for non-small cell lung cancer: focus on epidermal growth factor receptor mutation testing and mutation-positive patients. *Cancer Treat Rev* (2013) 39(8):839–50. doi: 10.1016/j.ctrv.2013.05.001
22. Renaud S, Seitlinger J, Guerrera F, Reeb J, Beau-Faller M, Voegeli A-C, et al. Prognostic Value of Exon 19 Versus 21 EGFR Mutations Varies According to Disease Stage in Surgically Resected Non-small Cell Lung Cancer Adenocarcinoma. *Ann Surg Oncol* (2018) 25(4):1069–78. doi: 10.1245/s10434-018-6347-3
23. Yang JC-H, Wu Y-L, Schuler M, Sebastian M, Popat S, Yamamoto N, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials.The Lancet. *Oncology* (2015) 16(2):141–51. doi: 10.1016/S1470-2045(14)71173-8
24. Kuan F-C, Kuo L-T, Chen M-C, Yang C-T, Shi C-S, Teng D, et al. Overall survival benefits of first-line EGFR tyrosine kinase inhibitors in EGFR-mutated non-small-cell lung cancers: a systematic review and meta-analysis. *Br J Cancer* (2015) 113(10):1519–28. doi: 10.1038/bjc.2015.356
25. Fenchel K, Dale SP, Dempke WCM. Improved overall survival following tyrosine kinase inhibitor (TKI) treatment in NSCLC-are we making progress? *Trans Lung Cancer Res* (2016) 5(4):373–6. doi: 10.21037/tlcr.2016.07.01
26. Ma H, Tian X, Zeng X-T, Zhang Y, Wang Y, Wang F, et al. The Efficacy of Erlotinib Versus Conventional Chemotherapy for Advanced Nonsmall-Cell Lung Cancer: A PRISMA-Compliant Systematic Review With Meta-Regression and Meta-Analysis. *Medicine* (2016) 95(2):e2495–5. doi: 10.1097/MD.0000000000002495
27. Guetz GD, Landre T, Uzzan B, Chouahnia K, Nicolas P, Morere J-F. Is There a Survival Benefit of First-Line Epidermal Growth Factor Receptor Tyrosine-Kinase Inhibitor Monotherapy Versus Chemotherapy in Patients with Advanced Non-Small-Cell Lung Cancer?: A Meta-Analysis. *Target Oncol* (2016) 11(1):41–7. doi: 10.1007/s11523-015-0373-x
28. Gao G, Ren S, Li A, Xu J, Xu Q, Su C, et al. Epidermal growth factor receptor-tyrosine kinase inhibitor therapy is effective as first-line treatment of advanced non-small-cell lung cancer with mutated EGFR: A meta-analysis from six phase III randomized controlled trials. *Int J Cancer* (2012) 131(5):E822–9. doi: 10.1002/ijc.27396
29. Mok TS, Cheng Y, Zhou X, Lee KH, Nakagawa K, Niho S, et al. Improvement in Overall Survival in a Randomized Study That Compared Dacomitinib With Gefitinib in Patients With Advanced Non-Small-Cell Lung Cancer and EGFR-Activating Mutations. *J Clin Oncol* (2018) 36(22):2244–50. doi: 10.1200/JCO.2018.78.7994
30. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *N Engl J Med* (2020) 382(1):41–50. doi: 10.1056/NEJMoa1913662
31. Ettinger DS, Wood DE, Aggarwal C, Aisner DL, Akerley W, Bauman JR, et al. NCCN Guidelines Insights: Non-Small Cell Lung Cancer, Version 1.2020. *J Natl Compr Canc Netw* (2019) 17(12):1464–72. doi: 10.6004/jnccn.2019.0059
32. Li T, Ling Y-H, Goldman ID, Perez-Soler R. Schedule-dependent cytotoxic synergism of pemetrexed and erlotinib in human non-small cell lung cancer cells. *Clin Cancer Res* (2007) 13(11):3413–22. doi: 10.1158/1078-0432.CCR-06-2923
33. Giovannetti E, Lemos C, Tekle C, Smid K, Nannizzi S, Rodriguez JA, et al. Molecular mechanisms underlying the synergistic interaction of erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor, with the multitargeted antifolate pemetrexed in non-small-cell lung cancer cells. *Mol Pharmacol* (2008) 73(4):1290–300. doi: 10.1124/mol.107.042382
34. Feng X, Zhang Y, Li T, Li Y. Sequentially administrated of pemetrexed with icotinib/erlotinib in lung adenocarcinoma cell lines in vitro. *Oncotarget* (2017) 8(69):114292–9. doi: 10.18632/oncotarget.23224
35. Oizumi S, Sugawara S, Minato K, Harada T, Inoue A, Fujita Y, et al. Updated survival outcomes of NEJ005/TCOG0902: a randomised phase II study of concurrent versus sequential alternating gefitinib and chemotherapy in previously untreated non-small cell lung cancer with sensitive EGFR mutations. *ESMO Open* (2018) 3(2):e000313. doi: 10.1136/esmoopen-2017-000313
36. Mok TS, Wu Y-L, Thongprasert S, Yang C-H, Chu D-T, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) 361(10):947–57. doi: 10.1056/NEJMoa0810699
37. Zhou C, Wu Y-L, Chen G, Feng J, Liu X-Q, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* (2011) 12(8):735–42. doi: 10.1016/S1470-2045(11)70184-X
38. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, 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):239–46. doi: 10.1016/S1470-2045(11)70393-X
39. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* (2010) 362(25):2380–8. doi: 10.1056/NEJMoa0909530
40. Li X, Wei S, Chen J. Critical appraisal of pemetrexed in the treatment of NSCLC and metastatic pulmonary nodules. *Onco Targets Ther* (2014) 7:937–45. doi: 10.2147/OTT.S45148
41. Pérez-Moreno MA, Galván-Banqueri M, Flores-Moreno S, Villalba-Moreno A, Cotrina-Luque J, Bautista-Paloma FJ. Systematic review of efficacy and safety of pemetrexed in non-small-cell-lung cancer. *Int J Clin Pharm* (2014) 36(3):476–87. doi: 10.1007/s11096-014-9920-2
42. Wu Q, Luo W, Li W, Wang T, Huang L, Xu F. First-generation EGFR-TKI plus chemotherapy versus EGFR-TKI alone as first-line treatment in advanced

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ORIGINAL RESEARCH
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Primary Tumor Resection Improves Survival for EGFR-TKI-Treated Patients With Occult M1a Lung Adenocarcinoma

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Background: The role of primary tumor resection in occult M1a lung adenocarcinoma remains unclear, especially for patients receiving targeted therapy. The purpose of this study is to assess the effect of primary tumor resection on overall survival (OS) in lung adenocarcinoma patients with occult pleural disseminations receiving targeted therapy.

Methods: Lung adenocarcinoma patients with intraoperatively-confirmed occult pleural dissemination (M1a), who hospitalized in the Department of Thoracic Surgery in Fudan Shanghai Cancer Center from May 2008 to December 2017 and received EGFR-TKIs therapy, were enrolled. Log-rank tests were used to compare the survival differences between groups.

Results: 34 patients receiving EGFR-TKIs were enrolled. The majority of them were never smokers (29/34, 85.3%). Among the enrolled patients, 20 (58.8%) patients underwent primary tumor resection, while 14 (41.2%) patients not. There was no distributional difference of baselines between patients undergoing and not undergoing primary tumor resection. Further analyses demonstrated that the patients undergoing primary tumor resection had a prolonged OS compared with those not (log-rank P= 0.042). The 2-year and 5-year OS for patients receiving primary tumor resection and EGFR-TKIs was 90.0% and 60.1%.

Conclusions: Primary tumor resection was associated with improved survival in patients with occult intraoperatively-confirmed M1a adenocarcinoma receiving EGFR-TKIs.

Keywords: lung adenocarcinoma, M1a, primary tumor resection, EGFR-TKIs, survival

Abbreviations: BMI, body mass index; NSCLC, non-small cell lung cancer; OS, overall survival.

INTRODUCTION

Lung cancer is the deadliest malignancy worldwide, accounting for the largest number of new cancer cases and cancer-related deaths (1). Stage IV lung cancer is responsible for 45% of newly diagnosed lung cancer patients in Surveillance, Epidemiology, and End Results (SEER) Program population-based registries (2). However, the 2-year and 5-year overall survival (OS) rate for stage IV non-small cell lung cancer (NSCLC) is only 17% and 6%, respectively (3). Over the past ten years, treatment for stage IV NSCLC has been revolutionized by the rapid development of targeted therapy. In the era of targeted therapy, lung cancer patients live a much longer life than before. Nowadays lung cancer seems to be a "chronic" disease thanks to the clinical application of targeted agents. However, patients will eventually develop drug resistance to targeted agents, and it is a critical issue to address methods improving survival of patients receiving target therapy. A previous study indicated that patients with initial disease progression in primary tumors accounted for 45% of progressed patients with targeted therapy (4). Moreover, in clinical practice, occult pleural dissemination is sometimes discovered intraoperatively. There is no consensus on whether we should perform primary tumor resection in that case. Therefore, we hypothesized that surgical resection of primary tumors could improve the survival of lung adenocarcinoma patients with occult pleural metastases in the era of targeted therapy.

Although surgery is not deemed as a treatment option, given the fact that therapeutic goals of stage IV disease have focused on optimization of quality of life and palliation, recent studies indicated local consolidative therapy were able to prolong progression-free survival and overall survival (OS) in stage IV NSCLC patients who received first-line systemic therapy (5, 6). Nevertheless, the role of primary lesion surgery in patients with occult M1a lung cancer receiving targeted therapy remains unclear.

To address this, we aimed to assess OS after primary tumor resection versus no resection in lung adenocarcinoma patients with occult pleural dissemination treated with EGFR-TKIs.

METHODS

Patients

Selected patients with occult M1a lung adenocarcinoma hospitalized in the Department of Thoracic Surgery, Fudan Shanghai Cancer Center (FUSCC) from May 2008 to December 2017 were reviewed retrospectively. The inclusion criteria were (1) pathologically confirmed primary lung adenocarcinoma, (2) occult pleural dissemination and pathologically confirmed M1a intraoperatively by frozen section examinations, and (3) receiving targeted therapy toward *EGFR* mutation. Age, gender, smoking history, body mass index (BMI), receiving primary tumor resection or not, mutation status, EGFR-TKIs therapy, and survival data were collected. Primary tumor resection was defined as the surgical removal of primary lung cancer lesion, which was usually the largest or first appeared radiologically. The study was approved by the Institutional Review Board of Fudan University Shanghai Cancer Center (IRB#090977-1), and the protocol number of this study was IRB2008223-9.

Mutational Analyses

The mutational analyses were conducted by the central laboratory of pathology in FUSCC using the resected primary tumor specimens. Genomic DNA was extracted for further amplification refractory mutation system.

Statistical Analyses

Overall survival was calculated from the date of the diagnosis to the date of death or last follow-up, and death from any cause was considered as an event. Chi-square tests were used to evaluate the difference of categorized variables between patients receiving and not receiving primary tumor resection. The Kaplan-Meier method was used to analyze OS, and log-rank tests were used to compare differences between groups. Data were analyzed by SPSS (version 25.0; IBM Corp, Armonk, NY). All tests were two-tailed, and statistical significance was set at $P < 0.05$.

RESULTS

Patient Characteristics

A total of 34 patients were enrolled in the study (**Table 1**). There are 22 (64.7%) females and 12 (35.3) males. A majority of patients (29/34, 85.3%) were never-smokers. Most of patients had cT1 and cT2 disease. 17 (50.0%) patients had cN0 disease, 5 (14.7%) had cN1, and 12 (35.3%) had cN2 disease. Among these patients, 20 (58.8%) patients underwent primary tumor resection, while the rest (14/34, 41.2%) not. Concerning firstly-used EGFR-TKIs, the most common agent was gefitinib (26/34, 67.2%), followed by erlotinib (6/34, 17.6%), icotinib (1/34, 2.9%), and osimertinib (1/34, 2.9%). Five (5/34, 14.7%) patients received osimertinib as the second therapy after developing drug resistance to gefitinib and erlotinib. Besides targeted therapy, 23 (67.6%) patients received platinum doublet chemotherapy, and 6 (17.6%) patients received radiotherapy.

Comparisons were made between patients who received primary tumor resection and those who did not (**Table 2**). There were no distributional differences between two groups in age (P=0.477), gender (P=0.066), smoking history (P=1.000), body mass index (BMI, P=1.000), cT (P=0.207), cN (P=0.512), firstly-used EGFR-TKIs (P=1.000), secondly-used osimertinib (P=0.627), platinum doublet chemotherapy (P=1.000), and radiotherapy (P=1.000). Previous studies have revealed the prognostic value of clinical T and N descriptors in patients with operable lung cancer (7, 8), so we also investigated it in patients with intraoperatively-confirmed M1a disease. The results demonstrated that there was no difference in survival between patients with different cT (P=0.96) and cN (P=0.87) descriptors (**Supplementary Figure 1**).

TABLE 1 | Clinicopathologic characteristics of patients with M1a lung adenocarcinoma.

Variables	Enrolled patients (N = 34)	
	N	%
Age (years)		
≤60	21	61.8
>60	13	38.2
Gender		
Male	12	35.3
Female	22	64.7
Smoking history		
Ever	5	14.7
Never	29	85.3
BMI		
≤24	23	67.6
>24	11	32.4
cT		
cT1	14	41.2
cT2	18	52.9
cT3	1	2.9
cT4	1	2.9
cN		
cN0	17	50.0
cN1	5	14.7
cN2	12	35.3
Primary tumor resection		
Yes	20	58.8
No	14	41.2
Firstly-used EGFR-TKIs		
Gefitinib	26	76.5
Erlotinib	6	17.6
Icotinib	1	2.9
Osimertinib	1	2.9
Secondly-used osimertinib		
Yes	5	14.7
No	29	85.3
Platinum doublet chemotherapy		
Yes	23	67.6
No	11	32.4
Radiotherapy		
Yes	6	17.6
No	28	82.4

BMI, Body Mass Index.

16 patients died during the follow-up period. With the median follow-up time of 65.0 months, the median OS was 60.0 months (95% confidential interval [CI], 33.4-88.6). The 2-year and 5-year OS was 85.3% and 45.5% respectively (**Figure 1**).

The Association Between Primary Tumor Resection and Improved Survival

To investigate the prognostic role of primary tumor resection, Kaplan-Meier curves were used. The patients undergoing primary tumor resection had a prolonged OS compared with those not (log-rank *P*= 0.042; **Figure 2**). The median OS for patients receiving primary tumor resection and those not was 83.0 months (95% CI: 41.2-124.8) and 43.7 months (95%CI: 30.6-56.7). The 2-year and 5-year OS for patients receiving primary tumor resection was 90.0% and 60.1%, while the 2-year and 5-year OS for those not was 78.6% and 26.8%.

DISCUSSION

Currently, there was no consensus on whether to perform surgical resection to lung cancer patients with occult pleural disseminations. Chemotherapy or targeted therapy was recommended as initial treatments for stage IV NSCLC, and radiotherapy could also be considered if necessary, without the recommendation of surgical resection. Gomez and his colleges (5, 6) reported local consolidative therapy, including surgery and/or radiotherapy, could prolong progression-free survival and overall survival significantly in stage IV lung cancer after receiving first-line systemic therapy. However, only patients with stable disease and partial response to chemotherapy were enrolled, and most of the patients received platinum doublet chemotherapy as the first-line treatment. Besides, there were only six patients (6/25, 24%) receiving surgery of metastatic and/or primary sites in their study. Therefore, the actual effect of surgery in patients with stage IV lung cancer remains unclear, especially for patients receiving targeted therapy. In our study, we investigated lung adenocarcinoma patients with occult pleural disseminations receiving EGFR-TKIs and found out upfront primary tumor resection was associated with improved survival. To the best of our knowledge, it is the first study to reveal the association between primary surgical resection and improved survival in lung adenocarcinoma patients with occult pleural metastases in the era of targeted therapy. The study provided novel evidence for the treatments of occult M1a lung adenocarcinoma.

In clinical practice, pleural disseminations might be occult for some patients and was discovered intraoperatively. There is no consensus on whether we should perform primary tumor resection in that case. Since surgical resection can result in a 5-year survival rate of 30% to 50% in patients with metastatic NSCLC (9), and the role of thoracic surgery in the management of metastatic NSCLC attracts our attention. Theoretically, surgical resection of primary lesions could reduce tumor burden, which was considered to be associated with targeted drug resistance and prognosis of patients. In our study, primary tumor resection could significantly prolong the survival of patients receiving EGFR-TKIs (*P*= 0.042). The 5-year OS of patients undergoing surgical resection and those not was 60.1% and 26.8%. These results supported the conclusion that surgical resection could improve survival in lung adenocarcinoma patients with occult pleural disseminations receiving EGFR-TKIs.

With the improved disease response and control rate with targeted therapy, patients with advanced-stage lung cancer live longer. According to previous real-world studies (10), the median OS for *EGFR*-mutant metastatic lung adenocarcinoma patients treated with EGFR-TKIs was 30.9 months. Especially for Asian patients, our previous study more than half of patients with lung adenocarcinoma harbored *EGFR* kinase domain mutations (11). The targeted agent is an ideal treatment for them, but drug resistance will occur sooner or later. Our study might provide a potential way to slow down the drug resistance to EGFR-TKIs. Further randomized clinical trials are urged on the possible combined use of surgery and targeted therapy.

TABLE 2 | Clinicopathologic characteristics of patients receiving and not receiving primary lesion resection.

Variables	Patients receiving primary tumor resection (N = 20)		Patients not receiving resection (N = 14)		*P*
	N	%	N	%	
Age (years)					0.477
≤60	11	55.0	10	71.4	
>60	9	45.0	4	28.6	
Gender					0.066
Male	10	50.0	12	85.7	
Female	10	50.0	2	14.3	
Smoking history					1.000
Ever	3	15.0	2	14.3	
Never	17	85.0	12	85.7	
BMI					1.000
≤24	14	70.0	9	64.3	
>24	6	30.0	5	35.7	
cT					0.207
cT1	6	30.0	8	57.1	
cT2	13	65.0	5	35.7	
cT3/4	1	5.0	1	7.1	
cN					0.512
cN0	10	50.0	7	50.0	
cN1	4	20.0	1	7.1	
cN2	6	30.0	6	42.9	
Firstly-used EGFR-TKIs					1.000
Gefitinib	15	75.0	11	78.6	
Erlotinib	3	15.0	3	21.4	
Icotinib	1	5.0	0	0	
Osimertinib	1	5.0	0	0	
Secondly-used osimertinib					0.627
Yes	2	10.0	3	21.4	
No	18	90.0	11	78.6	
Platinum doublet hemotherapy					1.000
Yes	14	70.0	9	64.3	
No	6	30.0	5	35.7	
Radiotherapy					1.000
Yes	3	15.0	3	21.4	
No	17	85.0	11	78.6	

BMI, Body Mass Index.

The survival benefit from surgical resection of primary tumors might be explained by the following potential mechanisms. Intratumor heterogeneity results in different subclones of tumor cells, some of which are resistant to targeted therapy or chemotherapy (12). After resection of the primary lesion, generally the largest of all lesions, the drug-resistant subclones could also be removed (13). Thus, in this scenario, patients may have a better drug response and longer survival. Another possible mechanism is that primary tumors seed circulating tumor cells *via* the bloodstream, resulting in micro-metastasis in distant sites (14). In that case, resection of primary tumors could slow the growth speed of micro-metastasis. Therefore, upfront surgical resection helped to maximize drug response of targeted therapy, suggesting a combination of surgical resection and targeted therapy might be an effective option.

In our study, surgical resection was performed before targeted therapy. Upfront surgery followed by targeted therapy could provide advantages in some ways. In our previous study, upfront surgery followed by adjuvant therapy may also provide favorable survival outcomes for selected patients with lung cancer (15). The resection of primary lesions spared patients from biopsies to confirm pathology and mutational status. Additionally, pathologic and mutational analyses based on surgically resected specimens were generally more precise than biopsy specimens. Therefore, upfront surgery followed by targeted therapy was feasible in clinical practice.

There are several limitations of this study. First, the number of patients seemed small. We only enrolled patients receiving EGFR-TKIs with intraoperatively-confirmed occult M1a lung adenocarcinoma, who have been hospitalized in the department of thoracic surgery. The multivariable analyses were inappropriate due to limited sample size. Nevertheless, the baselines of two groups were comparable, resulting in no confounding factors during the direct survival comparison by Kaplan-Meier method. Second, it is a retrospective study from a single institution, and selection bias was inevitable. Our results need to be validated in future multi-centered randomized controlled clinical trials. Third, progression-free survival was not calculated in the study, because some patients received surveillance in other institutions. Nevertheless, we believe that OS is a more important outcome and the study based on OS is

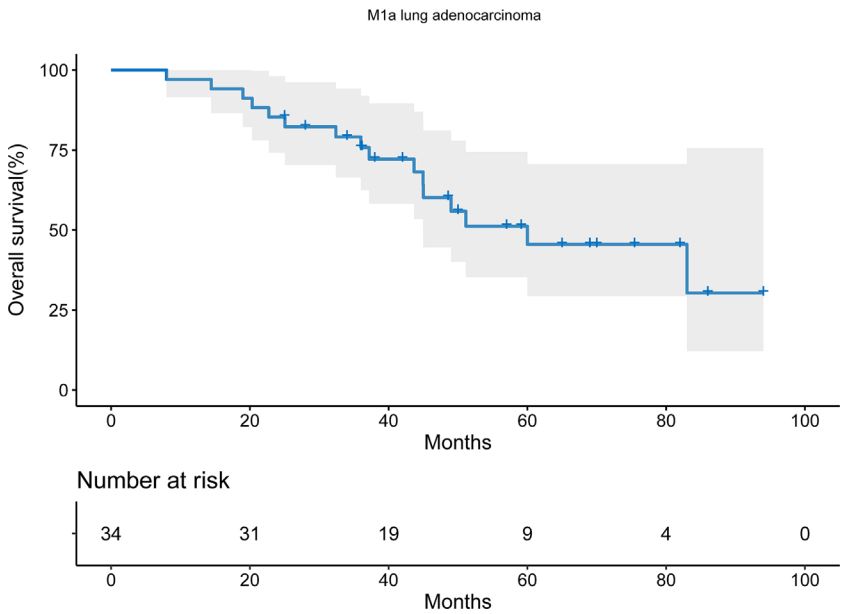

FIGURE 1 | Overall survival of M1a lung adenocarcinoma patients receiving targeted therapy.

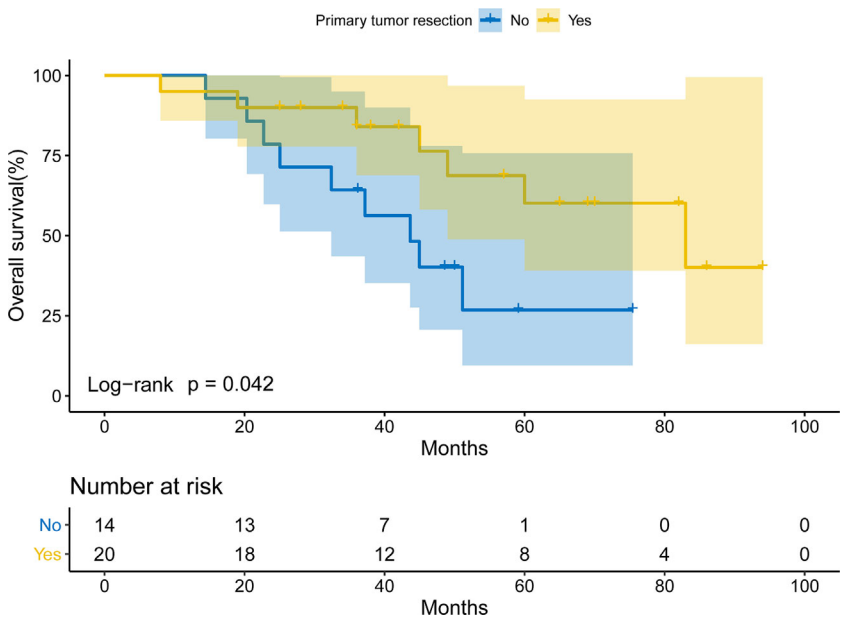

FIGURE 2 | Overall survival of EGFR-TKIs-treated patients with intraoperatively-confirmed M1a lung adenocarcinoma receiving or not receiving primary tumor resection.

more meaningful for patients. Fourth, the main agent of EGFR-TKIs in this study was gefitinib due to the use of histological data before the adoption of first-line osimertinib. However, the study provided a promising treatment for lung adenocarcinoma with occult pleural disseminations.

In summary, primary surgical resection improves survival of lung adenocarcinoma patients with intraoperatively-confirmed occult pleural metastases followed by EGFR-TKIs. Primary tumor resection might be a promising method for the treatment of patients with occult M1a lung adenocarcinoma receiving target therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Fudan University Shanghai Cancer Center (IRB#090977-1). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Study concepts: FF, ZW, YaZ, and HC. Study design: FF, ZW, YaZ, and HC. Literature research: FF, ZW, ZG, YuZ, YL, YaZ, and HC. Data acquisition: FF, ZW, ZG, YuZ, and YL. Data analysis/interpretation: FF, ZW, ZG, YuZ, and YL. Statistical analysis: FF, ZW, ZG, YuZ, and YL. Manuscript preparation: FF, ZW, YaZ, and HC. Manuscript editing: FF, ZW, YaZ, and HC. Manuscript final version approval: FF, ZW, ZG, YuZ, YL, YaZ, and HC. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.622723/full#supplementary-material

Supplementary Figure 1 | Overall survival of patients with intraoperatively-confirmed M1a lung adenocarcinoma stratified by cT **(A)** and cN **(B)**.

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. doi: 10.3322/caac.21492
2. Chen VW, Ruiz BA, Hsieh MC, Wu XC, Ries LA, Lewis DR. Analysis of stage and clinical/prognostic factors for lung cancer from SEER registries: AJCC staging and collaborative stage data collection system. *Cancer* (2014) 120:3781–92. doi: 10.1002/cncr.29045
3. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WE, et al. The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer. *J Thorac Oncol* (2016) 11:39–51. doi: 10.1016/j.jtho.2015.09.009
4. Al-Halabi H, Sayegh K, Digamurthy SR, Niemierko A, Piotrowska Z, Willers H, et al. Pattern of Failure Analysis in Metastatic EGFR-Mutant Lung Cancer Treated with Tyrosine Kinase Inhibitors to Identify Candidates for Consolidation Stereotactic Body Radiation Therapy. *J Thorac Oncol* (2015) 10:1601–7. doi: 10.1097/JTO.0000000000000648
5. Gomez DR, Blumenschein GRJr, Lee JJ, Hernandez M, Ye R, Camidge DR, et al. Local consolidative therapy versus maintenance therapy or observation for patients with oligometastatic non-small-cell lung cancer without progression after first-line systemic therapy: a multicentre, randomised, controlled, phase 2 study. *Lancet Oncol* (2016) 17:1672–82. doi: 10.1016/S1470-2045(16)30532-0
6. Gomez DR, Tang C, Zhang J, Blumenschein GRJr, Hernandez M, Lee JJ, et al. Local Consolidative Therapy Vs. Maintenance Therapy or Observation for Patients With Oligometastatic Non-Small-Cell Lung Cancer: Long-Term Results of a Multi-Institutional, Phase II, Randomized Study. *J Clin Oncol* (2019) 37:1558–65. doi: 10.1200/JCO.19.00201
7. Tsutani Y, Miyata Y, Nakayama H, Okumura S, Adachi S, Yoshimura M, et al. Solid tumor size on high-resolution computed tomography and maximum standardized uptake on positron emission tomography for new clinical T descriptors with T1 lung adenocarcinoma. *Ann Oncol* (2013) 24:2376–81. doi: 10.1093/annonc/mdt230
8. Suzuki K, Nagai K, Yoshida J, Nishimura M, Takahashi K, Nishiwaki Y. The prognosis of surgically resected N2 non-small cell lung cancer: the importance of clinical N status. *J Thorac Cardiovasc Surg* (1999) 118:145–53. doi: 10.1016/S0022-5223(99)70153-4
9. David EA, Clark JM, Cooke DT, Melnikow J, Kelly K, Canter RJ. The Role of Thoracic Surgery in the Therapeutic Management of Metastatic Non-Small Cell Lung Cancer. *J Thorac Oncol* (2017) 12:1636–45. doi: 10.1016/j.jtho.2017.08.008
10. Lin JJ, Cardarella S, Lydon CA, Dahlberg SE, Jackman DM, Janne PA, et al. Five-Year Survival in EGFR-Mutant Metastatic Lung Adenocarcinoma Treated with EGFR-TKIs. *J Thorac Oncol* (2016) 11:556–65. doi: 10.1016/j.jtho.2015.12.103
11. Wang R, Zhang Y, Pan Y, Li Y, Hu H, Cai D, et al. Comprehensive investigation of oncogenic driver mutations in Chinese non-small cell lung cancer patients. *Oncotarget* (2015) 6:34300–8. doi: 10.18632/oncotarget.5549
12. Cheng X, Chen H. Tumor heterogeneity and resistance to EGFR-targeted therapy in advanced nonsmall cell lung cancer: challenges and perspectives. *Onco Targets Ther* (2014) 7:1689–704. doi: 10.2147/OTT.S66502
13. Hittelman WN. Clones and subclones in the lung cancer field. *J Natl Cancer Inst* (1999) 91:1796–9. doi: 10.1093/jnci/91.21.1796
14. Mascaux C, Angelova M, Vasaturo A, Beane J, Hijazi K, Anthoine G, et al. Immune evasion before tumour invasion in early lung squamous carcinogenesis. *Nature* (2019) 571:570–5. doi: 10.1038/s41586-019-1330-0
15. Zheng D, Ye T, Hu H, Zhang Y, Sun Y, Xiang J, et al. Upfront surgery as first-line therapy in selected patients with stage IIIA non-small cell lung cancer. *J Thorac Cardiovasc Surg* (2018) 155:1814–22. doi: 10.1016/j.jtcvs.2017.10.075

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CASE REPORT
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Small Cell Lung Cancer Transformation as a Resistance Mechanism to Osimertinib in Epidermal Growth Factor Receptor-Mutated Lung Adenocarcinoma: Case Report and Literature Review

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Introduction: Small cell lung cancer (SCLC) transformation represents a mechanism of resistance to osimertinib in *EGFR*-mutated lung adenocarcinoma, which dramatically impacts patients' prognosis due to high refractoriness to conventional treatments.

Case Description: We present the case of a patient who developed a SCLC phenotypic transformation as resistance mechanism to second-line osimertinib for T790M-positive *EGFR*-mutated NSCLC. Our patient received platinum–etoposide doublet following SCLC switch and achieved a modest clinical benefit which lasted 4 months. NGS and IHC analyses for p53 and Rb were performed on subsequent liver biopsies, revealing baseline *TP53* mutation and complete absence of p53 and Rb expression. Primary cell cultures were established following a liver biopsy at the time of SCLC transformation, and drug sensitivity assays showed meaningful cell growth inhibition when osimertinib was added to platinum–etoposide compared with control ($p < 0.05$). A review of the current literature regarding SCLC transformation after failure of osimertinib was performed.

Conclusions: Based on retrospective data available to date, platinum–etoposide chemotherapy is the preferred treatment choice in the occurrence of SCLC transformation after osimertinib failure. The extension of osimertinib in combination with chemotherapy in the occurrence of SCLC transformation as resistance mechanism to osimertinib is a matter of debate. The combination of osimertinib and platinum–etoposide was effective in inhibiting cell growth in our primary cell cultures. Clinical studies are needed to further explore this combination in the occurrence of SCLC transformation as a resistance mechanism to osimertinib.

Keywords: NSCLC, EGFR, osimertinib resistance, SCLC transformation, phenotype switch

INTRODUCTION

The epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) osimertinib constitutes a milestone for the treatment of advanced *EGFR*-mutated non-small cell lung cancer (NSCLC), both in the second line after failure of the previous generation of EGFR-TKIs due to the onset of T790M mutation and in the first line, regardless of T790M status (1). Despite remarkable activity exerted by osimertinib in this clinical setting, several resistance mechanisms have been described (2). Among these, small cell lung cancer (SCLC) phenotypic transformation represents a critical issue for clinicians, since effective therapeutic strategies to apply in this circumstance are lacking to date.

Herein, we report the case of a patient who developed a SCLC switch as resistance mechanism to second-line osimertinib for T790M-positive *EGFR*-mutated NSCLC, whose pre-clinical studies revealed a promising activity of prolonged osimertinib in combination with chemotherapy. Moreover, we performed a literature review to summarize the underlying mechanism and clinical features of SCLC transformation following osimertinib treatment, including current and future therapeutic opportunities.

CASE DESCRIPTION

In September 2017, due to persistence of dry cough, a never-smoker 63-year-old woman underwent computed tomography (CT) scan which showed a lesion to the upper left pulmonary lobe associated with ipsilateral hilar lymph nodes. The subsequent positron emission tomography (PET) showed increased glucose uptake at both lesions. Left upper lobectomy and mediastinal lymphadenectomy were performed in November 2017, and the pathologic examination revealed an *EGFR*-mutated (exon 19 deletion) adenocarcinoma of the lung with stage pT3N2, R1 for microscopic residual disease at the bronchial margin. At the post-operative CT scan performed in January 2018, a recurrence of disease was documented, involving bilateral pulmonary metastases and left pleural effusion (**Figure 1**). Due to the presence of sensitizing *EGFR* mutation, the patient started gefitinib treatment, achieving partial response of the disease, with almost a complete disappearance of bilateral pulmonary nodules and a decrease of left pleural effusion. The benefit was maintained until October 2018, when the onset of multiple liver metastases and bone lesions was documented. A liver biopsy (liver biopsy 1, LB1) was subsequently performed in order to explore putative resistance mechanisms to gefitinib, revealing the presence of secondary T790M *EGFR* mutation in the context of exon 19 deletion. The presence of *EGFR* activating and T790M mutations was also confirmed on liquid biopsy (**Figure 2**). The patient promptly started osimertinib, which led to complete metabolic response on the liver and osteoblastic reaction of pre-existing bone lesions. Osimertinib therapy was continued until December 2019, when liver lesions increased. The patient underwent a liver biopsy on a new-onset lesion (LB2), which showed a phenotypic switch to SCLC.

Following LB2, primary cell line establishment was attempted. Tissue from liver metastasis was enzymatically digested using the Tumor Dissociation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), and the gentleMACS™ Dissociator (Miltenyi Biotec) was used for the mechanical dissociation in a closed and sterile system. The single-cell suspension was cultured in a 1:1 ratio of Ham's nutrient mixture F-12:DMEM, 10% FBS, 2 mM glutamine, 1× mammary epithelial growth supplement (MEGS, Life Technologies Corp., CA), and a Rho-associated protein kinase (ROCK) inhibitor.

A drug screening was performed in the primary cell culture testing osimertinib alone, cisplatin plus etoposide,

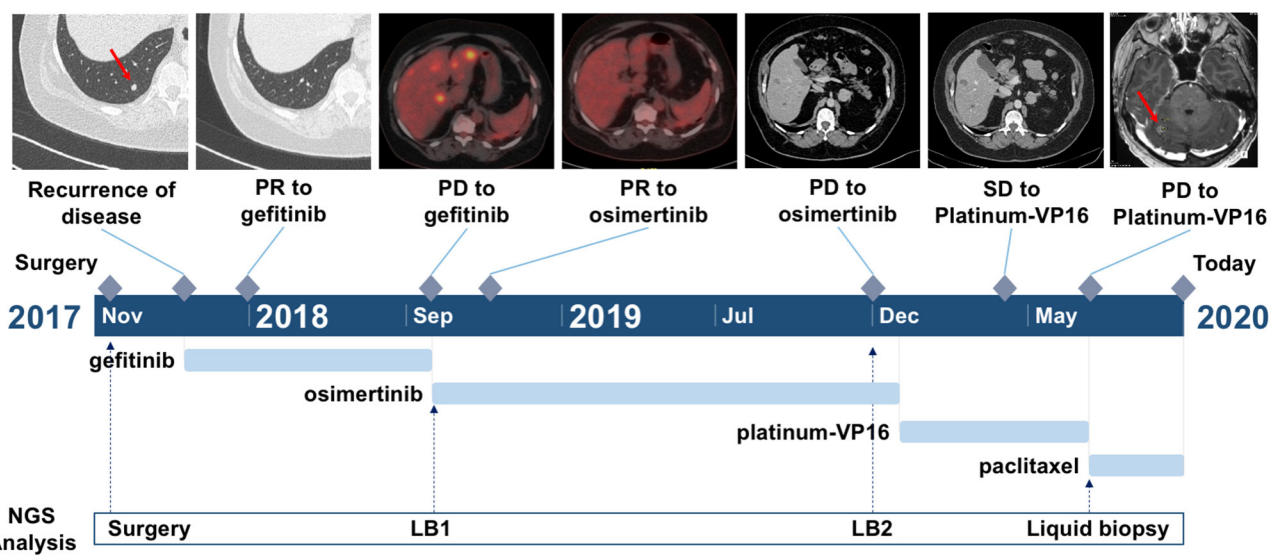

FIGURE 1 | Timeline of the clinical course of the patient. Arrows indicate the link between the clinical event and the date of liquid biopsy time point.

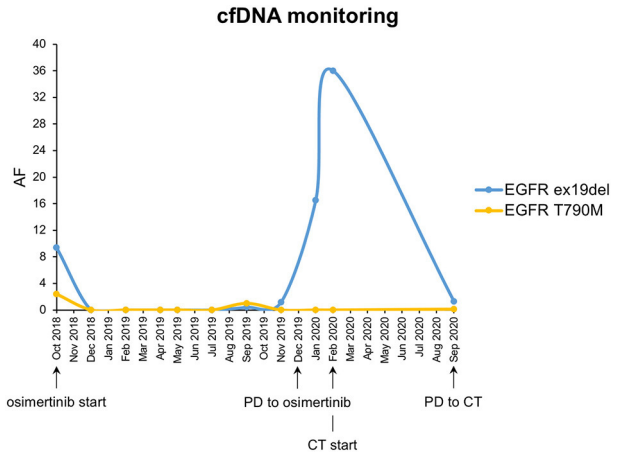

FIGURE 2 | CfDNA monitoring of *EGFR* ex19del and *EGFR* T790M mutations. Each dot corresponds to a different liquid biopsy time point. AF, allele frequency; CT, chemotherapy with platinum–etoposide.

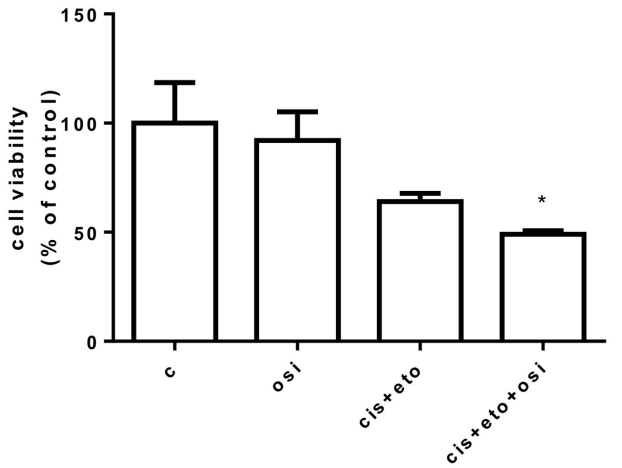

FIGURE 3 | Drug screening in a primary cell line following osimertinib resistance. Primary cells were treated with 250 nM osimertinib, 0.150 μg/ml etoposide, and 0.05 μg/ml cisplatin. After 72 h, cell proliferation was assessed by CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). Data are expressed as percent of cell viability vs. control cells and are means ± SD of three measurements. *$p < 0.05$.

and osimertinib combined with cisplatin plus etoposide. As shown in **Figure 3**, tumor cells were sensitive to the combination of osimertinib with chemotherapeutic agents ($p < 0.05$ vs. control) even if the results did not reach statistical significance vs. single drug treatments. Unfortunately, after a few weeks, the cells stopped their proliferation and it was not possible to perform additional experiments and to establish a stable cell line.

The patient underwent platinum–etoposide doublet in February 2020, and chemotherapy granted stability of the disease, as documented at the CT scan after three cycles. Unfortunately, following further three cycles, the patient experienced liver progression and central nervous system (CNS) progression due to the onset of multiple brain metastases (**Figure 1**). A further liver biopsy (LB3) was conducted on a new-onset liver lesion, with diagnosis of pure adenocarcinoma. At the time of writing the manuscript, the patient has been receiving weekly paclitaxel and whole-brain radiotherapy was performed. A liquid biopsy was carried out at the time of chemotherapy switch.

SCLC transformation clearly emerged as a mechanism of resistance to osimertinib. Nonetheless, next-generation sequencing (NGS) and immunohistochemistry (IHC) of Rb1 and p53 were performed in order to characterize the proficiency of the initial tumor to evolve in a neuroendocrine differentiated subtype. DNA was extracted from the liver biopsy undertaken before osimertinib treatment (LB1) and on SCLC-transformed liver lesion at osimertinib progression (LB2). Molecular analyses on LB3 were not conducted due to insufficient material. NGS study was performed with Solid Tumor Solution panel, Sophia Genetics, on MiSeq Platform, Illumina. No other putative resistance mechanisms to osimertinib were underlined (**Table 1**) and variants on *TP53* were found on LB1. The presence of those variants was retrospectively confirmed by NGS also in the lobectomy samples.

Expression of p53 and Rb1 was evaluated with IHC on lobectomy tissue, LB1 and LB2, as previously described (3). IHC analysis was not performed on LB3 due to insufficient material. The evaluation of Rb1 showed the complete absence of expression in all analyzed samples, while p53 presented an abnormal pattern of expression consistent with inactivation. In fact, p53 was negative in the surgery tissue, overexpressed in LB1, and scattered positive in LB2 (**Figure 4**).

Moreover, NGS analysis was carried out on liquid biopsy collected after disease progression to third-line platinum–etoposide with AVENIO ctDNA Expanded Panel, Roche, on NextSeq Platform, Illumina. Interestingly, *EGFR* T790M showed up again with the known activating mutation and with two *TP53* non-sense variants (**Table 1**). The presence of *EGFR* mutations on liquid biopsy were confirmed also with ddPCR (**Figure 2**).

LITERATURE REVIEW

Incidence of SCLC Transformation in Osimertinib-Resistant Population

Dissecting the mechanisms of acquired resistance to osimertinib and other third-generation EGFR-TKIs represents an area of active investigation (2, 4, 5). Nonetheless, the role of histologic transformations, and more specifically SCLC switch, remains partly uncovered. This could be ascribed to the lack of analyses conducted on tissue samples; in fact, in both registrational trials, AURA and FLAURA, the delineation of genomic profiles of osimertinib-resistant NSCLC has been performed by NGS on plasma samples (6–8). Tissue biopsy at the time of progression to osimertinib plays a critical role in order to unravel SCLC

TABLE 1 | NGS analyses on available samples.

	Surgery (allelic frequency, %)	Pre-osimertinib	PD to osimertinib	PD to platinum–etoposide
		Tissue_LB1 (allelic frequency, %)	Tissue_LB2 (allelic frequency, %)	Liquid biopsy (allelic frequency, %)
EGFR p.Glu746_Ala750del	49.60	64.50	45.30	1.30
EGFR T790M	–	18.40	–	0.13
TP53 p.Gln375*	50.60	66.70	38.10	0.47
TP53 p.His193Leu	25.4	31.80	19.70	–
BRAF p.Leu441Ile	–	5.70	–	–
NRAS p.Phe141Leu	–	5.40	–	–

The symbol "" means an amino acid change in a stop codon (Ter, *) according to the Sequence Variant Nomenclature of Human Genome Variation Society.*

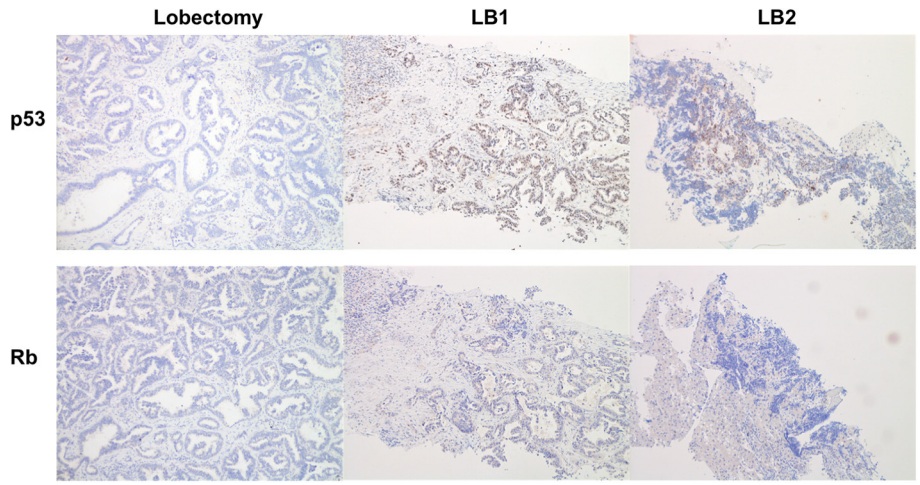

FIGURE 4 | Immunohistochemistry analysis for p53 (upper line) and Rb (lower line). The resected tumor (lobectomy) showed a negativity for both p53 and Rb. In the first liver biopsy (LB1), p53 was overexpressed, whereas Rb was negative; the histotype of LB1 is that of a NSCLC/adenocarcinoma with morphology similar to lobectomy. In the second liver biopsy (LB2), we detected only scattered cells positive for p53 and negative for Rb; the histotype is that of a SCLC also documented by positivity for neuroendocrine markers.

transformation (9). Recent data from the six largest series of osimertinib-resistant cases to date reported an overall incidence of SCLC transformation ranging from 2 to 15% (10). As documented by Oxnard et al., among 28 patients who developed disease progression to second-line osimertinib and lost T790M, SCLC transformation resulted the most frequent mechanism of resistance accounting for 21% of all the recognized causes (11). Along the same line, Lee et al. reported small cell transformation as EGFR-independent resistance process in 5 over 36 tested cases (12). A lower proportion of SCLC-switched cases (4–6%) was present in "first-line" and "latter-line" cohorts included in the study by Schoenfeld et al. (13) and in osimertinib-resistant population described by Piotrowska et al. (14) and Michels et al. (15). In the real-world study conducted by Le et al., although potentially affected by the pure retrospective nature of the analysis, the incidence significantly decreased to 2% (16).

Underlying Pathogenetic Mechanisms

Although histologic transformation is a well-known phenomenon, the comprehension of how it occurs and leads to EGFR-TKI resistance is still incomplete. SCLC transformation was first described in 2006 in a 45-year-old never-smoker woman with advanced *EGFR*-mutant adenocarcinoma after erlotinib failure (17). Since this initial observation, several additional cases have been reported (18), all confirmed by positive immunohistochemical staining for synaptophysin, chromogranin, or CD56/NCAM.

Different hypotheses have been proposed concerning the origin of SCLC as a mechanism of resistance to EGFR-TKIs. Initial studies on SCLC-transformed cancers have revealed relevant similarities to *de novo* SCLCs, most remarkably the inactivation of tumor suppression *via RB1* (19) and *TP53* (20). SCLC and NSCLC histologies might coexist within the same initial tumor, with the SCLC subtype becoming dominant after an initial response to EGFR-TKIs (18). Conversely, other lines of evidence support the assumption of a trans-differentiation process of the original *EGFR*-mutated adenocarcinoma under the pressure of TKIs (18, 21). Of interest, most of the SCLC-switched tumors retained the same *EGFR* mutation after transformation (22).

Potential Predictors of SCLC Transformation and Therapeutic Options

The identification of biomolecular mediators of treatment-dependent SCLC transformation represents a fundamental goal to subsequently develop therapeutic interventions. Triple mutant adenocarcinomas (*EGFR/RB1/TP53*) are considered at higher risk for transformation to SCLC (23). Moreover, a rapid increase in the serum levels of neurone specific enolase (NSE) together with a poor response to EGFR-TKIs usually indicates a transformation from adenocarcinoma to SCLC (24). Along the same line, the assessment of the pro-gastrin-releasing peptide (pro-GRP) has also been recommended for the early prediction of disease transformation (25).

Since most of the SCLC-transformed cases harbor typical neuroendocrine differentiation, platinum–etoposide chemotherapy remains the current standard treatment at the time of SCLC switch (26). Even though SCLC-transformed cases achieve similar objective response rate from chemotherapy than primary SCLC (around 80%), the prognosis of the former is usually worse than the latter even after a favorable response (27). Ferrer et al. recently performed a retrospective study on 61 SCLC-transformed cases, either with *EGFR* mutation or not (22). In this study, overall survival (OS) from the initial diagnosis was lower in the *EGFR*-mutated group compared with the non-*EGFR*-mutant group; however, OS from the time of transformation into SCLC was comparable between the two groups (22).

The early introduction of platinum–etoposide chemotherapy along with osimertinib may act as an effective therapeutic strategy to eradicate emerging SCLC subclones and prevent the phenotypic transformation in *EGFR*-mutated patients with a high risk of SCLC switch (ongoing clinical trial, Clinicaltrials.gov: NCT03567642). Other approaches that might be pursued involve targeting cell cycle vulnerabilities generated upon *RB1* loss through the use of Aurora kinase (AURKA or AURKB) inhibitors (28, 29) or applying epigenetic therapy, mainly directly against reprogramming factors such as *EZH2* (30).

DISCUSSION

In the present study, we reported a case of osimertinib resistance driven by SCLC switch in an *EGFR*-mutated NSCLC patient. At the time of progression to osimertinib, due to phenotypic transformation, our patient received standard platinum–etoposide chemotherapy, achieving only modest clinical benefit.

Overall, it could be difficult to determine whether SCLC arises by transformation from NSCLC, rather than being a new tumor or being present simultaneously with the NSCLC from the beginning. Since SCLC is characterized by rapid growth and is not controlled by EGFR-TKIs, a simultaneous SCLC–NSCLC mixed tumor is expected to recur quickly. Our patient benefited from ∼2 years of EGFR-TKIs; hence, it is unlikely that SCLC was part of the initial presentation.

A fundamental issue is represented by the identification of biomarkers able to predict SCLC transformation. Current evidence supports *TP53* and *RB1* mutations as potential predictors of phenotypic switch in *EGFR*-mutated NSCLC (23). In our case, histological examination at diagnosis showed the complete absence of p53 and Rb at IHC, likely underlying *TP53* and *RB1* baseline alterations. In addition, *TP53* mutations were detected by tissue NGS analysis before starting osimertinib, suggesting that the patient had a high risk of developing SCLC as a resistance mechanism.

To date, platinum–etoposide chemotherapy is the only viable treatment approach with a confirmed clinical efficacy in counteracting SCLC after failure of EGFR-TKIs. Given the positive results of the exploratory analysis of the IMpower150 trial in *EGFR*-mutated patients (31), a combination strategy of carboplatin–paclitaxel plus atezolizumab and bevacizumab after failure of previous EGFR-TKIs could be envisaged in this peculiar subset of patients, considering the proven efficacy of chemotherapy plus atezolizumab for the frontline treatment of extensive stage SCLC (32). Whether continued EGFR-TKI might gain additional clinical benefit is still a matter of debate, considering that SCLC is generally resistant to EGFR inhibition. Against this notion, a recent study reported a successful treatment with osimertinib in a synchronous SCLC and adenocarcinoma histology (33).

The continuation of osimertinib and its potential association with chemotherapy is still under investigation (34), also in view of the results obtained from the phase III IMPRESS study that did not demonstrate any PFS or OS improvement by continuing gefitinib vs. placebo in combination with second-line, platinum-based chemotherapy in *EGFR*-mutated NSCLC (35). In our report, drug screening assay on primary cell cultures from post-osimertinib biopsy showed increased sensitivity to the combination of osimertinib with chemotherapeutic drugs compared with control ($p < 0.5$), suggesting a potential effective therapeutic option. Moreover, it is likely that the progression to platinum–etoposide was driven by the *EGFR*-positive component in our case. To support this, the liver biopsy performed after chemotherapy (LB3) showed pure adenocarcinoma histology, and the liquid biopsy revealed the restoration of *EGFR* T90M. In this view, we assume that the interruption of EGFR pressure might have unleashed *EGFR*-positive clones resulting in inexorable treatment failure. Considering that our patient experienced CNS disease progression to platinum–etoposide, the excellent CNS penetration of osimertinib and its effectiveness on brain metastases might lead to continue osimertinib along with platinum-based chemotherapy in this occurrence.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found here: NCBI BioProject, Accession No: PRJNA698448.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided

their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AL, RM, and MT designed the manuscript. AL and GM collected the clinical data and performed the literature review. RM conducted the molecular analyses. LG and NC conducted the IHC analyses. SL and RA isolated tumor cells from biopsy tissue and performed the drug screening assay. AO performed the liver biopsies. LV performed the thoracic surgery. All the authors contributed to the writing of the manuscript and approved the submitted version.

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REFERENCES

1. *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) - Non-Small Cell Lung Cancer - Version 4.2021* (2021). Available online at: https://www.nccn.org/ (accessed April 9, 2021).
2. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E, Tiseo M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *Br J Cancer*. (2019) 121:725–37. doi: 10.1038/s41416-019-0573-8
3. Minari R, Bordi P, Del Re M, Facchinetti F, Mazzoni F, Barbieri F, et al. Primary resistance to osimertinib due to SCLC transformation: issue of T790M determination on liquid re-biopsy. *Lung Cancer*. (2018) 115:21–7. doi: 10.1016/j.lungcan.2017.11.011
4. Schmid S, Li JJN, Leighl NB. Mechanisms of osimertinib resistance and emerging treatment options. *Lung Cancer*. (2020) 147:123–9. doi: 10.1016/j.lungcan.2020.07.014
5. Schoenfeld AJ, Yu HA. The evolving landscape of resistance to osimertinib. *J Thorac Oncol*. (2020) 15:18–21. doi: 10.1016/j.jtho.2019.11.005
6. Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med*. (2017) 376:629–40. doi: 10.1056/NEJMoa1612674
7. Papadimitrakopoulou VA, Wu Y-L, Han J-Y, Ahn M-J, Ramalingam SS, John T, et al. Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. *Ann Oncol*. (2018) 29:viii741. doi: 10.1093/annonc/mdy424.064
8. Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. (2018) 378:113–25. doi: 10.1056/NEJMoa1713137
9. Piotrowska Z, Hata AN. Resistance to first-line osimertinib in EGFR-mutant NSCLC: tissue is the issue. *Clin Cancer Res*. (2020) 26:2441–3. doi: 10.1158/1078-0432.CCR-20-0097
10. Piper-Vallillo AJ, Sequist LV, Piotrowska Z. Emerging treatment paradigms for EGFR-mutant lung cancers progressing on osimertinib: a review. *J Clin Oncol*. (2020) 38:2926–36. doi: 10.1200/JCO.19.03123
11. Oxnard GR, Hu Y, Mileham KF, Husain H, Costa DB, Tracy P, et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. *JAMA Oncol*. (2018) 4:1527–34. doi: 10.1001/jamaoncol.2018.2969
12. Lee J, Kim HS, Lee B, Kim HK, Sun JM, Ahn JS, et al. Genomic landscape of acquired resistance to third-generation EGFR tyrosine kinase inhibitors in EGFR T790M-mutant non-small cell lung cancer. *Cancer*. (2020) 126:2704–12. doi: 10.1002/cncr.32809
13. Schoenfeld AJ, Chan JM, Kubota D, Sato H, Rizvi H, Daneshbod Y, et al. Tumor analyses reveal squamous transformation and off-target alterations as early resistance mechanisms to first-line osimertinib in EGFR-mutant lung cancer. *Clin Cancer Res*. (2020) 26:2654–63. doi: 10.1158/1078-0432.CCR-19-3563
14. Piotrowska Z, Isozaki H, Lennerz JK, Gainor JF, Lennes IT, Zhu VW, et al. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. *Cancer Discov*. (2018) 8:1529–39. doi: 10.1158/2159-8290.CD-18-1022
15. Michels S, Heydt C, van Veggel B, Deschler-Baier B, Pardo N, Monkhorst K, et al. Genomic profiling identifies outcome-relevant mechanisms of innate and acquired resistance to third-generation epidermal growth factor receptor tyrosine kinase inhibitor therapy in lung cancer. *JCO Precis Oncol*. (2019) 3:PO.18.00210. doi: 10.1200/PO.18.00210
16. Le X, Puri S, Negrao MV, Nilsson MB, Robichaux J, Boyle T, et al. Landscape of EGFR-dependent and -independent resistance mechanisms to osimertinib and continuation therapy beyond progression in EGFR-mutant NSCLC. *Clin Cancer Res*. (2018) 24:6195–203. doi: 10.1158/1078-0432.CCR-18-1542
17. Zakowski MF, Ladanyi M, Kris MG. EGFR mutations in small-cell lung cancers in patients who have never smoked [18]. *N Engl J Med*. (2006) 355:213–5. doi: 10.1056/NEJMc053610
18. Oser MG, Niederst MJ, Sequist LV, Engelman JA. Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin. *Lancet Oncol*. (2015) 16:e165–72. doi: 10.1016/S1470-2045(14)71180-5
19. Niederst MJ, Sequist LV, Poirier JT, Mermel CH, Lockerman EL, Garcia AR, et al. RB loss in resistant EGFR mutant lung adenocarcinomas that transform to small-cell lung cancer. *Nat Commun*. (2015) 6:6377. doi: 10.1038/ncomms7377
20. Lee J-K, Lee J, Kim S, Kim S, Youk J, Park S, et al. Clonal history and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. *J Clin Oncol*. (2017) 35:3065–74. doi: 10.1200/JCO.2016.71.9096
21. Shaurova T, Zhang L, Goodrich DW, Hershberger PA. Understanding lineage plasticity as a path to targeted therapy failure in EGFR-mutant non-small cell lung cancer. *Front Genet*. (2020) 11:1–15. doi: 10.3389/fgene.2020.00281
22. Ferrer L, Giaj Levra M, Brevet M, Antoine M, Mazieres J, Rossi G, et al. A brief report of transformation from NSCLC to SCLC: molecular and therapeutic characteristics. *J Thorac Oncol*. (2019) 14:130–4. doi: 10.1016/j.jtho.2018.08.2028
23. Offin M, Chan JM, Tenet M, Rizvi HA, Shen R, Riely GJ, 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) 14:1784–93. doi: 10.1016/j.jtho.2019.06.002
24. Liu Y. Small cell lung cancer transformation from EGFR-mutated lung adenocarcinoma: a case report and literatures review. *Cancer Biol Ther*. (2018) 19:445–9. doi: 10.1080/15384047.2018.1435222
25. Norkowski E, Ghigna MR, Lacroix L, Le Chevalier T, Fadel É, Dartevelle P, et al. Small-cell carcinoma in the setting of pulmonary adenocarcinoma: new insights in the era of molecular pathology. *J Thorac Oncol*. (2013) 8:1265–71. doi: 10.1097/JTO.0b013e3182a407fa
26. van Meerbeeck JP, Fennell DA, De Ruysscher DKM. Small-cell lung cancer. *Lancet*. (2011) 378:1741–55. doi: 10.1016/S0140-6736(11)60165-7
27. Jiang S-Y, Zhao J, Wang M-Z, Huo Z, Zhang J, Zhong W, et al. Small-cell lung cancer transformation in patients with pulmonary adenocarcinoma. *Medicine (Baltimore)*. (2016) 95:e2752. doi: 10.1097/MD.0000000000002752
28. Gong X, Du J, Parsons SH, Merzoug FF, Webster Y, Iversen PW, et al. Aurora a kinase inhibition is synthetic lethal with loss of the RB1 tumor suppressor gene. *Cancer Discov*. (2019) 9:248–63. doi: 10.1158/2159-8290.CD-18-0469
29. Oser MG, Fonseca R, Chakraborty AA, Brough R, Spektor A, Jennings RB, et al. Cells lacking the RB1 tumor suppressor gene are hyperdependent on aurora B kinase for survival. *Cancer Discov*. (2019) 9:230–47. doi: 10.1158/2159-8290.CD-18-0389

30. Ishak CA, Marshall AE, Passos DT, White CR, Kim SJ, Cecchini MJ, et al. An RB-EZH2 complex mediates silencing of repetitive DNA sequences. *Mol Cell*. (2016) 64:1074–87. doi: 10.1016/j.molcel.2016.10.021
31. Mok TSK, Socinski MA, Reck M, Jotte RM, Lim DW-T, Cappuzzo F, et al. IMpower150: an exploratory analysis of efficacy outcomes in patients with EGFR mutations. *Ann Oncol*. (2018) 29:ix177–8. doi: 10.1093/annonc/mdy483.008
32. Horn L, Mansfield AS, Szczesna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. *N Engl J Med*. (2018) 379:2220–9. doi: 10.1056/NEJMoa1809064
33. Batra U, Nathany S, Sharma M, Mehta A, Jain P, Bansal A. Successful treatment of EGFR-mutant synchronous SCLC and lung adenocarcinoma with osimertinib. *JTO Clin Res Rep*. (2021) 2:100098. doi: 10.1016/j.jtocrr.2020.100098
34. Jänne P, Planchard D, Howarth P, Todd A, Kobayashi K. OA07.01 osimertinib plus platinum/pemetrexed in newly-diagnosed advanced EGFRm-positive NSCLC; the phase 3 FLAURA2 study. *J Thorac Oncol*. (2019) 14:S222–3. doi: 10.1016/j.jtho.2019.08.441
35. Soria JC, Wu YL, Nakagawa K, Kim SW, Yang JJ, Ahn MJ, et al. Gefitinib plus chemotherapy versus placebo plus chemotherapy in EGFR-mutation-positive non-small-cell lung cancer after progression on first-line gefitinib (IMPRESS): a phase 3 randomised trial. *Lancet Oncol*. (2015) 16:990–8. doi: 10.1016/S1470-2045(15)00121-7

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Efficacy of Combination Docetaxel and Nintedanib in Advanced Non-Small Cell Lung Cancer in Thailand: A Multicenter Study

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Introduction: The mainstay systemic treatment for non-oncogenic addictive advanced stage non-small cell lung cancer is chemotherapy. Anti-angiogenic agents are additive compounds that enhance disease control and lead to improvement of overall survival benefit. Recently PD-(L)1 blockage, a checkpoint inhibitor, has been adopted as another line of treatment. A sequential strategy to enhance the efficacy of combination docetaxel and nintedanib after immunotherapy, correlated with genomic mutation, has been explored.

Method: A retrospective cohort study of 56 patients from 8 centers in Thailand who received combination docetaxel and nintedanib *via* the Thai nintedanib Named Patient Use program was conducted. Demographic characteristics, treatment details, and treatment responses were retrieved from medical records.

Results: The majority of patients were male (62.5%) with adenocarcinoma subtype (88%). Thirty-five percent had sensitizing *EGFR* mutation. Combination docetaxel and nintedanib was given as second to fourth line of treatment. Median PFS of docetaxel/nintedanib was 5.6 months [95% CI 4.8-6.9]. Median OS of the entire cohort was 22.5 months [95% CI 20.2-31.1]. Among them, only four patients received this combination after immunotherapy which limited the validity of efficacy analysis. Median PFS of those four patients was 7.9 months [range 5.2-9.1] which was slightly higher than the remaining cohort (median PFS 4.5 months, 95% CI: 4.0-6.0, *p*-value 0.09). Among the adenocarcinoma subtype, a relapse-time of platinum-doublet chemotherapy of more than 6 months was solely indicated as a benefit of combination docetaxel/nintedanib

treatment compared to the relapse-time of platinum-doublet chemotherapy of less than 6 months by multivariate HR of PFS 0.32 [95% CI: 0.14-0.68, *p*-value 0.003].

Conclusion: Combination docetaxel and nintedanib provided more benefit in relapse-time of platinum-doublet chemotherapy of more than 6 months in advanced stage adenocarcinoma lung cancer. Neither *EGFR* nor *ALK* alteration influenced the outcome of treatment.

Keywords: docetaxel, nintedanib, non-small cell lung cancer, sequential treatment, anti-angiogenesis therapy

INTRODUCTION

The development of current standard treatments of advanced non-small cell lung cancer has led to the improvement of survival outcome. Novel strategies adopting predictive biomarkers have guided treatment towards an era of precision medicine. Biomarker discoveries to define more targeted therapies have been explored in many clinical studies. For non-targetable advanced non-small cell lung cancer, which has no targeted therapy option, there seems to be fewer treatment opportunities and worse prognosis outcome (1). Combination antiangiogenic therapies and chemotherapy has improved the efficacy of treatment in non-small cell carcinoma lung cancer by normalizing abnormal tumor vasculature and enhancing tumor shrinkage. A combination of docetaxel/nintedanib was approved by the USFDA as a subsequent treatment after platinum-resistance in advanced adenocarcinoma lung cancer patients. Significant improvement in progression-free survival (PFS) with a median of 3.4 months vs. 2.7 months compared to placebo and docetaxel has been shown in the phase III LUME-lung 1 global study (2). Furthermore, PD-(L)1 blockage, a novel immunotherapy, has shown benefits for improving survival outcomes, either by monotherapy or combination with chemotherapy (3–5). Chemotherapy enhances the effect of immunotherapy by increasing recognition, eliminating tumor cells by the host immune system, and reducing the immunosuppressive microenvironment (6). Furthermore, preclinical reports revealed that VEGFR blockage inhibits suppressive immune cells (MDSC, Treg, macrophages) and increases mature dendritic cell results in delayed tumor growth (7, 8). Combination anti-angiogenic and PD-(L)1 blockage has shown significant tumor control (9). Sequence of immunotherapy before subsequent docetaxel/nintedanib treatment has also shown improved response to treatment in a retrospective cohort (10, 11).

The Thai non-squamous cell carcinoma of the lung has up to 57% predominated *EGFR* mutation (12), contrary to the Western non-squamous lung cancer population, which has less than 10% prevalence of *EGFR* mutation. Comparing the efficacy in our country to a global study that enrolled a majority of Caucasian patients might help us to understand the real benefits of treatment of Asian patients. We report a retrospective cohort study of advanced stage non-small lung cancer patients who received subsequent treatment of docetaxel/nintedanib after platinum-resistant advanced stage lung cancer to explore treatment efficacy in terms of *EGFR/ALK* alteration status and efficacy of treatment following immunotherapy.

Abbreviations: FDA, Food and Drug Administration; MDSC, Myeloid-derived suppressor cell; VEGFR, Vascular endothelial growth factor receptor.

MATERIALS AND METHODS

Study Participants

A retrospective study of fifty-six advanced non-small cell lung cancer patients who enrolled in the Thai nintedanib Named Patient Use program from eight centers across Thailand was conducted to evaluate the treatment efficacy of combination nintedanib and docetaxel as a treatment after platinum-doublet chemotherapy during 2017-2018. These eight centers included four hospitals in Bangkok: The King Chulalongkorn Memorial Hospital, Siriraj Hospital, Ramathibodi Hospital, and Rajavithi Hospital, and four provincial hospitals: Maharaj Nakorn Chiang Mai Hospital, Chiangmai, Srinakarin Hospital, Khon Kaen Hospital, and Songklanagarind Hospital, Songkhla. This study is a collaborative project of the Thai Society of Clinical Oncology: Lung Cancer Working Group.

All patients had either a cytologic or histologic confirmed diagnosis of NSCLC. Demographic characteristics were obtained from individual patients. Treatment decision, assessments, and follow-up were obtained from individual physicians as standard practice per institute through medical records. Patient death date was validated from The Bureau of Registration Administration, Ministry of Interior, Bangkok, Thailand. This study was approved by the Ethics Committee of each local institution: Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB 536/62), Faculty of Medicine, Siriraj Hospital, Mahidol University [IRB 349/2563 (EC4)], Faculty of Medicine, Khon Kaen University (HE631180), Faculty of Medicine, Ramathibodi Hospital, Mahidol University (IRB MURA2020/794), Faculty of Medicine, Chiang Mai University (IRB MED-2563-07205). Written informed consent was waived from individual study participants as permission from the director of each hospital was granted. Objective response and progression of disease were determined by local investigators using RECIST version 1.1 (13).

Statistical Analysis

Mann-Whitney *U* test was used to assess the difference between groups of non-parametric distributed variables. Chi-square or

Fisher's exact tests were used for categorical variables. There were varying lines of combination docetaxel/nintedanib treatment from second to fourth. Then progression free survival of docetaxel/nintedanib was defined as duration from start of docetaxel/nintedanib treatment to disease progression or death. Overall disease control rate was defined as the best response evaluation of complete remission and partial response by the provided physician. We applied RECIST criteria version 1.1 as the standard oncology practice in Thailand. Overall survival was defined as the duration from diagnosis of cancer to death from any cause or at censored time which was defined on December 31, 2019. A Survival curve comparison was performed using the Kaplan-Meier method and log-rank test. The cox proportion hazards regression analysis was used to estimate multivariate hazard ratios of progression-free survival and overall survival. A two-sided *p*-value of less than 0.05 was defined as statistically significant. All statistical analyses were carried out using R version 3.3.0.

RESULTS

Demographic Characteristics and Treatment Overview

From January 2017 to October 2018, 56 patients from eight centers who received combination docetaxel/nintedanib treatment in advanced stage were enrolled in this retrospective study. Patients in this retrospective cohort received nintedanib *via* Named Patient Use program following the criteria of having advanced non-small cell lung cancer with disease progression after platinum-doublet chemotherapy. Among them, 62.5% of patients were male with majority ECOG performance status of 0-1 (89.2%) and adenocarcinoma cell type (88%). Demographic characteristics and patient treatments are shown in **Tables 1**, **2**. *EGFR* and *ALK* testing were performed in 82.1% and 50%, respectively by using a standard platform of testing according to each institute. 35% had a sensitizing *EGFR* mutation that was composed of *EGFR* exon 19 deletion (n=12; 75%) and L858R (n=4; 25%). 61% of patients received more than three lines of treatment which included chemotherapy, EGFR TKI, and immunotherapy as combination or single agent. Pemetrexed, paclitaxel, and gemcitabine were commonly used as part of platinum-doublet chemotherapy at 34%, 30.3% and 23.2%, respectively. 88% of patients received 60 mg/m^2 instead of 75 mg/m^2 of docetaxel as the common Thai standard practice in advanced stage disease. Eight patients (14.2%) received immunotherapy as a line of standard treatment in advanced stage. Among them, four patients received immunotherapy before docetaxel/nintedanib combination treatment (**Table 2**).

TABLE 1 | Demographic characteristics of participants in this study.

Characteristic		N (%)
Sex	Male	35 (62.5%)
	Female	21 (37.5%)
Age at diagnosis	< 60 years	32 (57.1%)
	≥ 60 years	24 (42.9%)
ECOG performance status at recurrence/metastasis	0-1	50 (89.2%)
	≥2	6 (10.8%)
Smoking status	Current/Former smoker	27 (48%)
	Never smoker	29 (52%)
Histology	Adenocarcinoma	49 (88%)
	Squamous cell carcinoma	1(1.5%)
	Large cell carcinoma	1(1.5%)
	NSCLC NOS	5 (9%)
EGFR/ALK alteration	Exon 19 del (n=12)/ L858R (n=4)	16/46 (34.7%)
	ALK overexpression	2/28 (7%)
Reimbursement	Universal/social insurance	18 (32%)
	CSMBS/state enterprise	31 (55%)
	Out of pocket	7 (13%)
Initial stage at diagnosis	Advanced stage	46 (82%)
	Relapsed/recurrence	10 (18%)
Lines of treatment	< 3 regimens	22 (39.3%)
	≥ 3 regimens	34 (60.7%)

¶*ECOG performance status denotes the Eastern Cooperative Oncology Group (ECOG) scale; a performance status grade of 0 indicates asymptomatic; 1 restricted in strenuous activity but ambulatory; 2 ambulatory and capable of all self-care but unable to carry out any work activities.*

Outcome of Platinum-Doublet Chemotherapy

The majority of patients (80%) received platinum-doublet chemotherapy as a first-line metastatic setting. Among them, five patients received bevacizumab as part of a combination and maintenance treatment. The median cycle of platinum-doublet chemotherapy was five cycles [range 1-14]. Median PFS of platinum-based doublet chemotherapy was 5.6 months [range 0.5-48, 95% CI: 4.9-6.8]. 31 patients (55%) had disease progression in less than 6 months since the start of treatment, while 15 patients (26.7%) had disease progression in less than 3 months.

Outcome of Combination Docetaxel and Nintedanib

The median number of treatment cycles of docetaxel and nintedanib as part of combination docetaxel/nintedanib were 6

TABLE 2 | Summary treatment of participants in this study.

Details of treatment		N (%)
Previous platinum-doublet chemoRx regimen	Platinum-based/gemcitabine	13 (23.2%)
	Platinum-based/paclitaxel	17 (30.3%)
	Platinum-based/vinorelbine	1 (1.8%)
	Platinum-based/pemetrexed	19 (34%)
	Platinum-based/etoposide	1 (1.8%)
	Platinum-based/paclitaxel/bevacizumab	1 (1.8%)
	Platinum-based/gemcitabine/bevacizumab	2 (3.5%)
	Platinum-based/pemetrexed/bevacizumab	2 (3.5%)
Number of Rx lines before docetaxel/ nintedanib	One-line	36 (64%)
	Two-lines	11 (17%)
	Three-lines	5 (9%)
Relapse-time of platinum-doublet chemotherapy	< 3 months	15 (26.7%)
	< 6 months	31 (55%)
Sequence of immunoRx	Never received immunoRx	48 (86%%)
	Before docetaxel/nintedanib	4 (7%)
	After docetaxel/nintedanib	4 (7%)

[range 1-10] and 5 cycles [range 0-43], respectively. Overall disease control rate (DCR) of combination docetaxel/nintedanib was 57%. The median PFS was 5.6 months [range 0.25-45, 95% CI: 4.8-6.9] (**Figure 1B**) which was longer than median PFS in the LUME-lung 1 study (median PFS 3.4 months [95% CI 2.9-3.9]. Three patients (5%) stopped docetaxel/nintedanib after the first cycle due to intolerance/toxicities and response of treatment could not be evaluated. 10 patients (17%) and 20 patients (35%) had either interrupted or reduced doses of docetaxel and nintedanib, respectively. The prevalence of dose modification of nintedanib in our study was higher than the LUME lung I study (18.6%). Three patients continuing maintenance nintedanib beyond eight cycles of docetaxel and were censored on December 31, 2019.

Analysis According to Relapse-Time of Platinum-Double Chemotherapy

The efficacy of docetaxel/nintedanib disease control was categorized by relapse-time of platinum-doublet chemotherapy i.e. rapid (less than 3 months) or slow progressor (more than 3 months). For excluded patients who could not tolerate treatment, median PFS of combination docetaxel/nintedanib for rapid relapse-time of platinum-doublet chemotherapy was 3.6 months [range 1-3; 95% CI: 2.5-5.5] which was significantly shorter than slow progressor which had a median PFS of 6.4 months [range 1.2-43.9; 95% CI: 4.9-7.4, *p*-value 0.03]. Using a relapse-time of 6 months also represented shorter disease control from combination docetaxel/nintedanib than relapse-time of more than 6 months. Median PFS for patients who had a relapse-time of platinum-doublet chemotherapy of less than 6 months and more than 6 months were 3.8 months [range 1-24.4; 95% CI: 3.2-5.2] and 7.3 months [range 1.2-43.9; 95% CI: 5.1-8.6, *p*-value = 0.01), respectively (**Figure 1C**).

Analysis According to Sequence of Immunotherapy Treatment

The efficacy of docetaxel/nintedanib according to the sequence of immunotherapy, either before or after, was explored. Four patients who received combination docetaxel/nintedanib after immunotherapy had a median PFS of 7.9 months [range 5.2-9.1]. This duration seemed longer than the median PFS of the remaining patients (median PFS 4.5 months, range 0.25-43.9; 95% CI: 4.0-6.0, *p*-value 0.09). A response assessment was done in three patients and revealed a partial response rate of 50% and stable disease rate of 25%. One patient who received only one cycle of docetaxel/nintedanib after immunotherapy was not evaluated for response due to toxicity from treatment.

Cox Proportional Hazards Regression Model for Prognostic and Predictive Factors

The median OS of the entire cohort was 22.5 months [range 2.2-100.1; 95% CI 20.2-31.1] (**Figure 1A**). We evaluated prognostic factors of overall survival using cox proportional hazards regression model and applied it to all potential factors including age, ECOG, smoking status, histology, oncogenic alteration, relapse-time of platinum-doublet chemotherapy, line of treatment, and sequence of docetaxel/nintedanib after immunotherapy (**Table 3**) and found that none of them prognosticated survival in our study.

We further analyzed predictive factors of combination docetaxel/nintedanib to define which patient subgroup might benefit most from this treatment. Nevertheless, we restricted predictive factor analysis for combination docetaxel/nintedanib in only the adenocarcinoma subtype following the USFDA approval indication. Relapse-time of platinum-doublet of more than 6 months was correlated with longer PFS with the HR of 0.36 [95% CI 0.19-0.69]. It was also an independent predictive factor of progression-free survival from docetaxel/nintedanib with the multivariate HR of 0.32 [95% CI: 0.14-0.68, *p*-value 0.003] (**Table 4**). Relapse-time of platinum-doublet chemotherapy of more than 6 months provided benefits of combination docetaxel/nintedanib treatment compared to the relapse-time of platinum-doublet chemotherapy of less than 6 months.

DISCUSSION

Novel strategies to define treatment by adopting predictive biomarkers are currently accepted as standard practice. However,

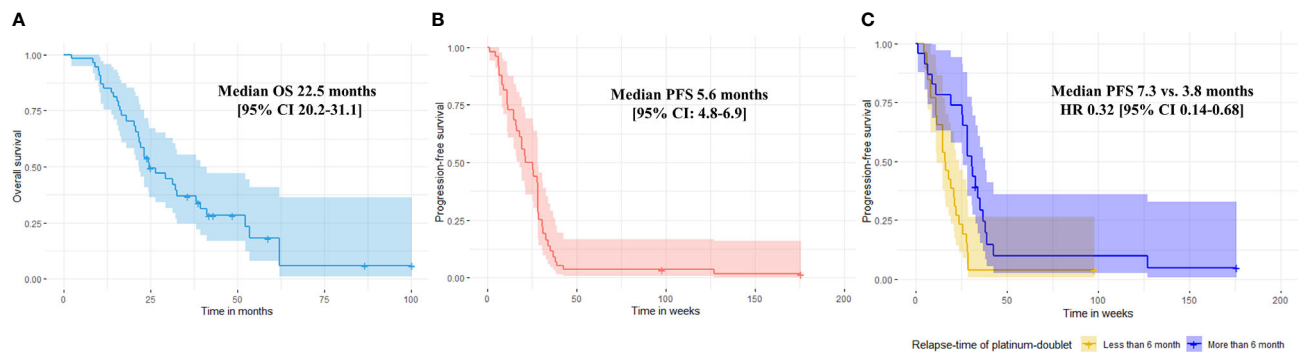

FIGURE 1 | Median overall survival **(A)** and median progression-free survival of combination docetaxel/nintedanib of the entire population **(B)**. Progression-free survival of combination docetaxel and nintedanib according to relapse-time of platinum-doublet chemotherapy. Median PFS were 7.3 months vs. 3.8 months for relapse-time of platinum doublet chemotherapy ≥ 6 months vs. < 6 months, respectively (multivariate HR 0.32 [95% CI: 0.14-0.68], *p*-value 0.003) **(C)**.

TABLE 3 | Univariate and multivariate analysis of prognostic factors to overall survival benefit including demographic characteristics, treatment by using Cox proportional hazards regression model.

Variables	Univariate, HR [95% CI]	*p*-value	Multivariate, HR [95% CI]	*p* value
Sex (Female vs. Male)	0.83 [0.45-1.53]	0.55	1.32 [0.34-5.13]	0.67
Age (≥ 60 vs. <60)	0.81 [0.45-1.48]	0.51	1.06 [0.40-2.75]	0.90
ECOG (0-1 vs. ≥2)	0.60 [0.25-1.43]	0.25	0.40 [0.12-1.26]	0.12
Smoking status (Never vs. Former/Current)	0.61 [0.33-1.13]	0.11	0.40 [0.12-1.37]	0.14
Histology (Adenocarcinoma vs. Non-adenocarcinoma)	0.44 [0.19-1.01]	0.05	0.47 [0.12-1.90]	0.29
EGFR/ALK alteration (Present vs. Absent)	0.58 [0.29-1.18]	0.13	0.79 [0.32-1.97]	0.62
Lines of treatment (≥ 3 vs.< 3)	0.59 [0.32-1.08]	0.08	0.64 [0.25-1.58]	0.33
Relapse-time of platinum-doublet (≥ 6 vs. < 6 months)	0.76 [0.42-1.38]	0.36	0.78 [0.34-1.79]	0.56
Sequence of docetaxel/nintedanib (after immunoRx vs. none)	1.26 [0.38-4.14]	0.69	3.72 [0.85-16.1]	0.07

TABLE 4 | Univariate and multivariate analyses of predictive factors of combination docetaxel/nintedanib treatment for adenocarcinoma subtype including demographic characteristics and treatment by using Cox proportional hazards regression model.

Variables	Univariate, HR [95% CI]	*p* value	Multivariate, HR [95% CI]	*p* value
Sex (Female vs. Male)	0.97 [0.53-1.80]	0.94	0.78 [0.27-2.23]	0.64
Age (≥ 60 vs. <60)	0.68 [0.38-1.22]	0.19	0.94 [0.43-2.07]	0.89
ECOG (0-1 vs. ≥2)	0.66 [0.23-1.86]	0.43	0.75 [0.24-2.39]	0.63
Smoking status (Never vs. Former/Current)	0.74 [0.41-1.34]	0.33	0.73 [0.27-1.96]	0.54
EGFR/ALK alteration (Present vs. Absent)	1.31 [0.67-2.57]	0.42	1.14 [0.53-2.44]	0.72
Relapse-time of platinum-doublet (≥ 6 vs. < 6 months)	0.36 [0.19-0.69]	0.001*	0.32 [0.14-0.68]	0.003*
Sequence of docetaxel/nintedanib (after immunoRx vs. none)	0.50 [0.15-1.65]	0.26	1.05 [0.30-3.72]	0.92

**Statistically significant.*

in the setting of subsequent treatment after disease progression, there are limitations of biomarker usage. Subsequent immunotherapy after platinum-doublet chemotherapy were explored in several randomized phase III trials to improve patient survival benefit and ensure quality of life (5, 14, 15). There was more progression disease and shorter PFS compared to the standard treatment arm of docetaxel. This could imply that a novel immune checkpoint inhibitor did have efficacy of long term durability and disease control in a limited number of patients in a second-line setting (11). Adding anti-angiogenesis such as nintedanib to docetaxel is another option that has been approved by the USFDA as second-line treatment after platinum-resistance in advanced NSCLC patients with adenocarcinoma subtype (2). However, there is no comparative efficacy of this combination to immunotherapy. The strategy to enhance treatment efficacy by modulating the sequence of treatment requires further elucidation. There are potential high objective response rates and PFS reports for the nintedanib/docetaxel treatment combination after immunotherapy in a case series from the Spanish Named patient used program (ORR 36%, median PFS 3.2 months [95%CI: 1.4-14.6]) (16) and the prospective non-interventional VARGADI cohort study (ORR 58%, PFS 5.5 months [95% CI: 1.9-8.7]) (10). In our series, albeit a small sample size to validate the results, the median PFS for patients who received combination nintedanib/docetaxel after immunotherapy (median PFS 7.9 months, range 5.2-9.1) was longer than the rest of the patients in this retrospective cohort (median PFS 4.5 months, range 0.25-43.9; 95% CI: 4.0-6.0, *p*-value 0.09). Among the adenocarcinoma subtype, the Cox proportional hazard regression analysis did not indicate superiority of sequential docetaxel/nintedanib after immunotherapy in terms of PFS with HR 0.50 [95% CI: 0.15-1.65, *p*-value 0.26] by univariate analysis and HR 1.05 [95% CI: 0.30-3.72, *p*-value 0.92] by multivariate analysis.

Advanced stage adenocarcinoma histology lung cancer patients who had rapid progression of platinum-doublet chemotherapy within 9 months had better outcomes when

adding nintedanib to docetaxel as a subsequent treatment, which can be translated to survival outcome (17, 18). Advanced stage non-small cell lung cancer with rapid progressive disease might not be fruitful for immunotherapy (19). However, none of our patients who received immunotherapy had rapid progression from platinum-doublet chemotherapy. This limits our ability to explore this issue. Furthermore, there was a smaller proportion than the general prevalence of sensitizing *EGFR* mutation in this cohort (34.7%) which represented physician selections of preferred non-oncogenic addicted advanced stage lung cancer for anti-angiogenic treatment. However, among the adenocarcinoma subtype, neither *EGFR* nor *ALK* alteration impacts the outcome of this combination. A relapse time of platinum-doublet chemotherapy of more than 6 months solely indicated the benefit of combination docetaxel/nintedanib treatment compared to the relapse time of platinum-doublet chemotherapy of less than 6 months by multivariate HR of PFS 0.32 [95% CI: 0.14-0.68, *p*-value 0.003].

We would like to declare our study limitations. First, the retrospective cohort prohibits us to retrieve complete information, for example, toxicity of treatment and precise time of imaging evaluation. There might be variations among each center that provided treatment for patients. The treatment lines of combination nintedanib/docetaxel varied from second to forth line. Heavy pretreatment chemotherapy might evolve resistance clones than limited-line treatment. Moreover, the median PFS of combination docetaxel/nintedanib in our study (5.6 months, 95% CI 4.8-6.9) was longer than the LUME lung-1 study (3.4 months, 95% CI 2.9-3.9). The higher frequency of imaging evaluation in LUME lung-1 [first at 4-weeks and then every 6- weeks after randomization compared to our usual standard practice (every 9-weeks)] probably explains this finding. Lastly, even though the general global recommended dosage of docetaxel is 75 mg/m2, in Thailand, the standard used is 60 mg/m2. No direct comparison between dosage efficacy has been reported, however, results from a prospective randomized phase IIb (SENECA study) revealed less toxicity, such as afebrile neutropenia and mucositis of combination nintedanib/docetaxel from the lower dosage (33 mg/m^2 D1,D8) of docetaxel, without any compromise of efficacy (20).

DATA AVAILABILITY STATEMENT

Due to confidentiality agreements, supporting data can only be made available to bona fide researchers subject to a non-disclosure agreement. Please contact the corresponding author (chanida.vi@chula.ac.th).

ETHICS STATEMENT

This study was approved by the Ethics Committee of each local institution: Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB 536/62), Faculty of Medicine, Siriraj Hospital, Mahidol University (IRB 349/2563 (EC4)), Faculty of Medicine, Khon Kaen University (HE631180), Faculty of Medicine, Ramathibodi Hospital, Mahidol University (IRB MURA2020/794), and Faculty of Medicine, Chiang Mai University (IRB MED-2563-07205). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conception and design: CV. Administrative support: CV. Provision of study materials or patients: KK, PD, TR, BC, JC, KM, CS, and LT. Collection and assembly of data: KK and CV. Data analysis and interpretation: CV. Manuscript writing: all authors. Final approval of manuscript: all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.572740/full#supplementary-material

REFERENCES

1. Kris MG, Johnson BE, Berry LD, Kwiatkowski DJ, Iafrate AJ, Wistuba II, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA* (2014) 311:1998–2006. doi: 10.1001/jama.2014.3741
2. Reck M, Kaiser R, Mellemgaard A, Douillard JY, Orlov S, Krzakowski M, et al. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. *Lancet Oncol* (2014) 15:143–55. doi: 10.1016/S1470-2045(13)70586-2
3. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* (2018) 378:2078–92. doi: 10.1056/NEJMoa1801005
4. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* (2015) 372:2018–28. doi: 10.1056/NEJMoa1501824
5. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* (2017) 389:255–65. doi: 10.1016/S0140-6736(16)32517-X

6. Leonetti A, Wever B, Mazzaschi G, Assaraf YG, Rolfo C, Quaini F, et al. Molecular basis and rationale for combining immune checkpoint inhibitors with chemotherapy in non-small cell lung cancer. *Drug Resist Updat* (2019) 46:100644. doi: 10.1016/j.drup.2019.100644
7. Roland CL, Lynn KD, Toombs JE, Dineen SP, Udugamasooriya DG, Brekken RA. Cytokine levels correlate with immune cell infiltration after anti-VEGF therapy in preclinical mouse models of breast cancer. *PloS One* (2009) 4: e7669. doi: 10.1371/journal.pone.0007669
8. Manning EA, Ullman JG, Leatherman JM, Asquith JM, Hansen TR, Armstrong TD, et al. A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. *Clin Cancer Res* (2007) 13:3951–9. doi: 10.1158/1078-0432.CCR-07-0374
9. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med* (2018) 378:2288–301. doi: 10.1056/NEJMoa1716948
10. Grohe C, Gleiber W, Haas S, Losem C, Mueller-Huesmann H, Schulze M, et al. Nintedanib plus docetaxel after progression on immune checkpoint inhibitor therapy: insights from VARGADO, a prospective study in patients with lung adenocarcinoma. *Future Oncol* (2019) 15:2699–706. doi: 10.2217/fon-2019-0262
11. Horn L, Spigel DR, Vokes EE, Holgado E, Ready N, Steins M, et al. Nivolumab Versus Docetaxel in Previously Treated Patients With Advanced Non-Small-Cell Lung Cancer: Two-Year Outcomes From Two Randomized, Open-Label, Phase III Trials (CheckMate 017 and CheckMate 057). *J Clin Oncol* (2017) 35:3924–33. doi: 10.1200/JCO.2017.74.3062
12. Sriuranpong V, Chantranuwat C, Huapai N, Chalermchai T, Leungtaweeboon K, Lertsanguansinchai P, et al. High frequency of mutation of epidermal growth factor receptor in lung adenocarcinoma in Thailand. *Cancer Lett* (2006) 239:292–7. doi: 10.1016/j.canlet.2005.08.029
13. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* (2000) 92:205–16. doi: 10.1093/jnci/92.3.205
14. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* (2015) 373:1627–39. doi: 10.1056/NEJMoa1507643
15. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* (2016) 387:1540–50. doi: 10.1016/S0140-6736(15)01281-7
16. Corral J, Majem M, Rodriguez-Abreu D, Carcereny E, Cortes AA, Llorente M, et al. Efficacy of nintedanib and docetaxel in patients with advanced lung adenocarcinoma treated with first-line chemotherapy and second-line immunotherapy in the nintedanib NPU program. *Clin Transl Oncol* (2019) 21:1270–9. doi: 10.1007/s12094-019-02053-7
17. Gaschler-Markefski B, Sikken P, Heymach JV, Gottfried M, Mellemgaard A, Novello S, et al. Time since start of first-line therapy as a predictive clinical marker for nintedanib in patients with previously treated non-small cell lung cancer. *ESMO Open* (2017) 2:e000102. doi: 10.1136/esmoopen-2016-000102
18. Gottfried M, Bennouna J, Bondarenko I, Douillard JY, Heigener DF, Krzakowski M, et al. Efficacy and Safety of Nintedanib Plus Docetaxel in Patients with Advanced Lung Adenocarcinoma: Complementary and Exploratory Analyses of the Phase III LUME-Lung 1 Study. *Target Oncol* (2017) 12:475–85. doi: 10.1007/s11523-017-0517-2
19. Garde-Noguera J, Martin-Martorell P, De Julian M, Perez-Altozano J, Salvador-Coloma C, Garcia-Sanchez J, et al. Predictive and prognostic clinical and pathological factors of nivolumab efficacy in non-small-cell lung cancer patients. *Clin Transl Oncol* (2018) 20:1072–9. doi: 10.1007/s12094-017-1829-5
20. Capelletto E, Migliorino MR, Morabito A, Chiari R, Grossi F, Tiseo M, et al. Final results of the SENECA (SEcond line NintEdanib in non-small cell lung CAncer) trial. *Lung Cancer* (2019) 134:210–7. doi: 10.1016/j.lungcan.2019.06.028

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Resistance Profile of Osimertinib in Pre-treated Patients With EGFR T790M-Mutated Non-small Cell Lung Cancer

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Background: Osimertinib efficacy in pre-treated patients with epidermal growth factor receptor (EGFR) T790M-mutated non-small cell lung cancer (NSCLC) has been demonstrated in clinical trials, but real-world data, particularly regarding resistance profile, remains limited. This study aims to analyze the resistance mechanisms acquired after treatment with Osimertinib.

Methods: Clinical outcomes and molecular results from re-biopsies at the time of osimertinib progression of EGFR T790M-mutated NSCLC patient were analyzed.

Results: Twenty-one patients with stage IV adenocarcinoma were included [median 69 years; 57.1% female; 85.7% never-smokers; 23.8% ECOG performance status (PS) ≥2]. Median PFS and OS were 13.4 (95% CI: 8.0–18.9) and 26.4 (95% IC: 8.9–43.8) months, respectively. At the time of analysis, 10 patients had tumor progression (47.6%). T790M loss occurred in 50%, being associated with earlier progression (median PFS 8.1 vs. 21.4 months, $p = 0.011$). Diverse molecular alterations were identified, including C797S mutation ($n = 1$), PIK3CA mutation ($n = 2$), MET amplification ($n = 1$), CTNNB1 mutation ($n = 1$), and DCTN1-ALK fusion ($n = 1$). Histological transformation into small cell carcinoma occurred in one patient.

Conclusions: This real-world life study highlights the relevance of re-biopsy at the time of disease progression, contributing to understand resistance mechanisms and to guide treatment strategies.

Keywords: non-small cell lung cancer, EGFR T790M mutation, osimertinib, resistance, real-world data, next generation sequencing

INTRODUCTION

Patients with advanced non-small-cell lung cancer (NSCLC) with activating mutations in the epidermal growth factor receptor (EGFR) gene are eligible for EGFR tyrosine kinase inhibitors (TKIs). Despite the high response rates to first-line TKIs and a median progression-free survival (PFS) of 10–14 months (1–8), the disease ultimately progresses. In about 50–60% of patients, the acquired mechanism of resistance to first-line TKIs is a p.Thr790Met point mutation (T790M) in the EGFR gene. This mutation increases the receptor affinity for ATP binding, drastically reducing the drug activity (9–12).

Osimertinib is an irreversible EGFR-TKI that is selective for both EGFR and T790M resistance mutations (13). In the Phase III AURA3 trial (AZD9291 vs. Platinum-Based Doublet-Chemotherapy in Locally Advanced or Metastatic Non-Small Cell Lung Cancer), osimertinib was superior to platinum therapy plus pemetrexed in patients with T790M whom the disease progressed during first-line EGFR-TKI therapy with a median PFS of 10.1 months, and objective response rate (ORR) of 71% (14). Moreover, osimertinib had significant efficacy in patients with central nervous system (CNS) metastases (15). Recently, in the Phase III FLAURA trial (AZD9291 vs. Gefitinib or Erlotinib in Patients With Locally Advanced or Metastatic Non-small Cell Lung Cancer), Osimertinib was also superior in first-line (16).

Despite the survival data and response rates for osimertinib, acquired resistance, unfortunately, occurs after about 10 months (17). The mechanisms that determine disease progression are heterogeneous and not fully understood, including on-target EGFR-dependent and off-target independent mechanisms. EGFR-dependent mechanisms include new tertiary mutations, like the exon 20 C797S mutation, EGFR amplification or T790M disappearance. EGFR independent mechanisms can occur with bypass pathway activation, such as erb-b2 receptor tyrosine kinase 2 (HER2) and MET amplification, PIK3CA activating mutations, PTEN deletion, RAS mutations, fusions affecting anaplastic lymphoma kinase (ALK), and RET and others. There is also the possibility of phenotypic alteration, such as the transformation in small-cell lung cancer (SCLC) (18–20).

Treatment approaches for patients progressing from third-generation EGFR TKIs have not been clearly established. However, in case of disease progression without targeted therapy available, chemotherapy is still indicated and maintaining osimertinib beyond progression, with or without adjunctive radiotherapy, can be a useful option (21, 22). Although, a considerable amount of data is published on 3rd generation EGFR-TKIs, real-world data is limited. In this sense, this study aims to analyze the resistance profile of Osimertinib in a T790M EGFR-mutated population.

MATERIALS AND METHODS

Study Design

A retrospective analysis of T790M-mutated NSCLC patients treated with osimertinib, at the Centro Hospitalar e Universitário de São João (CHUSJ), Porto, Portugal, was performed. This study was conducted under the Declaration of Helsinki and was approved by the Ethics Committee of CHUSJ (243/20).

Eligible patients were required to have histologically confirmed stage IV NSCLC (based on TNM staging AJCC 8th edition), with an activating EGFR mutation, treated with osimertinib after progression with at least one 1st or 2nd generation TKI and with confirmed EGFR T790M mutation identified by re-biopsy at the time of progression. Patients initiated osimertinib between August 2016 and April 2019. Last data analysis was performed on 30 April 2020.

Patient demographics and clinical features, tumor histology, disease stage, lines of treatment received before osimertinib, and pattern of progression were recorded.

Molecular analyses from initial biopsies and re-biopsies at osimertinib progression were reviewed. Digital protein chain reaction (PCR) was used for EGFR T790M detection, and next-generation sequencing (NGS) at the time of progression was performed using a validated amplicon-based NGS (Oncomine^{TM} Focus Assay, ThermoFisher). These assays allow the analysis of targeted regions in EGFR, KRAS, NRAS, BRAF, MET, HER2, HER4, PIK3CA, and ALK genes plus the detection of ALK, ROS1, RET and NTRK (1, 2, and 3) gene fusions.

Statistical Analysis

Most analysis was descriptive. Categorical variables are presented as relative frequencies and percentages, and continuous variables as median, interquartile range (IQR) and minimum and maximum values. Kaplan-Meier actuarial curves analysis was used to estimate OS, PFS and time to treatment discontinuation (TTD) for the entire cohort. Group comparisons were performed using the Mann-Whitney test. The significance level assumed was 0.05. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, IBM Corp, Chicago, IL, USA) software, version 25.0.

RESULTS

Patients' Characteristics

Twenty-one patients treated with osimertinib were included (**Table 1**), with median age of 69 (range 39–84) years, 12 (57.1%) were female, mostly never-smokers ($n = 18$; 85.7%). Of note, 13 (61.9%) patients were $\geq$65 years, and 5 (23.8%) had an ECOG performance status (PS) $\geq$2. All patients were diagnosed with stage IV adenocarcinoma [IVA $n = 9$ (42.9%); IVB $n = 12$ (57.1%)]. Exon 19 deletion and exon 21 L858R mutations were present, at initial biopsy, in 17 (85%) and 3 (15%) cases, respectively. The T790M mutation was detected by tissue biopsy in eight (38.1%), liquid biopsy in five (23.8%), and by both in 8 (38.1%) patients. Osimertinib was given as 2nd line treatment in 13 cases (61.9%), after a 1st or 2nd generation EGFR-TKI, and in 3rd or more line in eight cases (38.1%). The best ORR was 52.7% (nine partial responses; one complete response) and DCR 89.5% (seven with stable disease), respectively. Median PFS was 13.4 (95% CI: 8.0–18.9) months (**Figure 1A**). Of the 17 cases with an objective response/disease control, 10 subsequently progressed and underwent re-biopsy. There were eight patients with oligo-progression (80%) and two with systemic progression (20%).

TABLE 1 | Baseline patients' characteristics.

Characteristics	*n* (%)
Age, years	
Median (range)	69 (39–84)
Gender	
Female	12 (57.1)
Male	9 (42.9)
Performance status	
0–1	16 (76.2)
≥2	5 (23.8)
Smoking status	
Never	18 (85.7)
Smoker/former smoker	3 (14.3)
Type of EGFR sensitizing mutation	
Exon 19 deletion	18 (85.7)
L858R (exon 21)	3 (14.3)
Stage	
IVA	9 (42.9)
IVB	12 (57.1)
Metastasis	
CNS	3 (14.3)
Extra-thoracic	13 (61.9)
Previous treatment	
1	13 (61.9)
≥2	8 (38.1)
Previous TKIs	
1st generation (erlotinib/gefitinib)	18 (85.7)
2nd generation (afatinib)	1 (4.8)
Sequential TKIs	2 (9.5)

CNS, central nervous system.

The main sites of progression were bone ($n = 4$), lung ($n = 4$), pleura ($n = 2$), CNS ($n = 1$), and liver ($n = 1$). Thirteen (61.9%) patients died, with a median OS since osimertinib initiation of 26.4 months (95% IC: 8.9–43.8) (**Figure 1B**).

Osimertinib was well-tolerated, with only two cases reporting grade ≥3 AE, corresponding to pneumonitis resolved with definite discontinuation (9.5%) and corticosteroids treatment.

Post-osimertinib Resistance Profile and Progression Treatment

At the time of analysis, 10 patients had tumor progression (47.6%), and the resistance profile is summarized in **Figure 2** and **Table 2**.

A total of 12 re-biopsies were analyzed among the 10 patients who progressed (**Figure 2**). Molecular testing was performed in all cases, 10 on tissue biopsy (83.3%), including 8 computed tomography (CT)-guided percutaneous core needle biopsy (PCNB), one ultrasound-guided liver biopsy and one CT-guided soft tissue biopsy, and two (16.7%) on liquid biopsy. The patients that performed liquid biopsy presented no clinical conditions for tissue biopsy ($n = 1$) or inaccessible disease ($n = 1$).

T790M mutation loss occurred in 50% of cases ($n = 6$), but other molecular changes were also found among this group, including PIK3CA mutation ($n = 2$), MET amplification ($n = 1$), and CTNNB1 mutation ($n = 1$). In the T790M-persistent group, a patient presented a newly exon 20 C797S mutation and another a DCTN1-ALK fusion ($n = 1$). Histological transformation in SCLC occurred in one patient.

T790M mutation loss was associated with earlier progression [PFS: median 8.1 (range: 3.8–11.3) vs. 21.4 months (range: 20.3–45.0), $p = 0.011$] and worse OS [median 13.0 (range: 7.0–30.3) vs. 32.1 months (range: 29.6–45.7), $p = 0.019$].

Of the 10 progressing patients, nine received at least one subsequent treatment, three received osimertinib beyond progression (33.3%), two of them in association with local ablative treatment (LAT), and six initiated a new treatment line [ChT ($n = 4$); another EGFR-TKI ($n = 2$)]. One patient received best supportive care (BSC). The patient with DCTN1-ALK fusion started crizotinib, presenting a partial response at the 3-month CT evaluation. The three patients who received osimertinib beyond progression had a new re-biopsy at the time of 2nd progression (**Figure 2**), and all received a new treatment line (2 ChT). The median post-progression PFS (ppPFS) was 5.0 months (range: 3.2–13.1; all cases with progression). The ppPFS of those who received a new treatment line was 2.7 months (range: 0.5–4.8; 2 cases without progression), $p = 0.12$.

DISCUSSION

Randomized controlled trials are the gold standard in clinical research. Still, real-world data is essential to verify the effectiveness, safety, application of treatment in the general population and to understand the patient's evolution in daily clinical practice. This is the first report of osimertinib in pre-treated EGFR T790M-mutated NSCLC patients in our population, focusing on the resistance mechanisms, and progression profile.

Identifying the resistance profile is critical in selecting the appropriate treatment after osimertinib, as several biological mechanisms of acquired resistance have been identified. To fully capture the diversity of resistance mechanisms is essential to repeat a biopsy to obtain the best possible sample that harbors the alteration responsible for progression with the less invasive and safer technique. However, obtaining tissue samples from patients experiencing progressive disease after EGFR-TKI failure remains a challenge. Rate of patients submitted to re-biopsy ranges from 50 to 60% in different series (23–25). With the analysis of circulating tumor DNA (ctDNA), liquid biopsy is a promising technique considering its invasiveness, repeatability, and accessibility. Some studies proved the role of ctDNA based assays to detect EGFR activations mutations and the T790M. In the osimertinib progression setting, in AURA3 trial, ctDNA genomic profile detected several resistance mechanisms, including MET amplification (26). Nevertheless, the use of cfDNA presents some limitations and challenges, especially considering the occurrence of false-negative results associated with the absence or low DNA of tumoral origin present

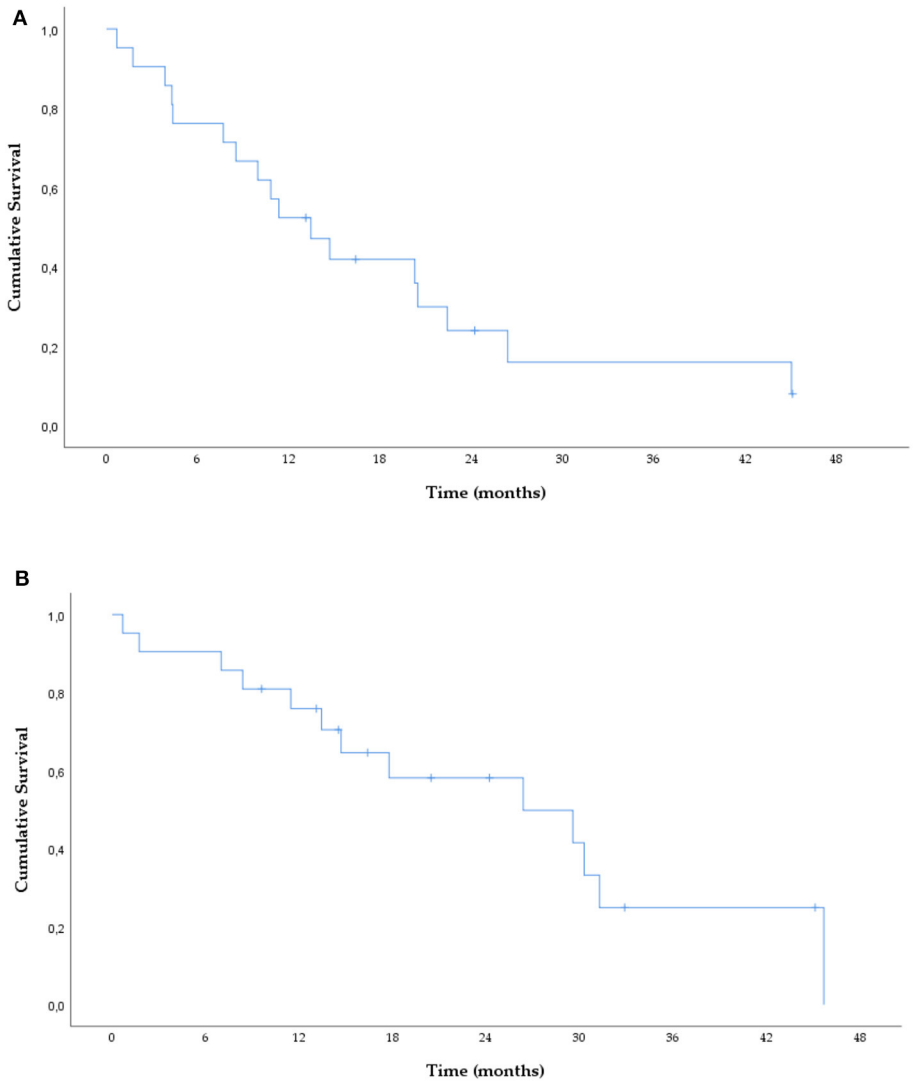

FIGURE 1 | PFS **(A)** and OS **(B)** under osimertinib treatment.

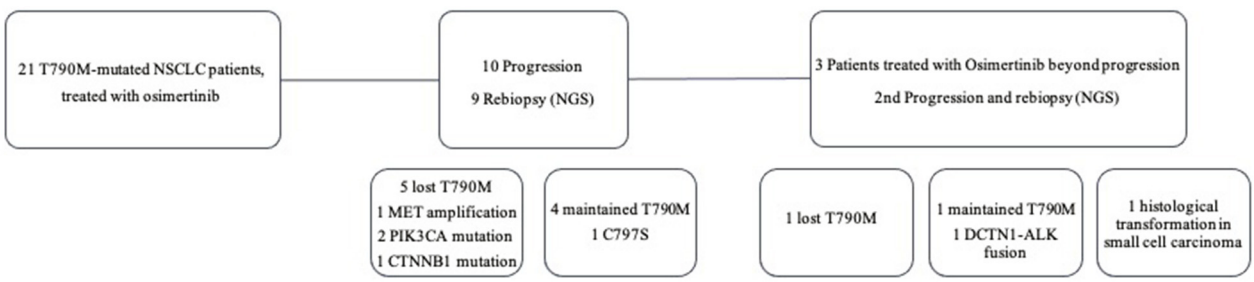

FIGURE 2 | Treatment and resistance profile of NSCLC patients post-osimertinib progression.

on plasma or analytical limitations and to the difficulty to detect small cell-transformation. In the setting of EGFR progressive disease, both tissue and liquid assays, are complementary.

Comprehensive NGS panels help to define the genomic diversity of resistance mechanisms and are particularly important in this setting, where there is no single alteration to detect.

TABLE 2 | Resistance profile of osimertinib.

Group	Initial biopsy	1st re-biopsy	PFS (months)	Post-progression treatment	Treatment response	2nd re-biopsy	2nd post-progression treatment	Treatment response
					Post-progression PFS (months)			Post-progression PFS 2 (months)
Loss of T790M	T790M Exon 19 del	Exon 19 del MET amp	3.8	ChT	Death 1.5			
	T790M Exon 19 del	Exon 19 del PIK2CA mut CTNNB1 mut	7.7	2nd generation TKI (afatinib)	Progression 2.0			
	T790M Exon 19 del	Exon 19 del	8.5	2nd generation TKI (afatinib)	Death 0.5			
	T790M Exon 19 del	Exon 19 del	4.3	ChT	Stable disease 3.8			
	T790M L858R	L858R PIK3CA mut	10.8	ChT	Stable disease 3.5			
Maintained T790M	T790M Exon 19 del	T790M Exon 19 del	20.3	Osimertinib LAT	Progression 3.2	Small cell carcinoma	ChT	Death 3.1
	T790M Exon 19 del	T790M Exon 19 del	11.3	Osimertinib LAT	Progression 13.1	Exon 19 del	ChT	Death 5.6
	T790M Exon 19 del	T790M Exon 19 del C797S	22.4	ChT	Progression 4.8			
	T790M* Exon 19 del	-	20.5	Osimertinib	Progression 5.0	T790M Exon 19 del DCTN1/ALK fusion	ALK inhibitor (crizotinib)	Partial response 5.0
	T790M Exon 19 del	T790M Exon 19 del	45.90	BSC	Death 0.6			

**Patient with 2 re-biopsies (1st: maintained T790M; 2nd: lost T790M).*

In this group, concerning osimertinib efficacy, ORR was 52.7%, median PFS 13.4 and median OS 26.4 months, similar to data from other real-world studies. All patients underwent a new biopsy at the time of progression, mainly tissue re-biopsy, and two liquid biopsies. All samples were evaluated with a targeted gene panel NGS.

We found that T790M mutation loss is common in Osimertinib-resistant cases (50%), consistent with previous studies (21, 27–30). Also, T790M mutation loss was associated with a shorter median PFS, which agrees with a previous study in which acquired resistance to osimertinib mediated by T790M mutation loss was associated with early progression, lower PFS and shorter TTD (28).

Molecular analyses from the AURA 3 trial revealed the presence of acquired EGFR mutations in 21% of patients, most commonly a new exon 20 point mutation C797S (14%) (26). In our series, only one patient acquired the C797S mutation. Most of the molecular alterations found were in EGFR-independent pathways, two PIK3CA mutation, one MET amplification, one CTNNB1 mutation, and one DCTN1-ALK fusion. In one patient occurred histological transformation into SCLC, a mechanism previously described in other studies (18, 20, 31).

Until today, no specific drug has been approved for the treatment of Osimertinib resistant patients, and a plethora of strategies are being explored. Rechallenge with 1st/2nd generation TKIS for C797S occurring in *trans* can be an option (32). Innumerous therapeutic combinations between osimertinib and antiangiogenics can be an option to overcome EGFR-dependent resistance mechanisms (33). Combination of Osimertinib and other inhibitors can help overcome resistance mediated through alternative kinase activation, as MET, MEK, and BRAF inhibitors (34–36). For most patients, platinum-based doublet chemotherapy is the only available option.

Regarding resistance to osimertinib, oligo-progression is frequent, being present in our series in more than 2/3 of patients. Schmid et al. (37) also reported this finding in 73% of cases. In this situation, LATs and osimertinib continuation beyond progression can be beneficial (37). In about one-third of cases, osimertinib treatment was continued beyond progression, with a longer ppPFS than patients who started a new treatment line, although not significant (5.0 vs. 2.0 months, $p = 0.22$). In the remaining cases, a new treatment line was started, mostly ChT. Two patients who lost the T790M and maintained the exon 19 deletion were treated with 2nd generation TKI afatinib, with a poor outcome. Crizotinib was initiated in a patient with DCTN1-ALK fusion with partial response.

Although, being a single center, retrospective study with small sample size, it illustrates the feasibility and relevance of performing re-biopsies, and NGS to study the resistance mechanisms at the time of progression, opening the window for new therapeutic strategies as demonstrated.

CONCLUSION

Re-biopsy at the time of disease progression is feasible outside clinical trials, being of extreme usefulness to understand the underlying resistance mechanisms, to guide treatment strategies and, consequently, contributing to increase patient's survival.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CHUSJ (approval n° 243/20).

AUTHOR CONTRIBUTIONS

CS and MGOF: conceptualization and writing—original draft preparation. CS, MGOF, and NC-M: methodology. CS and NC-M: formal analysis. CS, MJ, and LA: investigation and data curation. CS, MGOF, MJ, LA, and NC-M: writing—review and editing. VH and HQ: supervision. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med.* (2009) 361:947–57. doi: 10.1056/NEJMoa0810699
2. Hanna N, Johnson D, Temin S, Baker S Jr, Brahmer J, Ellis PM, et al. Systemic therapy for stage IV non-small-cell lung cancer: American Society of clinical oncology clinical practice guideline update. *J Clin Oncol.* (2017) 35:3484–515. doi: 10.1200/JCO.2017.74.6065
3. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, 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) 11:121–8. doi: 10.1016/S1470-2045(09)70364-X
4. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol.* (2011) 12:735–42. doi: 10.1016/S1470-2045(11)70184-X
5. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, 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) 13:239–46. doi: 10.1016/S1470-2045(11)70393-X
6. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol.* (2013) 31:3327–34. doi: 10.1200/JCO.2012.44.2806
7. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol.* (2014) 15:213–22. doi: 10.1016/S1470-2045(13)70604-1
8. Wu YL, Zhou C, Liam CK, Wu G, Liu X, Zhong Z, et al. First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. *Ann Oncol.* (2015) 26:1883–9. doi: 10.1093/annonc/mdv270
9. Arcila ME, Oxnard GR, Nafa K, Riely GJ, Solomon SB, Zakowski MF, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. *Clin Cancer Res.* (2011) 17:1169–80. doi: 10.1158/1078-0432.CCR-10-2277
10. Oxnard GR, Arcila ME, Sima CS, Riely GJ, Chmielecki J, Kris MG, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. *Clin Cancer Res.* (2011) 17:1616–22. doi: 10.1158/1078-0432.CCR-10-2692
11. Sun JM, Ahn MJ, Choi YL, Ahn JS, Park K. Clinical implications of T790M mutation in patients with acquired resistance to EGFR tyrosine kinase inhibitors. *Lung Cancer.* (2013) 82:294–8. doi: 10.1016/j.lungcan.2013.08.023
12. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res.* (2013) 19:2240–7. doi: 10.1158/1078-0432.CCR-12-2246
13. Cross DA, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov.* (2014) 4:1046–61. doi: 10.1158/2159-8290.CD-14-0337
14. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med.* (2017) 376:629–40. doi: 10.1056/NEJMoa1612674
15. Wu YL, Ahn MJ, Garassino MC, Han JY, Katakami N, Kim HR, et al. CNS efficacy of osimertinib in patients with T790M-positive advanced non-small-cell lung cancer: data from a randomized phase III trial (AURA3). *J Clin Oncol.* (2018) 36:2702–9. doi: 10.1200/JCO.2018.77.9363
16. Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non–small-cell lung cancer. *N Engl J Med.* (2017) 378:113–25. doi: 10.1056/NEJMoa1713137
17. Janne PA, Yang JC, Kim DW, Planchard D, Ohe Y, Ramalingam SS, et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *N Engl J Med.* (2015) 372:1689–99. doi: 10.1056/NEJMoa1411817
18. Minari R, Bordi P, Tiseo M. Third-generation epidermal growth factor receptor-tyrosine kinase inhibitors in T790M-positive non-small cell lung cancer: review on emerged mechanisms of resistance. *Transl Lung Cancer Res.* (2016) 5:695–708. doi: 10.21037/tlcr.2016.12.02
19. Lazzari C, Gregorc V, Karachaliou N, Rosell R, Santarpia M. Mechanisms of resistance to osimertinib. *J Thorac Dis.* (2019) 12:2851–8. doi: 10.21037/jtd.2019.08.30
20. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E, Tiseo M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *Br J Cancer.* (2019) 121:725–37. doi: 10.1038/s41416-019-0573-8
21. Le X, Puri S, Negrao MV, Nilsson MB, Robichaux J, Boyle T, et al. Landscape of EGFR-dependent and -independent resistance mechanisms to osimertinib and continuation therapy beyond progression in EGFR-mutant NSCLC. *Clin Cancer Res.* (2018) 24:6195–203. doi: 10.1158/1078-0432.CCR-18-1542
22. Cortellini A, Leonetti A, Catino A, Pizzutillo P, Ricciuti B, De Giglio A, et al. Osimertinib beyond disease progression in T790M EGFR-positive NSCLC patients: a multicenter study of clinicians' attitudes. *Clin Transl Oncol.* (2020) 22:844–51. doi: 10.1007/s12094-019-02193-w
23. Kawamura T, Kenmotsu H, Omori S, Nakashima K, Wakuda K, Ono A, et al. Clinical factors predicting detection of T790M mutation in rebiopsy for

EGFR-mutant non-small-cell lung cancer. *Clin Lung Cancer.* (2018) 19:e247–52. doi: 10.1016/j.cllc.2017.07.002
24. Ichihara E, Hotta K, Kubo T, Higashionna T, Ninomiya K, Ohashi K, et al. Clinical significance of repeat rebiopsy in detecting the EGFR T790M secondary mutation in patients with non-small cell lung cancer. *Oncotarget.* (2018) 9:29525–31. doi: 10.18632/oncotarget.25705
25. Zhou J, Zhao C, Zhao J, Wang Q, Chu X, Li J, et al. Re-biopsy and liquid biopsy for patients with non-small cell lung cancer after EGFR-tyrosine kinase inhibitor failure. *Thoracic cancer.* (2019) 10:957–65. doi: 10.1111/1759-7714.13035
26. Papadimitrakopoulou V, Wu YL, Han JY, Ahn MJ, Ramalingam S, John T, et al. LBA51 analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. *Ann Oncol.* (2018) 29:viii741. doi: 10.1093/annonc/mdy424.064
27. Piotrowska Z, Isozaki H, Lennerz JK, Gainor JF, Lennes IT, Zhu VW, et al. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. *Cancer Discov.* (2018) 8:1529–39. doi: 10.1158/2159-8290.CD-18-1022
28. Oxnard GR, Hu Y, Mileham KF, Husain H, Costa DB, Tracy P, et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. *JAMA Oncol.* (2018) 4:1527–34. doi: 10.1001/jamaoncol.2018.2969
29. Mehlman C, Cadranel J, Rousseau-Bussac G, Lacave R, Pujals A, Girard N, et al. Resistance mechanisms to osimertinib in EGFR-mutated advanced non-small-cell lung cancer: a multicentric retrospective French study. *Lung Cancer.* (2019) 137:149–56. doi: 10.1016/j.lungcan.2019.09.019
30. Lee J, Kim HS, Lee B, Kim HK, Sun JM, Ahn JS, et al. Genomic landscape of acquired resistance to third-generation EGFR tyrosine kinase inhibitors in EGFR T790M-mutant non-small cell lung cancer. *Cancer.* (2020) 126:2704–12. doi: 10.1002/cncr.32809
31. Zhang YC, Zhou Q, Wu YL. Clinical management of third-generation EGFR inhibitor-resistant patients with advanced non-small cell lung cancer: current status and future perspectives. *Cancer Lett.* (2019) 459:240–7. doi: 10.1016/j.canlet.2019.05.044
32. Niederst MJ, Hu H, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z, et al. The allelic context of the C797S mutation acquired upon treatment with third-generation EGFR inhibitors impacts sensitivity to subsequent treatment strategies. *Clin Cancer Res.* (2015) 21:3924–33. doi: 10.1158/1078-0432.CCR-15-0560
33. Janjigian YY, Smit EF, Groen HJ, Horn L, Gettinger S, Camidge DR, et al. Dual inhibition of EGFR with afatinib and cetuximab in kinase inhibitor-resistant EGFR-mutant lung cancer with and without T790M mutations. *Cancer Discov.* (2014) 4:1036–45. doi: 10.1158/2159-8290.CD-14-0326
34. Ou SI, Agarwal N, Ali SM. High MET amplification level as a resistance mechanism to osimertinib (AZD9291) in a patient that symptomatically responded to crizotinib treatment post-osimertinib progression. *Lung Cancer.* (2016) 98:59–61. doi: 10.1016/j.lungcan.2016.05.015
35. York ER, Varella-Garcia M, Bang TJ, Aisner DL, Camidge DR. Tolerable and effective combination of full-dose crizotinib and osimertinib targeting MET amplification sequentially emerging after T790M positivity in EGFR-mutant non-small cell lung cancer. *J Thorac Oncol.* (2017) 12:e85–e8. doi: 10.1016/j.jtho.2017.02.020
36. Ho CC, Liao WY, Lin CA, Shih JY, Yu CJ, Yang JC. Acquired BRAF V600E mutation as resistant mechanism after treatment with osimertinib. *J Thorac Oncol.* (2017) 12:567–72. doi: 10.1016/j.jtho.2016.11.2231
37. Schmid S, Klingbiel D, Aeppli S, Britschgi C, Gautschi O, Pless M, et al. Patterns of progression on osimertinib in EGFR T790M positive NSCLC: a swiss cohort study. *Lung Cancer.* (2019) 130:149–55. doi: 10.1016/j.lungcan.2019.02.020

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Current Evidence of the Efficacy and Safety of Neoadjuvant EGFR-TKIs for Patients With Non-small Cell Lung Cancer

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Purpose: Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been indicated to be an effective treatment for advanced EGFR-mutant NSCLC. However, the neoadjuvant application of EGFR-TKIs in resectable NSCLC needs further investigation. Here, we aimed to evaluate the efficacy and safety of neoadjuvant EGFR-TKIs for lung cancer.

Methods: Published studies on neoadjuvant EGFR-TKIs in NSCLC were identified in PubMed, Web of Science, and EMBASE until June 1, 2020. Data on surgical rates, objective response rates (ORRs), pathologic responses, and adverse event (AE) rates were retrieved for proportional meta-analysis.

Results: In total, 7 enrolled studies involving 129 EGFR-TKI-sensitive NSCLC patients were included in this analysis. The overall surgical rate in these studies was 95% (95% CI: 83% to 100%), with an ORR of 48% (95% CI: 39% to 57%) in the population with EGFR-TKI-sensitive mutations, whereas the ORR including wild-type EGFR patients was 28% (95% CI: 14% to 44%). The rate of grade 1-2 AEs was 69% (95% CI: 41% to 91%) but with an acceptable rate of grade 3-4 AEs of 0% (95% CI: 0% to 5%). The pooled rates of rash and diarrhea were 56% (95% CI: 31% to 79%) and 25% (95% CI: 6% to 51%), respectively. The impact of neoadjuvant EGFR-TKIs on survival remains inconclusive.

Conclusions: Neoadjuvant EGFR-TKIs showed objective responses in approximately half of EGFR-sensitive NSCLC patients with a tolerable adverse effect profile. The favorable impact of neoadjuvant EGFR-TKIs on NSCLC needs more evidence for validation, such as the comparison of survival improvement between EGFR-TKIs and chemotherapy. The efficacy of neoadjuvant next-generation EGFR-TKIs in clinical trials remains unclear.

Keywords: neoadjuvant therapy, EGFR-TKI, meta-analysis, NSCLC, surgery

INTRODUCTION

Surgery is an effective treatment for non-small cell lung cancer (NSCLC), but the 5-year overall survival (OS) rates of patients with stage II and IIIA disease are only 65% and 41%, respectively (1). Even when the tumors in these patients have been radically resected, micrometastasis may exist before surgery and is considered to be the main factor causing postoperative local or distant recurrence. In addition to the elimination of micrometastases, preoperative systemic treatment could result in tumor shrinkage and decreased lymph node enlargement, therefore reducing the TNM stage and tumor burden and facilitating the surgical procedure. Therefore, the optimal neoadjuvant therapy should be to reduce tumor burden without delaying the scheduled operation and have fewer adverse effects. Studies have shown that the use of neoadjuvant chemotherapy can improve the OS of NSCLC patients (2). Although targeted therapy led by epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) and immunotherapy led by PD-1 inhibitors have been proven to be effective treatments in advanced NSCLC, the application of those reagents as neoadjuvant therapy for lung cancer other than chemotherapy is still at the exploration stage.

For the large group of patients with EGFR gene mutations, the administration of EGFR-TKIs is preferred (3, 4). Compared to the controversial molecular markers for the prediction of the efficacy of immunotherapy, the limited application of EGFR-TKIs in patients with EGFR wild-type NSCLC and the low abundance of EGFR mutations (5) are widely accepted. However, the design of previous neoadjuvant EGFR-TKI clinical trials did not distinguish between populations that were sensitive and those with wild-type mutations (6, 7). In more recent studies, the safety and efficacy of neoadjuvant EGFR-TKI therapy have been more focused on populations with EGFR-TKI-sensitive mutations (8, 9). In theory, sensitive mutations may improve the efficacy of neoadjuvant EGFR-TKIs, but there is currently little evidence to support this hypothesis.

In addition to the above clinical trial designs, which are based on changes in EGFR mutation status, a more detailed design taking clinical staging into consideration is needed. From 2009 to 2016, clinical trials tried to cover a broad spectrum of TNM stages, including patients from stage I to stage IV, and wild-type EGFR status (6, 10). Since 2016, the study designs have tended to focus on NSCLC patients with stage II and stage III disease (8, 9) with EGFR-sensitive mutations. In addition, a comparison of neoadjuvant EGFR-TKIs and chemotherapy (11, 12) suggests that neoadjuvant EGFR-TKIs can improve patient prognosis compared with chemotherapy. Nevertheless, the chemotherapy group in the study by Zhong et al. (11) was administered gemcitabine plus cisplatin, while in the study by Xiong et al. (12), cisplatin-based doublet chemotherapies including vinorelbine, gemcitabine, paclitaxel, docetaxel or pemetrexed were administered. In the cases of limited sample sizes and different chemotherapy combinations contributing as a confounding factor, the level of evidence for this conclusion needs to be improved by adding more results in future studies.

Though a series of phase II trials on neoadjuvant EGFR-TKIs for NSCLC have been reported, the safety and efficacy of neoadjuvant EGFR-TKIs, especially in subgroups of EGFR mutation status or TNM staging, remain unclear. Considering that these trials have great potential to change current neoadjuvant practice in lung cancer surgery, we performed a meta-analysis incorporating the results of the surgical rates, clinical responses, pathologic responses, toxicities, and prognoses to evaluate the safety and efficacy of neoadjuvant EGFR-TKI therapy.

MATERIALS AND METHODS

We prospectively registered the protocol for this study in the International Prospective Register of Systematic Reviews (PROSPERO number: CRD42020187031). We reported the analysis by following the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) standards (**Table S1**) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Literature Retrieval

We performed a literature search in PubMed, Web of Science, and EMBASE until June 1, 2020. We used the following combination of keywords: "NSCLC", "EGFR-TKI", "neoadjuvant", "preoperative", and drug names of EGFR-TKIs. The detailed literature search criteria are listed in the supplementary files. We also performed an additional search through Google Scholar. Two authors (XY Dong and JX Zhai) removed the duplicated literature independently. Only studies reported in English were included.

Inclusion and Exclusion Criteria

We included studies based on the following criteria: (I) studies reported NSCLC patients with neoadjuvant EGFR-TKI therapy, and any generation of EGFR-TKIs was permissive; and (II) the surgical rate, objective response rate (ORR), and rate of adverse events (AEs) were available. Studies with the following characteristics were excluded from this meta-analysis: (I) studies from the same institutions or research group, studies with a close timeframe, and the same clinical trials (only the largest patient population was included); (II) comments, letters, and reviews; (III) incomplete data that are unable to be used for statistical analysis, such as studies that do not provide the ORR and rate of AEs; and (IV) case reports or studies with sample sizes less than 10.

Quality Assessment and Data Extraction

We (JX Zhai, XG Liu, Z Ni) used the Newcastle-Ottawa Scale to assess all the included studies (**Table S2**). Funnel plots were used to assess publication bias for outcomes reported by a minimum of 3 studies.

Data on the surgical rate, ORR, rate of AEs, and survival outcome were extracted by JX Zhai and Z Ni independently. In this study, the assessment of the surgical rate was limited to

patients with EGFR-sensitive mutations. Other measurements included patients with wild-type EGFR in the expanded analysis. We reached a final consensus if discrepancies occurred.

Statistical Analysis

The surgical rates of patients with EGFR-sensitive mutations receiving neoadjuvant EGFR-TKIs were calculated by the actual number of surgeries divided by the total number of patients. ORR was defined as the sum of the complete response plus partial response divided by the total number of included patients. Similarly, the number of pathological responses and grade 1-2 and grade 3-4 AEs were retrieved from the included literature and then transformed into rates by dividing by the total number of included patients. Survival data were retrieved by the methods reported by Tierney et al. (13). We performed a normality test for each rate in the proportional meta-analysis based on the raw rate, log transformation, logit transformation, arcsine transformation, and Freeman-Tukey double arcsine transformation to determine which method was best for the pooled analysis (**Table S3**). Finally, we applied the Freeman-Tukey double arcsine transformed proportion in the pooled analysis. As reported in our previous studies (14), heterogeneity was measured by the Cochran Q test and I^2 value. We reported values from the random effects model for studies with potential heterogeneity; otherwise, values from the fixed effects model were reported. All analyses were performed using R version 3.5.1, in which the proportional meta-analysis was performed with the "meta" package and the meta-regression analysis was performed with the "metafor" package. We considered a statistical test with a P value < 0.05 as significant.

RESULTS

Features of the Eligible Studies

We identified records based on the search strategy and finally enrolled 7 studies involving 129 NSCLC patients with clear EGFR-sensitive mutation status out of a total of 312 patients, with a summary provided in **Table 1**. The PRISMA 2009 flow diagram is shown in **Figure 1**. In this analysis, the exact number of patients with EGFR-TKI-sensitive mutations was not available in two studies and was partially available in two studies, so we included only the selected number in the relevant analysis.

Neoadjuvant EGFR-TKIs Are Feasible

We evaluated the feasibility of neoadjuvant EGFR-TKIs based on the pooled estimation of the surgical rate, pathologic response, ORR, rate of stable disease, and rate of grade 3-4 AEs. Overall, the surgical rate in the population with EGFR-TKI-sensitive mutations was 94% (95% CI: 83% to 100%, **Figure 2A**). Additionally, meta-regression analysis indicated that the surgical rate could decrease in the advanced stage population (**Figure 2B**). Other important measurements for the justification of neoadjuvant therapy are tumor response. The cutoff of 50% tumor necrosis and no more than 10% viable tumor cells were both considered as pathological response in this study. Only

TABLE 1 | Characteristics of the included studies.

Author	Public Year	Location	Research Center	Study Year	Trial phase	Stage	Group	Sample size	Age	EGFR-TKI	Preoperative treatment
Yang Zhang	2020	China	Single	2013.8-2015.10	II	II-IIIA	Single arm	35	57 (52-63) median (IQR)	Gefitinib	250 mg of oral gefitinib daily for 42 days
Liwen Xiong	2018	China	Single	2011.7- 2014.6	II	IIIA-N2	Single arm	19	59 (33–74) median (range)	Erlotinib	150 mg per day for 56 days
Wenzhao Zhong	2019	China	multiple	2011.12-2017.12	II	IIIA-N2	Erlotinib	37	59 (32-73) median (range)	Erlotinib	Patients received erlotinib 150 mg/d, 42 days
							Gemcitabine + Cisplatin	35	58 (33-76) median (range)	Chemo	gemcitabine 1,250 mg/m^2 plus cisplatin 75 mg/m^2
Tao Wang	2016	China	Single	2011.12-2014.12	Retrospective	IA–IIIA	Single arm	67	59 (37–78) median	Icotinib	125 mg thrice daily until the day before surgery
Eva E. Schaake	2012	Netherlands	multicenter	2006.12 - 2010.11	II	I-IV	Single arm	60	64 (37 -74) median (range)	Erlotinib	150 mg once daily for 3 weeks*
Lara-Guerra	2009	Canada	Single	NA	II	I	Single arm	36	65 (38 -81) median (range)	Gefitinib	250 mg orally once daily for up to 28 days
Haura	2010	USA	Single	NA	Pilot Study	IA-IIIB	Single arm	23	70 median	Gefitinib	250 mg daily for 4 weeks before surgical resection.

*Chemo, chemotherapy; *: dose was reduced due to toxicity.*

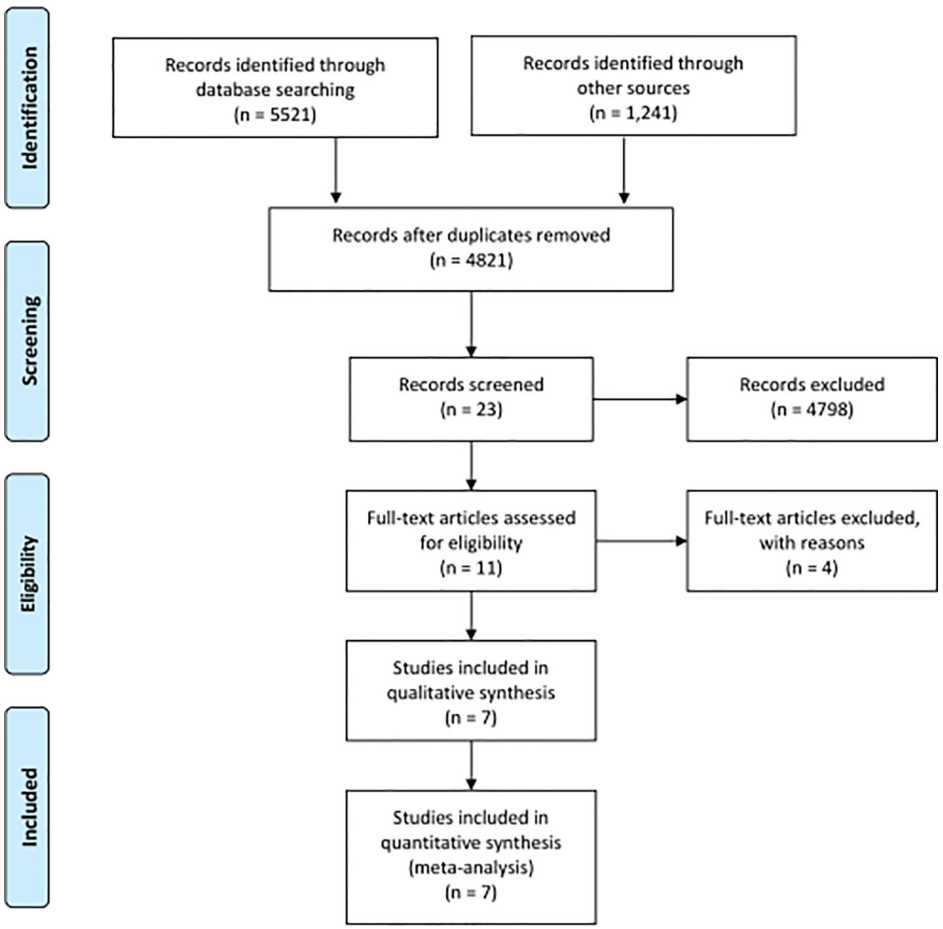

FIGURE 1 | The PRISMA 2009 flow diagram.

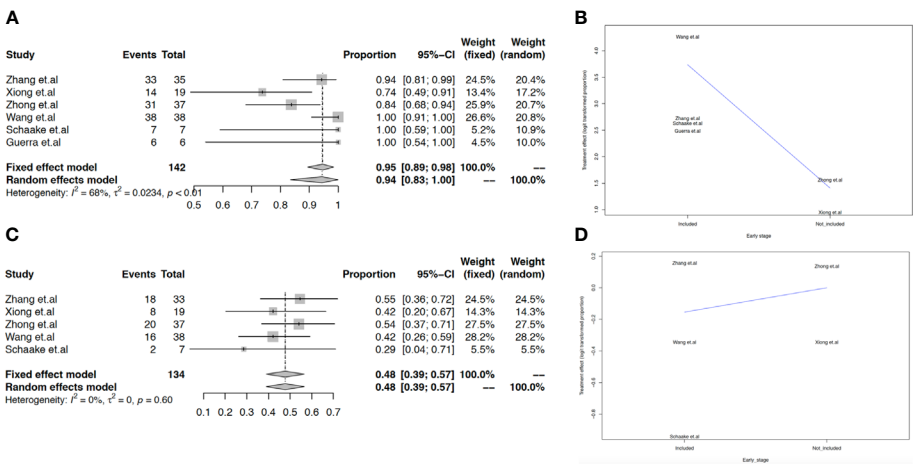

FIGURE 2 | Meta-analysis and meta-regression analysis of surgical rate and ORR. The pooled surgical rate in the population with EGFR-TKI-sensitive mutations **(A)**; meta-regression analysis of the surgical rate based on different advanced stages **(B)**; the pooled ORR in the population with EGFR-TKI-sensitive mutations **(C)**; meta-regression analysis of ORR based on different TNM stages **(D)**.

three studies reported pathological response, with a pooled estimated rate of 20% (95% CI: 6% to 38%, **Figure S1**). In our analysis, the ORR in the population with EGFR-TKI-sensitive mutations was 48% (95% CI: 39% to 57%, **Figure 2C**), while the ORR in the overall population including patients with wild-type EGFR status decreased to 28% (95% CI: 14% to 44%, **Figure S2**). In populations with EGFR-TKI-sensitive mutations, studies including early-stage NSCLC may decrease the ORR of neoadjuvant EGFR-TKIs (**Figure 2D**). Of note, the rate of stable disease in the population with EGFR-TKI-sensitive mutations was 45% (95% CI: 36% to 53%, **Figure S3**), which could more likely occur in early-stage NSCLC (**Figure S4**).

Next, we found that the rate of grade 1-2 AEs reached 69% (95% CI: 41% to 91%, **Figure 3A**), and the rate of grade 3-4 AEs was 0% (95% CI: 0% to 5%, **Figure 3B**). In the population with EGFR-TKI-sensitive mutations, rash and diarrhea were the most common adverse effects, with pooled rates of 56% (95% CI: 31% to 79%, **Figure 3C**) and 25% (95% CI: 6% to 51%, **Figure 3D**), respectively. However, the current evidence did not support the increased rate of rash (45%, 95% CI: 29% to 62%, **Figures S5** and **S6**) or diarrhea (22%, 95% CI: 12% to 34%, **Figures S7** and **S8**) when patients with wild-type EGFR were included. In the subgroup analysis, the rate of rash was higher while the rate of diarrhea was lower in the early TNM stage subgroup when neoadjuvant EGFR-TKIs were used in the overall population (**Figures S9** and **S10**).

The Impact of Neoadjuvant EGFR-TKIs on Survival

Detailed survival data were not available in the majority of current publications. Only two studies reported survival data

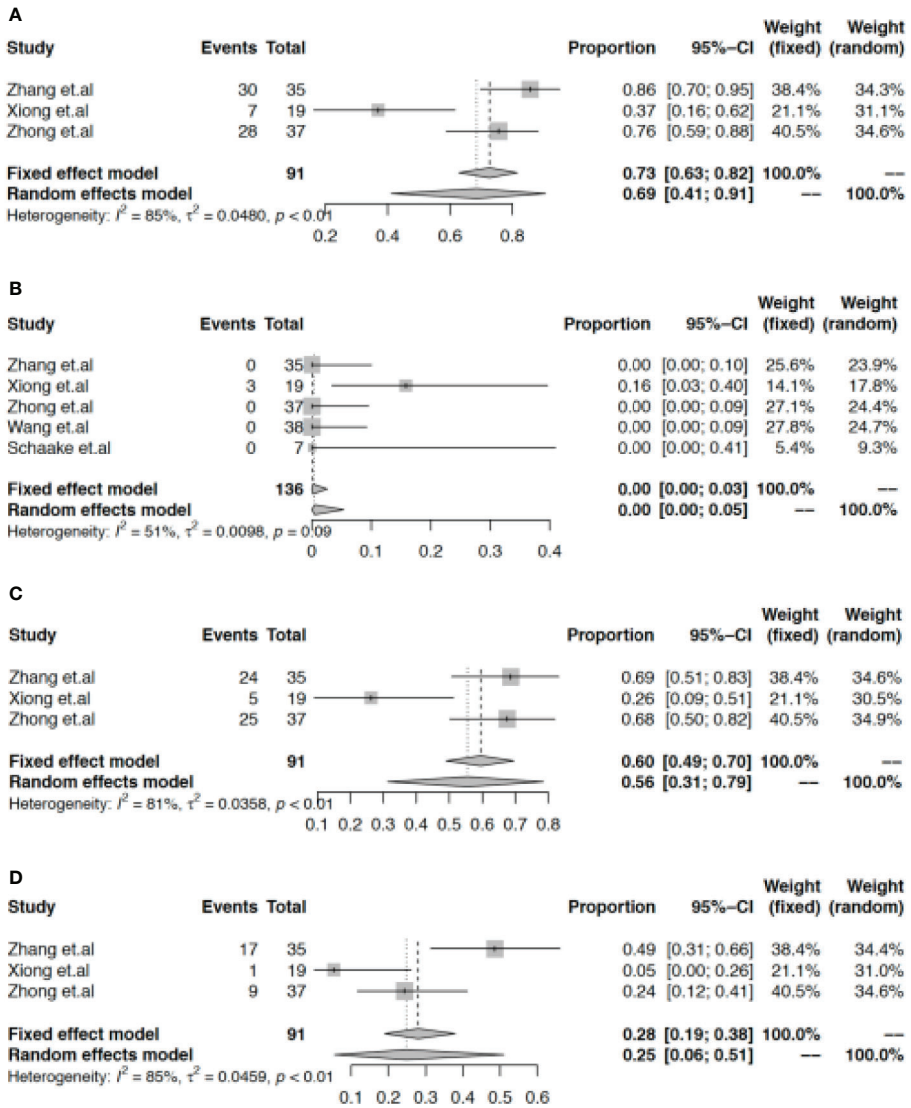

FIGURE 3 | Meta-analysis of the rate of adverse effects. The pooled rate of grade 1-2 AEs **(A)**, grade 3-4 AEs **(B)**, rash **(C)**, and diarrhea **(D)**.

related to neoadjuvant EGFR-TKIs compared with neoadjuvant chemotherapy. Zhang et al. (8) reported a median disease-free survival (DFS) of 33.5 months (95% CI, 19.7-47.3), while Xiong et al. (9) reported a median DFS of 10.5 months (95% CI, 7.7-29.9). For the comparison of the survival outcomes of neoadjuvant EGFR-TKIs versus neoadjuvant chemotherapy, Zhong et al. (11) reported that the median progression-free survival (PFS) and OS were significantly longer with erlotinib than with gemcitabine plus cisplatin chemotherapy (HR, 0.39; 95% CI, 0.23 to 0.67; P < 0.001; and HR, 0.77; 95% CI, 0.41 to 1.45; P = 0.417). Similar to these results, in one excluded study, Xiong et al. (12) reported that erlotinib may have a survival benefit compared with cisplatin-based doublet chemotherapy in terms of DFS (HR, 0.51; 95% CI, 0.13 to 2.01; P =0.39) and OS (HR, 0.45; 95% CI, 0.04 to 5.54; P =0.12), but a significant difference was not found. However, the chemotherapy arm in this study included vinorelbine, gemcitabine, paclitaxel, docetaxel or pemetrexed with limited participants (n=16). Moreover, different adjuvant therapy regimens and surgical procedures (segmentectomy, lobectomy, and pneumonectomy) may impose different impacts on individual survival. Therefore, the contribution of neoadjuvant EGFR-TKIs to survival remains inconclusive.

Assessment of Publication Bias

All publication bias was analyzed by Egger's test and visualized by funnel plots, as shown in **Figure S11**. No significant publication bias was found.

DISCUSSION

Currently, neoadjuvant therapy based on chemotherapy has been proven to be effective (2, 15). The unsatisfactory overall response, adverse effects, and sometimes delay of surgery or inoperability, especially in the middle and late stages of NSCLC, require a more effective adjuvant treatment option. In this analysis, neoadjuvant EGFR-TKI therapy was shown to be a potential alternative for NSCLC patients with EGFR-TKI-sensitive mutations.

Compared with the overall response rates ranging from 50 to 70% depending on the combination (16) in neoadjuvant chemotherapy studies, the 48% ORR in the population with EGFR-TKI-sensitive mutations in this analysis seems to be acceptable. When considering the 45% stable disease rate in the population with EGFR-TKI-sensitive mutations including those with early-stage NSCLC, we hypothesize that the small EGFR-sensitive mutation tumors have relatively low abundances of EGFR mutations. Therefore, the improvement is not apparent. Of note, patients with advanced TNM stages may benefit more from neoadjuvant EGFR-TKIs, while early-stage patients may not benefit much, suggesting that there would be an optimal cutoff TNM stage to achieve a better neoadjuvant EGFR-TKI outcome. Furthermore, this study also suggests that the ORR can be significantly reduced with mixed wild-type mutation studies. Although we were unable to reanalyze the EGFR mutation status of subgroups of patients in some of the previous neoadjuvant chemotherapy studies, Zhong et al. (11) reported that the ORR for neoadjuvant erlotinib is better than that of gemcitabine plus cisplatin chemotherapy (54.1% versus 34.3%) in the EGFR-sensitive population. This finding suggests that this choice of chemotherapy in the EGFR-sensitive population as a neoadjuvant therapy could be inferior to the use of EGFR-TKIs. Of course, more definite decision making depends on more evidence from clinical trials in the future.

Another indicator of whether a drug is suitable for neoadjuvant therapy is the occurrence and level of the AEs. Although preoperative chemotherapy has advantages, its toxicity and side effects cannot be ignored. In addition to affecting liver and kidney functions, chemotherapy drugs also present toxicities in the cardiovascular and nervous systems. Although the occurrence of side effects is closely related to the dose and combination of chemotherapy drugs, in general, EGFR-TKIs have fewer side effects. Similar to the findings of previous EGFR-TKI studies (17), the most common side effects in neoadjuvant EGFR-TKI studies were rash and diarrhea. Although more than half of the patients had grade 1-2 AEs, fortunately, only a small number of patients had grade 3-4 AEs, possibly avoiding the accumulating toxicity from the long-term use of EGFR-TKIs in previous clinical trials. This confers one of the essential factors to ensure that the surgery is performed as scheduled.

There are many limitations in current neoadjuvant EGFR-TKI studies. First, this study has not been included results from ongoing clinical trials on the neoadjuvant therapy with next-generation EGFR-TKIs, such as afatinib (NCT04201756) or osimertinib (NCT03433469). Second, most of the current clinical trials based on patients with EGFR-TKI-sensitive mutations are from China, and more evidence from the Caucasian population is needed. The current studies inconsistently reported the effect of neoadjuvant EGFR-TKIs on survival (only 2 out of 7 studies), so it is not rational to perform a pooled analysis for this outcome. Further studies on this limitation are warranted. It is unknown whether the combination of neoadjuvant EGFR-TKIs and chemotherapy or immunotherapy achieves a better response rate and prolongs NSCLC survival. Finally, for the intrinsic few studies have been reported in and the methods of controlling were different among studies, the heterogeneity cannot be ignored. An updated meta-analysis is needed in the future.

We first provided pooled estimates of the surgical rate, response rate, and drug toxicity rate in patients receiving neoadjuvant EGFR-TKIs. Our analysis revealed that EGFR-TKIs are a promising neoadjuvant option for NSCLC patients with EGFR-TKI-sensitive mutations. Potential factors that affect these estimates were also investigated. Our findings indicate that neoadjuvant EGFR-TKIs could be more effective in NSCLC patients with EGFR-TKI-sensitive mutations than in those with wild-type EGFR.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization: KC and XS. Data collection and literature screening: XD, JZ, XL, and ZN. Data curation: XS, XD, and HW. Data analysis: XS. Funding acquisition: XS and KC. Methodology: XS and XD. Writing—original draft: DL, JZ and XS. Writing—review and editing: DL, ZN, XD, and XL. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WE, et al. The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer. *J Thorac Oncol* (2016) 11:39–51. doi: 10.1016/j.jtho.2015.09.009
2. NSCLC Meta-analysis Collaborative Group. Preoperative Chemotherapy for non-Small-Cell Lung Cancer: A Systematic Review and Meta-Analysis of Individual Participant Data. *Lancet* (2014) 383:1561–71. doi: 10.1016/s0140-6736(13)62159-5
3. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or Chemotherapy for non-Small-Cell Lung Cancer With Mutated EGFR. *N Engl J Med* (2010) 362:2380–8. doi: 10.1056/NEJMoa0909530
4. Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, et al. Biomarker Analyses and Final Overall Survival Results From a Phase III, Randomized, Open-Label, First-Line Study of Gefitinib Versus Carboplatin/Paclitaxel in Clinically Selected Patients With Advanced non-Small-Cell Lung Cancer in Asia (Ipass). *J Clin Oncol* (2011) 29:2866–74. doi: 10.1200/jco.2010.33.4235
5. Zhou Q, Zhang XC, Chen ZH, Yin XL, Yang JJ, Xu CR, et al. Relative Abundance of EGFR Mutations Predicts Benefit From Gefitinib Treatment for Advanced non-Small-Cell Lung Cancer. *J Clin Oncol* (2011) 29:3316–21. doi: 10.1200/jco.2010.33.3757
6. Lara-Guerra H, Waddell TK, Salvarrey MA, Joshua AM, Chung CT, Paul N, et al. Phase II Study of Preoperative Gefitinib in Clinical Stage I non-Small-Cell Lung Cancer. *J Clin Oncol* (2009) 27:6229–36. doi: 10.1200/jco.2009.22.3370
7. Schaake EE, Kappers I, Codrington HE, Valdés Olmos RA, Teertstra HJ, van Pel R, et al. Tumor Response and Toxicity of Neoadjuvant Erlotinib in Patients With Early-Stage non-Small-Cell Lung Cancer. *J Clin Oncol* (2012) 30:2731–8. doi: 10.1200/jco.2011.39.4882
8. Zhang Y, Fu F, Hu H, Wang S, Li Y, Hu H, et al. Gefitinib as Neoadjuvant Therapy for Resectable Stage II-IIIA non-Small Cell Lung Cancer: A Phase II Study. *J Thorac Cardiovasc Surg* (2020) 16(2):434–42.e2. doi: 10.1016/j.jtcvs.2020.02.131
9. Xiong L, Li R, Sun J, Lou Y, Zhang W, Bai H, et al. Erlotinib as Neoadjuvant Therapy in Stage IIIA (N2) EGFR Mutation-Positive non-Small Cell Lung Cancer: A Prospective, Single-Arm, Phase II Study. *Oncologist* (2019) 24:157–e64. doi: 10.1634/theoncologist.2018-0120
10. Haura EB, Sommers E, Song L, Chiappori A, Becker A. A Pilot Study of Preoperative Gefitinib for Early-Stage Lung Cancer to Assess Intratumor Drug Concentration and Pathways Mediating Primary Resistance. *J Thorac Oncol* (2010) 5:1806–14. doi: 10.1097/JTO.0b013e3181f38f70
11. Zhong WZ, Chen KN, Chen C, Gu CD, Wang J, Yang XN, et al. Erlotinib Versus Gemcitabine Plus Cisplatin as Neoadjuvant Treatment of Stage IIIA-N2 EGFR-Mutant non-Small-Cell Lung Cancer (EMERGING-CTONG 1103): A Randomized Phase II Study. *J Clin Oncol* (2019) 37:2235–45. doi: 10.1200/jco.19.00075
12. Xiong L, Lou Y, Bai H, Li R, Xia J, Fang W, et al. Efficacy of Erlotinib as Neoadjuvant Regimen in EGFR-mutant Locally Advanced non-Small Cell Lung Cancer Patients. *J Int Med Res* (2019) 48(4):300060519887275. doi: 10.1177/0300060519887275
13. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical Methods for Incorporating Summary Time-to-Event Data Into Meta-Analysis. *Trials* (2007) 8:16. doi: 10.1186/1745-6215-8-16
14. Shi X, Dong X, Young S, Chen AM, Liu X, Zheng Z, et al. The Impact of Angiogenesis Inhibitors on Survival of Patients With Small Cell Lung Cancer. *Cancer Med* (2019) 8:5930–8. doi: 10.1002/cam4.2462
15. Pisters KM, Vallières E, Crowley JJ, Franklin WA, Bunn PAJr, Ginsberg RJ, et al. Surgery With or Without Preoperative Paclitaxel and Carboplatin in Early-Stage non-Small-Cell Lung Cancer: Southwest Oncology Group Trial S9900, an Intergroup, Randomized, Phase III Trial. *J Clin Oncol* (2010) 28:1843–9. doi: 10.1200/jco.2009.26.1685
16. Lewis J, Gillaspie EA, Osmundson EC, Horn L. Before or After: Evolving Neoadjuvant Approaches to Locally Advanced non-Small Cell Lung Cancer. *Front Oncol* (2018) 8:5. doi: 10.3389/fonc.2018.00005
17. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib Versus Chemotherapy as First-Line Treatment for Patients With Advanced EGFR Mutation-Positive non-Small-Cell Lung Cancer (OPTIMAL, CTONG-0802): A Multicentre, Open-Label, Randomised, Phase 3 Study. *Lancet Oncol* (2011) 12:735–42. doi: 10.1016/s1470-2045(11)70184-x

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REVIEW
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Immunotherapy in Treating EGFR-Mutant Lung Cancer: Current Challenges and New Strategies

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Lung cancer is the leading cause of cancer-related deaths worldwide. Immune checkpoint inhibitors, including monoclonal antibodies against programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1), have dramatically improved the survival and quality of life of a subset of non-small cell lung cancer (NSCLC) patients. Multiple predictive biomarkers have been proposed to select the patients who may benefit from the immune checkpoint inhibitors. EGFR-mutant NSCLC is the most prevalent molecular subtype in Asian lung cancer patients. However, patients with EGFR-mutant NSCLC show poor response to anti-PD-1/PD-L1 treatment. While small-molecule EGFR tyrosine kinase inhibitors (TKIs) are the preferred initial treatment for EGFR-mutant NSCLC, acquired drug resistance is severely limiting the long-term efficacy. However, there is currently no further effective treatment option for TKIs-refractory EGFR-mutant NSCLC patients. The reasons mediating the poor response of EGFR-mutated NSCLC patients to immunotherapy are not clear. Initial investigations revealed that EGFR-mutated NSCLC has lower PD-L1 expression and a low tumor mutational burden, thus leading to weak immunogenicity. Moreover, the use of PD-1/PD-L1 blockade prior to or concurrent with osimertinib has been reported to increase the risk of pulmonary toxicity. Furthermore, emerging evidence shows that PD-1/PD-L1 blockade in NSCLC patients can lead to hyperprogressive disease associated with dismal prognosis. However, it is difficult to predict the treatment toxicity. New biomarkers are urgently needed to predict response and toxicity associated with the use of PD-1/PD-L1 immunotherapy in EGFR-mutated NSCLC. Recently, promising data have emerged to suggest the potentiation of PD-1/PD-L1 blockade therapy by anti-angiogenic agents and a few other novel therapeutic agents. This article reviews the current investigations about the poor response of EGFR-mutated NSCLC to anti-PD-1/PD-L1 therapy, and discusses the new strategies that may be adopted in the future.

Keywords: targeted therapy, non-small cell lung cancer, immunotherapy, PD-1, PD-L1, EGFR mutation, tyrosine kinase inhibitor

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INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths worldwide (1). Non-small cell lung cancer (NSCLC) is the most common histological subtype which constitutes more than 85% of all lung cancer cases. The prognosis of advanced NSCLC is very poor. A few subsets of NSCLC patients harboring epidermal growth factor receptor (EGFR) mutation or anaplastic lymphoma kinase (ALK) rearrangement were known to respond well to the respective molecular targeted drugs with minimum adverse reaction (2). However, targeted therapies are ineffective in most NSCLC patients whose tumors lack the oncogenic driver alterations. On the other hand, despite excellent initial response to targeted therapies, essentially all EGFR-mutant NSCLC inevitably progress over time due to acquired drug resistance (3). There is currently no further effective therapeutic options for NSCLC who develop disease progression on EGFR tyrosine kinase inhibitors (TKIs) (4).

In recent years, immunotherapy has become integrated into the treatment plan of NSCLC patients, which tremendously improved survival and quality of life in some patients (5). Anti-CTLA-4 (e.g., ipilimumab) that changed the paradigm in melanoma treatment, when tested in clinical trials did not show the expected benefit in NSCLC patients (6, 7). On the other hand, monoclonal antibodies targeting programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) have demonstrated survival benefits, long lasting responses and good safety profile over chemotherapy in patients with advanced NSCLC in several recent Phase III trials (8–11). To date, four anti-PD-1/PD-L1 monoclonal antibodies (nivolumab, pembrolizumab (anti-PD-1); atezolimumab and durvalumab (anti-PD-L1)) have been approved as 1st or 2nd line therapy for NSCLC patients with metastatic and locally advanced NSCLC respectively (12, 13).

PD-1 is an inhibitory receptor expressed on activated T cells, B cells and natural killer cells, which normally function to blunt the immune response. The major ligand of PD-1, PD-L1, is expressed in tumor cells and infiltrating immune cells. When PD-L1 interacts with PD-1, they suppress the T cell-mediated cancer killing effect. Anti-PD-1/PD-L1 antibodies work by binding to inhibitory PD-1 receptor on tumor-reactive T cells and PD-L1 on tumor cells, respectively. The PD-1/PD-L1 interaction is then disrupted to reactivate the anti-tumor T cell-mediated cell cytotoxicity. Clinical benefit from anti-PD-1/PD-L1 therapy is associated with high tumor mutational load, high levels of pre-treatment tumor-infiltrating T cells, and high expression of pre-treatment PD-L1 on tumor cells and tumor-infiltrating immune cells (14).

EGFR-mutant NSCLC is the most prevalent molecular subtype in lung cancer patients. However, patients with EGFR-mutant NSCLC show poor response to anti-PD-1/PD-L1 treatment. The mechanisms mediating the poor response of EGFR-mutated NSCLC patients to immunotherapy are not clear. Initial investigations revealed that EGFR-mutated NSCLC has lower PD-L1 expression and a low tumor mutational burden (TMB), thus leading to weak immunogenicity.

This review recapitulates the underlying mechanisms contributing to the inferior clinical outcomes of anti-PD-1/PD-L1 immune-checkpoint inhibitors (ICIs) in NSCLC patients bearing EGFR mutations. Novel strategies to potentiate the use of PD-1 blockade therapy in EGFR mutant NSCLC are discussed.

PREDICTIVE BIOMARKERS FOR SELECTING NSCLC PATIENTS FOR IMMUNOTHERAPY

Identification of predictive biomarkers to select NSCLC patients most likely responding to anti-PD-1/PD-L1 ICIs is currently an area of intensive research. The response rates of anti-PD-1/PD-L1 ICIs were estimated to be around 14-20% in unselected patients (15). The most established predictive biomarker is PD-L1 expression status of tumor cells from biopsy. It is now routinely used in clinical practice for treatment decision to select patients who may benefit the most. In fact, a number of clinical studies have reported the association between PD-L1 expression and clinical outcome in NSCLC patients [reviewed in (16)]. PD-L1 expression on tumor cells is considered not only a predictive biomarker for response to PD-1/PD-L1 ICIs but also a prognostic factor in NSCLC patients (17).

However, a recent study reported significant discrepancy in the assessment of PD-L1 tumor expression in NSCLC patients and its association with prognosis (17). Multiple PD-L1 immunohistochemical (IHC) assays with various scoring systems and cutoff values have been developed for companion diagnostic use (18, 19). Thus, appreciable differences in the correlation observed in different clinical trials may arise from the different IHC assays, the antibodies used for the assays, positivity cutoff, type of biopsies (primary versus metastasis) and staining of tumor versus immune cells (17). It will be important to standardize a universal assay to assess tumoral PD-L1 expression and also to define appropriate cut-off points (20).

On the other hand, PD-L1 is also known to be highly expressed in circulating immune cells, including dendritic cells (21) and myeloid-derived suppressor cells (22). They regulate T cell activation during antigen presentation or excessive inflammation (23, 24). It has been postulated that the baseline distribution of PD-L1 expression in systemically circulating immune cells could contribute to the therapeutic responses to PD-L1/PD-1 blockade immunotherapy. While tumoral PD-L1 expression was assessed in most clinical trials investigating anti-PD-1/PD-L1 ICIs, PD-L1 level of tumor-infiltrating immune cells was also evaluated in atezolizumab's trials [POPLAR (25) and OAK (11)]. In both POPLAR and OAK trials, higher PD-L1 levels in both tumor cells and tumor-infiltrating immune cells were associated with improved patient survival after atezolizumab treatment. A more recent report also revealed that NSCLC patients with percentages of PD-L1+ CD11b+ myeloid cells above 30% before the start of anti-PD-L1 immunotherapy exhibited superior response rates of 50% (26). The data suggest that PD-L1 expression on myeloid cells in the systemic circulation could serve as a useful and accessible biomarker for patient stratification.

However, the utility of PD-L1 expression alone as an exclusive predictive biomarker for clinical efficacy of anti-PD-1/PD-L1

ICIs remains controversial (17). The determination of PD-L1 level alone is insufficient to understand the mechanisms of resistance to anti-PD-1/PD-L1 ICIs. It also does not explain why some PD-L1-negative patients can achieve response to treatment. PD-L2 is another ligand identified for PD-1 T cell receptor (TCR) (27). While PD-L1 is the predominant ligand for PD-1, PD-L2 could compete with PD-L1 with 2-6 fold higher affinity to PD-1 (28). However, the biological role of PD-L2 in the tumor microenvironment (TME) and as a predictive marker in NSCLC has not been definitively established. More investigations about the predictive and prognostic roles of PD-L2 are warranted.

Besides PD-L1 expression, TMB, DNA mismatch repair deficiency, extent of CD8+ cell infiltration, immune gene expression signatures and composition of the gut microbiome have also been proposed to correlate with clinical response to anti-PD-1/PD-L1 ICIs (29–32). NSCLC patients with high TMB and the smokers were found to respond better to anti-PD-1/PD-L1 ICIs (33). The potential use of TMB as a predictive marker of clinical response to anti-PD-1 therapy has been evaluated in the CheckMate026, CheckMate568 and CheckMate227 trials (34–37). NSCLC patients with high TMB showed prolonged clinical benefit and PFS to immunotherapy regardless of PD-L1 expression (38–40). It is noteworthy that lung cancer generally has higher TMB when compared with other tumor types (41). However, overall survival (OS) in anti-PD-1 ICI-treated NSCLC patients was not affected by TMB alone. Thus, further investigation about the role of TMB as a predictive biomarker is warranted before clinical implementation. Galectin-3 is a carbohydrate-binding lectin whose expression is associated with inflammatory cells including macrophage. Recently, NSCLC patients with negative or intermediate expression of galectin-3 in their tumor cells were found to demonstrate an early and durable response to pembrolizumab (42). A large multicenter clinical trial is underway to investigate the potential use of galectin-3 as a predictive marker for better patient selection for immunotherapy (42). Last but not least, NSCLC patients bearing EGFR mutations have been reported to show poor response to anti-PD-1/PD-L1 ICIs (43), which will be discussed in detail in the next section.

NSCLC PATIENTS HARBORING EGFR MUTATIONS SHOW POOR RESPONSE TO ANTI-PD-1/PD-L1 IMMUNOTHERAPY

Initial Enthusiasm About Using PD-1 ICIs in EGFR-Driven NSCLC According to Preclinical Studies

Early preclinical studies have reported that aberrant oncogenic EGFR signaling upregulates PD-L1 expression in NSCLC cell lines (44). PD-1 inhibitors were found to inhibit tumor cell proliferation in coculture systems of EGFR-mutant tumor and immune cells *in vitro* (44, 45). Moreover, PD-1 inhibitors were also shown to improve survival in EGFR-mutant mouse models (44). However, clinical studies have revealed an opposite result. NSCLC patients harboring EGFR mutation exhibited poorer response to PD-1/PD-L1 ICIs than those bearing wild-type EGFR (9, 11, 46, 47). More recently, a retrospective analysis conducted by Gainor et al. has revealed that EGFR mutations were associated with low clinical response to PD-1 blockade in NSCLC patients (48). The discrepancies between preclinical and clinical findings indicate a complex relationship among EGFR mutation, the immune microenvironment and therapeutic response from immunotherapy. Furthermore, EGFR TKI treatment in EGFR-driven NSCLC cell model was shown to cause PD-L1 downregulation (45), thus also deterring the utility of combining EGFR TKI with PD-1 inhibitor. In fact, the combination of EGFR TKI and PD-1 inhibitor did not lead to synergistic anticancer effect in EGFR-driven coculture system (45).

Key Clinical Trials Evaluating Anti-PD-1/PD-L1 ICIs in EGFR-Mutant NSCLC

Advanced NSCLC patients bearing EGFR mutations only account for about 5-14% of the total number of patients recruited in the major clinical trials investigating the four approved anti-PD-1/PD-L1 ICIs (**Table 1**) (8, 9, 11, 25, 46, 51, 52). Since these clinical trials were not designed solely to investigate the role of PD-1/PD-L1 blockade immunotherapy in EGFR mutant NSCLC patients, the efficacy in EGFR mutant patients was revealed by patient subgroup analysis. CheckMate-057 is the first Phase III trial to report the clinical efficacy of PD-1/PD-L1 inhibitors in NSCLC patients bearing EGFR mutant tumors. While this trial confirmed that patients with advanced non-squamous NSCLC and progress during or after platinum-based chemotherapy survived longer with nivolumab (an anti-PD-1 monoclonal antibody) than docetaxel, subgroup analysis revealed that there was no PFS or OS benefit in patients with activating EGFR mutation (9). Patient subgroup analysis in another Phase III trial (KEYNOTE-010) evaluating pembrolizumab (another PD-1 inhibitor) also indicated that EGFR mutant NSCLC did not achieve statistically significant OS benefit from immunotherapy over salvage chemotherapy (46). In another Phase III trial (OAK) evaluating atezolizumab (an anti-PD-L1 monoclonal antibody), NSCLC patients with EGFR-mutated tumor also did not achieve OS benefit from the immunotherapy over docetaxel (11). A pooled analysis evaluating data from 3 clinical trials (CheckMate-057, KEYNOTE-010 and POPLAR) confirmed that PD-1/PD-L1 ICIs did not enhance OS versus docetaxel in advanced NSCLC patients bearing EGFR mutation (n = 186, HR = 1.05, 95% CI: 0.70-1.55, P < 0.81) (47). Furthermore, another pooled analysis which covered 5 trials (CheckMate-017, CheckMate-057, KEYNOTE-010, OAK, and POPLAR) also verified that prolonged OS was only observed in the EGFR wild-type patient group but not in the EGFR-mutant subgroup (50).

A Phase II clinical trial (NCT0287994) was conducted to specifically evaluate the efficacy of pembrolizumab (anti-PD-1 monoclonal antibody) in TKI-naïve EGFR-mutant advanced NSCLC patients whose tumors have high PD-L1 expression (49). The trial enrolment was halted due to lack of efficacy after 11 of the 25 planned patients received the immunotherapy. Thus, the patient number is very small because of premature

TABLE 1 | Key clinical trials reporting efficacy and toxicity of PD-1/PD-L1 blockade immunotherapy in EGFR-mutant NSCLC patients.

PD-1/PD-L1 blockade therapy	Clinical trial #	Efficacy	Toxicity	Reference
Atezolizumab	OAK (Phase III; NCT02008227)	No OS benefit from atezolizumab over docetaxel in EGFR mutant versus wild-type patients (HR: EGFR mutant – 1.24 (0.71-2.18) versus EGFR wild-type – 0.69 (0.57-0.83)	Grade 3-4 treatment-related adverse events: 15% with atezolizumab group versus 43% with docetaxel group	(11)
Nivolumab	CheckMate 057 (Phase III; NCT01673867)	Median OS was 12.2 months (n=292) in nivolumab group versus 9.4 months in docetaxel group (n=290). However, subgroup analysis in EGFR mutated patients did not show PFS or OS benefit from nivolumab (HR=1.18 (0.69-2.00)).	Grade 3-5 treatment related adverse events were reported in 10% of nivolumab and 54% of docetaxel-treated patients	(9)
Pembrolizumab	KEYNOTE-010 (Phase III; NCT01905657)	No OS benefit from pembrolizumab over docetaxel in EGFR mutant versus wild-type patients (HR: EGFR mutant – 0.88 (0.45-1.70) versus EGFR wild-type 0.66 (0.55-0.80))	Grade 3-5 treatment-related adverse events: 13% with pembrolizumab group versus 35% with docetaxel group	(46)
Pembrolizumab	Phase II; NCT0287994	The efficacy of pembrolizumab was evaluated in TKI-naïve NSCLC patients with EGFR mutation and PD-L1 positive tumors. None of the patients with EGR-mutant NSCLC responded. Enrollment was ceased due to lack of efficacy after 11 of the 25 planned patients were treated.	–	(49)
Nivolumab, Pembrolizumab, Atezolizumab	Pooled analysis (CheckMate 057, KEYNOTE 010 and POPLAR)	PD-1/PD-L1 blockade immunotherapy did not enhance OS versus docetaxel in advanced NSCLC patients bearing EGFR mutation (n=186, HR=1.05, 95% CI: 0.70-1.55, P<0.81)	–	(47)
Nivolumab, pembrolizumab, atezolizumab	Pooled analysis (CheckMate 017, 057, 063, 003)	PD-1/PD-L1 blockade immunotherapy prolonged OS in EGFR wild-type subgroup (HR=0.67; 95% CI: 0.60=0.75; P<0.001) but not in EGFR mutant subgroup (HR=1.11; 95% CI: 0.80-1.53; P=0.54)	–	(50)

CI, confidence interval; HR, hazard ratio; OS, overall survival; PD-1, Programmed death-1; PD-L1, Programmed death ligand-1.

closure of the trial, none of the patients bearing EGFR mutations responded to pembrolizumab (49). Based on these clinical findings, the National Comprehensive Cancer Network (NCCN) clinical practice guidelines of NSCLC (version 4, 2021) did not recommend immunotherapy for the treatment of EGFR-mutant NSCLC patients.

Efficacy of Anti-PD-1/PD-L1 ICIs in NSCLC Bearing the Two Most Common EGFR Sensitizing Mutation Subtypes

Interestingly, the heterogeneity of EGFR mutation subtypes was found to cause variations in the therapeutic efficacy of anti-PD-1/PD-L1 ICIs. A multicenter retrospective study analyzed the clinical data of 171 EGFR mutant NSCLC patients on treatment with PD-1/PD-L1 ICI alone or in combination with CTLA4 inhibitor (53). While patients harboring EGFR exon 19 deletion or L858R mutation shown less benefit from immunotherapy than the EGFR wild-type group, the L858R group exhibited more favorable response than the exon 19 deletion group (ORR, 7% in EGFR 19 deletion subgroup versus 16% in L858R subgroup versus 22% in wild-type subgroup). However, EGFR T790M status and PD-L1 expression did not affect response and survival outcomes to anti-PD-1/PD-L1 ICIs. NSCLC tumors bearing EGFR exon 19 deletion was found to have a lower tumor mutation burden compared with the EGFR L858R subtype despite similar smoking history. Therefore, screening for EGFR mutation subtypes could be useful for personalized use of PD-1/PD-L1 ICIs in EGFR-mutant NSCLC patients. Further studies with larger patient cohorts are warranted.

Mechanisms Contributing to the Poor Efficacy of Anti-PD-1/PD-L1 Therapy in EGFR Mutant NSCLC

PD-L1 Expression

PD-L1 expression in tumor tissues is the most extensively studied predictive biomarker for clinical response to PD-1/PD-L1 inhibitors. Status of tumoral PD-L1 expression is now routinely used for selecting patients who could benefit the most. The contribution of PD-L1 expression to poor efficacy of anti-PD-1/PD-L1 therapy in EGFR mutant NSCLC is controversial. A number of studies have reported that high PD-L1 expression is found more frequently in EGFR-mutant than EGFR-wild type lung tumor tissues (44, 54–56). The activation of the PD-1 pathway is thus believed to contribute to immune escape in EGFR-driven NSCLC. In contrast, another study has found a decreased PD-L1 expression in tumor tissues from NSCLC patients bearing EGFR mutation (57). More recently, a pooled analysis of 15 clinical studies also revealed that patients with EGFR mutation have decreased PD-L1 expression (58). Analysis of The Cancer Genome Atlas (TCGA) and the Guangdong Lung Cancer Institute (GCLI) cohort have also confirmed an inverse correlation between EGFR mutation and PD-L1 expression in tumor tissues (58). On the other hand, other studies have reported a lack of correlation between the expression of PD-L1 and PD-L2 in patients with different EGFR mutation status (59). With these conflicting findings, the use of PD-L1 expression alone could not adequately predict and explain why EGFR-mutant NSCLC exhibits poor response to PD-1/PD-L1 inhibitors.

Tumor Mutational Burden (TMB)

In a recent study investigating the impact of TMB on clinical outcomes of NSCLC patients treated with EGFR TKIs, TMB was found to be remarkably lower in EGFR-mutated tumors (n = 153) than EGFR wild-type tumors (n = 1,849) (median 3.77 versus 6.12 mutations/Mb; $P < 0.0001$) (60). To this end, the association of higher TMB with tobacco smoking leading to better outcomes with PD-1/PD-L1 ICIs is well documented (9, 11, 34, 37, 46, 61). A recent meta-analysis also revealed that never smokers are less responsive to PD-1/PD-L1 inhibitors (62). Interestingly, EGFR mutant NSCLC is more enriched in the never smoking population (63).

Among the more common sensitizing EGFR mutations, TMB in the exon 19 deletion cohort was found to be lower than that in the L858R cohort (60). Hastings et al. has recently reported that clinical outcomes (OS and ORR) with PD-1 ICIs were worse in patients with exon 19 deletion than patients harboring L858R mutation (53). PD-L1 and smoking status were similar in the two patient subpopulations. Further TMB analyses of the two patient cohorts suggested that the higher TMB in EGFR L858R mutation could contribute to the differential responses to PD-1 ICIs.

In fact, specific subset of EGFR-mutant NSCLC patients have been shown to preferentially benefit from PD-1/PD-L1 ICIs to some extent (64–68). In a retrospective study evaluating NSCLC patients from the IMMUNOTARGET registry, the response of 125 pre-treated EGFR-mutated patients with ICI monotherapy was compared among patients with different EGFR mutation subgroups (69). The ORR was not notably affected by PD-L1 expression levels, smoking status or previous lines of treatment, but was significantly different in the various EGFR mutation subgroups (3.7%, 9.5%, 20.8% and 11.8%, respectively, in EGFR T790M, exon 19 deletion, L858R, and other EGFR mutation subgroups) (69). This is in line with the aforementioned findings of Hastings et al., demonstrating more favorable outcomes of L858R (coincident with higher TMB) than exon 19 deletion (coincident with lower TMB). The combination of nivolumab and erlotinib in 21 EGFR mutated NSCLC patients has been evaluated by Gettinger et al. (64). Intriguingly, patients bearing the EGFR L858R mutation were achieving longer survival benefit than those harboring other EGFR mutations (64). While TMB in the tumor tissues was not assessed in the study, the findings are consistent with the fact that TMB in EGFR L858R mutated NSCLC favors better response to PD-1 ICIs.

Immunosuppressive Tumor Microenvironment (TME) in EGFR-Mutant Tumors

A typical tumor mass consists of not only a heterogeneous population of cancer cells but also a variety of neighboring host cells, tumor-infiltrating lymphocytes, extracellular matrix proteins, and other secreted factors, which collectively referred to as the TME. Tumors have been classified into 4 different TME types according to the presence or absence of tumor-infiltrating lymphocytes (TIL) and PD-L1 expression (Type I: TIL^+, $PD\text{-}L1^+$; Type II: TIL^-, $PD\text{-}L1^-$; Type III: TIL^-, $PD\text{-}L1^+$; and Type IV: TIL^+, $PD\text{-}L1^-$) (70). Type I tumors were found to the only subtype that responds to PD-1/PD-L1 inhibitor (70). Therefore, besides PD-L1 expression, high level of TIL is critical to allow PD-1/PD-L1 inhibitors to work. Similarly, Chen et al. divided tumors into different immunity phenotypes (immune-desert phenotype; immune-excluded phenotype; and inflamed phenotype) according to a set of tumor, host, and environment factors (71). Using Chen's classification, tumors with the immune-desert and immune-excluded phenotypes are resistant to PD-1/PD-L1 inhibitors (71). Importantly, there is a close correlation between EGFR mutations and an uninflamed TME with immunological tolerance and weak immunogenicity (48, 58). This correlation may explain the inferior response of EGFR-mutant NSCLC to PD-1 blockade therapy. The overexpression of CD73 has also been reported in EGFR mutant NSCLC, thus resulting an immunosuppressive TME and reduced IFN gamma signature (72). **Figure 1** depicts the characteristic composition and function of the immunosuppressive TME in EGFR-mutated NSCLC cells.

EGFR-mutant NSCLC is characterized by its aberrant activation of the EGFR signaling pathway. To this end, activation of EGFR signaling has been reported in numerous studies to participate in immunosuppression and immune escape. Regulatory T cells (Tregs) play a critical role in suppressing the immune response to self and foreign particles, which help prevent autoimmune disease. They generally suppress the induction and proliferation of effector T cells. Amphiregulin (AREG) is an EGF-like growth factor and it is frequently upregulated in tumors (73). AREG is a known ligand of EGFR (74). Importantly, AREG is critical for Treg function *in vivo*, thus providing a mechanistic link between the EGFR signaling and regulation of the immune system (75). Wang et al. reported that AREG maintains the suppressive function of Tregs *via* the EGFR/GSK-3β/Foxp3 machinery *in vitro* and *in vivo*, thus confirming the importance of EGFR signaling in the regulation of Tregs (76). Recently, a long noncoding RNA lnc-EGFR has been shown to stimulate Treg differentiation and promote immune invasion of hepatocellular carcinoma *via* an EGFR-dependent signaling pathway (77). Moreover, the inhibition of EGFR signaling by gefitinib has been shown to alter the immune environment of the targeted cancer *in vitro* and *in vivo*, probably by reducing the number of Tregs in the tumors (78).

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that suppress immune responses. MDSCs expand during cancer, infection and inflammatory diseases. A recent study reported that EGFR TKI therapy alters the TME in EGFR mutant NSCLC and elevates the level of mononuclear MDSCs (79). The serum level of inflammatory factors IL-10 and CCL-2 was also found to be increased *in vivo* after EGFR TKI treatment (79). The increase in MDSC and inflammatory factors associated with EGFR TKI treatment has been proposed to explain why most EGFR TKI-resistant NSCLC patients are also refractory to anti-PD-1/PD-L1 ICIs (79). Moreover, MDSCs are known to inhibit IL-2 and anti-CD3/CD28 mAb-induced T cell amplification and Th1 polarization but induce apoptosis in T cells in an IDO-dependent manner (80). To this end, the activation of STAT3 (an important downstream signaling molecule of the EGFR pathway) is required for IDO expression (80). Therefore, STAT3 activation is essential for immune suppression of MDSCs. In fact, persistent activation of STAT3

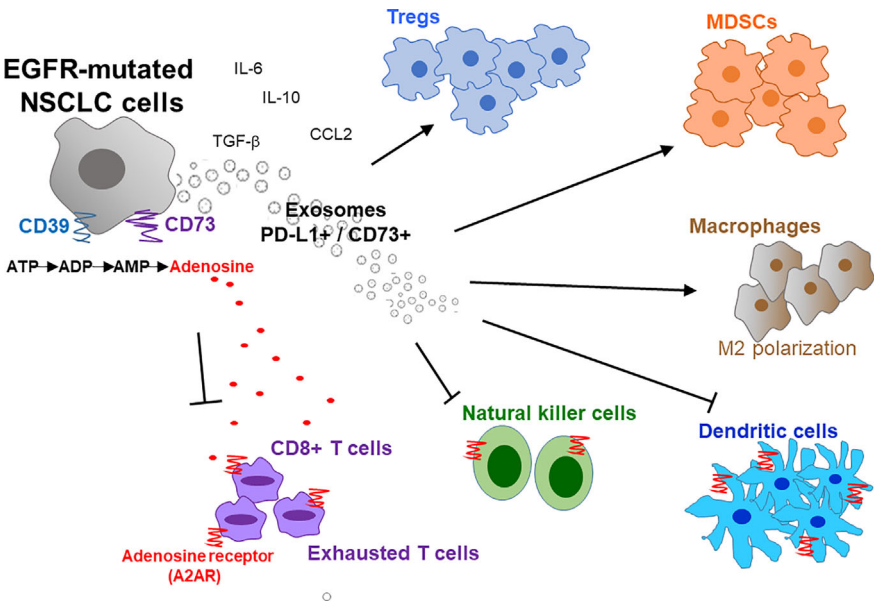

FIGURE 1 | Immunosuppressive tumor microenvironment (TME) in EGFR-mutated NSCLC. EGFR mutations promote an immunosuppressive TME by interfering with several intracellular pathways and modulating immune accessory cells including tumor-infiltrating lymphocytes (TILs), natural killer cells (NK), T-regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). Overexpression of CD39/CD73 in EGFR-mutated NSCLC induces high extracellular production and release of adenosine that inhibit the activity of innate and adaptive immune system cells and endothelial cells in TME. Activation of CD39 triggers the de-phosphorylation of ATP to ADP, and subsequently to AMP. On the other hand, CD73 catalyzes the hydrolysis of AMP to adenosine and phosphate. The increased level of extracellular adenosine bind to A2A adenosine receptor (A2AR) expressed by both adaptive and innate immunity, thereby inhibiting the activity of various immune cells. Moreover, exosomes secreted from EGFR-mutated NSCLC cells also increase PD-L1+/CD73+ expression and extracellular adenosine release to promote immunosuppression. IL, interleukin; M2, macrophages 2; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; CCL2, C-C motif chemokine ligand 2.

has been shown to promote MDSC-mediated immune suppression in lung cancer (81).

Yes-Associated Protein (YAP)

YAP is a major mediator of the Hippo pathway and it has been shown to promote cancer progression, drug resistance and metastasis in NSCLC (82). Accumulating evidence suggests that YAP also plays critical role in cancer immunity. YAP interacts with interferon regulatory factor 3 to negatively regulate innate immunity (83). YAP has also been reported to regulate tumor–associated immune cells (including MDSCs, macrophages, and Tregs) in the TME (83–85). In NSCLC tumor specimens, high nuclear YAP staining is associated with positive PD-L1 expression (86). Genetic knockdown or chemical inhibition of YAP was shown to reduce mRNA and protein expression of PD-L1 in NSCLC cell lines (86). On the other hand, forced expression of YAP was shown to increase PD-L1 protein expression in NSCLC A549 cells (87). Recently, a gefitinib-resistant PC9 cell line has been shown to express higher protein level of YAP and PD-L1 than the parental cells (88). Importantly, YAP knockdown could reduce PD-L1 expression in gefitinib-resistant PC9 cells (88). It is also noteworthy that the EGFR signaling pathway has crosstalk with the Hippo/YAP pathway, which positively regulating the YAP oncogenic function in various cancers including NSCLC (89).

Preferential Response of NSCLC Patients With Uncommon EGFR Mutations to PD-1 Blockade Therapy

Emerging evidence from a few recent studies suggest that the efficacy of PD-1 blockade therapy is relatively more favorable in NSCLC patients bearing uncommon EGFR mutations compared to those with the classical mutations (46, 69, 90). Approximately 10% of EGFR mutant NSCLC is classified as the uncommon subtypes (91), including G719X, L861Q, S768I, and exon 20 insertion, which have different clinicopathological characteristics and response to EGFR TKIs (92–96). Most recently, Chen et al. investigated the clinical response of Chinese NSCLC patients harboring uncommon EGFR mutations to PD-1/PD-L1 inhibitors and the underlying mechanisms (90). They tied the favorable response of the NSCLC patients with uncommon EGFR mutations to the high incidence of concomitant PD-L1 expression and CD8+ tumor-infiltrating lymphocytes within TME (90). In a retrospective efficacy analysis of PD-1 inhibitors conducted by a Japanese group, NSCLC patients harboring uncommon EGFR mutations and without T790M mutations were associated with significantly longer PFS than those with common EGFR mutations or with T790M mutation (97). This retrospective study was limited by the small sample size (n = 27). Further investigations are warranted to identify the clinical biomarkers useful for predicting the ICI responders with EGFR mutations.

TOXICITY EXPERIENCED ON COMBINATION TREATMENT WITH EGFR TKI AND IMMUNOTHERAPY

Immunotherapy generally has a lower incidence of adverse reactions than chemotherapy. However, ICIs are known to mediate inflammatory side effects commonly referred to as immune-related adverse events (irAEs) (98). The etiology leading to irAEs is largely unknown but it is believed to be caused by the disruption of immunologic homeostasis (99). The occurrence of irAEs generally predicts treatment efficacy of ICIs in NSCLC (100, 101), and it also triggers treatment discontinuation and premature termination of clinical trials. A Phase 1b TATTON trial (NCT02143466) investigating osimertinib (3rd generation EGFR TKI) plus nivolumab was stopped early because of the high incidence (38%) of interstitial lung disease (102). Another Phase III open-label CAURAL trial (NCT02454933) evaluating the combination of osimertinib and durvalumab in EGFR T790M positive NSCLC patients was also prematurely terminated due to safety concerns (103). irAEs can affect one or multiple organ systems. The incidence of grade 3 or above toxicities is around 7-13% in NSCLC patients treated with anti-PD-1/PD-L1 ICIs (104). It is noteworthy that toxicity experienced upon combination treatment with immunotherapy and EGFR TKIs can be severe, difficult to predict and with unusual forms of presentation. Specific laboratory tests and regular physical examinations should be conducted to facilitate early detection of irAEs and their effective management (105, 106).

HYPERPROGRESSIVE DISEASE (HPD) ASSOCIATED WITH PD-1/PD-L1 BLOCKADE IN NSCLC PATIENTS

Several recent studies have reported a paradoxical deleterious effect of anti-PD-1/PD-L1 immunotherapy, which is described as "hyperprogressive disease (HPD)", in a subset of patients (106, 107). HPD is characterized as an unexpected and fast progression in tumor volume and rate, poor survival of patients and early fatality (108).

HPD has been defined in different studies using 5 different criteria (109). Various parameters including tumor growth rate, tumor growth kinetics and time to treatment failure were used to define and quantify the incidence of HPD (110). In a recent retrospective cohort study of NSCLC patients, these 5 definitions of HPD were found to be associated with different tumoral behaviors. Kas et al. proposed a new definition of HPD, which is based on ΔTGR (tumor growth rate) greater than 100 (110). This new definition appeared to be more closely associated with the expected characteristics for HPD (i.e., rapid increase in tumor kinetics and poor patient survival). Numerous biomarkers associated with HPD have been proposed, which may be used to stratify patients for anti-PD-1/PD-L1 immunotherapy. Among them, EGFR mutation represents an important tumor cell biomarker linked with HPD after immunotherapy (108). HPD has been reported in 20% of patients with EGFR mutations and it was associated with worse clinical outcome. EGFR mutations were known to upregulate cell surface inhibitory receptors (e.g., PD-1/PD-L1 and CTLA-4), cytokines and immunosuppressive cells, subsequently driving innate immune resistance (44). The precise role of EGFR mutations in HPD warrants further investigation.

NOVEL STRATEGIES TO POTENTIATE PD-1/PD-L1 BLOCKADE IMMUNOTHERAPY IN EGFR MUTANT NSCLC

A promising therapeutic approach is to combine a PD-1/PD-L1 ICI with chemotherapy, EGFR TKI, or other type of ICI with an aim to increase the immunogenicity of tumor cells, or to inhibit immunosuppressive signaling in the TME (111).

Combination of PD-1/PD-L1 Blockade Therapy and Conventional Chemotherapy

In lung cancer patients without targetable mutations, the addition of PD-1/PD-L1 blockade therapy to standard chemotherapy has been shown to give rise to significantly longer OS and PFS than chemotherapy alone (10). Therefore, it is also speculated that addition of PD-1/PD-L1 immunotherapy to chemotherapy in NSCLC patients with EGFR mutation could also achieve desirable clinical outcomes.

Antitumor effect from conventional chemotherapy is not solely attributed to the direct tumor cell cytotoxicity, but it is also mediated by the restoration of immunosurveillance. To this end, antitumor immune response and re-establishment of immunosurveillance can be primed by immunogenic cell death (ICD). ICD comprises the release of damage-associated molecular patterns (DAMPs) from dying tumor cells that result in activation of tumor-specific immune responses (112). This can trigger long-term efficacy of anticancer drugs by combining direct cancer cell killing and antitumor immunity. ICD-induced DAMPs include surface-exposed calreticulin (CALR) and secreted ATP, annexin A1, type I interferon, and high mobility group box 1 (113). A number of classical chemotherapeutic drugs, including anthracyclines, cyclophosphamide, oxaliplatin, and paclitaxel, are known to elicit ICD (114). In mice bearing KRAS-positive and TP53-negative NSCLC tumor xenograft, two immunogenic chemotherapeutic drugs (oxaliplatin and cyclophosphamide) were shown to strongly enhance T cell infiltration of the tumors and sensitize them to subsequent checkpoint inhibition targeting both CTLA-4 and PD-1 (115).

A few recent clinical reports have corrobated the hypothesis that pretreatment with ICD-inducing anthracyclines or irradiation could potentiate the efficacy of ICIs in various tumor types including NSCLC (116) (**Table 2**). The PACIFIC study is a Phase III trial that evaluated durvalumab (PD-L1 antibody) as consolidation therapy in stage III NSCLC patients (51). The enrolled subjects did not present disease progression after 2 or more cycles of platinum-based chemotherapy (51). The study showed a significantly longer median PFS in the durvalumab cohort (16.8 months) than that in the placebo

TABLE 2 | Representative clinical trials evaluating the combination of PD-1/PD-L1 blockade immunotherapy and conventional chemotherapy in EGFR-mutant NSCLC patients.

Clinical trial #	PD-1/PD-L1 blockade therapy	Chemotherapy	Key findings	Reference
PACIFIC	Durvalumab (PD-L1 antibody)	Platinum-based chemotherapy	- Phase III trial evaluating durvalumab as consolidation therapy in stage III NSCLC patients who did not present disease progression after 2 or more cycles of chemotherapy. - In patient subgroup analysis, patients with EGFR mutations demonstrated slightly more clinical benefit from durvalumab after chemoradiotherapy.	(51)
Checkmate 722 (NCT02864251)	Nivolumab (PD-1 antibody)	Pemetrexed, cisplatin, or carboplatin	- Open-label phase III trial enrolling ~500 patients with confirmed stage IV or recurrent EGFR mutated NSCLC progressed on prior EGFR TKI therapy - Efficacy of nivolumab plus chemotherapy, nivolumab plus ipilimumab and chemotherapy alone was compared. - Final result has not been reported.	(117)
KEYNOTE-789 (NCT03515837)	Pembrolizumab (PD-1 antibody)	Pemetrexed, carboplatin or cisplatin	- Ongoing Phase II trial which compared efficacy of pembrolizumab and its combination with chemotherapy - It also recruited NSCLC patients bearing EGFR T790M who have acquired resistance to osimertinib	(118)

cohort (5.6 months). Importantly, in subgroup analysis, patients with EGFR-mutations demonstrated slightly more clinical benefit from durvalumab after chemoradiotherapy (platinum doublet chemotherapy administered with definitive-dose radiotherapy) (51). An open-label Phase III trial (Checkmate 722; NCT02864251) has also been conducted to compare the efficacy of nivolumab plus chemotherapy and nivolumab plus ipilimumab with chemotherapy alone (117). The Checkmate 722 study enrolled patients with EGFR-mutated metastatic or recurrent NSCLC who progressed on 1st or 2nd line EGFR TKIs (117). The final result has not been reported yet. Another ongoing Phase III trial (KEYNOTE-789; NCT03515837) is evaluating the efficacy of pembrolizumab in combination with chemotherapy (pemetrexed, carboplatin or cisplatin). Unlike the CheckMate 722 trial, KEYNOTE-789 will also recruit NSCLC patients bearing EGFR T790M who have acquired resistance to osimertinib (118).

Combination of PD-1/PD-L1 Immunotherapy With Targeted Therapy

High levels of intra-tumoral T cells and tumor antigenicity have been shown to correlate with favorable response from immunotherapy (119). Thus, therapies capable of increasing these factors may be combined effectively with ICIs to enhance the treatment outcome. In addition to the specific effects on oncogenic signaling pathways, a few targeted therapeutic agents are also known to increase tumor antigen presentation (120, 121), promote intra-tumoral T cell infiltration (122), or upregulate PD-1/PD-L1 expression (123). Therefore, it is logical to combine these targeted therapies with immunotherapies.

EGFR TKIs

EGFR TKIs have been reported to cause immunogenic apoptosis of tumor cells (124), and subsequently releasing aberrant intracellular antigens and recruiting T cells *via* interferon-γ-induced major histocompatibility complex class I presentation (120). A few clinical studies have been conducted to evaluate the combination of PD-1-based immunotherapy and EGFR targeted therapy in EGFR TKI-naïve and/or pretreated EGFR-mutant NSCLC patients (**Table 3**).

CheckMate 012 (NCT01454102) is a multi-arm Phase 1 study evaluating the combination of nivolumab and different agents including erlotinib in advanced NSCLC with small sample size (n = 20) (125). At least 4 out of the 20 patients with acquired resistance to EGFR TKI were shown to achieve clear benefit from the addition of nivolumab to EGFR TKI therapy [objective response rate (ORR) = 15%, including 1 complete response (CR)] (125). The combination of atezolizumab and erlotinib has been evaluated in another Phase I clinical trial in EGFR TKI-naïve and –treated NSCLC patients (NCT02013219; n = 28) (126). Atezolizumab plus erlotinib was shown to demonstrate a tolerable safety profile and favorable efficacy compared with prior reports of erlotinib monotherapy. ORR was 75% and median PFS was 15.4 months (126).

On the other hand, the combination of EGFR TKIs with PD-1/PD-L1 inhibitors did not demonstrate favorable clinical efficacy in EGFR-mutated NSCLC patients in a few other trials. In the Phase I/II KEYNOTE-021 trial (NCT02039674), the combination of erlotinib (n = 12) or gefitinib (n = 7) with pembrolizumab was evaluated in EGFR-mutant advanced NSCLC patients (129). While pembrolizumab plus erlotinib produced similar adverse events as erlotinib monotherapy, pembrolizumab plus gefitinib combination caused grade 3/4 liver toxicity in five out of seven patients and resulting in premature treatment discontinuation. Disappointingly, pembrolizumab plus erlotinib did not improve ORR compared with previous EGFR TKI monotherapy studies (129). Another open-label multicenter Phase I trial (NCT02088112) has also been recently conducted to evaluate the combination of gefitinib and duvalumab in patients with EGFR-mutant and EGFR TKI-naïve NSCLC (127). Durvalumab and gefitinib in combination was shown to produce higher toxicity than either drug alone. There was no significant improvement in PFS or ORR compared with gefitinib monotherapy previously reported in similar patient populations. To the best of our knowledge, no other Phase III trials investigating the combination EGFR TKI and PD-1 inhibitors in EGFR TKI-naïve patients are currently planned or actively recruiting.

TABLE 3 | Representative clinical trials evaluating the combination of PD-1/PD-L1 blockade immunotherapy and targeted therapy in EGFR-mutant NSCLC patients.

Clinical trial #	PD-1/PD-L1 blockade therapy	Targeted therapy	Key findings	Reference
CheckMate 012 (NCT01454102)	Nivolumab	Erlotinib (EGFR TKI)	- Phase I trial evaluating combination of nivoluman and various other agents including erlotinib - At least 4 out of the 20 recruited NSCLC patients with acquired resistance to EGFR TKI achieved clear benefit from combination of nivolumab and erlotinib (ORR = 15%, including 1 CR)	(125)
NCT02013219	Atezolizumab	Erlotinib (EGFR TKI)	- Phase I trial in EGFR TKI-naïve and –treated NSCLC patients - Combination of atezolizumab and erlotinib was well tolerated and it exhibited favorable efficacy compared with prior reports of erlotinib monotherapy. ORR = 75% and median PFS = 15.4 months	(126)
KEYNOTE-021 (NCT02039674)	Pembrolizumab	Gefitinib or erlotinib (EGFR TKI)	- Phase I/II trial evaluating the combination of pembrolizumab with erlotinib or gefitinib in advanced NSCLC patients bearing EGFR mutation - Pembrolizumab plus erlotinib did not improve ORR compared with previous EGFR TKI monotherapy - Pembrolizumab plus gefitinib combination caused grade 3/4 liver toxicity in 5 out of 7 patients, resulting in premature treatment discontinuation	(126)
NCT02088112	Durvalumab	Gefitinib (EGFR TKI)	- Open-label multicenter Phase I trial evaluating combination of gefitinib and durvalumab in patients with EGFR-mutant and EGFR TKI-naïve NSCLC - No significant improvement in PFS or ORR compared with gefitinib monotherapy previously reported in similar patient populations. - Gefitinib-naïve patients: ORR=63.6%; DoR=9.2 months; PFS=10.1 months - Gefitinib-pretreated patients: ORR=70.0%; DoR=12.6 months; PFS=12.0 months	(127)
TATTON (NCT02143466)	Durvalumab	Osimertinib (EGFR TKI)	- Phase Ib trial investigating the safety and tolerability of osimertinib and durvalumab combination - 38% of subjects developed serious interstitial pneumonitis	(102)
IMpower150 (NCT02366143)	Atezolizumab	Bevacizumab (anti-VEGF monoclonal antibody)	- Open-label Phase III study comparing atezolizumab + chemotherapy + bevacizumab (ABCP group) versus chemotherapy + bevacizumab (BCP group) in metastatic and chemotherapy-naïve NSCLC patients - ABCP group achieved significantly longer PFS (8.3 versus 6.8 months) and OS (19.2 versus 14.7 months) than BCP group, regardless of PD-L1 expression and EGFR/ALK genetic alteration status	(128)

CR, complete response; DoR, duration of response; ORR, objective response rate; OS, overall survival; PFS, progression free survival.

In preclinical studies, the 3rd generation EGFR TKI, osimertinib, has been shown to enhance the antitumor efficacy of PD-1/PD-L1 blockade therapy by increasing CD8+ T cell infiltration in tumors (130). However, in a Phase Ib trial TATTON (NCT02143466) investigating the safety and tolerability of combining osimertinib and durvalumab, 38% of patients developed serious interstitial pneumonitis and thus the poor safety profile renders the combination not feasible (102). In an animal study using EGFR mutated tumor-bearing mouse model, osimertinib (but not gefitinib) combined with anti-PD-L1 therapy was shown to cause pneumonitis and injury to lung tissues (124).

Vascular Endothelial Growth Factor Receptor (VEGFR) TKI

IMpower150 was an open-label Phase III study (NCT02366143) comparing atezolizumab ± chemotherapy + bevacizumab (ABCP group) versus chemotherapy + bevacizumab (BCP group) in metastatic NSCLC patients who had not previously received chemotherapy (128). Bevacizumab is an anti-VEGF monoclonal antibody and its combination with chemotherapy has been approved for the treatment of metastatic NSCLC (131). Apart from the well-known antiangiogenic effects of bevacizumab (132), the inhibition of VEGF has also been shown to mediate immunomodulatory effects (14, 133, 134). Thus, the efficacy of atezolizumab may be enhanced by the addition of bevacizumab to reverse VEGF-mediated immunosuppression (134, 135). Encouragingly, the ABCP group was shown to achieve significantly longer PFS (8.3 months versus 6.8 months) and OS (19.2 months versus 14.7 months) than the BCP group, regardless of PD-L1 expression and EGFR/ALK genetic alteration status (128) (**Table 3**).

Combination of PD-1/PD-L1 ICIs With Other Immunotherapies

Both CTLA-4 and PD-1 ICIs demonstrated impressive durable antitumor response and they had a manageable safety profile. However, benefits of monotherapy were limited by low response rates and only a fraction of patients were found to be responsive (136). Combination of CTLA-4 and PD-1/PD-L1 blockade was proposed to increase the response rates and patient survival rates. It was thought that blockade of CTLA-4 (primarily involved in regulation of T cell activation in lymph nodes and in suppression of DC activity *via* Treg cells) could act synergistically with blockade of PD-1 (mainly involved in inhibition of effect T cells and NK cell activation in peripheral tissues and in induction of Treg cell differentiation) (137).

Multiple studies have investigated the combination of PD-1/PD-L1 plus CTLA-4 antibodies in treating NSCLC (**Table 4**). The first study is a phase Ib trial that evaluated the safety and efficacy of durvalumab (anti-PD-L1) and tremelimumab (anti-CTLA-4) combination. Encouraging clinical activity was observed in NSCLC patients with PD-L1 positive as well as PD-L1 negative tumors, with investigator assessed confirmed ORR in 23% patients (138). Importantly, this study revealed that the antitumor effect of the combination does not depend on PD-L1 expression. Thus, this might provide a new treatment option for patients with negative PD-L1 expression (138). In a phase III

trial evaluating chemotherapy-naïve stage IV or recurrent NSCLC patients, the combination of nivolumab and ipilimumab achieved ORR of 45.3%, 1-year progressive free survival rate of 42.6% and median PFS of 7.2 months (139). The relative incidence of disease progression or death was significantly lower in nivolumab plus ipilimumab combination compared to chemotherapy alone group (HR for disease progression or death, 0.58, $p < 0.001$). In another phase II study, the efficacy and safety of nivolumab plus "low dose" ipilimumab as first line treatment for metastatic NSCLC was investigated (140). The association of efficacy with PD-L1 expression and TMB was also assessed. The ORR achieved by the combination was found to be higher in patients with TMB of at least 10 mutations per megabase and it was not dependent on PD-L1 expression (140).

Combination of PD-1/PD-L1 Blockade Immunotherapy With Other Miscellaneous Therapies

Interleukin-10 (IL-10) is known to possess anti-inflammatory and CD8+ T cell stimulating activities (141). Pegilodecakin (pegylated IL-10) is a first-in-class long-acting IL-10 receptor agonist that induces oligoclonal T cell expansion (142). It has demonstrated single-agent activity in advanced solid tumors (143) (**Table 5**). IVY is a multicenter, multicohort, open-label, phase Ib trial (NCT02009449) evaluating the combination of pegilodecakin and pembrolizumab or nivolumab for patients with advanced solid tumors (144). The combination of pegilodecakin with anti-PD-1 monoclonal antibodies demonstrated a manageable toxicity profile and promising antitumor activity. Th ORR was relatively higher for NSCLC (43%), than that in renal cell carcinoma (40%) and melanoma (10%) (144). The favorable responses were also observed when PD-1/PD-L1 blockade immunotherapy only produced limited benefit, such as low PD-L1 expression, low TMB and liver metastasis. Since subgroup analysis focusing on EGFR-mutant patients were not available from the study, further investigation is needed to verify its clinical usefulness for NSCLC patients with EGFR mutations.

As discussed above, the major Hippo regulator YAP plays critical role in regulating tumor immunity and PD-L1 expression (83–88). It follows that therapies targeting YAP may potentially enhance the efficacy of anti-PD-1/PD-L1 ICIs in EGFR-mutant NSCLC. A few small molecule compounds or drugs, including dasatinib, JQ1, norcantharidin, MLN8237 and dobutamine, have been shown to inhibit YAP (150). Further investigation is needed to verify the beneficial effect of combining YAP inhibitors with anti-PD-1/PD-L1 ICIs for treating EGFR TKI resistant NSCLC. Apart from inhibiting YAP, the modulation of YAP-related oncogenic pathways may also be evaluated. Inhibition of MEK1/2 is known to promote YAP degradation in NSCLC (146) (**Table 5**). Recently, the combination of MEK inhibitor and anti-PD-1/PD-L1 antibodies have been shown to produce synergistic anticancer effect and prolong survival of NSCLC tumor-bearing mice (147). On the other hand, cyclin-dependent kinase 9 (CDK9) is a key mediator promoting YAP-driven transcription of its downstream oncogenic effectors (148). Therefore, CDK9 inhibitors, such as dinaciclib and seliciclib, may be evaluated for potentiation of PD-1/PD-L1 blockade therapy. In fact, a recent animal study has shown that a highly selective CDK9 inhibitor (MC180295) sensitizes cancer cells to anti-PD-1 antibodies (149).

The combination of a few novel immune modulating agents and PD-1/PD-L1 ICIs have also been investigated. Eftilagimod alpha (IMP321) is a recombinant LAG-3Ig fusion protein that binds to MHC class II to activate antigen presenting cell and CD8 T-cell. The increase in activated T cells by IMP321 could potentially reduce the number of non-responders to

TABLE 4 | Representative clinical trials investigating the combination of PD-1/PD-L1 and CTLA-4 blockade immunotherapies in NSCLC.

Clinical trial #	PD-1/PD-L1 inhibitor	CTLA-4 inhibitor	Key findings	Reference
NCT02000947 (Phase Ib)	MEDI4736 (anti-PD-L1 mAb)	Tremelimumab	- Advanced NSCLC patients - ORR, 23% - Grade 3-4 AEs, 35%	(138)
NCT01454102 (Phase I)	Nivolumab	Ipilimumab	- Untreated advanced NSCLC - ORR, 47% - Median PFS, 8.1 months - 24-week PFS rate, 68% - Grade 3-4 AEs, 37%	(139)
NCT02659059 (Phase II)	Nivolumab	Ipilimumab	- Untreated advanced (Stage IV) NSCLC patients - In patients with TMB > 10 mutations/megabase: ORR, 44% Median PFS, 7.1 months 6-month PFS rate, 55% Grade 3-4 AEs, 29%	(140)
NCT02477826 (Phase III)	Nivolumab	Ipilimumab	- Untreated advanced (Stage IV) NSCLC patients - In patients with TMB > 10 mutations/megabase: ORR, 45% Median PFS, 7.2 months 12-month PFS rate, 43% HR for disease progression or death, 0.58 Grade 3-4 AEs, 31%	(139)

AE, adverse event; HR, hazard ratio; mAb, monoclonal antibody; ORR, objective response rate; PFS, progression free survival; TMB, tumor mutational burden.

TABLE 5 | Representative studies (clinical trials and animal studies) evaluating the combination of PD-1/PD-L1 blockade immunotherapy and other miscellaneous therapies in EGFR-mutant NSCLC.

Type of study	PD-1/PD-L1 blockade therapy	Other miscellaneous therapy	Key findings	Reference
Clinical trial: IVY (NCT02009449)	Pembrolizumab or nivolumab	Pegilodecakin (PEGylated IL-10) (first-in-class long-acting IL-10 receptor agonist that induces oligoclonal T cell expansion)	- Multicenter, multicohort, open-label, phase Ib trial evaluating the drug combination in patients with advanced solid tumors (including NSCLC, renal cell carcinoma, and melanoma) - ORR for the drug combination was higher for NSCLC (43%) than that in renal cell carcinoma (40%) and melanoma (10%) - However, patient subgroup analysis focusing on EGFR-mutant patients were not available from the study	(144)
Clinical trial: TACTI-002 (NCT03625323)	Pembrolizumab	IMP321 (recombinant LAG-3Ig fusion protein)	- Ongoing Phase II trial investigating the combination in patients with previously untreated unresectable or metastatic NSCLC, recurrent PD-X refractory NSCLC, or metastatic HNSCC - Pilot results demonstrated that combination achieved an ORR of 47% in advanced NSCLC	(145)
Clinical trial: NCT03835949	Atezolizumab	TJ004309 (anti-CD73 antibody)	- Ongoing Phase I trial investigating the combination in patients with advanced or metastatic cancer	
Animal study	Anti-PD-1 (RMP1-14) and anti-PD-L1 (10F.9G2) monoclonal antibodies	Trametinib (MEK inhibitor)	- Inhibition of MEK1/2 promoted YAP degradation in NSCLC - The drug combination was shown to produce synergistic anticancer effect and prolong survival of NSCLC tumor-bearing mice	(146, 147)
Animal study	Anti-PD-1 monoclonal antibodies	CDK9 inhibitor (MC180295)	- CDK9 promotes YAP-driven transcription of its downstream oncogenic effectors - MC180295 sensitizes NSCLC to anti-PD-1 antibody in C57Bl/6 mouse model	(148, 149)

CR, complete response; ORR, objective response rate; OS, overall survival; PFS, progression free survival.

pembrolizumab. Pilot results from the TACTI-002 trial showed that the combination of IMP321 and pembrolizumab achieved an ORR of 47% in advanced NSCLC (145) (**Table 5**). Activation of EGFR is associated with overactivation of Tregs. To this end, CD36 is known to make Tregs more adaptable to TME by serving as a metabolic modulator (151). Forced expression of CD63 by genetic approach in Tregs was shown to suppress tumor growth and enhance the antitumor efficacy of PD-1 therapy (151). CD73 is a cell surface nucleotidase, which catalyzes the hydrolysis of AMP into adenosine and phosphate. CD73-generated adenosine plays a critical role in tumor immunoescape (152). In an ongoing clinical trial (NCT03835949), the combination of an anti-CD73 drug (TJ004309) and atezolizumab is investigated in patients with advanced or metastatic cancer (153).

Local Co-Treatments

Besides the aforementioned systemic combination treatment for potentiating immunotherapy, a few local treatment options have also been investigated. These include thermal therapies, radiotherapy and minimal invasive intratumoral therapy.

Immunomodulation by Local Thermal Ablation of Cancer

Thermal ablation has been used for the management of localized tumors for patients not eligible for surgical resection. A growing body of evidence suggests that thermoablation could modulate both adaptive and innate immunity (154). However, the induced immune responses are mostly weak and not sufficient for the eradication of tumors or durable prevention of disease progression. In recent years, the combination of thermal ablation and ICIs therapy have been evaluated with promising results. Shi et al. reported that radiofrequency ablation (RFA) treatment of liver metastases increased not only T cell infiltration but also PD-L1 expression in primary human colorectal tumors (155). Using mouse tumor models, RFA treatment of one tumor was found to initially enhance a strong T cells mediated immune response in tumor. However, the tumor quickly overcame the immune responses by inhibiting CD8 and CD4 T cell function, subsequently driving a shift to higher regulatory T cell to Teff ratio (155). Importantly, the combination of RFA and anti-PD-1 antibodies was found to significantly enhance T cell immune responses and lead to prolonged animal survival (155).

Radiotherapy to Induce ICD

Radiotherapy is commonly used in cancer therapy to achieve a local control of the irradiated target tumor lesions regardless of clinical stage. Numerous preclinical studies have demonstrated that radiotherapy could activate anti-tumor immune response. Irradiation is known to activate host immunity by triggering ICD, which is characterized by the release of DAMPs to activate dendritic cells and to prime antigen-specific T cells in a dose-dependent manner (156). Procureur et al. has recently published an excellent review about the enhancement of ICIs by radiotherapy-induced immunogenic cell death (157). Radiotherapy-induced systemic immune activation could cause shrinkage of distant tumor lesions outside the irradiated field, a phenomenon known as abscopal effect (158). In the past, abscopal effect was believed to be a very rare phenomenon. However, recent clinical data revealed that the combination of radiotherapy and anti-PD-1/PD-L1 ICIs could induce the abscopal effect (159).

On the other hand, the induction of immunosuppressive cytokines and chemokines by radiotherapy contribute to immunosuppressive reactions (160). The PD-1/PD-L1 axis is one of the key factors in cancer immune escape induced by radiotherapy. Moreover, upregulation of PD-L1 expression has been reported in NSCLC patients who have undergone radiotherapy with or without

chemotherapy as preoperative treatment (161). In addition, EGFR signaling after irradiation leads to PD-L1 upregulation *via* the IL-6/JAK/STAT3 pathway (162). As anti-PD-1/PD-L1 antibodies could relieve this immunosuppression, it makes sense to combine PD-1/PD-L1 ICIs and radiotherapy (161).

The phase III PACIFIC trial (NCT02125461) provided the most remarkable clinical data to support the combination of radiotherapy and anti-PD-1/PD-L1 ICIs. In this study, progression-free survival was significantly prolonged by prescribing durvalumab as a consolidation therapy after concurrent chemoradiotherapy as compared with placebo (51). Based on this trial, durvalumab following chemoradiotherapy has been approved for the treatment of NSCLC by the US Food and Drug Administration in 2018.

Minimal Invasive Intratumoral Injection of ICD Inducer

Local immunotherapies such as the intratumoral injection of oncolytic compounds have been used to reinstate and enhance systemic anticancer immune responses. A recent animal study reported the use of local immunotherapy to sensitize the tumor to subsequent immune checkpoint blockade (163). LTX-401 is an oncolytic peptide designed for local immunotherapy. The sequential LTX-401 treatment combined with double checkpoint inhibition of PD-1 and CTLA-4 exhibited strong antineoplastic effects on both the primary lesions and distant tumors (163).

CONCLUSION

In this review, we summarize the recent investigations about the use of PD-1/PD-L1 blockade therapy in EGFR-mutated NSCLC. The underlying mechanisms leading to the inferior clinical efficacy of PD-1/PD-L1 inhibitors in EGFR-mutated NSCLC and new strategies for its potentiation are discussed. Currently, the NCCN guidelines do not recommend immunotherapy for treating NSCLC patients carrying EGFR mutations. The combinations of PD-1/PD-L1-based immunotherapy and several other treatment modalities are under active investigation in clinical trials. While outcomes of these trials are immature, the optimal sequence, schedule and dosing remain to be determined. Moreover, the possible risk of combined toxicity pose a major challenge for the drug combinations. Therefore, a thorough investigation about the mechanism of action and risks associated with drug combinations is needed. It will help identify specific patient population that can benefit from the drug combination, predict the likelihood of toxicities, and guide dosing/administration sequencing and clinical monitoring consideration.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2020. *CA Cancer J Clin* (2020) 70:7–30. doi: 10.3322/caac.21590
2. Jiang W, Cai G, Hu PC, Wang Y. Personalized Medicine in non-Small Cell Lung Cancer: A Review From a Pharmacogenomics Perspective. *Acta Pharm Sin B* (2018) 8:530–8. doi: 10.1016/j.apsb.2018.04.005
3. Gao J, Li HR, Jiang JH, Ding JY. Strategies to Overcome Acquired Resistance to EGFR TKI in the Treatment of non-Small Cell Lung Cancer. *Clin Transl Oncol* (2019) 21:1287–301. doi: 10.1007/s12094-019-02075-1
4. Yuan M, Huang LL, Chen JH, Wu J, Xu Q. The Emerging Treatment Landscape of Targeted Therapy in non-Small-Cell Lung Cancer. *Signal Tranduct Targeting Ther* (2019) 4:61. doi: 10.1038/s41392-019-0099-9
5. Passaro A, Attili I, Morganti S, Del Signore E, Gianoncelli L, Spitaleri G, et al. Clinical Features Affecting Survival in Metastatic NSCLC Treated With Immunotherapy: A Critical Review of Published Data. *Cancer Treat Rev* (2020) 89:102085. doi: 10.1016/j.ctrv.2020.102085
6. Govindan R, Szczesna A, Ahn MJ, Schneider CP, Gonzalez Mella PF, Barlesi F, et al. Phase III Trial of Ipilimumab Combined With Paclitaxel and Carboplatin in Advanced Squamous non-Small-Cell Lung Cancer. *J Clin Oncol* (2017) 35:3449–57. doi: 10.1200/JCO.2016.71.7629
7. Mooradian MJ, Gainor JF. Putting the Brakes on CTLA-4 Inhibition in Lung Cancer? *Transl Lung Cancer Res* (2018) 7(Suppl 1):S35–8. doi: 10.21037/tlcr.2018.01.05
8. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab Versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *N Engl J Med* (2015) 373:123–35. doi: 10.1056/NEJMoa1504627
9. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab Versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* (2015) 373:1627–39. doi: 10.1056/NEJMoa1507643
10. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab Plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* (2018) 378:2078–92. doi: 10.1056/NEJMoa1801005
11. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab Versus Docetaxel in Patients With Previously Treated Non-Small-Cell Lung Cancer (OAK): A Phase 3, Open-Label, Multicenter Randomized Controlled Trial. *Lancet* (2017) 389:255–65. doi: 10.1016/S0140-6736(16)32517-X
12. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab Versus Chemotherapy for PD-L1-positive Non-Small-Cell Lung Cancer. *N Engl J Med* (2016) 375:1823–33. doi: 10.1056/NEJMoa1606774
13. Langer CJ, Gadgeel SM, Borghaei H, Papadimitrakopoulou VA, Patnaik A, Powell SF, et al. Carboplatin and Pemetrexed With or Without Pembrolizumab for Advanced, non-Squamous Non-Small-Cell Lung Cancer: A Randomized, Phase 2 Cohort of the Open-Label KETNOTE-021 Study. *Lancet Oncol* (2016) 17:1497–508. doi: 10.1016/S1470-2045(16)30498-3
14. Kim JM, Chen DS. Immune Escape to PD-L1/PD-1 Blockade: Seven Steps to Success (or Failure). *Ann Oncol* (2016) 27:1492–504. doi: 10.1093/annonc/mdw217
15. Xia L, Liu Y, Wang Y. Pd-1/Pd-L1 Blockade Therapy in Advanced Non-Small-Cell Lung Cancer: Current Status and Future Directions. *Oncologist* (2019) 1:31–41. doi: 10.1634/theoncologist.2019-IO-S1-s05
16. Takada K, Okamoto T, Toyokawa G, Kozuma Y, Matsubara T, Haratake N, et al. The Expression of PD-L1 Protein as a Prognostic Factor in Lung Squamous Cell Carcinoma. *Lung Cancer* (2017) 104:7–15. doi: 10.1016/j.lungcan.2016.12.006

17. Takada K, Toyokawa G, Shoji F, Okamoto T, Maehara Y. The Significance of the PD-L1 Expression in non-Small-Cell Lung Cancer: Trenchant Double Swords as Predictive and Prognostic Markers. *Clin Lung Cancer* (2017) 19:120–9. doi: 10.1016/j.cllc.2017.10.014
18. Kerr K, Hirsch F. Programmed Death Ligand-1 Immunohistochemistry. Friend or Foe? *Arch Pathol Lab Med* (2016) 140:326–31. doi: 10.5858/arpa.2015-0522-SA
19. Ratcliffe MJ, Sharpe A, Midha A, Barker C, Scott M, Scorer P, et al. Agreement Between Programmed Cell Death Ligand-1 Diagnostic Assays Across Multiple Protein Expression Cutoffs in Non-Small Cell Lung Cancer. *Clin Cancer Res* (2017) 23:3585–91. doi: 10.1158/1078-0432.CCR-16-2375
20. Carbognin L, Pilotto S, Milella M, Vaccaro V, Brunelli M, Calio A, et al. Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A According to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers. *PloS One* (2015) 10:e0130142. doi: 10.1371/journal.pone.0130142
21. Karwacz K, Bricogne C, Macdonald D, Arce F, Bennett CL, Collins M, et al. Pd-L1 Co-Stimulation Contributes to Ligand-Induced T Cell Receptor Down-Modulation on CD8(+) T Cells. *EMBO Mol Med* (2011) 3:581–92. doi: 10.1002/emmm.201100165
22. Gato-Canas M, Martinez de Morentin X, Blanco-Luquin I, Fernandez-Irigoyen J, Zudaire I, Liechtenstein T, et al. A Core of Kinase-Regulated Interactomes Defines the Neoplastic MDSC Lineage. *Oncotarget* (2015) 6:27160–75. doi: 10.18632/oncotarget.4746
23. Ibanez-Vea M, Zuazo M, Gato M, Arasanz H, Fernandez-Hinojal G, Escors D, et al. Myeloid-Derived Suppressor Cells in the Tumor Microenvironment: Current Knowledge and Future Perspectives. *Arch Immunol Ther Exp* (2018) 66:113–23. doi: 10.1007/s00005-017-0492-4
24. Karwacz K, Arce F, Bricogne C, Kochan G, Escors D. Pd-L1 Co-Stimulation, Ligand-Induced TCR Down-Regulation and Anti-Tumor Immunotherapy. *Oncoimmunology* (2012) 1:86–8. doi: 10.4161/onci.1.1.17824
25. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, et al. Atezolizumab Versus Docetaxel for Patients With Previously Treated Non-Small-Cell Lung Cancer (POPLAR): A Multicentre, Open-Label, Phase 2 Randomized Controlled Trial. *Lancet* (2016) 387:P1837–1846. doi: 10.1016/S0140-6736(16)00587-0
26. Bocanegra A, Fernandez-Hinojal G, Zuazo-Ibarra M, Arasanz H, Garcia-Granda MJ, Hernandez C, et al. Pd-L1 Expression in Systemic Immune Cell Populations as a Potential Predictive Biomarker of Responses to PD-L1/PD-1 Blockade Therapy in Lung Cancer. *Int J Mol Sci* (2019) 20:1631. doi: 10.3390/ijms20071631
27. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. Pd-L2 Is a Second Ligand for PD-1 and Inhibits T Cell Activation. *Nat Immunol* (2001) 2:261–8. doi: 10.1038/85330
28. Tang S, Kim PS. A High-Affinity Human PD-1/PD-L2 Complex Informs Avenues for Small-Molecule Immune Checkpoint Drug Discovery. *Proc Natl Acad Sci USA* (2019) 116:24500–6. doi: 10.1073/pnas.1916916116
29. Kim JH, Kim HS, Kim BJ. Prognostic Value of Smoking Status in Non-Small-Lung Cancer Patients Treated With Immune Checkpoint Inhibitors: A Meta-Analysis. *Oncotarget* (2017) 8:93149–55. doi: 10.18632/oncotarget.18703
30. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-Tumor Genomic Biomarkers for PD-1 Checkpoint Blockade-Based Immunotherapy. *Science* (2018) 362:eaar3593. doi: 10.1126/science.aar3593
31. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of Tumor Mutation Burden as an Immunotherapy Biomarker: Utility for the Oncology Clinic. *Ann Oncol* (2019) 30:44–56. doi: 10.1093/annonc/mdy495
32. Maung T, Ergin H, Javed M, Inga EE, Khan S. Immune Checkpoint Inhibitors in Lung Cancer: Role of Biomarkers and Combination Therapies. *Cureus* (2020) 12:e8095. doi: 10.7759/cureus.8095
33. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational Landscape Determines Sensitivity to PD-1 Blockade in Non-Small Cell Lung Cancer. *Science* (2015) 348:124–8. doi: 10.1126/science.aaa1348
34. Peters S, Creelan B, Hellmann MD, Socinski MA, Reck M, Bhagavatheeswaran P, et al. Abstract Ct082: Impact of Tumor Mutation Burden on the Efficacy of First-Line Nivolumab in Stage IV or Recurrent non-Small Cell Lung Cancer: An Exploratory Analysis of Checkmate 026. *Cancer Res* (2017) 77:CT082. doi: 10.1158/1538-7445.AM2017-CT082
35. Chang H, Sasson A, Srinivasan S, Golhar R, Greenawalt DM, Geese WJ, et al. Bioinformatic Methods and Bridging of Assay Results for Reliable Tumor Mutational Burden Assessment in Non-Small-Cell Lung Cancer. *Mol Diagn Ther* (2019) 23:507–20. doi: 10.1007/s40291-019-00408-y
36. Ramalingam SS, Hellmann MD, Awad MM, Borghaei H, Gainor J, Brahmer J, et al. Abstract Ct078: Tumor Mutational Burden (Tmb) as a Biomarker for Clinical Benefit From Dual Immune Checkpoint Blockade With Nivolumab (Nivo) + Ipilimumab (Ipi) in First-Line (1l) Non-Small Cell Lung Cancer (Nsclc): Identification of Tmb Cutoff From Checkmate 568. *Cancer Res* (2018) 78:CT078. doi: 10.1158/1538-7445.AM2018-CT078
37. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab Plus Ipilimumab in Lung Cancer With a High Tumor Mutational Burden. *N Engl J Med* (2018) 378:2093–104. doi: 10.1056/NEJMoa1801946
38. Wang L, Zhao D, Qin K, Rehman FU, Zhang X. Effect and Biomarker of Nivolumab for Non-Small-Cell Lung Cancer. *BioMed Pharmacother* (2019) 117:109199. doi: 10.1016/j.biopha.2019.109199
39. Duma N, Santana-Davila R, Molina JR. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clin Proc* (2019) 94:1623–40. doi: 10.1016/j.mayocp.2019.01.013
40. Hirsch FR, Kerr KM, Bunn PA Jr, Kim ES, Obasaju C, Perol M, et al. Molecular and Immune Biomarker Testing in Squamous-Cell Lung Cancer: Effect of Current and Future Therapies and Technologies. *Clin Lung Cancer* (2018) 19:331–9. doi: 10.1016/j.cllc.2018.03.014
41. Pu X, Wu L, Su D, Mao W, Fang B. Immunotherapy for non-Small-Cell Lung Cancers: Biomarkers for Predicting Responses and Strategies to Overcome Resistance. *BMC Cancer* (2018) 1186:1082. doi: 10.1186/s12885-018-4990-5
42. Capalbo C, Scafetta G, Filetti M, Marchetti P, Bartolazzi A. Predictive Biomarkers for Checkpoint Inhibitor-Based Immunotherapy: The Galectin-3 Signature in Nsclcs. *Int J Mol Sci* (2019) 20:E1607. doi: 10.3390/ijms20071607
43. Proto C, Ferrara R, Signorelli D, Lo Russo G, Galli G, Imbimbo M, et al. Choosing Wisely First-Line Immunotherapy in Non-Small Cell Lung Cancer (NSCLC): What to Add and What to Leave Out. *Cancer Treat Rev* (2019) 75:39–51. doi: 10.1016/j.ctrv.2019.03.004
44. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 Pathway Contributes to Immune Escape in EGFR-Driven Lung Tumors. *Cancer Discovery* (2013) 3:1355–63. doi: 10.1158/1535-7163.TARG-13-B290
45. Chen N, Fang W, Zhan J, Hong S, Tang Y, Kang S, et al. Upregulation of PD-L1 by EGFR Activation Mediates the Immune Escape in EGFR-driven NSCLC: Implication for Optional Immune Targeted Therapy for NSCLC Patients With EGFR Mutations. *J Thorac Oncol* (2015) 10:910–23. doi: 10.1097/JTO.0000000000000500
46. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab Versus Docetaxel for Previously Treated, PD-L1-Positive, Advanced Non-Small-Cell Lung Cancer (KEYNOTE-010): A Randomized Controlled Trial. *Lancet* (2016) 387:1540–50. doi: 10.1016/S0140-6736(15)01281-7
47. Lee CK, Man J, Lord S, Links M, Gebski V, Mok T, et al. Checkpoint Inhibitors in Metastatic EGFR-mutated Non-Small Cell Lung Cancer- a Meta-Analysis. *J Thorac Oncol* (2017) 12:403–7. doi: 10.1016/j.jtho.2016.10.007
48. Gainor JF, Shaw AT, Sequist LV, Fu X, Azzoli CG, Piotrowska Z, et al. EGFR Mutations and ALK Rearrangements are Associated With Low Response Rates to PD-1 Pathway Blockade in Non-Small Cell Lung Cancer: A Retrospective Analysis. *Clin Cancer Res* (2016) 22:4585–93. doi: 10.1158/1078-0432.CCR-15-3101
49. Lisberg A, Cummings A, Goldman JW, Bornazyan K, Reese N, Wang T, et al. A Phase II Study of Pembrolizumab in EGFR-Mutant, Pd-L1+, Tyrosine Kinase Inhibitor Naïve Patients With Advanced NSCLC. *J Thorac Oncol* (2018) 13:1138–45. doi: 10.1016/j.jtho.2018.03.035
50. Lee CK, Man J, Lord S, Cooper W, Links M, Gebski V, et al. Clinical and Molecular Characteristics Associated With Survival Among Patients Treated With Checkpoint Inhibitors for Advanced Non-Small Cell Lung Carcinoma:

A Systematic Review and Meta-Analysis. *JAMA Oncol* (2018) 4:210–6. doi: 10.1001/jamaoncol.2017.4427
51. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab After Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* (2017) 377:1919–29. doi: 10.1056/NEJMoa1709937
52. Antonia SJ, Borghaei H, Ramalingam SS, Horn L, De Castro Carpeno J, Pluzanski A, et al. Four-Year Survival With Nivolumab in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer: A Pooled Analysis. *Lancet Oncol* (2019) 20:1395–408. doi: 10.1016/S1470-2045(19)30407-3
53. Hastings K, Yu HA, Wei W, Sanchez-Vega F, DeVeaux M, Choi J, et al. EGFR Mutation Subtypes and Response to Immune Checkpoint Blockade Treatment in Non-Small-Cell Lung Cancer. *Ann Oncol* (2019) 30:1311–20. doi: 10.1093/annonc/mdz141
54. D'Incecco A, Andreozzi M, Ludovini V, Rossi E, Capodanno A, Landi L, et al. PD-1 and PD-L1 Expression in Moleculely Selected Non-Small-Cell Lung Cancer Patients. *Br J Cancer* (2015) 112:95–102. doi: 10.1038/bjc.2014.555
55. Azuma K, Ota K, Kawahara A, Hattori S, Iwama E, Harada T, et al. Association of PD-L1 Overexpression With Activating EGFR Mutations in Surgically Resected Nonsmall-Cell Lung Cancer. *Ann Oncol* (2014) 25:1935–40. doi: 10.1093/annonc/mdu242
56. Tang Y, Fang W, Zhang Y, Hong S, Kang S, Yan Y, et al. The Association Between PD-L1 and EGFR Status and the Prognostic Value of PD-L1 in Advanced Non-Small Cell Lung Cancer Patients Treated With EGFR-Tkis. *Oncotarget* (2015) 6:14209–19. doi: 10.18632/oncotarget.3694
57. Ji M, Liu Y, Li Q, Ning Z, Zhao W, Shi H, et al. Pd-1/Pd-L1 Expression in non-Small-Cell Lung Cancer and its Correlation With EGFR/KRAS Mutations. *Cancer Biol Ther* (2016) 17:407–13. doi: 10.1080/15384047.2016.1156256
58. Dong ZY, Zhang JT, Liu SY, Su J, Zhang C, Xie Z, et al. EGFR Mutation Correlates With Uninflamed Phenotype and Weak Immunogenicity, Causing Impaired Response to PD-1 Blockade in Non-Small Cell Lung Cancer. *Oncoimmunology* (2017) 6:e1356145. doi: 10.1080/2162402X.2017.1356145
59. Koh J, Go H, Keam B, Kim MY, Nam SJ, Kim TM, et al. Clinicopathologic Analysis of Programmed Cell Death-1 and Programmed Cell Death-Ligand 1 and 2 Expressions in Pulmonary Adenocarcinoma: Comparison With Histology and Driver Oncogenic Alteration Status. *Mod Pathol* (2015) 28:1154–66. doi: 10.1038/modpathol.2015.63
60. Offin M, Rizvi H, Tenet M, Ni A, Sanchez-Vega F, Li BT, et al. Tumor Mutation Burden and Efficacy of EGFR-tyrosine Kinase Inhibitors in Patients With EGFR-mutant Lung Cancers. *Clin Cancer Res* (2019) 25:1063–9. doi: 10.1158/1078-0432.CCR-18-1102
61. Rizvi H, Plodkowski AJ, Tenet M, Halpenny D, Long N, Sauter JL, et al. Clinical and Molecular Features Predicting Long-Term Response (LTR) to anti-PD-(L)-1 Based Therapy in Patients With NSCLC. *J Clin Oncol* (2018) 36(Suppl 15):9022. doi: 10.1200/JCO.2018.36.15_suppl.9022
62. Mo J, Hu X, Gu L, Chen B, Khadaroo PA, Shen Z, et al. Smokers or non-Smokers: Who Benefits More From Immune Checkpoint Inhibitors in Treatment of Malignancies? An Up-to-Date Meta-Analysis. *World J Surg Oncol* (2020) 18:15. doi: 10.1186/s12957-020-1792-4
63. Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N Engl J Med* (2017) 377:2500–1. doi: 10.1056/NEJMc1713444
64. Gettinger S, Horn L, Jackman D, Spigel D, Antonia S, Hellmann M, et al. Five-Year Follow-Up of Nivolumab in Previously Treated Advanced Non-Small-Cell Lung Cancer: Results From the CA209-003 Study. *J Clin Oncol* (2018) 36:1675–84. doi: 10.1200/JCO.2017.77.0412
65. Hui R, Garon EB, Goldman JW, Leighl NB, Hellmann MD, Patnaik A, et al. Pembrolizumab as First-Line Therapy for Patients With PD-L1-positive Advanced Non-Small Cell Lung Cancer: A Phase I Trial. *Ann Oncol* (2017) 28:874–81. doi: 10.1093/annonc/mdx008
66. Garassino MC, Cho BC, Kim JH, Mazieres J, Vansteenkiste J, Lena H, et al. Durvalumab as Third-Line or Later Treatment for Advanced Non-Small-Cell Lung Cancer (ATLANTIC): An Open-Label, Single-Arm, Phase 2 Study. *Lancet Oncol* (2018) 19:521–36. doi: 10.1016/S1470-2045(18)30144-X
67. Leighl NB, Hellmann MD, Hui R, Carcereny E, Felip E, Ahn MJ, et al. Pembrolizumab in Patients With Advanced Non-Small-Cell Lung Cancer (KEYNOTE-001): 3-Year Results From an Open-Label, Phase I Study. *Lancet Respir Med* (2019) 7:347–57. doi: 10.1016/S2213-2600(18)30500-9
68. von Pawel J, Bordoni R, Satouchi M, Fehrenbacher L, Cobo M, Han JY, et al. Long-Term Survival in Patients With Advanced Non-Small-Cell Lung Cancer Treated With Atezolizumab Versus Docetaxel: Results From the Randomized Phase III OAK Study. *Eur J Cancer* (2019) 107:124–32. doi: 10.1016/j.ejca.2018.11.020
69. Mazieres J, Drilon A, Lusque A, Mhanna L, Cortot AB, Mezquita L, et al. Immune Checkpoint Inhibitors for Patients With Advanced Lung Cancer and Oncogenic Driver Alterations: Results From the Global IMMUNOTARGET Registry. *Ann Oncol* (2019) 30:1321–8. doi: 10.1093/annonc/mdz167
70. Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying Cancers Based on T-Cell Infiltration and PD-L1. *Cancer Res* (2015) 75:2139–45. doi: 10.1158/0008-5472.CAN-15-0255
71. Chen DS, Mellan I. Elements of Cancer Immunity and the Cancer-Immune Set Point. *Nature* (2017) 541:321–30. doi: 10.1038/nature21349
72. Streicher K, Higgs BW, Wu S, Coffman K, Damera G, Durham N, et al. Increased CD73 and Reduced IFNG Signature Expression in Relation to Response Rates to Anti-PD-1(L1) Therapies in EGFR-mutant Nsclc. *J Clin Oncol* (2017) 35:abstr 11505. doi: 10.1200/JCO.2017.35.15_suppl.11505
73. Busser B, Sancey L, Brambilla E, Coll JL, Hurbin A. The Multiple Roles of Amphiregulin in Human Cancer. *Biochim Biophys Acta* (2011) 1816:119–31. doi: 10.1016/j.bbcan.2011.05.003
74. Singh B, Carpenter G, Coffey RJ. EGF Receptor Ligands: Recent Advances. *F1000Res* (2016) 5:F1000 Faculty Rev–2270. doi: 10.12688/f1000research.9025.1
75. Zaiss DM, van Loosdregt J, Gorlani A, Bekker CP, Grone A, Sibilia M, et al. Amphiregulin Enhances Regulatory T Cell-Suppressive Function Via the Epidermal Growth Factor Receptor. *Immunity* (2013) 38:275–84. doi: 10.1016/j.immuni.2012.09.023
76. Wang S, Zhang Y, Wang Y, Ye P, Li J, Li H, et al. Amphiregulin Confers Regulatory T Cell Suppressive Function and Tumor Invasion Via the EGFR/GSK-3beta/Foxp3 Axis. *J Biol Chem* (2016) 291:21085–95. doi: 10.1074/jbc.M116.717892
77. Jiang R, Tang J, Chen Y, Deng L, Ji J, Xie Y, et al. The Long Noncoding RNA lnc-EGFR Stimulates T-Regulatory Cells Differentiation Thus Promoting Hepatocellular Carcinoma Immune Evasion. *Nat Commun* (2017) 8:15129. doi: 10.1038/ncomms15129
78. Mascia F, Schloemann DT, Cataisson C, McKinnon KM, Krymskaya L, Wolcott KM, et al. Cell Autonomous or Systemic EGFR Blockade Alters the Immune-Environment in Squamous Cell Carcinomas. *Int J Cancer* (2016) 139:2593–7. doi: 10.1002/ijc.30376
79. Jia J, Li X, Jiang T, Zhao S, Zhao C, Zhang L, et al. EGFR-Targeted Therapy Alters the Tumor Microenvironment in EGFR-Driven Lung Tumors: Implications for Combination Therapies. *Int J Cancer* (2019) 145:1432–44. doi: 10.1002/ijc.32191
80. Yu J, Du W, Yan F, Wang Y, Li H, Cao S, et al. Myeloid-Derived Suppressor Cells Suppress Antitumor Immune Responses Through IDO Expression and Correlate With Lymph Node Metastasis in Patients With Breast Cancer. *J Immunol* (2013) 190:3783–97. doi: 10.4049/jimmunol.1201449
81. Wu L, Du H, Li Y, Qu P, Yan C. Signal Transducer and Activator of Transcription 3 (STAT3C) Promotes Myeloid-Derived Suppressor Cell Expansion and Immune Suppression During Lung Tumorigenesis. *Am J Pathol* (2011) 179:2131–41. doi: 10.1016/j.ajpath.2011.06.028
82. Lo Sardo F, Strano S, Blandino G. YAP and TAZ in Lung Cancer: Oncogenic Role and Clinical Targeting. *Cancers (Basel)* (2018) 10:137. doi: 10.3390/cancers10050137
83. Wang G, Lu X, Dey P, Deng P, Wu CC, Jiang S, et al. Targeting YAP-dependent MDSC Infiltration Impairs Tumor Progression. *Cancer Discovery* (2016) 6:80–95. doi: 10.1158/2159-8290.CD-15-0224
84. Murakami S, Shahbazian D, Surana R, Zhang W, Chen H, Graham GT, et al. Yes-Associated Protein Mediates Immune Reprogramming in Pancreatic Ductal Adenocarcinoma. *Oncogene* (2017) 36:1232–44. doi: 10.1038/onc.2016.288
85. Ni X, Tao J, Barbi J, Chen Q, Park BV, Li Z, et al. YAP is Essential for Treg-Mediated Suppression of Antitumor Immunity. *Cancer Discovery* (2018) 8:1026–43. doi: 10.1158/2159-8290.CD-17-1124

86. Miao J, Hsu PC, Yang YL, Xu Z, Dai Y, Wang Y, et al. YAP Regulates PD-L1 Expression in Human NSCLC Expression in Human NSCLC Cells. *Oncotarget* (2017) 8:114576–87. doi: 10.18632/oncotarget.23051
87. Hsu PC, Miao J, Wang YC, Zhang WQ, Yang YL, Wang CW, et al. Inhibition of Yes-Associated Protein Down-Regulates PD-L1 (CD274) Expression in Human Malignant Pleural Mesothelioma. *J Cell Mol Med* (2018) 22:3139–48. doi: 10.1111/jcmm.13593
88. Lee BS, Park DI, Lee DH, Lee JE, Yeo MK, Park YH, et al. Hippo Effector YAP Directly Regulates the Expression of PD-L1 Transcripts in EGFR-TKI-Resistant Lung Adenocarcinoma. *Biochem Biophys Res Commun* (2017) 491:493–9. doi: 10.1016/j.bbrc.2017.07.007
89. McGowan M, Kleinberg L, Halvorsen AR, Helland A, Brustugun OT. NSCLC Depend Upon YAP Expression and Nuclear Localization After Acquiring Resistance to EGFR Inhibitors. *Genes Cancer* (2017) 8:497–504. doi: 10.18632/genesandcancer.136
90. Chen K, Cheng G, Zhang F, Zhu G, Xu Y, Yu X, et al. Pd-L1 Expression and T Cells Infiltration in Patients With Uncommon EGFR-Mutant Non-Small Cell Lung Cancer and the Response to Immunotherapy. *Lung Cancer* (2020) 142:98–105. doi: 10.1016/j.lungcan.2020.02.010
91. Brindel A, Althakfi W, Barritault M, Watkin E, Maury JM, Bringuier PP, et al. Uncommon EGFR Mutations in Lung Adenocarcinoma: Features and Response to Tyrosine Kinase Inhibitors. *J Thorac Dis* (2020) 12:4643–50. doi: 10.21037/jtd-19-3790
92. De Pas T, Toffalorio F, Manzotti M, Fumagalli C, Spitaleri G, Catania C, et al. Activity of Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors in Patients With Non-Small Cell Lung Cancer Harboring Rare Epidermal Growth Factor Receptor Mutations. *J Thorac Oncol* (2011) 6:1895–901. doi: 10.1097/JTO.0b013e318227e8c6
93. Yang JC, Sequist LV, Geater SL, Tsai CM, Mok TS, Schuler M, et al. Clinical Activity of Afatinib in Patients With Advanced Non-Small-Cell Lung Cancer Harbouring Uncommon EGFR Mutations: A Combined Post-Hoc Analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. *Lancet Oncol* (2015) 16:830–8. doi: 10.1016/S1470-2045(15)00026-1
94. Lund-Iversen M, Kleinberg L, Fjellbirkeland L, Helland A, Brustugun OT. Clinicopathological Characteristics of 11 NSCLC Patients With EGFR-Exon 20 Mutations. *J Thorac Oncol* (2012) 7:1471–3. doi: 10.1097/JTO.0b013e3182614a9d
95. Meseure D, Vacher S, Alsibai KD, Nicolas A, Chemlali W, Caly R, et al. Expression of ANRIL-polycomb complexes-CDKN2A/B/ARF Genes in Breast Tumors: Identification of a Two-Gene (EZH2/CBX7) Signature With Independent Prognostic Value. *Mol Cancer Res* (2016) 14:623–33. doi: 10.1158/1541-7786.MCR-15-0418
96. Massarelli E, Johnson FM, Erickson HS, Wistuba II, Papadimitrakopoulou V. Uncommon Epidermal Growth Factor Receptor Mutations in Non-Small Cell Lung Cancer and Their Mechanisms of EGFR Tyrosine Kinase Inhibitors Sensitivity and Resistance. *Lung Cancer* (2013) 80:235–41. doi: 10.1016/j.lungcan.2013.01.018
97. Yamada T, Hirai S, Katayama Y, Yoshimura A, Shiotsu S, Watanabe S, et al. Retrospective Efficacy Analysis of Immune Checkpoint Inhibitors in Patients With EGFR-Mutated Non-Small Cell Lung Cancer. *Cancer Med* (2019) 8:1521–9. doi: 10.1002/cam4.2037
98. Postow MA, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated With Immune Checkpoint Blockade. *N Engl J Med* (2018) 378:158–68. doi: 10.1056/NEJMra1703481
99. Das S, Johnson DB. Immune-Related Adverse Events and Anti-Tumor Efficacy of Immune Checkpoint Inhibitors. *J Immunother Cancer* (2019) 7:306. doi: 10.1186/s40425-019-0805-8
100. Haratani K, Hayashi H, Chiba Y, Kudo K, Yonesaka K, Kato R, et al. Association of Immune-Related Adverse Events With Nivolumab Efficacy in non-Small-Cell Lung Cancer. *JAMA Oncol* (2018) 4:374–8. doi: 10.1001/jamaoncol.2017.2925
101. Terraoka S, Fujimoto D, Morimoto T, Kawachi H, Ito M, Sato Y, et al. Early Immune-Related Adverse Events and Association With Outcome in Advanced Non-Small Cell Lung Cancer Patients Treated With Nivolumab: A Prospective Cohort Study. *J Thorac Oncol* (2017) 12:1798–805. doi: 10.1016/j.jtho.2017.08.022
102. Oxnard GR, Yang JC, Yu H, Kim SW, Saka H, Horn L, et al. TATTON: A Multi-Arm, Phase Ib Trial of Osimertinib Combined With Selumetinib, Savolitinib, or Durvalumab in EGFR-Mutant Lung Cancer. *Ann Oncol* (2020) 31:507–16. doi: 10.1016/j.annonc.2020.01.013
103. Yang JC, Shepherd FA, Kim DW, Lee GW, Lee JS, Chang GC, et al. Osimertinib Plus Durvalumab Versus Osimertinib Monotherapy in EGFR T790M-Positive NSCLC Following Previous EGFR TKI Therapy: CAURAL Brief Report. *J Thorac Oncol* (2019) 14:933–9. doi: 10.1016/j.jtho.2019.02.001
104. Puzanov I, Diab A, Abdallah K, Bingham CO3, Brogdon C, Dadu R, et al. Managing Toxicities Associated With Immune Checkpoint Inhibitors: Consensus Recommendations From the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer* (2017) 5:95. doi: 10.1186/s40425-017-0300-z
105. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-Related Adverse Events With Immune Checkpoint Blockade: A Comprehensive Review. *Eur J Cancer* (2016) 54:139–48. doi: 10.1016/j.ejca.2015.11.016
106. Champiat S, Lambotte O, Barreau E, Belkhir R, Berdelou A, Carbonnel F, et al. Management of Immune Checkpoint Blockade Dysimmune Toxicities: A Collaborative Position Paper. *Ann Oncol* (2016) 27:559–74. doi: 10.1093/annonc/mdv623
107. Ferrara R, Mezquita L, Texier M, Lahmar J, Audigier-Valette C, Tessonnier L, et al. Hyperprogressive Disease in Patients With Advanced non-Small Cell Lung Cancer Treated With PD-1/PD-L1 Inhibitors or With Single-Agent Chemotherapy. *JAMA Oncol* (2018) 4:1543–52. doi: 10.1001/jamaoncol.2018.3676
108. Wang X, Wang F, Zhong M, Yarden Y, Fu L. The Biomarkers of Hyperprogressive Disease in PD-1/PD-L1 Blockage Therapy. *Mol Cancer* (2020) 19:81. doi: 10.1186/s12943-020-01200-x
109. Fuentes-Antras J, Provencio M, Diaz-Rubio E. Hyperprogression as a Distinct Outcome After Immunotherapy. *Cancer Treat Rev* (2018) 70:16–21. doi: 10.1016/j.ctrv.2018.07.006
110. Kas B, Talbot H, Ferrara R, Richard C, Lamarque JP, Pitre-Champagnat S, et al. Clarification of Definitions of Hyperprogressive Disease During Immunotherapy for Non-Small Cell Lung Cancer. *JAMA Oncol* (2020) 6:1039–46. doi: 10.1001/jamaoncol.2020.1634
111. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. *Cancer Cell* (2015) 28:690–714. doi: 10.1016/j.ccell.2015.10.012
112. Ahmed A, Tait SWG. Targeting Immunogenic Cell Death in Cancer. *Mol Oncol* (2020) 14:2994–3006. doi: 10.1002/1878-0261.12851
113. Fucikova J, Kepp O, Kasikova L, Petroni G, Yamazaki T, Liu P, et al. Detection of Immunogenic Cell Death and its Relevance of Cancer Therapy. *Cell Death Dis* (2020) 11:1013. doi: 10.1038/s41419-020-03221-2
114. Wang YJ, Fletcher R, Yu J, Zhang L. Immunogenic Effects of Chemotherapy-Induced Tumor Cell Death. *Genes Dis* (2018) 5:194–203. doi: 10.1016/j.gendis.2018.05.003
115. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic Chemotherapy Sensities Tumors to Checkpoint Blockade Therapy. *Immunity* (2016) 44:343–54. doi: 10.1016/j.immuni.2015.11.024
116. Kepp O, Zitvogel L, Kroemer G. Clinical Evidence That Immunogenic Cell Death Sensitizes to PD-1/PD-L1 Blockade. *Oncoimmunology* (2019) 8:1637188. doi: 10.1080/2162402X.2019.1637188
117. Park K, Yang JC, Girard N, Mok T, Gainor J, Nakagawa K, et al. P1-182 – Nivolumab + Chemotherapy vs Chemotherapy in EGFR-mutated NSCLC After 1L or 2L EGFR Tkis (CheckMate 722). *Ann Oncol* (2019) 30(suppl_6): vi126. doi: 10.1093/annonc/mdz343.039
118. Riely G, Hui R, Carbone D, Park K, Carrigan M, Xu X, et al. P1.01-81 Phase 3 Study of Pemetrexed-Platinum With or Without Pembrolizumab for TKI-Resistant / EGFR-mutated Advanced NSCLC: Keynote-789. *J Thorac Oncol* (2018) 13(10):Supp S494. doi: 10.1016/j.jtho.2018.08.637
119. Plesca I, Tunger A, Muller L, Wehner R, Lai X, Grimm M-L, et al. Characteristics of Tumor-Infiltrating Lymphocytes Prior to and During Immune Checkpoint Inhibitor Therapy. *Front Immunol* (2020) 11:364. doi: 10.3389/fimmu.2020.00364
120. Li X, Lian Z, Wang S, Xing L, Yu J. Interactions Between EGFRand Pd-1/Pd-L1 Pathway: Implications for Treatment of NSCLC. *Cancer Lett* (2018) 418:1–9. doi: 10.1016/j.canlet.2018.01.005
121. Lizotte PH, Hong RL, Luster TA, Cavanaugh ME, Taus LJ, Wang S, et al. A High-Throughput Immune-Oncology Screen Identifies EGFR Inhibitors as Potent Enhancers of Antigen-Specific Cytotoxic T-lymphocyte Tumor Cell Killing. *Cancer Immunol Res* (2018) 6:1511–23. doi: 10.1158/1538-7445.AM2018-4935

122. Hughes PE, Caenepeel S, Wu LC. Targeted Therapy and Checkpoint Immunotherapy Combinations for the Treatment of Cancer. *Trends Immunol* (2016) 37:462–76. doi: 10.1016/j.it.2016.04.010
123. Moya-Horno I, Viteri S, Karachaliou N, Rosell R. Combination of Immunotherapy With Targeted Therapies in Advanced non-Small Cell Lung Cancer (NSCLC). *Ther Adv Med Oncol* (2018) 10:1758834017745012. doi: 10.1177/1758834017745012
124. Jia Y, Zhao S, Jiang T, Li X, Zhao C, Liu Y, et al. Impact of EGFR-TKIs Combined With PD-L1 Antibody on the Lung Tissue of EGFR-Driven Tumor-Bearing Mice. *Lung Cancer* (2019) 137:85–93. doi: 10.1016/j.lungcan.2019.09.016
125. Gettinger S, Hellmann M, Chow L, Borghaei H, Antonia S, Brahmer J, et al. Nivolumab Plus Erlotinib in Patients With EGFR-Mutant Advanced NSCLC. *J Thorac Oncol* (2018) 13:1363–72. doi: 10.1016/j.jtho.2018.05.015
126. Rudin C, Cervanters A, Dowlati A, Besse B, Ma B, Costa D, et al. Abstract MA15.02 Long-Term Safety and Clinical Activity Results From a Phase IB Study of Erlotinib Plus Atezolizumab in Advanced NSCLC. *J Thorac Oncol* (2018) 13:MA15.02. doi: 10.1016/j.jtho.2018.08.440
127. Creelan BC, Yeh TC, Kim SW, Nogami N, Kim DW, Chow LQ, et al. A Phase 1 Study of Gefitinib Combined With Durvalumab in EGFR TKI-Naïve Patients With EGFR Mutation-Positive Locally Advanced/Metastatic non-Small-Cell Lung Cancer. *Br J Cancer* (2021) 124:383–90. doi: 10.1038/s41416-020-01099-7
128. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med* (2018) 378:2288–301. doi: 10.1056/NEJMoa1716948
129. Yang J, Gadgeel S, Sequist L, Wu C, Papadimitrakopoulou V, Su W, et al. Pembrolizumab in Combination With Erlotinib or Gefitinib as First-Line Therapy for Advanced NSCLC With Sensitizing EGFR Mutation. *J Thorac Oncol* (2019) 14:553–9. doi: 10.1016/j.jtho.2018.11.028
130. Thress KS, Jacobs V, Angell HK, Yang JC, Sequist LV, Blackhall F, et al. Modulation of Biomarker Expression by Osimertinib: Results of the Paired Tumor Biopsy Cohorts of the AURA Phase I Trial. *J Thorsc Oncol* (2017) 12:1588–94. doi: 10.1016/j.jtho.2017.07.011
131. Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, et al. Overall Survival With Cisplatin-Gemcitabine and Bevacizumab or Placebo as First-Line Therapy for Nonsquamous Non-Small-Cell Lung Cancer: Results From a Randomized Phase III Trial (Avail). *Ann Oncol* (2010) 21:1804–9. doi: 10.1093/annonc/mdq020
132. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and Development of Bevacizumab, an anti-VEGF Antibody for Treating Cancer. *Nat Rev Drug Discovery* (2004) 3:391–400. doi: 10.1038/nrd1381
133. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet AL, et al. VEGF-a Modulates Expression of Inhibitory Checkpoints on CD8+ T Cells in Tumors. *J Exp Med* (2015) 212:139–48. doi: 10.1084/jem.20140559
134. Hedge PS, Wallin JJ, Mancao C. Predictive Markers of anti-VEGF and Emerging Role of Angiogenesis Inhibitors as Immunotherapeutics. *Semin Cancer Biol* (2018) 52:117–24. doi: 10.1016/j.semcancer.2017.12.002
135. Chen DS, Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* (2013) 39:1–10. doi: 10.1016/j.immuni.2013.07.012
136. Rotte A, Jin JY, Lemaire V. Mechanistic Overview of Immune Checkpoints to Support the Rational Design of Their Combinations in Cancer Immunotherapy. *Ann Oncol* (2018) 29:71–83. doi: 10.1093/annonc/mdx686
137. Rotte A. Combination of CTLA-4 and PD-1 Blockers for Treatment of Cancer. *J Exp Clin Cancer Res* (2019) 38:255. doi: 10.1186/s13046-019-1259-z
138. Antonia S, Goldberg SB, Balmanoukian A, Chaft JE, Sanborn RE, Gupta A, et al. Safety and Antitumor Activity of Durvalumab Plus Tremelimumab in Non-Small Cell Lung Cancer: A Multicentre, Phase 1B Study. *Lancet Oncol* (2016) 17:299–308. doi: 10.1016/S1470-2045(15)00544-6
139. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab Plus Ipilimumab in Lung Cancer With a High Tumor Mutational Burden. *N Engl J Med* (2018) 378:2093–104. doi: 10.1056/NEJMoa1801946
140. Ready N, Hellmann MD, Awad MM, Otterson GA, Gutierrez M, Gainor JF, et al. First-Line Nivolumab Plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer (CheckMate 568): Outcomes by Programmed Death Ligand 1 and Tumor Mutational Burden as Biomarkers. *J Clin Oncol* (2019) 37:992–1000. doi: 10.1200/JCO.18.01042
141. Mumm JB, Emmerich J, Zhang X, Chan I, Wu L, Mauze S, et al. Il-10 Elicits IFNgamma-dependent Tumor Immune Surveillance. *Cancer Cell* (2011) 20:781–96. doi: 10.1016/j.ccr.2011.11.003
142. Naing A, Infante JR, Papadopoulos KP, Chan IH, Shen C, Ratti NP, et al. Pegylated IL-10 (Pegilodecakin) Induces Systemic Immune Activation, CD8 (+) T Cell Invigoration and Polyclonal T Cell Expansion in Cancer Patients. *Cancer Cell* (2018) 34:775–91. doi: 10.1016/j.ccell.2018.10.007
143. Oft M. Immune Regulation and Cytotoxic T Cell Activation of IL-10 Agonists – Preclinical and Clinical Experience. *Semin Immunol* (2019) 44:101325. doi: 10.1016/j.smim.2019.101325
144. Naing A, Wong DJ, Infante JR, Korn WM, Aljumaily R, Papadopoulos KP, et al. Pegilodecakin Combined With Pembrolizumab or Nivolumab for Patients With Advanced Solid Tumors (IVY): A Multicenter, Multicohort, Open-Label, Phase Ib Trial. *Lancet Oncol* (2019) 20:1544–55. doi: 10.1016/S1470-2045(19)30514-5
145. Peguero JA, Bajaj P, Carcereny E, Clay TD, Doger B, Felip E, et al. A Multicentre, Phase II Study of Soluble LAG-3 (Eftilagimod Alpha) in Combination With Pembrolizumab (TACTI-002) in Patients With Advanced non-Small Cell Lung Cancer (NSCLC) or Head and Neck Squamous Cell Carcinoma (HNSCC). *J Clin Oncol* (2019) 3715_suppl: TPS2667–TPS2667. doi: 10.1200/JCO.2019.37.15_suppl.TPS2667
146. You B, Yang YL, Xu Z, Dai Y, Liu S, Mao JH, et al. Inhibition of ERK1/2 Down-Regulates the Hippo/YAP Signalling Pathway in Human NSCLC Cells. *Oncotarget* (2015) 6:4357–68. doi: 10.18632/oncotarget.2974
147. Lee JW, Zhang Y, Eoh KJ, Sharma R, Sanmamed MF, Wu J, et al. The Combination of MEK Inhibitor With Immunomodulatory Antibodies Targeting Programmed Death 1 and Programmed Death Ligand 1 Results in Prolonged Survival in Kras/p53-driven Lung Cancer. *J Thorac Oncol* (2019) 14:1046–60. doi: 10.1016/j.jtho.2019.02.004
148. Galli GG, Carrara M, Yuan WC, Valdes-Quezada C, Gurung B, Pepe-Mooney B, et al. YAP Drives Growth by Controlling Transcriptional Pause Release From Dynamic Enhancers. *Mol Cell* (2015) 60:328–37. doi: 10.1016/j.molcel.2015.09.001
149. Zhang H, Pandey S, Travers M, Sun H, Morton G, Madzo J, et al. Targeting CDK9 Reactivates Epigeneitically Silenced Genes in Cancer. *Cell* (2018) 175:1244–58. doi: 10.1016/j.cell.2018.09.051
150. Tang Z, Ma Q, Wang L, Liu C, Gao H, Yang Z, et al. A Brief Review: Some Compounds Targeting YAP Againist Malignancies. *Future Oncol* (2019) 15:1535–43. doi: 10.2217/fon-2019-0035
151. Wang H, Franco F, Tsui YC, Xie X, Trefny MP, Zappasodi R, et al. CD36-Mediated Metabolic Adaptation Supports Regulatory T Cell Survival and Function in Tumors. *Nat Immunol* (2020) 21:298–308. doi: 10.1038/s41590-019-0589-5
152. Ghiringhelli F, Bruchard M, Chalmin F, Rebe C. Production of Adenosine by Ectonucleotides: A Key Factor in Tumor Immunoescape. *J BioMed Biotech* (2012) 2012:473712. doi: 10.1155/2012/473712
153. ClinicalTrials.gov. *Study of TJ004309 in Combination With Atezolizumab (Tecentriq) in Patients With Advanced or Metastatic Cancer*. Available at: https://clinicaltrials.gov/ct2/show/NCT03835949 (Accessed 15 Apr 2021).
154. Haen SP, Pereira PL, Salih HR, Rammensee HG, Gouttefangeas C. More Than Just Tumor Destruction: Immunomodulation by Thermal Ablation of Cancer. *Clin Dev Immunol* (2011) 2011:160250. doi: 10.1155/2011/160250
155. Shi L, Chen L, Wu C, Zhu Y, Xu B, Zheng X, et al. PD-1 Blockade Boosts Radiofrequency Ablation-Elicited Adaptive Immune Responses Against Tumor. *Clin Cancer Res* (2016) 22:1173–84. doi: 10.1158/1078-0432.CCR-15-1352
156. Golden EB, Frances D, Pellicciotta I, Demaria S, Barcellos-Hoff MH, Formenti SC. Radiation Fosters Dose-Dependent and Chemotherapy-Induced Immunogenic Cell Death. *Oncoimmunology* (2014) 3:28518. doi: 10.4161/onci.28518
157. Procureur A, Simonaggio A, Bibault JE, Oudard S, Vano YA. Enhance the Immune Checkpoint Inhibitors Efficacy With Radiotherapy Induced Immunogenic Cell Death: A Comprehensive Review and Latest Developments. *Cancers* (2021) 13:678. doi: 10.3390/cancers13040678
158. Yilmaz MT, Elmali A, Yazici G. Abscopal Effect, From Myth to Reality: From Radiation Oncologists' Perspective. *Cureus* (2019) 11:e3860. doi: 10.7759/cureus.3860
159. Ashrafizadeh M, Farhood B, Musa AE, Taeb S, Rezaeyan A, Najafi M. Abscopal Effect in Radioimmunotherapy. *Int Immunopharmacol* (2020) 85:106663. doi: 10.1016/j.intimp.2020.106663
160. Shevtsov M, Sato H, Multhoff G, Shibata A. Novel Approaches to Improve the Efficacy of Immune-Radiotherapy. *Front Oncol* (2019) 9:156. doi: 10.3389/fonc.2019.00156

161. Sato H, Okonogi N, Nakano T. Rationale of Combination of Anti-PD-1/PD-L1 Antibody Therapy and Radiotherapy for Cancer Treatment. *Int J Clin Oncol* (2020) 25:801–9. doi: 10.1007/s10147-020-01666-1
162. Zhang N, Zeng YY, Du WW, Zhu JJ, Shen D, Liu Z, et al. The EGFR Pathway is Involved in the Regulation of PD-L1 Expression Via the IL-6/JAK/STAT3 Signaling Pathway in EGFR-mutated Non-Small Cell Lung Cancer. *Int J Oncol* (2016) 49:1360–8. doi: 10.3892/ijo.2016.3632
163. Xie W, Mondragon L, Mauseth B, Wang Y, Pol J, Levesque S, et al. Tumor Lysis With LTX-401 Creates Anticancer Immunity. *Oncoimmunology* (2019) 8:1594555. doi: 10.1080/2162402X.2019.1594555

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c-Src Increases the Sensitivity to TKIs in the EGFR-Mutant Lung Adenocarcinoma

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c-Src and the epidermal growth factor receptor (EGFR) are key apical kinases that govern cell responses to microenvironmental cues. How c-Src affects EGFR-related signaling and targeted therapy remains elusive. Initially, caspase-8 phosphorylated at tyrosine 380 by c-Src predominantly enhancing c-Src activation to facilitate metastasis through attaining epithelial-mesenchymal transition (EMT) phenotype in lung adenocarcinoma. Mechanistically, the linkage of c-Src SH2 domain with phosphotyrosine 380 of caspase-8 and SH3 domain with "PDEP" motif of caspase-8 overactivates c-Src as compared with other c-Src-partner proteins. c-Src is incapable of triggering EGFR-related signaling. This is reflected by the levels of phosphotyrosine 1068, 1086, and 1145, which have no impact on c-Src activation. Tyrosine kinase inhibitors (TKIs) suppress EGFR-related signaling to yield cell deaths of lung adenocarcinoma by both necroptosis and intrinsic apoptosis. Given that c-Src activation is frequent in lung adenocarcinoma, blocking c-Src activation through dasatinib can seal the survival-signaling-related phosphotyrosines of EGFR by its SH2 domain, which in turn increases the antitumor activity of TKIs in EGFR-mutant lung adenocarcinoma. Collectively, c-Src inactivation by dasatinib administration sensitizes EGFR-mutant lung adenocarcinoma to TKIs.

Keywords: c-Src, caspase-8, non-small cell lung cancer, tyrosine kinase inhibitor, resistance

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HIGHLIGHTS

- c-Src exclusively phosphorylated caspase-8 at tyrosine 380. Caspase-8 was predominant for c-Src overactivation by phosphotyrosine 380 and "PDEP" motif docking to SH2 domain and SH3 domain of c-Src in lung adenocarcinoma;
- c-Src inactivation through dasatinib was able to seal the survival-signaling-related phosphotyrosines of EGFR to increase the TKIs-induced necroptosis in the EGFR-mutant lung adenocarcinoma.

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide, with a surprising increase in the incidence of lung adenocarcinoma; traditional chemotherapeutic drugs are only modestly effective (1–4). Recent advances with targeted therapies have provided a marked benefit to subsets of patients whose tumors harbor specific genetic abnormalities (5, 6). In particular, lung adenocarcinomas with mutations in the gene encoding the epidermal growth factor receptor (EGFR) are uniquely sensitive to EGFR blockade with specific tyrosine kinase inhibitors (TKIs) (7, 8). Majority of cancers with EGFR-sensitive mutations achieve the marked and durable responses to treatment with EGFR TKIs, including gefitinib or erlotinib. However, lung adenocarcinoma inevitably acquires resistance to these inhibitors after approximately 1 year. Multiple mechanisms of acquired resistance to first- and second-generation EGFR-TKIs have been identified thus far (7), in which 30% of EGFR TKI resistance was because of an unknown mechanism (8). This sheds light on the lack of research on the underlying mechanism of EGFR TKIs, which must be investigated to enhance the therapeutic potency in lung adenocarcinoma.

Caspase-8, an apical sensory protease, is recruited into the death-inducing signaling complexes following death-related receptor ligation to initiate an extrinsic apoptosis cascade (9, 10). The inactivating mutation of caspase-8 is surprisingly infrequent among various human cancers (11–13). The study that reported the association of caspase-8 to adhesion and metastasis of human tumors is actually recent (14–16). It is of note that caspase-8 has also been reported to be involved in the focal adhesion complexes (17). The interplay between caspase-8 and c-Src is confirmed by the observation that cells stimulated with the survival-promoting factors lead to c-Src–mediated caspase-8 phosphorylation on tyrosine 380 (pY380 caspase-8 or p-Casp8), which then inhibits its apoptotic function (18–20). Consistently, we found that p-Casp8 reversely activated c-Src (pY416 c-Src or p-Src) *via* docking of phosphotyrosine 380 to the SH2 domain to restrain chemotherapy efficacy in lung adenocarcinoma (21). c-Src overactivation was ubiquitously detected in human tumors and involved in the resistance of TKIs (7, 22). Therefore, it is of interest to explore the interaction between caspase-8 and c-Src and their effect on the clinical efficacy of TKIs in EGFR-mutant lung adenocarcinoma.

In our study, caspase-8 phosphorylated by c-Src predominantly enhanced c-Src activation to facilitate metastasis through attaining EMT phenotypic features in lung adenocarcinoma. We found that EGFR activation and c-Src activation did not mutually interact with one another. TKIs suppressed EGFR-related signaling to yield cell deaths of lung adenocarcinoma by necroptosis and intrinsic apoptosis. Surprisingly, c-Src inactivation through caspase-8 knockdown or dasatinib was able to block the survival-signaling-related tyrosine phosphorylation of EGFR, which, in turn, increased the antitumor activity of TKIs in EGFR-mutant lung adenocarcinoma.

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

The procedures of this study, which included seven references, were approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University. The experiments were performed upon receiving written consent from each subject. The study methodologies conformed to the standards set by the Declaration of Helsinki.

Patients and Treatments

Human lung adenocarcinoma and adjacent paracancerous tissues (≥ 2.0 cm from the primary tumor site) from 84 patients were collected following surgeries at the Department of Pathology of the First and Second Affiliated Hospital of Xi'an Jiaotong University from 2009 to 2012. Lung adenocarcinoma was determined by two individual pathologists and classified as pathological stages I to IIIA according to the American Joint Committee on Cancer 2018 (AJCC 2018). Parallel to this, tissues from metastatic lung adenocarcinomas with EGFR-sensitive mutations of patients from our cancer center were retrospectively collected. EGFR mutation was performed by the Amplification-refractory mutation system (ARMS) in Big Science (China, HuaDa gene). The patients with EGFR-mutant lung adenocarcinoma received the first-generation TKIs gefitinib or erlotinib, in accordance with the guidelines. The response to treatment, including complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD), was evaluated according to response evaluation criteria in solid tumors (RECIST 1.1) until disease progression. The eligible patients were routinely scheduled for lifelong follow-up at the outpatient clinic every 3 months during the first 2 years and every 6 months for the next 3 years. Whenever recurrent or metastatic events were suspected, radiologic, endoscopic, and histologic confirmation was compulsory. The calculation of duration of response started at the date of treatment and ended at the date of the following events: recurrence, disease progression, or oncological death. The calculation of overall survival (OS) started at the date of treatment and ended at the date of death. Unless it was reported here, no participants were lost during follow-up. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University. Informed consent was obtained from the patients before the study implementation.

Cell Culture, DNA/Short Hairpin RNA Transfection, and Stable Cell Line Generation

Lung adenocarcinoma cell lines, including A549 and National Cancer Institute (NCI)-H522, were kind gifts from Chen Huang from the Department of Cell Biology, Xi'an Jiaotong University, Shaanxi Province, P.R. China. H1650, H3255 and PC9 cells were purchased from the American Type Culture Collection (ATCC) and cultured in RPMI 1640 supplemented with 10% fetal bovine

serum (FBS, Hyclone Laboratories Inc., Logan, UT, USA) and penicillin/streptomycin/L-glutamine (Sigma-Aldrich, St. Louis, MO, USA). The cell lines in our study were authenticated by short tandem repeat (STR) analysis. To observe the morphological features of EMT, we cultured cells for 5 days on fibronectin (10 μg/ml)-coated dishes. EGFR-nonaddictive lung adenocarcinoma cells were assayed for expression and viability following treatment with reagents or drugs at 24 h after attachment to the fibronectin-coated dishes. EGFR-addictive lung adenocarcinoma cells were not attached on fibronectin-coated dishes for 24 h. The knockdown of Fyn, Yes, Lyn, Hck, EGFR, Her-2, Her-3, Her-4, FAK, and caspase-8 or c-Src was performed using lentivirus-delivered short hairpin RNAs (shRNAs; GenePharma, Shanghai, China), with the shRNAs corresponding to the siRNAs. Following lentiviral transfection, stable cell lines were selected *via* culturing in the presence of 500 μg/ml G418 (Grand Island Biological Company, Waltham, MA, USA). The open reading frames of genes of interest, including Fgr, Lck, Blk, wild-type caspase-8, EGFR and its mutants, and c-Src and its mutants, were cloned into the MSCV-IRES-zeo plasmid with a hemagglutinin (HA) tag to allow the expression of HA-tagged proteins. The subsequent DNA was transfected into packaging cells. Virus-containing supernatants were removed, and debris was pelleted by centrifugation. Target cells were cultured in virus-containing supernatants for 48 h before selection for a stable cell line with 10 mg/ml zeocin (Invitrogen, Waltham, MA, USA) for 14 days.

RESULTS

Tyrosine 380 of Caspase-8 Was Pivotal for Lung Adenocarcinoma Metastasis Through EMT

The recent evidence has been reported that supports the role of caspase-8 in tumor cell migration under the nonapoptotic condition (15, 17, 23, 24). Our team found that c-Src phosphorylated caspase-8 at tyrosine 380 hampered the apoptosis of caspase-8 responding to chemotherapy in lung adenocarcinoma (25). We initially probed the interactive effects of caspase-8 and c-Src on the aggressive properties of lung adenocarcinoma. The expressions of caspase-8 and c-Src were examined using immunoblotting in the lung adenocarcinoma-derived cell lines, including A549, H522, PC9, H1975, H1650, H3255, and H23 (**Figure 1A**). $Casp8^{-}Src^{+}$ H522 and $Casp8^{+}Src^{+}$A549 with wild type (WT) EGFR were selected as experimental cell lines. Given the role of tyrosine 380 of caspase-8, we constructed the hemagglutinin (HA)-tagged wild type/Y380A mutant caspase-8 (**Figure 1B**) and reconstituted the physiological level of these constructions in the caspase-8-deficient H522 cells (**Figure 1C**). In addition, the lentivirus-delivered shRNAs of caspase-8 and c-Src were applied to knockdown endogenous caspase-8 and c-Src in A549 cells (**Figure 1C**).

The chick embryo model has been useful in investigating angiogenesis and tumorigenesis *in vivo* (26, 27). To approximate the actual number of tumor cells in each tissue sample, a standard curve was generated through quantitative real-time PCR amplification of genomic DNA extracted from a serial dilution of A549/H522 cells mixed with individual chick lung homogenates (**Figure 1D**). By interpolating the alu signal from real-time PCR with the standard curve (**Figure 1E**), the actual number of tumor cells/lung could be determined over a linear range. Knockdown of endogenous caspase-8 or c-Src by lentivirus-delivered shRNAs of caspase-8 and c-Src efficiently decreased A549 cells metastasis as compared with control shRNA (**Figure 1F**). A disproportionate increase in the metastasis was also detected in the H522 cells re-expressing WT caspase-8 (Casp8 WT) (**Figure 1F**), whereas Y380A mutation in the holoprotein of caspase-8 obviously attenuated the metastasis of H522 cells (**Figure 1F**). We subsequently explored the role of caspase-8 and c-Src in spontaneous distant metastasis after primary tumors that weighed the same size were removed from nude mice. As measured by bioluminescence (**Figure 1G**), mice implanted with A549 + control shRNA cells and H522 + Casp8 WT cells had a significantly increased tumor burden, and metastasis occurred in all 10 mice in the group (**Figures 1H, I**). Markedly increased metastatic incidences and extended tumor distributions were observed in the mice inoculated with A549 + control shRNA cells and H522 + Casp8 WT cells (**Figures 1J, K**), which corresponded with worse OS (**Figures 1L, M**). EMT was characterized as a prometastatic process in the human cancers (28, 29). It was observed that c-Src and caspase-8 was pivotal for EMT in lung adenocarcinoma (**Figures 1N, O**). It was of note that Y380A mutation in the holoprotein of caspase-8 attenuated the spontaneous metastasis and impaired EMT process in lung adenocarcinoma (**Figures 1G–K, M–O**). Collectively, tyrosine 380 of caspase-8 in the c-Src-caspase-8 interaction was pivotal for metastasis through EMT in lung adenocarcinoma.

c-Src Was Overactivated in Lung Adenocarcinoma Dependently on Caspase-8

As a non-receptor tyrosine kinase, activated c-Src underpinned the EMT of cancer cells following the multiple cell signaling (30). Caspase-8 was phosphorylated on tyrosine 380 (Tyr-380) by c-Src following the attachment to fibronectin (**Figure 2A**), which was consistent with previous report (25). It was of importance that c-Src was overactivated in a caspase-8–dependent manner (**Figure 2A**). It had been proven that integrin $\alpha_V\beta_3$ is expressed on the surfaces of A549 and H522 cells (data not shown). With the attachment to fibronectin, A549 and H522 cells expressing caspase-8 displayed c-Src overactivation relative to those lacking caspase-8 or c-Src (**Figures 2B–E**). Fibronectin was deemed necessary to induce caspase-8-dependent c-Src overactivation. In the consonance with immunoblottings, caspase-8 knockdown or deficiency remarkably dampened c-Src activity in A549 and H522 cells attached to fibronectin (**Figures 2F, G**). It was intriguing that the basic activity of c-Src was maintained in A549 and H522 cells with caspase-8 knockdown or deficiency following fibronectin attachment (**Figures 2C, D, F, G**).

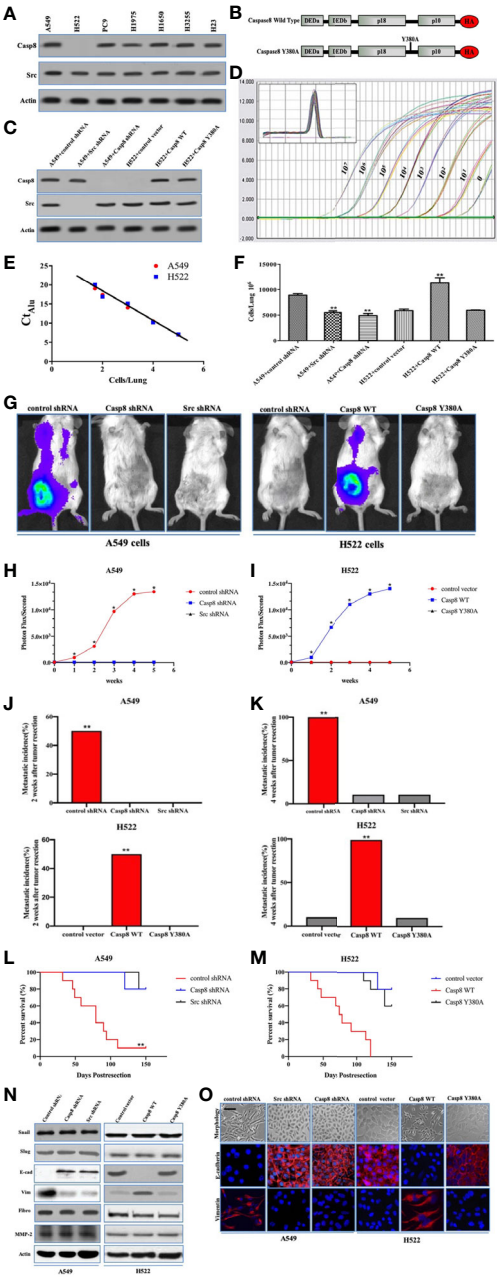

FIGURE 1 | Tyrosine 380 of Caspase-8 was pivotal for lung adenocarcinoma metastasis through EMT. **(A)** Immunoblotting analysis of Caspase-8, c-Src and β-actin in various human lung adenocarcinoma cell lines, A549, NCI-H522, NCI-H1975, NCI-H1650, NCI-H3255, NCI-H23 and PC9. **(B)** Schematic representation of various mutants of Caspase-8 that were used in this study. **(C)** Immunoblotting analysis of Caspase-8, c-Src and β-actin in A549 + control/Src/Casp8 shRNA and H522 + control vector/Casp8 WT/Casp8 Y380A. **(D)** Alu PCR was used to amplify alu repeat sequences in human (A549 cells) DNA. Quantitative real-time alu PCR was performed on genomic DNA extracted from the indicated number of A549 cells serially diluted into individual chick embryo lung homogenates. **(E)** Real-time quantitative alu PCR was used to generate a standard curve from A549 cells/lung by plotting the Ct against the number of cells per lung. **(F)** A quantitative analysis of spontaneous metastasis in the chick embryo using different human tumor cells. *vs*. control, $^{**}p < 0.01$. **(G–I)** Ectopically grown A549/H522 tumors were surgically removed. Biweekly quantification of bioluminescence showed accelerated tumor growth and increased spontaneous metastasis in the mice implanted with A549 + control shRNA **(H)** and H522 + Casp8 WT **(I)** ($^{*}p < 0.05$). Data were presented as mean ± SD. **(J, K)** Total cumulative incidences of metastasis confirmed by immunostaining in the tumor-implanted mice cohorts by 2 or 4 weeks after tumor removal ($^{**}p < 0.01$). Metastatic events were confirmed by immunostaining in various organs of BALB/c nude mice. Tissue sections were scored as positive or negative based on the presence or absence of detectable metastasis. **(L, M)** Kaplan-Meier survival curves for each group of mice with A549 cells **(L)** and H522 cells **(M)**. **(N)** Immunoblotting analysis of Snail, Slug, E-cadherin (E-cad), Vimentin (Vim), Fibronectin (Fibro), MMP-2 and β-actin in A549 + control/Src/Casp8 shRNA and H522 + control vector/Casp8 WT/Casp8 Y380A. **(O)** Immunofluorescence of E-cadherin and Vimentin. Decreased E-cadherin and increased Vimentin in the $Casp8^{+}Src^{+}$ cells with spindle and dendritic shapes (scale bars = 50 μm).

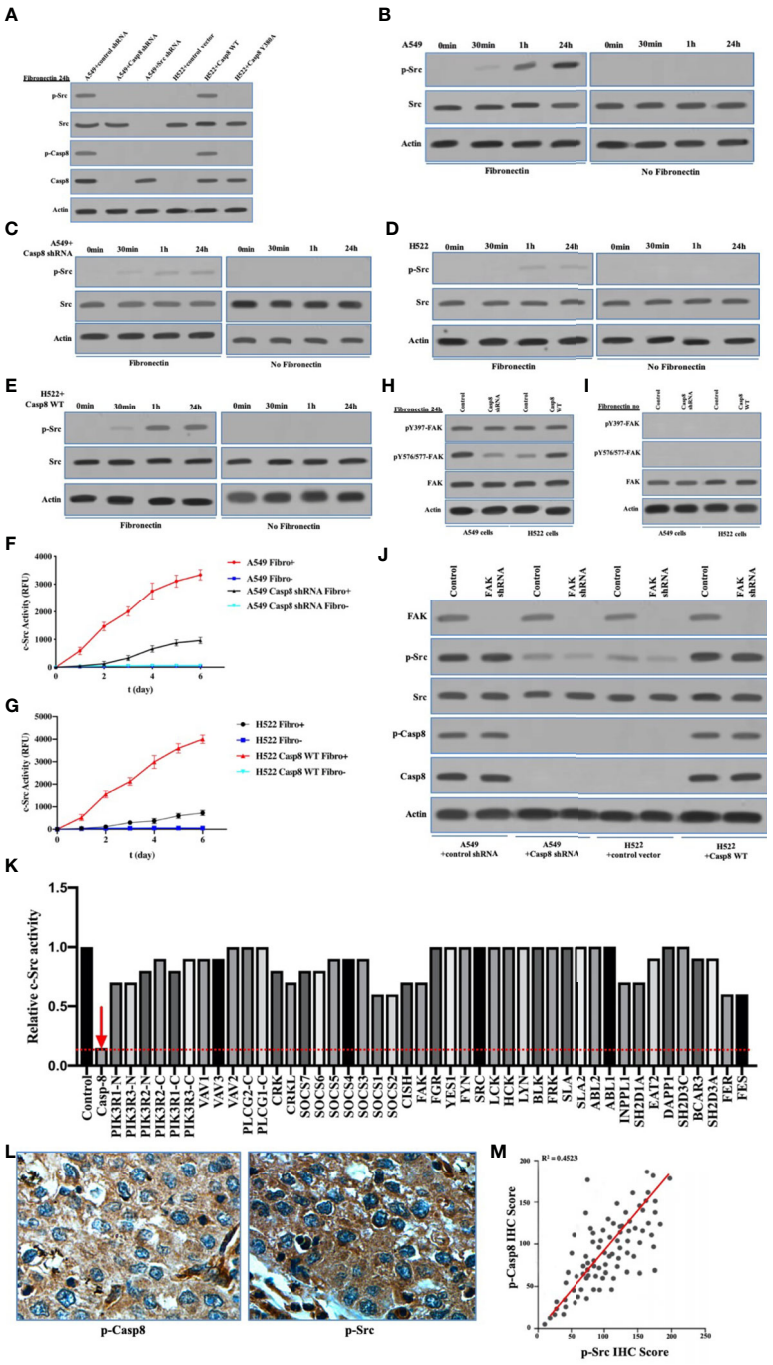

FIGURE 2 | c-Src was overactivated in lung adenocarcinoma dependently on Caspase-8. **(A)** Immunoblotting analysis of p-Src, c-Src, p-Casp8, Caspase-8 and β-actin in A549 + control/Src/Casp8 shRNA and H522 + control vector/Casp8 WT/Casp8 Y380A attached on fibronectin for 24 h. **(B–E)** Immunoblotting analysis of p-Src, c-Src and β-actin in A549 + control **(B)** /Casp8 shRNA **(C)** and H522 + control vector **(D)** /Casp8 WT **(E)** attached on fibronectin or not for 24 h. **(F, G)** A549 cells with a lentiviral delivery of either control shRNA **(F)** or Casp8 shRNA and H522 cells with an adenoviral delivery of either control vector or Casp8 WT **(G)** attached on fibronectin or not were in the presence of a c-Src-specific fluorescent substrate, and fluorescence was recorded as the function of time (*$p < 0.05$). **(H, I)** Immunoblotting analysis of pY397-FAK, pY567/577-FAK, FAK and β-actin in A549 + control/Casp8 shRNA and H522 + control vector/Casp8 WT attached on fibronectin **(H)** or not **(I)** for 24 h. **(J)** Immunoblotting analysis of FAK, p-Src, c-Src, p-Casp8, Caspase-8 and β-actin in A549 + control/Casp8 shRNA and H522 + control vector/Casp8 WT with or without FAK knockdown attached on fibronectin for 24 h. **(K)** c-Src activity analysis in A549 cells with a set of siRNAs for targets (**Table S1**) as indicated attached on fibronectin for 24 h. **(L)** Positive expressions of p-Casp8 and p-Src examined by immunohistochemistry (IHC) of specific antibody in cancerous tissue (×400). **(M)** Correlation between p-Casp8 and p-Src expression in cancerous tissues.

This indicated that the extracellular stimuli maintained the basic activity of c-Src that initiated c-Src–caspase-8 interaction, while caspase-8 virtually overactivated c-Src in lung adenocarcinoma.

To confirm the specific role of caspase-8 on c-Src overactivation relative to other intracellular c-Src activators, such as focal adhesion kinase (FAK), we tested the interaction between c-Src and FAK, which has been reported to contribute to c-Src activation in various human tumors (31). It has also been reported that fibronectin-integrin triggered the autophosphorylation of Tyr 397 of FAK to facilitate c-Src activation, while Tyr 576/577 of FAK was phosphorylated by activated c-Src (30, 31). Autophosphorylation of Tyr 397 of FAK was dependent on fibronectin rather than on caspase-8, which was consistent. Caspase-8 knockdown or deficiency attenuated Tyr 576/577 phosphorylation of FAK (**Figures 2H, I**). We then knocked down FAK in A549 and H522 cells. It was of note that FAK phosphorylation was completely eliminated without fibronectin attachment (**Figure 2I**). Surprisingly, FAK knockdown slightly decreased c-Src activation in the caspase-8–lacking A549 and H522 cells (**Figure 2J**), while it was unable to remarkably affect caspase-8 phosphorylation and c-Src overactivation in the caspase-8-expressing A549 and H522 cells (**Figure 2J**). To further confirm the role of caspase-8 on c-Src overactivation in lung adenocarcinoma, we profiled a set of the potential activator of c-Src through the siRNA library (**Table S1**). Accordingly, caspase-8 was strongly associated with c-Src overactivation in lung adenocarcinoma (**Table S2** and **Figure 2K**). We retrospectively examined the association between p-Casp8 and p-Src in the patients with resectable lung adenocarcinoma (**Table 1**). Our examination revealed that p-Casp8 was positively correlated with p-Src in the lung adenocarcinoma tissues (**Figures 2L, M**). Together, caspase-8 was able to exclusively overactivate c-Src in lung adenocarcinoma.

Phosphorylated Caspase-8 by c-Src Overactivated c-Src Through Its Phosphotyrosine 380 and "PDEP" Motif Docking to SH2 and SH3 Domain of c-Src

To clarify the interplay between c-Src and caspase-8, we depicted the specificity of c-Src-induced caspase-8 phosphorylation and phosphorylated caspase-8–induced c-Src overactivation. Initially, we explored the expressions of other c-Src kinase family members in the lung adenocarcinoma cells. Fyn and Yes were frequently expressed in the lung adenocarcinoma cell lines (**Figure 3A**). The other c-Src kinase family members did not affect caspase-8 phosphorylation in A549 cells (**Figures 3B, C**). We applied immunofluorescence to reveal the subcellular locations of c-Src/caspase-8 and p-Src/p-Casp8. p-Src/caspase-8 and c-Src/p-Casp8 were co-localized in A549 cells (**Figures 3D, E**). There were 18 tyrosines in the holoprotein of caspase-8 (**Figure 3F**). We aimed to clarify whether tyrosine 380 of caspase-8 was specific for c-Src kinase substrate. Three mutants of caspase-8 were constructed, including caspase-8 with all tyrosine mutations (caspase-8 tyr-mut), all tyrosine mutations except for tyrosine 380 (caspase-8 tyr [380]-mut) and tyrosine 380 mutation (caspase-8 Y380A), all of which were stably transfected into H522 cells with caspase-8 deficiency. caspase-8 tyr(380)-mut and WT caspase-8 (caspase-8 WT) were able to similarly maintain caspase-8 tyrosine phosphorylation and c-Src overactivation instead of Casp8 Y380A (**Figure 3G**). This suggested that tyrosine 380 of caspase-8 was specific for c-Src-induced phosphorylation.

We explored the capability of the SH2 domains of human diverse proteins (**Table S1**) to bind to phosphotyrosine 380 of caspase-8. Phosphotyrosine 380 of caspase-8 docked to a huge number of human SH2 domains, including c-Src (**Figure 3H**), implying that the phosphotyrosine 380 site of caspase-8 was not specific for the SH2 domain of c-Src. A question was then raised as to how caspase-8 most potently overactivated c-Src in lung adenocarcinoma. Our team reanalyzed and scrutinized the sequence of caspase-8 holoprotein. The amino acid motif of "PDEP" (site 193-196) of caspase-8 might potentially bind to the SH3 domain (**Figure 3F**). A set of caspase-8 mutants as shown in **Figure 3I** were constructed and stably transfected into the caspase-8-lacking H522 cells by adenoviral vectors. Caspase-8 with "PDEP" motif deletion did not impair the phosphotyrosine 380 of caspase-8 in H522 cells (**Figure 3J**), whereas "PDEP"-deleted caspase-8 was unable to overactivate c-Src with no impacts on c-Src-induced caspase-8 phosphorylation (**Figure 3J**). The "PDEP" motif of caspase-8 was not required for its interaction with c-Src as tyrosine 380 of c-Src (**Figure 3K**). Collectively, caspase-8 phosphorylated by c-Src reversely overactivated c-Src through its phosphotyrosine 380 and "PDEP" motif docking to the SH2 and SH3 domains of c-Src, respectively.

c-Src Was Unable to Trigger EGFR-Related Signaling Reflected by the Phosphotyrosines 1068, 1086, and 1145 of EGFR

EGFR signaling was critical to maintain proliferation and metastasis of NSCLC in particular for EGFR-mutant NSCLC (32, 33). The interaction between EGFR and other tyrosine kinases amplified the survival signaling through the phosphoinositide-3 kinase (PI3K)-protein kinase B (AKT) and the mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK1/2 or ERK) pathway (32–34). Therefore, we hoped to uncover the relationship between c-Src activation and EGFR signaling in lung adenocarcinoma. We showed the activation model of EGFR in the EGFR-nonaddictive H522 cells lacking c-Src activation, in which the autophosphorylation of EGFR at tyrosine 1173 was paralleled with the phosphorylated tyrosine 1068, 1086, and 1148 with the epidermal growth factor (EGF) addition (**Figure 4A**), while the phosphorylation of tyrosine 845, 1101, 974, and 992 was undetectable (**Figure 4A**). It seemed that the tyrosine 1173 was more sensitive to reflect EGF-triggered EGFR activation (**Figure 4A**). EGFR activation was completely eliminated with no EGF addition in H522 cells (**Figure 4A**). In addition, tyrosine 1045 was slightly phosphorylated following EGF stimulation (**Figures 4A, B**). It was explicable that the phosphotyrosine 1045 initiated the ubiquitination and degradation of EGFR (35). It was of

interest to clarify whether c-Src overactivation was able to affect EGFR activation in lung adenocarcinoma. In the EGFR-nonaddictive H522 cells and A549 cells with caspase-8 expression, the phosphorylation of tyrosine 845 and 1101 was significantly increased under the stimulation of EGF (**Figure 4B**), suggesting that the phosphorylation of tyrosine 845 and 1101 was associated with c-Src overactivation and EGF stimulation. EGFR activation depended on EGF in the EGFR-nonaddictive lung adenocarcinoma. EGF triggered the EGFR-associated downstream signaling including PI3K-AKT and MAPK-ERK (**Figure 4C**). It was noteworthy that EGF triggered survival signaling and did not affect c-Src activity in lung adenocarcinoma cells (**Figure 4C**).

We next evaluated the potency of phosphorylated tyrosine sites of EGFR to trigger the EGFR-related signaling. Our team constructed the tyrosine site mutants (tyrosine [Y] to alanine [A]) of EGFR tagged with HA tag in the EGFR-deleted A549 cells (**Figure 4D**). Accordingly, tyrosine 1068, 1086, and 1148 rather than tyrosine 1173 had the synergic effect to trigger PI3K-AKT and MAPK-ERK signaling (**Figure 4D**). It was of notes that c-Src activation led to two tyrosine sites (tyrosine 845 and 1101) of EGFR being phosphorylated and did not contribute to EGFR-related signaling. A previous study uncovered that EGFR with sensitive mutation was self-active through the form of homodimerization to trigger survival signaling (36). To explore the EGFR activation through EGFR homo- or hetero-multimerization, we synthesized shRNAs for targets on Her-2, Her-3, and Her-4 (**Figure 4E**). The knockdown of Her-2, Her-3, or Her-4 was incapable of affecting EGFR-related signaling as EGFR knockdown in the EGFR-nonaddictive lung adenocarcinoma cells (**Figure 4F**). This indicated that EGFR homodimerization might trigger EGFR-related signaling. We extended the molecular machinery for EGFR-related signaling in the EGFR-addictive lung adenocarcinoma cells with the sensitive mutation of EGFR (PC9 and H1650 with exon 19 deletion mutation [deletion E746-A750], H3255 with exon 21 L858R). In PC9, H1650, and H3255 cells, EGFR activation and EGFR-related signaling were similar regardless of EGF stimulation (**Figures 4G, H**). Together, the phosphorylation of tyrosine 1068, 1086, and 1148 was reflective of the EGFR activation accompanied by the EGFR-related signaling in lung adenocarcinoma.

TKIs Blocked EGFR-Related Signaling to Facilitate Cell deaths of EGFR-Mutant Lung Adenocarcinoma

Targeted therapy with TKIs is characterized as a standard treatment for patients with EGFR-mutated lung adenocarcinoma (37, 38). It was deemed necessary to determine the antitumor effects and molecular mechanisms of TKIs in lung adenocarcinoma. As shown in **Figures 5A, B**, TKIs did not lead to the marked decrease in tumor cell viability in the EGFR-nonaddictive A549 and H522 cells with or without c-Src overactivation. By contrast, PC9, H1650, and H3255 cells with the sensitive mutation of EGFR showed the potent antitumor activity corresponding to distinct TKIs (**Figure 5C**). TKIs had the great efficacy to inhibit the phosphorylation of tyrosine 1068, 1086, and 1145 of EGFR in the EGFR-mutant lung adenocarcinoma cells (**Figure 5D**). In turn, TKIs efficiently suppressed PI3K-AKT and MAPK-ERK signaling, particularly for osimertinib (**Figure 5E**). It was intriguing that osimertinib could attenuate c-Src activation to a lesser extent (**Figure 5E**). Together, this indicated that TKIs induced the antitumor activity through the inhibition of EGFR-related signaling in the EGFR-mutant lung adenocarcinoma cells.

Sequentially, we sought to disclose the association between the inhibitions of PI3K-AKT/MAPK-ERK signaling and cell deaths. Our team procured a set of the inhibitors for PI3K-AKT and MAPK-ERK signaling (MAPK/ERK kinase; MEK) as shown in **Table S3**. In the EGFR-mutant cell lines, the variable inhibitors of PI3K-AKT and MAPK-ERK presented variable antitumor activity (**Figures 5F, H, J**), indicating that the ability of TKIs to eliminate tumor cells accounted for the inhibitor of

TABLE 1 | Association of phosphotyrosine 380 Caspase-8 (p-Casp8) expression with clinicopathological characteristics of patients with operable lung adenocarcinoma (n = 84).

Clinicopathological characteristics	p-Casp8 expression n (%)		*P*
	Positive	Negative	
Age (y)			
≤60	19 (42.2%)	16 (41.0%)	>0.05
>60	26 (57.8%)	23 (59.0%)	
Sex			
Male	35 (77.8%)	29 (74.3%)	>0.05
Female	10 (22.2%)	10 (25.7%)	
Differentiation			
Well	24 (53.3%)	23 (58.9%)	>0.05
Moderate	15 (33.3%)	13 (33.3%)	
Poor	6 (13.4%)	3 (7.8%)	
Lymphnodes metastasis			
N_{0-1}	22 (48.9%)	16 (41.0%)	>0.05
N_{2-3}	23 (51.1%)	23 (59.0%)	
pTNM stage			
I-II	21 (46.7%)	17 (43.6%)	>0.05
III-IV	24 (53.3%)	22 (56.4%)	

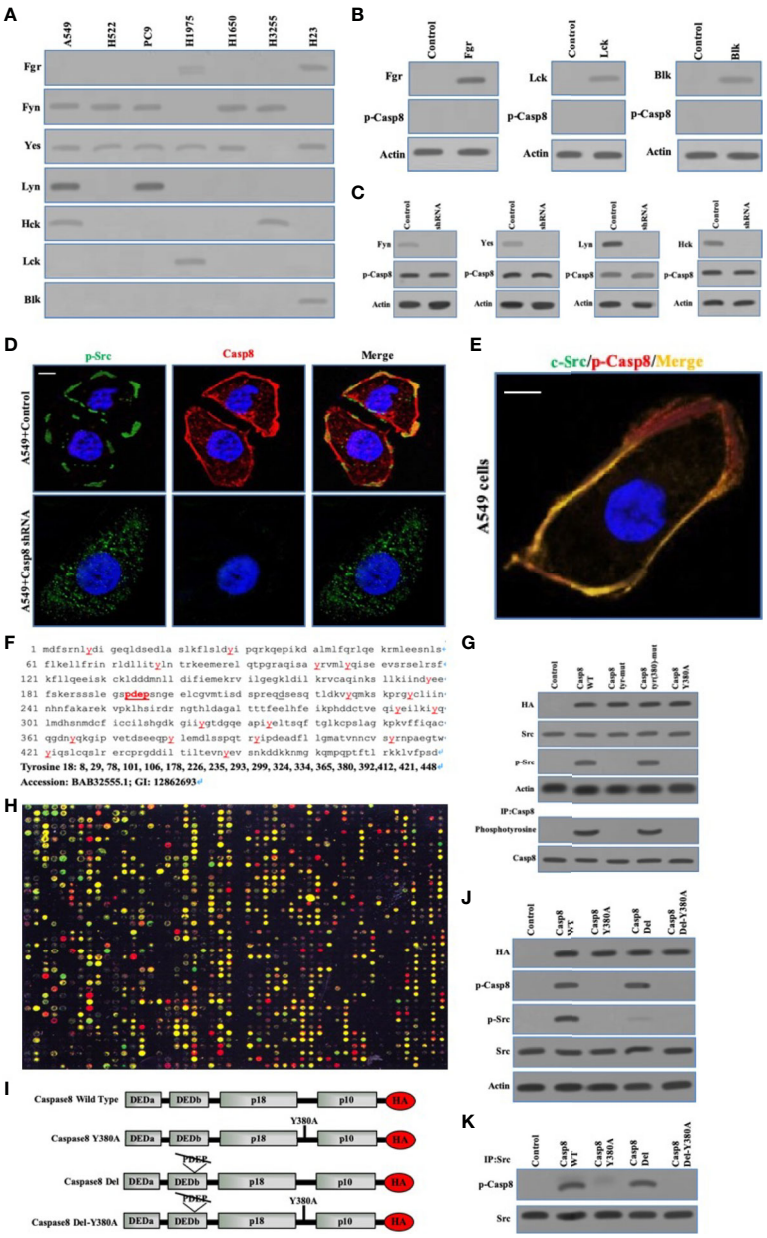

FIGURE 3 | Phosphorylated Caspase-8 by c-Src overactivated c-Src through its phosphotyrosine 380 and "PDEP" motif docking to SH2 and SH3 domain of c-Src. **(A)** Immunoblotting analysis of Fgr, Fyn, Yes, Lyn, Hck, Lck and Blk in lung adenocarcinoma cell lines including A549, H522, PC9, H1975, H1650, H3255 and H23 cells attached on fibronectin for 24 h. **(B)** Immunoblotting analysis of Fgr, Lck, Blk and p-Casp8 in A549 cells transfected by adenoviral delivery of Fgr, Lck and Blk attached on fibronectin for 24 h. **(C)** Immunoblotting analysis of Fyn, Yes, Lyn, Hck and p-Casp8 in A549 cells transfected by lentiviral delivery of shRNAs of Fyn, Yes, Lyn and Hck attached on fibronectin for 24 h. **(D, E)** A549 cells were allowed to attach onto fibronectin-coated dish for 24 h and assessed by confocal microscopy using antibodies against p-Src, Casp8, c-Src and p-Casp8. Scale bars, 10 μm. **(F)** The amino acid sequence and tyrosine position of Caspase-8. **(G)** Immunoblotting analysis of HA, p-Casp8, c-Src, p-Src and β-actin in H522 cells with adenovirus encoding control vector, Casp8 WT, Casp8 tyr-mut, Casp8 tyr (380)-mut and Casp8 Y380A attached on fibronectin for 24 h. The TCLs of H522 cells with adenovirus encoding control vector, Casp8 WT, Casp8 tyr-mut, Casp8 tyr(380)-mut and Casp8 Y380A attached on fibronectin for 24 h were subjected to immunoprecipitation (IP) using anti-Caspase-8 antibody. The blot was then stripped and reprobed for phosphotyrosine and Casp8. **(H)** Microarray analysis of SH2 domains of intracellular proteins binding to p-Casp8 in A549 cells. Green and yellow represented equal signal and downregulation; red represented upregulation. **(I)** Schematic representation of various HA-tagged mutants of Caspase-8. **(J)** Immunoblotting analysis of HA, p-Src, p-Casp8, c-Src and β-actin in H522 cells with adenovirus encoding Caspase-8 mutants attached on fibronectin for 24 h. **(K)** The TCLs of H522 cells with adenovirus encoding Caspase-8 mutants in 3I were subjected to IP using anti-c-Src antibody. The blot was then stripped and reprobed for c-Src and p-Casp8.

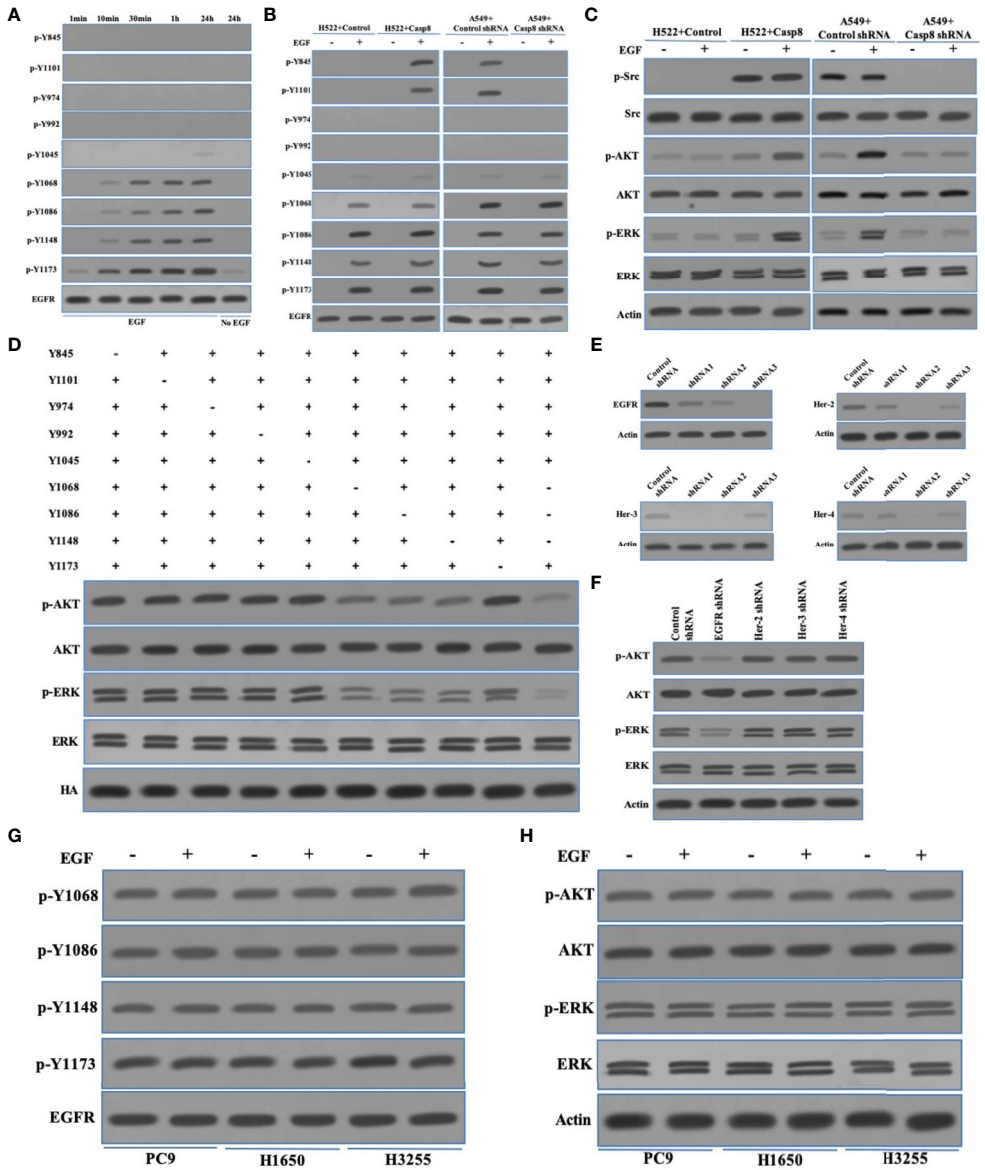

FIGURE 4 | c-Src was unable to trigger EGFR-related signaling reflected by the phosphotyrosines 1068, 1086, and 1145 of EGFR. **(A)** Immunoblotting analysis of p-Y845, p-Y1101, p-Y947, p-Y992, p-Y1045, p-Y1068, p-Y1086, p-Y1148, p-Y1173 of EGFR and EGFR in H522 cells with Casp8 WT attached on fibronectin for 24 h stimulated by EGF or not as indicated times. **(B)** Immunoblotting analysis of p-Y845, p-Y1101, p-Y947, p-Y992, p-Y1045, p-Y1068, p-Y1086, p-Y1148, p-Y1173 of EGFR and EGFR in H522 cells with control vector or Casp8 WT and A549 cells with control shRNA or Casp8 shRNA attached on fibronectin for 24 h stimulated by EGF or not for 24 h. **(C)** Immunoblotting analysis of p-Src, c-Src, p-AKT, AKT, p-ERK, ERK and β-actin in H522 cells with control vector or Casp8 WT and A549 cells with control shRNA or Casp8 shRNA attached on fibronectin for 24 h stimulated by EGF or not for 24 h. **(D)** Immunoblotting analysis of p-AKT, AKT, p-ERK, ERK and HA in EGFR-deleted A549 cells transfected with lentiviral tyrosine mutants of EGFR attached on fibronectin for 24 h stimulated by EGF for 24 h. **(E)** Immunoblotting analysis of EGFR, Her-2, Her-3, Her-4 and β-actin in A549 cells with lentiviral shRNAs specific for control, EGFR, Her-2, Her-3 and Her-4. **(F)** Immunoblotting analysis of p-AKT, AKT, p-ERK, ERK and β-actin in A549 cells with lentiviral shRNAs specific for control, EGFR, Her-2, Her-3 and Her-4 attached on fibronectin for 24 h stimulated by EGF for 24 hr. **(G)** Immunoblotting analysis of p-Y1068, p-Y1086, p-Y1148, p-Y1173 of EGFR and EGFR in PC9, H1650 and H3255 cells stimulated by EGF or not for 24 hr. **(H)** Immunoblotting analysis of p-AKT, AKT, p-ERK, ERK and β-actin in PC9, H1650 and H3255 cells attached on fibronectin for 24 h stimulated by EGF or not for 24 hr.

survival signaling, including PI3K-AKT and MAPK-ERK. Nevertheless, PI3K-AKT and MAPK-ERK inhibition was unable to kill tumor cells as TKIs did (**Figures 5G, I, K**). This implied the possibility that the applied inhibitors at the concentration could not inhibit the survival signaling as TKIs alone did. We then attempted to clarify the effects of the survival signaling blockade on the viability of EGFR-nonaddictive cell lines. It was intriguing that the PI3K-AKT and MAPK-ERK

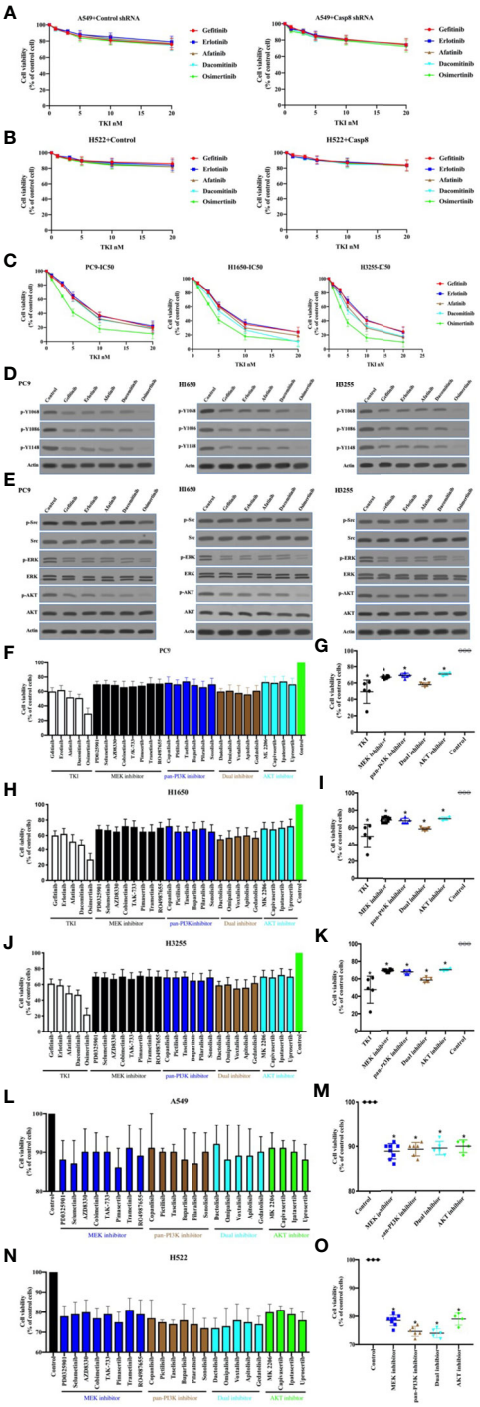

FIGURE 5 | TKIs blocked EGFR-related signaling to facilitate cell deaths of EGFR-mutant lung adenocarcinoma. **(A)** Viability assay of A549 cells with control shRNA or Casp8 shRNA attached on fibronectin for 24 h treated by TKIs at indicated concentrations for 48 h (n = 3). **(B)** Viability assay of H522 cells with control vector or Casp8 WT attached on fibronectin for 24 h treated by TKIs at indicated concentrations for 48 h (n = 3). **(C)** Viability assay of PC9, H1650 and H3255 cells treated by TKIs at indicated concentrations for 48 h (n = 3). **(D)** Immunoblotting analysis of p-Y1068, p-Y1086, p-Y1148 of EGFR and β-actin in PC9, H1650 and H3255 cells. **(E)** Immunoblotting analysis of p-Src, c-Src, p-AKT, AKT, p-ERK, ERK and β-actin in PC9, H1650 and H3255 cells. **(F, G)** Viability assay of PC9 cells treated by drugs indicated for 48 h (n = 3). * *vs.* control, $p < 0.05$. **(H, I)** Viability assay of H1650 cells treated by drugs indicated for 48 h (n = 3). * *vs.* control, $p < 0.05$. **(J, K)** Viability assay of H3255 cells treated by drugs indicated for 48 h (n = 3). * *vs.* control, $p < 0.05$. **(L, M)** Viability assay of A549 cells attached on fibronectin for 24 h treated by drugs indicated for 48 h (n = 3). * *vs.* control, $p < 0.05$. **(N, O)** Viability assay of H522 cells attached on fibronectin for 24 h treated by drugs indicated for 48 h (n = 3). * *vs.* control, $p < 0.05$.

blockade induced A549 and H522 cell deaths (**Figures 5L–O**). It seemed that H522 cells with caspase-8 deficiency were more sensitive to the inhibition of survival signaling (**Figures 5L, N**). Therefore, it was rationalized that TKIs could induce to antitumor activity in the EGFR-mutant lung adenocarcinoma cells through the inhibition of survival signaling.

The Necroptosis Through FADD Complex Was Predominant for TKIs-Induced Cell Death in the EGFR-Mutant Lung Adenocarcinoma

It has been reported that TKIs induced apoptosis through the inhibition of survival pathways in EGFR-mutant lung adenocarcinoma (39, 40). We previously reported that necroptosis was predominant for chemotherapy-induced cell death in lung adenocarcinoma cells, owing to c-Src-induced caspase-8 phosphorylation to block apoptosis (21). In line with this, the inhibitor for caspase-8–induced apoptosis (z-IETD-fmk) did not impact the antitumor activity of TKIs in PC9, H1650, and H3255 cells expressing p-Casp8 (**Figures 6A–C**). We found that caspase-9 inhibitor (intrinsic apoptosis inhibitor: z-LEHD-fmk) and pan-caspase inhibitor (z-VAD-fmk) reduced approximately 8%, 18%, and 4% (4%–18%) of cell deaths in the EGFR-sensitive lung adenocarcinoma cells, while necrostatin-1 (necroptosis inhibitor; nec-1) salvaged the most cell deaths in lung adenocarcinoma (**Figures 6A–C**). To further dissect the models of TKI-induced cell death, flow cytometry analysis *via* Annexin V-propidium iodide (PI) staining was applied. Dual inhibitors for intrinsic apoptosis and necroptosis completely rescued the cell deaths of EGFR-mutated lung adenocarcinoma cells (Annexin V-positive: apoptosis; PI-positive: necroptosis; **Figures 6D–F**). This indicated that necroptosis was predominant in the TKI-induced cell deaths of lung adenocarcinoma, whereas the intrinsic apoptosis was highly variable. Caspase-9 inhibitor and nec-1 were able to prevent tumor cells from apoptosis and necroptosis, respectively, in the EGFR-mutant lung adenocarcinoma cells (**Figures 6G–I**).

It was shown that receptor-interacting serine/threonine-protein kinase (RIPK) 1 and RIPK3 were obviously phosphorylated in PC9, H1650, and H325 cells treated with TKIs (**Figures 6J–L**). It was previously reported that Fas-associated death domain (FADD) complex was the crux to balance between caspase-8–induced apoptosis and RIPK1-induced necroptosis (41–43). In line with that, FADD was coimmunoprecipitated with sharply increased RIPK1 under the stimulation of TKIs (**Figures 6M–O**). Our team harvested 33 pairs of EGFR-mutant lung adenocarcinoma and noncancerous tissues, of which 13 pairs were accessible to patient-derived xenografts (PDXs) or tumors. We sought to determine the cell death pattern in the patient-derived tumor cells with the sensitive-mutation EGFR (**Table S4**). The necroptosis accompanied by apoptotic inhibition contributed to a greater portion of TKI-induced cell deaths as compared with intrinsic apoptosis alone (**Figures 6P, Q**). This, therefore, indicated that the necroptosis was critical for TKI-induced antitumor activity in the EGFR-mutant lung adenocarcinomas.

Inactivated c-Src Blocked EGFR-Related Survival Pathway Through Sealing the Phosphotyrosines of EGFR

Although activated c-Src did not enhance EGFR-related survival pathways in lung adenocarcinoma, the possibility of c-Src inactivation resulting in caspase-8 dephosphorylation to facilitate cell death was considered. p-Src and p-Casp8 were ubiquitous in EGFR-mutant lung adenocarcinomas (**Figure 5E** and **Table S4**). It was expected that c-Src inactivation was attained by dasatinib in the EGFR-mutant lung adenocarcinoma cells (21). The addition of dasatinib in the EGFR-mutant lung adenocarcinoma cells efficiently attenuated c-Src activation (**Figure 7A**). It was of note that c-Src inactivation had the potential to suppress EGFR-related survival pathways (**Figure 7A**). We then explored the interaction between c-Src and EGFR through the coimmunoprecipitation assay. Coimmunoprecipitated c-Src was remarkably reduced with the mutations of tyrosine 1068, 1086, and 1145 of EGFR in the EGFR-lacking A549 cells (**Figure 7B**), while the interplay between c-Src and EGFR was not affected in the other mutants of EGFR (**Figure 7B**). It was more likely that c-Src was binding to phosphotyrosine 1068, 1086, and 1145 to block the EGFR-induced survival pathways. Then, we constructed various c-Src mutants (**Figure 7C**). Moreover, the deletion of the SH2 domain of c-Src completely eliminated its interaction with EGFR in the EGFR-mutant lung adenocarcinoma (**Figures 7D–F**). We constructed the microarray of SH2 domains of intracellular proteins in the lung adenocarcinoma according to **Table S1**. Our data showed that a huge number of SH2 domains could bind to phosphorylated tyrosine 1068, 1086, and 1145 of EGFR, in which the SH2 domain of c-Src could more effectively bind to the phosphotyrosines of EGFR (**Figure 7G**). It seemed that other members of the c-Src family were not binding to the phosphotyrosines of EGFR as c-Src did (**Figure 7G**). We analyzed the correlation between c-Src activation and the therapeutic effect. It was intriguing that c-Src inactivation promoted the clinical response of TKIs (**Figure 7H**).

To answer the question of whether dasatinib facilitated caspase-8–induced apoptosis through caspase-8 dephosphorylation, dasatinib obviously increased TKI-induced cell deaths in PC9, H1650, and H3255 cells (**Figure 7I**). Strikingly, apoptosis was not significantly increased, compared with necrosis (**Figure 7J**). Dasatinib inactivated c-Src to dephosphorylate caspase-8 without the apoptotic cleavage of caspase-8 (**Figures 7A, K**), whereas RIPK1/RIPK3 phosphorylation was obviously enhanced (**Figure 7K**). This suggested that dasatinib promoted the necroptosis of TKIs through RIPK1/RIPK3 activation. On the other hand, caspase-8 knockdown with the impairment of c-Src activation significantly increased the response of the xenografts to gefitinib (**Figure 7L**), whereas dasatinib achieved a similar effect (**Figure 7M**). In the sequence, we observed the response of human lung adenocarcinoma with EGFR mutation to gefitinib and dasatinib in the PDX model. The addition of dasatinib consistently had the marked impacts on promoting the response of the EGFR-mutant lung adenocarcinoma to gefitinib (**Figures 7N–P**). Furthermore, our team retrospectively analyzed all the patients with EGFR-mutant lung adenocarcinoma in our center.

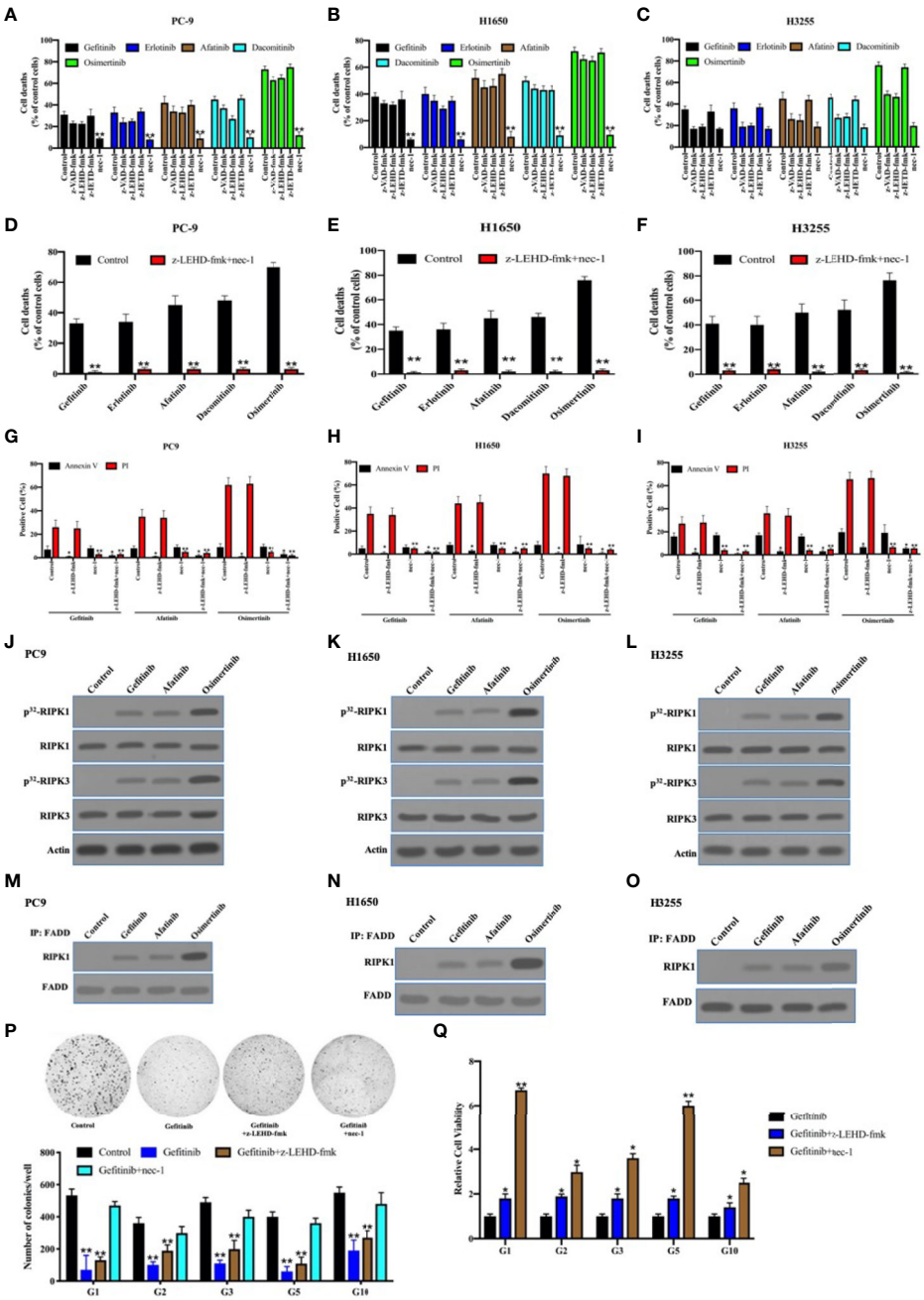

FIGURE 6 | The necroptosis through FADD complex was predominant for TKIs-induced cell death in the EGFR-mutant lung adenocarcinoma. **(A–C)**, PC9, H1650, and H3255 cells were treated with TKIs with the addition of DMSO (Control), zIETD-fmk, zVAD-fmk, and nec-1 for 48 h. Cell viability was determined by measuring ATP levels using Cell Titer-Glo kit. Data were represented as mean ± standard deviation of duplicates. **, $p < 0.01$. **(D–F)** PC9, H1650, and H3255 cells were treated with TKIs with the addition of DMSO (Control) and zIETD-fmk+nec-1 for 48 h. Cell viability was determined by measuring ATP levels using Cell Titer-Glo kit. Data were represented as mean ± standard deviation of duplicates. ** *vs.* control, $p < 0.01$. **(G–I),** PC9, H1650, and H3255 cells were treated with TKIs with the addition of DMSO (Control), zIETD-fmk, nec-1 and zIETD-fmk+nec-1 for 48 h. Cells were analyzed for Annexin V/PI staining by flow cytometry. All experiments were repeated 3 times with similar results. **, *vs.* Control, $p < 0.01$. **(J–L)**, Immunoblotting analysis of RIPK1, RIPK3 and β-actin in PC9, H1650 and H3255 cells treated with TKIs 48 h. Cells were labelled with [^{32}P]-orthophosphate. Phosphorylated RIPK1 and RIPK3 were measured by Cyclone Plus Phosphor Imager. **(M–O)** The immunocomplexes of PC9, H1650 and H3255 cells treated with TKIs for 48 h were eluted with antibody against FADD, and whole elution was used to measure RIPK1. **(P)** Soft agar colony formation assay of patient-derived tumor cells with DMSO (Control), gefitinib, gefitinib+zIETD-fmk and gefitinib+nec-1 in 12-well dish (5×10^3 cells per well) for 1 weeks ($n = 3$). Representative images (*upper*) and average number of colonies (*lower*) are shown. **, *vs.* Control, $p < 0.01$. **(Q)** PC9, H1650, and H3255 cells were treated with gefitinib, gefitinib+zIETD-fmk and gefitinib+nec-1 for 48 h. Cell viability was determined by measuring ATP levels using Cell Titer-Glo kit. Data were represented as mean ± standard deviation of duplicates. * *vs.* control, $p < 0.05$. ** *vs.* control, $p < 0.01$.

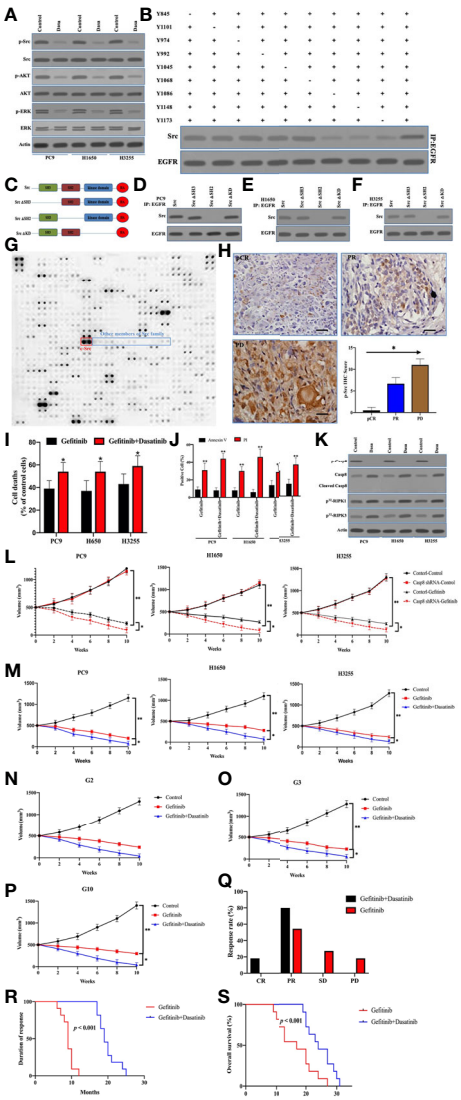

FIGURE 7 | Inactivated c-Src blocked EGFR-related survival pathways through sealing the phosphotyrosines of EGFR triggering cell deaths. **(A)** Immunoblotting analysis of Caspase-8, p-Src, c-Src, p-AKT, AKT, p-ERK, ERK, and β-actin in PC9, H1650, and H3255 cells treated with control and dasatinib for 48 h. **(B)** The immunocomplexes of EGFR-deficient A549 cells transfected the adenoviral HA-tagged tyrosine mutants of EGFR treated with EGF were eluted with antibody against EGFR, and whole elution was used to measure c-Src. **(C)** Schematic representation of various mutants of c-Src used in this study. **(D–F)** The immunocomplexes of PC9, H1650, and H3255 cells transfected lentiviral Casp8 shRNA were eluted with antibody against EGFR, and whole elutions were used to measure HA. **(G)** Microarray analysis of SH2 domains of intracellular proteins binding to EGFR in A549 cells attached on fibronectin for 24 h. **(H)** Immunohistochemistry for p-Src expression in patients with EGFR-mutant lung adenocarcinoma representing CR, PR, and PD to TKIs by specific antibody (×400). The relationship between p-Src IHC score and chemotherapy response was showed. * vs. intergroup, $p < 0.05$. **(I)** PC9, H1650, and H3255 cells were treated with gefitinib or gefitinib+dasatinib for 48 h. Cell viability was determined by measuring ATP levels using Cell Titer-Glo kit. Data were represented as mean ± standard deviation of duplicates. * vs. Gefitinib, $p < 0.05$. **(J)** PC9, H1650, and H3255 cells were treated with gefitinib or gefitinib+dasatinib for 48 h. Cells were analyzed for Annexin V/PI staining by flow cytometry. All experiments were repeated 3 times with similar results. **, vs. Gefitinib, $p < 0.01$. **(K)** Immunoblotting analysis of Caspase-8, p-Casp8, cleaved Casp8, and β-actin in PC9, H1650 and H3255 cells attached on fibronectin for 24h treated with gefitinib+control (Control) and gefitinib+dasatinib (Dasa). Cells were labelled with [^{32}P]-orthophosphate. Phosphorylated RIPK1, RIPK3 and MLKL were measured by Cyclone Plus Phosphor Imager. **(L)** Subcutaneous xenograft assay of PC9, H1650, and H3255 cells transfected with adenoviral control shRNA and Casp8 shRNA in nude mice treated with control or dasatinib. Volumes of tumors were shown ($n = 10$ per group). **$p < 0.01$. *$p < 0.05$. **(M)** Subcutaneous xenograft assay of PC9, H1650 and H3255 cells in nude mice treated with control or gefitinib or gefitinib+dasatinib. Volumes of tumors were shown ($n = 10$ per group). **, $p < 0.01$. *, $p < 0.05$. **(N–P)** Representative patient-derived tumor xenografts in nude mice treated with control or gefitinib or gefitinib+dasatinib. Volumes of tumors were shown ($n = 10$ per group). **$p < 0.01$. *$p < 0.05$. **(Q)** Response to gefitinib or gefitinib +dasatinib of patients with metastatic EGFR-mutant lung adenocarcinoma. **$p < 0.01$. **(R, S)** Kaplan-Meier analysis of duration of response and overall survival in the cohort of PDX models, $p < 0.001$.

18 patients with metastatic lung adenocarcinoma who received gefitinib/erlotinib plus dasatinib were screened out. Of these patients, 10 received no treatment except for the combined treatment in **Table S5**. It was daunting that all eligible lung adenocarcinomas were p-Src-positive (**Table S5**). After being compared with the results of the EGFR-mutant lung adenocarcinoma patients treated by gefitinib alone, dual drugs of gefitinib and dasatinib achieved the better response rate and duration as compared with gefitinib alone (**Figures 7Q, R**), which led to a better prognosis for OS (**Figure 7S**). In sum, inactivated c-Src by dasatinib sealed the phosphorylated tyrosine 1068, 1086, and 1145 of EGFR to inhibit the survival pathway to sensitize EGFR-mutant lung adenocarcinoma to TKI.

DISCUSSION

NSCLC is the most commonly diagnosed human cancer as well as the leading cause of cancer-related deaths in 2019, of which lung adenocarcinoma accounted for 55% with a significant increase (3). The clinical utility of using a single gene-based biomarker as a therapeutic focus for lung adenocarcinoma was first realized with the discovery of mutations in the tyrosine kinase domain of EGFR in 2004; this enabled the identification of patients with greater sensitivity to TKIs (38, 44). The first-generation EGFR TKIs, gefitinib and erlotinib, designed to reversibly compete for the adenosine triphosphate binding sites and, thus, block EGFR-induced downstream signaling activation in the lung adenocarcinoma treatment (38, 45). A layer of complexity in EGFR signaling is the potential for cross-talk with cytoplasmic tyrosine kinases, particularly the ubiquitously expressed kinases c-Src (46). Therefore, we expected to uncover a novel path to sensitize TKIs in EGFR-mutant lung adenocarcinomas.

To date, there is increasing striking evidence that supports the nonapoptotic roles of caspase-8 (14, 47). This was substantiated by our observation that caspase-8 was rarely lacking in lung adenocarcinoma (21, 25). The linkage between caspase-8 and c-Src has been confirmed by the observation that cell stimulations with the survival-promoting factors led to c-Src-mediated caspase-8 phosphorylation on Tyr-380, and thus inhibiting its apoptotic function (18, 25). It was more likely that c-Src–caspase-8 interaction had a key role in human cancers. Therefore, we further dissected the interaction between c-Src and caspase-8 in lung adenocarcinoma cell lines. Strikingly, only tyrosine 380 out of 18 tyrosines of caspase-8 was clarified to be phosphorylated by activated c-Src. On the other hand, phosphorylated caspase-8 was the most powerful in inducing c-Src overactivation as compared with other putative activators of c-Src, such as EGFR and FAK. To answer the question as to why caspase-8 can furiously overactivate c-Src and surpass other putative activators, prior studies uncovered that c-Src was activated by docking to the SH2 domain or the SH3 domain with a higher affinity (35, 48). Caspase-8 had the phosphotyrosine 380 docking to the SH2 domain of c-Src and "PDEP" motif binding to the SH3 domain of c-Src to overactivate c-Src in lung adenocarcinoma. It was reasonable that EGFR and FAK could not activate c-Src due to a lack of binding to the SH3 domain of c-Src despite its phosphorylated tyrosines docking to the SH2 domain of c-Src.

EMT is a trans-differentiation characterized as a key step toward cancer metastasis with decreased epithelial markers, such as E-cadherin, and increased mesenchymal markers, such as vimentin (49, 50). Activated c-Src has been identified as a potent inducer for EMT (49, 50). Following phosphotyrosine 380 of caspase-8–mediated c-Src overactivation, we detected the downregulation of E-cadherin and upregulation of vimentin with morphological characteristics of spindle and dendritic shapes that could promote tumor metastasis in lung adenocarcinoma. Our previous data demonstrated that RNF43 was able to ubiquitinate and degrade E-cadherin phosphorylated by c-Src to facilitate the EMT process in lung adenocarcinoma, indicating the mechanism that activated c-Src induced the EMT phenotype (25). c-Src overactivation by caspase-8 triggered EMT to facilitate tumor metastasis and yielded resistance to therapies in lung adenocarcinoma. Hence, the blockade of caspase-8-induced c-Src overactivation shed a light on this topic in clinical practice.

EGFR is a receptor tyrosine kinase that is frequently upregulated in human cancers, such as in NSCLC (51, 52).

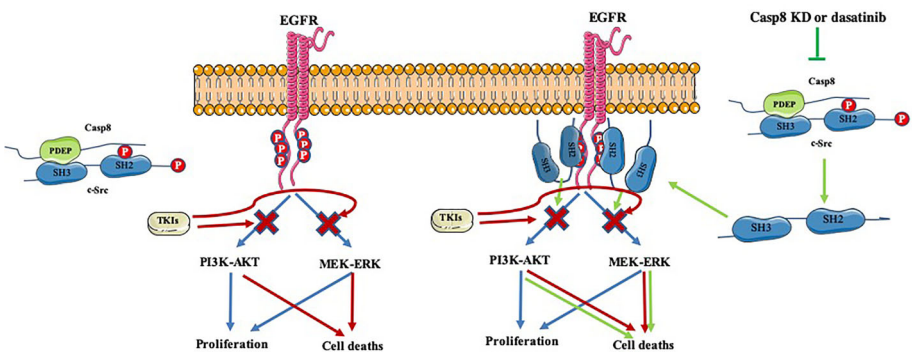

FIGURE 8 | A schematic diagram of synergic effects of inactivated c-Src and TKIs.

Diverse mechanisms augmented EGFR activity, including the EGFRvIII truncations, as well as to its kinase domain, such as the L858R mutations (52). These EGFR aberrations overactivated downstream pro-oncogenic signaling pathways, including the MAPK-ERK and PI3K-AKT pathways (51). These survival pathways activated many biological outputs that were beneficial to cancer cell proliferation. Nevertheless, the EGFR activation model remained elusive. Tyrosine 1068, 1086, and 1143 of EGFR were characterized to reflect EGFR activation in lung adenocarcinoma, and were irreplaceable for EGFR-related signaling. The notion has been supported by previous reports that tyrosine 1068, 1086, and 1143 of EGFR played a dominant role on EGFR activation in lung cancer (40). Our observation differed from a previous study in that EGFR was homo- or heteromultimerized with Her-2, Her-3, and Her-4. Her-2 was an important effector that leads to the resistance of TKIs to tumor cells. We found that Her-2, Her-3, or Her-4 had less contribution to EGFR activation. It was inferred that EGFR homomultimerization was critical for EGFR activation.

Surprisingly, EGFR signaling was different in the capability to maintain tumor growth in the different lung adenocarcinoma cells. The EGFR-mutation lung adenocarcinomas were more addictive to EGFR-induced survival signaling than the EGFR-WT lung adenocarcinomas. TKIs efficiently blocked EGFR-induced PI3K-AKT and MAPK-ERK in order to initiate necroptosis to the majority and intrinsic apoptosis to a lesser extent. This implied that EGFR addiction for tumor growth might underlie the clinical value of TKIs. Accordingly, intrinsic apoptosis was highly variable in the TKI-induced cell deaths of EGFR-mutant lung adenocarcinoma. This may be because the intrinsic apoptotic pathway was too entangled to disentangle, involving too many molecular factors. Dauntingly, dasatinib inactivated c-Src to seal the survival signaling-related phosphorylated tyrosines of EGFR by the SH2 domain of c-Src to facilitate necroptosis instead of caspase-8–induced apoptosis. Our team has done great work in exploring why caspase-8 dephosphorylation could not initiate apoptosis during dasatinib treatment in lung adenocarcinoma (data unpublished). However, dasatinib had limited benefit for advanced EGFR-mutant NSCLC (53, 54). It was more likely that dasatinib was unable to efficiently inactivate c-Src kinase to maintain its clinical therapeutic value.

Collectively, caspase-8 phosphorylated at tyrosine 380 by c-Src predominantly enhanced c-Src activation to induce EMT phenotypic features in lung adenocarcinoma. Mechanistically, the linkage of the c-Src SH2 domain with phosphotyrosine 380 of caspase-8 and SH3 domain with "PDEP" motif of caspase-8 furiously overactivated c-Src. In addition, activated EGFR reflected by the levels of phosphotyrosine 1068, 1086, and 1145 of EGFR had no impact on c-Src activation, while TKIs attenuated EGFR activation to induce cell deaths of lung adenocarcinoma. Surprisingly, blocking c-Src activation through dasatinib was able to inhibit the EGFR survival signaling by sealing tyrosine 1068, 1086, and 1145 of EGFR, which in turn increased the antitumor activity of TKIs in EGFR-mutant lung adenocarcinoma. Together, inactivated c-Src by dasatinib administration sensitized EGFR-mutant lung adenocarcinoma to TKIs (**Figure 8**).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. The animal study was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University. Written informed consent was obtained from the owners for the participation of their animals in this study. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Conception and design: YZ. Administrative support: SZ. Provision of study materials or patients: YZ and CH. Collection and assembly of data: YZ, WM, and CH. Manuscript writing: YZ. Final approval of manuscript: YZ. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.602900/full#supplementary-material

REFERENCES

1. Zheng R, Zeng H, Zuo T, Zhang S, Qiao Y, Zhou Q, et al. Lung Cancer Incidence and Mortality in China, 2011. *Thorac Cancer* (2016) 7(1):94–9. doi: 10.1111/1759-7714.12286
2. Herbst RS, Morgensztern D, Boshoff C. The Biology and Management of Non-Small Cell Lung Cancer. *Nature* (2018) 553(7689):446–54. doi: 10.1038/nature25183
3. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2018. *CA Cancer J Clin* (2018) 68(1):7–30. doi: 10.3322/caac.21442
4. Deng Y, Zhao P, Zhou L, Xiang D, Hu J, Liu Y, et al. Epidemiological Trends of Tracheal, Bronchus, and Lung Cancer at the Global, Regional, and National Levels: A Population-Based Study. *J Hematol Oncol* (2020) 13(1):98. doi: 10.1186/s13045-020-00915-0
5. Wheeler DL, Dunn EF, Harari PM. Understanding Resistance to EGFR Inhibitors-Impact on Future Treatment Strategies. *Nat Rev Clin Oncol* (2010) 7(9):493–507. doi: 10.1038/nrclinonc.2010.97
6. Zhong WZ, Wang Q, Mao WM, Xu ST, Wu L, Shen Y, et al. Gefitinib Versus Vinorelbine Plus Cisplatin as Adjuvant Treatment for Stage II-IIIA (N1-N2) EGFR-Mutant NSCLC (ADJUVANT/CTONG1104): A Randomised, Open-Label, Phase 3 Study. *Lancet Oncol* (2018) 19(1):139–48. doi: 10.1016/S1470-2045(17)30729-5
7. Nukaga S, Yasuda H, Tsuchihara K, Hamamoto J, Masuzawa K, Kawada I, et al. Amplification of EGFR Wild-Type Alleles in Non-Small Cell Lung Cancer Cells Confers Acquired Resistance to Mutation-Selective EGFR Tyrosine Kinase Inhibitors. *Cancer Res* (2017) 77(8):2078–89. doi: 10.1158/0008-5472.CAN-16-2359
8. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors. *Sci Trans Med* (2011) 3(75):75ra26. doi: 10.1126/scitranslmed.3002003
9. Wang L, Du F, Wang X. TNF-Alpha Induces Two Distinct Caspase-8 Activation Pathways. *Cell* (2008) 133(4):693–703. doi: 10.1016/j.cell.2008.03.036
10. Morgan MJ, Kim YS, Liu ZG. Membrane-Bound Fas Ligand Requires RIP1 for Efficient Activation of Caspase-8 Within the Death-Inducing Signaling Complex. *J Immunol* (2009) 183(5):3278–84. doi: 10.4049/jimmunol.0803428
11. Son JW, Kang HK, Chae MH, Choi JE, Park JM, Lee WK, et al. Polymorphisms in the Caspase-8 Gene and the Risk of Lung Cancer. *Cancer Genet Cytogenet* (2006) 169(2):121–7. doi: 10.1016/j.cancergencyto.2006.04.001
12. Xu B, Zhou ZG, Li Y, Wang L, Yang L, Zhou B, et al. Clinicopathological Significance of Caspase-8 and Caspase-10 Expression in Rectal Cancer. *Oncology* (2008) 74(3-4):229–36. doi: 10.1159/000151392
13. Wu Y, Alvarez M, Slamon DJ, Koeffler P, Vadgama JV. Caspase 8 and Maspin are Downregulated in Breast Cancer Cells Due to CpG Site Promoter Methylation. *BMC Cancer* (2010) 10:32. doi: 10.1186/1471-2407-10-32
14. Maelfait J, Beyaert R. Non-Apoptotic Functions of Caspase-8. *Biochem Pharmacol* (2008) 76(11):1365–73. doi: 10.1016/j.bcp.2008.07.034
15. Barbero S, Barila D, Mielgo A, Stagni V, Clair K, Stupack D. Identification of a Critical Tyrosine Residue in Caspase 8 That Promotes Cell Migration. *J Biol Chem* (2008) 283(19):13031–4. doi: 10.1074/jbc.M800549200
16. Finlay D, Howes A, Vuori K. Caspase-8 as a Potential Mediator of Pro-Tumorigenic Signals. *Cell Cycle* (2009) 8(21):3441–2. doi: 10.4161/cc.8.21.9649
17. Barbero S, Mielgo A, Torres V, Teitz T, Shields DJ, Mikolon D, et al. Caspase-8 Association With the Focal Adhesion Complex Promotes Tumor Cell Migration and Metastasis. *Cancer Res* (2009) 69(9):3755–63. doi: 10.1158/0008-5472.CAN-08-3937
18. Cursi S, Rufini A, Stagni V, Condo I, Matafora V, Bachi A, et al. Src Kinase Phosphorylates Caspase-8 on Tyr380: A Novel Mechanism of Apoptosis Suppression. *EMBO J* (2006) 25(9):1895–905. doi: 10.1038/sj.emboj.7601085
19. Finlay D, Howes A, Vuori K. Critical Role for Caspase-8 in Epidermal Growth Factor Signaling. *Cancer Res* (2009) 69(12):5023–9. doi: 10.1158/0008-5472.CAN-08-3731
20. Finlay D, Vuori K. Novel Noncatalytic Role for Caspase-8 in Promoting SRC-Mediated Adhesion and Erk Signaling in Neuroblastoma Cells. *Cancer Res* (2007) 67(24):11704–11. doi: 10.1158/0008-5472.CAN-07-1906
21. Diao Y, Ma X, Min W, Lin S, Kang H, Dai Z, et al. Dasatinib Promotes Paclitaxel-Induced Necroptosis in Lung Adenocarcinoma With Phosphorylated Caspase-8 by C-Src. *Cancer Lett* (2016) 379(1):12–23. doi: 10.1016/j.canlet.2016.05.003
22. Crystal AS, Shaw AT, Sequist LV, Friboulet L, Niederst MJ, Lockerman EL, et al. Patient-Derived Models of Acquired Resistance Can Identify Effective Drug Combinations for Cancer. *Science* (2014) 346(6216):1480–6. doi: 10.1126/science.1254721
23. Helfer B, Boswell BC, Finlay D, Cipres A, Vuori K, Bong Kang T, et al. Caspase-8 Promotes Cell Motility and Calpain Activity Under Nonapoptotic Conditions. *Cancer Res* (2006) 66(8):4273–8. doi: 10.1158/0008-5472.CAN-05-4183
24. Senft J, Helfer B, Frisch SM. Caspase-8 Interacts With the P85 Subunit of Phosphatidylinositol 3-Kinase to Regulate Cell Adhesion and Motility. *Cancer Res* (2007) 67(24):11505–9. doi: 10.1158/0008-5472.CAN-07-5755
25. Zhang Y, Sun L, Gao X, Guo A, Diao Y, Zhao Y. RNF43 Ubiquitinates and Degrades Phosphorylated E-Cadherin by C-Src to Facilitate Epithelial-Mesenchymal Transition in Lung Adenocarcinoma. *BMC Cancer* (2019) 19(1):670. doi: 10.1186/s12885-019-5880-1
26. Kim J, Yu W, Kovalski K, Ossowski L. Requirement for Specific Proteases in Cancer Cell Intravasation as Revealed by a Novel Semiquantitative PCR-Based Assay. *Cell* (1998) 94(3):353–62. doi: 10.1016/S0092-8674(00)81478-6
27. Zijlstra A, Mellor R, Panzarella G, Aimes RT, Hooper JD, Marchenko ND, et al. A Quantitative Analysis of Rate-Limiting Steps in the Metastatic Cascade Using Human-Specific Real-Time Polymerase Chain Reaction. *Cancer Res* (2002) 62(23):7083–92.
28. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in Cancer. *Nat Rev Cancer* (2018) 18(2):128–34. doi: 10.1038/nrc.2017.118
29. Dongre A, Weinberg RA. New Insights Into the Mechanisms of Epithelial-Mesenchymal Transition and Implications for Cancer. *Nat Rev Mol Cell Biol* (2018) 20(2):69–84. doi: 10.1038/s41580-018-0080-4
30. Frame MC. V-SRC Informs Integrin Signalling. *Nat Rev Mol Cell Biol* (2013) 14(9):548. doi: 10.1038/nrm3647
31. Avizienyte E, Frame MC. Src and FAK Signalling Controls Adhesion Fate and the Epithelial-to-Mesenchymal Transition. *Curr Opin Cell Biol* (2005) 17(5):542–7. doi: 10.1016/j.ceb.2005.08.007
32. Irmer D, Funk JO, Blaukat A. EGFR Kinase Domain Mutations - Functional Impact and Relevance for Lung Cancer Therapy. *Oncogene* (2007) 26(39):5693–701. doi: 10.1038/sj.onc.1210383
33. Pao W, Chmielecki J. Rational, Biologically Based Treatment of EGFR-Mutant Non-Small-Cell Lung Cancer. *Nat Rev Cancer* (2010) 10(11):760–74. doi: 10.1038/nrc2947
34. Behan FM, Iorio F, Picco G, Goncalves E, Beaver CM, Migliardi G, et al. Prioritization of Cancer Therapeutic Targets Using CRISPR-Cas9 Screens. *Nature* (2019) 568(7753):511–6. doi: 10.1038/s41586-019-1103-9
35. Ferguson FM, Gray NS. Kinase Inhibitors: The Road Ahead. *Nat Rev Drug Discovery* (2018) 17(5):353–77. doi: 10.1038/nrd.2018.21
36. Huang Y, Bharill S, Karandur D, Peterson SM, Marita M, Shi X, et al. Molecular Basis for Multimerization in the Activation of the Epidermal Growth Factor Receptor. *Elife* (2016) 5:e14107. doi: 10.7554/eLife.14107
37. Leduc C, Merlio JP, Besse B, Blons H, Debieuvre D, Bringuier PP, et al. Clinical and Molecular Characteristics of Non-Small-Cell Lung Cancer (NSCLC) Harboring EGFR Mutation: Results of the Nationwide French Cooperative Thoracic Intergroup (IFCT) Program. *Ann Oncol* (2017) 28(11):2715–24. doi: 10.1093/annonc/mdx404
38. Skoulidis F, Heymach JV. Co-Occurring Genomic Alterations in Non-Small-Cell Lung Cancer Biology and Therapy. *Nat Rev Cancer* (2019) 19(9):495–509. doi: 10.1038/s41568-019-0179-8
39. Deng J, Shimamura T, Perera S, Carlson NE, Cai D, Shapiro GI, et al. Proapoptotic BH3-Only BCL-2 Family Protein BIM Connects Death Signaling From Epidermal Growth Factor Receptor Inhibition to the Mitochondrion. *Cancer Res* (2007) 67(24):11867–75. doi: 10.1158/0008-5472.CAN-07-1961
40. Rotow J, Bivona TG. Understanding and Targeting Resistance Mechanisms in NSCLC. *Nat Rev Cancer* (2017) 17(11):637–58. doi: 10.1038/nrc.2017.84
41. Tourneur L, Chiocchia G. FADD: A Regulator of Life and Death. *Trends Immunol* (2010) 31(7):260–9. doi: 10.1016/j.it.2010.05.005
42. Dillon CP, Oberst A, Weinlich R, Janke LJ, Kang TB, Ben-Moshe T, et al. Survival Function of the FADD-CASPASE-8-cFLIP(L) Complex. *Cell Rep* (2012) 1(5):401–7. doi: 10.1016/j.celrep.2012.03.010

43. Thapa RJ, Nogusa S, Chen P, Maki JL, Lerro A, Andrake M, et al. Interferon-Induced RIP1/RIP3-Mediated Necrosis Requires PKR and Is Licensed by FADD and Caspases. *Proc Natl Acad Sci USA* (2013) 110(33):E3109–18. doi: 10.1073/pnas.1301218110
44. Socinski MA, Villaruz LC, Ross J. Understanding Mechanisms of Resistance in the Epithelial Growth Factor Receptor in Non-Small Cell Lung Cancer and the Role of Biopsy at Progression. *Oncologist* (2017) 22(1):3–11. doi: 10.1634/theoncologist.2016-0285
45. Lin Y, Wang X, Jin H. EGFR-TKI Resistance in NSCLC Patients: Mechanisms and Strategies. *Am J Cancer Res* (2014) 4(5):411–35.
46. Cantor AJ, Shah NH, Kuriyan J. Deep Mutational Analysis Reveals Functional Trade-Offs in the Sequences of EGFR Autophosphorylation Sites. *Proc Natl Acad Sci USA* (2018) 115(31):E7303–12. doi: 10.1073/pnas.1803598115
47. Zhao Y, Sui X, Ren H. From Procaspase-8 to Caspase-8: Revisiting Structural Functions of Caspase-8. *J Cell Physiol* (2010) 225(2):316–20. doi: 10.1002/jcp.22276
48. van Leeuwen RW, van Gelder T, Mathijssen RH, Jansman FG. Drug-Drug Interactions With Tyrosine-Kinase Inhibitors: A Clinical Perspective. *Lancet Oncol* (2014) 15(8):e315–26. doi: 10.1016/S1470-2045(13)70579-5
49. Aiello NM, Kang Y. Context-Dependent EMT Programs in Cancer Metastasis. *J Exp Med* (2019) 216(5):1016–26. doi: 10.1084/jem.20181827
50. Lu W, Kang Y. Epithelial-Mesenchymal Plasticity in Cancer Progression and Metastasis. *Dev Cell* (2019) 49(3):361–74. doi: 10.1016/j.devcel.2019.04.010
51. Wee P, Wang Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. *Cancers (Basel)* (2017) 9(5):52. doi: 10.3390/cancers9050052
52. Cocco E, Scaltriti M, Drilon A. NTRK Fusion-Positive Cancers and TRK Inhibitor Therapy. *Nat Rev Clin Oncol* (2018) 15(12):731–47. doi: 10.1038/s41571-018-0113-0
53. Johnson FM, Bekele BN, Feng L, Wistuba I, Tang XM, Tran HT, et al. Phase II Study of Dasatinib in Patients With Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* (2010) 28(30):4609–15. doi: 10.1200/JCO.2010.30.5474
54. Li J, Rix U, Fang B, Bai Y, Edwards A, Colinge J, et al. A Chemical and Phosphoproteomic Characterization of Dasatinib Action in Lung Cancer. *Nat Chem Biol* (2010) 6(4):291–9. doi: 10.1038/nchembio.332

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GLOSSARY

EMT	Epithelial-mesenchymal transition
Tyr	Tyrosine
NSCLC	Non–small cell lung cancer
WT	Wild type
EGFR	Epidermal growth factor receptor
PI3K	Phosphoinositide-3 kinase
AKT	Protein kinase B
MAPK	Mitogen-activated protein kinase
ERK1/2 or ERK	Extracellular signal-regulated kinase
MEK	MAPK/ERK kinase
FAK	Focal adhesion kinase
HA	Hemagglutinin
TKI	Tyrosine kinase inhibitor
PDX	Patient-derived xenografts
CR	Complete remission
PR	Partial remission
SD	Stable disease
PD	Progressive disease
AJCC	American Joint Committee on Cancer
RECIST	Response evaluation criteria in solid tumors
IHC	Immunohistochemistry
NCI	National cancer institute
ATCC	American type culture collection
FBS	Fetal bovine serum
shRNAs	Short hairpin RNAs
Ct	Threshold cycle
nec-1	Necrostatin-1
IP	Immunoprecipitation
PI	Propidium iodide
IHC	Immunohistochemistry
RIPK	Receptor-interacting serine/threonine-protein kinase
FADD	Fas-associated death domain
ARMS	Amplification refractory mutation system

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Modelled Economic Analysis for Dacomitinib–A Cost Effectiveness Analysis in Treating Patients With EGFR-Mutation-Positive Non-Small Cell Lung Cancer in China

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Objectives: To establish the cost-effectiveness of dacomitinib compared to gefitinib from the Chinese healthcare system perspective.

Patients: Advanced non-small cell lung cancer (NSCLC) harbouring epidermal growth factor receptor (EGFR) mutations.

Methods: Partitioned survival analysis was undertaken to examine the cost-effectiveness of dacomitinib utilising individual patient data (IPD) from the pivotal randomised controlled trial (RCT) (ARCHER 1050). The three health states modelled were progression-free, post-progression, and death. Parametric survival distributions were fitted to IPD against the Kaplan-Meier survival curves corresponding to progression-free survival (PFS) and overall survival (OS) outcomes by randomised groups. Costs included drug acquisition and administration, outpatient management (outpatient consultation and examinations), and best supportive care costs. Utility weights were sourced from the pivotal trial and other published literature. The incremental cost-effectiveness ratio (ICER) was calculated with costs and quality-adjusted life years (QALYs) discounted at an annual rate of 5%. Both deterministic and probabilistic sensitivity analyses were undertaken.

Results: In the base case, dacomitinib (CNY 265,512 and 1.95 QALY) was associated with higher costs and QALY gains compared to gefitinib (CNY 247,048 and 1.61 QALYs), resulting in an ICER of CNY 58,947/QALY. Using the empirical WTP/QALY threshold, dacomitinib is a cost-effective treatment strategy for patients with EGFR-mutation-positive advanced NSCLC. The probabilistic sensitivity analysis suggested that dacomitinib had a 97% probability of being cost-effective.

Conclusions: Dacomitinib is a cost-effective treatment strategy in treating patients with EGFR-mutation-positive NSCLC from the Chinese healthcare system perspective. The uncertainty around the cost-effectiveness of dacomitinib could be reduced if long-term survival data become available.

Clinical Trial Registration: NCT01024413

Keywords: epidermal growth factor receptor (EGFR) mutations, NSCLC, cost-effectiveness analysis (CEA), partitioned survival analysis, economic model

INTRODUCTION

With more than 2.1 million new cases and 11.6% of the total cancer incidence in 2018, lung cancer is the leading cause of cancer-related mortality (1.8 million deaths, 18.4% of the total) worldwide (1). Among these patients, more than a third of all newly diagnosed lung cancers were from China, constituting a substantial burden for patients, families, and society as a whole (2). In 2014, the China annual cancer report revealed that there were 782,000 patients with newly identified lung cancer (3), including 521,000 male and 261,000 female patients, which represented a significant increase (20%) from 651,053 total new cases in 2011 (4). It was estimated that the total national medical cost attributable to lung cancer was US$10.31 billion, accounting for 2% of the total medical cost in China in 2015 (5).

Of all lung cancer cases, approximately 85% are non-small cell lung cancer (NSCLC), and the majority of these patients are diagnosed at the advanced or metastatic stage, losing their opportunities for surgery. Epidermal growth factor receptor (EGFR) mutations are observed in approximately 50% of Asian and 20% of non-Asian patients (6). EGFR mutations occurred more frequently in patients who had never smoked, women, adenocarcinomas, and Asian patients (7–9).

First-line treatment options of NSCLC patients harbouring EGFR-mutation include the EGFR tyrosine kinase inhibitors (TKIs), gefitinib, erlotinib, and afatinib all demonstrating improvements in progression-free survival (PFS) and quality of life, compared with platinum-based doublet chemotherapy. There are approved second (treating NSCLC harbouring activating EGFR mutations) and third (i.e., osimertinib, targeting NSCLC carrying EGFR-TKI–sensitising and EGFR p.Thr790Met (T790M) resistance mutations) (10) generation EGFT-TKIs in China. Dacomitinib is a second-generation, irreversible EGFR TKI that was approved in China in 2019. It is a pan-HER irreversible inhibitor that has activity against all three kinase-active members of the ErbB family (EGFR/HER1, HER2, and HER4). The FDA and China Food and Drug Administration (CFDA) granted dacomitinib market access based on a randomised, multicentre, open-label trial (ARCHER 1050). This trial evaluated the efficacy and safety of dacomitinib versus gefitinib as a first-line therapy in patients with advanced EGFR-mutation-positive NSCLC. The outcome from this phase III trial showed that dacomitinib significantly improved PFS compared to gefitinib in first-line treatment of patients with EGFR-mutation-positive NSCLC, with median PFS of 14.7 vs 9.2 months, respectively (hazard ratio 0.59, 95% confidence interval, CI: 0.47-0.74, p<0.0001). also showed clinically meaningful improvement in overall survival (OS) with dacomitinib (11).

The National Drug Reimbursement List (NDRL) has four first-line EGFR TKIs (i.e., gefitinib, erlotinib, icotinib, and afatinib) currently registered to treat patients with EGFR-mutation-positive NSCLC dating back to 2016. However, given that the marked gap in the health outcome for patients with EGFR-mutation-positive NSCLC still exists and the availability of more effective treatment options, the next critical question to address is whether more effective treatment (i.e., dacomitinib) represents value-for-money, in other words, whether the increased benefits justify the increased costs. This is pivotal for the Chinese government since there is always a constraint between ever-increasing healthcare demand and the already stretched healthcare budget. In response, we aimed to undertake a modelled economic evaluation of dacomitinib in treating patients with EGFR-mutation-positive NSCLC from the Chinese healthcare system perspective using the ARCHER 1050 trial and local costing data.

METHODS

Model Structure

Partitioned survival analysis was utilised to model the long-term cost-effectiveness of dacomitinib versus gefitinib. A proportion of patients can move among progression-free (PF), post-progression (PP), and death states. The progressed patient cannot return to the PF health state. This modelling approach was chosen because it is most widely used to summarise the overall impact of treatments on survival and health-related quality of life (HRQoL) in the context of clinical trials (12–15). The survival curves of progression-free survival (PFS) and overall survival (OS) were used independently to derive the proportion of the cohort at PF and PP (i.e., the difference in survival at the same timepoint from PFS and OS curves) health states by various timepoints. Thus, the proportion of patients in each modelled health state are time-dependent.

Population

Patients diagnosed with EGFR-mutation-positive advanced NSCLC (stages IIIB/IV or recurrent) and at least one documented EGFR mutation (exon 19 deletion or the Leu858Arg mutation, with or without the Thr790Met mutation) were modelled. The baseline characteristics were defined as per the published clinical trial. Briefly, the modelled cohort had a median age of 62 years, with female participants overrepresented (>50%) and predominantly

stage IV cancer (81%). Exon 19 deletion (59%) and Leu858Arg (41%) are the key EGFR mutation types.

Long-Term Extrapolation

Treatment-specific PFS and OS curves from the pivotal trial were used to track the proportion of patients who stayed in the PF, PP, and death health states. Since the median duration of follow-up was 22.1 versus 23.0 months in the dacomitinib and gefitinib-treated patients, respectively, extrapolation of survival curves observed from the trial is necessary to assess the long-term cost-effectiveness of dacomitinib.

The ARCHER 1050 patient-level data were analysed to generate the within-trial Kaplan-Meier survival curves for PFS (assessed by the independent review committee) and OS by randomised groups. The recommended parametric survival distributions, including exponential, Weibull, log-normal, log-logistic, generalised-gamma, and Gompertz, were fitted to the within-trial Kaplan-Meier curves (16). The best fit curve for long-term extrapolation was selected based on the goodness-of-fit statistics (AIC and BIC values), visual inspection (17), and clinical validation. Input from clinical experts was sought to assess the plausibility of the extrapolation.

Treatment Protocol

Hypothetical patients started either dacomitinib or gefitinib treatment in the first cycle of the partitioned survival analysis (PartSA) model. It was assumed that patients only discontinued the dacomitinib/gefitinib treatment (i.e., first-line treatment) upon disease progression (i.e., transition from PF to PP state). Those who progressed are eligible for second- and third-line treatment incorporating gefitinib, erlotinib, afatinib, osimertinib, and other standard chemotherapy (i.e., pemetrexed, and platinum-based chemotherapy). Around 71% of patients underwent the second-line treatment since the disease progression, and a further 48% of them received the third-line anti-cancer treatment. The duration of second-, third-, and subsequent treatment are summarised in **Supplementary Table 1**. The dosing regimen for each treatment is supplied in **Table 1**.

Costs

Since the healthcare system perspective was adopted to measure the cost and benefits, only direct medical costs were considered in the modelled economic analysis. Primary cost components included first-line treatment (drug acquisition and administration cost relating to dacomitinib and gefitinib), second- and third-line treatment, outpatient visit, and costs due to adverse events. The costs related to the treatment of commonly reported adverse events are included: for example, diarrhoea (56%), alanine aminotransferase increase (39%), and aspartate aminotransferase increase (36%) (details summarised in **Supplementary Table 2**). A 28-day cycle was adopted to estimate the costs according to the treatment regimen. The costs are expressed in Chinese yuan (CNY) valued in the year 2018. EGFR-TKIs drug cost used in the model is the national reimbursement price. All the unit costs of treatment are listed in **Table 1**.

Utility Weights

The utility weights associated with being in the PF health states were sourced from the pivotal trial. For the PF state, patients who received dacomitinib (0.783) reported lower quality of life compared to those who were treated with gefitinib (0.828); using this, differentiated utility weights by treatment status are considered not favouring the intervention. Different utility weights were assigned for patients

TABLE 1 | Unit cost of healthcare resources included in the analysis.

Treatment	Unit price	Dosing regimen	Frequency per 28-day	Cost per cycle	Reference
First-line					
Dacomitinib	¥88/15 mg	45 mg/day	1	¥7,418	Local charge
Gefitinib	¥236/25mg	25 mg/day	1	¥6,608	Online resource (16)
Second- & third-line					
Erlotinib	¥195/150mg	150mg/day	1	¥5,460	Online resource (16)
Afatinib	¥200/40mg	40mg/day	1	¥5,600	Online resource (16)
Osimertinib	¥510/80mg	80mg/day	1	¥14,280	Online resource (16)
Docetaxel*	¥97/20 mg	120 mg	1.33	¥5082.40	Zeng et al 2012 (17)
Pemetrexed^	¥321/200 mg	850 mg	1.33	¥11212.71	Zeng et al 2012 (17)
Platinum-based therapy			1.33	¥18,174.39	Zeng et al 2012 (17)
Docetaxel+ platinum-based	–	120 mg+ 120 mg	1.33	¥13,282.55	Zeng et al 2012 (17)
Chemotherapy drug					
Cisplatin	¥64.31	128 mg	1.33	¥10,932.70	Zeng et al 2012 (17)
Docetaxel	¥39.86	128 mg	1.33	¥6,776.20	Zeng et al 2012 (17)
Pemetrexed	¥26.79	500 mg	1.33	¥17,861.33	Gu et al 2019 (18)
Chemotherapy administration					
Platinum-based	¥596.74/day		1.33		Zeng et al 2012 (17)
Single drug	¥270.87/day		1.33		Zeng et al 2012 (17)
Management					
Outpatient consult	¥382.68	–	1	¥382.68	Zeng et al 2012 (17)
CT	¥484.92	–	0.5	¥242.46	Zeng et al 2012 (17)
MRI	¥1101.34	–	0.5	¥550.67	Zeng et al 2012 (17)
Ultrasound	¥402.73	–	0.5	¥201.37	Zeng et al 2012 (17)
Best support care	¥1902.33	–	1	¥1902.33	Zeng et al 2012 (17)
Terminal care	¥17,423.00	–	1	¥17,423.00	Lu S et al, 2017 (19)

**77% of patients received platinum-based chemotherapy; ^23% of patients received pemetrexed.*

receiving second- or third-line TKI treatment, chemotherapy, or best supportive care to account for the different profiles associated with treatment-related adverse events post-progression. The utility weights applied in the modelled economic analysis are outlined in **Supplementary Table 3**.

Cost-Effectiveness Analysis

The primary outcome measure was the quality-adjusted life year (QALY), which combines morbidity and mortality. Gefitinib was selected as the sole comparator since it has been reimbursed in China and adopted as the comparator for the economic evaluation of osimertinib in Australia that underpinned the reimbursement decision-making (18). In addition, there was no significant difference in effectiveness between erlotinib and gefitinib (and other first-line EGFR TKIs (19)). The incremental cost-effectiveness ratio (ICER) was calculated as the ratio between the incremental costs and incremental QALYs. All the costs and QALYs were accrued over a 15-year time period, given the relatively poor prognosis of the modelled population. In the absence of an official willingness-to-pay (WTP) per QALY threshold in China, three times the Gross Domestic Production (GDP) per capita (CNY 64,644×3) (20) from 2018 was adopted to examine the cost-effectiveness of dacomitinib. All the costs and benefits were discounted at a 5% rate per year (21).

Sensitivity Analysis

Deterministic and probabilistic sensitivity analyses (DSA and PSA) were undertaken to test the robustness of base care results. In the DSA, a series of one-way sensitivity analyses were performed to examine the variation in ICER by varying one key parameter within a range at a time. The results were presented in the form of a Tornado diagram. In the PSA, the distribution of key uncertainty parameters was incorporated. The second-order Monte Carlo simulation technique was adopted to sample 1000 iterations from each distribution to parameterise the model and calculate the average across these 1000 iterations (and the 95% confidence interval). The results from the PSA were plotted in the incremental cost-effectiveness plane. The parameters that varied in the DSA and PSA are shown in **Supplementary Table 4**. Further, the various WTP/QALY thresholds were tested.

RESULTS

Long-Term Extrapolation

In consultation with clinical experts and the AIC/BIC values and visual inspection, for PFS, Weibull and generalised gamma distribution were considered most plausible, while for OS, Weibull, Gompertz, and generalised gamma distribution were deemed reasonable. Following the NICE DSU recommendations, the same type of distribution for both arms of each endpoint is preferred. The different distributions have differential tail characteristics and therefore, utilising the same distribution could potentially avoid bias in the comparison generated by these differences. Moreover, the two treatment modalities compared are both TKIs, which have a similar mode of action. Hence, the Weibull distribution was chosen to extrapolate the PFS and OS curve regardless of treatment groups. Extrapolation of PFS and OS curves by alternative parametric survival functions is shown in **Supplementary Figures 1, 2**.

Published economic evaluations for similar EGFR TKIs were also reviewed. In the CEA of afatinib vs. gefitinib by Chouaid et al. (2017) (based on the LUX-Lung 7 trial), the authors used the Weibull distribution for both PFS and OS based on the AIC (22). Gefitinib was a common comparator between the LUX-Lung 7 and ARCHER 1050 trials, and afatinib and dacomitinib have analogous mechanisms of action. As a result, it is reasonable to assume that their long-term survival curves would follow a similar distribution. It is acknowledged that the LUX-Lung 7 trial had a complete follow-up period (i.e., 27.3 months) (23), which is more informative for model selection in lieu of long-term extrapolation. It is considered as an external data point justifying the selection of the Weibull distribution in the current economic evaluation.

The goodness-of-fit statistics for fitting PFS and OS curves are provided in **Table 2**, and fitted parametric curves are shown in **Figure 1**. The PFS results based on the independent review committee (IRC) were used for the modelled cost-effectiveness analysis.

Cost-Effectiveness Analysis

Over a 15-year time period, dacomitinib (CNY 265,512 and 1.96 QALY) was associated with higher costs and QALY gains compared to gefitinib (CNY 247,048 and 1.61 QALY), resulting in an ICER of CNY 58,947/QALY. Using the empirical WTP/QALY threshold, it is considered that dacomitinib is a cost-effective treatment strategy for patients with EGFR-mutation-positive advanced NSCLC. The key cost components included costs related to first-line medications (CNY 108,795 and 83,414), outpatient care (CNY 81,944 and 73,215), second- and third-line medications (CNY 59,446 and 74,699), terminal care (CNY15,290 and 15,690), and AE (CNY 37 and 30) in dacomitinib and gefitinib groups, respectively (**Table 3**).

TABLE 2 | Goodness-of-Fit Statistics for the PFS and OS by treatment groups.

Treatment	Curve (Weibull)	AIC	BIC	Mean (month)	Median (month)
Dacomitinib	PFS (IRC)	545.20	552.04	18.67	14.74
	PFS (INV)	530.36	537.21	19.06	15.70
	OS	465.03	471.88	38.92	33.36
Gefitinib	PFS (IRC)	514.46	521.29	11.80	10.25
	PFS (INV)	513.42	520.25	13.25	11.60
	OS	461.29	468.12	32.01	28.55

AIC, Akaike information criterion; BIC, Bayesian information criterion; IRC, independent review committee; INV, investigator; OS, overall survival.

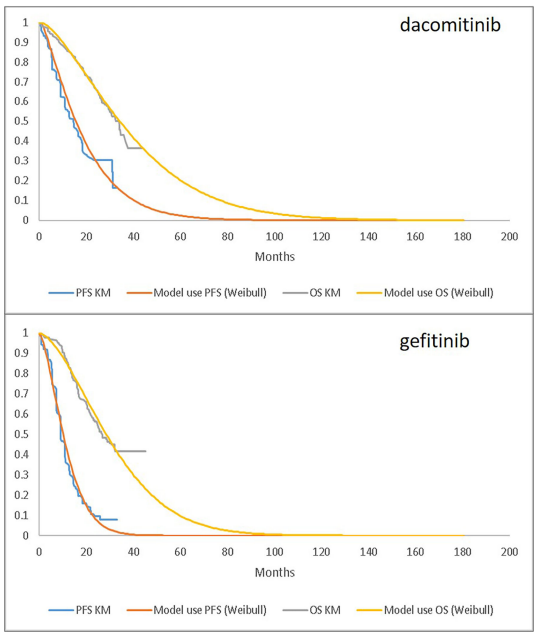

FIGURE 1 | Parametric Fitting (Weibull) Compared to Observed KM Data: PFS (based on independent review committee) and OS for dacomitinib (upper) and gefitinib (lower). PFS, progression-free survival; OS, overall survival; KM, Kaplan-Meier.

Sensitivity Analyses

The DSA identified that drug acquisition cost for dacomitinib and gefitinib, dacomitinib OS extrapolation, second-line treatment duration and probability of receiving second-line treatment post-gefitinib, and second-line treatment duration post-dacomitinib are the key determinants for the ICER. At the same time, probability of receiving third-line treatment post-dacomitinib, the medical resource use (i.e., outpatient care) cost per cycle for both dacomitinib and gefitinib, and gefitinib/dacomitinib PFS extrapolation are less determinant for the ICER (**Figure 2**).

The base case ICER was moderately sensitive to the parametric survival distributions adopted (i.e., for extrapolating survival curves for gefitinib); for example, if the generalised gamma distribution was selected for the PFS curve while Gompertz distribution was used for the OS curve, the ICER increased to CNY 70,152/QALY (**Supplementary Table 4**).

The PSA showed that most of the results demonstrated that dacomitinib contributed to greater costs and QALYs, suggesting a probability of 97% of being cost-effective compared to gefitinib (**Figure 3**). The cost-effective acceptability curve is shown in **Figure 4**, which shows that when the WTP/QALY was over three times the GDP/Capita in China, dacomitinib becomes highly likely to be cost-effective (over 90%). Lowering the WTP/QALY to two or one times the GDP/Capita and reducing cost-effective probability to 89% and 54%, respectively (**Supplementary Figures 3, 4**).

DISCUSSION

The modelled cost-effectiveness analysis of dacomitinib as a first-line treatment for patients with locally advanced or metastatic EGFR-mutation-positive NSCLC in China was associated with an ICER of CNY 58,947/QALY compared with gefitinib over a 15-year time period. The incremental cost and QALYs were CNY 18,463 and 0.3132, respectively. Using the empirical WTP/QALY threshold in China, dacomitinib is considered a cost-effective treatment modality in this population from the Chinese healthcare payer's perspective.

It is acknowledged that the parametric survival models for long-term extrapolation play a key role in determining the cost-effectiveness of the intervention. Sensitivity analyses were thus undertaken by varying the model parameters and testing the alternative distributions (i.e., generalised gamma). Not surprisingly, the sensitivity analyses indicated that the OS parameters for dacomitinib and gefitinib were critical drivers for

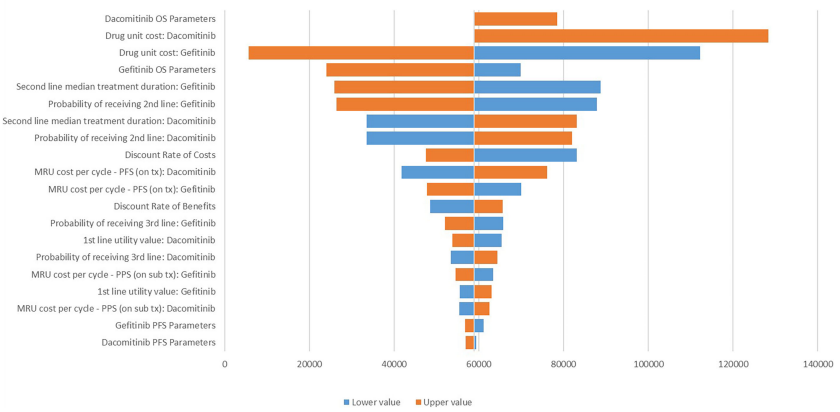

FIGURE 2 | Tornado diagram for the one-way sensitivity analysis. MRU, medical resource use; tx, treatment; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival. The lower values were not tested for the dacomitinib OS parameters and its unit cost due to the negative ICER generated.

TABLE 3 | Base case results of the cost-effectiveness analysis.

Treatment	Cost (CNY)	QALYs	Incremental Cost (CNY)	Incremental QALYs	ICER
Dacomitinib	265,512	1.9548			
Gefitinib	247,048	1.6067	18,463	0.3132	58,947

CNY, Chinese Yuan; QALY, quality-adjusted life year; ICER, incremental cost-effectiveness ratio.

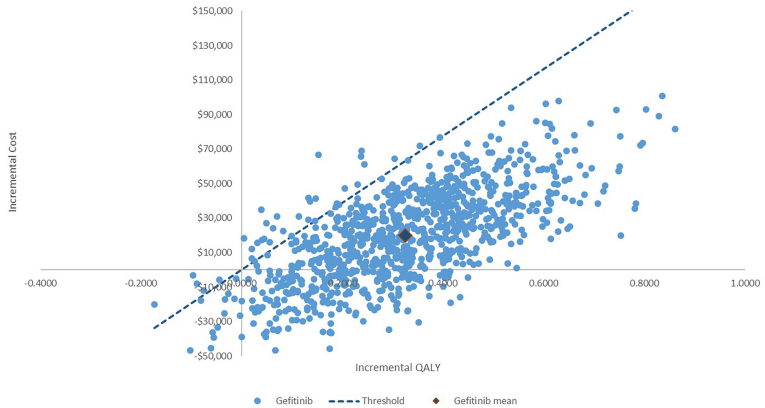

FIGURE 3 | Incremental cost-effectiveness plane: Dacomitinib vs. gefitinib. The probability of dacomitinib being cost-effective is 97%.

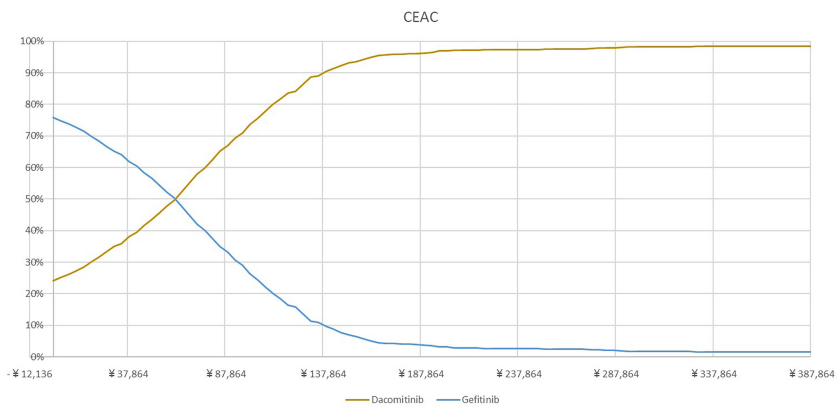

FIGURE 4 | Cost-effectiveness acceptability curve.

the ICER. The sensitivity analyses by adopting alternative parametric distribution showed that even after adopting alternative distribution to extrapolate the within-trial observation, dacomitinib was still a cost-effective treatment strategy compared to gefitinib.

The patient-level data were utilised to derive the long-term extrapolation, which captured all the possible covariates that might have influenced the OS and PFS over the trial duration and reflected the time dependence. The PartSA approach directly applies the primary outcomes from the pivotal trial (i.e., PFS and OS) and derivation of the state membership from the survival function directly. As the OS curve was utilised directly to estimate the proportion of patients in the death state over time, the OS from the PartSA was a perfect match to the observed OS within-trial in this approach. In a 2017 NICE DSU review of NICE oncology technology appraisals, 73% (22/30) of the appraisal for cancer interventions adopted the PartSA to assess the long-term cost-effectiveness of the intervention. It is believed that this is an appropriate modelling technique in this case as well.

Of particular importance, the base case cost-effectiveness results were based on the PFS outcome assessed by the independent review committee, which is considered conservative. The pivotal trial showed median PFS was 14.7 (95%CI: 11.1-16.6) vs 9.2 months

(95%CI: 9.1-11.0) from the independent review committee (HR 0.59, 95% CI: 0.47-0.74), while the same outcome was 16.6 (95%CI: 12.9-18.4) vs 11.0 months (95%CI: 9.4-12.1) from the investigators' judgement (HR 0.62, 95%CI: 0.50-0.78).

The QALY outcome of dacomitinib from the current study (i.e., when a 10-year time period was adopted, the QALY gain was 1.938 and 1.629 in dacomitinib and gefitinib groups) was similar to other published cost-effectiveness analyses concerning similar therapies. The modelled economic analysis of afatinib versus gefitinib reported a QALY of 1.857 and 1.687, respectively, which also extrapolated the OS and PFS curves using the Weibull distribution whereas they adopted a shorter time frame (i.e., 10 years) and a 4% discount rate (22). Another report had slightly lower QALY gains for the assessed TKIs conducted in the United States (i.e., 1.50 QALY for afatinib, 1.51 QALY for erlotinib, and 1.47 QALY for gefitinib), compared with the current results. However, since the US study did not have access to the individual-level patient data, the long-term extrapolation may be less accurate than this presented study which extrapolated the within-trial data based on individual patient data. In terms of the incremental costs, the previous studies reported €7,700 and $7,714 respectively in the base case scenarios. With the simple currency conversion, incremental costs were similar across these modelled economic evaluations. Another economic analysis that compared afatinib with pemetrexed-cisplatin in the same population reported an ICER of SG$137,648/QALY (24) (the QALY gain was 1.69 in the afatinib treatment group) based on the PartSA technique. A French study compared afatinib versus erlotinib as a second-line treatment (patient failed platinum-based therapy) for NSCLC, the QALY gain was lower than those in the first-line treatment setting (0.94 versus 0.78 in these patients with more advanced disease), but concluded it was highly likely to be cost-effective over a 10-year time period with a corresponding ICER of €30,277/QALY (25).

The empirical WTP/QALY is established using the WHO recommendation of one to three times of GDP/Capita, and we also examined the cost-effectiveness conclusion by varying such a threshold in the sensitivity analyses. Three times the GDP/Capita is usually adopted for non-developed countries, and this threshold is consistent with prior published economic evaluation in China (26–28).

This study is not without limitations. First, only the PartSA modelling technique was utilised to simulate the long-term costs and QALY associated with dacomitinib treatment. Because the primary assumption underlying the PartSA approach (i.e., PFS and OS are independent, so PFS is not predictive of OS), this assumption cannot hold sometimes. Second, the treatment with dacomitinib/gefitinib was discontinued upon disease progression in the model; however, in actual clinical practice, patients may continue such treatment with the treating physician's discretion. Third, the patients recruited in the trial may not be the same as the characteristics of patients in China. For example, ARCHER 1050 had more women and non-smokers and patients with less advanced NSCLC compared to the real-world patients (29). Therefore, the cost-effectiveness analysis based on the trial population may not be directly applicable for the Chinese patient population (30). Nevertheless, this is the first published economic evaluation of dacomitinib in treating patients with EGFR-mutation-positive NSCLC, which was performed based on the individual patient data that can maximise the accuracy of the long-term extrapolation for the OS and PFS curves, which bears important implications for policy decision-making. The economic evaluation was performed from the Chinese healthcare system perspective; however, the results may be helpful for other countries with similar economic status.

CONCLUSIONS

Dacomitinib is a cost-effective treatment strategy in the first-line treatment of patients with EGFR-mutation-positive NSCLC from the Chinese healthcare payer's perspective. The uncertainty around the cost-effectiveness of dacomitinib could be reduced if long-term survival data become available.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data used for the current study can be requested on a reasonable basis. Requests to access these datasets should be directed to shunlu@sjtu.edu.cn.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Shanghai Chest Hospital, Shanghai, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-FY, LL, F-FZ, PD, L-HM, L-TL, LG, and SL contributed to the conception and design of the study. LL undertook the analysis. All the authors contributed to the result interpretation and critically reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.564234/full#supplementary-material

REFERENCES

1. World Health Organisation. *Latest Global Cancer Data: Cancer Burden Rises to 18.1 Million New Cases and 9.6 Million Cancer Deaths in 2018*. Available at: https://www.who.int/cancer/PRGlobocanFinal.pdf. 2018.
2. Hong QY, Wu GM, Qian GS, Hu CP, Zhou JY, Chen LA, et al. Prevention and Management of Lung Cancer in China. *Cancer* (2015) 121(Suppl 17):3080–8. doi: 10.1002/cncr.29584
3. Chen W, Sun K, Zheng R, Zeng H, Zhang S, Xia C, et al. Cancer Incidence and Mortality in China, 2014. *Chin J Cancer Res* (2018) 30(1):1–12. doi: 10.21147/j.issn.1000-9604.2018.01.01
4. Chen WQ, Zheng RS, Zeng HM, Zhang SW, He J. Annual Report on Status of Cancer in China, 2011. *Chin J Cancer Res* (2015) 27:2–12. doi: 10.1186/s40880-015-0001-2
5. Fan S, Mao Z, Lee AH, Teh-wei H. Economic Costs of Lung Cancer in China. *Int J Oncol Res* (2018) 1:007. doi: 10.23937/ijor-2017/1710007
6. Ha SY, Choi S-J, Cho JH, Choi HJ, Lee J, Jung K, et al. Lung Cancer in Never-Smoker Asian Females Is Driven by Oncogenic Mutations, Most Often Involving EGFR. *Oncotarget* (2015) 6(7):5465–74. doi: 10.18632/oncotarget.2925
7. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for Epidermal Growth Factor Receptor Mutations in Lung Cancer. *N Engl J Med* (2009) 361(10):958–67. doi: 10.1056/NEJMoa0904554
8. Ettinger DS, Wood DE, Akerley W, Bazhenova LA, Borghaei H, Camidge DR, et al. Non-Small Cell Lung Cancer, Version 6.2015. *J Natl Compr Canc Netw* (2015) 13(5):515–24. doi: 10.6004/jnccn.2015.0071
9. Sandelin M, Berglund A, Sundstrom M, Micke P, Ekman S, Bergqvist M, et al. Patients With Non-Small Cell Lung Cancer Analyzed for EGFR: Adherence to Guidelines, Prevalence and Outcome. *Anticancer Res* (2015) 35(7):3979–85 Available at: https://ar.iiarjournals.org/content/anticanres/35/7/3979.full.pdf.
10. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall Survival With Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *New Engl J Med* (2019) 382:41–50. doi: 10.1056/NEJMoa1913662
11. Wu YL, Cheng Y, Zhou XD, Lee KH, Nakagawa K, Niho S, et al. Dacomitinib Versus Gefitinib as First-Line Treatment for Patients With EGFR-Mutation-Positive Non-Small-Cell Lung Cancer (ARCHER 1050): A Randomised, Open-Label, Phase 3 Trial. *Lancet Oncol* (2017) 18(11):1454–66. doi: 10.1016/S1470-2045(17)30608-3
12. Cole BF, Gelber RD, Anderson KM. Parametric Approaches to Quality-Adjusted Survival Analysis. International Breast Cancer Study Group. *Biometrics* (1994) 50(3):621–31. doi: 10.2307/2532777
13. Goldhirsch A, Gelber RD, Simes RJ, Glasziou P, Coates AS. Costs and Benefits of Adjuvant Therapy in Breast Cancer: A Quality-Adjusted Survival Analysis. *J Clin Oncol* (1989) 7:36–44. doi: 10.1200/JCO.1989.7.1.36
14. Glasziou PP, Cole BF, Gelber RD, Hilden J, Simes RJ. Quality Adjusted Survival Analysis With Repeated Quality of Life Measures. *Stat Med* (1998) 17:1215–29. doi: 10.1002/(SICI)1097-0258(19980615)17:11<1215::AID-SIM844>3.0.CO;2-Y
15. Glasziou PP, Simes RJ, Gelber RD. Quality Adjusted Survival Analysis. *Stat Med* (1990) 9:1259–76. doi: 10.1002/sim.4780091106
16. *NICE DSU Technical Support Document 14. Undertaking Survival Analysis for Economic Evaluations Alongside Clinical Trials - Extrapolation With Patient-Level Data* (2011). Available at: http://www.nicedsu.org.uk.
17. Williams C, Lewsey JD, Mackay DF, Briggs AH. Estimation of Survival Probabilities for Use in Cost-Effectiveness Analyses: A Comparison of a Multi-State Modeling Survival Analysis Approach With Partitioned Survival and Markov Decision-Analytic Modeling. *Med Decis Making* (2017) 37:427–39. doi: 10.1177/0272989X16670617
18. Pharmaceutical Benefits Advisory Committee Australia. *Public Summary Pharmaceutical Benefits Advisory Committe (PBAC) Meeting. Osimertinib* (2018). Available at: https://www.health.gov.au/sites/default/files/document-pbac-meeting-for-medicines-relating-to-astrazeneca.pdf.
19. Krawczyk P, Kowalski DM, Ramlau R, et al. Comparison of the Effectiveness of Erlotinib, Gefitinib, and Afatinib for Treatment of Non-Small Cell Lung Cancer in Patients With Common and Rare EGFR Gene Mutations. *Oncol Lett* (2017) 13:4433–44. doi: 10.3892/ol.2017.5980
20. *National Bureau of Statistics of China*. National Data 2018 (2018).
21. Gold MR, Siegel JE, Russell LB, Weinstein MC. *Cost-Effectiveness in Health and Medicine*. New York: Oxford University Press (1995).
22. Pignata M, Luciani L, McConnachie C, Chouaid C, Roze S, Lay KLE. Cost-Effectiveness of Afatinib Versus Erlotinib for the Treatment of Squamous Non-Small Cell Lung Cancer in France After a First-Line Platinum Based Therapy. *Eur Respir J* (2017) 50. doi: 10.1183/1393003.congress-2017.PA2795
23. Park K, Tan EH, O'Byrne K, Zhang L, Boyer M, Mok T, et al. Afatinib Versus Gefitinib as First-Line Treatment of Patients With EGFR Mutation-Positive Non-Small-Cell Lung Cancer (LUX-Lung 7): A Phase 2B, Open-Label, Randomised Controlled Trial. *Lancet Oncol* (2016) 17:577–89. doi: 10.1016/S1470-2045(16)30033-X
24. Tan PT, Aziz MIA, Pearce F, Lim WT, Wu DB, Ng K. Cost Effectiveness Analysis of Afatinib Versus Pemetrexed-Cisplatin for First-Line Treatment of Locally Advanced or Metastatic EGFR Mutation Positive Non-Small-Cell Lung Cancer From the Singapore Healthcare Payer's Perspective. *BMC Cancer* (2018) 18:352. doi: 10.1186/s12885-018-4223-y
25. Pignata M, Le Lay K, Luciani L, McConnachie C, Gordon J, Chouaid C, et al. Cost-Effectiveness of Afatinib Versus Erlotinib for the Treatment of Squamous Non-Small Cell Lung Cancer in France After a First-Line Platinum Based Therapy. *Ann Oncol* (2017) 28. doi: 10.1183/1393003.congress-2017.PA2795
26. Lu S, Ye M, Ding L, Tan F, Fu J, Wu B. Cost-Effectiveness of Gefitinib, Icotinib, and Pemetrexed-Based Chemotherapy as First-Line Treatments for Advanced Non-Small Cell Lung Cancer in China. *Oncotarget* (2017) 8:9996–10006. doi: 10.18632/oncotarget.14310
27. Li X, Li W, Hou L. A Trial-Based Cost-Effectiveness Analysis of Bevacizumab and Chemotherapy Versus Chemotherapy Alone for Advanced Nonsquamous Non–Small-Cell Lung Cancer in China. *Value Health Regional Issues* (2019) 18:1–7. doi: 10.1016/j.vhri.2018.05.001
28. Peng Z, Hou X, Huang Y, Xie T, Hua X. Cost-Effectiveness Analysis of Fruquintinib for Metastatic Colorectal Cancer Third-Line Treatment in China. *BMC Cancer* (2020) 20:990. doi: 10.1186/s12885-020-07486-w
29. Liang H, Song X, Zhang Y, Zhang S, Li F, Fang J, et al. Real-World Data on EGFR/ALK Gene Status and First-Line Targeted Therapy Rate in Newly Diagnosed Advanced Non-Small Cell Lung Cancer Patients in Northern China: A Prospective Observational Study. *Thorac Cancer* (2019) 10:1521–32. doi: 10.1111/1759-7714.13090
30. Soo RA, Loh M, Mok TS, Ou SH, Cho BC, Yeo WL, et al. Ethnic Differences in Survival Outcome in Patients With Advanced Stage Non-Small Cell Lung Cancer: Results of a Meta-Analysis of Randomized Controlled Trials. *J Thorac Oncol* (2011) 6:1030–8. doi: 10.1097/JTO.0b013e3182199c03

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An updated network meta-analysis of EGFR-TKIs and combination therapy in the first-line treatment of advanced EGFR mutation positive non-small cell lung cancer

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Objectives: Tyrosine kinase inhibitors (TKIs) are a standard care option in patients with non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) mutation. TKI-based combination treatment modes show encouraging outcomes. However, it remains unknown which is the optimal treatment as the first-line regimen for these patients on overall survival (OS).

Materials and methods: Randomized controlled trials and meeting abstracts that investigated EGFR-TKIs alone or in combination as front-line care for patients with NSCLC were systematically searched in relevant databases and reviewed. Fixed and random effects network meta-analysis models were used to estimate progression-free survival (PFS), OS, overall response rate, and grade three and higher adverse events (AEs). Surface under the cumulative ranking curves (SUCRAs) were used to rank treatment effects.

Results: Eighteen studies covering six treatments and involving a total of 4389 patients were included in this network meta-analysis. On OS, the top three treatment were first-generation EGFR-TKIs (1G EGFR-TKIs) plus chemotherapy (SUCRA, 88.1%), osimertinib (SUCRA, 65.8%) and second-generation EGFR-TKIs (2GEGFR-TKIs) (SUCRA, 63.3%). On PFS, the top three treatments were osimertinib (SUCRA, 96.0%), 1G EGFR-TKIs plus chemotherapy (SUCRA, 67.1%), and 1G EGFR-TKIs plus antiangiogenesis (SUCRA, 48.2%). Two types of TKI-

based combination therapy have significantly higher risk of grade three and higher AEs than TKI alone.

Conclusion: 1G EGFR-TKIs plus chemotherapy and osimertinib seem to be the two better options as first-line care in advanced NSCLC patients with EGFR-mutation. Osimertinib caused the lowest incidence of AEs. However, TKIs-based combination therapy significantly increased AEs.

KEYWORDS

non-small-cell Lung cancer, epidermal growth factor receptor tyrosine kinase inhibitors, EGFR-TKIs, anti-angiogenesis, first line, overall survival, network meta-analysis

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide (1). Non-small-cell lung cancer (NSCLC) accounts for nearly 85% of all lung cancer cases. Most patients with NSCLC are diagnosed at an advanced stage and have a poor prognosis (2). With the development of new drugs and novel therapeutic strategies, patients with NSCLC harboring epidermal growth factor receptor (EGFR) mutations have prolonged survival and improved prognosis. Since 2004, several important trials have established EGFR tyrosine kinase inhibitor (TKI) therapy as the standard first-line care for patients with EGFR mutations (3–5). First-generation EFGR-TKIs, including gefitinib and erlotinib, improved progression-free survival (PFS) to 9–13.7 months (3–5). Compared with the first-generation EGFR-TKIs, second- and third-generation drugs prolong PFS to 11.0 months (afatinib), 14.7 months (dacomitinib), and 18.9 months (osimertinib), which is significantly better than platinum-based chemotherapy (6–8). Unfortunately, patients with EGFR mutations inevitably develop progression as a result of acquired resistance (3–8), especially among patients with the L858R mutation, who develop resistance earlier than patients with exon 19 deletion.

In order to improve survival, combination therapy strategies are considered and emerging with promising results. The JO25567 trial (JapicCT-111390) identified that the addition of bevacizumab to erlotinib demonstrates significant clinical benefit in improving PFS(16.0 vs. 9.7 months, HR 0.54, 96% CI 0.36–0.79) (9). Similarly, the NEJ009 study (UMIN000006340) shows that concurrent combined treatment of gefitinib and chemotherapy significantly extends both PFS (20.9 vs. 11.9 months, HR 0.49, 95% CI 0.39–0.62) and overall survival (OS) (50.9 vs. 38.8months, HR 0.72, 95% CI 0.55–0.95) compared with EGFR-TKI monotherapy (10). Studies exploring EGFR-TKIs plus the anti-PD-1/PD-L1 antibody in the treatment of EGFR-mutation positive NSCLC are on the way (TATTON, NCT02143466).

Currently, there is a diverse array of treatment strategies under development for metastatic NSCLC (mNSCLC) with sensitizing EGFR mutation. The National Comprehensive Cancer Network and European Society for Medical Oncology (ESMO) guidelines recommend first line osimertinib as the preferred option and other treatment strategies as alternative candidates (11, 12). Also, several previous network meta-analyses compared these multiple treatments in terms of PFS, and the results showed a favorable efficacy of osimertinib compared with other EGFR-TKIs and combination treatments in PFS. As a result, osimertinib is indicated as a preferable option as up-front therapy in patients with activating EGFR mutation mNSCLC (13–15). However, it still remains unclear which treatment showed favorable efficacy in OS and how patients can benefit the most. As the maturity of OS from relevant clinical studies, it is necessary to make a comparison in terms of OS among these available candidates to guide clinicians. This review also aims to develop personalized treatment plans for each patient with activating EGFR mutation NSCLC in an advanced stage by subgroup analysis and provide some valuable clues to guide further studies.

Abbreviations: NSCLC, non-small cell lung cancer; RCTs, randomizesponded controlled trials; OS, overall survival; PFS, progression-free survival; ORR, objective response rate; AEs, adverse effects; HR, hazard ratio; RR, relative risk; CI, confidence intervals; FDA, Food and Drug Administration; VEGF, vascular endothelial growth factor; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement; ECOG, Eastern Cooperative Oncology Group; NCCN, National Comprehensive Cancer Network; ESMO, European Society for Medical Oncology; WCLC, The World Conference on Lung Cancer; SUCRAs, Surface under the cumulative ranking curves; 1G EGFR-TKI, first-generation EGFR-TKI; 2G EGFR-TKI, second-generation EGFR-TKI; 1L, first line; 2L, second line.

Materials and methods

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines and extension for network meta-analysis (16) were strictly followed in this study.

Literature search strategy

In this network meta-analysis, two authors independently searched PubMed, Web of Science, Embase, MEDLINE, the Cochrane Central Register of Controlled Trials, ClinicalTrial.gov, and the Chinese Biomedical Literature Database (in Chinese) for all studies published before December 31, 2021. The terms used for the search included "non-small-cell lung cancer, NSCLC, erlotinib, gefitinib, icotinib, afatinib, dacomitinib, osimertinib, epidermal growth factor receptor tyrosine kinase inhibitors, EGFR-TKI, anti-angiogenic drugs, bevacizumab, ramucirumab, vascular endothelial growth factor receptor (VEGFR) inhibitors, apatinib and chemotherapy" as well as their synonyms and variations. The full literature search strategy.

In addition, the abstracts from annual meetings and meetings related to lung cancer of the American Society of Clinical Oncology, ESMO, and The World Conference on Lung Cancer were reviewed to identify related studies.

Study inclusion and exclusion criteria

The inclusion criteria were as follows:

(1). Patients: Patients aged 18 years or older and who were histologically or cytologically confirmed as having NSCLC with clinical stage IIIb or IV harboring EGFR mutation. Patients had no prior antitumor treatment (chemotherapy, radiotherapy, and surgery).

(2). Intervention: 2G EGFR-TKIs (afatinib or dacomitinb) or third-generation EGFR-TKIs (osimertinib) or 1G EGFR-TKIs (erlotinib, gefitinib, and icotinib) plus bevacizumab or ramucirumab or apatinib or plus chemotherapy.

(3). Comparison: the 1G EGFR-TKIs (erlotinib, gefitinib, and icotinib).

(4). Outcome: PFS, OS, objective response rate (ORR), and incidence of adverse events (AEs).

(5). Study design: high-quality randomized controlled trials (RCTs).

Duplication information, animal experimental studies, single-arm clinical trials, retrospective clinical analysis, case reports, and review commentaries were excluded.

Data extraction and quality assessments

Two reviewers independently assessed each RCT according to the predetermined criteria, and a third reviewer was consulted if there were some disagreements. The same two reviewers independently extracted the data from the selected studies using a standardized data extraction method, including study name, publication year, author information, trial phase, study design, sample size, intervention, primary end points, participant characteristics, response rate, median PFS, median OS, and number of patients who suffered grade three and higher AEs. Hazard ratios (HRs) and 95% confidence intervals (CIs) were directly extracted from qualified trials.

The Cochrane Collaboration Tool was adopted to assess the risk of bias for each RCT, and it is based on various kinds of bias from the following five domains: randomization sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases (17). The quantitative Jadad scale was used to assess study quality (18).

Statistical analysis

All data analysis is based on the intention-to-treatment principle. The primary outcomes of interest were PFS, OS, ORR, and AEs. For time-to-events variables, PFS and OS were synthesized by HR with corresponding 95% CIs. For dichotomous variables, ORR and AEs were measured by relative risks (RRs) with 95% CIs. A two-tailed P value of less than 0.05 was considered statistically significant.

Heterogeneity across studies was evaluated by the Cochran Q total statistic and the inconsistency index (I^2 statistic) (19). If $I^2 > 50\%$ or the P value for the Q test < 0.1 indicated significant heterogeneity (20), a random effects model was applied to synthesize the available evidence; otherwise, a fixed effects model was used. A sensitivity analysis was also performed to investigate the influence of each single study on the overall estimate size by omitting each one by one if there was significant heterogeneity.

A Bayesian network meta-analysis was performed for all outcome measures in R software (R v4.1.2., https://www.r-project.org) using the package "gemtc" (v1.0-1, https://cran.r-project.org/web/packages/gemtc/index.html), which calls upon JAGS software (v4.3.0., https://mcmc-jags.sourceforge.io) using the rjags package (v4-12, https://cran.r-project.org/web/packages/rjags/index.html) for Markov chain Monte Carlo methods. Cox proportional HRs and their corresponding CIs were used as the summary estimates of relative treatment effects. Log HRs and their corresponding standard errors were used as inputs in the fixed-effect models, which were run with four chains, at least 5000 burns-ins, and 10,000 inferential iterations

per chain to ensure model convergence. All analyses were replicated in WinBUGS software (version 1.4.3) for comparative validation in R software in order to double-check the results.

Rank probabilities for each treatment were also produced on Bayesian NMA by calculating the probability of each treatment that could achieve the best rank among the included treatments (21). Surface under the cumulative ranking curves (SUCRAs) were calculated to rank probabilities of all treatments in R software (R v4.1.2.). Each statistical test was considered two-sided.

Results

Search results and study selection

As shown in Figure 1, after reviewing abstracts and titles, 80 potentially eligible studies were assessed carefully by full-text review. Among them, 62 studies were excluded for the following reasons: 15 studies lacked outcomes of interest, 14 studies were just trial protocols (study designs) without study results, 14 studies referred to second-line treatments; 10 studies were single-arm studies, five trials included patients without selecting EGFR mutation, and four trials failed to extract data. Finally, 18 RCTs involving 4389 participates were considered to meet the inclusion criteria and included in the network meta-analysis to compare five treatments, including the 1G EGFR-TKIs, 2G EGFR-TKIs, third-generation EGFR-TKIs (3G-EGFR-TKIs), and the 1G EGFR-TKIs plus chemotherapy or plus antiangiogenic drugs (6–10, 22–39). Among these 18 trials, 17 were reported as publications (6–10, 22–39), and some data of interest in one study was extracted from a meeting abstract (10). Two RCTs compared afatinib or dacomitinib with gefitinib, respectively (7, 8, 36, 37), one RCT compared osimertinib with erlotinib or gefitinib (6, 38), eight RCTs compared erlotinib or gefitinib plus chemotherapy with erlotinib or gefitinib alone (10, 29–35). Six RCTs compared erlotinib plus bevacizumab or ramucirumab with erlotinib alone (9, 22–27). One RCT compared gefitinib plus apatinib, a VEGFR 2 TKI, with gefitinib alone (28). One RCT compared high-dose icotinib with routine-dose icotinib in patients with the L858R mutation (39).

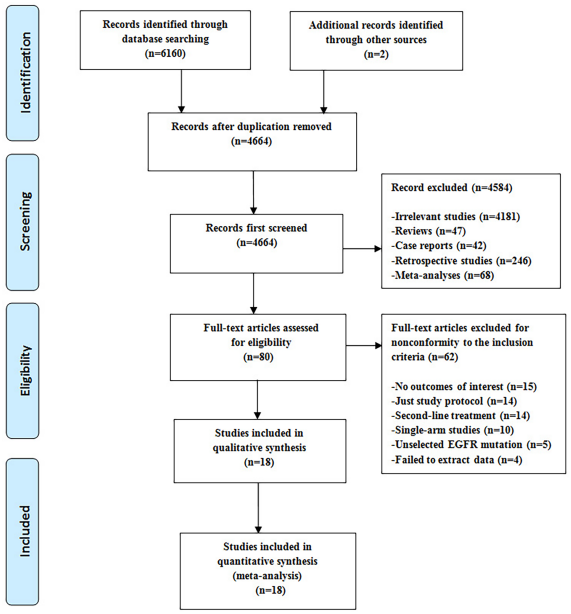

FIGURE 1
Search strategy and follow chat of the included studies.

Population characteristics

In each trial, the demographic characteristic of participates were generally well-balanced between different trial arms, within each trial, and across trials. The sample size of included studies ranged from 50 to 556. The basic characteristics of the included 18 RCTs are summarized in Table 1. Median age ranged from 55 to 67.5 years. Most of the patients were in stage IIIb and IV of the disease. Exon 19 deletion and exon 21-L858R were mainly EGFR mutations. The majority of the included trials in two combination treatment divisions and the INCREASE trial were conducted in Asia (9, 10, 22–24, 26, 30–35, 39). A graphic network structure shows the network of trials for PFS and OS (Figure 2). Each circle node represents a special type of treatment. Direct comparisons are represented by the black lines connecting treatments. The width of lines is proportionate to the number of studies that perform head-to-head comparisons in the same study (40) (Figure 2).

Quality assessment and publication bias

All 18 included trials were judged to have low risk of bias through using the risk of bias tool described in the Cochrane Handbook for Systematic Reviews of Interventions (17). All included trials generated an adequate randomization sequence without observable allocation concealment and selective outcome reporting.

Overall survival

There were 15 trials contributing to network meta-analysis for OS. The 2G EGFR-TKIs (HR 0.81, 95%CI 0.67–0.98), 1G EGFR-TKIs plus chemotherapy (HR 0.73, 95%CI 0.63–0.85), and osimertinib (HR 0.80, 95%CI 0.64–1.00) were all more effective in comparison with 1G EGFR-TKIs in improving OS except 1G EGFR-TKIs plus anti-VEGF drugs (HR 0.95, 95%CI

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TABLE 1 Characteristics of the included randomized trials in the meta-analysis.

Study	Region	Phase	Treatment	Sample size (no.)	No. Of EGFR mutation		Efficacy			Grade≥3 AEs (%)
					ex19del	L858R	ORR (%)	PFS (months)	OS (months)	
JO25567 (9, 22)	Japan	II	Erlotinib+bevacizumab	75	40	35	69	16.0	47.0	91
(JapicCTI-111390)	multicenter		Erlotinib	77	40	37	64	9.7	47.4	53
NEJ026 (23, 24)	Japan	III	Erlotinib+bevacizumab	112	56	56	81	16.9	NA	98
(*UMIN000017069)*	multicenter		Erlotinib	112	55	57	74	13.3	NA	46
Stinchcombe et al (25)	USA	II	Erlotinib+bevacizumab	43	29	14	81	17.9	32.4	NR
(NCT01532089)	multicenter		Erlotinib	45	30	15	83	13.5	50.6	NR
CTONG1509 (26)	China	III	Erlotinib+bevacizumab	157	82	75	86.3	18.0	NR	53.5
(NCT02759614)	multicenter		Erlotinib	154	79	75	87.4	11.3	NR	25.5
RELAY (27)	worldwide	III	Erlotinib+ramucirumab	224	123	99	76	19.4	NR	72
(NCT02411448)	multicenter		Erlotinib+placebo	225	120	105	75	12.4	NR	54
CTONG1706 (28)	China	III	Gefitinib+Apatinib	157	81	74	77.1	13.7	NR	84.1
(NCT02824458)	multicenter		Gefitinib+placebo	156	83	73	73.7	10.2	NR	37.7
CALGB30406 (29)	USA	II	Paclitaxel+carboplatin +erlotinib	33	16	17	73	17.2	38.1	NA
(NCT00126581)			Erlotinib	33	23	10	70	14.1	31.3	NA
Yang et al. (30)	East Asia	III	Pemetrexed+cisplatin +gefitinib	26	14	10	65.4	12.9	32.4	34
(NCT01017874)			Gefitinib	24	11	13	70.8	16.6	45.7	16
Cheng et al. (31)	East Asia	II	Pemetrexed+gefitinib	126	65	52	80.2	15.8	43.4	53
(NCT01469000)			Gefitinib	65	40	23	73.8	10.9	36.8	12
An et al. (32)	China	II	Pemetrexed+gefitinib	45	16	29	80.0	18.0	34.0	NR
			Gefitinib	45	17	28	73.3	14.0	32.0	NR
Han et al. (33)	China	II	Pemetrexed+carboplatin +gefitinib	40	21	19	82.5	17.5	32.6	NR
(NCT02148380)			Gefitinib	41	21	20	65.9	11.9	25.8	NR
Noronha (34)	India	III	Pemetrexed+carboplatin +gefitinib	174	107	60	84	20.9	50.9	65.3
(CTRI/2016/08/ 007149)			Gefitinib	176	109	60	67	11.9	38.8	31.0
NEJ009 (10)	Japan	III	Pemetrexed+carboplatin +gefitinib	170	93	69	75.3	20.9	50.9	75
(UMIN000006340)			Gefitinib	172	95	67	68.3	11.9	38.8	49.4
Xu et al. (35)	China	II	Pemetrexed+carboplatin +icotinib	90	51	38	77.8	16.0	36.0	NR
(NCT02031601)			Icotinib	89	52	37	64.0	10.0	34.0	NR
LUX-Lung7 (7, 36)	Worldwide	II	Afatinib	160	93	67	70.0	11.0	27.9	31.0
(NCT01024413)	multicenter		Gefitinib	159	93	66	56.0	10.9	24.5	18.0
ARCHER1050 (8, 37)	Japan, Korea	III	Dacomitinib	227	134	93	75.0	14.7	34.1	63
(NCT01774721)	multicenter		Gefitinib	225	133	92	72.0	9.2	26.8	41
FLAURA (6, 38)	Worldwide	III	Osimertinib	279	158	97	80.0	18.9	38.6	32.0
(NCT02296125)	multicenter		Gefitinib/erlotinib	277	155	90	76.0	10.2	31.8	41.0
INCREASE (39)	China	II	Icotinib high dose	90	0	90	73.0	12.9	6.67	NR
(NCT02404675)	multicenter		Icotinib routine dose	86	0	86	48.0	9.2	8.20	NR

NA, not available; Outcomes: progression-free survival (PFS); objective response rate (ORR); adverse events (AEs); overall survival (OS). NR, not reach.

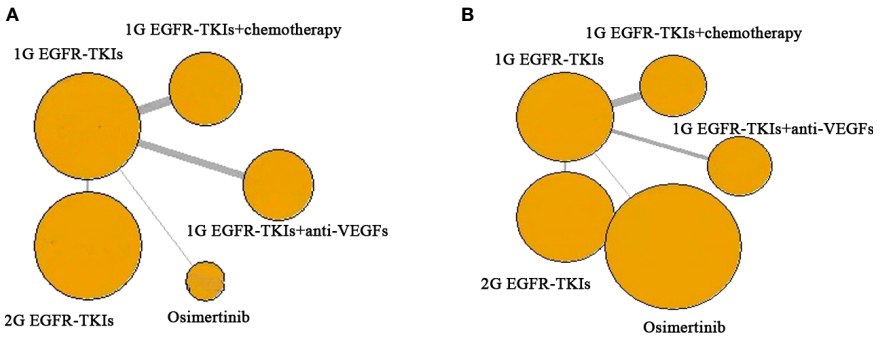

FIGURE 2
Network of the comparisons for the network meta-analysis. **(A)** PFS; **(B)** OS. Each circular node represents a type of treatment. The circle size is proportional to the total number of studies. The width of lines is proportional to the number of studies performing a head-to-head comparison in the same study. Abbreviations: First-generation EGFR-TKIs (1G EGFR-TKIs); Second-generation EGFR-TKIs (2G EGFR-TKIs); anti-vascular endothelial growth factors drugs (anti-VEGFs).

0.78–1.20). Osimertinib was not clearly superior to 2G EGFR-TKIs (HR 0.99, 95%CI 0.74–1.30), 1G EGFR-TKIs plus anti-VEGF drugs (HR 0.84, 95%CI 0.63–1.10) or plus chemotherapy (HR 1.10, 95%CI 0.84–1.40). According to SUCRAs, the rank probability of OS was as follows: 1G EGFR-TKIs plus chemotherapy (88.1%) > osimertinib (65.8%) > 2G EGFR-TKIs (63.3%) > 1G EGFR-TKIs plus anti-VEGF agents (24.5%) > 1G EGFR-TKIs (8.3%).

There were nine trials that reported OS and corresponding HRs in patients with specific mutations. For patients with the ex19del mutation, osimertinib (HR 0.80, 95%CI 0.64–1.00), 2G EGFR-TKIs (HR 0.81, 95%CI 0.67–0.98), and 1G EGFR-TKIs plus chemotherapy (HR 0.73, 95%CI 0.63–0.85) were all more effective in comparison with 1G EGFR-TKIs monotherapy in improving OS. Osimertinib was not clearly superior to 2G EGFR-TKIs (HR 0.99, 95%CI 0.74–1.30), 1G EGFR-TKIs plus anti-VEGF agents (HR 0.84, 95%CI 0.63–1.10), or plus chemotherapy (HR 1.10, 95%CI 0.84–1.40). 1G EGFR-TKIs plus anti-VEGF agents did not improve OS (HR 0.95, 95%CI 0.78–1.20) compared with 1G EGFR-TKIs. According to SUCRAs, 1G EGFR-TKIs plus chemotherapy (86.7%), osimertinib (80.7%), and 2G EGFR-TKIs (47.5%) were the top three treatments in terms of OS for patients with the ex19del mutation (Figure 3 and Table 2). For patients with the L858R mutation, 13 trials with five treatments reported OS and contributed to the meta-analysis of OS. Only 1G EGFR-TKIs plus chemotherapy tended to improve OS (HR 0.71, 95%CI 0.50–1.00) in comparison with 1G EGFR-TKIs. However, osimertinib was not clearly superior to 2G EGFR-TKIs (HR 1.20, 95%CI 0.80–1.90), 1G EGFR-TKIs plus anti-VEGF agents (HR 1.10, 95%CI 0.69–1.90), and 1G EGFR-TKIs plus chemotherapy (HR 1.40, 95%CI 0.87–2.30). According to SUCRAs, 1G EGFR-TKIs plus chemotherapy (84.6%), 2G EGFR-TKIs (67.9%), and 1G EGFR-TKIs plus anti-VEGF agents (50.5%) were the top three treatments in terms of OS for patients with the L858R mutation (Figure 3 and Table 2).

Exploration of OS in potential subgroups of interest (based on the existence of CNS metastasis, gender, and ECOG PS) are calculated but that of other interests (based on age, ethnicity, and smoking status) was not feasible due to inconsistent reporting of group data across the trials. In subgroup analysis, two combination treatments, 1G EGFR-TKIs plus chemotherapy (HR 0.57, 95%CI 0.36–0.9, SUCRA 85.6%) and plus antiangiogenic drugs (HR 0.62, 95%CI 0.38–1.00, SUCRA 77.9%) showed a significant improvement of OS in patients with CNS metastasis compared with 1G EGFR-TKIs alone. They were ranked the top two treatments for patients with brain metastasis. Better efficacy of osimertinib was observed in the female group (HR 0.79, 95%CI 0.60–1.04, SUCRA 73.4%) as well as 1G EGFR-TKIs plus chemotherapy (HR 0.66, 95%CI 0.44–0.99, SUCRA 75.9%) and osimertinib (HR 0.70, 95%CI 0.54–0.91, SUCRA 69.3%) in the ECOG PS 1 group.

Progress-free survival

There were 18 trials contributing to the network meta-analysis for PFS analysis. As shown in Figure 4 and Table 3, comparing the five treatments, osimertinib (HR 0.43, 95%CI 0.29–0.64), 2G EGFR-TKIs (HR 0.64, 95%CI 0.48–0.86), 1G EGFR-TKIs plus anti-VEGF agents (HR 0.62, 95%CI 0.49–0.77), and 1G EGFR-TKIs plus chemotherapy (HR 0.55, 95%CI 0.44–0.69) were all more effective in comparison with 1G EGFR-TKI monotherapy in improving PFS. Osimertinib was clearly superior to 2G EGFR-TKIs (HR 0.71, 95%CI 0.54–0.93) and 1G EGFR plus anti-VEGF agents (HR 0.75, 95%CI 0.53–1.00), but it was not more effective than 1G EGFR-TKIs plus chemotherapy (HR 0.81, 95%CI 0.57–1.10). According the

TABLE 2 Results of network meta-analysis for PFS and OS.

a. Hazard ratios (HR) with 95% confidence interval (CI) for progress-free survival (PFS) in patients with ex19del.

Osimertinib				
0.67 (0.41, 1.10)	**2G-TKIs**			
0.70 (0.45, 1.10)	1.00 (0.72, 1.50)	**1G-TKIs+anti-VEGFs**		
0.78 (0.49, 1.20)	1.20 (0.80, 1.70)	1.10 (0.81, 1.50)	**1G-TKIs+CT**	
0.43 (0.29, 0.64)	**0.64 (0.48, 0.86)**	**0.62 (0.49, 0.77)**	**0.55 (0.44, 0.69)**	**1G-TKIs**

b. Hazard ratios(HR) with 95% confidence interval(CI) for overall survival (OS) in patients with ex19del.

Osimertinib				
0.99(0.74, 1.30)	**2G-TKIs**			
0.84(0.63, 1.10)	0.85(0.65, 1.10)	**1G-TKIs+anti-VEGFs**		
1.10(0.84, 1.40)	1.10(0.87, 1.40)	1.30(1.00, 1.70)	**1G-TKIs+CT**	
0.80(0.64, 1.00)	**0.81(0.67, 0.98)**	0.95(0.78, 1.20)	**0.73(0.63, 0.85)**	**1G-TKIs**

c.Hazard ratios (HR) with 95% confidence interval(CI) for progress-free survival(PFS) in patients with L858R.

Osimertinib					
0.77(0.50, 1.20)	**2G-TKIs**				
0.80(0.55, 1.20)	1.00(0.76, 1.40)	**1G-TKIs+anti-VEGFs**			
0.97(0.65, 1.50)	1.30(0.90, 1.80)	1.20(0.92, 1.60)	**1G-TKIs+CT**		
0.68(0.42, 1.10)	0.89(0.58, 1.40)	0.85(0.58, 1.20)	**0.70(0.47, 1.00)**	**High 1G-TKIs**	
0.51(0.36, 0.72)	**0.66(0.51, 0.86)**	**0.64(0.54, 0.76)**	**0.53(0.42, 0.65)**	0.75(0.53,1.10)	**1G -TKIs**

d.Hazard ratios (HR) with 95% confidence interval(CI) for overall survival (OS) in patients with L858R.

Osimertinib					
1.20(0.80, 1.90)	**2G-TKIs**				
1.10(0.69, 1.90)	0.92(0.58, 1.40)	**1G-TKIs+anti-VEGFs**			
1.40(0.87, 2.30)	1.10(0.72, 1.80)	1.20(0.74, 2.00)	**1G-TKIs+CT**		
1.00(0.71, 1.40)	0.80(0.61, 1.10)	0.88(0.61, 1.30)	**0.71(0.50, 1.00)**	**1G -TKIs**	

OS, overall survival; PFS, progression-free survival; ORR, objective response rate. First-generation EGFR-TKIs (1G-TKIs); Second-generation EGFR-TKIs (2G-TKIs); anti-vascular endothelial growth factor (anti-VEGF), Chemotherapy (CT). Significant hazard ratios are in bold.

SUCRAs, the rank probability of PFS was as follows: osimertinib (96.0%) > 1G EGFR-TKIs plus chemotherapy (67.1%) > 1G EGFR-TKIs plus anti-VEGF agents (48.2%) > 2G EGFR-TKIs (38.7%) > 1G EGFR-TKIs (0.03%).

There were 13 trials that reported HRs in patients with specific mutations, 2284 (52.0%) patients had an ex19del mutation, and 1892 (39.8%) had an L858R mutation. For patients with the ex19del mutation, osimertinib (HR 0.43, 95% CI 0.29–0.64), 2G EGFR-TKIs (HR 0.64, 95%CI 0.48–0.86), 1G EGFR-TKIs plus anti-VEGF agents (HR 0.62, 95%CI 0.49–0.77), and 1G EGFR-TKIs plus chemotherapy (HR 0.55, 95%CI 0.44–0.69) were all more effective in comparison with 1G EGFR-TKI monotherapy in improving PFS. Osimertinib was not clearly superior to 2G EGFR-TKIs (HR 0.67, 95%CI 0.41–1.10), 1G EGFR-TKIs plus anti-VEGF agents (HR 0.70, 95%CI 0.45–1.10), or 1G EGFR-TKIs plus chemotherapy (HR 0.78, 95%CI 0.49–1.20). According to SUCRAs, the top three treatments were osimertinib (94.2%), 1G EGFR-TKIs plus chemotherapy (67.6%), and the 1G EGFR-TKIs plus anti-VEGF agents (46.8%) in terms of PFS. For patients with the L858R mutation, in addition to the above 13 trials, there was a special treatment reported by a trial for patients with the L858R mutation, which increased the dose of incotinib, a kind of first-generation EGFR-TKI, to improve the efficacy. All 14 trials with six treatments were included in the network meta-analysis for PFS analysis. Osimertinib (HR 0.51, 95%CI 0.36–0.72), 2G EGFR-TKIs (HR 0.66, 95%CI 0.51–0.86), 1G EGFR-TKIs plus anti-VEGF agents (HR 0.64, 95%CI 0.54–0.76), and 1G EGFR-TKIs plus chemotherapy (HR 0.53, 95%CI 0.42–0.65) were all more effective in comparison with 1G EGFR-TKI monotherapy with routine dosage in improving PFS. No treatment was clearly superior to others among the four treatments. However, a high dose of 1G EGFR-TKIs (HR 0.75, 95%CI 0.53–1.10) was not more effective than the normal dose of 1G EGFR-TKIs. According to the SUCRAs, osimertinib (85.3%), 1G EGFR-TKIs plus chemotherapy (84.7%), and 1G EGFR-TKIs plus anti-VEGF agents (52.3%) were the top three in terms of PFS (Figure 3 and Table 2).

Objective response rate

For network meta-analysis of ORR, there were 17 trials that covered five treatments included. As shown in Figure 4 and Table 3, 1G EGFR-TKIs plus chemotherapy was considered the highest probability of being the best treatment to achieve a

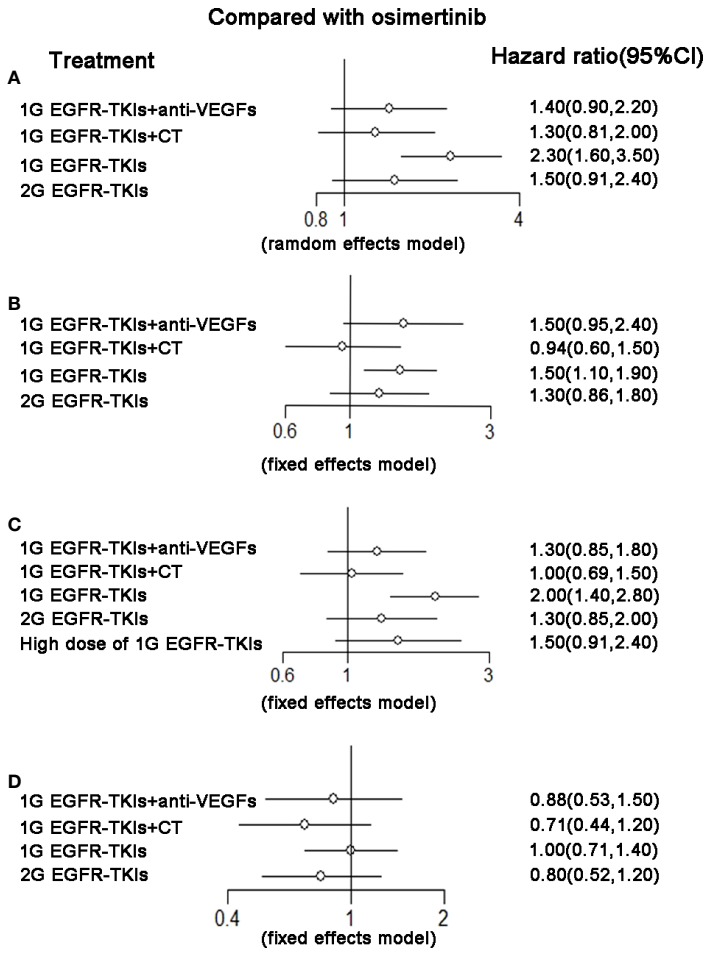

FIGURE 3
(A) PFS, forest plot of hazard ratio (HRs) for progression-free survival; **(B)** OS, forest plot of hazard ratio (HRs) for overall survival;**(C)** ORR, forest plot of hazard ratio (HRs) for objective response rate; **(D)** SAE, forest plot of hazard ratio (HRs) for Serious Adverse Events. Results were based on fixed effects or random effects method. First-generation EGFR-TKIs (1G EGFR-TKIs); Second-generation EGFR-TKIs (2G EGFR-TKIs); anti-vascular endothelial growth factor (anti-VEGF).

response (92.3%), followed by 2G EGFR-TKIs (68.4%), osimertinib (47.3%), 1G EGFR-TKIs plus anti-VEGF drugs (33.3%) and 1G EGFR-TKIs (8.7%).

Serious Adverse Events (SAEs)

As shown in Figure 4 and Table 3, regarding grade three or worse AEs, compared with osimertinib, 1G EGFR-TKIs plus anti-VEGF drugs (HR 2.40, 95%CI 1.70–3.40) and 1G EGFR-TKIs plus chemotherapy (HR 2.50, 95%CI 1.60–4.60) led to a significantly higher risk of grade three and worse AEs. Both 1G EGFR-TKIs plus anti-VEGF drugs and 1G EGFR-TKIs plus chemotherapy have a significantly higher risk of grade three and worse AEs than 1G EGFR-TKIs alone. But there were no significant differences between these two kinds of combined therapies (HR 0.93, 95%CI 0.47–1.70). According to SUCRAs, osimertinib had the lowest risk of grade three and worse AEs and the rank probability was as follows: osimertinib (96.1%) > 1G EGFR-TKIs (76.7%) > 2G EGFR-TKIs (42.7%) > 1G EGFR-TKIs plus anti-VEGF agents (22.5%) > 1G EGFR-TKIs plus chemotherapy (12.2%).

Discussion

In patients with advanced EGFR-mutant NSCLC, EGFR-TKIs are approved as first-line options because all of them show superior efficacy and prolonged PFS compared with platinum-based chemotherapy (3–8). The second-generation TKIs (afatinib and dacomitinib) and third-generation TKIs (osimertinib) were more effective in comparison with first-

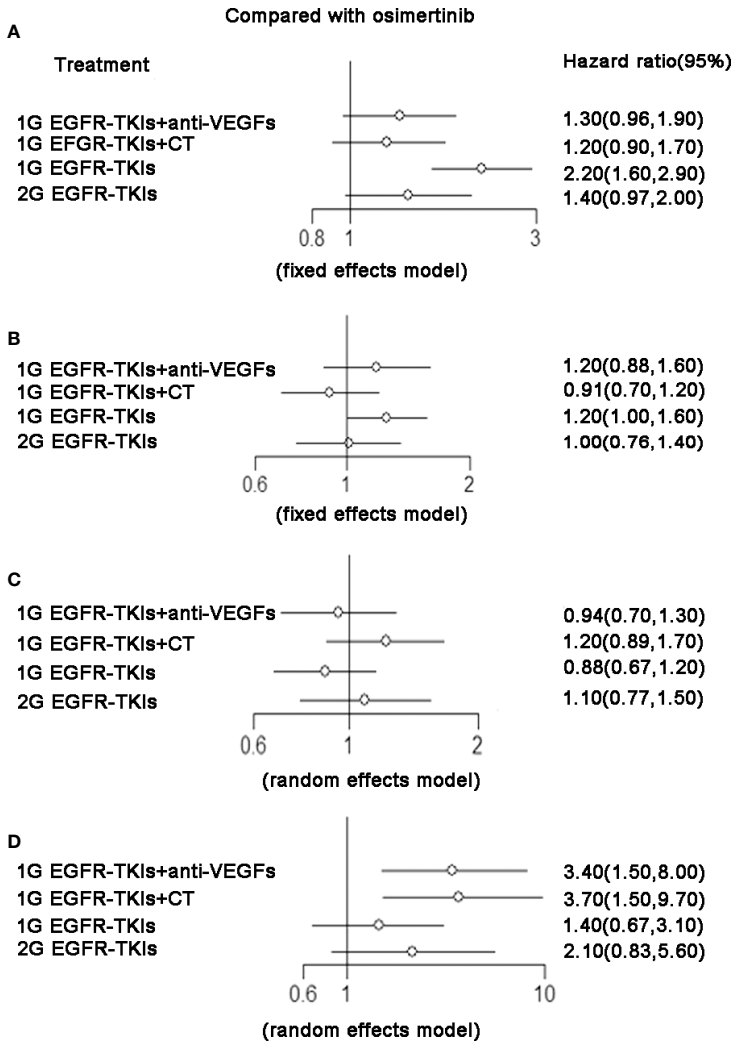

FIGURE 4
(A) Forest plot of hazard ratio (HRs) for progression-free survival (PFS) in patients with 19 deletion mutation; **(B)** Forest plot of hazard ratio (HRs) for progression-free survival (PFS) in patients with L858R mutation; **(C)** Forest plot of hazard ratio (HRs) for overall survival (OS) in patients with 19 deletion mutation; **(D)** Forest plot of hazard ratio (HRs) for overall survival (OS) in patients with L858R mutation. Results were based on fixed effects or random effects methods. First-generation EGFR-TKIs (1G EGFR-TKIs); Second-generation EGFR-TKIs (2G EGFR-TKIs); anti-vascular endothelial growth factor drugs (anti-VEGFs.).

generation TKIs at improving PFS (7–9) in the first-line setting. Survival of advanced NSCLC with activating EGFR-mutation is significantly improved due to the introduction of osimertinib. The FLAURA trial (NCT02296125) demonstrated that osimertinib significantly extended the mPFS (18.9 months) compared with the first-generation EGFR-TKIs (gefitinib or erlotinib, 10.2 months) (7). In combined treatment strategies, both the addition of chemotherapy or anti-angiogensis to 1G EGFR-TKIs demonstrate considerable clinical benefit with improved PFS (9, 10, 23–35). The precise network meta-analysis demonstrated first-line osimertinib is superior to 1G and 2G EGFR-TKIs as well as the combination of anti-VEGF agents and 1G EGFR-TKIs and ranked top in terms of PFS (13–15). The results of our study are consistent with these previous meta-analyses. In the FLAURA study (NCT02296125), first-line osimertinib also has significant OS improvement compared with 1G EGFR-TKIs, which established the foundation of osimertinib as the standard first-line care in advanced NSCLC with activating EGFR-mutations (38). The AURA3 study (NCT02151981) demonstrated that 2L osimertinib exceeded mPFS (10.1 vs. 4.4 months; HR 0.30, 95%CI 0.23–0.41) compared with chemotherapy in patients with T790M

TABLE 3 Results of network meta-analysis for PFS, OS, ORR and SAEs.

a. Hazard ratios(HR) with 95% confidence interval(CI) for progress-free survival (PFS)

Osimertinib				
0.71 (0.54, 0.93)	**2G-TKIs**			
0.75 (0.53, 1.00)	1.10 (0.80, 1.40)	**1G-TKIs+anti-VEGFs**		
0.81 (0.57, 1.10)	1.10 (0.86, 1.50)	1.10 (0.86, 1.30)	**1G-TKIs+CT**	
0.46 (0.34, 0.62)	**0.65 (0.52, 0.81)**	**0.62 (0.53, 0.72)**	**0.57 (0.49, 0.67)**	**1G-TKIs**

b. Hazard ratios(HR) with 95% confidence (CI) for overall survival (OS).

Osimertinib				
0.99 (0.74, 1.30)	**2G-TKIs**			
0.84 (0.63, 1.10)	0.85 (0.65, 1.10)	**1G-TKIs+anti-VEGFs**		
1.10 (0.84, 1.40)	1.10 (0.87, 1.41)	**1.30 (1.00, 1.70)**	**1G-TKIs+CT**	
0.80 (0.64, 1.00)	**0.81 (0.67, 0.98)**	0.95 (0.78, 1.20)	**0.73 (0.63, 0.85)**	**1G-TKIs**

c.Odds ratios(OR) with 95% confidence interval(CI) for objective response (ORR).

Osimertinib				
0.92 (0.65, 1.30)	**2G-TKIs**			
1.10 (0.78, 1.40)	1.20 (0.88, 1.50)	**1G-TKIs+anti-VEGFs**		
0.82 (0.60, 1.10)	0.89 (0.69, 1.20)	**0.78 (0.63, 0.96)**	**1G-TKIs+CT**	
1.10 (0.87, 1.50)	**1.20 (1.00, 1.50)**	1.10 (0.93, 1.20)	**1.40 (1.20, 1.60)**	**1G-TKIs**

c.Odds ratios(OR) with 95% confidence interval(CI) for serious adverse events (SAEs).

Osimertinib				
0.47 (0.18, 1.20)	**2G-TKIs**			
0.29 (0.12, 0.66)	0.63 (0.32, 1.20)	**1G-TKIs+anti-VEGFs**		
0.27 (0.10, 0.65)	0.58 (0.26, 1.20)	0.93 (0.47, 1.70)	**1G-TKIs+CT**	
0.69 (0.32, 1.50)	1.50 (0.84, 2.70)	**2.40 (1.70, 3.40)**	**2.50 (1.60, 4.60)**	**1G-TKIs**

OS, overall survival; PFS, progression-free survival; ORR, objective response rate. SAEs, serious adverse events; First-generation EGFR-TKIs (1G-TKIs); Second-generation EGFR-TKIs (2G-TKIs); anti-vascular endothelial growth factor (anti-VEGF). Significant hazard ratios are in bold

mutations followed by 1G/2G EGFR-TKIs as 1L therapy, which established osimertinib as the standard of care for patients who develop a T790M mutation after 1G/2G EGFR-TKI therapy as a first line (41). There is a concern raised as to which setting of osimertinib is most beneficial as the lL or 2L therapy. Some clinicians may worry that, if osimertinib is set in the first line, there are no targeted drugs available in the 2L treatment after osimertinib resistance. In fact, if osimertinib was reserved in 2L therapy, a portion of patients have a probability to not be tested for and found to be positive for T790M mutation and lose the opportunity to accept osimertinib therapy. Also, not all patients develop a resistance mechanism to the T790M mutation after earlier generation EGFR-TKI therapy, and some patients do not survive to accept 2L therapy. A real-world study shows that only 72% of patients were tested for the T790M mutation after 1G/2G EGFR-TKI resistance, and the remaining nearly 30% of patients were untested. About half of the tested patients were T790M-positive. Only one third of the patients received osimertinib upon progression on 1G/2G EGFR-TKIs (42). Moreover, the FLAURA trial demonstrated that a significant OS improvement with osimertinib in the 1L setting exists in spite of the fact that 47% of patients assigned to division of first line 1G/2G EGFR-TKIs received osimertinib as the second line therapy (38). Therefore, setting osimertinib as the first-line treatment seems to be more favored. Further trials need to provide more evidence to determine which line osimertinib set in is more efficient and rational. The APPLE study (NCT02856893), an ongoing phase II trial, was designed to evaluate the best strategy for sequencing gefitinib and osimertinib in patients with an EGFR mutation and EGFR TKI treatment-naive advanced NSCLC in 1L treatment, which could help to determine when osimertinib is most beneficial as 1L or 2L treatment (43).

OS is considered the gold standard for choosing the optimal therapy. As far as we are aware, this study is the first network meta-analysis to compare the mature OS of these multiple treatments. Results show the combined treatments of 1G EGFR-TKIs and chemotherapy surpassed osimertinib and was ranked the top in terms of OS in both all population and patients with CNS metastasis. It indicates that combination therapy with osimertinib and chemotherapeutic drugs seems to be a promising strategy to further improve survival and even to approach a cure. However, a randomized phase 2 clinical trial (jRCTs071180062) showed that, as a second-line therapy after initial EGFR-TKI resistance, the addition of carboplatin-pemetrexed to osimertinib failed to improve PFS (14.6 vs. 15.8 months; HR 1.09, 95%CI 0.51–2.32) and OS (HR 2.42, 95%CI

0.82–7.15) compared with standard osimertinib monotherapy (44). Outcomes of ongoing FLAURA 2 (NCT04035486), a phase 3 clinical trial, evaluate osimertinib and platinum-pemetrexed versus osimertinib in treatment-naive advanced NSCLC patients with EGFR-mutation, are eagerly awaited to assess whether this combination confers a significant survival benefit in a first line setting.

The EGFR and VEGF pathways share downstream signaling targets, and dual blockade of EGFR and angiogenic caused synergetic effects (45). Clinically, the addition of bevacizumab and remucirumab to 1G EGFR-TKIs significantly improved PFS in advanced NSCLC with EGFR mutation (10, 22–28). In a first line setting, the combination of erlotinib and bevacizumab demonstrates an improved PFS of 16.0, 16.9, and 18.0 months in JO25567 (JapicCTI-111390), NEJ026 (UMIN000017069) and CTONG1509 (NCT02759614) trials, respectively (9, 23–28). But the significant PFS benefit observed with erlotinib plus bevacizumab failed to translate into a significant OS benefit (22, 24, 26). The combination of erlotinib and ramucirumab showed a significantly improved PFS of 19.4 months in the RELAY trial, and the OS remains immature. In 2L treatment, both the WJOG 8715L (UMIN000023761) and BOOSTER (NCT03133546) trials demonstrate the addition of bevacizumab to osimertinib in advanced NSCLC patients with the EGFR mutation and acquired T790M mutation after failure of 1L EGFR-TKI treatment was not associated with an improvement in both PFS and OS, which suggests this combination strategy may not be able to increase efficacy over osimertinib monotherapy (46, 47). Outcomes of ongoing studies in EGFR-TKI naive patients accepting osimertinib plus bevacizumab (NCT4181060) or ramucirumab (NCT03909334) may further examine the role of an antiangiogenic-included combination strategy in 1L treatment.

Ex19del and L858R are two of the most common types of EGFR mutations, but they have biological differences and specific mechanisms that account for their different efficacy to treatment (48). Subgroup analyses of major studies reveal a tendency for patients with ex19del to benefit more from treatment with three generations of EGFR-TKI candidates than patients with L858R. Taking into account the subgroup analysis in each landmark trial, patients with both ex19del and L858R could significantly benefit from treatment of afatinib, dacomitinib, and osimertinib compared with first-generation EGFR-TKIs in terms of PFS (3–8). However, only osimertinib improved the OS of patients with the ex19del mutation (HR 0.68, 95%CI 0.51–0.90) (38). No significant OS benefit from treatment with second-generation EGFR-TKIs (afatinib, HR 0.80, 95%CI 0.64–1.00; dacomitinib, HR 0.80, 95%CI 0.64–1.00) and even osimertinib (HR 1.00, 95%CI 0.71–1.40) was observed in the subgroup of patients with the L858R mutation (36–38). The INCREASE trial (NCT02404675), a randomized phase II trial, demonstrated high-dose icotinib improved PFS in comparison with routine-dose icotinib in mNSCLC patients harboring the L858R mutation (HR 0.75, 95%CI 0.53–1.05) (39). In combination treatments, NEJ009 (UMIN000006340) showed significant improvements in PFS from a combination of EGFR-TKIs and chemotherapy for patients harboring both the ex19del mutation (HR 0.47, 95%CI 0.34–0.64) and the L858R mutation (HR 0.55, 95%CI 0.38–0.80) in IL treatment, but subgroup data on OS are not available (10). A number of meta-analyses offer strong evidence that patients with both ex19del (HR 0.61, 95%CI 0.49–0.75, p = 0.00) and patients with L858R (HR 0.59, 95%CI 0.47–0.73, p = 0.00) benefit from a combination of elortinib and antiangiogenesis therapy on PFS (49, 50). In the CTONG1509 trial (NCT02759614), the PFS of patients with the L858R mutation achieved 19.5 months in the combination group, which is the best PFS observed to date (26). The result was approximately double that of the erlotinib-alone group (9.7 months) and even exceeded the 14.4 months PFS of patients receiving osimertinib, which is followed by erlotinib and ramucirumab (19.4 months) in the RELAY (NCT02411448) trial and erlotinib and bevacizumab (17.4 months) in NEJ026 (6, 23, 27). The data suggest that patients with L858R derive more benefit from the addition of an anti-angiogensis to erlotinib. Unfortunately, this significant prolonged PFS did not translate into a significant OS benefit in patients with the L858R mutation in both NEJ026 and CTONG1509, and OS data are awaited from the RELAY trial to further evaluate the role of this combination strategy for patients with the L858R mutation (24, 26). A group of prospective trials focuses on the combination of osimertinib and anti-angiogenic drugs (UMIN000028071, NCT 0281579) is expected to further improve the efficacy and break though the treatment bottleneck of patients with L858R mutation in the first line setting.

EGFR-TKIs remains the standard care of advanced NSCLC patients with sensitizing EGFR mutations. The molecular mechanism of acquired resistance in up-front treatments are of great importance because choosing the optimal subsequent therapies after disease progression on 1L therapy depends largely on the mechanisms driving resistance. T790M mutation is the most common resistance mechanism to 1G and 2G EGFR-TKIs, occurring in up to two thirds of patients and for whom osimertinib is the standard of care (51). In the NEJ026 and JO25567 studies, the frequency of T790M mutation in progression patients after 1L treatments was similar between the bevacizumab plus erlotinib and erlotinib alone groups, which identified that the combination of bevacizumab and erlotinib had no effect on the acquired T790M mutation, which allowed patients in both groups to have same chance to use osimertinib in a second line setting (9, 22–24). For patients who are T790M mutation-negative, there is a lack of effective options in the second line setting and where there remains an urgent unmet medical need. Continuing with EGFR-TKIs, local therapy and systemic chemotherapy are current alternative options, and

clinical determination depends on patients' characteristics. Current explorations cover bevacizumab plus chemotherapy and atezolizumab plus bevacizumab plus chemotherapy for these T790M-negative patients after 1G/2G EGFR-TKI treatment (51, 52).

The molecular mechanisms of resistance to osimertinib are complex and still under study. Patterns of molecular resistance vary depending on whether osimertinib is given in a first line setting or in a subsequent line. It seems that the resistance mechanism spectrum of osimertinib in the second line is more complex than that in in the first line setting (53). However, the resistance mechanism of osimertinib in both clinical contexts could be grouped into two categories: on-target EGFR-dependent and off-target EGFR-independent mechanisms (54). EGFR-dependent resistance typically is related to alterations in the banding site caused by additional EGFR-mutations, which disrupt the osimertinib binding. The most common EGFR-dependent resistance mutation of osimertinib is the EGFR exon 20 C797S mutation, and other EGFR alterations include C797X, L718O, and S768I in the front line and T790M absence, L792H/L792V, G796S/G796C, and G724S in the second line (53–55). EGFR-independent mechanisms are mostly associated with aberrant downstream signaling or alternative pathway activation and histological transformations. MET amplification is the most frequent off-target mechanism of resistance to osimertinib, which activates the MET-related downstream PI3K/AKT and MAPK pathways. Other mechanisms include HER2 amplification and the emergence of NRAS, PI3KCA, BRAF, and KRAS mutations (56). Currently, platinum-based combination chemotherapy, platinum plus pemetrexed in most cases, is approved as the standard of care in patients after osimertinib resistance. For patients with transformation to SCLC and squamous cell carcinoma, treatments preferred are platinum-etoposide and platinum-gemcitabine, respectively. A treatment strategy of combined MET and EGFR inhibition in the setting of MET amplification–driven osimertinib resistance seems a promising and compelling approach in preliminary results of the INSIGHT 1 trial (NCT01982955) assessing the combination of tepotinib and gefitinib and in the CHRYSALIS-1 study (NCT02609776) evaluating lazertinib, a 3G EGFR-TKI, in combination with amivantamab, which is a special antibody that can inhibit both EGFR and MET receptors (57, 58). As with the MET amplification, a combination of EGFR-TKIs and an inhibitor of the acquired mutation is an emerging trend in the treatment strategy for patients with acquired HER2, ALK, RET, BRAF, and other oncogenes. Brigatinib plus cetuximab could be of benefit and may be potentially effective to improve outcomes in patients with acquired co-mutations in C797S and EGFR T790M–driven resistance (59). The prospective ELIOS trial (NCT03239340) will provide a more complete picture of osimertinib resistance in the 1L setting and help to develop a more reasonable treatment strategy for sequential treatment.

Several potential limitations should be considered when interpreting the results of this study. First, heterogeneity exists in network meta-analyses, especially in subgroup analyses. The main intrinsic sources of heterogeneity were from different trial designs, including different treatments, races, and designs. It was difficult to resolve even using the individual patient data. Second, one study was only presented as abstract, which led to insufficient data in subgroups being available. This limitation built a barrier to reach a definitive conclusion about the superiority between different treatments. Finally, most of the included RCTs in the EGFR-TKIs plus chemotherapy group (30–35) and EGFR-TKIs plus anti-angiogenesis group (9, 22–24, 26, 28) were performed in Asian countries; therefore, the vast majority of participants were Asians. And data on other races were not available.

Conclusions and perspectives

In summary, our study is, to our knowledge, the first network meta-analysis to estimate and compare the mature OS of five treatments as the first-line treatment in advanced NSCLC patients who are EGFR mutation-sensitive. IG EGFR-TKIs plus chemotherapy and osimertinib had high SUCRAs for PFS and OS and ranked as the top two best treatments. With regard to AEs, osimertinib had an obvious advantage due to a significantly low risk of SAEs. However, limitations of the study, including a single RCT investigating osimertinib and lacking data on the combination regimens from other races than Asian. Further investigations and updated analyses are needed to provide additional evidence to verify the most favorable first-line management in patients harboring activated EGFR-mutated NSCLC. From our perspective, further direction of effort includes next-generation EGFR-TKIs, the resistance mechanisms of EGFR-TKIs and new agents to target these resistances, novel combination modes, and control of AEs.

Data availability statement

All data generated or analyzed in this study are included in this article/Supplementary Material. Further enquires can be directed to the corresponding authors.

Author contributions

YQ and SW designed and conceived the study. LG, LX and YD collected the data. LS analyze the data and performed the statistical analysis. Prof. JT gave the important guidance for statistical analysis and methodology.XX and RN provided critical intellectual contributions. And YQ drafted the manuscript. All authors reviewed and approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.616546/full#supplementary-material

References

1. Vachani A, Sequist LV, Spira A. AJRCCM: 100-year anniversary. the shifting landscape for lung cancer: Past, present, and future. *Am J Respir Crit Care Med* (2017) 195(9):1150–60. doi: 10.1164/rccm.201702-0433CI

2. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). *Am J Cancer Res* (2015) 5(9):2892–911.

3. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non small-cell lung cancer with mutated EGFR. *N Engl J Med* (2010) 362(25):2380–8. doi: 10.1056/NEJMoa0909530

4. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* (2011) 12(8):735–42. doi: 10.1016/S1470-2045(11)70184-X

5. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-linetreatment 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):239–46. doi: 10.1016/S1470-2045(11)70393-X

6. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non-Small-Cell lung cancer. *N Engl J Med* (2018) 378(2):113–25. doi: 10.1056/NEJMoa1713137

7. Park K, Tan EH, O'Byrne K, Zhang L, Boyer M, Mok T, et al. Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-lung 7): a phase 2B, open-label, randomised controlled trial. *Lancet Oncol* (2016) 17(5):577–89. doi: 10.1016/S1470-2045(16)30033-X

8. Wu YL, Cheng Y, Zhou X, Lee KH, Nakagawa K, Niho S, et al. Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomised, open-label, phase 3 trial. *Lancet Oncol* (2017) 18(11):1454–66. doi: 10.1016/S1470-2045(17)30608-3

9. Seto T, Kato T, Nishio M, Goto K, Atagi S, Hosomi Y, et al. Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring EGFR mutations (JO25567): an open-label, randomised, multicentre, phase 2 study. *Lancet Oncol* (2014) 15(11):1236–44. doi: 10.1016/S1470-2045(14)70381-X

10. Hosomi Y, Morita S, Sugawara S, Kato T, Fukuhara T, Gemma A, et al. Gefitinib alone versus gefitinib plus chemotherapy for non-Small-Cell lung cancer with mutated epidermal growth factor receptor: NEJ009 study. *J Clin Oncol* (2020) 38(2):115–23. doi: 10.1200/JCO.19.01488

11. https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1450.

12. https://www.esmo.org/guidelines/lung-and-chest-tumours/clinical-practice-living-guidelines-metastatic-non-small-cell-lung-cancer.

13. Zhang H, Chen J, Liu T, Dang J, Li G. First-line treatments in EGFR-mutated advanced non-small cell lung cancer: A network meta-analysis. *PloS One* (2019) 14(10):e0223530. doi: 10.1371/journal.pone.0223530

14. Li XY, Lin JZ, Yu SH. Front-line therapy in advanced non-small cell lung cancer with sensitive epidermal growth factor receptor mutations: A network meta-analysis. *Clin Ther* (2020) 42(2):338–350.e4. doi: 10.1016/j.clinthera.2019.12.006

15. Batson S, Mitchell SA, Windisch R, Damonte E, Munk VC, Reguart N. Tyrosine kinase inhibitor combination therapy in first-line treatment of non-small-cell lung cancer: systematic review and network meta-analysis. *Onco Targets Ther* (2017) 10:2473–82. doi: 10.2147/OTT.S134382

16. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* (2010) 8(5):336–41. doi: 10.1016/j.ijsu.2010.02.007

17. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The cochrane collaboration's tool for assessing risk of bias in randomised trials. *BMJ* (2011) 343:d5928. doi: 10.1136/bmj.d5928

18. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: Is blinding necessary? *Control Clin Trials* (1996) 17:1–12. doi: 10.1016/0197-2456(95)00134-4

19. Demets DL. Methods for combining randomized clinical trials: strengths and limitations. *Stat Med* (1987) 6(3):341–50. doi: 10.1002/sim.4780060325

20. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* (2003) 327(7414):557–60. doi: 10.1136/bmj.327.7414.557

21. Cipriani A, Higgins JP, Geddes JR, Salanti G. Conceptual and technical challenges in network meta-analysis. *Ann Intern Med* (2013) 159(2):130–7. doi: 10.7326/0003-4819-159-2-201307160-00008

22. Yamamoto N, Seto T, Nishio M, Goto K, Yamamoto N, Okamoto I, et al. Erlotinib plus bevacizumab vs erlotinib monotherapy as first-line treatment for advanced EGFR mutation-positive non-squamous non-small-cell lung cancer: Survival follow-up results of the randomized JO25567 study. *Lung Cancer* (2021) 151:20–4. doi: 10.1016/j.lungcan.2020.11.020

23. Saito H, Fukuhara T, Furuya N, Watanabe K, Sugawara S, Iwasawa S, et al. Erlotinib plus bevacizumab versus erlotinib alone in patients with EGFR-positive advanced non-squamous non-small-cell lung cancer (NEJ026): interim analysis of an open-label, randomised, multicentre, phase 3 trial. *Lancet Oncol* (2019) 20(5):625–35. doi: 10.1016/S1470-2045(19)30035-X

24. Kawashima Y, Fukuhara T, Saito H, Furuya N, Watanabe K, Sugawara S, et al. Bevacizumab plus erlotinib versus erlotinib alone in Japanese patients with advanced, metastatic, EGFR-mutant non-small-cell lung cancer (NEJ026): overall survival analysis of an open-label, randomised, multicentre, phase 3 trial. *Lancet Respir Med* (2022) 10(1):72–82. doi: 10.1016/S2213-2600(21)00166-1

25. Stinchcombe TE, Jänne PA, Wang X, Bertino EM, Weiss J, Bazhenova L, et al. Effect of erlotinib plus bevacizumab vs erlotinib alone on progression-free

survival in patients with advanced EGFR-mutant non-small cell lung cancer: A phase 2 randomized clinical trial. *JAMA Oncol* (2019) 5(10):1448–55. doi: 10.1001/jamaoncol.2019.1847

26. Zhou Q, Xu CR, Cheng Y, Liu YP, Chen GY, Cui JW, et al. Bevacizumab plus erlotinib in Chinese patients with untreated, EGFR-mutated, advanced NSCLC (ARTEMIS-CTONG1509): A multicenter phase 3 study. *Cancer Cell* (2021) 39(9):1279–1291.e3. doi: 10.1016/j.ccell.2021.07.005

27. Nakagawa K, Garon EB, Seto T, Nishio M, Ponce Aix S, Paz-Ares L, et al. Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* (2019) 20(12):1655–69. doi: 10.1016/S1470-2045(19)30634-5

28. Zhao H, Yao W, Min X, Gu K, Yu G, Zhang Z, et al. Apatinib plus gefitinib as first-line treatment in advanced EGFR-mutant NSCLC: The phase III ACTIVE study (CTONG1706). *J Thorac Oncol* (2021) 16(9):1533–46. doi: 10.1016/j.jtho.2021.05.006

29. Janne PA, Wang X, Socinski MA, Crawford J, Stinchcombe TE, Gu L, et al. Randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 trial. *J Clin Oncol* (2012) 30:2063–9. doi: 10.1200/JCO.2011.40.1315

30. Yang JC, Kang JH, Mok T, Ahn MJ, Srimuninnimit V, Lin CC, et al. First-line pemetrexed plus cisplatin followed by gefitinib maintenance therapy versus gefitinib monotherapy in East Asian patients with locally advanced or metastatic non-squamous non-small cell lung cancer: a randomised, phase 3 trial. *Eur J Cancer* (2014) 50(13):2219–30. doi: 10.1016/j.ejca.2014.05.011

31. Cheng Y, Murakami H, Yang PC, He J, Nakagawa K, Kang JH, et al. Randomized phase II trial of gefitinib with and without pemetrexed as first-line therapy in patients with advanced nonsquamous non-Small-Cell lung cancer with activating epidermal growth factor receptor mutations. *J Clin Oncol* (2016) 34:3258–66. doi: 10.1200/JCO.2016.66.9218

32. An C, Zhang J, Chu H, Gu C, Xiao F, Zhu F, et al. Study of gefitinib and pemetrexed as first-line treatment in patients with advanced non-small cell lung cancer harboring EGFR mutation. *Pathol Oncol Res* (2016) 22:763–8. doi: 10.1007/s12253-016-0067-4

33. Han B, Jin B, Chu T, Niu Y, Dong Y, Xu J, et al. Combination of chemotherapy and gefitinib as first-line treatment for patients with advanced lung adenocarcinoma and sensitive EGFR mutations: A randomized controlled trial. *Int J Cancer* (2017) 141:1249–56. doi: 10.1002/ijc.30806

34. Noronha V, Patil VM, Joshi A, Menon N, Chougule A, Mahajan A, et al. Gefitinib versus gefitinib plus pemetrexed and carboplatin chemotherapy in EGFR-mutated lung cancer. *J Clin Oncol* (2020) 38:124–36. doi: 10.1200/JCO.19.01154

35. Xu L, Qi Q, Zhang Y, Cui J, Liu R, Li Y. Combination of icotinib and chemotherapy as first-line treatment for advanced lung adenocarcinoma in patients with sensitive EGFR mutations: A randomized controlled study. *Lung Cancer* (2019) 133:23–31. doi: 10.1016/j.lungcan.2019.05.008

36. Paz-Ares L, Tan EH, O'Byrne K, Zhang L, Hirsh V, Boyer M, et al. Afatinib versus gefitinib in patients with EGFR mutation-positive advanced non-small-cell lung cancer: Overall survival data from the phase IIb LUX-lung 7 trial. *Ann Oncol* (2017) 28:270–7. doi: 10.1093/annonc/mdw611

37. Mok TS, Cheng Y, Zhou X, Lee KH, Nakagawa K, Niho S, et al. Improvement in overall survival in a randomized study that compared dacomitinib with gefitinib in patients with advanced non-Small-Cell lung cancer and EGFR-activating mutations. *J Clin Oncol* (2020) 38(31):3725. doi: 10.1200/JCO.2018.78.7994

38. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, et al. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N Engl J Med* (2020) 382:41–50. doi: 10.1056/NEJMoa1913662

39. Li X, Zhang L, Jiang D, Wang Y, Zang A, Ding C, et al. Routine-dose and high-dose icotinib in patients with advanced non-smallcell lung cancer harboring EGFR exon 21-L858R mutation: The randomized, phase II, INCREASE trial. *Clin Cancer Res* (2020) 26:3162–71. doi: 10.1158/1078-0432.CCR-19-3064

40. Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med* (2017) 376(7):629–40. doi: 10.1056/NEJMoa1612674

41. Cuppens K, Lodewyckx L, Demedts I, Decoster L, Colinet B, Deschepper K, et al. Real-world treatment patterns, epidermal growth factor receptor (EGFR) testing and outcomes in EGFR-mutated advanced non-small cell lung cancer patients in Belgium: Results from the REVEAL study. *Drugs Real World Outcomes* (2021) 8(2):141–52. doi: 10.1007/s40801-021-00243-w

42. Remon J, Menis J, Hasan B, Peric A, De Maio E, Novello S, et al. The APPLE trial: Feasibility and activity of AZD9291 (Osimertinib) treatment on positive PLasma T790M in EGFR-mutant NSCLC patients. EORTC 1613. *Clin Lung Cancer* (2017) 18(5):583–8. doi: 10.1016/j.cllc.2017.02.005

43. Tanaka K, Asahina H, Kishimoto J, Miyata Y, Uchida T, Watanabe K, et al. Osimertinib versus osimertinib plus chemotherapy for non-small cell lung cancer with EGFR (T790M)-associated resistance to initial EGFR inhibitor treatment: An open-label, randomised phase 2 clinical trial. *Eur J Cancer* (2021) 149:14–22. doi: 10.1016/j.ejca.2021.02.019

44. Byers LA, Heymach JV. Dual targeting of the vascular endothelial growth factor and epidermal growth factor receptor pathways: rationale and clinical applications for non-small-cell lung cancer. *Clin Lung Cancer* (2007) 8 Suppl 2: S79–85. doi: 10.3816/clc.2007.s.006

45. Akamatsu H, Toi Y, Hayashi H, Fujimoto D, Tachihara M, Furuya N, et al. Efficacy of osimertinib plus bevacizumab vs osimertinib in patients with EGFR T790M-mutated non-small cell lung cancer previously treated with epidermal growth factor receptor-tyrosine kinase inhibitor: West Japan oncology group 8715L phase 2 randomized clinical trial. *JAMA Oncol* (2021) 7(3):386–94. doi: 10.1001/jamaoncol.2020.6758

46. Soo RA, Han JY, Dafni U, Cho BC, Yeo CM, Nadal E, et al. A randomised phase II study of osimertinib and bevacizumab versus osimertinib alone as second-line targeted treatment in advanced NSCLC with confirmed EGFR and acquired T790M mutations: the European thoracic oncology platform (ETOP 10-16) BOOSTER trial. *Ann Oncol* (2022) 33(2):181–92. doi: 10.1016/j.annonc.2021.11.010

47. Li WQ, Cui JW. Non-small cell lung cancer patients with ex19del or exon 21 L858R mutation: distinct mechanisms, different efficacies to treatments. *J Cancer Res Clin Oncol* (2020) 146(9):2329–38. doi: 10.1007/s00432-020-03296-6

48. Chen F, Chen N, Yu Y, Cui J. Efficacy and safety of epidermal growth factor receptor (EGFR) inhibitors plus antiangiogenic agents as first-line treatments for patients with advanced EGFR-mutated non-small cell lung cancer: A meta-analysis. *Front Oncol* (2020) 10:904. doi: 10.3389/fonc.2020.00904

49. Peravali M, Wang H, Kim C, Veytsman I. Combined inhibition of EGFR and VEGF pathways in patients with EGFR-mutated non-small cell lung cancer: A systematic review and meta-analysis. *Curr Oncol Rep* (2020) 22(12):119. doi: 10.1007/s11912-020-00981-0

50. Yang JC, Ahn MJ, Kim DW, Ramalingam SS, Sequist LV, Su WC, et al. Osimertinib in pretreated T790M-positive advanced non-Small-Cell lung cancer: AURA study phase II extension component. *J Clin Oncol* (2017) 35(12):1288–96. doi: 10.1200/JCO.2016.70.3223

51. Reck M, Mok TSK, Nishio M, Jotte RM, Cappuzzo F, Orlandi F, et al. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. *Lancet Respir Med* (2019) 7(5):387–401. doi: 10.1016/S2213-2600(19)30084-0

52. Schoenfeld AJ, Yu HA. The evolving landscape of resistance to osimertinib. *J Thorac Oncol* (2020) 15(1):18–21. doi: 10.1016/j.jtho.2019.11.005

53. Schmid S, Li JJN, Leighl NB. Mechanisms of osimertinib resistance and emerging treatment options. *Lung Cancer* (2020) 147:123–9. doi: 10.1016/j.lungcan.2020.07.014

54. Schmid S, Früh M, Peters S. Targeting MET in EGFR resistance in non-small-cell lung cancer-ready for daily practice? *Lancet Oncol* (2020) 21(3):320–2. doi: 10.1016/S1470-2045(19)30859-9

55. Schoenfeld AJ, Chan JM, Kubota D, Sato H, Rizvi H, Daneshbod Y, et al. Tumor analyses reveal squamous transformation and off-target alterations as early resistance mechanisms to first-line osimertinib in EGFR-mutant lung cancer. *Clin Cancer Res* (2020) 26(11):2654–63. doi: 10.1158/1078-0432.CCR-19-3563

56. Wu YL, Cheng Y, Zhou J, Lu S, Zhang Y, Zhao J, et al. Tepotinib plus gefitinib in patients with EGFR-mutant non-small-cell lung cancer with MET overexpression or MET amplification and acquired resistance to previous EGFR inhibitor (INSIGHT study): an open-label, phase 1b/2, multicentre, randomised trial. *Lancet Respir Med* (2020) 8(11):1132–43. doi: 10.1016/S2213-2600(20)30154-5

57. Park K, Haura EB, Leighl NB, Mitchell P, Shu CA, Girard N, et al. Amivantamab in EGFR exon 20 insertion-mutated non-Small-Cell lung cancer progressing on platinum chemotherapy: Initial results from the CHRYSALIS phase I study. *J Clin Oncol* (2021) 39(30):3391–402. doi: 10.1200/JCO.21.00662

58. Wang Y, Yang N, Zhang Y, Li L, Han R, Zhu M, et al. Effective treatment of lung adenocarcinoma harboring EGFR-activating mutation, T790M, and cis-C797S triple mutations by brigatinib and cetuximab combination therapy. *J Thorac Oncol* (2020) 15(8):1369–75. doi: 10.1016/j.jtho.2020.04.014

59. Cai Z, Yin Y, Zhao Z, Xin C, Cai Z, Yin Y, et al. Comparative effectiveness of neoadjuvant treatments for resectable gastroesophageal cancer: A network meta-analysis. *Front Pharmacol* (2018) 9:872. doi: 10.3389/fphar.2018.00872

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