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Efficacy of erlotinib as neoadjuvant regimen in EGFR-mutant locally advanced non-small cell lung cancer patients

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Abstract

Background: The optimal neoadjuvant regimen for locally advanced resectable non-small cell lung cancer (NSCLC) remains controversial. EGFR inhibitors have significantly improved survival in patients with *EGFR*-mutant advanced NSCLC. However, their efficacy in neoadjuvant settings, particularly for treating locally advanced NSCLC, remains unclear. We compared the clinical benefits of chemotherapy and erlotinib as neoadjuvant therapy for stage IIIA NSCLC.

Method: Thirty-one treatment-naïve Chinese patients with stage IIIA NSCLC were enrolled. Patients without *EGFR* mutation received cisplatin-based doublet chemotherapy (n = 16; N-chemo group) while *EGFR*-mutant patients received erlotinib (n = 15; N-TKI group) as neoadjuvant therapy.

Results: After completing neoadjuvant treatment, 12 and 8 patients from the N-TKI and N-chemo groups underwent surgery, respectively. Our data revealed that patients who received erlotinib had a marginally better clinical objective response rate (67% vs. 19%), pathological response rate (67% vs. 38%), and overall survival (51.0 months vs. 20.9 months) compared

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with those who received chemotherapy. Furthermore, patients in the N-TKI group had a significantly greater reduction in tumor diameter, serum carcinoembryonic level, and maximum allelic fraction.

Conclusion: Our findings demonstrate that erlotinib is an effective neoadjuvant regimen in patients with *EGFR*-mutant locally advanced NSCLC, paving the way for its extended use in neoadjuvant settings.

[ClinicalTrials.gov identifier: NCT01217619]

Keywords
Adenocarcinoma, erlotinib, locally advanced, neoadjuvant, stage IIIA, chemotherapy, EGFR-TKI

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Introduction

Lung cancer is the leading cause of cancer-related death in China and worldwide.[1,2] Non-small cell lung cancer (NSCLC) accounts for about 80% of lung cancer cases. With symptoms presenting late in the disease, the majority of patients already have locally advanced (stage III) or metastatic (stage IV) disease at diagnosis and have an extremely limited possibility of being cured.[3] Clinical management of lung cancer depends on the histological type, pathological stage, presence of sensitizing gene mutations, and overall medical condition of the patient. As the most heterogeneous of all pathological stages, treatment strategies for stage III lung cancer are controversial. Currently, treatment regimens for patients with potentially resectable stage III NSCLC involve cisplatin or carboplatin-containing neoadjuvant therapy; complete surgical removal of the tumor, if feasible; and subsequent adjuvant chemotherapy and/or radiotherapy to prevent rapid relapse.[3] Despite the survival benefit associated with adjuvant chemotherapy in patients with completely resected NSCLC,[4–6] patients remain at risk of relapse and death. Currently, the estimated 5-year survival rate for patients with stage I to IIA disease range from 90% to 60%, and the rate falls to 53% and 36% for stage IIB and IIIA disease, respectively. The estimated 5-year survival rate drops further to 26%, 13%, and 10% for stage IIIB, IIIC and IV disease, respectively.[7] Significant efforts have been directed at developing new therapies and refining existing treatment strategies to control the disease and to maximize the survival outcomes of patients with NSCLC.

Treatment strategies including neoadjuvant therapies have been widely adopted and can markedly improve the prognosis of patients with various types of cancer. Neoadjuvant therapy, also termed induction therapy, involves the introduction of therapeutic agents prior to the main treatment, which is typically surgery. The aim of neoadjuvant therapy is to shrink the tumor size and increase the success rate of the main treatment. Current neoadjuvant regimens include local and systemic agents such as radiotherapy, chemotherapy, molecular targeted therapies, immunotherapy, and a combination of chemotherapy and radiotherapy. Neoadjuvant therapy in responsive patients may increase the possibility of adequate delivery of a systemic dose of the

main therapy and result in tumor shrinkage, pathological downstaging, complete resection, and long-term survival.[8–11]

For patients with locally advanced stage III NSCLC, the use of chemotherapy, chemoradiotherapy, or molecular targeted therapy in the neoadjuvant setting has been explored. However, the optimal neoadjuvant regimen remains controversial. Randomized clinical trials[8,12–15] and meta-analyses[9,10,16,17] have demonstrated the survival benefit of neoadjuvant chemotherapy in patients with resectable NSCLC. One of the earliest clinical studies on the survival benefits of neoadjuvant chemotherapy in stage IIIA NSCLC showed a median overall survival (OS) of 21 months in the neoadjuvant chemotherapy group compared with 14 months in patients who received surgery up front.[12–14] With neoadjuvant chemotherapy, a pathological response and complete resection based on pathological downstaging have been reported in 48.5% of NSCLC patients.[15] Furthermore, previous studies have shown that neoadjuvant chemotherapy confers a 5% improvement in OS at 5 years.[17] However, a study comparing chemotherapy and chemoradiotherapy as neoadjuvant regimens yielded a comparable OS but lower residual nodal disease (RND) in favor of chemoradiotherapy.[18]

On the basis of improved prognosis in patients with metastatic NSCLC harboring sensitizing mutations, the benefit of molecular targeted therapy as first-line therapy either as post-operative adjuvant or pre-operative neoadjuvant treatment in resectable NSCLC is gaining increasing attention. In several clinical trials, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) as adjuvant therapy has been shown to significantly improve survival compared with chemotherapy in patients with completely resected *EGFR*-mutant NSCLC.[19,20] However, limited studies have investigated the efficacy of EGFR-TKI in the neoadjuvant setting, with only two phase II clinical trials to date having explored its clinical benefits in this context.[21,22] One previous study reported the feasibility of neoadjuvant EGFR-TKI use but failed to show any survival benefit of EGFR-TKI in *EGFR*-mutant stage IIIA-N2 NSCLC patients.[21] However, our earlier single-arm study of the EGFR-TKI erlotinib as neoadjuvant therapy showed promising results.[22] In the present study, we compared the clinical benefits of erlotinib versus chemotherapy as neoadjuvant treatment in Chinese patients with stage IIIA NSCLC.

Patients and methods

Patient selection and study procedures

Thirty-one treatment-naïve patients with stage IIIA NSCLC were recruited to the study from February 2011 to July 2015 at the Shanghai Chest Hospital in Shanghai, People's Republic of China. Inclusion criteria included: 1) age 18 years or older; 2) no previous treatment for lung cancer; 3) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and 4) pathologically-confirmed locally advanced and surgically resectable stage IIIA NSCLC. Upon enrollment, relevant clinical information and baseline tumor biopsy and blood samples were obtained from all patients. The stage of the primary lung tumor (T), affected lymph node (N), and metastasis (M) were evaluated based on the American Joint Committee on Cancer 7th edition TNM staging system for NSCLC.[23] Stage III lung cancer is defined as locoregionally advanced disease due to primary tumor extension into extrapulmonary structures (T3 or T4) or mediastinal lymph node involvement (N2 or N3) without evidence of distant metastases (M0).[23] *EGFR* mutation status was also evaluated in enrolled patients and was the basis for neoadjuvant treatment grouping.

EGFR-positive patients received erlotinib 150 mg once daily for 4 to 7 weeks as neoadjuvant therapy (N-TKI group), while patients with wild-type *EGFR* received two cycles of cisplatin-based doublet chemotherapy in combination with vinorelbine, gemcitabine, paclitaxel, docetaxel or pemetrexed (N-chemo group). Tumor diameter was measured at baseline and at the post-neoadjuvant pre-surgery stage using endobronchial ultrasonography (EBUS) or CT-scanning. Objective response rates (ORR) to the neoadjuvant therapies were assessed based on the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1.[24] After the designated neoadjuvant treatment period, each patient underwent pre-surgical evaluation including assessment of best overall ORR. Patients who consented to receive surgery underwent radical resection (R0) within 0 to 2 weeks after their designated neoadjuvant regimen. Conventional post-surgery adjuvant chemotherapy or chemoradiotherapy was offered to all patients according to their status. Data on survival outcomes were obtained during follow-up of evaluable patients every 3 months until December 31, 2017. The study design is shown in Figure 1. The study was approved by the ethics committee of Shanghai Chest Hospital (approval number: KS1453) and was conducted in

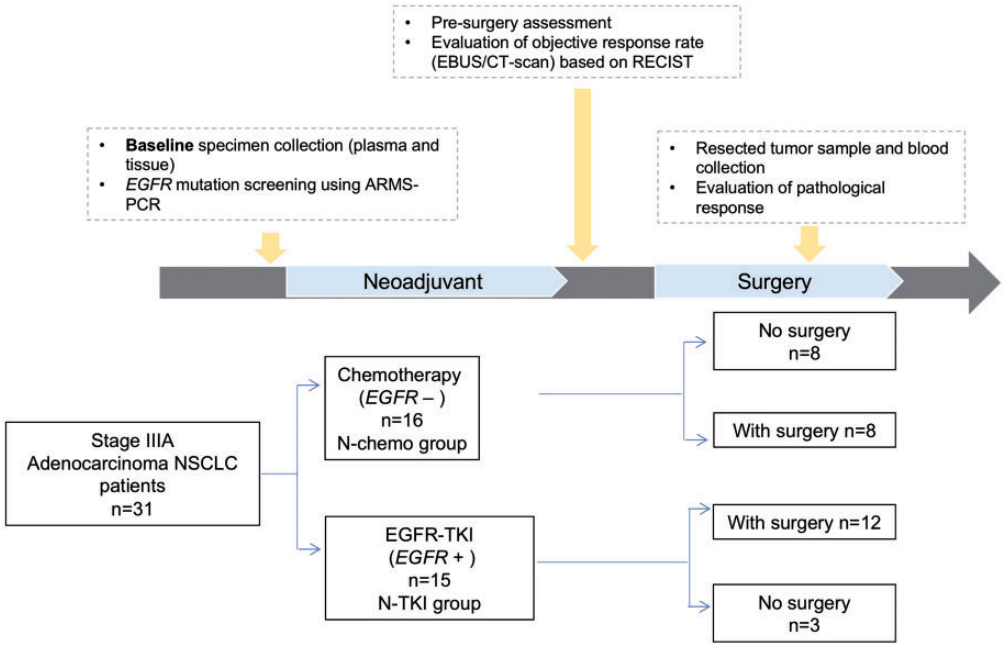

Figure 1. Schematic diagram of the study design. Paired baseline blood and tumor tissue samples were obtained from each patient. The neoadjuvant treatment regimen was assigned based on *EGFR* mutation status. After the completion of neoadjuvant treatment, pre-surgical evaluation was conducted. Paired blood and tumor tissue samples were obtained from patients who underwent surgery. Post-surgery evaluations were conducted every 3 months. Abbreviations: NSCLC, non-small cell lung cancer; N-chemo, neoadjuvant chemotherapy group; N-TKI, neoadjuvant EGFR-tyrosine kinase inhibitor (TKI); ARMS-PCR, amplification refractory mutation polymerase chain reaction; *EGFR* −, wild-type *EGFR*; *EGFR* +, *EGFR* mutant; EGFR-TKI, EGFR tyrosine kinase inhibitor; EBUS, endobronchial ultrasonography; CT-scan, computed tomography scan; RECIST, Response Evaluation Criteria in Solid Tumors.

accordance with the Declaration of Helsinki. All patients provided written informed consent prior to participation.

Tissue DNA extraction

DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissues using a QIAamp DNA FFPE tissue kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions.

Detection of EGFR mutation status

Tissue DNA was used to assess the common *EGFR* mutations in exon 21 L858R and exon 19 deletions (19del) by direct sequencing and amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) methods, respectively, using an AmoyDx Super-ARMS® EGFR mutation test kit (Amoy Diagnostics, Xiamen, China).

Capture-based targeted DNA sequencing

A minimum of 50 ng of DNA was required for next-generation sequencing (NGS) library construction. Tissue DNA was sheared using a Covaris M220 focused-ultrasonicator (Covaris Ltd., Coburn, MA, USA) and then subjected to end repair, phosphorylation, and adaptor ligation. Fragments of 200 to 400 bp in size were purified (Agencourt AMPure XP Kit, Beckman Coulter, CA, USA) followed by hybridization with capture probes baits, hybrid selection with magnetic beads, and PCR amplification. The quality and size of fragments were assessed using a Qubit 2.0 Fluorimeter with a dsDNA high-sensitivity assay kit (Life Technologies, Carlsbad, CA). Indexed samples were sequenced on a Nextseq500 system (Illumina, Inc., San Diego, CA, USA) with paired-end reads. A panel of 52 genes, of which 7 genes were related to NSCLC targeted therapy and 45 were cancer-related genes, was used in this study.

Sequence data analysis

Sequence data were mapped to the reference human genome (hg19) using Burrows–Wheeler Aligner software v.0.7.10.[25] Local alignment optimization, variant calling, and annotation were performed using Genome Analysis Tool Kit v.3.2[26] and VarScan.[27] Variants were filtered using the VarScan fpfilter pipeline, and loci with a depth less than 100 were filtered out. Base calling in tissue samples required at least five and eight supporting reads for small insertion-deletions (INDELs) and single nucleotide variants (SNVs), respectively. INDELs and SNVs with population frequency over 0.1% in the ExAC, 1000 Genomes, dbSNP, or ESP6500SI-V2 databases were grouped as SNPs and excluded from further analysis. The remaining variants were annotated using ANNOVAR[28] and SnpEff v3.6.[29] DNA translocation analysis was performed using Factera v.1.4.3.[30]

Statistical analysis

All data were analyzed using R software (R v.3.4.0; The R-Project for Statistical Computing, Vienna, Austria). Survival data were analyzed using Kaplan–Meier estimates and a log-rank test was used to compare differences between the survival groups. Progression-free survival (PFS) and recurrence-free survival (RFS) were defined as the time from the date of neoadjuvant treatment to the first date of disease progression or death, respectively. Disease-free survival (DFS) was defined as the time from surgery to tumor recurrence or death from any cause, whichever occurred earlier. Overall survival (OS) was defined as the time from the date of diagnosis to death from any cause. Differences in the N-chemo and N-TKI groups were calculated and presented using two-tailed Student's *t*-test with *p*-values. Significance in the best response rate between the groups was calculated

using Fisher's exact test. For all statistical tests, values of $p < 0.05$ were considered statistically significant.

Results

Patient characteristics

Thirty-one stage IIIA NSCLC patients were recruited. The patient demographics and clinical characteristics are summarized in Table 1. Invasive staging of the mediastinum was performed using EBUS-guided or computed tomography (CT)-guided transbronchial needle biopsy in 23 (74%) and 8 (26%) patients, respectively. The majority of patients were diagnosed with adenocarcinoma subtype (84%, 26/31), while 16% (5/31) had an unspecified NSCLC subtype. Upon enrollment, all patients underwent testing for *EGFR* mutation status to determine the neoadjuvant

Table 1. Demographic characteristics according to neoadjuvant regimen.

Group	*EGFR*-mutant (N-TKI) n = 15, n (%)	Wild-type *EGFR* (N-chemo) n = 16, n (%)
Histology (initial diagnosis)		
Adenocarcinoma	12 (80%)	14 (87.5%)
Others	3 (20%)	2 (12.5%)
Histology (confirmed after surgical resection)		
Adenocarcinoma	15 (100%)	16 (100%)
Gender		
Male	3 (20%)	10 (62.5%)
Female	12 (80%)	6 (37.5%)
Smoking status		
Yes	2 (13.3%)	6 (7.5%)
Non/Ever	13 (86.7%)	10 (62.5%)
Age (median and range)	60 (34–74)	60 (30–76)
Stage		
IIIA	15 (100%)	16 (100%)
TNM stage at diagnosis		
T1N2M0	0	1 (6.25%)
T2N2M0	12 (80%)	9 (56.25%)
T3N2M0	2 (13.3%)	5 (31.25%)
T3N1M0	1 (6.7%)	1 (6.25%)
Best response (post-neoadjuvant, pre-surgery)		
PR	10 (66.7%)	3 (18.7%)
SD	5 (33.3%)	12 (75%)
PD	0	1 (6.3%)
Pathologic response		
pCR	0	1 (12.5%)
pPR	8 (66.7%)	2 (25%)
Tumor diameter (mean and range)		
Baseline	4.1 (2.7–6.2)	5.15 (1.3–8.6)
Post-neoadjuvant pre-surgery	2.57 (0.9–4.2)	4.7 (1.3–9.1)

Abbreviations: N-TKI, neoadjuvant EGFR-TKI group; N-chemo, neoadjuvant chemotherapy group; T, tumor; N, node; M, metastasis; PR, partial response; SD, stable disease; PD, progressive disease; pCR, pathological complete response; pPR, pathological partial response.

treatment regimen. As a result, 16 patients with wild-type (WT) *EGFR* were assigned to receive cisplatin-based doublet chemotherapy as neoadjuvant (N-chemo) while the remaining 15 patients who tested positive for either EGFR L858R or 19del received erlotinib as neoadjuvant therapy (N-TKI).

In the N-chemo group, the majority of patients (88%, 14/16) were diagnosed with adenocarcinoma while two patients had an unspecified NSCLC subtype. The baseline TNM stages of the patients included one T1N2M0, nine T2N2M0, five T3N2M0, and one T3N1M0. All patients completed the assigned neoadjuvant chemotherapy, and eight patients underwent surgery while the remaining eight patients did not consent to the planned resection for personal reasons. Moreover, the two patients initially diagnosed with unspecified NSCLC subtype were confirmed to have adenocarcinoma after pathologic examination of surgically-resected tissues.

Among the 15 patients in the N-TKI group, the majority (80%, 12/15) of patients had adenocarcinoma while three patients had an unspecified NSCLC subtype. The baseline TNM stages of the patients included 12 T2N2M0, 2 T3N2M0, and 1 T3N1M0. Erlotinib treatment was well-tolerated and no toxicity-related symptoms were observed among the 15 patients who completed the planned neoadjuvant therapy. Twelve patients in the N-TKI group (12/15, 80%) underwent surgery. The remaining three patients, who achieved a partial clinical response (PR), refused the planned surgical resection for personal reasons and instead continued with erlotinib treatment until disease progression. Of note, the three patients with an unspecified NSCLC subtype were confirmed to have adenocarcinoma after pathologic examination of surgically resected tissues.

Clinical and pathological responses to neoadjuvant therapies

Among the 16 patients in the N-chemo group, 3 achieved a partial response (PR), 12 had stable disease (SD), and 1 had disease progression (PD), resulting in an ORR of 19%. Among the 15 patients in the N-TKI group, 10 had PR and the remaining 5 had SD, resulting in an ORR of 67%. Our data show that patients in the N-TKI group had a marginally better ORR than those in the N-chemo group, although the difference was not statistically significant (Figure 2a).

In addition to clinical responses, pathologic responses were determined by histologic examination of tissues obtained during surgery to identify residual malignant cells, thus providing a more accurate evaluation of neoadjuvant treatment efficacy. Of the eight patients who underwent surgery in the N-chemo group, one patient achieved a pathological complete response (pCR) and two patients achieved a pathological partial response (pPR), resulting in a major pathological response of 38%. Meanwhile, among the 12 patients who underwent surgery in the N-TKI group, 8 patients achieved a pPR, resulting in a major pathological response of 67%. Collectively, patients in the N-TKI group had a higher rate of major pathological response rate compared with those in the N-chemo group, although the difference was not statistically significant.

Maximum tumor diameter was measured by EBUS or CT-scanning in all patients. The mean tumor diameter in the N-chemo group was 5.2 cm (range: 1.3–8.6 cm) at baseline and 4.7 cm (range: 1.3–9.1 cm) after neoadjuvant therapy, representing a significant reduction in mean diameter of 16% after neoadjuvant treatment ($p = 0.005$, Figure 2b). In the N-TKI group, the mean tumor diameter was 4.1 cm (range: 2.7–6.2 cm) at baseline and 2.6 cm (range: 0.9–4.2 cm) after neoadjuvant

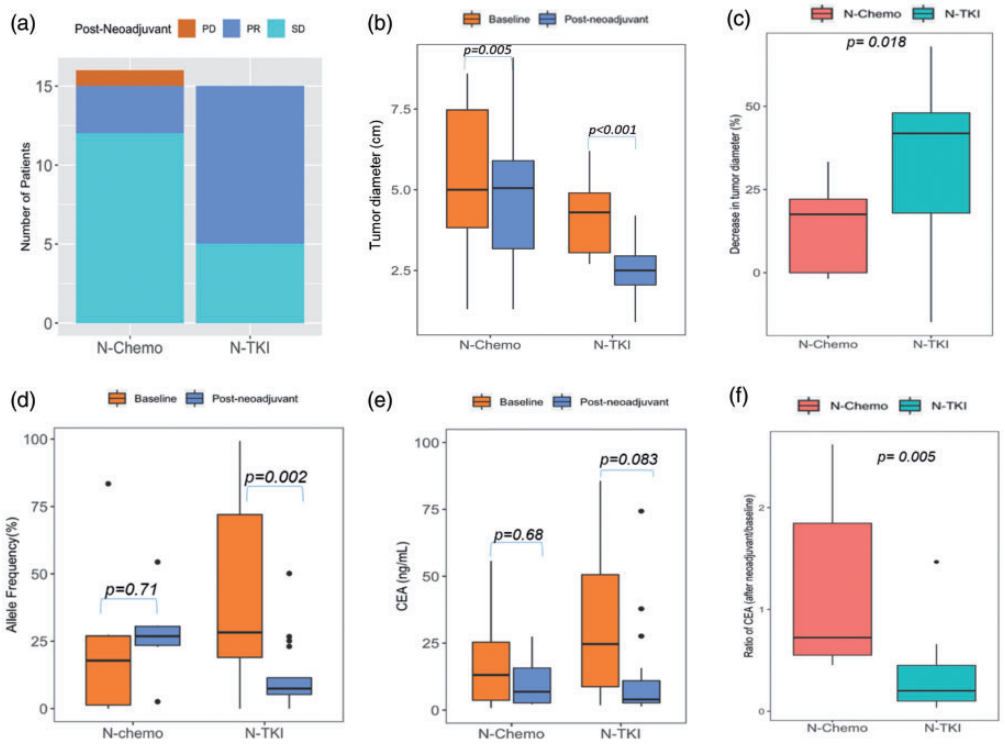

Figure 2. Clinical responses to neoadjuvant treatment. (a). EGFR-TKI neoadjuvant therapy provides better clinical responses compared with chemotherapy. Post-neoadjuvant therapy objective response rate (ORR) for N-chemo and N-TKI groups. Cyan represents the number of patients with SD. Blue represents the number of patients who achieved PR. Orange represents patients whose best response was evaluated as PD. (b)–(c). EGFR-TKI neoadjuvant therapy significantly reduces tumor diameter. (b). Average tumor diameter for patients in each treatment regimen. (c). Average changes in tumor diameter. *p*-values were calculated using a t-test. (d). EGFR-TKI neoadjuvant therapy significantly reduces maximum allele fraction (maxAF). MaxAF detected in tumor biopsy samples at baseline and surgically resected tumor specimens post-neoadjuvant therapy in the N-chemo and N-TKI groups. Absolute levels of maxAF. A t-test was used to calculate statistical significance. (e)–(f). Serum carcinoembryonic antigen (CEA) levels were significantly reduced in patients who received neoadjuvant EGFR-TKI therapy. (e). Absolute serum CEA levels (ng/ml). F. Ratio of serum CEA after neoadjuvant therapy relative to baseline. Abbreviations: N-chemo, neoadjuvant chemotherapy group; N-TKI, neoadjuvant EGFR-tyrosine kinase inhibitor (TKI); ORR, overall objective response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CEA, carcinoembryonic antigen.

therapy, representing a significant reduction of 35% ($p<0.001$, Figure 2b). Moreover, three patients with T2N2M0 in the N-TKI group showed a dramatic reduction in tumor diameter after neoadjuvant therapy and were pathologically downstaged to T1aN2M0, T1bN2M0, and T1aN0M0, respectively. Furthermore, patients treated with erlotinib as neoadjuvant therapy (N-TKI group) had a significantly greater reduction in tumor diameter than patients who received chemotherapy ($p=0.018$, Figure 2c).

We also compared the maximum allelic fractions (maxAF) derived from capture-based ultra-deep sequencing results between

baseline tissue biopsies and surgically resected tumor specimens to clarify the effect of neoadjuvant treatment on the maxAF of patients in both the N-chemo and N-TKI groups. MaxAF was defined as the highest allelic fraction among all mutations detected per patient sample from the panel used, regardless of gene or mutation site. The average maxAF remained constant among the N-chemo patients (Figure 2d) while an 83% reduction in average maxAF was detected among the N-TKI patients ($p = 0.002$, Figure 2d). These data suggest that, as neoadjuvant therapy, erlotinib was more effective than chemotherapy in killing cancer cells and thus reducing the fraction of mutant alleles detected in the resected tissues.

Serum carcinoembryonic antigen (CEA) levels were also measured in blood samples from all patients prior to and after neoadjuvant treatment to monitor treatment efficacy. Compared with baseline, the levels of CEA in the two groups did not change significantly after neoadjuvant therapy (Figure 2e). However, the CEA level in erlotinib-treated patients after neoadjuvant therapy was significantly lower than that in patients treated with chemotherapy ($p = 0.005$, Figure 2f).

Taken together, these data demonstrate that erlotinib was a more effective neoadjuvant therapy because it was associated with a better ORR trend and significantly greater reductions in tumor diameter, serum CEA level, and maxAF compared with chemotherapy.

Survival outcome following neoadjuvant therapy

We further analyzed the survival outcomes of all patients who underwent radical resection. In total, 12 N-TKI patients and 8 N-chemo patients underwent surgery. Our data revealed a comparable progression-free survival (PFS) (Figure 3a) and disease-free survival (DFS) (Figure 3b) between the groups. Median PFS in the N-TKI and N-chemo groups was 12.1 months and 11.0 months, respectively, while median DFS was 10.2 months and 8.0 months, respectively. Furthermore, our data revealed a trend of improved overall survival (OS) in the N-TKI group compared with the N-chemo group (51.0 months vs. 20.9 months), although the difference was not statistically significant (Figure 3c).

In addition, there were three patients in the N-TKI group that achieved best response of PR during pre-surgical assessments but did not consent to surgery and chose instead to remain on erlotinib treatment until disease progression. PFS in these patients was 11, 14, and 16 months, respectively.

Benefits of radical resection

The benefits of surgery in patients with stage IIIA NSCLC remain controversial. Hence, we explored the effect of radical resection on survival outcomes in the N-chemo patients. Of the 16 patients in the N-chemo group, only 8 underwent radical resection, while the other 8 refused to pursue surgery and continued to receive chemotherapy. Our data revealed a comparable RFS between the two groups (Figure 3d). Patients with and without surgery had an average RFS of 8.0 and 4.8 months, respectively, and those who underwent surgery had a marginally longer OS (20.9 vs. 15.3 months, Figure 3e), although the difference was not statistically significant. These data suggest that radical resection resulted in only a marginal OS benefit in patients with stage IIIA disease.

Discussion

In the present study, we compared the efficacy of erlotinib, a first-generation

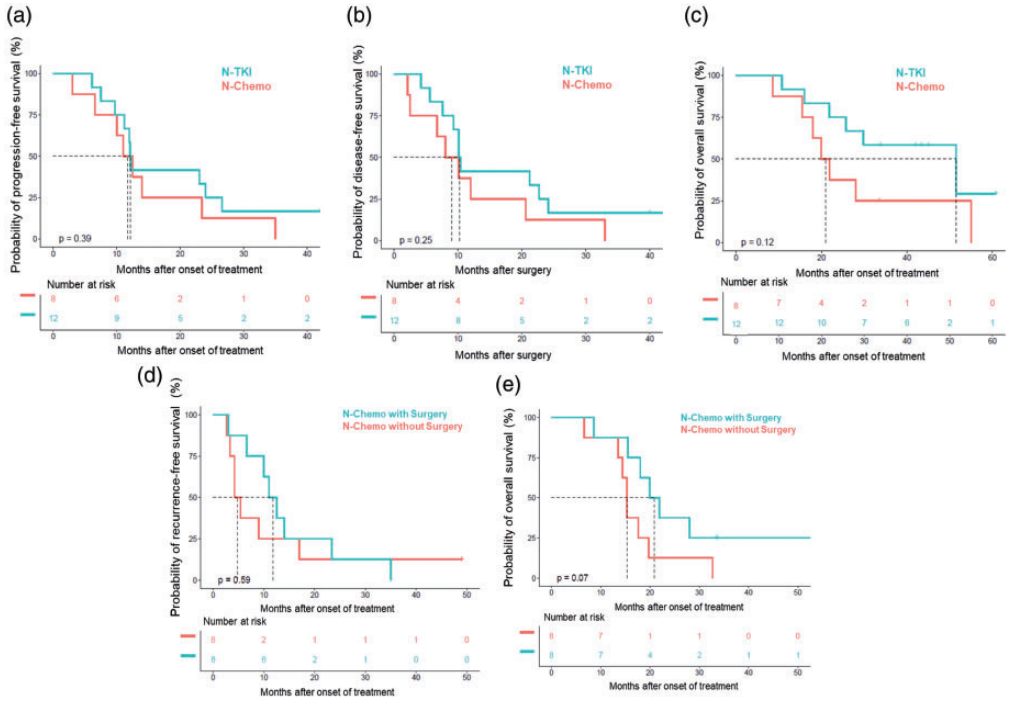

Figure 3. Kaplan–Meier estimation of the survival of neoadjuvant-treated patients. (a)–(c). Analysis of progression-free survival (PFS) (a), disease-free survival (DFS) (b), and overall survival (OS) (c) for resected, neoadjuvant chemotherapy, and erlotinib-treated patients. (d)–(e). Analyses of recurrence-free survival (RFS) (d) and overall survival (OS) (e) in chemotherapy-treated patients with or without subsequent surgery. Abbreviations: N-chemo, neoadjuvant chemotherapy group; N-TKI, neoadjuvant EGFR-tyrosine kinase inhibitor (TKI); DFS, disease-free survival; OS, overall survival; RFS, recurrence-free survival.

EGFR-TKI, with conventional chemotherapy in the neoadjuvant setting in patients with resectable stage IIIA NSCLC adenocarcinoma. Our earlier single-arm study (NCT01217619) showed that erlotinib as neoadjuvant therapy was well-tolerated and beneficial in *EGFR*-mutant patients with stage IIIA NSCLC.[22] In the present study, patients treated with erlotinib had a trend toward better clinical ORR, pathological response rate, and OS; however, given the small size of our study cohort, these results did not reach statistical significance. Furthermore, patients treated with erlotinib had a significantly greater reduction in tumor diameter ($p = 0.018$), serum CEA level ($p = 0.005$), and maxAF ($p = 0.002$) compared with patients treated with chemotherapy. Collectively, our findings support the use of erlotinib as neoadjuvant therapy in *EGFR*-mutant patients. Contrary to our observation, a previous clinical trial (NCT00600587) with a similar study design to that in the present study reported a median DFS and OS of 6.9 and 14.5 months, respectively, for erlotinib-treated patients with *EGFR*-mutation compared with 9.0 and 28.1 months, respectively, for gemcitabine/carboplatin-treated patients with wild-type *EGFR*.[21] Given the limited number of patients enrolled to the present study, further multi-center studies with larger study populations are needed to validate our results.

The value of surgery as the optimal treatment option for patients with locally advanced stage IIIA NSCLC is widely debated, given the heterogeneity of N2 involvement in stage III disease.[31] Some evidence has supported the benefit offered by surgery,[31–34] while other reports showed that surgery after neoadjuvant chemotherapy, radiotherapy, or chemoradiotherapy offered no benefit in terms of overall survival[11,35–38] and might even increase the risk of mortality.[38–40] Our data show that radical resection afforded only a marginal survival benefit after neoadjuvant chemotherapy and could therefore remain an optional strategy in the management of patients with locally advanced NSCLC.

Initial *EGFR* mutation status screening for neoadjuvant eligibility was determined by direct sequencing or ARMS-PCR and determined neoadjuvant treatment stratification in our study. However, targeted NGS of tissue DNA from all patients identified a patient in the chemotherapy group with *EGFR* 19del who was not identified using ARMS-PCR. This case illustrates the limitation of traditional *EGFR* screening methods and highlights the accuracy and sensitivity of NGS to detect low allele fractions.

Interestingly, *EML4-ALK* fusion was detected after neoadjuvant treatment in one patient who had received two cycles of neoadjuvant chemotherapy followed by surgery within 50 days of the start of treatment (data not shown). The fusion was detected in all resected tissues, including lymph node stations 4 and 7, but was absent in the baseline lymph node biopsies. There are two potential reasons for this observation. One is that the rearrangement could have been a chemotherapy-induced event, while the other more compelling possibility is intrinsic genetic heterogeneity in the tumor.[41] Baseline tissue samples were obtained by needle biopsy from lymph nodes, while tumor samples assayed after neoadjuvant therapy were surgically resected samples obtained from the primary tumor and four lymph nodes stations, with tumor content ranging from 5% to 95%. It is possible that the *EML4-ALK* fusion clone existed at baseline; however, for reasons ranging from intrinsic tumor heterogeneity, low allelic fraction, and detection limitations, it was not detected in the tissue. Our tissue and plasma assays, with a targeted sequencing depth of 1,000× and 10,000× respectively, have a detection limit of 2% for tissue samples and 0.2% for plasma samples.

In conclusion, our findings demonstrate that erlotinib was an effective neoadjuvant regimen in patients with *EGFR*-mutant locally advanced NSCLC. From our observations, we suggest stratifying patients for neoadjuvant therapy regimen based on their *EGFR* status. Our study represents a foundation for the use of erlotinib in neoadjuvant settings. However, given the limited number of patients in our cohort, larger multi-center studies are necessary to confirm our findings. Furthermore, the efficacy of EGFR-TKI as neoadjuvant therapy may be extrapolated to other molecular targeted therapies, such as ALK-TKI.

Abbreviations

NSCLC, non-small cell lung cancer; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; TNM, tumor, node and metastasis; TKI, neoadjuvant TKI group; N-chemo, neoadjuvant chemotherapy group; PFS, progression-free survival; OS, overall survival; ORR, overall objective response rate; PD, disease progression; SD, stable disease; PR, partial response; CR, complete response; pCR, pathological complete response; pPR, pathological partial response

Author contributions

L. Xiong, Y. Lou, A. Gu, and B. Han were responsible for the conception and design of the study. L. Xiong, Y. Lou, H. Bai, R. Li, J. Xia, and W. Fang collected the data. B. Li assisted with the statistical analysis. L. Xiong, Y. Lou, J. Zhang, H. Han-Zhang, and A. Lizaso analyzed the data. L. Xiong, Y. Lou, H. Han-Zhang, and A. Lizaso wrote the manuscript in consultation with A. Gu and B. Han. All authors approved the manuscript prior to submission.

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Declaration of conflicting interest

H. Han-Zhang, A. Lizaso, and B. Li are employees of Burning Rock Biotech. The other authors declare no conflicts of interest.

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