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Clinical Trial Results

Erlotinib as Neoadjuvant Therapy in Stage IIIA (N2) *EGFR* Mutation-Positive Non-Small Cell Lung Cancer: A Prospective, Single-Arm, Phase II Study

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Trial Information

- **ClinicalTrials.gov Identifier:** NCT01217619
- **Sponsor(s)**: Shanghai Roche Pharmaceuticals Limited, China Leading Talent Project Funding 2013, Shanghai Health and Family Plan Commission Research Projects, 201440032
- **Principal Investigator**: Baohui Han
- **IRB Approved**: Yes

Lessons Learned

- The findings of this prospective, single-arm, phase II study showed that neoadjuvant erlotinib was well tolerated and might improve the radical resection rate in patients with stage IIIA-N2 epidermal growth factor receptor mutation-positive non-small cell lung cancer (NSCLC).
- Erlotinib shows promise as a neoadjuvant therapy option in this patient population.
- Next-generation sequencing may be useful for predicting outcomes with preoperative tyrosine kinase inhibitors (TKIs) in patients with NSCLC.
- Large-scale randomized controlled trials investigating the role of TKIs in perioperative therapy, combining neoadjuvant and adjuvant treatments to enhance personalized therapy for patients in this precision medicine era, are warranted.

Abstract

Background. Information on epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) as neoadjuvant therapy in non-small cell lung cancer (NSCLC) is scarce. We evaluated whether neoadjuvant erlotinib improves operability and survival in patients with stage IIIA-N2 *EGFR* mutation-positive NSCLC.

Methods. We conducted a prospective, single-arm, phase II study. Patients received erlotinib 150 mg per day for 56 days in the neoadjuvant period. The primary endpoint was the radical resection rate.

Results. Nineteen patients were included in the final analysis. After erlotinib treatment, 14 patients underwent surgery. The radical resection rate was 68.4% (13/19) with a 21.1% (4/19) rate of pathological downstaging. The objective response rate was 42.1%; 89.5% (17/19) of patients achieved disease control, with a 10.3-month median disease-free survival among patients who underwent surgery. Among all 19 patients who received neoadjuvant therapy, median progression-free survival (PFS) and overall survival were 11.2 and 51.6 months, respectively. Adverse events (AEs) occurred in 36.8% (7/19) of patients, with the most common AE being rash (26.3%); 15.8% experienced grade 3/4 AEs. Quality of life (QoL) improvements were observed after treatment with erlotinib for almost all QoL assessments. Effects of *TP53* mutation on prognosis were evaluated in eight patients with adequate tissue samples. Next-generation sequencing revealed that most patients had a *TP53* gene mutation (7/8) in addition to an *EGFR* mutation. No *TP53* mutation, or very low abundance, was associated with longer PFS (36 and 38 months, respectively), whereas high abundance was associated with short PFS (8 months).

Conclusion. Neoadjuvant erlotinib was well tolerated and may improve the radical resection rate in this patient population. Next-generation sequencing may predict outcomes with preoperative TKIs. ***The Oncologist*** 2019;24:157–e64

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Discussion

Patients with stage IIIA-N2 NSCLC have a poor prognosis, especially those with *EGFR* mutations. Sufficient clinical benefit cannot be achieved with chemoradiotherapy without serious safety concerns. EGFR TKI therapy has shown good efficacy and favorable tolerability in patients with *EGFR* mutation-positive NSCLC. The TKI erlotinib is approved for first-line treatment of *EGFR* mutation-positive NSCLC; however, its usefulness as neoadjuvant therapy in patients with resectable *EGFR* mutation-positive NSCLC remains unclear.

We performed a single-arm trial to evaluate whether neoadjuvant erlotinib improves operability and survival in patients with stage IIIA-N2 *EGFR* mutation-positive NSCLC. The primary endpoint was the radical resection rate, and the estimated sample size was 30 cases. Of 44 patients diagnosed with stage IIIA-N2 NSCLC, 25 with *EGFR* mutation-positive disease were enrolled. Subsequent retesting with improved sequencing technologies reduced the sample size to 19 patients after an additional 6 patients were excluded (4 were negative for *EGFR* mutation by amplification-refractory mutation system polymerase chain reaction sequencing and 2 by next-generation sequencing [NGS]).

Most patients were female (12/19, 63.2%), the median age was 59 (range, 33–74) years, and all patients had an Eastern Cooperative Oncology Group performance status of 1 and adenocarcinoma histology. After erlotinib treatment, 73.7% of patients (14/19) underwent surgery; the radical resection rate was 68.4% (13/19). Most patients who underwent surgery benefited from neoadjuvant treatment prior to surgical resection and achieved disease control of the target lesion. The objective response rate was 42.1% (8/19) with a 21.1% (4/19) rate of clinical downstaging to T0–3N0M0; 89.5% of patients (17/19) achieved disease control after neoadjuvant therapy (Figure 1). In patients who underwent surgery, postoperative pathology showed that seven patients (50.0%) achieved partial response, seven (50.0%) achieved stable disease, and no patients had progressive disease. Among patients who underwent surgery, median disease-free survival calculated from the date of surgery and the date of neoadjuvant therapy was 10.3 months and 12.1 months, respectively. Among all patients who received neoadjuvant therapy, the median PFS and overall survival were 11.2 and 51.6 months, respectively.

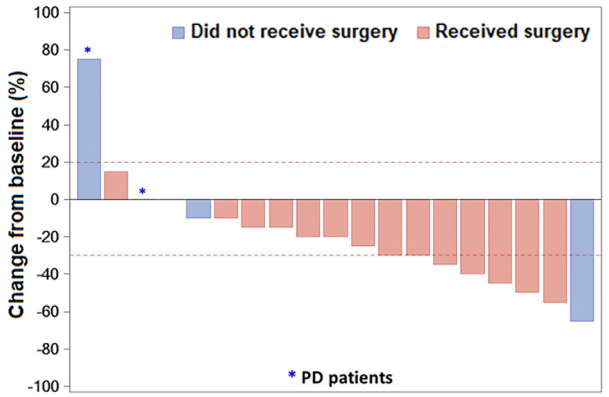

Figure 1. Waterfall plot of response to erlotinib neoadjuvant therapy. Bars show data from individual patients. Negative values suggest tumor shrinkage and positive values suggest PD; the dashed lines show the thresholds for a partial response (shrinkage by 30%) or for progressive disease (growth by 20%) according to RECIST criteria.
Abbreviation: PD, progressive disease.

To the best of our knowledge, this is the first study to evaluate the role of neoadjuvant EGFR TKI therapy with erlotinib in patients with stage IIIA-N2 *EGFR* mutation-positive NSCLC. Our findings are encouraging in view of the poor prognosis and limited treatment options available for patients with stage IIIA-N2 NSCLC.

Trial Information	
Disease	Lung cancer – NSCLC
Stage of Disease/Treatment	Neoadjuvant
Prior Therapy	None
Type of Study - 1	Phase II
Type of Study - 2	Single arm
Primary Endpoint	Radical resection rate
Secondary Endpoint	Pathological complete remission
Secondary Endpoint	Objective response rate
Secondary Endpoint	Disease-free survival
Secondary Endpoint	Overall survival
Secondary Endpoint	Quality of life
Secondary Endpoint	Safety
Secondary Endpoint	Biomarkers (exploratory)

Additional Details of Endpoints or Study Design

Patients

Patients who were at least 18 years of age with stage IIIA-N2 NSCLC, a confirmed activating mutation of *EGFR*, and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1 were eligible for this study. Tissue confirmation of N2 disease was required. Clinical and pathological stages of NSCLC were assessed in accordance with the

American Joint Committee on Cancer 7th edition TNM staging system. Diagnoses were histologically demonstrated in every case and confirmed from medical history, chest computed tomography (CT) scan, whole-body positron emission tomography scan (if not possible, abdomen CT scan and bone single-photon emission computed tomography were employed instead), or endobronchial ultrasound transbronchial needle aspiration examination. EGFR mutation testing was done by polymerase chain reaction (PCR)-based direct sequencing at screening and confirmed by amplification-refractory mutation system PCR after enrollment. Prior to study entry, all patients were evaluated by a multidisciplinary team including a medical oncologist, thoracic surgeon, radiologists, and endoscopists.

Study Design and Treatment

This was a prospective, single-arm, phase II clinical trial conducted at Shanghai Chest Hospital in Shanghai, People's Republic of China. Patients were evaluated for progressive disease (PD) after the first treatment cycle (4 weeks). Patients without PD continued with the next treatment cycle, whereas those with PD were managed with standard chemotherapy or chemoradiotherapy. Patients were evaluated by a surgical team to determine whether the tumor was resectable: a second chest CT scan was performed 56 days after erlotinib monotherapy to assess benefits from neoadjuvant therapy, but there were no predefined criteria for resectability. Patients who benefited from erlotinib and were determined to have resectable tumors received surgery followed by a platinum-based chemotherapy regimen. Follow-up evaluations were performed every 3 months for 2 years, or until death. The criteria for nodal downstaging was based on both imaging and postoperative tissue assessment. Tumor response and progression were assessed according to RECIST version 1.0. AEs were graded by the National Cancer Institute Common Terminology Criteria for AEs version 3.0. This study is registered at clinicaltrials.gov (NCT01217619, ESTERN). The planned sample size was 30 patients (to meet the primary endpoint of the radical resection rate).

Quality of Life

Quality of life was evaluated using the Functional Assessment of Cancer Therapy-Lung (FACT-L) Scale (version 4). Assessments included the lung cancer subscale (LCS), physical well-being, social (functional and emotional) well-being, and the Lung Cancer Symptom Scale. Patients completed questionnaires as part of the FACT-L Scale before the study began (day 1), in the middle of the treatment period (day 29), and after treatment with erlotinib (day 56). The trial outcome index was evaluated using physical and functional well-being and LCS subscales.

Gene Mutation Analysis by Next-Generation Sequencing

Eight patients with adequate formalin-fixed, paraffin-embedded (FFPE) tumor sections, both prior to commencing erlotinib treatment and after surgery, underwent NGS analysis. In total, 52 genes were screened on the Illumina NextSeq500 sequencing platform (Illumina, Inc., San Diego, CA); seven mutated genes related to NSCLC therapy, and 45 genes related to other cancer treatments. In all samples, more than 97% of regions were covered by >500× with mean depth over 1,200×.

Tissue DNA Extraction

DNA was extracted and isolated with the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was measured using Qubit dsDNA assay (Thermo Fisher Scientific, Waltham, MA).

Next-Generation Sequencing Library Preparation

Extracted tumor DNA was sheared using the Covaris M220 instrument (Covaris Inc., Woburn, MA), followed by end repair, phosphorylation, and adaptor ligation. Fragments sized 200–400 bp were selected using Agencourt AMPure beads (Beckman Coulter, Brea, CA) followed by hybridization with capture probes baits, hybrid selection with magnetic beads, and PCR amplification. A bioanalyzer high-sensitivity DNA assay was performed to assess the quality and size of the fragments, and indexed samples were sequenced on the NextSeq500 sequencer with pair-end reads.

Sequencing Data Analysis

The sequencing data in the FASTQ format were mapped to reference sequence hg19 (Human Genome version 19) using Burrows-Wheeler Aligner 0.7.10. Local alignment optimization, variant calling, and annotation were performed using Genome Analysis Toolkit 3.2, MuTect, and VarScan, respectively. DNA translocation analysis was performed using both Tophat2 and Factera 1.4.3. Gene-level copy number variation was assessed using a *t* test statistic after normalizing read depths at each region by total reads number and region size, and correcting GC bias using a LOESS model.

Panel Design

The panel consisted of all exons and critical introns of 56 NSCLC-related genes, spanning 330 kb of human genome. All driver genes and genes associated with targeted therapies (not limited to NSCLC) were included in the panel.

Statistical Methods

Overall response rate was defined as the proportion of patients who achieved a complete response or a partial response. Disease-free survival (DFS) was defined as the time from the date of surgery to tumor recurrence or death from any cause, whichever occurred earlier. PFS was defined as the time from the date of neoadjuvant treatment to the first date of disease progression or death. Overall survival was defined as the time from the date of diagnosis to death from any cause. Efficacy analyses were based on the intention-to-treat (ITT) population, which comprised enrolled patients with confirmed EGFR mutation who received at least one dose of erlotinib. Therefore, response rate, radical resection rate, PFS, and overall survival were analyzed in the ITT population, and DFS was analyzed in patients who underwent surgery.

Continuous variables were summarized by mean, standard deviation, median, minimum and maximum, and binary variables were summarized by frequencies and percentages. Student's *t* test was used to assess change from baseline in quality of life measures, and an analysis of covariance model was used to assess the mean carcinoembryonic antigen differences between patients who did and did not have surgery. All *p* values were two-sided. Median DFS and median PFS were calculated using the Kaplan-Meier method. SAS 9.2 software (SAS Institute, Cary, NC) was used for statistical analyses.

Investigator's Analysis

Active and should be pursued further.

Drug Information	
Drug 1	
Generic/Working Name	Erlotinib
Trade Name	Tarceva
Company Name	Roche Pharmaceuticals
Drug Type	Small molecule
Drug Class	EGFR
Dose	150 milligrams (mg) per flat dose
Route	Oral (po)
Schedule of Administration	150 mg per day for two 4-week cycles

Patient Characteristics	
Number of Patients, Male	7
Number of Patients, Female	12
Stage	IIIA
Age	Median (range): 59 (33–74) years
Number of Prior Systemic Therapies	Median (range): 0 (treatement-naive)
Performance Status: ECOG	0 — 1 — 19 2 — 3 — Unknown —
Other	Smoking/nonsmoking: 7/12
Cancer Types or Histologic Subtypes	Histology (adenocarcinoma), *n* = 19 (100%) EGFR mutation (exon 19 deletion), *n* = 12 (63%) EGFR mutation (exon 21 L858R mutation), *n* = 8 (42%)

Primary Assessment Method	
Number of Patients Screened	155
Number of Patients Enrolled	19
Number of Patients Evaluable for Toxicity	19
Number of Patients Evaluated for Efficacy	19
Evaluation Method	RECIST version 1.1
Response Assessment CR	*n* = 0 (0%)
Response Assessment PR	*n* = 8 (42%)
Response Assessment SD	*n* = 9 (47%)
Response Assessment PD	*n* = 2 (11%)
Response Assessment OTHER	*n* = 0 (0%)
(Median) Duration Assessments PFS	11 months
(Median) Duration Assessments OS	52 months

Adverse Events

See Table 5.

Serious Adverse Events

Name	Grade	Attribution
Cerebral infarction	4	Unrelated
Acute abnormal liver function	4	Unlikely

Assessment, Analysis, and Discussion	
Completion	Study completed
Investigator's Assessment	Active and should be pursued further

Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases and more than 15% of patients with NSCLC present with locally advanced clinical stage IIIA-N2 disease [1]. Patients with stage IIIA-N2 NSCLC whose tumors harbor epidermal growth factor receptor (EGFR) mutations are particularly challenging to treat and have a poor prognosis despite preoperative chemoradiotherapy [2]. Several phase III randomized controlled trials evaluated EGFR tyrosine kinase inhibitor (TKI) therapy in patients with *EGFR* mutation-positive NSCLC and demonstrated good efficacy and a favorable tolerability profile for EGFR TKI therapy in these patients [3–12]. Erlotinib was one of the first TKIs approved for the initial treatment of *EGFR* mutation-positive NSCLC. In this setting, first-line erlotinib therapy demonstrated superior efficacy over standard chemotherapy, with a more favorable tolerability profile, without the life-threatening toxicities associated with chemotherapies [11, 13, 14]. However, there is no consensus on the optimal treatment strategy for patients with stage IIIA-N2 NSCLC, including those with *EGFR* mutation-positive disease. The efficacy and tolerability of preoperative EGFR TKI therapy, although promising, requires further research. Therefore, this clinical trial aimed to evaluate whether erlotinib, as a neoadjuvant therapy, improves operability and overall survival (OS) in patients with stage IIIA-N2 *EGFR* mutation-positive NSCLC.

In total, 155 patients were screened from July 2011 to June 2014; 44 were diagnosed with stage IIIA-N2 NSCLC. Of these 44 patients, 25 patients with *EGFR* mutation-positive disease were enrolled. Next-generation sequencing (NGS) was repeated during the 3-year recruitment period, during which four patients were found to be negative for *EGFR* mutation (amplification-refractory mutation system polymerase chain reaction sequencing) and removed from the study. Further NGS analysis reduced our sample to 19 patients with activating *EGFR* mutations eligible for inclusion in the study (Fig. 1). Most patients (73.7%; 14/19) underwent surgical resection. Baseline demographic and clinical characteristics are shown in Table 1.

The tumor was resectable in 14 of 19 patients who received neoadjuvant erlotinib, and the radical resection rate in the overall population was 68.4% (13/19; Table 2). Among surgical patients, 35.7% of patients (5/14) had pathological downstaging from N2 to N1/N0, and 50% (7/14) experienced a reduction in tumor size (Table 3). The disease control rate was 89.5% (17/19) after neoadjuvant therapy. A waterfall plot of response to erlotinib neoadjuvant therapy is shown in Figure 2. There was a greater change from baseline in carcinoembryonic antigen level among patients who underwent surgery versus those who did not (Table 4). At the end of follow-up, most patients received platinum-based adjuvant chemotherapy (maximum six cycles, mean three cycles); no patients received postoperative radiotherapy. The Kaplan-Meier curves of disease-free survival, progression-free survival (PFS), and OS are shown in Figure 3.

To date, the selection of an optimal treatment regimen for patients with stage IIIA-N2 NSCLC remains controversial, and there is no standard of care for these patients. Our results suggest that neoadjuvant erlotinib improved the likelihood of surgery and was associated with a good resection rate as shown by the high rate of prospective downstaging in patients with stage IIIA-N2 NSCLC. In a study by Noh et al., 52.3% of patients with stage IIIA-N2 NSCLC experienced progressive disease with concurrent chemoradiotherapy, and 7 of 65 patients died from pulmonary radiation therapy-related adverse events (AEs). In this study, the authors reported a resection rate of 86.4% (456/528) among patients judged as medically operable [15]. Dy et al. found that chemotherapy achieved a relatively positive induction response and a radical resection rate of 94% among patients with early-stage IB to IIIA NSCLC who underwent surgery [16]. A small study of patients with resectable IIIA-N2 NSCLC that compared erlotinib versus gemcitabine/carboplatin as neoadjuvant therapy reported response rates of 58.3% versus 25.0% (p = .18), and 54.2% (13/24) patients underwent surgical resection [17]. Compared with these studies, our study demonstrated a strong disease control rate (89.5%) and downstage tumor rate (21.1%). Of note, among the 14 patients who underwent surgery, all but one (93%, 13/14) underwent radical resection. Importantly, patients achieved a median PFS of 11.2 months and median OS of 51.6 months. Taken together, these findings support the use of erlotinib prior to surgery to improve the radical resection rate and long-term survival of patients with IIIA-N2 NSCLC.

Erlotinib as neoadjuvant therapy was well tolerated. AEs occurred in 36.8% (7/19) of patients (Table 5). No patients died from treatment-related AEs, and only one patient had a grade 4 AE; these findings are comparable with those of a previous study [13]. A comparison of Functional Assessment of Cancer Therapy-Lung and Lung Cancer Symptom Scale scores before and after erlotinib treatment demonstrated improved quality of life after treatment with neoadjuvant erlotinib (Table 6).

Adequate tissue samples from before neoadjuvant therapy and after surgery were available for eight patients only. Tumor samples for these patients were analyzed for 68 genes by NGS (Tables 7 and 8 and Fig. 4). Patients who benefited most from neoadjuvant erlotinib therapy (cases 6 and 7), with PFS lasting over 36 months, had no *TP53* mutation or very low *TP53* abundance. Conversely, the patient with the greatest *TP53* abundance (case 8) had limited benefits in PFS (8 months) and OS (16 months). These findings indicate that additional biomarkers may play an important role in predicting response to TKI therapy in the neoadjuvant setting. These results are consistent with, and supportive of, previous

findings [18], suggesting that *TP53* gene mutation is independently associated with shorter OS in NSCLC.

We acknowledge that the small sample size of our study represents a major limitation. We recruited a specific subset of patients with NSCLC who, in addition to having stage IIIA-N2 disease, were positive for activating *EGFR* mutations. Our center is one of the largest for clinical lung cancer research in China, yet we encountered challenges with recruiting patients. Nevertheless, we believe that the findings from this study raise important clinical questions and will be helpful for informing larger controlled trials in the future.

In our study, neoadjuvant erlotinib was well tolerated and improved the rate of radical resection in patients with stage IIIA-N2 *EGFR* mutation-positive NSCLC. NGS analysis allowed us to identify markers that may help predict the clinical course of IIIA-N2 NSCLC. If of predictive value, markers of *TP53* abundance could be used to guide preoperative application of TKIs. Given our findings and those of other small studies, we look forward to large-scale randomized controlled trials investigating the role of TKIs in perioperative therapy, combining neoadjuvant and adjuvant treatments to enhance personalized therapy for patients in this precision medicine era.

Acknowledgments

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Disclosures

Baohui Han: Boehringer Ingelheim (C/A), AstraZeneca (speaker's bureau). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

References

1. National Cancer Institute, National Institutes of Health. Surveillance, Epidemiology and End Results (SEER) [database]. Available at https://seer.cancer.gov/about/.

2. Ahn HK, Choi YL, Han JH et al. Epidermal growth factor receptor mutation and treatment outcome of mediastinoscopic N2 positive non-small cell lung cancer patients treated with neoadjuvant chemoradiotherapy followed by surgery. Lung Cancer 2013;79:300–306.

3. Chen G, Feng J, Zhou C et al. Quality of life (QoL) analyses from OPTIMAL (CTONG-0802), a phase III, randomised, open-label study of first-line erlotinib versus chemotherapy in patients with advanced EGFR mutation-positive non-small-cell lung cancer (NSCLC). Ann Oncol 2013;24:1615–1622.

4. Costa C, Molina MA, Drozdowskyj A et al. The impact of EGFR T790M mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EURTAC trial. Clin Cancer Res 2014;20:2001–2010.

5. Fukuoka M, Wu YL, Thongprasert S 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;29:2866–2874.

6. Han JY, Park K, Kim SW et al. First-SIGNAL: First-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol 2012;30:1122–1128.

7. Maemondo M, Inoue A, Kobayashi K et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. New Engl J Med 2010;362:2380–2388.

8. Mitsudomi T, Morita S, Yatabe Y et al.; West Japan Oncology Group. 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;11:121–128.

9. Sequist LV, Yang JC, Yamamoto N et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013;31:3327–3334.

10. Wu YL, Zhou C, Hu CP 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;15:213–222.

11. Wu YL, Zhou C, Liam CK 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;26:1883–1889.

12. Yang JJ, Zhou Q, Yan HH et al. A randomized controlled trial of erlotinib versus gefitinib in advanced non-small-cell lung cancer harboring EGFR mutations (CTONG0901). J Thorac Oncol 2015;(suppl 2)10:16.13A.

13. Rosell R, Carcereny E, Gervais R et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239–246.

14. Zhou C, Wu YL, Chen G 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;12:735–742.

15. Noh JM, Ahn YC, Lee H et al. Definitive bimodality concurrent chemoradiotherapy in patients with inoperable N2-positive stage IIIA non-small cell lung cancer. Cancer Res Treat 2015;47:645–652.

16. Dy GK, Bogner PN, Tan W et al. Phase II study of perioperative chemotherapy with cisplatin and pemetrexed in non-small-cell lung cancer. J Thorac Oncol 2014;9:222–230.

17. Zhong W, Yang X, Yan H 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.

18. Molina-Vila MA, Bertran-Alamillo J, Gascó A et al. Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. Clin Cancer Res 2014;20:4647–4659.

Figures and Tables

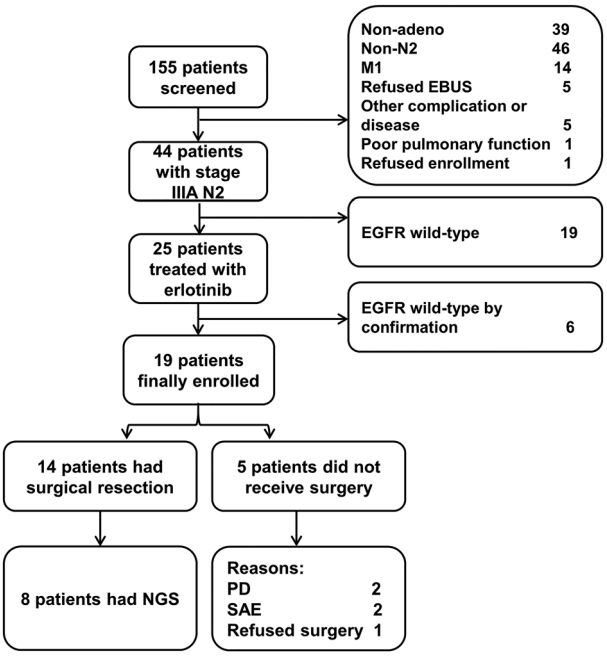

Figure 2. Patient flowchart.

Abbreviations: EBUS, endobronchial ultrasound; EGFR, epidermal growth factor receptor; NGS, next-generation sequencing; PD, progressive disease; SAE, serious adverse event.

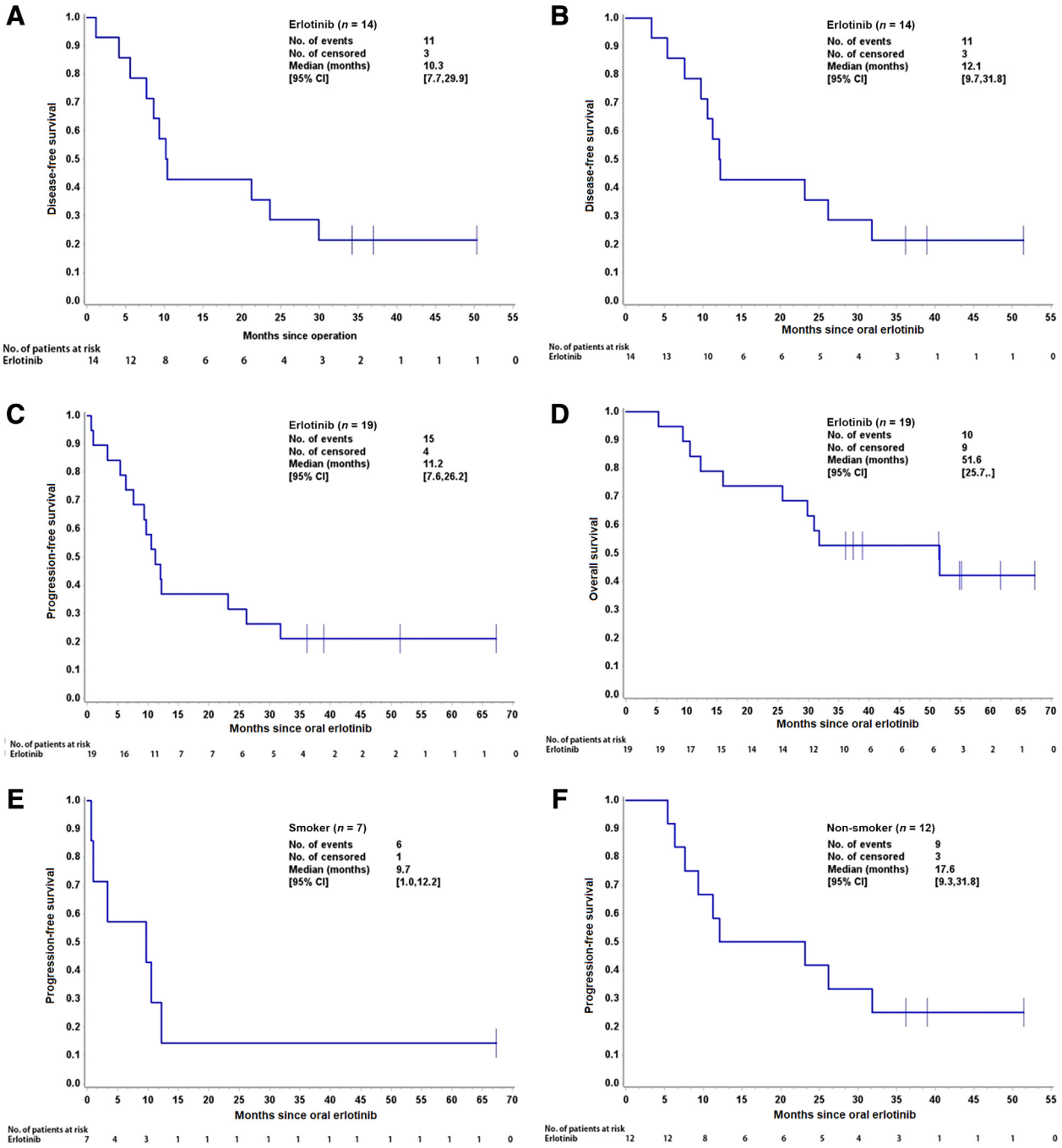

Figure 3. Kaplan-Meier curves of disease-free survival (DFS), progression-free survival (PFS), and overall survival (OS). **(A):** DFS for 14 patients who underwent surgery (calculated from the date of oral EGFR tyrosine kinase inhibitor to the first date of disease progression). **(B):** The DFS time of 14 patients who had surgery (calculated from the date of operation to the first date of disease progression). **(C):** PFS for all 19 patients. **(D):** OS for all 19 patients. **(E):** PFS for patients who were smokers. **(F):** PFS for patients who were nonsmokers.

Abbreviation: CI, confidence interval.

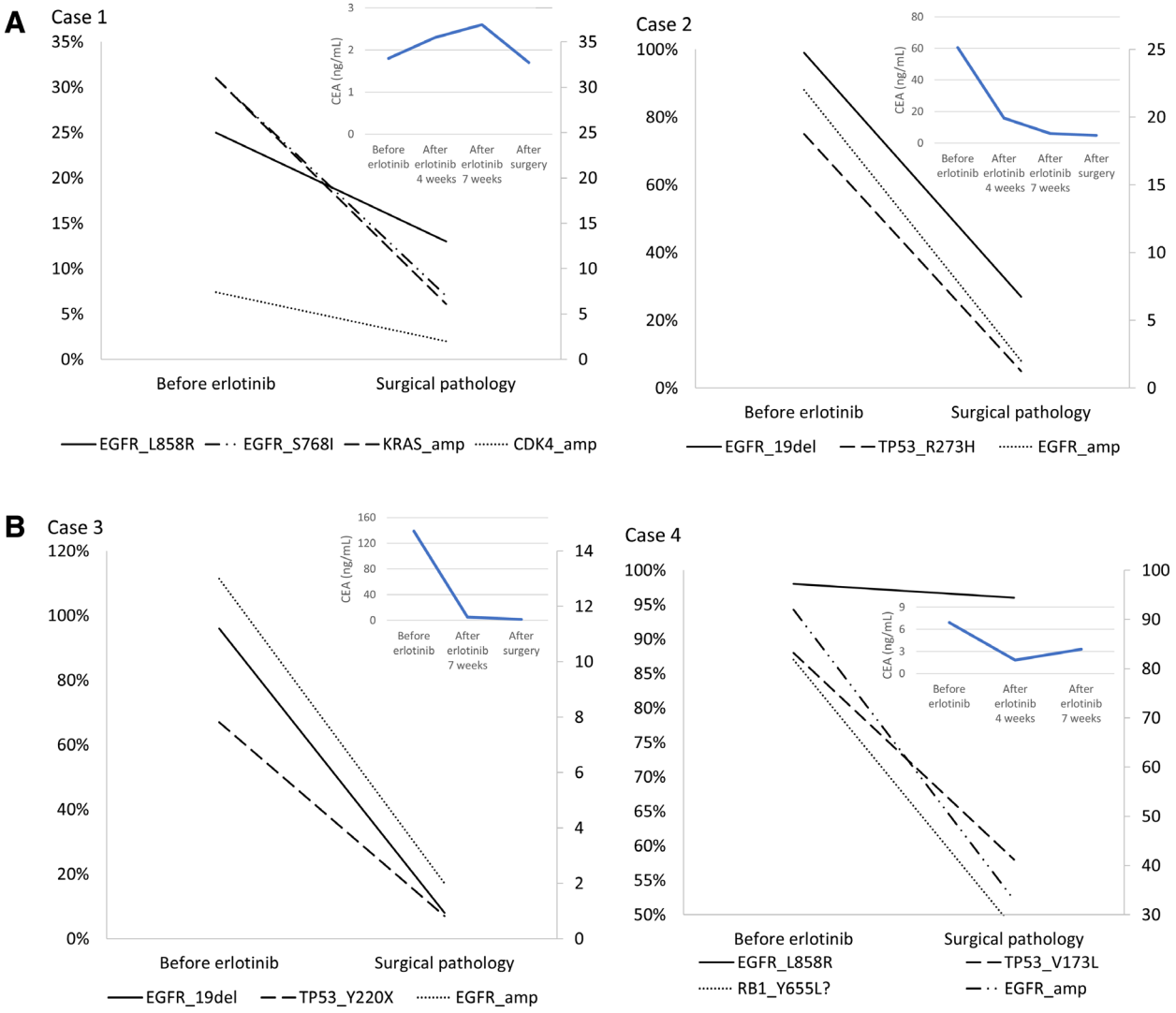

Figure 4. Next-generation sequencing analysis of mutation abundance and CEA level for eight cases **(A)–(D)**.
Abbreviations: CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor.

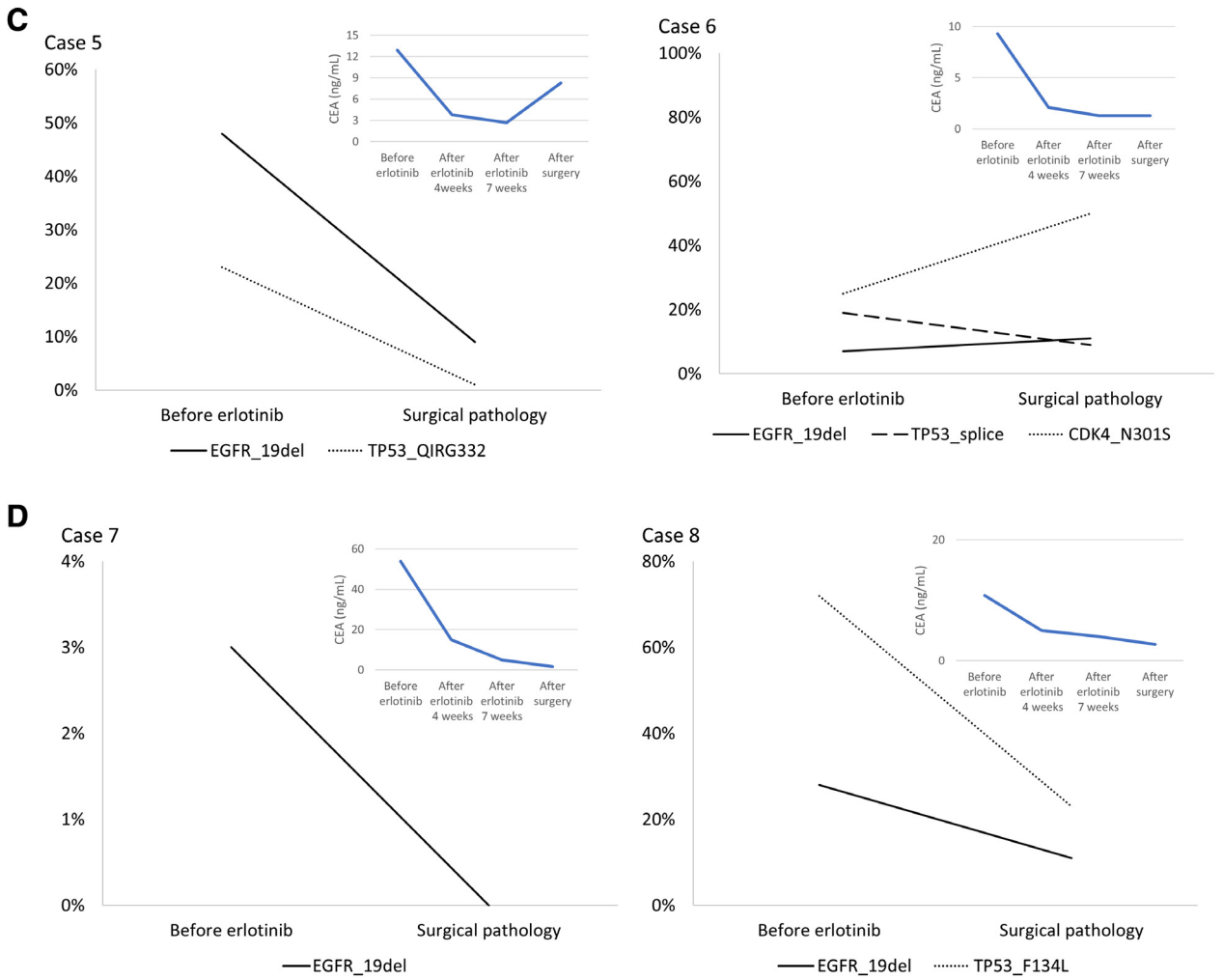

Figure 4. (Continued).

Table 1. Baseline demographic and clinical characteristics

Parameter	ITT population (n = 19), n (%)	No surgery (n = 5), n (%)	Surgery (n = 14), n (%)
Age, y, median (range)	59 (33–74)	55 (33–74)	61.5 (38–73)
Sex, *n*			
Male	7	3	4
Female	12	2	10
ECOG performance status 1	19 (100)	5 (100)	14 (100)
Histology: adenocarcinoma	19 (100)	5 (100)	14 (100)
Smoking status, *n*			
Smoking	7	3	3
Nonsmoking	12	2	11
EGFR mutation type[a]			
Exon 19 deletion	12 (63.2)	3 (60.0)	9 (64.3)
Exon 21 L858R mutation	8 (42.1)[a]	3 (60.0)[a]	5 (35.7)

[a]One patient had exon 19 deletion and exon 21 L858R point mutation.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; ITT, intention-to-treat.

Table 2. Response and radical resection rates.

Outcome	ITT (n = 19), n (%)	Surgery (n = 14), n (%)
Radical resection rate	13 (68.4)	13 (92.9)
Pathological downstaging	4 (21.1)	4 (28.6)
Partial response	8 (42.1)	7 (50.0)
Stable disease	9 (47.4)	7 (50.0)
Progressive disease	2 (10.5)	0 (0)
Objective response rate	8 (42.1)	7 (50.0)

The median (range) time from surgery to discharge was 8 (3–15) days.
Abbreviation: ITT, intention-to-treat.

Table 3. TNM stage list for patients who underwent surgical resection.

Patients	Before erlotinib	After surgery	Stage	Downgrade N	Downgrade T
1	T2bN2M0	T2aN1M0	IIA	N1	T2a
2	T2bN2M0	T2aN2M0	IIIA		T2a
3	T2bN2M0	T3N0M0	IIIA	N0	
4	T2aN2M0	T2aN2M0	IIIA		
5	T2aN2M0	T2aN3M0	IIIB		
6	T2aN2M0	T2aN2M0	IIIA		
7	T2aN2M0	T1aN2M0	IIIA		T1a
8	T2aN2M0	T1aN2M0	IIIA		T1a
9	T2aN2M0	T2aN0M0	IIB	N0	
10	T2bN2M0	T2aN2M0	IIIA		T2a
11	T2aN2M0	T2aN2M0	IIIA		
12	T2aN2M0	T1bN0M0	IA	N0	T1b
13	T2aN2M0	T3N2M0	IIIA		
14	T2aN2M0	T1bN0M0	IA	N0	T1b

Abbreviation: TNM, tumor, node, metastasis (cancer staging).

Table 4. Trend of serum CEA level before and after neoadjuvant erlotinib treatment.

CEA (ng/mL)	Before erlotinib mean ± SD (max, min)	After 4 weeks mean ± SD (max, min)	After erlotinib mean ± SD (max, min)	After surgery	Model-based estimate, mean ± SD (%)[a]
All patients (n = 19)	94.76 ± 160.78 (489.22, 0.95)	44.66 ± 100.06 (386.06, 1.28)	31.98 ± 67.64 (254.83, 1.34)		
Nonsurgery population (n = 5)	188.84 ± 251.64 (489.22, 0.95)	113.78 ± 166.10 (386.06, 1.28)	162.34 ± 130.80 (254.83, 69.86)		−7 ± 58
Surgery population (n = 14)	61.18 ± 108.12 (415.08, 1.80)	13.24 ± 22.46 (78.90, 1.83)	10.26 ± 15.76 (57.49, 1.34)	3.2 ± 2.36 (8.30, 1.34)	−60 ± 30

[a]Estimate of change in CEA level from baseline after 4 weeks. Between-group difference in change was −53%; p = .031.
Abbreviations: CEA, carcinoembryonic antigen; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; SD, standard deviation.

Table 5. Adverse events during erlotinib therapy[a]

Adverse events	CTCAE grade 1, *n* (%)	CTCAE grade 2, *n* (%)	CTCAE grade 3, *n* (%)	CTCAE grade 4, *n* (%)
Skin and subcutaneous tissue disorders				
Rash	5 (26.3)	—	—	—
Gastrointestinal disorders				
Diarrhea	1 (5.3)	—	—	—
Hepatobiliary disorders				
Abnormal liver function	—	—	—	1 (5.3)[b]
Blood and lymphatic system disorders				
Leukopenia	—	—	1 (5.3)	—
Vascular disorders				
Cerebral infarction	—	—	—	1 (5.3)[c]

[a]Adverse events occurred in 36.8% (7/19) of patients.
[b]Patient had hepatitis.
[c]Adverse event was not related to erlotinib induction.
Abbreviations: —, not occurred; CTCAE, Common Terminology Criteria for Adverse Events.

Table 6. Quality of life in all patients and in patients who underwent surgery

	All patients (*n* = 19)		Surgery patients (*n* = 14)		
Quality of life scores	Baseline mean (95% CI)	Day 29 mean (95% CI)	Baseline mean (95% CI)	Day 29 mean (95% CI)	Before surgery mean (95% CI)
FACT-L	78.3(73.4, 83.3)	93.6(82.2, 105.1)	79.3(73.3, 85.4)	96.9(83.8, 110.1)	91.8(78.3, 105.3)[a]
TOI	47.4(44.4, 50.5)	59.3(52.0, 66.6)	48.5(45.0, 52.0)	62.3(54.1, 70.4)	57.8(49.3, 66.2)[a]
LCSS	26.0(22.9, 29.0)	25.3(19.6, 31.1)	25.8(21.9, 29.7)	20.2(16.5, 23.9)	21.5(16.2, 26.9)[b]

[a]FACT-L data were missing for two patients prior to surgery.
[b]LCSS data were missing for one patient prior to surgery.
Abbreviations: CI, confidence interval; FACT-L, Functional Assessment of Cancer Therapy-Lung; LCSS, Lung Cancer Symptom Scale; TOI, Trial Outcome Index.

Table 7. List of 68 genes in next-generation sequencing analysis

Gene symbol	Gene name	Target region
AKT1	V-akt murine thymoma viral oncogene homolog 1	Whole exons
ALK	Anaplastic lymphoma receptor tyrosine kinase	Whole exons plus intron 19
APC	Adenomatous polyposis coli	Whole exons
AR	Androgen receptor	Whole exons
ARAF	A-Raf proto-oncogene, serine/threonine kinase	Whole exons
ATM	ATM serine/threonine kinase	Whole exons
BCL2L11	BCL2 like 11	Intron 2
BRAF	B-Raf proto-oncogene, serine/threonine kinase	Whole exons
BRCA1	Breast cancer 1	Whole exons
BRCA2	Breast cancer 2	Whole exons
CCND1	Cyclin D1	Whole exons
CD74	CD74 molecule	Whole exons
CDK4	Cyclin-dependent kinase 4	Whole exons
CDK6	Cyclin-dependent kinase 6	Whole exons
CDKN2A	Cyclin-dependent kinase inhibitor 2A	Whole exons
CTNNB1	Catenin beta 1	Whole exons
CYP2C19	Cytochrome P450 family 2 subfamily C member 19	rs4244285; rs4986893
CYP2D6	Cytochrome P450 family 2 subfamily D member 6	rs1065852; rs1135840; rs1058164
CYP3A4	Cytochrome P450 family 3 subfamily A member 4	rs55951658; rs4985908; rs28371759
DDR2	Discoidin domain receptor tyrosine kinase 2	Whole exons

(continued)

Table 7. (continued)

Gene symbol	Gene name	Target region
DPYD	Dihydropyrimidine dehydrogenase	rs3918290; rs1801159
EGFR	Epidermal growth factor receptor	Whole exons
ERBB2	Erb-b2 receptor tyrosine kinase 2	Whole exons
ERBB3	Erb-b2 receptor tyrosine kinase 3	Whole exons
ERBB4	Erb-b2 receptor tyrosine kinase 4	Whole exons
ESR1	Estrogen receptor 1	Whole exons
FGF19	Fibroblast growth factor 19	Whole exons
FGF3	Fibroblast growth factor 3	Whole exons
FGF4	Fibroblast growth factor 4	Whole exons
FGFR1	Fibroblast growth factor receptor 1	Whole exons
FGFR2	Fibroblast growth factor receptor 2	Whole exons
FGFR3	Fibroblast growth factor receptor 3	Whole exons plus introns 17–19
FLT3	Fms-related tyrosine kinase 3	Whole exons
HRAS	Harvey rat sarcoma viral oncogene homolog	Whole exons
IDH1	Isocitrate dehydrogenase 1 (NADP+)	Whole exons
IDH2	Isocitrate dehydrogenase 2 (NADP+), mitochondrial	Whole exons
IGF1R	Insulin-like growth factor 1 receptor	Whole exons
JAK1	Janus kinase 1	Whole exons
JAK2	Janus kinase 2	Whole exons
KDR	Kinase insert domain receptor	Whole exons
KIT	KIT proto-oncogene receptor tyrosine kinase	Whole exons
KRAS	Kirsten rat sarcoma viral oncogene homolog	Whole exons
MAP2K1	Mitogen-activated protein kinase kinase 1	Whole exons
MET	MET proto-oncogene, receptor tyrosine kinase	Whole exons
MTOR	Mechanistic target of rapamycin (serine/threonine kinase)	Whole exons
MYC	V-myc avian myelocytomatosis viral oncogene homolog	Whole exons
NF1	Neurofibromin 1	Whole exons
NOTCH1	Notch 1	Whole exons
NRAS	Neuroblastoma RAS viral	Whole exons
NTRK1	Neurotrophic tyrosine kinase, receptor, type 1	Whole exons plus intron 9
NTRK2	Neurotrophic tyrosine kinase, receptor, type 2	Whole exons
NTRK3	Neurotrophic tyrosine kinase, receptor, type 3	Whole exons
PDGFRA	Platelet-derived growth factor receptor alpha	Whole exons
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	Whole exons
PTCH1	Patched 1	Whole exons
PTEN	Phosphatase and tensin homolog	Whole exons
RAF1	Raf-1 proto-oncogene, serine/threonine kinase	Whole exons
RB1	Retinoblastoma 1	Whole exons
RET	Ret proto-oncogene	Whole exons plus intron 11
ROS1	ROS proto-oncogene 1, receptor tyrosine kinase	Whole exons plus introns 31–35
SMAD4	SMAD family member 4	Whole exons
SMO	Smoothened, frizzled class receptor	Whole exons
STK11	Serine/threonine kinase 11	Whole exons
TOP2A	Topoisomerase (DNA) II alpha	Whole exons
TP53	Tumor protein p53	Whole exons
TSC1	Tuberous sclerosis 1	Whole exons
TSC2	Tuberous sclerosis 2	Whole exons

Table 8. Clinical information of next-generation sequencing analysis

Case	Sex	Smoker	Tumor diameter, cm			LN	Evaluation response		PFS, mo	OS, mo
			Before erlotinib	After erlotinib	Surgical pathology	After surgery	After erlotinib	After surgery		
1	M	Yes	4.8	4.2	4	N0	SD	p-SD	12	55
2	M	Yes	3	2.3	3.5	N2	SD	p-SD	10	52
3	F	No	3.8	2	2	N2	PR	p-PR	11	26
4	F	No	4.3	3.8	3	N0	SD	p-PR	27	30
5	F	No	2.7	3.1	2.9	N2	SD	p-SD	12	39
6	F	No	3.1	1.8	2.1	N0	PR	p-PR	>38	39
7	F	No	5	2.2	3	N0	PR	p-PR	>36	36
8	F	No	5.4	2.8	3.5	N2	PR	p-PR	8	16

Abbreviations: F, female; M, male; OS, overall survival; p, pathological; PFS, progression-free survival; PR, partial response; SD, stable disease; LN: lymph node.