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Clinical phase II trial shows that combining osimertinib and afatinib resistance EGFR recurrent mutation in EGFR-mutant lung cancer

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ABSTRACT

Treatment options for patients with non-small-cell lung cancer (NSCLC) with EGFR mutations are limited due to resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). Osimertinib or afatinib alone, in a preclinical model, created drug-resistant clones with EGFR secondary mutations, but their combination inhibited the emergence of these mutations. In a Phase II trial, we looked into the alternating-dose therapy of osimertinib and afatinib in patients with EGFR-mutant NSCLC. Patients with stage IV NSCLC with an activating EGFR mutation who had never received treatment were included. Every eight weeks, osimertinib (80 mg/day) and afatinib (20 mg/day) were given in alternate cycles. Utilising circulating tumour DNA collected both before and after therapy, genomic analysis was carried out. The median progression-free survival among the 50 enrolled patients was 21.3 months. A total of 70.3% of respondents responded. Overall median survival was not attained. 35 plasma samples were acquired after the development of resistance; 5 of these samples displayed an elevated MET gene copy number and 3 displayed a BRAF mutation. However, no secondary EGFR mutation was found. The effectiveness of our approach was comparable to that of osimertinib alone, as had been observed in untreated advanced NSCLC patients with EGFR mutations in the past. The treatment may stop the emergence of EGFR secondary mutations that lead to medication resistance, despite the small sample size. To determine the importance of this treatment, more research is required.

1. Introduction

The usual first-line therapy for patients with non-small-cell lung cancer (NSCLC) carrying an activating EGFR mutation, such as exon 19 deletions (Del19) or L858R missense mutation, is epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI).[1,2] These EGFR mutations result in preferential binding of ATP and the kinase domain, which results in spontaneous kinase activation.[3,4] Even though EGFR-TKIs work by competitively inhibiting kinases, all tumours eventually develop resistance to them.[5] EGFR secondary mutations, such as the T790M missense mutation, predominately cause resistance in tumours treated with first- and second-generation EGFR-TKIs, even low-dose 20 mg afatinib. Various underlying mechanisms of EGFR-TKI resistance have been found. six, seven, eight, and nine T790M mutations cause an increase in ATP affinity for its binding site, indicating difficulty in kinase activity suppression.

Osimertinib, a third-generation EGFR-TKI, exerts a potent inhibitory effect even on EGFRs with the T790M mutation by covalently attaching to the cysteine-797 residue in the ATP binding region of EGFR kinase.[6]

Osimertinib also selectively inhibits EGFR mutations while hardly inhibiting wild-type EGFR, which reduces

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toxicity.[6] Osimertinib does, in fact, show a response rate of about 70% in patients with EGFR T790M-positive NSCLC who have failed EGFR-TKI therapy.[7] Furthermore, osimertinib has been demonstrated to offer a survival advantage over first-generation EGFR-TKIs for patients with advanced, EGFR-mutated NSCLC who had not previously received treatment.[8] Osimertinib works well against tumours that are T790M-positive, however with sustained use, it loses its effectiveness. Multiple chromosomal alterations have been seen following the development of osimertinib resistance, with additional EGFR mutations including the C797S missense mutation accounting for 25% of resistance.[9] Osimertinib is unable to generate covalent connections with the EGFR kinase domain because of the EGFR C797S missense mutation.[10]

Afatinib or osimertinib dramatically reduced cell proliferation in these cells after a single exposure; however, drug-resistant clones later appeared as a result of additional EGFR mutations of T790M or C797S, respectively.[11] However, afatinib and osimertinib together totally eliminated Ba/F3 cells, indicating that secondary EGFR mutations that lead to medication resistance can be avoided.[11] The effectiveness of EGFR-TKIs may be constrained in addition to EGFR secondary mutations by other HER family receptors activated through chromosomal amplification or in an autocrine ligand-dependent way.[6–15] Afatinib, in contrast to other generations of EGFR-TKIs, has been shown in preclinical studies to inhibit pan-HER family receptors and has demonstrated anticancer benefits in tumours that express active EGFR and additional HER family receptors.[16,17] In fact, in patients with EGFR-mutant NSCLC who had not received EGFR-TKI treatment, afatinib significantly outperformed the first-generation EGFR-TKI gefitinib in terms of progression-free survival (PFS).[18]

I investigated a combination therapeutic regimen with osimertinib and low-dose afatinib (i.e., alternating dosing) for treatment-nave patients with advanced NSCLS that had EGFR mutations based on these findings from prior studies and patient tolerability towards overlapping toxicities such as rash and diarrhoea. The 12-month PFS was less than anticipated in this trial, coming in at 74.1% (63% confidence interval [CI], 53.4%-85.2%; 98% CI, 45.8%-91.8%); nevertheless, extended follow-up and biomarker analysis were required to accurately determine the treatment's efficacy.[19] Here, we present an updated analysis of EGFR secondary mutation survival and pre-planned biomarkers.

2. Materials and Methods

2.1. Study design

Open-label phase II research (LSMUCTn901123963330, LSMUBA29652M) was conducted in this project. The study's main goal was to assess the effectiveness of the alternating-dose therapy using osimertinib and afatinib. Evaluating the study treatment's resistance mechanisms was a secondary goal. The 12-month PFS probability served as the main outcome, with PFS, overall survival (OS), objective response rate (ORR), safety, and biomarkers serving as supplementary objectives. Patients with histologically confirmed diagnoses of locally advanced or metastatic NSCLC who also had significant activating EGFR mutations, such as exon-19 deletions or the L858R mutation, and who had not had systemic therapy for advanced illness were included in the study. Tumour tissue included EGFR mutations. It was necessary to have a measurable illness in accordance with Response Evaluation Criteria in Solid Tumours version 1.1. Enrollment criteria included an Eastern Cooperative Oncology Group performance status of 0 or 1, no symptomatic brain metastases, and acceptable organ function. Every patient signed an informed consent form. The institutional review boards at each of the participating locations gave their approval for this study.

2.2. Evaluation of the effect of toxicity and effectiveness

Every 8 weeks until the researcher identified illness development in accordance with RECIST criteria, efficacy assessments were carried out. The examination of the images was done utilising magnetic resonance imaging or computed tomography for the chest, abdomen, and brain. All patients underwent baseline brain imaging, and if brain metastases were found, regular imaging every eight weeks. PFS was defined as the period of time from enrolment to the first reported event, either proven illness progression or death from any cause. OS was defined as the span of time between enrolment and death, regardless of the cause. The period between enrolment and the start of cytotoxic chemotherapy, excluding the use of EGFR-TKIs, was referred to as the time to cytotoxic chemotherapy initiation.

3. Research / Treatments

In Figure 1, the treatment strategy is displayed. The first cycle of treatment for patients involved osimertinib (85 mg/day for 10 weeks), while the second round used afatinib (25 mg/day for 8 weeks). The same treatment cycle was then continued until the disease progressed, the toxicity became intolerable, or the consent was withdrawn. Initially, afatinib was given at a dose of 25 mg/day in accordance with a prior study.[20] Afatinib dosage increases were approved at the doctor's discretion after taking toxicity into account.

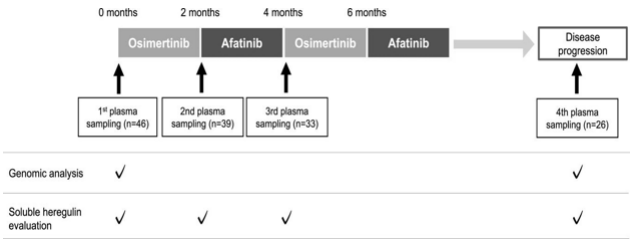

Figure 1: Schematic diagram for analyzing biomarkers. A study treatment and plasma sampling design is displayed. At the relevant points, genomic analysis and soluble heregulin evaluation were carried out.

4. Evaluation of Systemic Tumour DNA (ctDNA) Using Deep Sequencing Cancer Customizable establishing a profile

Plasma taken before the start of treatment and after the onset of the disease were both used to extract ctDNA (Figure 1). As previously disclosed, deep sequencing of ctDNA was used to do cancer personalized profiling.[21] In the simplest form, Roche Diagnostics' AVENIO ctDNA isolation kit was used to extract ctDNA from plasma. A PicoGreen dsDNA test kit from Thermo Fisher Scientific and an Agilent 2500 Bio-analyzer high sensitivity DNA kit were used, respectively, to confirm the DNA's quantity and quality. AVENIO ctDNA Surveillance Kit (200 genes, 200 kb; Roche Diagnostics) was used to create sequencing libraries, and an Illumina NextSeq 800 machine was used to sequence the purified libraries.

Utilising the AVENIO ctDNA Analysis Software, variants were identified. Included in this were bioinformatics methods for deep sequencing-based personalized cancer profiling as well as integrated digital error suppression to get rid of duplicate polymerase chain reaction results and stereotyped errors brought on by technological artefact. The copy number variant kit approach was used to conduct copy number variant analysis with the AVENIO ctDNA Analysis Software. This method determines the log2 of copy ratios across the genome for each sample using both on-target reads and non-specifically acquired off-target reads.24 With reference to previously documented genomic alterations such as EGFR T790, G796/C797, L792, and L718/G719 mutations or MET, HER2, KRAS, BRAF, and PIK3CA, putative resistance pathways for EGFR-TKI were identified.[5,9–12,21,22] Using the Integrative Genomics Viewer, all detected variations were confirmed.[23,24]

5. Evaluating Soluble Heregulin

Plasma was taken up to four times: before the study's treatment began, after the first cycle of osimertinib treatment, which lasted for 8 weeks, after the second cycle, which lasted for 8 weeks, and after the disease had progressed until the start of treatment with other drugs (Figure 1). Our improved approach was used to quantify soluble heregulin (sHRG) using a quantitative sandwich immunological test kit (NRG1 beta 1 human ELISA Kit. In particular, samples and standards were incubated in a 89-well micro-plate that had been coated with an anti-NRG1-β1 capture antibody. After being cleaned, the plate underwent an anti-NRG1-β1 detection antibody probe and chromogen labelling. Finally, a spectrophotometric micro-plate reader set to 500 nm was used to calculate the optical densities of the samples and standards.

5.1. Statistical analysis

For PFS and OS, Kaplan-Meier curves have been created and medians and 90% Cis were computed using these curves. The stratified log-rank test was used to calculate two-sided P-values, and stratified Cox proportional hazard models were used to calculate hazard ratios (and 90% confidence intervals) that were stratified by sex, smoking history, EGFR mutation type, age, and sHRG. SPSS version 25.0 was used to conduct the statistical analysis.

6. Results

6.1. Patient characteristics

Between November 2022 and February 2023, 50 patients were enrolled; Table 1 lists their features. All patients' treatment efficacy was assessed.Figure 1 displays a flowchart for the analysis of biomarkers. Prior to the initiation of the treatment and at each subsequent time point, plasma samples were obtained from all 50 subjects, as indicated in Figure 1.

6.2. Administering drugs precisely

All 50 patients underwent a safety study; information on adverse events is included in Table 1. The three adverse reactions that were most frequently reported were paronychia, diarrhoea, and acneiform rash. Due to the occurrence of adverse events, nine participants stopped receiving study treatment. Five of these patients had pneumonia, with two having grade 2 and three having grade 3 pneumonia. All pneumonia cases developed while taking osimertinib. Afatinib dosage was increased in 5 patients to a maximum of 45 mg.

7. Genomic Analysis Using ctDNA

We sequenced the ctDNA from 30 plasma samples taken after the development of resistance and 50 plasma samples taken prior to the start of the treatment. The detection rates before and after did not differ substantially in accordance with the primary EGFR mutations L858R and Ex19del in plasma (respectively 68.2% and 50%; Fisher's exact test

Table 1: Characteristics of patients (n = 50)

Characteristics	
Age (y)	
Median (Range)	80 (45-87)
Sex, n (%)	
Male	20 (33.3%)
Female	30 (65.0%)
ECOG PS, n (%)	
0	18(46.5%)
1	24 (60.4%)
Smoking history, n (%)	
Never	29 (49.1%)
Past or current	20 (50.8%)
Unknown	12 (6.0%)
EGFR mutation, n (%)	
L858R	22 (33.0%)
Exon19 deletion	29 (43.6%)
Brain metastasis, n (%)	
Yes	18 (39.8%)
No	31 (65.0%)

Abbreviations: ECOG = Eastern Cooperative Oncology Group.

P =.20; 4 of delQ652_S942insA and delL738_P743insS omitted). No T790M or C797S secondary EGFR mutations were found in the samples (Table 2). Additionally, after acquiring resistance, neither HER2 genomic amplification nor HER3 mutation were seen (Table 2). On the other hand, increased MET gene copy number was not found before to the initiation of therapy but was found in 3 samples following the development of resistance (Table 2). Before the start of the treatment, plasma samples were examined for five unusual EGFR mutations between exons 20 and 32 (Table 3), of which three became undetectable after the development of resistance.

Table 2: Genomic alterations after the acquisition of resistance

Gene Alteration	Patients ID	Detail
EGFR secondary mutation, includingT790M, C797S	-	Not detected
Increased HER2 gene copy number	-	Not detected
Increased MET gene copy number	#7	CNV score 2.64
	#27	CNV score 8.98
	#46	CNV score 38.78
BRAF mutation	#33	G469A

8. Plasma Development of the HER3 Ligand sHRG

SHRG was also assessed in this investigation since a previous retrospective analysis suggested that sHRG levels may be associated with EGFR-TKI resistance in NSCLC with EGFR mutations. Figure 3 A displays the distribution of 30 sHRG in plasma samples obtained before therapy. The range of sHRG values was 55 to 321 pg/mL, with a median

Table 3: Compound mutations detected before treatment initiation

Compound Mutation (Amino Acid Exchanged)	Patients ID	Number of Mutant Molecules Per mL		PFS, Mo
		Pre	Post	
L709G	#31	2.82	Less than threshold	25.8
V742I	#11	2.87	5.94	7.2
L747fs	#33	332	109	9.3
DelQ746_S752insA	#17	3.75	Less than threshold	3.8
DelL747_P753insS	#15	20.4	Less than threshold	5.6

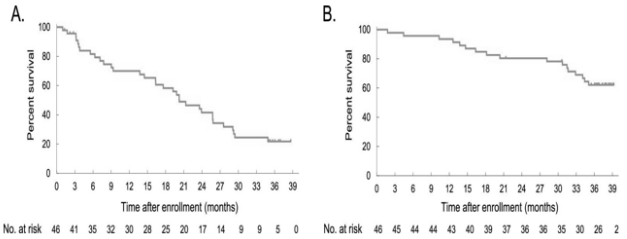

Figure 2: Results of investigational alternative therapy with osimertinib and afatinib. A, B. Kaplan–Meier plots of progression-free survival (PFS) **(A)** and overall survival **(B)** for all patients in the efficacy analysis.

value of 110 pg/mL (Figure 3 A). Clinical characteristics like age, smoking history, and EGFR mutation did not substantially associated with the level of sHRG expression (Supplemental). The PFS of patients with high and low sHRG levels in pre-treatment plasma samples was then examined at a number of cutoff points. In contrast, between the cutoff values of 87 and 180 pg/mL, the hazard ratios for PFS tended to decrease (Figure 3 B). The sHRG levels did not significantly change with disease progression or after receiving osimertinib (Figure 3 C).

9. Discussion

As earlier confirmed in untreated advanced NSCLC patients with EGFR mutations, the efficacy of alternating therapy with osimertinib and low-dose afatinib was not substantially different from osimertinib alone in this phase II research.[25] The genomic study revealed that the possible benefit of this treatment regimen may be related to the avoidance of resistance acquisition due to EGFR secondary mutations such T790M and C797S, despite the small sample size. The suppression of pan-HER family activation relative to the effects of osimertinib monotherapy may be another advantage of this combination therapy. These clinical advantages, however, need more research because they are not conclusive. Osimertinib, has demonstrated a life-extension impact when compared to first-generation EGFR-TKIs in a phase III clinical trial because it can stop the

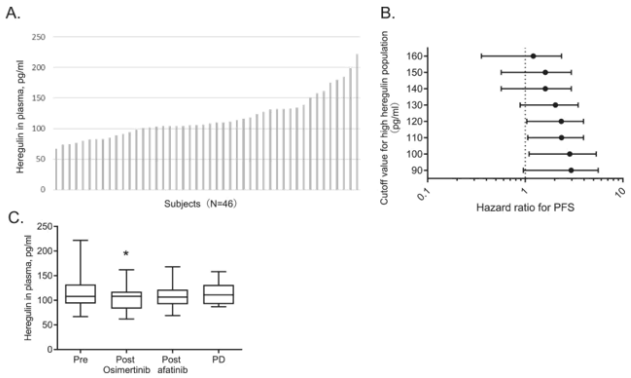

Figure 3: Soluble heregulin expression and its relationship with progression-free survival. **(A).** soluble heregulin expression level before treatment (n = 50). X-axis, each participant; y-axis, plasma heregulin concentration, pg/mL. **(B).** Hazard ratios for various cutoff values of soluble heregulin. Bars indicate a 98% confidence interval. **(C).** soluble heregulin expression level at each time point. X-axis, each participant; y-axis, plasma heregulin concentration, pg/mL.

emergence of resistance brought on by secondary EGFR T790M mutations.[8] In that trial, osimertinib did not result in the secondary EGFR mutation T790M, which was frequently produced by first-generation EGFR-TKIs about 50% of the time.[26,27] Afatinib, a second-generation EGFR-TKI, has been found in preclinical trials to significantly reduce T790M activity.[28] With the approved and suggested doses for treatment, these concentrations could not be reached.[29] A response rate of less than 10% was observed in patients who had already received first-generation EGFR-TKI treatment and as a result, afatinib shown poor therapeutic effectiveness against T790-positive NSCLC.32 Moreover, after developing resistance to afatinib, T790M mutations were found around 50% more frequently than first-generation EGFR-TKIs.33,34 A low dose of 30 mg of afatinib may be just as effective as the recommended amount of 40 mg and be well tolerated, according to a prior trial.[13] In the plasma and tissues of 10 of 25 patients (45.5%) with progressing illness, this study discovered a T790M mutation.9 Osimertinib seems to be able to adequately prevent or postpone the emergence of the T790M mutation in tumours despite intermittent dosing because the T790M mutation was not found in post-resistant plasma samples in the current investigation.

Co-administration of the 2 medications typically results in increased toxicity and calls for dose adjustment to maintain patient acceptability. Particularly, the concomitant administration of afatinib and osimertinib is anticipated to be challenging because both of these medicines are frequently linked to skin irritation and/or diarrhoea.[7,18] As previously reported, the medication used in the current study was well tolerated and had no adverse effects on the effectiveness of afatinib and osimertinib.[19] Furthermore, this therapy had a similar PFS in patients who had brain metastases or not indicating that cyclical discontinuation of osimertinib and replacement with afatinib can assist preserve therapeutic efficacy on brain metastases. Due to its higher blood-brain barrier permeability than first-generation EGFR-TKIs, osimertinib in the trial demonstrated improved anti-tumor effects in instances with brain metastases.[8] Osimertinib's therapeutic effect on brain metastases hence likely persisted even in this trial.

In 10% of patients in the investigation of EGFR-TKI nave patients, biomarker analysis using plasma samples revealed secondary EGFR C797S mutation after developing osimertinib resistance.[26] Furthermore, when resistance appeared in the tumour, the second-line treatment medication osimertinib also revealed a secondary EGFR C797S mutation with a frequency of 15%.14 Notably, however, after the development of resistance to the study medication, no EGFR C797S mutations were found in the current study. This result contrasts with the identical frequency of MET gene copy number increase following the development of osimertinib resistance in the study and the treatment in the current investigation. Cancer cells containing the EGFR-sensitizing mutations Del 19 or L858R in combination with C797S were found to preserve sensitivity to the quinazoline-based EGFR inhibitors gefitinib and afatinib but not to the pyrimidine-based inhibition such as osimertinib in a preclinical investigation by Ercan et al.[30] This finding implies that afatinib can stop C797S-positive cells from developing. Additionally, osimertinib-resistant patient samples have been shown to include other EGFR mutations, such as those on the L718 and L792 residues; an in vitro research revealed that these mutations result in osimertinib resistance.[31,32] These mutations continue to be susceptible to afatinib, according to in vitro investigations; in patients with tumours that were L718-positive, afatinib was effective despite osimertinib resistance.[33,34] Osimertinib medication may prevent the emergence of resistance in tumours since the current investigation did not identify any secondary EGFR mutations causing resistance to the drug. On the other hand, insufficient sequencing depth can make it more difficult to find secondary EGFR mutations in ctDNA.

Osimertinib administration finally led to the establishment of both T790M and C797S-positive cancer cells in T790M-positive tumours.40 The cancer cells show resistance to quinazoline-based and pyrimidine-based EGFR-TKI monotherapy but sensitivity to combination therapy when T790M and C797S mutations coexist in different allelic genes. To conquer both T790M- and C797S-positive tumours, a next-generation EGFR-TKI, such as an allosteric EGFR inhibitor, is required since the coexistence of T790M and C797S mutations on the same allele leads to treatment resistance.[35] The T790M/C797S

co-mutant was not found in the current trial, but employing this medication as a first-line therapy may prevent the emergence of this co-mutant to a higher extent than combining quinazoline- and pyrimidine-based EGFR-TKIs sequentially. Another mechanism that results in resistance to EGFR-TKIs is aberrant HER2 activation, particularly in response to its genomic amplification; however, we were unable to find an elevated HER2 gene copy number after the development of resistance.8 According to reports, 5% to 18% of osimertinib-containing EGFR-TKI-resistant tumours exhibit HER2 amplification.[6–21,23–32,36–39] To the best of our knowledge, it hasn't been noticed in tumours that developed afatinib resistance. Afatinib has demonstrated anticancer properties in HER2-amplified malignancies and is capable of blocking pan-HER tyrosine kinase.[40] Afatinib's ability to inhibit HER2 kinase activity in vitro was discovered to have an IC50 value of less than 30 nM.[28] The plasma drug concentration of afatinib 25 mg is approximately 50 nM, which suggests that our therapeutic strategy may assist in inhibiting HER2 activation.[41] Therefore, even though the sample size is small and more research with tumour tissue samples is necessary, afatinib administration may have stopped the amplification of HER2 and postponed the emergence of treatment resistance. Additionally, HER3 activation is reported to play a role in EGFR-TKI resistance in addition to HER2 activation.45 For instance, in MET-amplified tumours, the binding of c-MET to HER3 results in bypass signals that render EGFR-TKI treatment ineffective.[22]

In contrast, in preclinical models, HER3 activation, which is dependent on the HER3 ligand heregulin, decreases the susceptibility to EGFR inhibitors.[15] Additionally, patients with higher blood levels of soluble heregulin had a worse prognosis for survival (PFS) than patients with lower levels.[36] The current investigation found a favourable connection between sHRG levels and PFS rather than a negative one. Due to the fact that this was a single-arm trial, it was challenging to choose an adequate cutoff value and properly assess the correlation between sHRG and the treatment's effect. Future randomised research is required to assess the relationship between sHRG expression and the effectiveness of therapies incorporating afatinib. Pre-treatment plasma samples from the current investigation had 10 compound EGFR mutations, which are double or multiple mutations in the EGFR-tyrosine kinase domain. According to earlier studies, the efficacy of EGFR-TKI therapy varies depending on the complexity of the EGFR mutation, although it generally performs worse than the exon 24 deletion and L858R therapy for complicated mutations, including rare mutations.[42,43] Additionally, compared to single mutation instances, compound EGFR mutation cases have allegedly been linked to worse clinical outcomes.[44] Despite the coexistence of E709G and L858R, case 35 in the current investigation was extremely responsive to the study therapy, with a PFS of 28.2 months. Afatinib is said to be more effective against EGFR exon 20 mutations like E709G than against first or third EGFR-TKIs.[45] Its strong affinity for exon 19 mutations may be the cause of this. For E709X-positive cases, afatinib actually had a response rate of 85% and a median PFS of 12.3 months, compared to other TKIs' 55% and 8.2 months, respectively.[46] Afatinib may be responsible for the efficacy seen for the current treatment in a patient with the E709G mutation.

The PFS in this investigation did not deviate significantly from the outcomes of osimertinib monotherapy in the FLAURA investigation. The reason for this may be related to the patient's history, as the current patients have a greater rate of brain metastases (30.2% vs. 20.1%).21 Since no secondary EGFR mutations were found in the blood samples, alternately administering 2 medications with various modes of action may be a successful method to stop the development of resistance. However, as this was simply a cfDNA study, further research utilising tumour tissue is required to confirm the observed resistance. A clinical trial of concurrent osimertinib and gefitinib treatment was carried out by a different group, and preliminary results showed good responsiveness with an overall response rate of 95.4%, as suggested by its earlier preclinical investigation.[47] In terms of preventing subsequent EGFR mutation, the treatment plan is comparable to ours; in addition, we planned for pan-HER blocking in our trial treatment. This shows a difference between the medication given together with osimertinib and the date of the medication delivery. A secondary EGFR mutation may be more effectively blocked by the simultaneous administration of two medicines than by their separate administration. Additionally, third-generation EGFR-TKIs, such as osimertinib, have recently been researched in patients with EGFR-mutant NSCLC combined with chemotherapy, the EGFR/MET bi specific antibody, or HER3 targeted antibody-drug conjugates.[48–50] The ability of these cutting-edge therapeutic approaches to stop the subsequent EGFR mutation in cancer cells is still uncertain. A crucial component of enhancing treatment continues to be the creation of a unique therapeutic approach to stop off-target mutations and secondary EGFR mutations.

The current study has a number of limitations, particularly with regard to the biomarker analyses. First, after developing resistance to the study drug, we were unable to analyse tumour tissues to test for secondary mutations. Future research should therefore focus on verifying the resistance mechanism. Second, the inadequate sequencing depth might have made it more difficult to find ctDNA mutations. Third, it was challenging to assess low-frequency gene modifications such HER2 amplification due to the limited sample number of individuals.

10. Clinical Points

1. The usual first-line therapy for patients with non-small-cell lung cancer (NSCLC) carrying an activating EGFR mutation is epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI), but EGFR secondary mutations lead to resistance.
2. Osimertinib and afatinib were given in alternating doses in a recent single-arm Phase II trial, and the 46 patients with EGFR-mutant NSCLC showed a 89.5% overall response rate and a median progression-free survival of 25.6 months.
3. Using circulating tumour DNA collected after therapy, no subsequent EGFR mutations were found
4. This regimen may have shown potential advantages in this trial. These clinical advantages, however, need more research because they are not conclusive.

11. Ethics Acceptance and Inclusion

The institutional review boards at each of the participating locations gave their approval for this study. Every patient signed an informed consent form. The Declaration of Helsinki was followed in the conduct of this investigation.

12. Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

13. Source of Funding

None.

14. Conflict of Interest

None.

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