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Review RRJoOH

Efficacy of Combination Therapy with Osimertinib and Afatinib in epidermal growth factor receptor-mutant non-small-cell lung cancer Patients

Tshetiz Dahal[1,*], Bonish Raj Subedi[2]

Abstract

Treatment options for patients with non-small-cell lung cancer who have epidermal growth factor receptor mutations are constrained by the emergence of resistance to epidermal growth factor receptor tyrosine kinase inhibitors. Osimertinib or afatinib alone, when tested in a laboratory model, led to the formation of drug-resistant clones harboring epidermal growth factor receptor secondary mutations. However, combining these drugs prevented the emergence of such mutations. In a Phase II clinical trial, we investigated the efficacy of alternating-dose therapy involving Osimertinib and afatinib in patients diagnosed with epidermal growth factor receptor-mutant non-small-cell lung cancer. Eligible participants included individuals with stage IV non-small-cell lung cancer carrying an activating epidermal growth factor receptor mutation who had not previously undergone treatment. Every eight weeks, Osimertinib (80 mg/day) and afatinib (20 mg/day) were given in alternate cycles. Genomic analysis was carried out by utilizing circulating tumors DNA collected both before and after therapy. The median duration of progression-free survival for the 50 patients who participated in the study was 21.3 months. A total of 70.3% of respondents responded. Overall median survival was not attained. About 35 plasma samples were acquired after the development of resistance; five of these samples displayed an elevated MET gene copy number and three displayed a BRAF mutation. However, no secondary epidermal growth factor receptor mutation was found. The effectiveness of our approach was comparable to that of Osimertinib alone, as had been observed in untreated advanced non-small-cell lung cancer patients with epidermal growth factor receptor mutations in the past. The treatment may stop the emergence of epidermal growth factor receptor secondary mutations that lead to medication resistance, despite the small sample size. To determine the importance of this treatment, more research is required.

Keywords: non-small-cell lung cancer, mutation, BRAF mutation, secondary epidermal growth factor receptor mutation, efficacy, clinical trial, treatment strategy, medication resistance

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INTRODUCTION

The typical initial treatment for individuals diagnosed with non-small-cell lung cancer (NSCLC) and possessing an activating epidermal growth factor receptor (EGFR) mutation, such as exon 19 deletions (Del19) or the L858R missense mutation, involves the use of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) [1, 2]. These EGFR mutations result in preferential binding of adenosine triphosphate (ATP) and the kinase domain, which results in spontaneous kinase activation [3, 4]. Even though EGFR-TKIs work by competitively inhibiting kinases, all tumors eventually develop resistance to them. EGFR secondary mutations, including the T790M

missense mutation, predominantly lead to resistance in tumors treated with first- and second-generation EGFR-TKIs, including low-dose 20 mg Afatinib. Various underlying mechanisms contributing to EGFR-TKI resistance have been identified. The presence of six, seven, eight, and nine T790M mutations results in an increased affinity of ATP for its binding site, leading to challenges in suppressing kinase activity. Osimertinib, classified as a third-generation EGFR-TKI, demonstrates potent inhibitory effects even on EGFRs containing the T790M mutation by forming a covalent bond with the cysteine-797 residue within the ATP binding region of EGFR kinase [5–10].

Osimertinib also selectively inhibits EGFR mutations while hardly inhibiting wild-type EGFR, which reduces toxicity [11]. Osimertinib does, in fact, show a response rate of about 70% in patients with EGFR T790M-positive NSCLC who have failed EGFR-TKI therapy [12]. 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 [13]. 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 [14]. Osimertinib is unable to generate covalent connections with the EGFR kinase domain because of the EGFR C797S missense mutation [15].

Afatinib or Osimertinib significantly decreased cell proliferation in these cells upon initial exposure. However, subsequent emergence of drug-resistant clones was observed due to additional EGFR mutations, namely T790M or C797S, respectively [16]. However, Afatinib and Osimertinib together totally eliminated Ba/F3 cells, indicating that secondary EGFR mutations that lead to medication resistance can be avoided. 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 [8, 17]. 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 [18, 19]. 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) [20].

The present study investigated a combination therapeutic regimen with Osimertinib and low-dose Afatinib (that is, 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 [21]. Here, we present an updated analysis of EGFR secondary mutation survival and pre-planned biomarkers.

METHODS

Study Design

Open-label phase II research (LSMUCTn901123963330, LSMUBA29652M) was conducted in the present project.

The primary objective of the present study was to evaluate the efficacy of alternating-dose therapy involving Osimertinib and Afatinib. Investigating the resistance mechanisms of the treatment was a secondary aim. The primary outcome measure was the 12-month PFS probability, while PFS, overall survival (OS), objective response rate (ORR), safety profile, and biomarker analysis served as additional 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 present 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. Participants meeting the enrollment criteria had an Eastern Cooperative Oncology Group performance status of either 0 or 1, no symptomatic brain metastases, and demonstrated acceptable organ function. Each patient provided informed consent by signing a consent form. Approval for the study was granted by the institutional review boards at all participating sites.

Evaluation of the Effect of Toxicity and Effectiveness

Efficacy assessments were carried out every 8 weeks until the researcher identified illness development in accordance with RECIST criteria. The examination of images was done by utilising magnetic resonance imaging (MRI) or computed tomography (CT-scan) for the chest, abdomen, and brain. All patients underwent baseline brain imaging, and if brain metastases were found, regular imaging was done every eight weeks. PFS was described as the duration from enrollment until the occurrence of the initial documented event, which could be either confirmed disease progression or mortality 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.

Research / Treatments

The treatment strategy employed in the present study is displayed in Figure 1. 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 [22]. Increase in Afatinib dosage was approved at the doctor's discretion after taking toxicity into account.

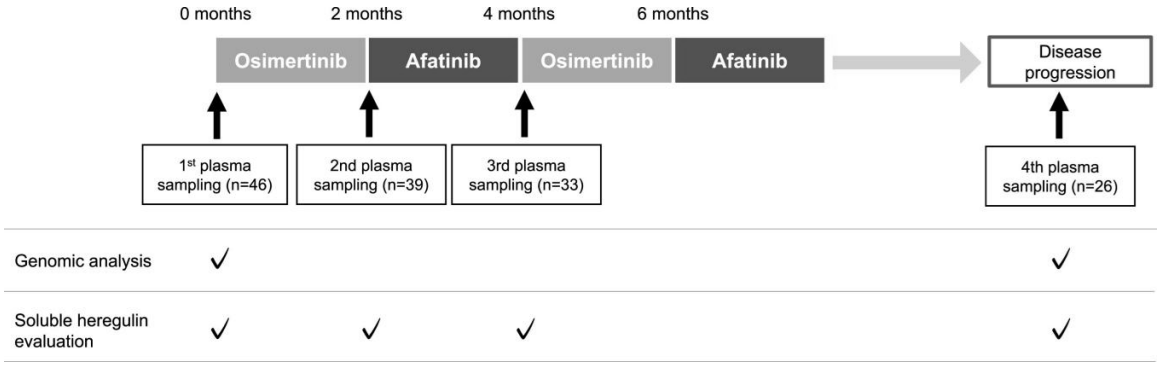

Figure 1. Schematic diagram for analyzing biomarkers. A study treatment and plasma sampling design is displayed. At the relevant points, genomic analysis and soluble heregulin evaluation were carried out.

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 perform cancer personalized profiling [23]. 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.

Variants were identified by utilising the AVENIO ctDNA Analysis Software. 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 (PCR) 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 [5–8, 14–16, 23]. All detected variations were confirmed using the Integrative Genomics Viewer [25–27].

Evaluation of 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 enhanced methodology was utilized to measure soluble heregulin (sHRG) levels by employing a quantitative sandwich immunological assay kit (NRG1 beta 1 human ELISA Kit). In particular, samples and standards were incubated in an 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.

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 statistical analysis.

RESULTS

Patient Characteristics

A total of 50 patients were enrolled between November 2022 and February 2023. Table 1 lists the features of all the enrolled patients. The treatment efficacy of all patients was assessed. Figure 1 displays a flowchart for the analysis of biomarkers. Plasma samples were obtained from all 50 subjects prior to the initiation of treatment and at each subsequent time point (Figure 1).

Table 1. Characteristics of patients (n=50).

Characteristics	
Age (in years)	
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%)
EGFR mutation, n (%)	
L858R	22 (33%)
Exon19 deletion	29 (43.6%)
Brain metastasis, n (%)	
Yes	18 (39.8%)

No	31 (65%)

ECOG = Eastern Cooperative Oncology Group.

Administering Drugs Precisely

All 50 patients underwent a safety study and information on adverse events were collected and tabulated (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 five patients to a maximum of 45 mg.

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 (68.2% and 50%, respectively; Fisher's exact test P =0.20; 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 the initiation of therapy but was found in three 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 acquisition of resistance.

Gene alteration	Patients ID	Detail
EGFR secondary mutation, including T790M, 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

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	
E709G	#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

PFS: progression-free survival

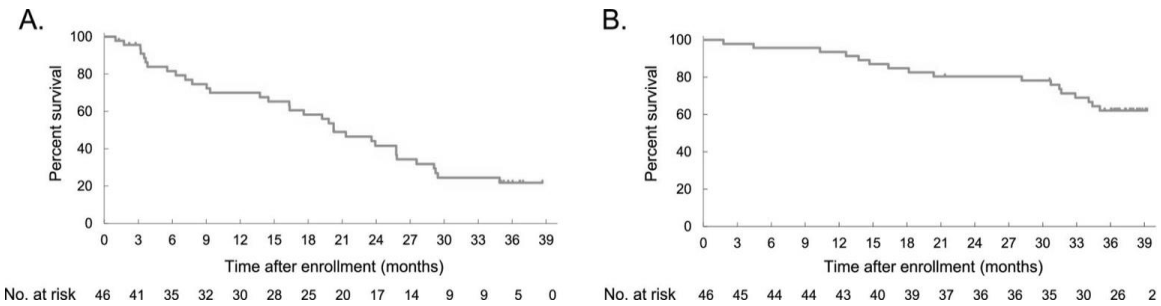

Figure 2. Results of investigational alternative therapy with Osimertinib and Afatinib. Kaplan–Meier plots of (A) progression-free survival (PFS) and (B) overall survival for all patients in the efficacy analysis.

Plasma Development of the HER3 Ligand sHRG

Investigational alternative therapy with Osimertinib and Afatinib demonstrated significant outcomes in terms of PFS and OS as illustrated in Figures 2A and 2B, respectively. The Kaplan–Meier plots in Figure 2A depict PFS for all patients in the efficacy analysis, while Figure 2B represents the overall survival outcome.

Furthermore, the investigation also assessed sHRG expression and its relationship with PFS. Figure 3A displays the distribution of sHRG levels in plasma samples obtained before therapy, showing a range of 55–321 pg/ml with a median value of 110 pg/ml. Clinical characteristics such as age, smoking history, and EGFR mutation did not show substantial associations with sHRG expression (Figure 3A).

The impact of sHRG levels on PFS was examined at various cutoff points, as depicted in Figure 3B. Notably, hazard ratios for PFS tended to decrease between the cutoff values of 87 and 180 pg/ml. Additionally, Figure 3C illustrates that sHRG levels did not significantly change with disease progression or after receiving Osimertinib treatment.

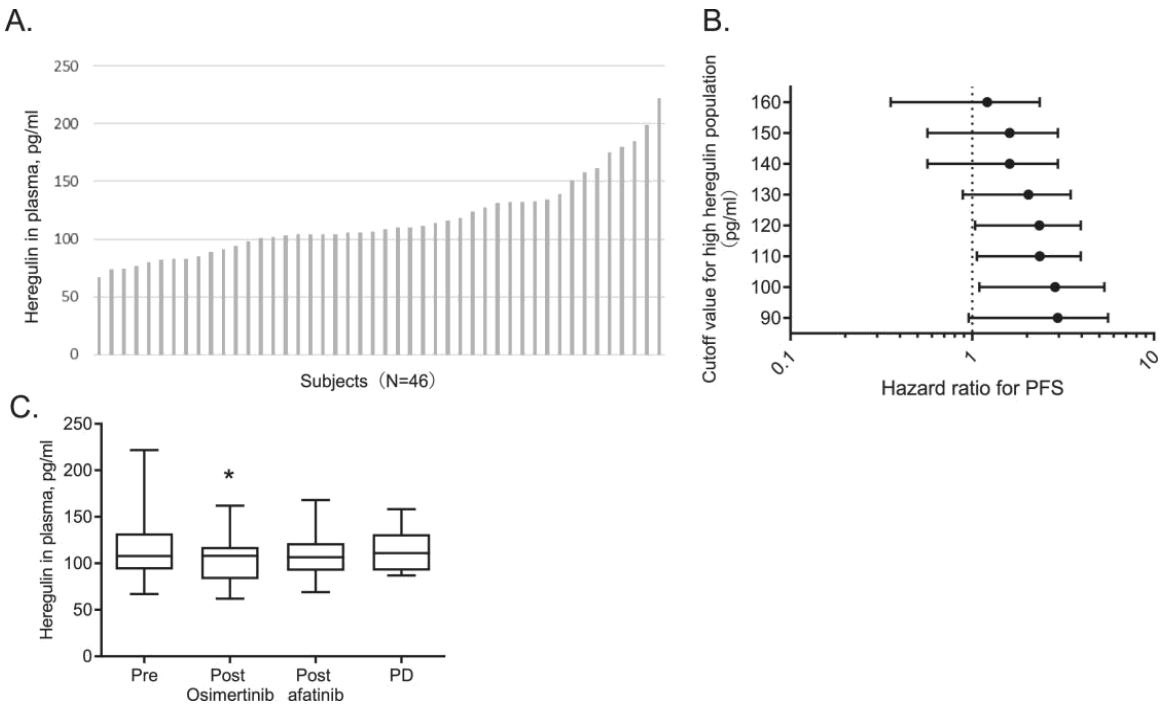

Figure 3. Soluble heregulin expression and its relationship with progression-free survival.

A. Soluble heregulin expression level before treatment (n = 50);
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 represent each participant and y-axis represent plasma heregulin concentration (pg/ml).

DISCUSSION

As previously validated in untreated advanced NSCLC patients with EGFR mutations, the effectiveness of alternating treatment involving Osimertinib and low-dose Afatinib showed no significant deviation from Osimertinib alone in this phase II investigation. Genomic analysis suggested that the potential advantage of this therapeutic approach could be linked to preventing the emergence of resistance caused by EGFR secondary mutations such as T790M and C797S, despite the limited size of the study cohort. 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 emergence of resistance brought on by secondary EGFR T790M mutations [13]. 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 [28, 29]. Afatinib—a second-generation EGFR-TKI—has been found in preclinical trials to significantly reduce T790M activity [30]. With the approved and suggested doses for treatment, these concentrations could not be reached [31]. 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 showed 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 [9]. In the plasma and tissues of 10 out 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 T790M mutation in tumours despite intermittent dosing because the T790M mutation was not found in post-resistant plasma samples in the current investigation.

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 [13].

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 [28]. 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 [35]. This finding implied that Afatinib can stop C797S-positive cells from development. 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 [36, 37]. According to in vitro investigations, these mutations continue to be susceptible to Afatinib. Afatinib was found to be effective in patients with tumours that were L718-positive, despite Osimertinib resistance [38, 39]. 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 [41]. 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 [41, 42]. 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, an elevated HER2 gene copy number was not found after the development of resistance [8]. According to reports, 5–18% of Osimertinib-containing EGFR-TKI-resistant tumours exhibit HER2 amplification [8, 37]. To the best of our knowledge, it has not 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 [43]. Afatinib's ability to inhibit HER2 kinase activity in vitro was discovered to have an IC50 value of less than 30 nM [30]. The plasma drug concentration of Afatinib 25 mg is approximately 50 nM, which suggests that our therapeutic strategy may assist in inhibiting HER2 activation [44]. 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 [7].

In contrast, in preclinical models, HER3 activation, which is dependent on the HER3 ligand heregulin, decreases the susceptibility to EGFR inhibitors [17]. Additionally, patients with higher blood levels of sHRG had a worse prognosis for survival (PFS) than patients with lower levels [26]. 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. Plasma samples collected before treatment in the present study exhibited 10 compound EGFR mutations, involving dual or multiple mutations within the EGFR-TKI 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 [46, 47]. Additionally, compared to single mutation instances, compound EGFR mutation cases have allegedly been linked to worse clinical outcomes [48]. 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 [49]. Its strong affinity for exon 19 mutations may be the cause of this behaviour. For E709X-positive cases, Afatinib actually had a response rate of 85% and a median PFS of 12.3 months, as compared to other TKIs with a response rate of 55% and median PFS of 8.2 months, respectively. Afatinib may be responsible for the efficacy seen for the current treatment in a patient with the E709G mutation [50].

CONCLUSION

PFS in the present 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% versus 20.1%) [21]. Since no secondary EGFR mutations were found in the blood samples, alternately administering two 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. 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. 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 present study is subject to several limitations, especially concerning the analysis of biomarkers. 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 as HER2 amplification due to the limited sample number of individuals.

Clinical Points

- The standard initial treatment for individuals diagnosed with NSCLC harboring an activating EGFR mutation typically involves the administration of EGFR-TKIs. However, resistance often develops due to EGFR secondary mutations.
- 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 PFS of 25.6 months.
- Using circulating tumour DNA collected after therapy, no subsequent EGFR mutations were found.
- This regimen may have shown potential advantages in this trial. These clinical advantages, however, need more research because they are not conclusive.

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 the present investigation.

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 the present paper.

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