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Efficacy of first-line treatment with epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) alone or in combination with chemotherapy for advanced non-small cell lung cancer (NSCLC) with low-abundance mutation

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ABSTRACT

Objective: The objective of this study was to investigate whether first-line treatment with epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) in combination with chemotherapy improves the prognosis of patients with advanced non-small cell lung cancer (NSCLC) who harbour low-abundance EGFR mutations.

Patients and methods: We retrospectively analysed the clinical data of 76 patients with advanced NSCLC who harboured low-abundance EGFR mutations. The patients were divided into the combination group and the monotherapy group. The combination group received EGFR-TKI combined with a platinum-based regimen. After the end of chemotherapy, EGFR-TKI was administered daily. The monotherapy group was administered EGFR-TKI therapy daily.

Results: No significant difference was observed in response rate between the different groups. The median PFS and OS were significantly longer in the combination group than in the monotherapy group (PFS: 7.9 months [95% CI,5.73–10.07] vs 5.9 months [95% CI, 4.99–6.81], p = 0.015; OS: 25.8 months[95% CI,16.27–35.33] vs 19.8 months [95% CI, 18.60–21.00], p = 0.047). Subgroup analysis showed that, for patients with the exon 21 L858R mutation, the PFS and OS were significantly longer in the combination group than in the monotherapy group (PFS: 7.2 months vs 5.8 months, p = 0.013; OS: 22.0 months vs 18.7 months, p = 0.024). The incidence of adverse events was significantly higher in the combination group.

Conclusion: For patients with advanced NSCLC and low-abundance EGFR mutations, first-line treatment with EGFR-TKI plus chemotherapy significantly improved PFS and OS. The combination therapy increased the incidence of adverse reactions, but all adverse reactions were expected and tolerated.

1. Introduction

In the past decade, dramatic progress has been made in the treatment of advanced non-small cell lung cancer (NSCLC). The Eastern Cooperative Oncology Group (ECOG)1594 concluded that there was a treatment bottleneck of 7.4–8.2 months in the median overall survival with conventional chemotherapy for advanced NSCLC [1]. Moreover, the Iressa Pan-Asia Study (IPASS) demonstrated the effect of the epidermal growth factor receptor (EGFR) driver gene on lung cancer treatment. EGFR-tyrosine kinase inhibitors (EGFR-TKIs) significantly improve the progression-free survival (PFS) of patients with EGFR mutations [2]. Today, with advancements in molecular and histological testing and drug treatment and strategies for NSCLC, the overall survival (OS) of patients with advanced NSCLC who harbour sensitive EGFR mutations can exceed 20 months [3,4]. The improvements in treatment outcome and prognosis have ushered in a new era of targeted therapy for advanced NSCLC.

In clinical practice, however, 20%–30% of NSCLC patients with sensitive EGFR mutations do not respond to EGFR-TKI therapy, or they develop early resistance to this therapy. In 2011, Wu and his colleagues first proposed the concept of individual variation in the abundance of EGFR mutations in NSCLC patients and reported that individual variation affects the efficacy of EGFR-TKIs. PFS is significantly longer in patients with a high-abundance of EGFR mutations after EGFR-TKI therapy [5]. Due to limitations in testing methods, Wu and his colleagues did not perform a quantitative analysis of the abundance of EGFR

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mutations. Later, Zhou and her colleagues conducted an in-depth study and quantitatively analysed the abundance of EGFR mutations. The results showed that high-abundance EGFR mutations contribute to better outcomes after targeted therapy and to improvements in median OS [6]. In contrast, the efficacy and median OS were not satisfactory in patients with low-abundance EGFR mutations after EGFR-TKI therapy. Therefore, the abundance of EGFR mutations is an important predictor of the efficacy of EGFR-TKI therapy.

In summary, numerous studies have demonstrated the effect of the abundance of EGFR mutations on treatment outcomes. To date, however, no clinical studies have evaluated this topic in detail. Given this, we conducted this study to evaluate the clinical efficacy and tolerability of an EGFR-TKI alone or in combination with chemotherapy in patients with low-abundance EGFR mutations in order to provide a reference for more rational and effective treatment strategies for patients with low-abundance EGFR mutations.

2. Patients and methods

2.1. Clinical data

We retrospectively analysed the clinical data of patients with advanced non-squamous NSCLC who harboured low-abundance sensitive EGFR mutations (exon 19 deletion and exon 21 L858R mutation) and who were treated at Henan Cancer Hospital between January 2013 and December 2015. Advanced NSCLC was defined as stage IV according to the UICC (The Union for International Cancer Control) 7th lung cancer TNM classification. All cases of NSCLC were confirmed by cytology or histology of at least one evaluable lesion. A baseline evaluation was completed one week before treatment.

2.2. Detection of EGFR mutations

All specimens were obtained by biopsy from primary or metastatic tumors. The EGFR mutational sites and mutation abundance in tumor specimens were quantified by amplification refractory mutation system (ARMS) method. Genomic DNA was extracted with a Qiagen 56,404 QIAamp DNA FFPE tissue kit (Qiagen Bioscience Co. Ltd., Hilden, Germany). The DNA concentration was measured by a broad-spectrum ultraviolet spectrometer and adjusted to 20–50 ng/mL. DNA samples were stored at −20 °C. EGFR mutations were detected using a Human EGFR gene mutation quantitative detection kit for 45 hot genes (Contain EGFR wild-type standard products). Beijing ACCB Biotech Ltd, China.) by fluorescence PCR. PCR was performed using a Stratagene Mx3000 P real-time PCR system (Agilent Technologies, Santa Clara, USA). The abundance of the wild-type and mutant EGFR genes in the specimens was calculated according to standard curves after the EGFR wild type and mutant alleles were individually amplified. The relative mutation rate was calculated according to the following equation: relative mutation abundance = mutant gene concentration / (mutant gene concentration + wild-type gene concentration) × 100% [7]. Wu and his colleagues first defined “low abundance of EGFR mutations” as those tumors were mutation positive by ARMS but negative by sequencing [5]. Sequencing can detect EGFR mutations in samples with a greater than 10% EGFR mutation frequency [8]. So a ratio of < 10% was considered low abundance in this study.

2.3. Treatment methods

The combination group received EGFR-TKI therapy (po, day 8 of each chemotherapy cycle to day1 of the next chemotherapy cycle) combined with a platinum-based regimen (cisplatin or carboplatin plus paclitaxel, docetaxel, pemetrexed, or gemcitabine). After the end of 2–6 cycles of chemotherapy, the EGFR-TKI was administered daily. The monotherapy group was administered EGFR-TKI therapy daily. The first efficacy evaluation was performed in the combination group after 2 cycles of chemotherapy. The EGFR-TKI was gefitinib 250 mg po qd, or erlotinib150 mg po qd, or icotinib 125 mg po tid. The number of patients received three different drugs in the combination therapy group were 18, 5 and 11, respectively. And there were 20, 8 and 14 in the monotherapy group.

2.4. Evaluation of response and adverse events

The response rate and adverse reactions were evaluated. Short-term efficacy was rated as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to the Response Evaluation Criteria in Solid Tumours (RECIST 1.1). The overall response rate (ORR) included CR and PR. The disease control rate (DCR) included CR, PR, and SD. Adverse reactions were rated from grade I to V according to the Common Terminology Criteria for Adverse Events (CTCAE 4.0). In this study, grade III-IV adverse reactions were considered moderate to severe reactions (grade V: death).

2.5. Survival

PFS was defined as the time from the start of treatment to disease progression or death. Cases with no disease progression or those that were lost to follow-up were censored, and the time point for statistical processing was considered the last follow-up time. OS was defined as the time from the start of treatment to death. Patients who survived and those who werelost to follow-up were censored, and the time point for statistical processing was considered the last follow-up time.

2.6. Statistical analysis

SPSS v17.0 was used for the statistical analysis. Continuous variables were analysed by t-test, while categorical variables were analysed by chi-square test. The Kaplan-Meier curve was used to analyse the PFS and OS. The multivariate Cox regression model was used for the multivariate survival analysis. $\alpha = 0.05$ was set as the significance level.

3. Results

3.1. Tumour response

In all, 76 patients, including 34 patients in the combination group and 42 in the EGFR-TKI monotherapy group, were included in this study. No significant between-group difference was observed in the baseline characteristics, which included sex, age, smoking history, ECOG performance status (ECOG PS) score, pathological type, mutation site, brain metastasis, and hepatic metastasis.

Among the 76 patients, 25 (31.6%) had an exon 19 deletion (E19 del), and 52 (68.4%) had the exon 21 L858R mutation(E21 L858R). The baseline characteristics are shown in Table A1.

Among the 34 patients in the combination group (EGFR-TKI plus chemotherapy), 2 achieved CR, 18 achieved PR, 10 achieved SD, and 4 achieved PD. The ORR was 55.9% and the DCR was 88.2%. Among the 42 patients in the EGFR-TKI monotherapy group, 0 patients achieved CR,17 achieved PR, 15 achieved SD, and 10 achieved PD. The ORR was 40.5% and the DCR was 76.2%. No significant between-group difference was observed in the ORR or the DCR (p = 0.181, 0.178). Please refer to Table A2 for details.

3.2. PFS

The median PFS was 7.9 months (95% CI,5.73–10.07) in the combination group and 5.9 months (95% CI, 4.99–6.81) in the monotherapy group, and the difference was statistically significant (p = 0.015) (Fig. 1). Among the patients with E19 del, the PFS was 11.5 months (95% CI,5.89–17.11) and 7.4 months (95% CI, 4.10–10.70)in the combination group and in the monotherapy group, respectively

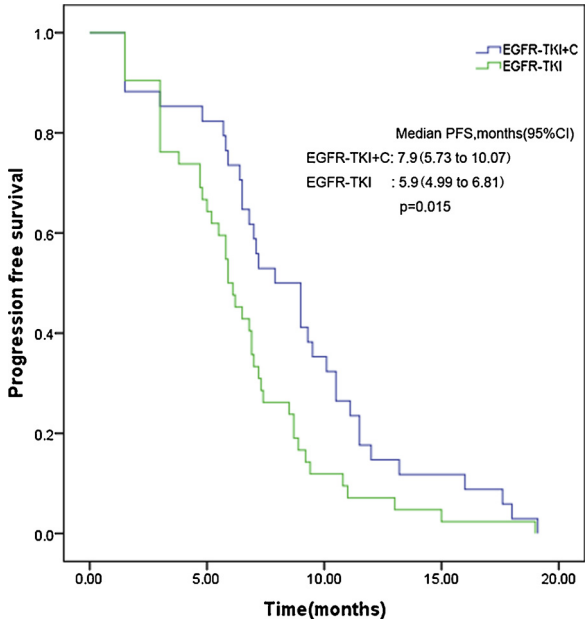

Fig. 1. PFS in both groups.

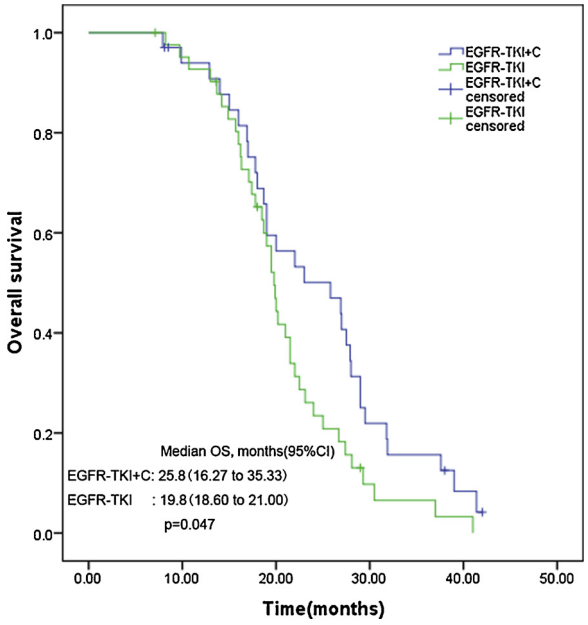

Fig. 3. OS in both groups.

(p = 0.171) (Fig. 2A). Among the patients with E21 L858R mutation, the PFS was 7.2 months (95% CI,5.79–8.61) and 5.8 months (95% CI, 4.89–6.71) in the combination group and the monotherapy group, respectively (p = 0.013) (Fig. 2B).

3.3. OS

The median OS was 25.8 months (95% CI,16.27–35.33) in the combination group and 19.8 months (95% CI, 18.60–21.00) in the monotherapy group, and the difference was statistically significant (p = 0.047) (Fig. 3). Among the patients with E19 del, the OS was 29.0 months (95% CI,21.41–36.59) and 27.4 months (95% CI, 23.45–31.35) in the combination group and the monotherapy group, respectively (p = 0.133) (Fig. 4A). Among the patients with the E21 L858R mutation, the OS was 22.0 months (95% CI,18.07–25.93) and 18.7 months (95% CI, 16.54–20.86)in the combination group and the monotherapy group, respectively (p = 0.024) (Fig. 4B).

The Cox regression model was used for the multivariate analysis. The results showed that among the factors including sex, smoking history, PS score, pathological type, mutation site, brain metastasis, hepatic metastasis, the factor of the treatment regimen, mutation site, hepatic metastasis, and brain metastasis were significantly correlated with OS. The risk of death was 0.417 times higher in the combination group(EGFR-TKI combined with chemotherapy) compared with the EGFR-TKI monotherapy group (HR = 0.417; 95% CI 0.243-0.716; p = 0.002), while the risk was 7.793 times higher in patients with E21 L858R mutation compared with patients with E19 del(HR = 7.793; 95% CI 3.844–15.798; p = 0.000). The risk of death was 4.717 times higher in patients with brain metastasis compared with patients with no brain metastasis(HR = 4.717; 95% CI 2.451–9.091; p = 0.000) and was 2.747 times higher in patients with hepatic metastasis compared with patients with no hepatic metastasis (HR = 2.747; 95% CI 1.481–5.102; p = 0.001). Please refer to Table A3 for details.

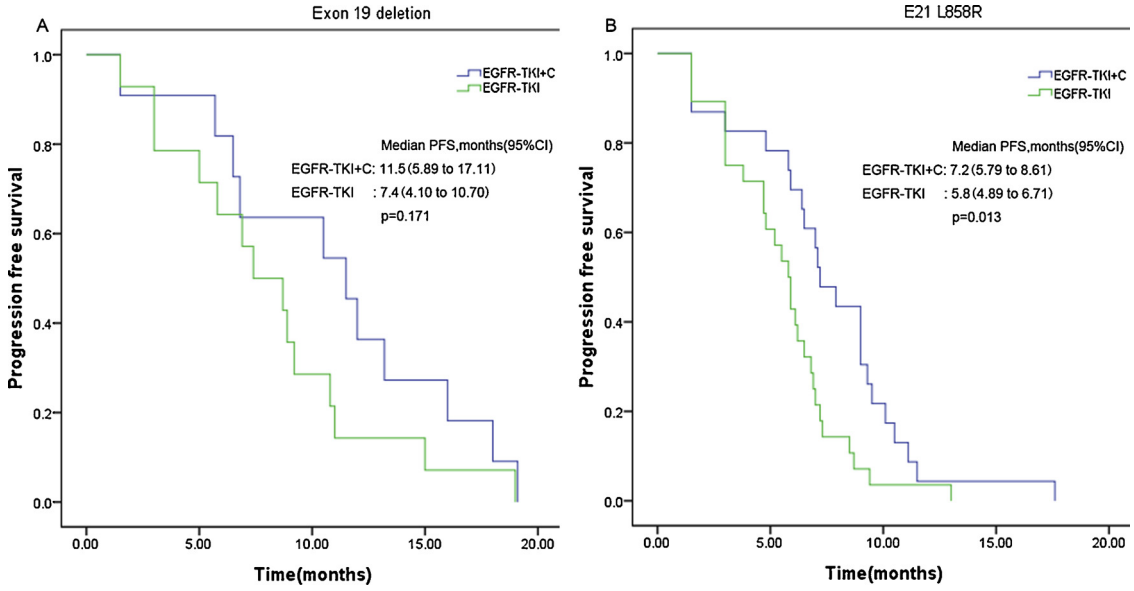

Fig. 2. PFS according to the subgroup analysis by EGFR mutation type. (A) exon 19 deletion subgroup; (B) exon 21 L858R point mutation subgroup.

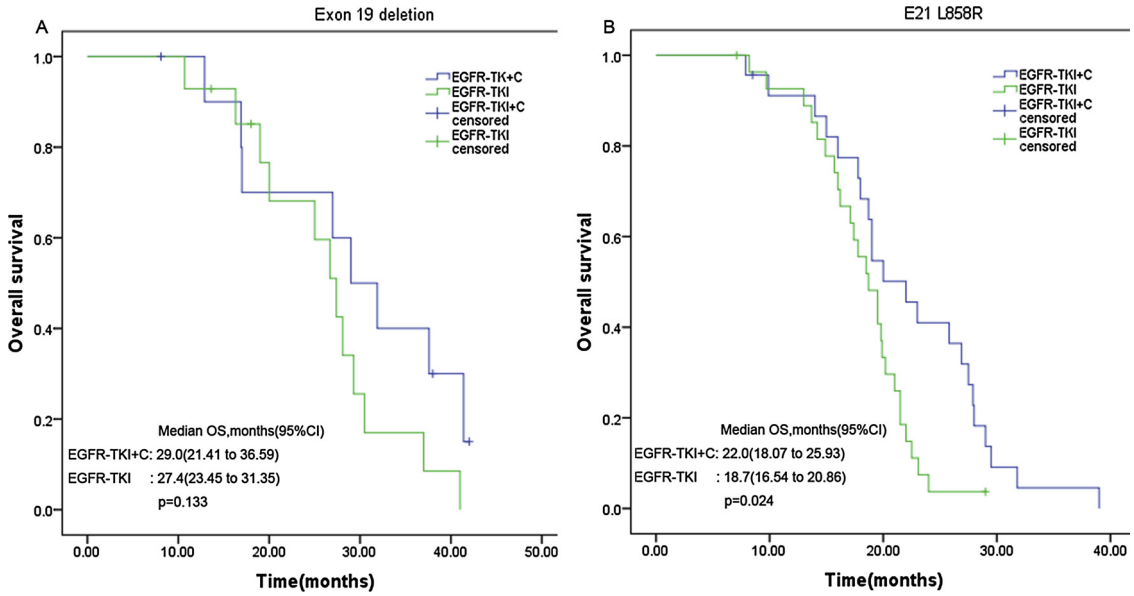

Fig. 4. OS according to the subgroup analysis by EGFR mutation type. (A) exon 19 deletion subgroup; (B) exon 21 L858R point mutation subgroup.

3.4. Adverse events

No unexpected adverse events occurred in this study. For non-hematologic adverse events, the incidence of elevated liver enzymes was significantly higher in the combination group (EGFR-TKI combined with chemotherapy) than in the EGFR-TKI monotherapy group (76.5% vs 50%, p = 0.018). However, no significant between-group difference was observed in the incidence of grade III-IV elevated liver enzymes (11.8%vs9.5%,p = 0.519). Liver function returned to normal in all cases with clinical intervention. In addition, the incidences of nausea, vomiting, and fatigue were significantly higher in the combination group than in the monotherapy group. For hematologic adverse events,the incidences of neutropenia, anaemia, and thrombocytopenia were also significantly higher in the combination group(EGFR-TKI combined with chemotherapy) than in the monotherapy group. Please refer to Table A4 for details.

4. Discussion

Combination therapy with an EGFR-TKI and chemotherapy has long been a hot topic in research of targeted therapy for lung cancer. At the beginning, no consideration was given to population selection before the discovery of the EGFR driver gene, which is an important predictor of efficacy. As a result, no significant difference was observed in the overall response rate, disease control rate, or survival between the standard platinum-based chemotherapy combined with EGFR-TKI and chemotherapy alone [9]. The results were unexpected at that time, but subsequent studies reached similar conclusions [10,11]. Then, the discovery of driver genes gave a new viewpoint to combination therapy for patients with lung cancer. Wu et al first used an EGFR-TKI during chemotherapy in patients with advanced NSCLC who harboured EGFR mutations. In particular, the EGFR-TKI was given on days 15–28 of the chemotherapy cycle, whereas placebo was given to the control group. Moreover, the EGFR-TKI or placebo was given alone after 6 cycles of chemotherapy were completed. A subgroup analysis showed that, among the patients with EGFR mutations, the PFS was16.8 months in the EGFR-TKI group and 6.9 months in the control group (p < 0.0001); the OS was 31.4 and 20.6 months, in the EGFR-TKI group and the control group, respectively (p = 0.0092), which indicates significant survival benefits [12]. Another recent study on chemotherapy combined with an EGFR-TKI also showed that first-line treatment with EGFR-TKI therapy and chemotherapy significantly improved the PFS (15.8vs10.9months, p = 0.029) [13]. This year, the American Society of Clinical Oncology (ASCO) published the data of NEJ009 [14], a phase III clinical study of gefitinib plus carboplatin/ pemetrexed versus gefitinib alone. The results showed that the median PFS was 20.9 months in the combination group, which was longer than that in the monotherapy group (11.2months). Moreover, the OS was significantly longer in the combination group than in the monotherapy group (52.2vs 38.8 months, HR 0.695, p = 0.013). Despite certain advantages, first-line treatment with EGFR-TKI and chemotherapy has not been widely used in clinical practice because of a lack of long-term survival data and concerns about potential increases in toxicity. With advancements in clinical testing for the abundance of EGFR mutations, the clinical outcome differs greatly between patients with high-abundance EGFR mutations and those with low-abundance EGFR mutations (ORR:62.7%vs44.4%; PFS:11.3vs 6.9 months) [6]. Thus, combination therapy with EGFR-TKI and chemotherapy maybe a better choice to improve the clinical outcome of patients with low-abundance EGFR mutations.

In this study, we retrospectively analysed the clinical data of EGFR-TKI therapy combined with chemotherapy in patients with advanced NSCLC who harboured low-abundance EGFR mutations. The results showed that the median PFS and OS were both longer in the combination group than in the monotherapy group. The median PFS was 7.9 months (95% CI,5.73–10.07) in the combination group and 5.9 months (95% CI, 4.99–6.81) in the monotherapy group (p = 0.015). The median OS was 25.8 months (95% CI,16.27–35.33) in the combination group and 19.8 months (95% CI, 18.60–21.00) in the monotherapy group (p = 0.047).The subgroup analysis showed that for patients with the E21 L858R mutation, the PFS and OS were significantly longer in the combination group compared with the monotherapy group. The PFS was 7.2 months (95% CI,5.79–8.61) and 5.8 months (95% CI, 4.89–6.71) (p = 0.013) in the combination and monotherapy groups, respectively, while the OS was 22.0 months (95% CI,18.07–25.93) and 18.7 months (95% CI, 16.54–20.86) (p = 0.024) in the combination and monotherapy groups, respectively. For patients with E19 del, the PFS and OS were longer in the combination group than in the monotherapy group, but the differences did not reach statistical significance. This is because, for patients with low-abundance EGFR mutations, E21 L858R was much more common than E19 del (68.4% vs 31.6%), this resulted in a smaller sample size of patients with E19del, which may have affected the statistics. In general, an EGFR-TKI combined with chemotherapy revealed benefits in the PFS and OS and significantly

improved the survival prognosis of NSCLC patients with low-abundance EGFR mutations.

This retrospective study is the first study to evaluate the combination of EGFR-TKIs and chemotherapy as first-line therapy in NSCLC patients who harboured low-abundance EGFR mutations, and the results are promising. Current clinical guidelines recommend first-line EGFR-TKI therapy in patients with EGFR mutations [2,15,16], however, the clinical outcome and survival prognosis are not satisfactory in patients with low-abundance EGFR mutations. This study provides a new treatment strategy for these patients. Pemetrexed, gemcitabine, or docetaxel (pemetrexed in particular) maintenance therapy after first-line platinum-containing chemotherapy is a proven and widely accepted treatment model [17–19]. In this study, most patients in the combination group received co-administration of pemetrexed, gemcitabine, or docetaxel but did not receive maintenance chemotherapy along with EGFR-TKI therapy after the end of induction chemotherapy, which was a limitation. In addition, patients with rare EGFR mutations were excluded from this study because of the low incidence and the great difference in clinical outcome with common mutations [4,15]. Another limitation of this study is the cut-off value to divide high abundance and low abundance groups of EGFR mutations. The definition of cut-off value is based on detection technique in this study. Therefore, we have less consideration for clinical efficacy in defining cut-off values.

A safety analysis showed that in the combination group, the adverse events reflected both the EGFR-TKI and the chemotherapy toxicity, with no superposition, which was consistent with findings in the literature [20]. Despite a higher incidence of adverse events in the combination group, all events were expected and were controlled after clinical intervention. These results suggested that EGFR-TKI therapy combined with chemotherapy was well-tolerated and safe.

Growing numbers of studies are evaluating first-line treatment with EGFR-TKIs plus chemotherapy for advanced NSCLC, which indirectly demonstrate the great potential of this treatment model. For patients with common EGFR mutations, combination therapy may bring additional clinical benefits. For patients with low-abundance EGFR mutations, combination therapy may be life-changing. With advancements in molecular and histological testing, target populations can be more precisely identified.

5. Conclusion

In this study, the target population was patients with advanced NSCLC and low-abundance EGFR mutations. EGFR-TKI therapy combined with chemotherapy significantly improved the PFS and OS relative to EGFR-TKI therapy alone. Moreover, the combination therapy did not cause unexpected adverse events and was well-tolerated. This study provides a more effective treatment strategy for patients with advanced NSCLC who harbour low-abundance EGFR mutations. Nevertheless, large, randomized, controlled, prospective studies are needed to further validate these results.

Conflicts of interests and source of funding

All authors declared no potential conflicts of interest. No funding.

Ethical approval

All procedures in studies involving human participants were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Henan Cancer Hospital Human Research Ethics Committee.

Conflict of interest

All authors declared no potential conflicts of interest.

Acknowledgments

The American Journal Experts (AJE) provided English edit. All of the authors contributed to the study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Appendix A

Table A1
The baseline characteristics of patients in the different groups.

Characteristic	EGFR-T KI + C(n = 34)	EGFR-TKI(n = 42)	*P*
Sex(Female/Male)	15/19	18/24	0.091
Median age(range), years	59.5(39-68)	61(42-69)	0.144
Smoking history, yes	13	17	0.842
ECOG performance status			0.990
0	21	26	
1	13	16	
Histologic subtype			0.827
Adenocarcinoma	26	33	
Others	8	9	
EGFR mutation type			0.859
Exon 19 deletion	11	14	
Exon 21 L858R point Mutation	24	28	
Brain metastasis	10	11	0.755
Hepatic metastasis	8	13	0.472

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR-T KI, Epidermal growth factor receptor-tyrosine kinase inhibitor; C, Chemotherapy.

Table A2
Efficacy outcomes.

Result	EGFR-T KI + C (n = 34)	EGFR-TKI (n = 42)	*P*
PFS(months)			
Median PFS (95% CI)	7.9(5.73-10.07)	5.9(4.99-6.81)	0.015
Exon 19 deletion	11.5(5.89-17.11)	7.4(4.10-10.70)	0.171
Exon 21 L858R point Mutation	7.2(5.79-8.61)	5.8(4.89-6.71)	0.013
OS(months)			
Median OS (95% CI)	25.8(16.27-35.33)	19.8(18.60-21.00)	0.047
Exon 19 deletion	29(21.41-36.59)	27.4(23.45-31.35)	0.133
Exon 21 L858R point Mutation	22(18.07-25.93)	18.7(16.54-20.86)	0.024
Tumour response, No. (%)			
CR	2(5.9)	0(0)	0.197
PR	18(52.9)	17(40.5)	0.278
SD	10(29.4)	15(35.7)	0.561
PD	4(11.8)	10(23.8)	0.178
ORR(CR + PR)	19(55.9)	17(40.5)	0.181
DCR(CR + PR + SD)	30(88.2)	32(76.2)	0.178

Abbreviations: EGFR-TKI, Epidermal growth factor receptor-tyrosine kinase inhibitor; C, Chemotherapy.

Table A3
The multivariate analysis by coxregression model.

Variables in the Equation									
		B	SE	Wald	df	Sig.	Exp(B)	95.0% CI Exp(B)	
								Lower	Upper
step 5 [a]	Treatment Regimen	.875	.276	10.063	1	.002	2.399	1.397	4.119
	Mutation site	2.053	.361	32.424	1	.000	7.793	3.844	15.798
	Brain metastasis	−1.551	.334	21.531	1	.000	.212	.110	.408
	Hepatic metastasis	−1.012	.315	10.297	1	.001	.364	.196	.675

[a] Variables entered on step 5: Treatment regimen, mutation site, brain metastasis, hepatic metastasis.

Table A4
Drug-related adverse events, by CTCAE grade.

Adverse events	EGFR-T KI + C (n = 34)		EGFR-TKI (n = 42)	
	All grade,No. (%)	III-IV,No. (%)	All grade,No. (%)	III-IV,No. (%)
Diarrhoea	14(41.2)	2(5.9)	21(50)	2(4.8)
Skin rash	25(73.5)	2(5.9)	32(76.2)	1(2.4)
Increased ALT or AST	26(76.5)	4(11.8)	21(50)	4(9.5)
Nausea	20(58.8)	4(11.8)	4(9.5)	0(0)
Vomiting	6(17.6)	3(8.8)	2(4.8)	0(0)
Fatigue	12(35.3)	4(11.8)	5(11.9)	0(0)
Neutropenia	19(55.9)	5(14.7)	2(4.8)	0(0)
Anaemia	9(26.5)	4(11.8)	3(7.1)	0(0)
Thrombocytopenia	5(14.7)	1(2.9)	1(2.4)	0(0)

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; EGFR-TKI, Epidermal growth factor receptor-tyrosine kinase inhibitor; C, Chemotherapy.

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