SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 13: 4433-4444, 2017

Comparison of the effectiveness of erlotinib, gefitinib, and afatinib for treatment of non-small cell lung cancer in patients with common and rare *EGFR* gene mutations

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Received October 26, 2016; Accepted January 4, 2017

DOI: 10.3892/ol.2017.5980

Abstract. Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are routinely used to treat non-small cell lung cancer (NSCLC) in patients with common activating mutations of the *EGFR* gene. The aim of the study was to compare the efficacies of EGFR-TKIs in patients with common (exon 19 deletions and exon 21 p.Leu858Arg) and rare *EGFR* mutations. A retrospective analysis of 180 NSCLC patients with common (n=167) and rare (n=13) *EGFR* mutations treated with erlotinib (n=98), gefitinib (n=66) and afatinib (n=16) was performed. *EGFR* mutations were determined using RT-PCR and the EntroGen EGFR Mutations Analysis kit. Partial and complete response (PR and CR), progression-free survival (PFS), and overall survival (OS) were analyzed. Demographic and clinical factors had no impact on PFS or OS in patients treated with EGFR-TKIs. Erlotinib, gefitinib, and afatinib showed similar efficacies based on treatment response, median PFS, and OS. The type of *EGFR* mutation had no impact on median OS; however, median PFS was significantly longer in patients with the exon 19 deletion compared to patients with the exon 21 p.Leu858Arg substitution and rare *EGFR* gene mutations (P=0.013). Patients with common *EGFR* mutations showed significantly longer median PFS than those with rare *EGFR* mutations (10 vs. 5 months; P=0.009). Erlotinib, gefitinib, and afatinib show similar efficacies in NSCLC patients with both common and rare *EGFR* mutations. When undergoing EGFR-TKI treatment, patients with rare *EGFR* mutations showed similar OS but poorer PFS. Further investigation into the associations between particular rare *EGFR* mutations and EGFR-TKIs treatment outcomes is required.

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Key words: non-small cell lung cancer, *EGFR* mutations, EGFR tyrosine kinase inhibitors

Introduction

Approximately 10-15% of Caucasian patients with advanced non-small cell lung cancer (NSCLC) have mutations in the epidermal growth factor receptor (*EGFR)* gene. Several clinical studies have demonstrated the efficacy of EGFR-tyrosine kinase inhibitors (EGFR-TKIs) for treatment of NSCLC patients with activating *EGFR* mutations (1-5). Currently, three EGFR-TKIs (erlotinib, gefitinib, and afatinib) have proven efficacy in the treatment of NSCLC in patients with common activating *EGFR* mutations. Erlotinib and gefitinib are reversible EGFR-TKIs, while afatinib is an irreversible EGFR-TKIs. However, despite extensive knowledge about the mechanism of action of EGFR-TKIs in NSCLC, some serious problems remain unsolved.

First of all, few prospective and randomized studies have directly compared the efficacy of the various classes of EGFR-TKIs in patients with NSCLC harboring activating *EGFR* mutations (6-8). There are the preliminary results of a single-center randomized phase II trial comparing the efficacy of gefitinib and erlotinib in second-line therapy of Asian NSCLC patients with activating *EGFR* mutations (6), as well as the results of the global, multi-center LUX-Lung 7 trial comparing the efficacy of afatinib and gefitinib in first-line treatment of NSCLC patients with common activating *EGFR* mutations (7). The results of the multinational, randomized

ARCHER 1009 trial indicate that gefitinib and daconitinib have similar efficacy. However, the ARCHER 1050 phase III trial comparing the efficacy of these two drugs is ongoing (8). We have preliminary results of phase III clinical studies comparing the efficacy of third-generation inhibitors (i.e., osimertinib in the FLAURA clinical trial, ASP8273 in the SOLAR clinical trial, and rociletinib in the TIGER-1 clinical trial) and erlotinib or gefitinib in first-line treatment of NSCLC patients with *EGFR* mutations (see clinicaltrials.gov). In the meantime, our knowledge of the effectiveness of various EGFR-TKIs comes from a limited number of retrospective studies comparing gefitinib, erlotinib, and afatinib.

The second serious problem related to EGFR-TKIs administration is the lack of reliable knowledge about their efficacy in patients with rare *EGFR* mutations. While gefitinib, erlotinib, and afatinib have proven efficacy in patients with the two major mutations in the *EGFR* gene (i.e., the classical Glu746-Ala750 deletion in exon 19 and the common p.Leu858Arg substitution in exon 21), their effectiveness in NSCLC cases with rare *EGFR* mutations remains unclear. Molecular tests based on real-time PCR techniques detect several rare *EGFR* mutations, including: Different substitutions in codons 709 and 719 in exon 18, substitutions and insertions in exon 20, as well as different substitutions in codons 858 and 861 in exon 21 (9). Such tests have confirmed that rare *EGFR* mutations occur more frequently than previously thought Results from the French National Cancer Institute network (ERMETIC-IFCT) indicated that ~10% of *EGFR*-mutated NSCLC patients may have rare *EGFR* gene mutations (10). Similarly, in our recent multicenter study in Poland, we showed that 14.77% of patients with *EGFR*-mutated NSCLC had rare mutations (11). Despite this, only a few retrospective analyses have investigated the efficacy of EGFR-TKIs in patients with rare *EGFR* mutations. Therefore, the predictive value of rare *EGFR* mutations for deciding on the first-line treatment option in patients with NSCLC remains unclear.

In this study, we conducted a retrospective analysis of the effectiveness of different EGFR-TKIs in NSCLC patients with common and rare *EGFR* mutations. To the best of our knowledge, this is the first study worldwide to compare the efficacy of erlotinib, gefitinib, and afatinib in patients with rare and common *EGFR* mutations.

Materials and methods

Study population. This study was approved by the Local Bioethics Committee of Medical University of Lublin. We retrospectively analyzed clinical outcomes in 180 NSCLC patients (95% with adenocarcinoma diagnosis) with different *EGFR* mutations, who had received erlotinib (n=98), gefitinib (n=66), or afatinib (n=16) therapy in four oncology centers in Poland (Warsaw, Lublin, Poznan, and Lodz). All patients had clinically proven recurrent or locally advanced or metastatic NSCLC. Patients with brain metastases controlled with radiotherapy or neurosurgery without intensive steroid therapy were included in the study. EGFR-TKIs were administered orally at a daily dose of 150 mg for erlotinib, 250 mg for gefitinib, and 40 mg for afatinib, and the cycle repeated every 28 days. The clinical parameters collected at the beginning of EGFR-TKIs treatment included: Age, gender, smoking status (including pack-years assessment), performance status (PS), stage of disease, pathomorphological diagnosis, line of EGFR-TKIs treatment, and information about prior surgical treatment.

Treatment was continued until progression or unacceptable toxicity. After discontinuation of EGFR-TKIs treatment, patients could receive chemotherapy or palliative radiotherapy. During this study, third-generation of EGFR-TKIs (e.g. osimertinib) have not been available in Poland. Also, therapeutic programs in Poland did not allow any possibility of switching the type of EGFR-TKIs in patients after progression on EGFR-TKIs. Five patients (2.8%) had early, no treatment-related toxicity of grade 4 and required discontinuation of EGFR-TKIs treatment. They were not included in our survival analysis.

Response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) and evaluation was performed by computed tomography every 2 months of EGFR-TKIs treatment. The treatment toxicity was assessed by Common Toxicity Criteria (CTC) scale (version 4.0). Performance status was evaluated according to Eastern Cooperative Oncology Group (ECOG) scale.

EGFR gene mutations analysis. DNA was extracted from tumor tissue or tumor cells obtained during routine diagnostic or therapeutic procedures (bronchoscopy, endobronchial ultrasound-guided transbronchial needle aspiration, mediastinoscopy, or surgical resection). Formalin-fixed paraffin-embedded materials, or cytological slides containing at least 10% of tumor cells, were used for molecular examination. Mutations of the *EGFR* gene (NM_0,05228.3) were tested using routine real-time PCR procedures and the EntroGen EGFR Mutations Analysis kit (USA). The mutations in exons 18 to 21 were examined (Table I). Non-classical deletions in exon 19 were distinguished from the classical deletion in exon 19 by a direct sequencing method.

Statistical analysis. Treatment outcomes included response rate, disease control rate, progression free survival (PFS), and overall survival (OS). PFS and OS were defined as the time elapsed between the date of EGFR-TKIs treatment beginning and the date of disease progression or death, respectively. In the absence of information about the progression or death, data were classified as censored (time was calculated to the last observation). Statistical analysis was performed using Statistica 10 (Statsoft, USA) and MedCalc 10 (MedCalc Software, Belgium). A $P<0.05$ was considered statistically significant. Using the Fisher's exact test, we assessed the associations between clinical factors and response rate or disease control rate. The Kaplan-Meier log-rank test was used to draw a comparison curve evaluating the survival probability (PFS and OS). Cox regression model with a stepwise selection with minimum AIC factor (Akaike Information Criterion) was used to determine the influence of clinical and genetic factors on PFS and OS.

Results

Patient characteristics. The groups of patients treated with different EGFR-TKIs were comparable with respect to their

Table I. Type of examined mutations in the *EGFR* gene.

Exon	Type of mutations
18	p.Gly719Ala (c.2156G>C), p.Gly719Ser (c.2155G>A), p.Gly719Cys (c.2155G>T)
19	c.2235-2249 del 15, c.2235-2252>AAT del 18, c.2236-2253 del 18, c.2237-2251 del 15, c.223c.7-2254 del 18, c.2237-2255>T del 19, c.2236-2250 del 15, c.2238-2255 del 18, c.2238-2248>GC del 11, c.2238-2252>GCA del 15, c.2239-2247 del 9, c.2239-2253 del 15, c.2239-2256 del 18, c.2239-2248>C del 10, c.2239-2258>CA del 20, c.2240-2251 del 12, c.2240-2257 del 18, c.2240-2254 del 15, c.2239-2251>C del 13
20	p.Thr790Met (c.2369C>T), p.Ser768Ile (c.2303G>T), c.2307-2308 ins GCCAGCGTG, c.2319-2320 ins CAC, c.2310-2311 ins GGT
21	p.Leu858Arg (c.2573T>G), p.Leu861Gln (c.2582T>A)

demographic, clinical, and molecular factors (Table II). The median age of all patients was 67 years and 55% patients were 67 years of age or older. 71% of EGFR-TKIs treated patients were women, and 43%-non-smokers. Smokers with *EGFR* mutations were rather heavy smokers, with a median pack-year history of 20. Most patients had a diagnosis of adenocarcinoma (95%) and distant metastases (81%), including brain metastases (15%). NSCLC recurrence after surgery was found in 17% of patients. All patients were in very good or good performance status. EGFR-TKIs were used in the first (72%), second (24%), or third-line (3.9%) of treatment. In progression after EGFR-TKIs therapy, 41% of patients obtained palliative radiotherapy and 39% of patients received one (30%) or more (9.4%) lines of chemotherapy.

Classical exon 19 deletions were found in 64% of patients, exon 21 p.Leu858Arg substitution was found in 29% of patients, and a relatively large population of patients (7.2%) had rare *EGFR* mutations. Among the rare *EGFR* mutations, insertions in exon 20 were most frequently diagnosed (23% of rare mutations), while double mutations of substitutions in codon 719 and 861 or 768 were found in two patients (15% of rare mutations). Detailed characteristics of the 13 NSCLC patients with rare *EGFR* gene mutations are outlined in Table II.

Response rates. Partial response (PR), complete response (CR), and disease control was achieved in 55, 3.3, and 80.5% of patients, respectively, while early progression occurred in 14.5% of patients treated with EGFR-TKIs (Table III). Demographic and clinical factors had no significant impact on the risk of NSCLC progression (Table III). Response to EGFR-TKIs was significantly ($P<0.05$) more frequent in patients with a deletion in exon 19 than in patients with rare mutations in *EGFR* gene. All patients with an insertion in exon 20 of *EGFR* gene showed early disease progression. All other patients with rare *EGFR* gene mutations (including patients with p.Ser768Ile substitution in exon 20) responded to treatment, except for a female patient with substitution at codon 747 of exon 19 and a female patient with double mutations in codons 719 and 768 in exons 18 and 20, in whom short stabilization of the disease occurred. The detailed characteristics of the response to treatment in the 13 NSCLC patients with rare *EGFR* gene mutations are provided in Table III.

Progression-free survival. The median PFS of patients receiving EGFR-TKIs treatment was 10 months, and 34.3% had no disease progression during observation. A comparison of the probability of PFS in NSCLC patients with *EGFR* gene mutations treated with erlotinib, gefitinib or afatinib is shown in Fig. 1. The median PFS was 10 months in patients treated with erlotinib, 9 months in patients treated with gefitinib, and 15 months in patients treated with afatinib (Table IV). Although patients treated with afatinib showed the longest median PFS, it was not significantly different from that observed with the other EGFR-TKIs.

Demographic and clinical factors had no significant impact on the PFS of patients treated with EGFR-TKIs. While, molecular factors did influence the clinical outcomes of EGFR-TKIs treatment (Table IV). Patients with common *EGFR* mutations showed significantly longer median PFS than patients with rare *EGFR* mutations (10 vs. 5 months; $P=0.009$). The significant difference in median PFS occurred between the group of patients with exon 19 deletion and group of patients with rare *EGFR* mutations ($P<0.005$). The median PFS was only slightly longer in patients with substitution p.Leu858Arg compared to patients with rare *EGFR* mutations. Moreover, insignificant ($P=0.095$) longer median PFS was observed in patients with exon 19 deletion than in patients with p.Leu858Arg substitution (Table IV, Fig. 2).

Overall survival. Demographic, clinical, and molecular factors did not affect the median OS (27 months) in our study (Table V). One-year and two-years OS for patients treated with EGFR-TKIs was 66.3 and 26.3%, respectively. A comparison of the probability of OS in NSCLC patients with *EGFR* gene mutations treated with erlotinib, gefitinib or afatinib is shown in Fig. 3. The median OS was 26 months in the gefitinib and erlotinib groups, whereas in the afatinib group, the median OS had not been reached at the time of the analysis (Table V). However, these differences in OS were not statistically significant among the three treatment arms.

Adverse events. Severe, no treatment-related toxicity (grade 4) resulting in discontinuation of EGFR-TKIs treatment only occurred in five patients in our study. Afatinib and erlotinib showed significantly more frequent rash and other skin toxicities, as well as diarrhea, compared to gefitinib. All patients

Table II. Patients' characteristics.

Characteristic	Total (n=180)	Erlotinib (n=98)	Gefitinib (n=66)	Afatinib (n=16)
Age				
Age (years, median ± SD)	67±11.8	67±11.09	69±13.06	62±9.84
≥67 years (n, %)	99,55	52,53.1	42,63.6	5,31.25
<67 years (n, %)	81,45	46,46.9	24,36.4	11,68.75
Gender				
Female (n, %)	128,71.1	72,73.5	46,69.7	10,62.5
Male (n, %)	52,28.9	26,26.4	20,30.3	6,37.5
Histopathological diagnosis				
Adenocarcinoma (n, %)	171,95.0	92,93.9	64,97.0	15,94.0
Adenosquamous carcinoma (n, %)	3,1.7	2,2.0	1,1.5	0,0.0
Large cell carcinoma (n, %)	2,1.1	1,1.0	0,0.0	1,6.0
NOS (n, %)	4,2.2	3,3.1	1,1.5	0,0.0
Performance status (PS)				
PS=0 (n, %)	33,18.3	23,23,5	8,12.1	2,12.5
PS≥1 (n, %)	147,81.7	75,76.5	58,87.9	14,87.5
Stage of disease				
IIIB (n, %)	33,18.3	22,22.4	8,12.1	3,18.8
IV (n, %)	147,81.7	76,77.6	58,87.9	13,81.2
CNS metastases				
Yes (n, %)	28,15.6	16,16.3	12,18.2	0,0.0
No (n, %)	152,84.4	82,83.7	54,81.8	16,100.0
Prior surgical treatment				
Yes (n, %)	31,17.2	14,14.3	13,19.7	4,25
No (n, %)	149,82.8	84,85.7	53,80.3	12,75
Smoking history				
Yes (n, %)	59,32.8	30,30.6	23,34.8	6,37.5
No (n, %)	78,43.3	47,48.0	22,33.3	9,56.25
No data (n, %)	43,23.9	21,21.4	21,31.8	1,6.25
Pack-years (median ± SD)	20±12.4	20±11.14	20±14.35	22.5±9.91
Line of EGFR-TKI therapy				
I (n, %)	129,71.7	53,54.1	63,95.5	13,81.2
II or III (n, %)	51,28.3	45,45.9	3,4.5	3,18.8
Early EGFR-TKI therapy discontinuation due to grade 3-4 toxicities				
Yes (n, %)	175,97.2	96,98	64,97	15,94.0
No (n, %)	5,2.8	2,2	2,3	1,6.0
Chemotherapy after EGFR-TKI treatment				
Yes (n, %)	71,39.4	38,38.8	28,42.4	5,31.3
No (n, %)	109,60.4	60,61.2	38,57.6	11,68.7
Palliative radiotherapy after EGFR-TKI treatment				
Yes (n, %)	73,40.6	42,42.9	30,45.5	1,6.0
No (n, %)	107,59.4	56,57.1	36,54.5	15,94.0
EGFR mutations status				
Deletion in exon 19 (n, %)	115,63.9	59,60.2	46,69.7	10,62.5
Substitution p.Leu858Arg (n, %)	52,28.9	32,32.7	16,24.2	4,25.0
Rare mutations (n, %)	13,7.2	7,7.1	4,6.1	2,12.5

Table III. Response to EGFR-TKI treatment according to demographic and clinical characteristics.

Characteristic	Partial response+complete response (n=105)	Stable disease (n=49)	Progressive disease (n=26)	p; χ^2
Age				
≥67 years, n=99 (n, %)	57,57.5	25,25.3	17,17.2	0.479;1.468
<67 years, n=81 (n, %)	48,59.3	24,29.6	9,11.1	
Gender				
Female, n=128 (n, %)	78,60.9	35,27.3	15,11.7	0.247;2.796
Male, n=52 (n, %)	27,51.9	14,26.9	11,21.2	
Histopathological diagnosis				
Adenocarcinoma, n=171 (n, %)	99,57.9	47,27.5	25,14.6	0.872;0.273
Other, n=9 (n, %)	6,66.7	2,22.2	1,11.1	
Performance status (PS)				
PS=0, n=33 (n, %)	24,72.7	7,21.2	2,6.1	0.14;3.938
PS≥1, n=147 (n, %)	81,55.1	42,28.6	24,16.3	
Stage of disease				
IIIB, n=33 (n, %)	22,66.7	7,21.2	4,12.1	0.558;1.168
IV, n=147 (n, %)	83,56.5	42,28.5	22,15.0	
CNS metastases				
Yes, n=28 (n, %)	16	7	5	0.845;0.336
No, n=152 (n, %)	89	42	21	
Prior surgical treatment				
Yes, n=31 (n, %)	21,67.7	6,19.4	4,12.9	0.477;1.481
No, n=149 (n, %)	84,56.4	43,28.9	22,14.8	
Smoking history				
Yes, n=59 (n, %)	39,66.1	13,22.0	7,11.9	0.643;0.883
No, n=78 (n, %)	46,59.0	19,24.4	13,16.6	
Line of EGFR-TKI therapy				
I, n=129 (n, %)	75,58.1	37,28.7	17,13.2	0.649;0.865
II or III, n=51 (n, %)	30,58.8	12,23.5	9,17.6	
Type of EGFR-TKI (reversible vs. irreversible)				
Erlotinib and gefitinib, n=164 (n, %)	95,58.0	46,28.0	23,14.0	0.69;0.741
Afatinib, n=16 (n, %)	10,62.5	3,18.75	3,18.75	
Type of EGFR-TKI (only reversible)				
Erlotinib, n=98 (n, %)	58,59.2	26,26.5	14,14.3	0.87;0.278
Gefitinib, n=66 (n, %)	37,56.1	20,30.3	9,13.6	
Type of EGFR-TKI (erlotinib vs. afatinib)				
Erlotinib, n=98 (n, %)	58,59.2	26,26.5	14,14.3	0.764;0.537
Afatinib, n=16 (n, %)	10,62.5	3,18.75	3,18.75	
Type of EGFR-TKI (gefitinib vs. afatinib)				
Gefitinib, n=66 (n, %)	37,56.1	20,30.3	9,13.6	0.626;0.936
Afatinib, n=16 (n, %)		10,62.5	3,18.75	3,18.75
EGFR mutations status (only common)				
Deletion in exon 19, n=115 (n, %)	66,57.4	36,31.3	13,11.3	0.302;2.396

Table III. Continued.

Characteristic	Partial response+complete response (n=105)	Stable disease (n=49)	Progressive disease (n=26)	p; χ^2
Substitution p.Leu858Arg, n=52 (n, %)	32,61.5	11,21.2	9,17.3	
EGFR mutations status (common vs. rare)				
Common mutations, n=167 (n, %)	98,58.7	47,28.1	22,13.2	0.178;3.355
Rare mutations, n=13 (n, %)	7,53.8	2,15.4	4,30.8	
EGFR mutations status (deletion in exon 19 vs. rare)				
Deletion in exon 19, n=115 (n, %)	66,57.4	36,31.3	13,11.3	0.113;4.355
Rare mutations, n=13 (n, %)	7,53.8	2,15.4	4,30.8	
EGFR mutations status (substitution p.Leu858 arg vs. rare)				
Substitution p.Leu858Arg, n=52 (n, %); 1.218	32,61.5	11,21.2	9,17.3	0.544
Rare mutations, n=13 (n, %)	7,53.8	2,15.4	4,30.8	

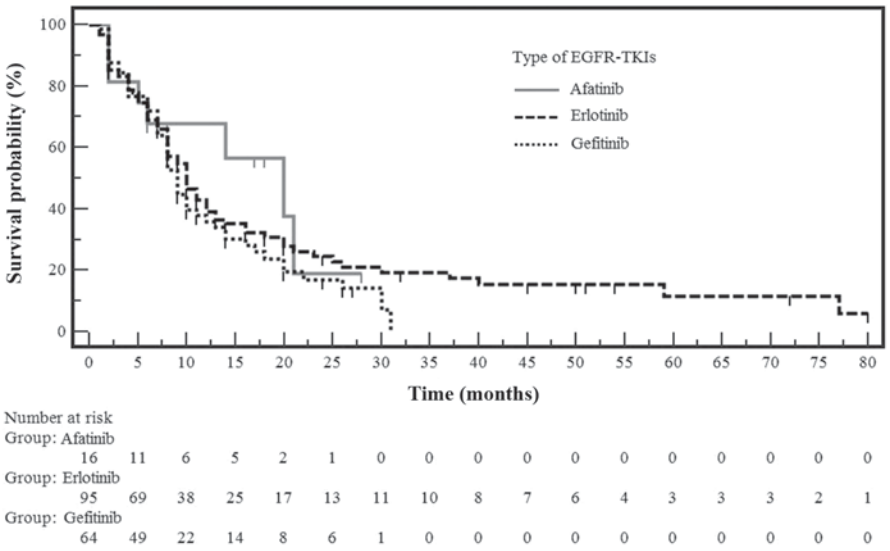

Figure 1. Probability of progression free survival in NSCLC patients with *EGFR* gene mutations treated with erlotinib, gefitinib or afatinib.

treated with afatinib showed mild diarrhea. Hepatotoxicity occurred only in three patients treated with gefitinib.

Discussion

This study was the first to directly compare EGFR-TKIs treatment efficacy in patients with NSCLC harboring common and rare activating *EGFR* mutations. Erlotinib, gefitinib, and afatinib had similar effectiveness in patients with common and rare *EGFR* mutations, although patients treated with afatinib had a slightly longer PFS. A relatively large proportion of our patients (7.2%) had rare *EGFR* gene mutations, and these patients had significantly poorer median PFS than those with common *EGFR* mutations (P<0.05). Moreover, patients with a rare insertion in exon 20 of *EGFR* gene showed early disease progression. Therefore, detection of specific *EGFR* mutations is important for EGFR-TKIs treatment outcomes.

Similar to our results, a previous meta-analysis by Liang *et al* indicated that erlotinib, gefitinib, and afatinib have equivalent efficacy. This meta-analysis included twelve phase III global clinical trials involving 1812 NSCLC patients with activating *EGFR* gene mutations (12). Authors reported a

Table IV. Progression-free survival (PFS) in NSCLC patients treated with EGFR-TKIs.

Characteristic	Median PFS (months)	Univariate analysis		Multivariate analysis	
		P	HR (95% CI)	P	HR (95% CI)
Age					
≥67 years	10	0.799	0.959 (0.679-1.354)	0.664	0.921 (0.638-1.331)
<67 years	10				
Gender					
Female	10	0.094	1.345 (0.914-1.981)	0.107	1.374 (0.935-2.02)
Male	8				
Histopathological diagnosis					
Adenocarcinoma	10	0.997	1.001 (0.468-2.143)	0.532	1.294 (0.579-2.893)
Other	11				
Performance status (PS)					
PS=0	10	0.399	1.208 (0.785-1.858)	0.896	1.036 (0.611-1.757)
PS≥1	10				
Stage of disease					
IIIB	13	0.191	1.343 (0.879-2.053)	0.242	1.37 (0.811-2.317)
IV	9				
CNS metastases					
Yes	9	0.256	1.277 (0.79-2.065)	0.421	1.214 (0.758-1.945)
No	10				
Prior surgical treatment					
Yes	16	0.094	1.432 (0.956-2.145)	0.285	1.307 (0.802-2.128)
No	9				
Smoking history					
Yes	9	0.448	1.139 (0.806-1.608)	0.859	1.035 (0.713-1.501)
No	10				
Line of EGFR-TKI therapy					
I	10	0.325	0.841 (0.588-1.204)	0.23	0.787 (0.533-1.162)
II or III	10				
Type of EGFR-TKI (reversible vs. irreversible)					
Erlotinib and gefitinib	10	0.533	1.243 (0.648-2.382)	0.454	1.333 (0.63-2.819)
Afatinib	18				
Type of EGFR-TKI (only reversible)					
Erlotinib	10	0.25	1.219 (0.847-1.754)		
Gefitinib	9				
Type of EGFR-TKI (erlotinib vs. afatinib)					
Erlotinib	10	0.623	1.191 (0.586-2.44)		
Afatinib	18				
Type of EGFR-TKI (gefitinib vs. afatinib)					
Gefitinib	9	0.431	1.329 (0.655-2.694)		
Afatinib	18				
EGFR mutations status (only common)					
Deletion in exon 19	11	0.095	1.361 (0.909-2.036)		
Substitution p.Leu858Arg	8				

Table IV. Continued.

Characteristic	Median PFS (months)	Univariate analysis		Multivariate analysis	
		P	HR (95% CI)	P	HR (95% CI)
EGFR mutations status (deletion in exon 19 vs. rare)					
Deletion in exon 19	11	0.0043	0.429 (0.099-0.65)		
Rare mutations	5				
EGFR mutations status (substitution p.Leu 858Arg vs. rare)					
Substitution p.Leu858Arg	8	0.115	0.616 (0.236-1.17)		
Rare mutations	5				
EGFR mutations status (common vs. rare)					
Common mutations	10	0.009	2.155 (0.907-5.122)	0.008	2.437 (1.263-4.702)
Rare mutations	5				

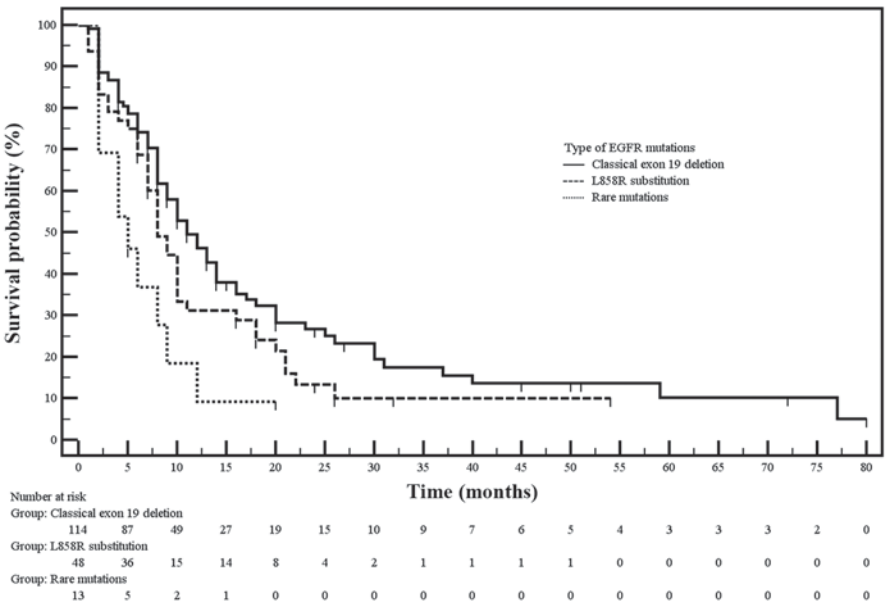

Figure 2. Probability of progression free survival in NSCLC patients treated with EGFR-TKIs depending on the type of mutation in *EGFR* gene.

1-year PFS of 43%, compared to 34% in our study. Moreover, 1-and 2-year OS rates were 79 and 50% in Liang study, compared to 66 and 26% in our study. The slight improvement in EGFR-TKIs efficacy shown by Liang *et al* (12) compared to us may be due to the fact that the patients in the clinical trials were closely matched. Moreover, most clinical trials enrolled an Asian patients compared to the Caucasian population used in our study. However, the nonrandomized design of our study did not allow a reliable assessment of the efficacy of particular EGFR-TKIs.

Despite the lower efficacy of the EGFR-TKIs observed in our study, the type and severity of adverse events was similar to those described in previous clinical trials (12). For example, afatinib and erlotinib resulted in a more severe rash and diarrhea in patients compared with gefitinib. Therefore, our results in a Caucasian cohort indicate that EGFR-TKIs are effective and show similar side effect profiles to previous studies in Asian populations.

Similar to our findings, Lim *et al* showed no difference in PFS between the erlotinib- and gefitinib-treated groups (11.7 vs. 14.5 months; P=0.507) in a retrospective case-control study of matched Asian patients (121 pairs) with NSCLC (13). These patients were young (median age 58 years), mostly non-smokers (64%), in very good or good performance status (91%), and received EGFR-TKIs treatment mainly in the second-line (74%) Patients showed excellent overall response rates to erlotinib and gefitinib (77 and 74.5%, respectively) (13). However, Kim *et al* showed the drugs were not as efficient in a randomized phase II study of 96 Asian patients with advanced NSCLC: In the erlotinib- and gefitinib-treatment arms the

Table V. Overall survival (OS) in NSCLC patients treated with EGFR-TKIs.

Characteristic	Median OS (months)	Univariate analysis		Multivariate analysis	
		P	HR (95% CI)	P	HR (95% CI)
Age					
≥67 years	32	0.482	0.853 (0.536-1.357)	0.373	0.797 (0.485-1.309)
<67 years	25				
Gender					
Female	32	0.25	1.322 (0.784-2.228)	0.224	1.405 (0.814-2.427)
Male	23				
Histopathological diagnosis					
Adenocarcinoma	26	0.686	0.815 (0.27-2.46)	0.896	0.929 (0.308-2.797)
Other	22				
Performance status (PS)					
PS=0	32	0.447	1.263 (0.713-2.239)	0.598	0.82 (0.394-1.706)
PS≥1	26				
Stage of disease					
IIIB	Not reached	0.077	1.829 (1.038-3.222)	0.092	2.013 (0.895-4.527)
IV	26				
CNS metastases					
Yes	23	0.405	1.282 (0.668-2.458)	0.553	1.235 (0.617-2.469)
No	26				
Prior surgical treatment					
Yes	51	0.181	1.477 (0.867-2.517)	0.333	1.39 (0.716-2.699)
No	25				
Smoking history					
Yes	26	0.706	1.089 (0.692-1.714)	0.873	0.96 (0.581-1.584)
No	26				
Line of EGFR-TKI therapy					
I	26	0.841	0.955 (0.602-1.517)	0.807	0.939 (0.568-1.552)
II or III	26				
Type of EGFR-TKI (reversible vs. irreversible)					
Erlotinib and gefitinib	26	0.408	1.77 (0.603-5.2)	0.455	1.739 (0.41-7.376)
Afatinib	Not reached				
Type of EGFR-TKI (only reversible)					
Erlotinib	26	0.353	1.238 (0.768-1.997)		
Gefitinib	26				
Type of EGFR-TKI (erlotinib vs afatinib)					
Erlotinib	26	0.418	1.754 (0.513-5.008)		
Afatinib	Not reached				
Type of EGFR-TKI (gefitinib vs afatinib)					
Gefitinib	26	0.425	1.739 (0.497-5.255)		
Afatinib	Not reached				
Chemotherapy after EGFR-TKI treatment					
Yes	26	0.677	0.909 (0.576-1.436)	0.679	1.11 (0.679-1.813)
No	26				

Table V. Continued.

Characteristic	Median OS (months)	Univariate analysis		Multivariate analysis	
		P	HR (95% CI)	P	HR (95% CI)
Palliative radiotherapy after EGFR-TKI treatment					
Yes	26	0.434	1.191 (0.757-1.874)	0.8	0.931 (0.538-1.611)
No	32				
EGFR mutations status (only common)					
Deletion in exon 19	27	0.604	1.143 (0.669-1.954)		
Substitution p.Leu858Arg	26				
EGFR mutations status (deletion in exon 19 vs. rare)					
Deletion in exon 19	27	0.201	0.588 (0.166-1.459)		
Rare mutations	22				
EGFR mutations status (substitution p.Leu858 arg vs. rare)					
Substitution p.Leu858Arg	26	0.799	0.885 (0.304-2.504)		
Rare mutations	22				
EGFR mutations status (common vs. rare)					
Common mutations	26	0.251	1.605 (0.576-4.473)	0.31	1.592 (0.652-3.885)
Rare mutations	22				

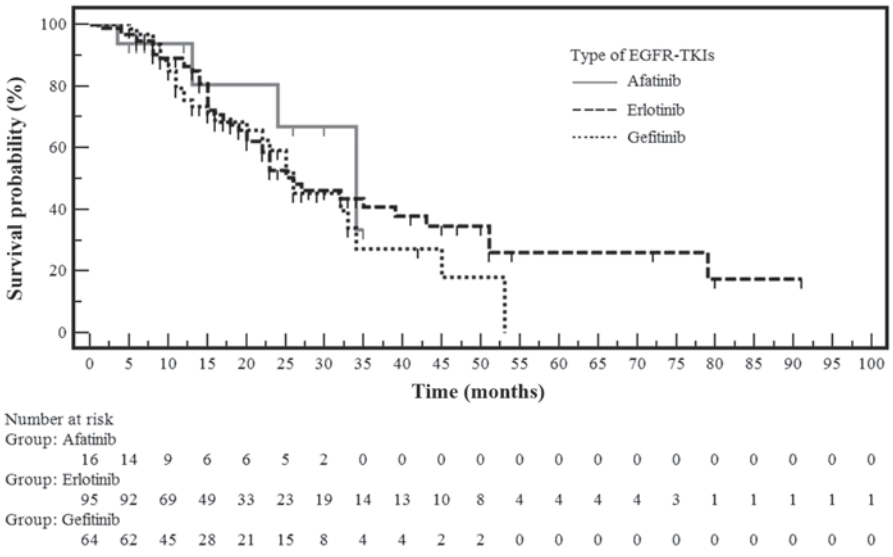

Figure 3. Probability of overall survival in NSCLC patients with *EGFR* gene mutations treated with erlotinib, gefitinib or afatinib.

response rates were 40 and 48%, and the median PFS was only 3.1 and 4.9 months, respectively (6). The authors concluded that the reason for treatment failure was including patients with unknown *EGFR* gene mutations with at least two out of three clinical factors associated with a higher incidence of *EGFR* gene mutations (6). Similarly, in a recent randomized phase III study of 562 pretreated patients with lung adenocarcinoma (including 401 with *EGFR* mutations), the response rates were 44 and 46% and the median PFS was 7.5 and 6.5 months in erlotinib- and gefitinib-treatment arms, respectively (14).

The first head-to-head comparison of afatinib and gefitinib was recently reported in the prospective phase IIb LUX-Lung 7 clinical trial (7). In this trial, 319 Caucasian and Asian NSCLC patients with common *EGFR* gene mutations were

randomized to first-line therapy with afatinib or gefitinib (7). Afatinib showed significant improvement in PFS, with a median duration of response of 10.1 months compared to 8.4 months with gefitinib (HR=0.73; 95% CI, 0.57-0.95; P=0.0165) (7). Similarly, in our study, we found a slight improvement in PFS with afatinib (18 months) compared to the two reversible EGFR-TKIs (10 months) although this was not statistically significant (HR=1.243; 95% CI, 0.648-2.382; P=0.533). However, due to the small sample size (n=16) of the afatinib-treated group, we cannot make any definitive conclusions about the observed difference in PFS.

We found that demographic and clinical factors did not affect the effectiveness of the EGFR-TKIs treatment of patients harboring *EGFR* gene mutations. While some authors have emphasized the impact of patients' performance status on the effectiveness of EGFR-TKIs treatment. The differences in EGFR-TKIs effectiveness in past studies were only found when groups of patients in good and very good performance status (PS=0 or 1) were compared with groups of patients with satisfactory performance status (PS=2) (15). Such comparison was not performed in the current study (only patients with PS=0 or 1 were included). Therefore, the impact of performance status on EGFR-TKIs requires further investigation.

We found that patients with the common exon 19 deletion in *EGFR* had a slightly longer PFS after treatment with EGFR-TKIs than patients with exon 21 p.Leu858Arg substitution (11 vs. 8 months; P=0.095) or rare *EGFR* mutations (11 vs. 5 months; P<0.005). Urata *et al* found no significant difference in the PFS among patients with the *EGFR* p.Leu858Arg mutation (n=172), the *EGFR* exon 19 deletion (n=192), or those with rare *EGFR* mutations (n=25) who were treated with gefitinib and erlotinib (14). Zhang *et al* also showed that the patients with the exon 19 deletion in *EGFR* receiving first-line EGFR-TKIs had longer PFS than those with exon 21 substitution (16). Similarly, Urata *et al* identified patients with the *EGFR* exon 19 deletion subgroup had slightly longer PFS when treated with gefitinib and erlotinib than those with p.Leu858Arg mutation (14). Furthermore, analysis of two phase III trials, LUX-Lung 3 and LUX-Lung 6, indicated that the first-line afatinib compared to chemotherapy improved OS for patients with the *EGFR* exon 19 deletion but not for patients with p.Leu858Arg substitution (17). We found that the common *EGFR* mutations (exon 19 deletion and p.Leu858Arg) did not impact the OS in this study. However, we did not specifically investigate these differences in the afatinib-treated group due to the small sample size (n=16).

Beau-Faller *et al* recently proved that rare *EGFR* gene mutations could be associated with resistance to EGFR-TKIs treatment (distal exon 20 insertions) or sensitivity to EGFR-TKIs treatment (exon 18 substitution or complex *EGFR* mutations) in Caucasian NSCLC patients (10). When investigating 50 NSCLC patients with rare *EGFR* gene mutations treated with EGFR-TKIs, they found that primary resistance to EGFR-TKIs was diagnosed in 54% of patients with exon 20 mutations, in 66% of patients with exon 18 substitutions, and in 14% of patients with more complex *EGFR* mutations (10). However, median OS from EGFR-TKIs was better for patients with exon 18 (22 months) than for patients with exon 20 mutations (9.5 months) (10). Our results fully agree with those of Beau-Faller *et al* (10) primarily finding that patients with exon 20 insertions of *EGFR* failed to respond to EGFR-TKIs treatment. Resistance to EGFR TKIs therapy has been associated with a Thr790Met substitution in exon 20 of *EGFR* (18). However, no patients with primary Thr790Met mutation were enrolled in our study. By contrast, patients with non-classical exon 19 deletions (especially deletions of greater than 15 bp) and rare substitutions (i.e., mutations in codon 858 and 861 in exon 21) had good response to EGFR-TKIs treatment (19,20). Our study confirms results indicating that rare *EGFR* mutations are important for EGFR-TKIs treatment outcomes (21). However, further research is required to build a database of all *EGFR* mutations and their individual impact on the differing EGFR-TKIs treatments.

To combat treatment resistance, third-generation EGFR-TKIs against the p.Thr790Met substitution in exon 20 of *EGFR* have been developed, including osimertinib, rociletinib, HM61713, ASP8273, EGF816, and PF-0,674,7775 (22). Recently, osimertinib has been registered for treatment of p.Thr790Met positive patients after failure of first- or second-generations EGFR-TKIs therapy. Clinical trials on these third-generation EGFR-TKIs are currently underway. However, further research is required to develop novel inhibitors that combat resistance in some of the other rare *EGFR* mutations.

Our study confirms that EGFR-TKIs treatment is effective in NSCLC patients with *EGFR* gene mutations, irrespective of demographic and clinical factors. We found no significant differences in the effectiveness of erlotinib, gefitinib, and afatinib among our Caucasian cohort of patients. However, qualification of patients with rare *EGFR* gene mutations, especially those with exon 20 insertions, to EGFR-TKIs treatment requires special attention due to the varied effectiveness of EGFR-TKIs treatment in this group of patients.

Acknowledgements

We thank all participating patients and their families. We also thank Proper Medical Writing Sp. z o.o, Poland, for editorial support and linguistic corrections.

References

1. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, *et al*: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362: 2380-2388, 2010.
2. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, *et al*: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361: 947-957, 2010.
3. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, *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 13: 239-246, 2012.
4. Wu WS, Wu CH, Lai SL, Chiu CH, Shih JF, Lee YC and Chen YM: Erlotinib salvage therapy in pulmonary adenocarcinoma patients with disease progression after previous EGFR-TKI treatment. Am J Clin Oncol 39: 556-562, 2016.
5. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, Li W, Hou M, Shi JH, Lee KY, *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 15: 213-222, 2014.

6. Kim ST, Uhm JE, Lee J, Sun JM, Sohn I, Kim SW, Jung SH, Park YH, Ahn JS, Park K and Ahn MJ: Randomized phase II study of gefitinib versus erlotinib in patients with advanced non-small cell lung cancer who failed previous chemotherapy. Lung Cancer 75: 82-88, 2012.
7. Park K, Tan E-H, Zhang L, Hirsh V, O'Byrne K, Boyer M *et al:* LBA2_PR Afatinib (A) vs gefitinib (G) as first-line treatment for patients (pts) with advanced non-small cell lung cancer (NSCLC) harboring activating EGFR mutations: Results of the global, randomized, open-label, Phase IIb trial LUX-Lung 7 (LL7). Ann Oncol 26 (Suppl 9): ix161-ix162, 2015.
8. Ramalingam SS, O'Byrne K, Boyer M, Mok T, Janne PA, Zhang H, Liang J, Taylor I, Sbar EI and Paz-Ares L: Dacomitinib versus erlotinib in patients with EGFR-mutated advanced nonsmall-cell lung cancer (NSCLC): Pooled subset analyses from two randomized trials. Ann Oncol 27: 423-410, 2016.
9. Karachaliou N, Molina-Vila MA and Rosell R: The impact of rare EGFR mutations on the treatment response of patients with non-small cell lung cancer. Expert Rev Respir Med 9: 241-244, 2015.
10. Beau-Faller M, Prim N, Ruppert AM, Nanni-Metéllus I, Lacave R, Lacroix L, Escande F, Lizard S, Pretet JL, Rouquette I, *et al*: Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: A multicentre observational study by the French ERMETIC-IFCT network. Ann Oncol 25: 126-131, 2014.
11. Krawczyk P, Reszka K, Ramlau R, PowrÓzek T, Pankowski J, Wojas-Krawczyk K, Kalinka-Warzocha E, Szczęsna A, Nicoś M, Jarosz B, *et al*: Prevalence of rare EGFR gene mutations in nonsmall-cell lung cancer: A multicenter study on 3856 Polish Caucasian patients. Ann Oncol 27: 358-359, 2016.
12. Liang W, Wu X, Fang W, Zhao Y, Yang Y, Hu Z, Xue C, Zhang J, Zhang J, Ma Y, *et al*: Network meta-analysis of erlotinib, gefitinib, afatinib and icotinib in patients with advanced non-small-cell lung cancer harboring EGFR mutations. PLoS One 9: e85245, 2014.
13. Lim SH, Lee JY, Sun JM, Ahn JS, Park K and Ahn MJ: Comparison of clinical outcomes following gefitinib and erlotinib treatment in non-small-cell lung cancer patients harboring an epidermal growth factor receptor mutation in either exon 19 or 21. J Thorac Oncol 9: 506-511, 2014.
14. Urata Y, Katakami N, Morita S, Kaji R, Yoshioka H, Seto T, Satouchi M, Iwamoto Y, Kanehara M, Fujimoto D, *et al*: Randomized phase III study comparing gefitinib with erlotinib in patients with previously treated advanced lung adenocarcinoma: WJOG 5108L. J Clin Oncol 34: 3248-3257, 2016.
15. Zhou C and Yao LD: Strategies to improve outcomes of patients with EGFR-mutant non-small cell lung cancer: Review of the literature. J Thorac Oncol 11: 174-186, 2016.
16. Zhang Y, Sheng J, Kang S, Fang W, Yan Y, Hu Z, Hong S, Wu X, Qin T, Liang W and Zhang L: Patients with exon 19 deletion were associated with longer progression-free survival compared to those with L858R mutation after first-line EGFR-TKIs for advanced non-small cell lung cancer: A meta-analysis. PLoS One 9: e107161, 2014.
17. Yang JC, Wu YL, Schuler M, Sebastian M, Popat S, Yamamoto N, Zhou C, Hu CP, O'Byrne K, Feng J, *et al*: Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): Analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol 16: 141-151, 2015.
18. Wu SG, Liu YN, Tsai MF, Chang YL, Yu CJ, Yang PC, Yang JC, Wen YF and Shih JY: The mechanism of acquired resistance to irreversible EGFR tyrosine kinase inhibitor-afatinib in lung adenocarcinoma patients. Oncotarget 7: 12404-12413, 2016.
19. De Pas T, Toffalorio F, Manzotti M, Fumagalli C, Spitaleri G, Catania C, Delmonte A, Giovannini M, Spaggiari L, de Braud F and Barberis M: Activity of epidermal growth factor receptor-tyrosine kinase inhibitors in patients with non-small cell lung cancer harboring rare epidermal growth factor receptor mutations. J Thorac Oncol 6: 1895-1901, 2011.
20. Lohinai Z, Hoda MA, Fabian K, Ostoros G, Raso E, Barbai T, Timar J, Kovalszky I, Cserepes M, Rozsas A, *et al*: Distinct epidemiology and clinical consequence of classic versus rare EGFR mutations in lung adenocarcinoma. J Thorac Oncol 10: 738-746, 2015.
21. Klughammer B, Brugger W, Cappuzzo F, Ciuleanu T, Mok T, Reck M, Tan EH, Delmar P, Klingelschmitt G, Yin AY, *et al*: Examining treatment outcomes with erlotinib in patients with advanced non-small cell lung cancer whose tumors harbor uncommon EGFR mutations. J Thorac Oncol 11: 545-555, 2016.
22. Wang S, Cang S and Liu D: Third-generation inhibitors targeting EGFR T790M mutation in advanced non-small cell lung cancer. J Hematol Oncol 9: 34, 2016.