Pemetrexed *Versus* Erlotinib in the Second-line Treatment of Patients with Advanced-stage Non-squamous NSCLC Harboring Wild-type *EGFR* Gene

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Abstract. *Background: Pemetrexed and erlotinib represent different agents commonly used for the second-line treatment of patients with advanced-stage non-small cell lung cancer (NSCLC). Patients and Methods: We analyzed data of 137 patients with advanced-stage non-squamous NSCLC treated with pemetrexed or erlotinib in the second line. All patients harbored a wild-type epidermal growth factor receptor gene. Genetic testing was performed using a combination of denaturing capillary electrophoresis and direct Sanger sequencing. Results: overall response rate and disease control rate in patients treated with pemetrexed was 20.8% and 62.5% vs. 6.3% and 53.2% in patients treated with erlotinib (p=0.022; p=0.358). Median progression-free and overall survival in patients treated with pemetrexed was 1.6 and 11.3 months vs. 1.9 and 11.4 months in patients treated with erlotinib (p=0.470 and p=0.942, respectively). Erlotinib was associated with skin rash and diarrhea; pemetrexed was associated with hematological toxicity and fatigue. Conclusion: A similar efficacy and different, although well-tolerated, toxicity profile of both pemetrexed and erlotinib was shown.*

Non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer (1), which is one of the most common human malignant diseases and the leading cause of cancer-related death worldwide (2). Pemetrexed and erlotinib represent new effective agents that have been approved for the treatment of patients with advanced-stage NSCLC in recent years. Pemetrexed is an intravenously administered cytostatic antifolate targeting several folate-dependent enzymatic pathways. Phase III randomized clinical trials demonstrated efficacy of pemetrexed in combination with platinum derivative in the first-line treatment of patients with advanced-stage non-squamous NSCLC (3, 4) and also as a single-agent in previously treated patients (5). Erlotinib is an orally administered low-molecular weight tyrosine kinase inhibitor (TKI) targeting epidermal growth factor receptor (EGFR). Phase III clinical trials demonstrated the efficacy of erlotinib in the treatment of advanced-stage NSCLC after failure of previous chemotherapy (6, 7) and also for the first-line treatment of patients harboring activating *EGFR* mutations (8, 9).

The aim of the present study was to compare the efficacy and safety of pemetrexed and erlotinib in second-line treatment of a selected population of patients with advanced-stage non-squamous NSCLC harboring a wild-type *EGFR* gene.

Patients and Methods

Study design and patients. We analyzed data of 137 patients with cytologically or histologically confirmed locally advanced (IIIB) or metastatic stage (IV) non-squamous NSCLC whose disease had progressed after first-line chemotherapy and were treated with pemetrexed or erlotinib in the second line between years 2005 and 2012. The primal objectives of the study were progression-free survival (PFS) and the safety profile, the secondary objectives were overall survival (OS), overall response rate (ORR) and disease control rate (DCR). All patients enrolled in this study were

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successfully genetically tested for the presence of activating *EGFR* mutation (exon 19 deletion or a point mutation in exon 21 termed L858R) and all of them harbored wild-type *EGFR* gene. We compared the outcomes of two groups of patients. The first group involved 49 patients treated with pemetrexed monotherapy in the second line. Pemetrexed was administered to this group intravenously at the standard approved dose of 500 mg/m^2 on day 1 every 3 weeks. The treatment was scheduled for up to six cycles unless there was development of intolerable toxic effects or disease progression occurred. The second group involved 88 patients treated with erlotinib in the second line. Erlotinib was administered to this group orally at the standard approved dose of 150 mg daily; dose interruption or reduction was permitted in the event of treatment-related toxicity. The treatment was continued until disease progression or development of intolerable toxic effects. The baseline patient characteristics are summarized in Table I.

Table I. *Basic clinical characteristics of patients.*

Characteristic	Pemetrexed (n=49)	Erlotinib (n=88)	*p*-Value
Gender, n (%)			
Male	28 (57.1)	45 (51.1)	0.593
Female	21 (42.9)	43 (48.9)	
Age (years)			
Median (5-95% range)	61 (49-76)	65 (48-77)	0.131
Smoking status, n (%)			
Current or former-smoker	45 (91.8)	58 (65.9)	0.001
Never-smoker	4 (8.2)	30 (34.1)	
Histology, n (%)			
Adenocarcinoma	44 (89.8)	84 (95.5)	0.281
Not otherwise specified	5 (10.2)	4 (4.5)	
Stage, n (%)			
IIIB	6 (12.2)	15 (17.0)	0.622
IV	43 (87.8)	73 (83.0)	
ECOG PS, n (%)			
0 or 1	45 (91.8)	49 (55.7)	<0.001
2 or 3	4 (8.2)	39 (44.3)	
Third-line treatment			
Erlotinib or chemotherapy	37 (75.5)	47 (53.4)	0.012
None	12 (24.5)	41 (46.6)	
Fourth-line treatment			
Chemotherapy	14 (28.6)	25 (28.4)	0.999
None	35 (71.4)	63 (71.6)	

ECOG PS: Eastern Cooperative Oncology Group (ECOG) performance status (PS).

Clinical assessment and statistical analysis. The treatment was prospectively monitored and the clinical course of patients was continuously assessed at specific time points. Clinical follow-up including physical examination, plain chest X-ray and routine laboratory tests were performed every 3-4 weeks; computed tomography (CT) or positron-emission tomography-CT (PET-CT) were performed after two or three cycles of pemetrexed and after 2 or 3 months of treatment with erlotinib, respectively. Standard summary statistics were used to describe the sample data set. The significance of differences in baseline characteristics were estimated using Fisher's exact test (in the case of categorical variables) or Mann–Whitney test (in the case of continuous variables). PFS was defined as the time from the date of second-line treatment initiation until the date of documented progression or death. OS was defined as the time from the date of second-line treatment initiation until the date of patient's death. The visualizations of PFS and OS, as well as the estimations, of survival probabilities were performed using Kaplan–Meier survival curves; all point estimates were accompanied by 95% confidence intervals. The differences in survival were tested using the log-rank test. As the acceptable level of statistical significance, p=0.05 was used.

The best treatment response was assessed in terms of complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) using Response Evaluation Criteria in Solid Tumors (RECIST) (10). ORR was defined as the sum of CR and PR. DCR was defined as the sum of CR, PR and SD. Adverse events were recorded and classified by grade according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (11). Comparison of ORR or DCR as well as the occurrence of adverse events (AEs) were performed using Fisher's exact test.

EGFR mutation analysis. The tumor specimens acquired during initial bronschoscopy examination were evaluated by a senior cytologist using standard giemsa staining. In a few cases, a tumor biopsy was processed into formalin-fixed paraffin-embedded (FFPE) histological sections. The cytology slides or, eventually, the FFPE sections, were submitted for molecular genetic testing, which included detection of somatic mutations in *EGFR* gene. If necessary, tumor cells were carefully selected and removed from the samples by laser microdissection using a P.A.L.M. microlaser instrument (Carl Zeiss MicroImaging GmbH, Jena, Germany). The microdissected cells were collected directly into the polymerase chain reaction (PCR) buffer and processed without a special DNA extraction step. In all other cases, the DNA was extracted from tissue cells by a standard spin-column procedure using JetQuick Tissue DNA Isolation Kit (Genomed GmbH, Loehne, Germany). Mutations in exons 19 and 21 of *EGFR* gene were tested by Genoscan mutation detection kits (Genomac International, Prague, Czech Republic) utilizing a denaturing capillary electrophoresis technique on an ABI PRISM 3100 16-capillary genetic analyzer (Applied Biosystems, Foster City, CA, USA). Detected mutations were confirmed by Sanger DNA sequencing using a BigDye v 3.0 chemistry (Applied Biosystems). In rare cases, where the overall fraction of mutated DNA was below the 20% threshold for DNA sequencing, mutation was identified indirectly after forming only a homoduplex fragment with a reference standard for a known mutation.

Results

Survival and response. The median PFS for patients treated with pemetrexed was 1.6 months *vs*. 1.9 months for patients treated with erlotinib (p=0.470) (Figure 1A). The median OS in patients treated with pemetrexed was 11.3 months *vs*. 11.4 months for patients treated with erlotinib (p=0.942) (Figure 1B). The summary of survival data for both compared groups is listed in Table II. In patients treated with pemetrexed, CR was achieved in one (2.1%) patient and PR was achieved in nine (18.8%). In patients treated with erlotinib, CR was not

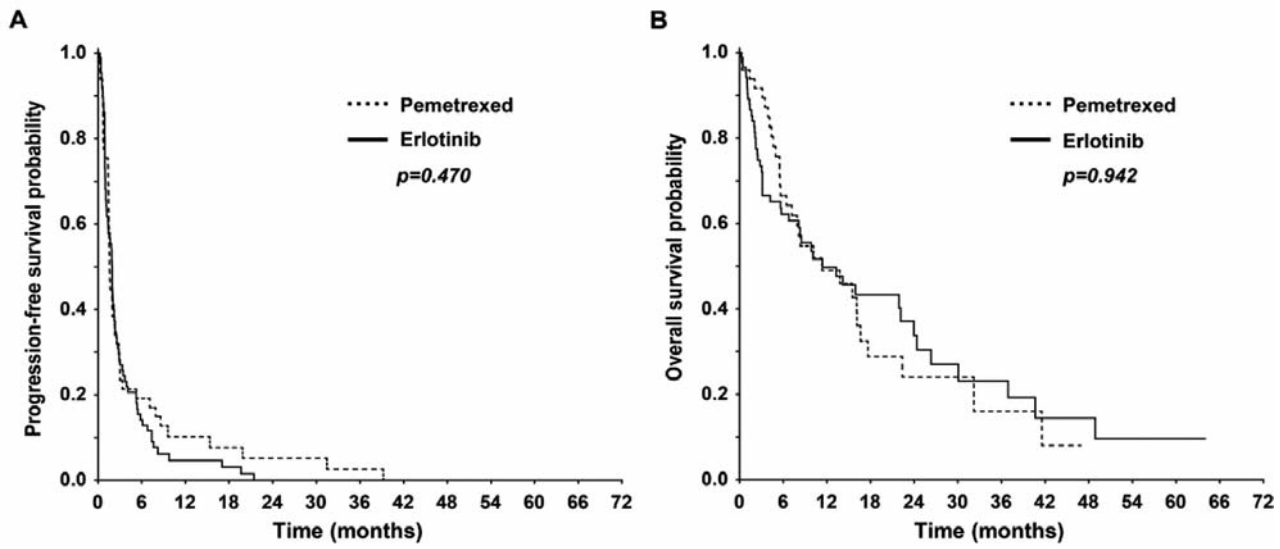

Figure 1. *Comparison of progression-free survival (A) and overall survival (B) between patients treated with pemetrexed and those treated with erlotinib in the second line.*

Table II. *Comparison of patient survival.*

	PFS				OS		
	n	Median (95% CI), months	6-Month rate (95% CI%)	*p*-Value*	Median (95% CI), months	6-Month rate (95% CI%)	*p*-Value*
All patients							
Pemetrexed	49	1.6 (1.4-1.8)	23.4% (11.4-35.5%)	0.470	11.3 (3.6-19.1)	66.5% (52.7%) 80.4)	0.942
Erlotinib	88	1.9 (1.7-2.2)	27.0% (17.2-36.8%)		11.4 (4.0-18.8)	62.1% (51.2%) 73.1)	
Patients with ECOG PS 0/1							
Pemetrexed	45	1.6 (1.4-1.7)	23.3% (10.7-35.8%)	0.812	11.3 (2.7-19.9)	68.8% (54.7%) 82.9)	0.043
Erlotinib	49	2.3 (1.6-3.1)	36.3% (22.2-50.4%)		26.3 (22.3-30.4)	83.3% (71.9%) 94.6)	

CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group (ECOG) performance status (PS). *Log-rank test.

achieved in any patient, and PR was achieved in five (6.3%) patients. The ORR in patients treated with pemetrexed was 20.8% *vs*. 6.3% in patients treated with erlotinib (p=0.022). The DCR in patients treated with pemetrexed was 62.5% *vs*. 53.2% in patients treated with erlotinib (p=0.358). The summary of the best response data for both groups is listed in Table III. Both groups were well balanced according to sex, age, histology and clinical stage. The group treated with pemetrexed included more patients with better Eastern Cooperative Oncology Group (ECOG) performance status (PS) (PS 0/1: 91.8% *vs*. 55.7%; p<0.001) and the group treated with erlotinib included more never-smokers (34.1% *vs*. 8.2%; p=0.001).

Safety and tolerability. The treatment was accompanied by an AE in 20 (40.8%) patients treated with pemetrexed *vs*. 55 (62.5%) patients treated with erlotinib; treatment termination due to AEs occurred in four (8.3%) patients treated with pemetrexed *vs*. five (6.3%) treated with erlotinib. Treatment with pemetrexed was associated with a higher incidence of hematological AEs (12.2% *vs*. 1.1%; p=0.009) and fatigue (16.3% *vs*. 1.1%; p=0.001). Treatment with erlotinib was associated with higher incidence of skin rash (52.3% *vs*. 2.0%; p<0.001) and diarrhea (20.5% *vs*. 0%; p<0.001). Grade 3/4 AEs did not significantly differ between the two groups. No cases of febrile neutropenia, interstitial lung disease-like events or toxic deaths were

Table III. *Comparison of best treatment response*

	n	CR, n (%)	PR, n (%)	SD, n (%)	PD, n (%)	NE, n (%)	ORR, n (%)	*p*-Value*	DCR, n (%)	*p*-Value*
All patients										
Pemetrexed	49	1 (2.1%)	9 (18.8%)	20 (41.7%)	15 (31.3%)	3 (6.3%)	10 (20.8%)	0.022	30 (62.5%)	0.358
Erlotinib	88	0	5 (6.3%)	37 (46.8%)	33 (41.8%)	4 (5.1%)	5 (6.3%)		42 (53.2%)	
Patients with ECOG PS 0/1										
Pemetrexed	45	1 (2.3%)	8 (18.2%)	18 (40.9%)	14 (31.8%)	3 (6.8%)	9 (20.5%)	0.258	27 (61.4%)	0.509
Erlotinib	49	0	5 (11.1%)	26 (57.8%)	12 (26.7%)	2 (4.4%)	5 (11.1%)		31 (68.9%)	

CR: Complete response; PR: partial response; SD: stable disease; PD: progressive disease; NE: not evaluated; ORR: overall response rate; DCR: disease control rate. *Fisher exact *p*-value.

Table IV. *Comparison treatment-related adverse events (AE).*

	Any AE (all grades), n (%)			AE grade 3/4, n (%)		
	Pemetrexed (n=49)	Erlotinib (n=88)	*p*-Value*	Pemetrexed (n=49)	Erlotinib (n=88)	*p*-Value*
Overall	20 (40.8%)	55 (62.5%)	0.020	3 (6.1%)	15 (17.0%)	0.111
Hematological toxicity	6 (12.2%)	1 (1.1%)	0.009	1 (2.0%)	0 (0)	0.358
Neutropenia	2 (4.1%)	0	0.126	0	0	0.999
Anemia	4 (8.2%)	1 (1.1%)	0.055	0	0	0.999
Pancytopenia	1 (2.0%)	0	0.358	1 (2.0%)	0	0.358
Non-hematological toxicity	16 (32.7%)	55 (62.5%)	0.012	3 (6.1%)	15 (17.0%)	0.111
Skin rash	1 (2.0%)	46 (52.3%)	<0.001	0	5 (5.7%)	0.160
Diarrhea	0	18 (20.5%)	<0.001	0	2 (2.3%)	0.537
Anorexia	6 (12.2%)	14 (15.9%)	0.623	2 (4.1%)	3 (3.4%)	0.999
Increased AST/ALT	0	5 (5.7%)	0.160	0	0	0.999
Nausea/vomiting	5 (10.2%)	4 (4.5%)	0.281	2 (4.1%)	3 (3.4%)	0.999
Soft-tissue infection	0	2 (2.3%)	0.537	0	2 (2.3%)	0.537
Fatigue	8 (16.3%)	1 (1.1%)	0.001	1 (2.0%)	1 (1.1%)	0.999
Arthralgia	1 (2.0%)	0	0.358	0	0	0.999
Polyneuropathy	1 (2.0%)	0	0.358	0	0	0.999

AST/ALT: Aspartate aminotransferase/alanine aminotransferase. *Fisher exact *p*-Value.

recorded in either group. The summary of AEs recorded in both groups is given in Table IV.

Survival and response in the sub-group of patients with ECOG PS 0 or 1. The median PFS for patients with ECOG PS of 0 or 1 treated with pemetrexed was 1.6 months *vs*. 2.3 months for patients treated with erlotinib (p=0.812) (Figure 2A). The median OS for patients treated with pemetrexed was 11.3 months *vs*. 26.3 months for those treated with erlotinib (p=0.043) (Figure 2B). The summary of survival data for both groups is listed in Table II. The ORR in patients treated with pemetrexed was 20.5% *vs*. 11.1% in patients treated with erlotinib (p=0.258). The DCR in patients treated with pemetrexed was 61.4% *vs*. 68.9% in patients treated with erlotinib (p=0.509). The summary of best response data for both groups is listed in Table III.

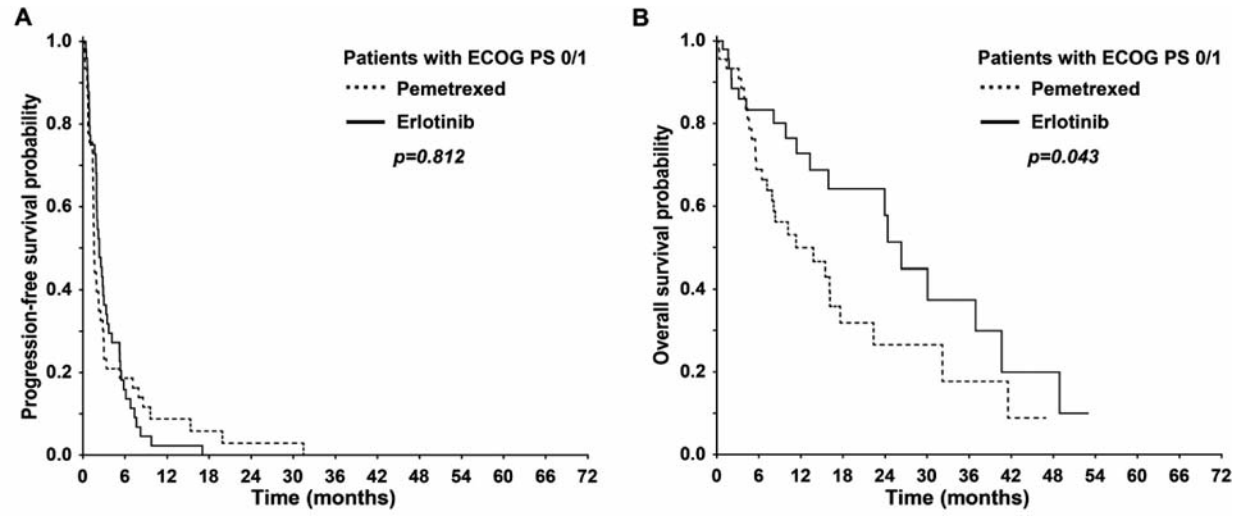

Figure 2. *Comparison of progression-free survival (A) and overall survival (B) between patients treated with pemetrexed and those treated with erlotinib in the second line in a subgroup of patients with Eastern Cooperative Oncology Group (ECOG) performance status (PS) (ECOG PS) 0/1.*

Discussion

Pemetrexed and erlotinib are agents that differ with respect to their mechanism of action, method of administration and treatment-related toxicity profile. Considerable progress in the field of molecular biology led to the identification of several biomarkers predicting the treatment efficacy of these agents. The presence of non-squamous histology is a predictor of a good response to pemetrexed (3, 12-14) and currently patients are selected for this treatment according to histological type of NSCLC (15). In the case of EGFR-TKIs, including erlotinib, the presence of activating *EGFR* mutations (predominantly exon 19 deletions or a point mutation in exon 21 termed *L858R*) represents the strongest predictor of a good treatment response (16-25) and currently patients are selected for first-line treatment according to the presence of activating *EGFR* mutations (15). Pemetrexed and erlotinib have both commonly been used for the second-line treatment of patients with advanced-stage non-squamous NSCLC, although there is still a lack of data comparing the efficacy and safety of these two agents reflecting histology and *EGFR* mutation status

In the present study, we recorded a significantly higher ORR for patients treated with pemetrexed compared to those treated with erlotinib (20.8% *vs*. 6.3%; p=0.022), although the difference in DCR between both groups was not significant (62.5% *vs*. 53.2%; p=0.358). The comparison of PFS did not prove to be significantly different between these groups (1.6 *vs*. 1.9 months; p=0.470) nor did the difference in OS (11.3 *vs*. 11.4 months; p=0.942). Similar findings were recently reported by Zugazagoitia *et al*. (26). When comparing our PFS data with those from the phase III clinical trial JMEI (5) in the case of pemetrexed and the phase III clinical trial BR.21 (6) in the case of erlotinib, we recorded shorter PFS for both patient groups. Similar differences are usually seen when results from clinical trials are compared to those from routine clinical practice. Moreover, our study focused on a selected population of patients harboring wild-type *EGFR* gene, which undoubtedly affected the efficacy of erlotinib. This fact could also have affected the efficacy of pemetrexed, referring to results from the phase III clinical trial IPASS, which showed lower efficacy of chemotherapy in patients harboring wild-type *EGFR* gene (27). When evaluating the treatment efficacy in a whole study population, it should be mentioned that there was a significant difference in PS between our two groups, which could have also affected our results. The group treated with pemetrexed included more patients with better PS compared to the group treated with erlotinib (PS 0/1: 91.8% *vs*. 55.7%; p<0.001). It is well-known that PS is a strong prognostic factor. For this reason, we compared the treatment efficacy for the sub-group of patients with PS 0 or 1. The results of this sub-analysis did not show any significant difference between pemetrexed and erlotinib in ORR (20.5% *vs*. 11.1%; p=0.258), DCR (61.4% *vs*. 68.9%; p=0.509) nor PFS (1.6 *vs*. 2.3 months; p=0.812), but interestingly, a significant difference in OS (11.3 *vs*. 26.3 months; p=0.043) was recorded. Although there was no significant difference in PFS, we found OS to be approximately two-fold longer in patients treated with erlotinib in the second line. This finding could be explained by a different efficacy of subsequent treatment in the third and fourth line, respectively. We recently reported significant improvement of both PFS and OS for patients treated with second-line erlotinib followed

by third-line pemetrexed as compared to the reverse sequence among patients with lung adenocarcinoma harboring wild-type *EGFR* gene (28).

The treatment with pemetrexed and with erlotinib was tolerated well and no cases of severe toxicity were recorded. Most recorded AEs were mild and well-manageable with supportive care. In conclusion, our findings showed comparable efficacy and good tolerability of both compared agents. Further studies should be performed to elucidate the optimal choice of second-line treatment and the role of sequential treatment with pemetrexed and erlotinib for previously treated patients with advanced-stage non-squamous NSCLC harboring wild-type *EGFR* gene.

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Conflicts of Interest

JF has received honoraria from Astra Zeneca, Roche and Novartis for consultations and lectures unrelated to this project. OF, MP, MS, MM, LB, ZB, RK and OT declare that they have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence this work.

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