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RESEARCH ARTICLE

Efficacy of platinum combination chemotherapy after first-line gefitinib treatment in non-small cell lung cancer patients harboring sensitive *EGFR* mutations

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Abstract
Purpose Gefitinib is an effective first-line chemotherapy for advanced non-small cell lung cancer (NSCLC) patients harboring sensitive *EGFR* mutations. However, whether second-line platinum combination chemotherapy after first-line gefitinib treatment shows similar effects to first-line platinum combination chemotherapy in these patients remains unclear. Therefore, we here aimed to investigate the efficacy of platinum combination chemotherapy after first-line gefitinib treatment in NSCLC patients harboring sensitive *EGFR* mutations.
Methods/patients We retrospectively evaluated the clinical effects of second-line platinum combination chemotherapy after first-line gefitinib treatment in NSCLC patients harboring sensitive *EGFR* mutations (exon 19 deletion or exon 21 L858R mutation) at five institutions. All patients were initially treated with gefitinib (250 mg/day) followed by platinum combination chemotherapy as second-line chemotherapy.
Results Between January 2006 and December 2012, 42 patients [8 men, 34 women; median age, 63 years (range 39–75 years)] were enrolled. The overall response rate, disease control rate, and median progression-free survival (PFS) were 26.2, 61.9 %, and 5.1 months, respectively, after the second-line treatment. The corresponding values for first-line gefitinib treatment were 69.0, 95.2 %, and 11.1 months, respectively. Moreover, second-line platinum combination chemotherapy with pemetrexed or bevacizumab-containing regimens was independently associated with improved PFS.
Conclusions Second-line platinum combination chemotherapy after first-line gefitinib treatment in NSCLC patients harboring sensitive *EGFR* mutations was effective and showed equivalent outcomes to first-line platinum combination chemotherapy. After failure of first-line gefitinib therapy, second-line platinum combination chemotherapy with pemetrexed or bevacizumab might result in improved PFS.

Keywords Advanced non-small cell lung cancer · *EGFR* mutations · Gefitinib · Non-small cell lung cancer · Second-line platinum-based chemotherapy

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Abbreviations

CR	Complete response
EGFR	Epidermal growth factor receptor
NSCLC	Non-small cell lung cancer
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PPS	Post-progression survival
PR	Partial response
RR	Response rate
SD	Stable disease
TKI	Tyrosine kinase inhibitor

Introduction

Lung cancer is the most common cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85 % of all lung cancer cases [1]. Most patients with NSCLC are diagnosed at the advanced stages (stages IIIb and IV), which are associated with particularly poor prognoses. First-line platinum-based chemotherapy has been documented to improve overall survival and quality of life, and is recommended for advanced-stage NSCLC [2, 3]; however, it is associated with several toxic effects [2].

Additionally, clinical trials have identified gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), as a first-line treatment for patients with NSCLC harboring sensitive *EGFR* mutations [4–6]. Although many patients initially achieve clinical remission or disease control with first-line chemotherapy, most subsequently experience disease progression and death. The extremely high response rate (RR) for gefitinib is associated with the presence of active *EGFR* mutations in the tumor cells, such as in-frame deletions in exon 19 or point mutations in exon 21 (e.g., L858R) [7–9]. Several phase III trials have compared platinum-containing chemotherapy to gefitinib in a first-line setting, and demonstrated that gefitinib improved the progression-free survival (PFS) in patients with *EGFR*-activating mutations [4–6]. Accordingly, gefitinib is one of the mainstay first-line treatments for NSCLC; however, once first-line gefitinib fails, the appropriate succeeding regimen is unknown. Yoshino et al. [10] analyzed the associations of PFS, post-progression survival (PPS), and tumor response with overall survival (OS) in patients with advanced NSCLC harboring sensitive *EGFR* mutations. They found that PPS after second-line therapy initiation strongly correlated with OS, unlike PFS and tumor shrinkage, suggesting that PPS may be a surrogate for OS in this patient population and that further therapy after disease progression following first-line treatment may significantly affect the OS rate. There are several options after first-line gefitinib, including platinum-based combination chemotherapy, non-platinum-based chemotherapy, or erlotinib, another approved EGFR-TKI, with NSCLC patients harboring an *EGFR* mutation treated with gefitinib, platinum, and pemetrexed or docetaxel having a median survival of approximately 3 years [11].

Platinum combination chemotherapy may be reserved for patients experiencing progression after first-line EGFR-TKI treatment. However, the mechanism of EGFR-TKI resistance is complex [12], and the post-EGFR-TKI treatment cancer cells may display different characteristics compared with the treatment-naïve cells. A recent report showed that the treatment efficacy of second-line platinum combination chemotherapy after first-line gefitinib treatment in NSCLC patients harboring sensitive *EGFR* mutations was lower [13], while another report demonstrated that prior EGFR-TKI treatment did not influence the efficacy of subsequent pemetrexed plus platinum chemotherapy [14]. Therefore, it remains unclear if clinical resistance to EGFR-TKI might also confer resistance to subsequent platinum combination therapy. Herein, we aimed to assess whether prior gefitinib use influences the efficacy of subsequent platinum combination therapy in advanced chemotherapy-naïve NSCLC patients harboring sensitive *EGFR* mutations.

Patients and methods

We retrospectively evaluated 42 patients with advanced NSCLC harboring sensitive *EGFR* mutations treated with first-line gefitinib and second-line platinum combination chemotherapy between January 2006 and December 2012 at five Japanese institutions (Gunma University Hospital, Gunma Prefectural Cancer Center, National Hospital Organization Nishigunma Hospital, Isesaki Municipal Hospital, and Maebashi Red Cross Hospital). The histological diagnosis and staging of NSCLC were based on the World Health Organization classification and the TNM staging system [15], respectively. The eligibility criteria were histologically or cytologically confirmed NSCLC, unresectable stage III/IV disease, and a drug-sensitive *EGFR* mutation (exon 19 deletion, or exon 21 L858R). Before chemotherapy, each patient underwent physical examination, chest radiography, thorax and abdomen computed tomography, bone scintigraphy or ^{18}F-fluorodeoxyglucose positron emission tomography, and brain computed tomography or magnetic resonance imaging to determine the TNM stage. For the identified subjects, clinical chart reviews were performed. The institutional review boards of each institution approved the study protocol, and the requirement for written informed consent was waived.

Genomic DNA was extracted from the tumor samples, and *EGFR* mutations in exons 18–21 were analyzed as previously described [16, 17]. All patients were EGFR-TKI-naïve, received first-line gefitinib (250 mg orally, once daily), and subsequently received platinum combination therapy as second-line treatment. The second-line regimen was determined by the treating physician and continued until disease progression, the appearance of intolerable toxicity, or withdrawal of consent.

The best overall response and maximum tumor shrinkage were recorded as the tumor responses. Radiographic tumor responses were defined according to the Response Evaluation Criteria In Solid Tumors, version 1.1 [18] as follows: complete response (CR), the disappearance of all target lesions; partial response (PR), a decrease in the sum of the target lesion diameters by at least 30 % compared to baseline; progressive disease (PD), an increase of at least 20 % in the sum of the target lesion diameters compared to the smallest sum during the study; and stable disease (SD), insufficient shrinkage or expansion to qualify as PR or PD. PFS was calculated from the treatment initiation until PD or all-cause death, and OS was recorded from the treatment initiation until death, or was censored on the date of the last follow-up. Kaplan–Meier survival curves were created and compared using the log-rank test. All categorical variables were analyzed using Fisher's exact test. The Cox proportional hazards model with stepwise regression was applied to determine the prognostic factors for PFS at second-line treatment and OS after the start of second-line therapy, and to estimate the hazard ratios and 95 % confidence intervals. p values <0.05 were considered statistically significant for both one-tailed and two-tailed tests. All statistical analyses were performed using JMP version 11.0 for Windows (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

The patient characteristics are listed in Table 1. Forty-two patients [8 men, 34 women; median age, 63 years (range 39–75 years)] were included. According to the Eastern Cooperative Oncology Group criteria, 39 (92.9 %) and 3 (7.1 %) patients had a performance status of 0–1 and 2, respectively. According to the TNM staging system, 2 and 36 patients were classified as stage IIIB and IV, respectively. Four patients experienced postoperative recurrence. The tumor type was adenocarcinoma in 41 patients, and was not specified in the remaining patient. Thirty-one patients had never smoked. Regarding the *EGFR* mutation types, 26 and 16 patients exhibited exon 19 deletions and exon 21 L858R mutations, respectively. After starting first-line gefitinib therapy, the median PFS was 11.1 months with an OS of 33.1 months (Fig. 1a, b). The median follow-up was 26.1 months (range 8.4–65.3 months).

Table 1 Patient characteristics

Characteristics	Number of patients (%)
Sex	
Male	8 (19.1)
Female	34 (80.9)
Age (years), median (range)	63 (39–75)
Performance status	
0	22 (52.4)
1	17 (40.5)
2	3 (7.1)
3	0 (0)
4	0 (0)
Clinical stage	
IIIB	2 (4.8)
IV	36 (85.7)
Postoperative recurrence	4 (9.6)
Histology	
Adenocarcinoma	41 (97.6)
Other/not specified	1 (2.4)
Smoking history	
Current or former	11 (26.2)
Never	31 (73.8)
EGFR mutation	
Exon 19 deletion	26 (61.9)
Exon 21 L858R	16 (38.1)
Number of courses of second-line chemotherapy, median (range)	4 (1–6)

Performance status was determined using the Eastern Cooperative Oncology Group criteria

EGFR epidermal growth factor receptor

The responses to first-line gefitinib of these 42 patients are listed in Table 2.

Treatment efficacy of second-line platinum combination chemotherapy after first-line gefitinib

The distributions of the second-line platinum combination chemotherapy regimens are listed in Table 3. Thirteen and 29 patients received cisplatin and carboplatin-based regimens, respectively, including gemcitabine, pemetrexed (±bevacizumab), and paclitaxel (±bevacizumab). The objective tumor RR for second-line platinum combination chemotherapy after first-line gefitinib is described in Table 2. During the observation period, no patients exhibited CR, while 11, 15, and 11 patients met the criteria

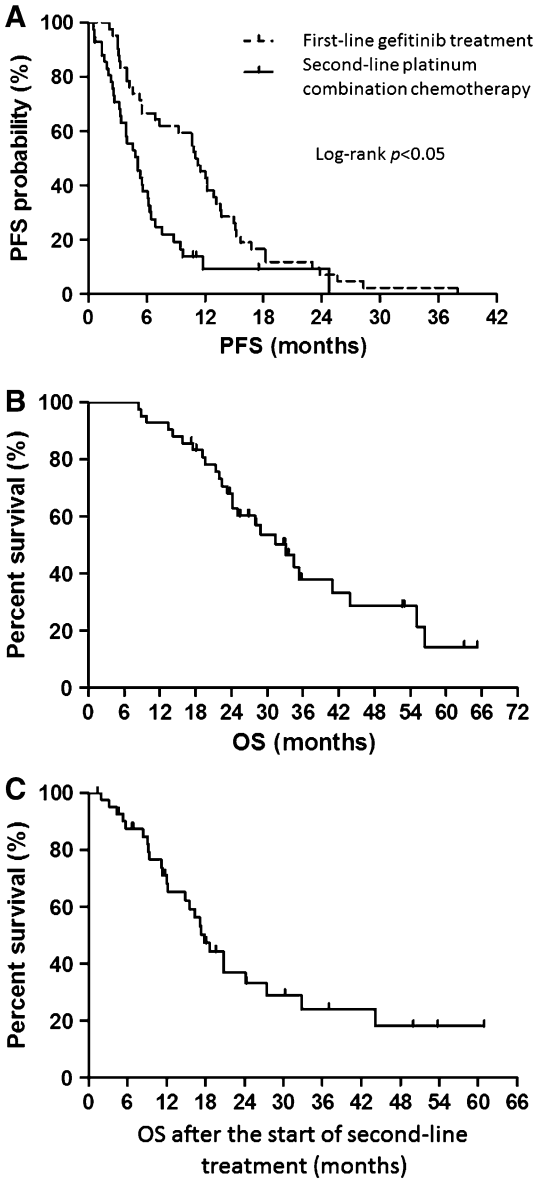

Fig. 1 A Kaplan–Meier analyses of **(a)** progression-free survival (PFS) and **(b)** overall survival (OS) after the start of first-line gefitinib in patients with subsequent platinum combination chemotherapy as second-line treatment. **a** First-line gefitinib treatment, median PFS: 11.1 months. Second-line platinum combination chemotherapy, median PFS: 5.1 months; **b** Median OS: 33.1 months, median follow-up interval: 26.1 months. **c** OS after the start of second-line platinum combination chemotherapy. Median OS: 17.8 months

for PR, SD, and PD, respectively. Thus, the overall RR and disease control rate were 26.2 and 61.9 %, respectively. Next, factors including sex, age, clinical stage, smoking history, *EGFR* mutation types, best response at first-line treatment, and second-line regimens were analyzed for associations with the response to second-line platinum combination chemotherapy (Table 4). In the univariate analysis, no factors were significantly associated with the treatment response.

Table 2 Response to first-line gefitinib and second-line platinum combination chemotherapy in patients with epidermal growth factor receptor-mutated non-small-cell lung cancer

	Number of patients (%)	
	First-line gefitinib	Second-line platinum combination chemotherapy
Complete response	3 (7.1)	0 (0)
Partial response	26 (61.9)	11 (26.2)
Stable disease	11 (26.2)	15 (35.7)
Progressive disease	1 (2.4)	11 (26.2)
Not evaluable	1 (2.4)	5 (11.9)
Response rate (%)	69.0	26.2
Disease control rate[a] (%)	95.2	61.9

[a] Calculated as the number of patients with complete response, partial response, and stable disease, divided by the whole study population

Table 3 Distribution of the second-line platinum combination chemotherapy regimens after first-line gefitinib

Regimen	Number of patients (%)
CDDP-based chemotherapy	13 (31.0)
CDDP + GEM	7 (16.7)
CDDP + PEM	5 (11.9)
CDDP + PEM + BEV	1 (2.4)
CBDCA-based chemotherapy	29 (69.0)
CBDCA + PEM	13 (31.0)
CBDCA + PEM + BEV	7 (16.7)
CBDCA + PTX	5 (11.9)
CBDCA + PTX + BEV	2 (4.8)
CBDCA + GEM	2 (4.8)

CDDP cisplatin, *GEM* gemcitabine, *PEM* pemetrexed, *BEV* bevacizumab, *CBDCA* carboplatin, *PTX* paclitaxel

Survival according to second-line platinum combination chemotherapy after first-line gefitinib

The median PFS of second-line chemotherapy was 5.1 months (Fig. 1a), and the median OS after the start of second-line platinum combination chemotherapy was 17.8 months (Fig. 1c). The PFS after second-line platinum combination chemotherapy was shorter than that after first-line gefitinib treatment (log-rank, $p < 0.05$) (Fig. 1a). The predictive value of various clinical factors on PFS at second-line therapy and OS after second-line therapy was subsequently assessed (Table 5). In the univariate analysis, second-line platinum combination chemotherapy with pemetrexed or bevacizumab was significantly associated with better PFS. In the multivariate analysis, adjusted for various clinical factors, second-line platinum combination chemotherapy with pemetrexed ($p = 0.01$) and

Table 4 Univariate analyses of the response rates in various patient subgroups at second-line platinum combination chemotherapy

Factors	Responders (*n*)	Response rate (%)	*p* value[a]
Sex			
Male	2	25.0	0.93
Female	9	26.4	
Age (years) at the beginning of second-line treatment			
≥60	7	24.1	0.65
<60	4	30.7	
PS at the beginning of second-line treatment			
0–1	10	25.6	0.77
2	1	33.3	
Clinical stage			
IIIB + IV	10	26.3	0.95
Postoperative recurrence	1	25.0	
Smoking history			
Current or former	4	36.3	0.37
Never	7	22.5	
EGFR mutation type			
Exon 19 del	8	30.7	0.38
Exon 21 L858R	3	18.7	
Best response at first-line treatment			
CR + PR	7	24.1	0.69
SD + PD	4	33.3	
Platinum use at second-line chemotherapy			
CDDP	5	38.4	0.22
CBDCA	6	20.6	
Gemcitabine			
With	4	44.4	0.16
Without	7	21.2	
Pemetrexed			
With	6	23.0	0.55
Without	5	31.2	
Paclitaxel			
With	1	14.2	0.43
Without	10	28.5	
Bevacizumab			
With	3	30.0	0.75
Without	8	25.0	

PS performance status, *EGFR* epidermal growth factor receptor, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *CDDP* cisplatin, *CBDCA* carboplatin

[a] Fisher's exact test

bevacizumab ($p = 0.01$) was independently associated with improved PFS. Second-line platinum combination chemotherapy with pemetrexed was associated with a longer median PFS than treatment without (5.3 vs. 4.2 months, log-rank, $p = 0.02$). Similarly, patients treated with second-line platinum combination chemotherapy with bevacizumab had a longer median PFS than those without (7.6 vs. 4.0 months, log-rank, $p = 0.02$). In the univariate and multivariate analyses, platinum combination chemotherapy with bevacizumab was the only factor significantly associated with better OS after the start of second-line therapy, with patients receiving platinum combination chemotherapy with bevacizumab having a longer median survival than those without (17.1 months, log-rank, $p = 0.01$).

Discussion

In this study, second-line platinum-based combination chemotherapy regimens including pemetrexed or bevacizumab after first-line gefitinib were identified as favorable prognostic factors for PFS. Moreover, bevacizumab-containing regimens were also linked to better OS after the induction of second-line platinum-based chemotherapy, and our results suggest that neither the efficacy of prior gefitinib therapy nor the *EGFR*-mutation type influenced the outcome of subsequent platinum combination chemotherapy.

Several studies have suggested that chemotherapy and EGFR-TKIs may influence the efficacy of each other. Recently, Chang et al. [19] demonstrated that chemotherapy-naïve patients showed a higher RR to gefitinib than chemotherapy-treated patients, and hypothesized that tumor cells evolve into a more heterogeneous and resistant phenotype over time. Bai et al. [20] also showed that chemotherapy may reduce the *EGFR*-mutation frequency in both plasma and tumor tissue, and accordingly suspected a reduction in the overall clinical benefit of subsequent EGFR-TKI treatment after chemotherapy. One recent study reported that platinum-based chemotherapy after initial gefitinib achieved a low response of only 7 % [13], whereas other studies have documented the effectiveness of cytotoxic agents after EGFR-TKIs against NSCLC with *EGFR* mutations [21–23]. Conversely, Deng et al. [24] used the lung adenocarcinoma cell lines PC9 and PC9/G, which have acquired resistance to gefitinib, to explore the influence of acquired resistance of EGFR-TKIs on the sensitivity of tumor cells to chemotherapeutic drugs, and showed no significant differences between these two cell lines, and several other studies have suggested that prior EGFR-TKI therapy does not influence the efficacy of subsequent platinum combination chemotherapy in NSCLC patients harboring sensitive *EGFR* mutations [14, 22]. Furthermore, Maemondo et al. [6] compared the efficacy of gefitinib and carboplatin plus paclitaxel as first-line treatment for patients with advanced NSCLC harboring sensitive *EGFR* mutations and found similar RRs of carboplatin plus paclitaxel in the first-line setting and as subsequent therapy after progression to first-line gefitinib

Table 5 Associations between clinical factors, and progression-free survival (PFS) at second-line chemotherapy and overall survival (OS) after second-line platinum combination chemotherapy

Factors	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	PFS at second-line			PFS at second-line			OS after second-line			OS after second-line		
	HR	95 % CI	*p* value	HR	95 % CI	*p* value	HR	95 % CI	*P* value	HR	95 % CI	*p* value
Sex (male/female)	1.27	0.50–2.79	0.57	1.61	0.62–3.71	0.30	1.00	0.29–2.65	0.99	1.21	0.33–3.54	0.73
Age (years) at the beginning of second-line treatment	1.02	0.98–1.07	0.24	1.04	0.99–1.09	0.10	0.99	0.95–1.05	0.94	1.00	0.95–1.07	0.79
PS at the beginning of second-line treatment (0–1/2)	0.40	0.13–1.70	0.18	0.28	0.08–1.25	0.08	0.78	0.22–4.92	0.75	0.89	0.21–6.20	0.89
Clinical stage (IIIB + IV/recurrence)	1.02	0.36–4.31	0.96				0.67	0.39–8.43	1.34			
Smoking history (current or former/never)	1.57	0.72–3.20	0.23				0.75	0.29–1.72	0.51			
EGFR type (exon 19 del/exon 21 L858R)	1.16	0.58–2.48	0.67				0.59	0.27–1.33	0.20			
Best response at first-line treatment (CR + PR/SD + PD)	1.15	0.57–2.48	0.68				1.04	0.45–2.59	0.92			
Platinum use at second-line chemotherapy (CDDP/CBDCA)	0.46	0.21–1.03	0.06				1.01	0.42–2.24	0.97			
Gemcitabine (with/without)	1.75	0.74–3.81	0.18				1.08	0.44–2.43	0.84			
Pemetrexed (with/without)	0.44	0.21–0.91	**0.02**	0.37	0.17–0.80	**0.01**	0.76	0.34–1.69	0.50	0.80	0.33–1.89	0.61
Paclitaxel (with/without)	1.88	0.74–4.17	0.16				1.40	0.46–3.46	0.51			
Bevacizumab (with/without)	0.36	0.13–0.85	**0.01**	0.30	0.10–0.74	**<0.01**	0.20	0.03–0.70	**<0.01**	0.20	0.03–0.71	**0.01**

Bold *p* values are statistically significant ($p < 0.05$)

HR hazard ratio, *CI* confidence interval, *PS* performance status, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *CDDP* cisplatin, *CBDCA* carboplatin

(30.7 vs. 28.8 %). In the present study, in the gefitinib group, 67.5 % of the patients received carboplatin plus paclitaxel as second-line therapy, and based on the above-mentioned findings, we speculate that there is no clinically meaningful interference between gefitinib and chemotherapy.

Our study focused on second-line treatment after EGFR-TKI, and our results revealed that the effects of second-line platinum combination chemotherapy were equal to those in previous reports on first-line platinum combination chemotherapy. Currently, platinum combination chemotherapy is the standard first-line therapy for NSCLC, with reported RRs of 20–30 % [2]. Ohe et al. [25] reported that the RR in a Japanese large phase III trial for advanced NSCLC was approximately 30 % for platinum combination chemotherapy. Although the number of patients in our study was relatively small, this study was comparable to the first-line setting for metastatic NSCLC. We found that the median PFS at second-line treatment and OS from the start of the second-line platinum combination chemotherapy were 5.1 and 17.8 months, respectively. Further, the PFS at second-line treatment was similar to that of a Japanese large phase III trial of first-line platinum combination chemotherapy, in which the time to progression and OS ranged between 4.0–4.7 and 11.4–14.0 months, respectively [25].

Despite initial responses to EGFR-TKIs (gefitinib, erlotinib, and afatinib), most NSCLC patients ultimately experience treatment failure. The majority of failures result from an acquired *EGFR* mutation (T790 M) or amplification of the *MET* oncogene [26]. However, the effectiveness of platinum combination chemotherapy did not appear to be influenced by gefitinib failure in our study, and our results indicate that the indications and timing of second-line platinum combination regimens after gefitinib failure could be the same as for first-line therapy. Although this was not a prospective study, our observations suggest that platinum combination chemotherapy should be used after first-line gefitinib if there is no obvious contraindication.

Nonetheless, it remains unknown which regimen after first-line gefitinib failure results in better survival outcomes. Our results revealed that second-line platinum combination chemotherapy with a pemetrexed or bevacizumab-containing regimen was associated with favorable outcomes. Interestingly, a previous study reported that lung adenocarcinoma patients with *EGFR* mutations receiving pemetrexed showed a better RR and longer PFS than those with wild-type *EGFR* [27]. Pemetrexed is an inhibitor of thymidylate synthase (TS), and increased TS expression may cause resistance to pemetrexed [28, 29]. In breast cancer, low pretreatment TS expression levels are associated with a better response to pemetrexed [30], and the TS mRNA levels are also predictive of the disease response to neoadjuvant gemcitabine and pemetrexed chemotherapy for NSCLC [31]. Patients with lung adenocarcinoma have lower baseline TS mRNA and protein levels than those with squamous cell carcinoma [32], and Giovannetti et al. [33], furthermore, showed different TS gene expression levels in NSCLC cell lines. Interestingly, among six cell lines investigated, H1650, which harbors *EGFR* mutations, had lower TS gene expression than the other NSCLC cell lines with wild-type *EGFR* [33]. Thus, it is possible that *EGFR* mutations may be associated with lower TS gene expression levels, which in turn may cause the NSCLC cells to become more sensitive to pemetrexed, and we speculate that this might be the reason for the favorable effects of combination platinum and pemetrexed treatment in our study.

On the other hand, bevacizumab-containing platinum combination chemotherapy is usually not administered to patients with tumor cells invading major blood vessels or to patients with cavitation, hemoptysis, or with a history of coagulation disorders or therapeutic anticoagulation and brain metastasis, and this might be why this regimen was associated with a good prognosis. Furthermore, Naumov et al. [34] reported that bevacizumab shows antitumor effects on the NSCLC cell line T790 M, which harbors a resistant *EGFR* mutation. It is possible that prolonged treatment with an EGFR inhibitor shifts the tumor cell population towards a less EGFR-dependent phenotype and more towards vascular endothelial growth factor-dependent angiogenesis, and this might be another reason for why the PFS at second-line chemotherapy and OS after the start of second-line chemotherapy were good in the present study.

Moreover, it has been discussed whether EGFR-TKIs should be immediately changed to another therapy or continued upon treatment failure. The results of a recent phase III study (IMPRESS trial; IRESSA Mutation Positive Multicentre Treatment Beyond Progression Study) were recently presented at the European Society for Medical Oncology 2014 conference; in this trial, in EGFR-mutated patients, gefitinib was continued beyond disease progression in combination with platinum-based chemotherapy (cisplatin and pemetrexed). However, this combination failed to prolong the PFS and had a deleterious effect on OS [35]. Pending the full publication of this trial and the results of similar studies, the continuation of EGFR TKIs in combination with chemotherapy should only be addressed in the setting of clinical trials. Monotherapies with newer generation EGFR-TKIs with more specific activity for the T790 M mutation, such as CO-1686 and AZD9291, seem to have better toxicity profiles in early clinical trials, and the results are very encouraging in patients with advanced NSCLC who develop resistance to EGFR-TKIs with a secondary T790 M mutation [36]. For the final decision regarding second-line therapy, re-biopsy might be important. As an alternative to tissue samples, there is a growing interest in studying liquid biopsies or blood samples by means of molecular characterization of circulating tumor cells and by examining circulating free DNA in the serum.

The study has several limitations. First, this was a retrospective study with selected groups of patients. Second, the use of gefitinib as first-line treatment and the selection of second-line therapy were made at the treating physician's discretion. Selection bias might exist in these decisions, and this may have influenced the survival after second-line therapy. Third, the planned chemotherapy was reduced, skipped, or delayed at the attending physician's discretion. To minimize this bias, all consecutive patients who were treated at our institutions were included in our analysis, and the patients' original charts were thoroughly reviewed. Lastly, another limitation is the relatively small population of our study. Further larger, prospective studies are mandatory for adaptation of our findings to clinical practice.

In conclusion, our results indicate that second-line platinum combination chemotherapy after first-line gefitinib treatment in NSCLC patients harboring sensitive *EGFR* mutations was effective and showed equivalent results to first-line platinum combination chemotherapy. After failure of first-line gefitinib therapy, second-line platinum combination chemotherapy with pemetrexed or bevacizumab might be associated with better PFS. Despite the retrospective design, our results suggest that second-line platinum combination chemotherapy should be considered a standard treatment after gefitinib failure for patients with advanced NSCLC harboring *EGFR* mutations.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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