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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Exceptional pemetrexed sensitivity can predict therapeutic benefit from subsequent chemotherapy in metastatic non-squamous non-small cell lung cancer

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Abstract

Purpose Based on an exceptionally durable response to pemetrexed observed in some patients with metastatic NSCLC, the predictive value of pemetrexed sensitivity to outcomes of subsequent systemic treatment was investigated.

Methods We retrospectively reviewed the patients with metastatic non-squamous NSCLC treated with pemetrexed monotherapy as their first- or second-line chemotherapy between November 2006 and February 2015. Good (top 5% longest) and poor responders (bottom 12% shortest) were defined according to the duration of pemetrexed maintenance. The first and second post-pemetrexed (PP) systemic treatments were defined as PP1 and PP2 therapies, respectively, to define their progression-free survivals (PFS) as PFS1 and PFS2.

Results In a total of 100 patients, 86% of patients received pemetrexed as their second-line chemotherapy, and 34% were classified as good responders. Good and poor responder groups showed 20.5 months and 0.7 months of the median duration of responses, respectively. PP1 and PP2 therapies were done in 74% and 41.9% of patients after failure to pemetrexed. To our surprise, disease control rate (DCR) was significantly higher in the good responder group than poor responder group (69.6% vs 37.3%, $p = 0.010$) in patients treated with PP1 therapy, and median PFS1 was also significantly longer (5.2 vs 2.2 months, $p < 0.01$) regardless of the type of subsequent systemic treatment. Meanwhile, pemetrexed sensitivity did not affect DCR or PFS of patients who received PP2 therapies.

Conclusions Patients who achieved durable response to pemetrexed might obtain greater therapeutic benefits from subsequent systemic treatment in metastatic non-squamous NSCLC without targets, which could potentiate more effective post-pemetrexed treatment strategy.

Keywords Pemetrexed · Sensitivity · Durable · Predict · Post-pemetrexed · Outcomes

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Introduction

The mortality rate of lung cancer, especially non-small cell lung cancer (NSCLC), has markedly fallen in the last decade with the introduction of newer targeted agents (Mok et al. 2009; Rosell et al. 2012). More recently, understanding of immune checkpoint and its anti-tumor activity has decisively changed the landscape and paradigm of NSCLC treatment (Reck et al. 2016; Borghaei et al. 2015). However, despite this great success, the majority of patients do not harbor appealing targets (Barlesi et al. 2016) or only present disappointing responses to novel immunotherapeutic agents, and even in a good responder, an inevitable resistance eventually develops (Barlesi et al. 2016; Restifo et al. 2016; Koyama et al. 2016). Thus, most of these patients still rely on conventional cytotoxic chemotherapy. Meanwhile, the recent enthusiasm for immunotherapy may lead us to underestimate the benefit of old but good drugs.

Pemetrexed, an inhibitor of thymidylate synthesis, has been widely used for induction and maintenance therapy in non-targetable non-squamous NSCLC with enriched efficacy and relatively mild toxicity compared with previous cytotoxic chemotherapy (Paz-Ares et al. 2013). We previously reported that some patients show an extraordinarily durable response to maintenance treatment with pemetrexed (Park et al. 2016). The median progression-free survival (PFS) in the top 5% of long-term responders to pemetrexed was 29.9 months (IQR, interquartile range 20.9–90.0) and the median number of cycles was 37 (IQR 18–129). Considering that the median PFS of the first-generation epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) is approximately 10–12 months in NSCLC patients having EGFR mutations (Mok et al. 2009; Rosell et al. 2012), these results seem very promising, which is reminiscent of durable responses in responders of immune checkpoint inhibitors (Gettinger et al. 2015). Although the status of smoking, tumor burden, and genetic alterations including anaplastic lymphoma kinase (ALK) translocation were suggested to influence the efficacy of pemetrexed (Park et al. 2016), they could not completely explain the unproportional benefit of pemetrexed in long-term responders. Given that there might be more crucial determining factors of exceptionally durable responses to pemetrexed, we investigated the treatment outcomes of post-pemetrexed systemic treatment according to their responses to pemetrexed, to see if pemetrexed sensitivity influences the therapeutic benefit of subsequent drugs.

Materials and methods

Study patients

From November 2006 to February 2015, we retrospectively reviewed the medical records of patients with metastatic non-squamous NSCLC who received pemetrexed monotherapy in Asan Medical Center (Seoul, South Korea). We ordered these patients according to the duration of pemetrexed maintenance treatment as in our previous study, and the top 5% patients (good responder) and the bottom 12% (poor responder) were screened (Park et al. 2016). Patients were finally eligible if they had metastatic non-squamous NSCLC and were administered single-agent pemetrexed as their first- or second-line chemotherapy. Exclusion criteria were as follows: patients who received less than two cycles of pemetrexed; received pemetrexed as combination chemotherapy with platinums; discontinued pemetrexed for reasons other than disease progression, such as toxicity, infection, poor performance status, or patients' refusal; harbored evident EGFR mutation or ALK translocation; only had insufficient information as transferred to another hospital or dropped out.

The study protocol was reviewed and approved by the Institutional Review Board (IRB Approval Number: 2018-0661) of Asan Medical Center and was conducted in full accordance with the Guidelines for Good Clinical Practice and the Declaration of Helsinki.

Post-pemetrexed chemotherapy and response evaluation

We further investigated patients who received additional systemic treatment after failure to pemetrexed treatment. In the study, the first post-pemetrexed (PP) systemic treatment was defined as PP1 therapy, and the second post-pemetrexed systemic treatment was defined as PP2 therapy. Patients who were done with PP1 and PP2 therapies were classified into four subgroups according to the type of later line drugs as follows: docetaxel, gemcitabine, vinorelbine, and first-generation EGFR-TKIs gefitinib or erlotinib. For these patients, we evaluated the overall response rate (ORR) and disease control rate (DCR). Treatment response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (Eisenhauer et al. 2009). Disease control rate (DCR) was defined including a patient who achieved complete response, partial response, and stable disease for at least 6 months.

Statistical analysis

Primary end point was progression-free survival (PFS) of post-pemetrexed systemic treatments including cytotoxic or targeted agents, which was defined as the time from the date

of treatment initiation to the date of disease progression. In the study, we defined PFS1 as PFS of PP1 therapy, and PFS2 as PP2 therapy. Secondary end point included DCR, ORR and overall survival (OS). OS was defined as the time from the curative resection to death due to any cause. Categorical variables were analyzed using Chi square or Fisher's exact test, and continuous variables were analyzed using Student's *t* test or Mann–Whitney test. Kaplan–Meier methods with the log-rank test were used for survival estimation. Patients whose survival data were not available or who were still alive at the time of analysis were censored. All tests of significance were two-sided; p values of < 0.05 were considered statistically significant. All statistical analysis was performed with SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA).

Results

Patients and comparison between good and poor responders

A total of 100 patients were included during the study period. We initially reviewed a total of 387 patients who had been at least once treated with pemetrexed-containing chemotherapies during the screening period. After that, we excluded 195 patients who were positive for EGFR-activating mutations or ALK fusion, and 54 patients who received pemetrexed-based chemotherapies beyond the second line of treatment. Among 138 patients remaining, we additionally excluded 38 patients who were treated with doublet of pemetrexed and platinums, which resulted in a total of 100 patients finally enrolled according to our eligibility criteria (Fig. S1). Most of the patients (86%) received pemetrexed chemotherapy as their second-line systemic treatment. Among them, 34% of patients were classified to the good responder group with a median duration of 20.5 (interquartile range, IQR 13.7–29.3) months of pemetrexed treatment, whereas 66 (66.0%) were poor responders with a median duration of 0.7 (IQR 0.7–0.7) months. Distribution of patients to either good or poor responder group was not significantly influenced by the line of pemetrexed treatment (Table S1). In addition, for 86 patients who received pemetrexed as their second-line chemotherapy, there was no significant difference of treatment response according to the type of the first-line treatment (Table S2). The median age was 60.0 years (range 52.0–67.0), and 96.0% of patients had Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. The majority of patients (95.0%) showed histologically confirmed adenocarcinoma, but patients with poorly differentiated NSCLC were also included (4.0%). Eighty-eight (88.0%) patients showed locally advanced or initially metastatic disease. These clinicopathologic characteristics were comparable between both groups except smoking habit (Table 1). Current or ex-smokers were more frequently observed in poor responders compared to good responders ($p = 0.015$).

Post-pemetrexed chemotherapies and treatment responses

After failure to pemetrexed, 74 (74.0%) patients received subsequent systemic treatments, and among them 44 (59.5%) received PP2 therapies. EGFR-TKI (41.9%) and vinorelbine (27.3%) were the most commonly chosen chemotherapy regimens for the PP1 and PP2 chemotherapeutic agents after discontinuation of pemetrexed, respectively. In addition, vinorelbine and EGFR-TKIs were the most frequently used PP1 therapies in good and poor responders, respectively. (Table 2). Meanwhile, none of the 39 patients who were treated with EGFR-TKI either as PP1 or PP2 therapy had activating EGFR mutations in the cohort.

ORR and DCR in the treatment groups are summarized in Table 3. In patients who received PP1 therapy, DCR was significantly higher in the good responder group than in the poor responder group (69.6% vs 37.3%, $p = 0.010$). ORR was also better in good responders, although it did not meet statistical significance (37.3% vs 19.6%, $p = 0.159$). However, neither DCRs nor ORRs showed significant differences in PP2 therapies between good and poor responder groups. The type of regimens did not significantly affect post-pemetrexed response outcomes both in PP1 and PP2 therapies (Table 3). Also, there were no significant differences in DCR and ORR of PP1 therapies in the line of pemetrexed administration (Table S3).

Survival outcomes of subsequent post-pemetrexed chemotherapies

The median PFS for the first systemic treatment after pemetrexed failure (PFS1) was significantly longer in the good responder group (5.2 months, 95% CI 2.9–7.5) than in the poor responder group (2.2 months, 95% CI 1.2–3.2, $p < 0.01$). However, in the analysis for PP2 therapy, the median PFS2 did not show a significant difference between good and poor responders (2.1 months vs 2.2 months; $p = 0.995$, Fig. 1, Table 4). There was no significant difference of PFS1 according to the line of pemetrexed treatment (Table S4). Additionally, when analyzed by each regimen, no difference in median PFS1 or PFS2 was observed between the two response groups except for EGFR-TKI administered as PP2 therapy (3.1 months vs 1.0 months, $p = 0.027$, Table 4). Good responders to pemetrexed also showed longer median OS for PP1 therapy compared with poor responders [60.0 (95% CI 36.1–81.9) months vs 10.2 (95% CI 8.1–12.4 months, $p < 0.01$, Fig. 2].

Table 1 Clinical characteristics of 100 patients with non-squamous NSCLC according to the response to pemetrexed chemotherapy

	Total ($n=100$)	Good responder ($n=34$)	Poor responder ($n=66$)	p value
Age (years)	60.0 (52.0–67.0)	60.5 (52.0–66.3)	59.5 (52.0–67.3)	1.000
Sex				0.277
Male	66 (66.0%)	20 (58.8%)	46 (69.7%)	
Female	40 (36.7%)	19 (46.3%)	21 (30.9%)	
History of smoking				0.015
Current or ex-smoker	57 (65.5%)	13 (48.1%)	44 (73.3%)	
Never smoker	30 (34.5%)	14 (51.9%)	16 (26.7%)	
ECOG PS				0.629
0	23 (23.2%)	9 (27.3%)	14 (21.2%)	
1	58 (58.6%)	18 (54.5%)	40 (60.6%)	
2	14 (14.1%)	5 (15.2%)	9 (13.6%)	
3	4 (4.0%)	1 (3.0%)	3 (4.5%)	
Histologic diagnosis				0.624
Adenocarcinoma	95 (95.0%)	32 (94.1%)	63 (95.5%)	
Large-cell carcinoma	1 (1.0%)	0 (0.0%)	1 (1.5%)	
Unclassified	4 (4.0%)	2 (5.9%)	2 (3.0%)	
Clinical stage				–
IIIA	3 (3.0%)	3 (8.8%)	0 (0.0%)	
IIIB	7 (7.0%)	3 (8.8%)	4 (6.1%)	
IV	78 (78.0%)	22 (64.7%)	56 (84.8%)	
Recurrence	12 (12.0%)	6 (17.6%)	6 (9.1%)	
Line of pemetrexed				0.451
First	14 (14.0%)	6 (17.6%)	8 (12.1%)	
Second*	86 (86.0%)	28 (82.4%)	58 (87.9%)	
No. of chemotherapies after pemetrexed	1.0 (0.0–2.0)	1.0 (0.0–3.0)	1.0 (1.0–2.0)	0.952

Data are reported as the median (interquartile range) and numbers (%)

NSCLC non-small cell lung cancer, *ECOG PS* Eastern Cooperative Oncology Group

*The chemotherapeutic regimens before pemetrexed were as follows: gemcitabine + cisplatin (carboplatin), 55 (64.0%); paclitaxel + cisplatin (carboplatin), 19 (22.1%); first-line EGFR-TKIs, 5 (5.8%, gefitinib in 4, Tarceva in 1); gemcitabine, 5 (5.8%); docetaxel + carboplatin, 1 (1.2%); carboplatin, 1 (1.2%)

Discussion

Despite unprecedented advances in the treatment of lung cancer, conventional cytotoxic chemotherapy still holds a pivotal role in the management of NSCLC, particularly in case of patients with no actionable target. Even in the era of immunotherapy, substantial patients with high programmed death ligand-1 (PD-L1) expression do not respond well to single immunotherapy and eventually encounter disease progression which mostly requires subsequent cytotoxic chemotherapy. Pemetrexed is one of the most commonly used front-line chemotherapeutic agents for non-squamous NSCLC without targets, either as monotherapy or combination. We previously suggested the prognostic significance and several clinicopathologic predictive factors of durable response to pemetrexed in these patients including genetic alterations (Park et al. 2016). The present study otherwise focused on the predictive value of pemetrexed sensitivity to the treatment outcomes of post-pemetrexed chemotherapy, and demonstrated that good responders to pemetrexed obtained significantly greater disease control rate and also benefit of PFS and OS with subsequent systemic treatment.

Pemetrexed inhibits thymidylate synthase and folate-dependent enzymes involved in purine and pyrimidine synthesis, suppressing RNA replication and consequently disrupting cancer DNA (Wilson et al. 2014; Chattopadhyay et al. 2007; Chen et al. 2014). It theoretically depletes the resources of DNA synthesis and induces S-phase growth arrest, which was also suggested in NSCLC cell lines (Tièche et al. 2016; Kano et al. 2006). In these studies, pemetrexed and cisplatin were administered with various schedules to evaluate the efficacy according to the treatment sequence. Surprisingly, cell cycle arrest was maximally prolonged to 14 days by pretreatment with pemetrexed for 48 h prior to cisplatin administration. Also, recovery of DNA damage was mostly delayed with pemetrexed pretreatment, and more interestingly, refractory subset with stem cell-like phenotype was exclusively

Table 2 Subsequent chemotherapeutic regimen after pemetrexed failure

PP1 therapy	Total ($n=74$)	Good responder ($n=23$)	Poor responder ($n=51$)
EGFR-TKI	31 (41.9%)	5 (21.7%)	26 (51.0%)
Gemcitabine based	16 (21.6%)	5 (21.7%)	11 (21.6%)
Vinorelbine	10 (13.5%)	7 (30.4%)	3 (5.9%)
Docetaxel	8 (10.8%)	2 (8.7%)	6 (11.8%)
Other*	9 (12.2%)	4 (17.4%)	5 (9.8%)
PP2 therapy	Total ($n=44$)	Good responder ($n=14$)	Poor responder ($n=30$)
EGFR-TKI	8 (18.2%)	4 (28.6%)	4 (13.3%)
Gemcitabine based	6 (13.6%)	2 (14.3%)	4 (13.3%)
Vinorelbine	12 (27.3%)	1 (7.1%)	11 (36.7%)
Docetaxel	10 (22.7%)	2 (14.3%)	8 (26.7%)
Other**	8 (18.2%)	5 (35.7%)	3 (10.0%)

Data are reported as number (%)

PP1 first post-pemetrexed, *PP2* 2nd post-pemetrexed, *EGFR-TKI* epidermal growth factor-tyrosine kinase 3 inhibitor

*The chemotherapeutic regimens include as follows: carboplatin singlet, 4 (44.4%); irinotecan + carboplatin, 2 (22.2%); crizotinib, 2 (22.2%); paclitaxel + carboplatin, 1 (11.1%); paclitaxel singlet, 1 (11.1%)

**The chemotherapeutic regimens include as follows; carboplatin singlet, 3 (37.5%); paclitaxel singlet, 3 (37.5%); paclitaxel + carboplatin, 1 (12.5%); irinotecan singlet, 1 (12.5%)

Table 3 Overall response rate (ORR) and disease control rate (DCR) according to the type of subsequent systemic treatment

PP1	Total ($n=74$)	Good responder ($n=23$)		Poor responder ($n=51$)		*p* value*	*p* value**
		DCR	ORR	DCR	ORR		
Total	74 (100.0%)	16/23 (69.6%)	8/23 (34.8%)	19/51 (37.3%)	10/51 (19.6%)	0.010	0.159
EGFR-TKIs	31 (41.9%)	3/5 (60.0%)	2/5 (40.0%)	8/26 (30.8%)	2/26 (7.7%)	0.317	0.112
Gemcitabine	16 (21.6%)	4/5 (80.0%)	1/5 (20.0%)	6/11 (54.5%)	4/11 (36.4%)	0.588	1.000
Vinorelbine	10 (13.5%)	4/7 (57.1%)	2/7 (28.6%)	0/3 (0.0%)	0/3 (0.0%)	0.200	1.000
Docetaxel	8 (10.8%)	1/2 (50.0%)	1/2 (50.0%)	2/6 (33.3%)	2/6 (33.3%)	1.000	1.000
PP2	Total ($n=44$)	Good responder ($n=14$)		Poor responder ($n=30$)		*p* value*	*p* value**
		DCR	ORR	DCR	ORR		
Total	44 (100.0%)	7/14 (50.0%)	4/14 (28.6%)	12/30 (40.0%)	4/30 (13.3%)	0.533	0.242
EGFR-TKIs	8 (18.2%)	3/4 (75.0%)	1/4 (25.0%)	1/4 (25.0%)	1/4 (25.0%)	0.486	1.000
Gemcitabine	6 (13.6%)	1/2 (50.0%)	1/2 (50.0%)	1/4 (25.0%)	0/4 (0.0%)	1.000	0.333
Vinorelbine	12 (27.3%)	0/1 (0.0%)	0/1 (0.0%)	4/11 (36.4%)	0/11 (0.0%)	1.000	–
Docetaxel	10 (22.7%)	1/2 (50.0%)	0/2 (0.0%)	4/8 (50.0%)	2/8 (25.0%)	1.000	1.000

Data are reported as *n* (%)

PP post-pemetrexed, *EGFR-TKI* epidermal growth factor receptor-tyrosine kinase inhibitor, *DCR* disease control rate, *ORR* overall response rate

p value* for DCR

P value** for ORR

sensitized by pemetrexed. Therefore, long-term pemetrexed maintenance in good responders with a median duration of 21 months in our study might enhance cell cycle arrest and sustain blockade of DNA synthesis, which could contribute to enriched chemosensitivity to subsequent drugs.

More importantly, from an immunological point of view, pemetrexed might potentially interact with host immune

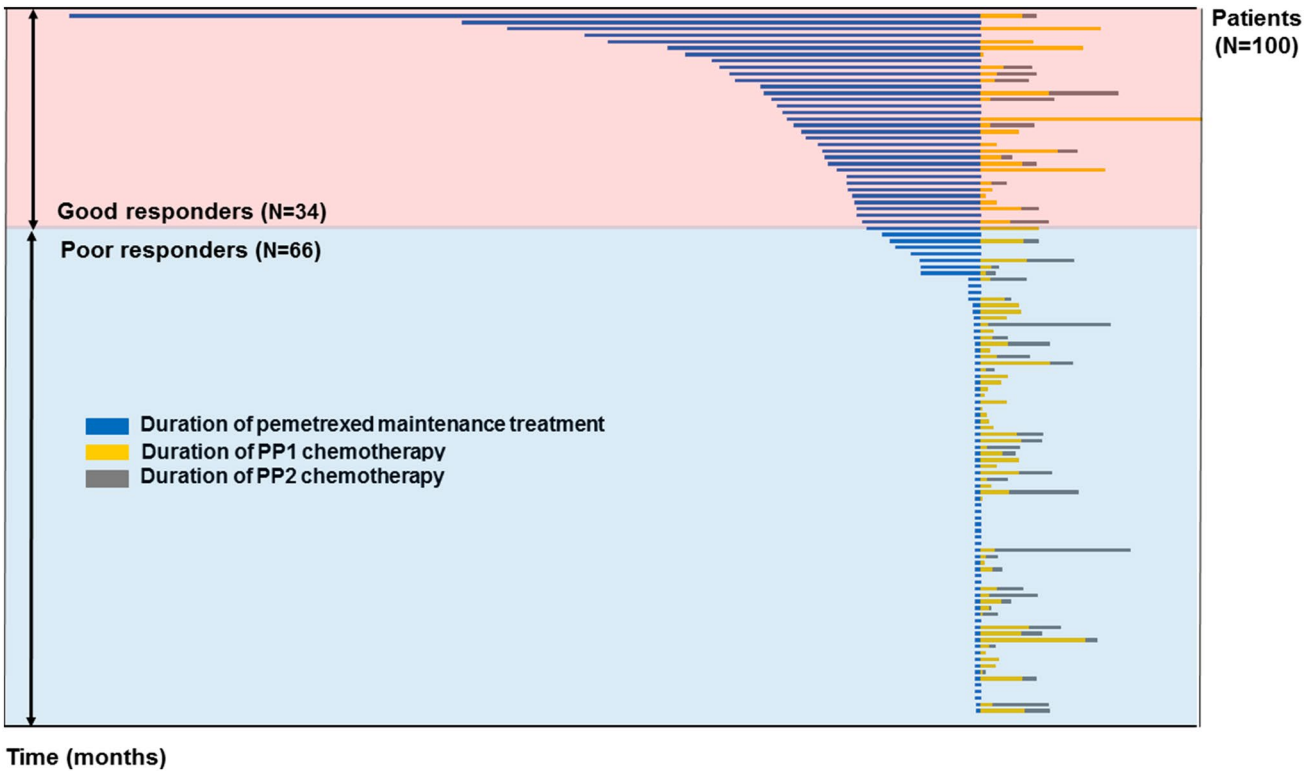

Fig. 1 Schematic plot for treatment durations of pemetrexed maintenance and following PP1 and PP2 therapies in the cohort ($n = 100$)

Table 4 Median progression-free survival (PFS) according to the type of subsequent systemic treatment

PP1	Total (n, %)	PFS1 (months)	Response to pemetrexed		p value
			Good responder ($n = 23$)	Poor responder ($n = 51$)	
All patients	74 (100.0)	2.7 (1.9–3.6)	5.2 (2.9–7.5)	2.2 (1.2–3.2)	< 0.001
EGFR-TKIs	31 (41.9)	2.2 (0.4–4.0)	6.4 (3.5–9.3)	1.5 (0.4–2.6)	0.150
Gemcitabine	16 (21.6)	2.4 (2.0–2.9)	2.5 (0.0–7.1)	2.3 (1.2–3.4)	0.132
Vinorelbine	10 (13.5)	3.2 (0.0–7.1)	4.1 (0.1–8.1)	1.6 (0.0–4.0)	0.093
Docetaxel	8 (10.8)	1.3 (0.0–3.3)	3.3*	1.3 (0.9–1.7)	0.646
PP2	Total (n, %)	PFS2 (months)	Good responder ($n = 14$)	Poor responder ($n = 30$)	p value
All patients	44 (100.0)	2.2 (1.2–3.3)	2.1 (0.0–4.3)	2.2 (1.0–3.5)	0.995
EGFR-TKIs	8 (18.2)	2.1 (0.6–3.6)	3.1 (0.5–5.7)	1.0 (0.1–1.8)	0.027
Gemcitabine	6 (13.6)	1.6 (1.2–2.0)	1.9*	1.5 (1.3–1.8)	0.198
Vinorelbine	12 (27.3)	2.8 (0.9–4.7)	1.5*	3.5 (2.5–4.4)	0.146
Docetaxel	10 (22.7)	1.0 (0.0–4.2)	0.8*	1.0 (0.0–4.0)	0.699

Data are reported as median (months, interquartile range), but median PFS could not be calculated in * columns owing to insufficient patient numbers

PP post-pemetrexed, *PFS* progression-free survival, *EGFR-TKI* epidermal growth factor-tyrosine kinase inhibitor

signature and affect both innate and adaptive immunities (Emens and Middleton 2015; Galluzzi et al. 2015). In earlier studies, the immunological effect of pemetrexed via folate deficiencies seemed controversial. Its immunosuppressive effect was suggested mainly by depleting cytotoxic T cells and NK cells, but hyperhomocysteinemia induced by folate and vitamin B12 deficiencies seemed to enhance cellular immune activity (Davis et al. 2012). However, based on previous studies which attempted to explain exceptional sensitivity to pemetrexed with molecular perspectives (Adjei

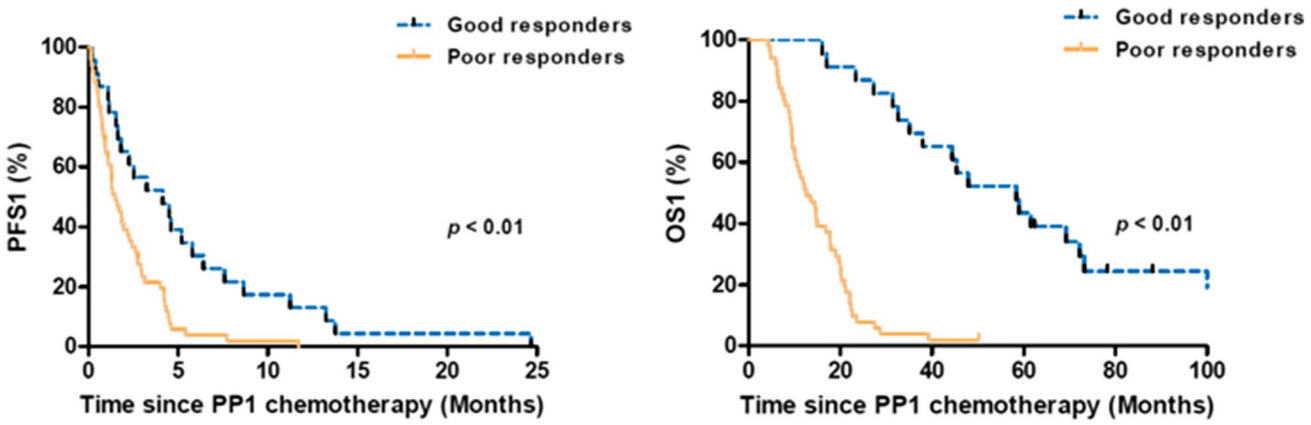

Fig. 2 Progression-free survival (PFS1) and overall survival (OS1) of the first post-pemetrexed (PP1) therapy according to the response to pemetrexed treatment

2004; Kuo et al. 2017; Yoshida et al. 2016), we can hypothesize that patients with exceptional pemetrexed sensitivity might interact with relatively more abundant molecular aberrations during exposure to pemetrexed. These enriched molecular interaction in good responders might possibly contribute to augmentation of the host's immunogenicity. Another preclinical study identified that downregulation of IDO, an immune-suppressive molecule contributing to immune escape, was significantly correlated with enhanced sensitivity to pemetrexed, suggesting active interaction of pemetrexed with immune mediators (Maleki Vareki et al. 2015; Prendergast et al. 2014). More recently, Denise et al. attempted to evaluate the relationship with individual innate immune function and response to combination chemotherapy of pemetrexed and cisplatin. They reported that patients with the longest PFS showed increased neutrophil expression and activity after pemetrexed and cisplatin treatment, suggesting a favorable modulation of the tumor microenvironment and stimulation of the intrinsic immunogenicity by pemetrexed. Immunosuppressive profiles were also observed in these patients, but the authors suggested it as a consequence of immune homeostasis after intrinsic immunogenic boost (Putri et al. 2018). In other recent studies, PD-L1 expression was induced by pemetrexed in NSCLC patients which was relevantly associated with favorable treatment outcomes, additionally providing a rationale of combining pemetrexed with immune-checkpoint inhibitors (Lin et al. 2017; Zhang et al. 2017). Collectively, pemetrexed-sensitive patients could achieve favorable treatment outcomes with post-pemetrexed systemic treatment, which might be attributable to a beneficial modulation of the tumor microenvironment and the host immune signature by prolonged pemetrexed treatment. However, controversies on the immunomodulatory effect of pemetrexed need to be further studied (Davis et al. 2012).

This study has several limitations. First, because this is a retrospectively designed, single center study with small sample size, it inevitably contains selection bias. Particularly, the possibility of clinical selection bias cannot be excluded in the enrollment of patients who were treated with single pemetrexed treatment without platinum. Second, although we excluded patients treated with platinum-based doublet therapy to minimize the confounding effect of platinum, patients were heterogeneously treated with pemetrexed in different settings of chemotherapy; the majority received it as their second-line regimen, but nine (8.3%) as their first-line treatment. However, there were no significant differences of distribution of responders for pemetrexed according to the lines of treatment, and more importantly, the response and survival outcomes of PP1 therapy were not influenced by the lines of pemetrexed treatment. Also, we exclusively enrolled non-targetable patients unlike our previous study which also included EGFR-mutant or ALK-positive patients, to enhance the homogeneity of the cohort by excluding potential biologic and clinical influence of these molecular aberrations in the pemetrexed treatment.

In conclusion, patients who achieved durable response to pemetrexed might obtain greater therapeutic benefits from subsequent systemic treatment regarding both PFS and DCR. Pemetrexed, in a new era of immuno-oncology, will be more widely applied for NSCLC patients in a tight linkage with upfront immunotherapy, and it has already been accepted as a standard chemotherapy partner of immune-checkpoint inhibitor in NSCLC without targets (Gandhi et al. 2018; Reck et al. 2016; Herbst et al. 2016). Therefore, based on its intrinsic cytotoxic effect and potential immunomodulatory role, pemetrexed sensitivity itself, followed by the prediction of subsequent treatment outcomes might give us another clue to design optimal treatment sequence in these patients.

Compliance with ethical standards

Conflict of interest No author has any financial disclosures to declare related to this study.

Research involving human participants It was conducted in full accordance with the Guidelines for Good Clinical Practice and the 1964 Declaration of Helsinki.

Informed consent The study protocol was reviewed and approved by the Institutional Review Board (IRB Approval Number: 2018-0661) of Asan Medical Center, and informed consents were obtained from all individual participants included in the study.

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