Original Article

Predictive factors for long-term responders of pemetrexed maintenance treatment in non-small cell lung cancer

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Abstract

Background: We determined the clinical characteristics and predictive factors of long-term response to pemetrexed maintenance therapy as first-line treatment for non-small cell lung cancer (NSCLC).

Methods: A total of 950 advanced NSCLC patients received pemetrexed (500 mg/m²) plus cisplatin (60 mg/m²) (Pem-Cis) induction chemotherapy every three weeks as first-line treatment between January 2010 and August 2018. Patients who did not show progression after four cycles of Pem-Cis and received at least one cycle of pemetrexed maintenance were recruited (n = 199).

Results: Patients were divided into subgroups according to total cycles of pemetrexed: ≤10 (F10, n = 134) and >10 (M10, n = 65). The M10 group had a higher proportion of patients with stage M1a (intrathoracic metastasis alone) and exhibited lower levels of thymidylate synthase (TS) than the F10 group (median H-score 10.0% vs. 60.0%; P = 0.031). Further subgrouping identified extreme responders: ≤7 (F7, n = 101) and ≥20 (M20, n = 26) cycles. The M20 group showed lower mean serum CEA levels before (17.5 vs. 147.0; P = 0.099) and after (6.9 vs. 53.2; P = 0.001) Pem-Cis treatment, and a higher incidence of normalization after Pem-Cis (abnormal 41.7% vs. 68.5%; P = 0.015). M1a stage, normalization of CEA levels after Pem-Cis, and lower TS H-score were predictors of progression-free survival in patients administered pemetrexed maintenance.

Conclusion: M1a stage and lower TS expression were predictors of long-term response to pemetrexed maintenance. CEA normalization after Pem-Cis could be an additional surrogate marker of positive response to long-term treatment.

Introduction

Over the past decades, significant improvements have been made in lung cancer treatment. Targeted therapies, including EGFR-tyrosine kinase inhibitors (TKIs) and ALK inhibitors, have improved the survival and quality of life of patients with advanced non-small cell lung cancer (NSCLC) who harbor driver mutations.1,2 In addition, immune checkpoint inhibitors (ICIs) targeting PD-1 and PD-L1 established a new paradigm for lung cancer treatment and increased survival in selected and unselected patients.1,2 However, few patients have such druggable targets at baseline, and options for subsequent treatment after failure of front-line treatments with TKIs and ICIs are still necessary. Thus, cytotoxic chemotherapy remains an essential component of lung cancer treatment.

Pemetrexed, a potent inhibitor of thymidylate synthase (TS) and other folate-dependent enzymes, is approved as first-line treatment of NSCLC in combination with cisplatin. In patients with chemotherapy-naïve non-squamous (NSq)-NSCLC, the JMDB trial showed that combination chemotherapy with pemetrexed plus cisplatin (Pem-Cis) was superior to therapy with gemcitabine plus cisplatin (Gem-Cis) in terms of efficacy and toxicity.3 A similar trend and more favorable survival outcomes in patients with NSq-NSCLC were described in subsequent exploratory subgroup analysis of East Asian patients.4 Thus,
Pem-Cis is considered one of the best therapeutic regimens for advanced NSq-NSCLC patients without targetable driver mutations or strong PD-L1 expression. Moreover, pemetrexed continuation maintenance therapy has been demonstrated as an efficacious strategy for patients with advanced NSq-NSCLC who do not show disease progression during Pem-Cis induction therapy.\textsuperscript{5–8}

Physicians often encounter patients who have been treated with multiple rounds of pemetrexed. Previous studies of pemetrexed treatment have reported that several factors are related to efficacy: non-squamous histology,\textsuperscript{3} female gender,\textsuperscript{9,10} no history of smoking,\textsuperscript{9,10} ALK gene rearrangement,\textsuperscript{10–13} low levels of TS,\textsuperscript{14–16} TTF-1 expression,\textsuperscript{14,15} and low tumor burden.\textsuperscript{10} However, studies on the factors associated with long-term response to pemetrexed maintenance treatment are limited, and most studies have investigated the efficacy of pemetrexed without considering the lines of treatment.

This study aimed to determine the clinical characteristics and predictive factors of long-term response to pemetrexed maintenance therapy as first-line treatment for NSCLC.

Methods

Study participants and chemotherapy

We retrospectively investigated patients with stage III or IV NSCLC treated with pemetrexed at Chonnam National University Hwasun Hospital between January 2010 and August 2018. A total of 950 patients were administered induction chemotherapy of pemetrexed (500 mg/m\textsuperscript{2}) plus cisplatin (60 mg/m\textsuperscript{2}) every three weeks as first-line treatment. Among them, 236 patients who did not show progression after the completion of four cycles of induction chemotherapy and received at least one cycle of maintenance therapy of pemetrexed (500 mg/m\textsuperscript{2} every 3 weeks) were recruited. A daily dose of oral folic acid (1 mg per day) was administered a week before pemetrexed was initiated until the end of treatment. In addition, 1 mg of vitamin B12 was administered via intramuscular injection within seven days of the first dose of pemetrexed and once every three cycles thereafter. Patients aged ≥ 18 years who had not been administered prior systemic therapy, with an Eastern Cooperative Oncology Group performance status of 0–2 were included. Patients with either an inconclusive response assessment after induction therapy or continuing maintenance treatment were excluded (Fig 1).

All data were gathered in accordance with the amended Declaration of Helsinki following independent institutional review board approval (No. CNUHH-2018-166). The need for written informed consent was waived because of the retrospective design of the study.

Analysis of tumor tissue biomarkers

Expression of TS, a therapeutic target of pemetrexed, was investigated by immunohistochemical (IHC) staining (1:50 dilution, DAKO clone M3614, Glostrup, Denmark) of available formalin-fixed paraffin-embedded tissues from enrolled patients. The degree of TS expression was graded using a scale of 0–3 according to the extent of cytoplasmic or nuclear staining. The percentage of positive tumor cells

Figure 1  Patient enrollment process. NSCLC, non-small cell lung cancer.
in each specimen was calculated and then multiplied by the staining intensity to obtain a final semi-quantitative H-score (possible range: 0–300).

Response assessment and statistical analysis

Treatment response of induction chemotherapy was evaluated according to the revised Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Progression-free survival (PFS) of pemetrexed was measured in two ways: from the first date of Pem-Cis induction chemotherapy (PFSi) or pemetrexed maintenance treatment (PFSm) to the first date of objective disease progression or death from any cause. The cut off serum CEA level was 5.0 ng/mL.

We collected baseline and clinical information of the enrolled patients at the start of Pem-Cis induction chemotherapy. Drug-related adverse events during induction chemotherapy and maintenance treatment were recorded and graded based on the level of severity using National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. All data were expressed as mean ± standard deviation and median (range), or as numbers with percentages. Intergroup comparisons were performed using the Mann–Whitney U test for continuous variables and Pearson's χ² or Fisher’s exact test for categorical variables. Survival times were estimated for each group using the Kaplan–Meier method. The predictive factors for PFS were analyzed using a Cox proportional hazard regression model to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Correlation between the number of pemetrexed cycles and TS H-score was evaluated using Spearman’s correlation method. Statistical analysis was performed using SPSS version 25, and P values < 0.05 were considered significant.

Results

Participants and baseline characteristics

After excluding 37 patients with either an inconclusive response assessment after induction chemotherapy or continuing maintenance treatment, 199 patients were enrolled and divided into two subgroups according to the total cycles of pemetrexed: ≤ 10 (F10, n = 134) and > 10 (M10, n = 65) (Fig 1). There were higher proportions of younger patients (mean 64.7 vs. 67.2; P = 0.046) and patients with stage IV lung cancer with intrathoracic metastasis alone (M1a as per International Association for the Study of Lung Cancer the 8th edition Tumor Node Metastasis classification, 40.0% vs. 22.4%; P = 0.010) in the M10 than in the F10 subgroup. However, there were no significant differences between the two subgroups in terms of gender, smoking history, brain metastasis, driver mutations, or previous treatment (Table 1).

Outcomes of pemetrexed treatment

The mean cycle of pemetrexed treatment was 11.1 ± 6.7 days (range: 5–43) (Table 1). The PFSi was 7.3 months (95% CI 6.2–8.3), while the PFSm was 4.4 months (95% CI 3.3–5.4). The median overall survival (OS) was 29.5 months (95% CI 14.9–44.0); the OS rate in the M10 subgroup has not yet been reached. There was no significant difference in the best response to induction chemotherapy between the subgroups (Table 1).

Characteristics of biomarkers in pemetrexed treatment

There were no significant differences in the CEA level at pre-induction and post-induction chemotherapy between the subgroups (Table 2). Among 92 patients with available tumor tissue, the M10 group showed lower TS expression compared to the F10 group, represented by a lower median H-score (10.0% vs. 60.0%; P = 0.031) (Fig 2a). There was a negative correlation between TS H-score and cycles of pemetrexed (rs = −0.285, P = 0.092) (Fig 2c).

Predictive factors for extreme responders

When subgroups were created to identify extremely poor (≤ 7 cycles, F7) and good (≥ 20, M20) responders, the M20 group showed a trend similar to that of the M10 group with higher numbers of M1a stage patients (42.3% vs. 20.8%; P = 0.024), a lower incidence of brain metastasis (23.1% vs. 42.6%; P = 0.069), and lower levels of TS expression (median H-score 5.0% vs. 50.0%; P = 0.030) (Fig 2b, Table 2). In addition, the M20 subgroup showed lower baseline levels of CEA before (mean 17.5 vs. 147.0; P = 0.099) and after (mean 6.9 vs. 53.2; P = 0.001) induction chemotherapy (Fig 3), and higher incidence of normalization after Pem-Cis treatment (abnormal 41.7% vs. 68.5%; P = 0.015) (Table 2). The Cox proportional hazard regression model identified M1a stage, normalization of CEA level after induction chemotherapy, and lower TS H-score as predictive factors of PFS (Table 3).

Toxicity

The common side effects of chemotherapy were anemia (71.7%), leukopenia (45.5%), and neutropenia (46.5%), but there was no significant difference in these rates between the two subgroups (Table 4).
Table 1 Comparison of baseline characteristics and treatment outcome according to total cycles of pemetrexed treatment

| Characteristics, N (%) | Total (n = 199) | 5–10 (F10) (n = 134) | > 10 (M10) (n = 65) | P |
|------------------------|-----------------|-----------------------|---------------------|---|
| Age, mean (± std dev)  | 66.5 (±10.1)    | 67.3 (±10.4)          | 64.7 (±9.3)         | 0.047 |
| Male/Female            | 136/63          | 90/44                 | 46/19               | 0.608 |
| Ever/Neve smoker       | 129/70          | 85/49                 | 44/21               | 0.555 |
| Histology: ADC/SQC/LCC/NSCLC NOS | 190/1/7/1 | 126/1/6/1             | 64/0/1/0            | 0.545 |
| Stage (III/IV) by IASLC 8th TNM | 23/176 | 19/115                | 4/61                | 0.555 |
| IV – M1α               | 56 (28.1)       | 30 (22.4)             | 26 (40.0)           | 0.010 |
| IV – M1β               | 40 (20.1)       | 31 (23.1)             | 9 (13.8)            | 0.125 |
| IV – M1c               | 80 (40.2)       | 54 (40.3)             | 26 (40.0)           | 0.968 |
| Brain metastasis       | 76 (38.2)       | 53 (39.6)             | 23 (35.4)           | 0.570 |
| Brain RT               | 40 (20.1)       | 30 (22.4)             | 10 (15.4)           | 0.248 |
| EGFR mutation (19del/20ins/L858R/T790M others) | 17/17 (8.5) (2/4/7/2/3) | 12 (9.0) (1/4/4/0/3) | 5/5 (7.1) (1/0/3/2/0) | 0.754 |
| ALK translocation (FISH-positive) | 21 (10.6) | 13 (9.7)              | 8 (12.3)            | 0.795 |
| Pre-chemotherapy treatment (surgery/radiotherapy) | 29 (14.6) (19/10) | 20 (14.9) (12/8) | 9 (13.8) (7/2) | 0.642 |
| Cycles of pemetrexed, mean (± std dev) | 11.1 (±7.6) | 7.0 (±1.4)            | 19.6 (±8.2)         | 0.000 |
| Induction cycles, mean (± std dev) | 4.0 (±0.3) | 4.0 (±0.3)            | 4.0 (±0.3)          | 0.346 |
| PFSi, months, median (95% CI) | 7.3 (6.2–8.3) | 6.0 (5.6–6.3) | 16.6 (13.5–19.7) | 0.000 |
| PFSm, months, median (95% CI) | 4.4 (3.3–5.4) | 2.8 (2.6–2.9) | 13.4 (10.5–16.3) | 0.000 |
| OS, months, median (95% CI) | 29.5 (14.9–44.0) | 8.4 (11.9–25.0) | NR                  | 0.000 |
| Best response of induction: PR/SD, N (%) | 88 (44.2)/111 (55.8) | 60 (44.8)/74 (55.0) | 28 (43.1)/37 (56.9) | 0.821 |
| Subsequent treatment, N (%) | 134 (67.3) | 93 (69.4)             | 41 (63.1)           | 0.372 |
| Chemotherapy           | 96 (50.5)       | 68 (51.9)             | 28 (47.5)           | 0.570 |
| EGFR or ALK-TKIs       | 67 (35.3)       | 48 (36.6)             | 19 (32.2)           | 0.554 |
| ICIs                   | 24 (12.6)       | 16 (12.2)             | 8 (13.6)            | 0.796 |

†Metastasis within the thoracic cavity; single and multiple extrathoracic metastasis; L858R and T790M occurred simultaneously in one patient. ADC, adenocarcinoma; ICIs, immune checkpoint inhibitors; LCC, large cell carcinoma; NSCLC NOS, non-small cell lung carcinoma not otherwise specified; IASLC, International association for the study of lung cancer; RT, radiotherapy; FISH, fluorescence in situ hybridization; OS, overall survival; NR, not reached; PFSi, progression-free survival from the first day of induction chemotherapy; PFSm, PFS from the first day of maintenance therapy; PR, partial response; SD, stable disease; SQC, squamous carcinoma; std dev, standard deviation; TKIs, tyrosine kinase inhibitors.

Discussion

In this study, we retrospectively investigated the clinical characteristics and predictive factors of long-term responders of pemetrexed maintenance therapy as first-line treatment for NSCLC. The subgroup that achieved a long-term response (pemetrexed >10 cycles, M10) consisted of higher proportions of younger and M1a stage patients, and exhibited lower levels of TS expression. In the analysis of extreme responders, lower levels of CEA at baseline and normalization of CEA after Pem-Cis were identified as potential additional markers of long-term response, especially in the subgroup administered ≥20 cycles of pemetrexed (M20).

The outcome of pemetrexed treatment in our study was comparable to those reported in previous landmark trials. The PFSm was 4.4 months (95% CI 3.3–5.4), and the median OS from the start of induction chemotherapy was 29.5 months (95% CI 14.9–44.0). In subgroup analysis in the JMEN study, the PFSm in the East Asian group was 4.4 months (95% CI 4.1–6.4) and the median OS was 19.7 months (95% CI 16.4–22.7). In the PARAMOUNT study, the PFSm was 4.4 months (95% CI 4.1–5.7), and the median OS rates from the start of maintenance treatment and induction chemotherapy were 13.9 (95% CI 12.8–16.0) and 16.9 months (95% CI 15.8–19.0), respectively. Excluding the duration of induction chemotherapy, the OS in our study was superior to those reported in the above-mentioned previous trials. This may be a result of the active application of subsequent treatments and the development of novel and high-performance drugs, including TKIs and ICIs.

High tumor burden and brain metastasis are considered typical predictors of poor treatment outcome in lung cancer. In a Korean study, two or more metastatic sites and intra-abdominal metastasis were poor prognostic factors for pemetrexed treatment. However, this study enrolled a majority of patients previously treated with other chemotherapeutic agents (n = 270, 86.0%). In other words, the high tumor burden might not have affected the poor response to pemetrexed alone and to other drugs used before pemetrexed treatment. In our study, stage IV NSCLC with intrathoracic metastasis alone (M1a) was a consistent predictive factor in the M10 (HR 0.65, 95% CI 0.47–0.91; P = 0.012) and M20 (HR 0.60, 95% CI 0.39–0.94; P = 0.024) subgroups. In addition, the
Table 2 Comparison of biomarkers according to total cycles of pemetrexed treatment

| Characteristics                              | Total  | 5–10 (F10) | > 10 (M10) | P     | 5–7 (F7) | ≥ 20 (M20) | P   |
|----------------------------------------------|--------|------------|------------|-------|----------|------------|-----|
| CEA, ng/ml, mean (± std dev)                | N = 171| N = 117    | N = 54     | 0.900 | N = 87   | N = 22     | 0.099|
| Pre-induction abnormal, N (%)               | 124 (67.8) | 85 (67.5) | 39 (68.4)  | 0.898 | 64 (67.4) | 14 (63.6)  | 0.738|
| Post-induction abnormal, N (%)              | 113 (63.1) | 81 (65.9) | 32 (57.1)  | 0.263 | 63 (68.5) | 10 (41.7)  | 0.051|
| Difference between pre & post               | −73.4 (±451.5) | −78.5 (±482.3) | −62.3 (±380.3) | 0.827 | −94.5 (±556.3) | −10.6 (±27.2) | 0.767|
| ≥ 50% decrease, N (%)                       | 47 (27.6) | 32 (27.6) | 15 (27.8)  | 0.979 | 24 (27.6) | 7 (31.8)   | 0.694|
| TS, N (%)                                   | N = 92 | N = 66    | N = 26     | 1.000 | N = 51   | N = 11     | 1.000|
| IHCS - high intensity (3+)                  | 10 (10.9) | 7 (10.6) | 3 (11.5)   | 1.000 | 6 (11.8) | 1 (9.1)    | 1.000|
| IHCS - low intensity (0–2+)                 | 82 (89.1) | 59 (89.4) | 23 (88.5)  | 1.000 | 45 (88.2) | 10 (90.9)  | 1.000|
| H-score, %, median (range)                  | 45.0 (0.0–300.0) | 60.0 (0.0–300.0) | 10.0 (0.0–160.0) | 0.031 | 50.0 (0.0–300.0) | 5.0 (0.0–60.0) | 0.030|

std dev, standard deviation; TS, thymidylate synthase; IHCS, immunohistochemical stain.

Figure 2 Correlation between cycles of pemetrexed and thymidylate synthase (TS) expression represented by H-score. Differences in TS H-score between pemetrexed cycles of (a) ≤10, (b) ≥10, and (c) >10, and (d) ≤7, and ≥20. (e) Correlation between thymidylate synthase (TS) expression represented by H-score and cycles of pemetrexed (Spearman's rho, r = −0.285, P = 0.092).
prevalence of brain metastasis in the M20 subgroup was lower than in the F7 subgroup. Thus, M1a stage is a reliable prognostic factor for long-term response to pemetrexed maintenance treatment.

TS was a predictive marker for clinical outcomes of pemetrexed-based chemotherapy, especially in NSq-NSCLC. However, TS-positive tumors have been defined differently in several studies, using an H-score, an IHC score, or the percentage of positive tumor cells. In present study, we analyzed the levels of TS expression using a graded scale according to the extent of cytoplasmic or nuclear staining. There was no difference in the level of TS expression according to IHC intensity between the F10 and M10 subgroups. However, there was a significant difference in the TS H-score between the subgroups. In addition, when we divided the patients into subgroups based on median TS H-score (45.0), a value < 45.0 correlated with long-term response to pemetrexed maintenance therapy (HR 0.42, 95% CI 0.19–0.92; P = 0.031). In previous studies, TS expression was investigated in patients treated with pemetrexed plus platinum or pemetrexed monotherapy. Most of these studies did not enroll patients administered continuation maintenance therapy after induction chemotherapy. Thus, the results of our study could serve as a guide for the continuation of maintenance therapy as first-line treatment of patients with NSCLC according to TS expression when the response of induction Pem-Cis is equivocal.

Although the use of CEA as predictive marker of lung cancer treatment is debated, serial follow-up of the serum level is widely performed in real practice, especially for NSq-NSCLC. In a systematic review of previous studies, a high CEA serum level was considered as a risk factor for brain metastasis in treatment-naïve NSCLC, and increments in the serum CEA level during chemotherapy was a predictive marker for early relapse, progression, or PFS. In present study, there were no differences in the serum CEA level at the time of pre-induction or post-induction chemotherapy between the F10 and M10 subgroups. However, in a comparison between F7 and M20, lower serum levels of CEA at baseline and normalization before the start of pemetrexed maintenance could be predictive markers.

Figure 3  Differences in serum CEA levels between pre-induction and post-induction chemotherapy according to total cycles of pemetrexed treatment. *P = 0.099; **P = 0.001. (a) Pre and (p) Post.

Table 3  Predictive factors of progression-free survival

| Factors                                      | PFSi, HR (95% CI) F10† vs. M10‡ | P | F7§ vs. M20¶ | P | PFSm, HR (95% CI) F10† vs. M10‡ | P | F§ vs. M20¶ | P |
|----------------------------------------------|---------------------------------|---|--------------|---|---------------------------------|---|--------------|---|
| M stage (by 8th TNM)                         |                                 |   |              |   |                                 |   |              |   |
| M1a (vs. M1b or M1c)                         | 0.65 (0.47–0.91)                | 0.012 | 0.60 (0.39–0.94) | 0.024 | 0.67 (0.48–0.93)                | 0.017 | 0.62 (0.40–0.96) | 0.030 |
| CEA, post-induction, Normal (vs. abnormal)   | 0.70 (0.49–0.98)                | 0.040 | 0.62 (0.40–0.96) | 0.031 | 0.70 (0.49–0.99)                | 0.042 | 0.63 (0.41–0.98) | 0.040 |
| TS H-score                                   | ≤ 45.0†† (vs. > 45.0)           | 0.42 (0.19–0.92) | 0.031 | 0.31 (0.11–0.85) | 0.024 | 0.42 (0.19–0.94) | 0.035 | 0.31 (0.11–0.88) | 0.028 |

Pemtrexed cycles: †≤ 10; ‡>10; §≤ 7; ¶> 20. ††Median thymidylate synthase (TS) H-score. CI, confidence interval; HR, hazard ratio; PFSi, progression-free survival from the first day of induction chemotherapy; PFSm, PFS from the first day of maintenance therapy.
for long-term response (HR 0.62, 95% CI 0.40–0.96; P = 0.031).

According to RECIST v1.1, tumor markers such as CEA alone cannot be used to assess tumor response and only a few disease-specific markers, such as CA-125 (recurrent ovarian cancer) and PSA (recurrent prostate cancer), are incorporated into guidelines for objective tumor assessment.17 In the present study, no correlation was observed between pre- and post-induction changes (%) in serum CEA levels and tumor size calculated based on RECIST version 1.1 (rs = −0.134, P = 0.139, data not shown). Moreover, no significant difference was observed in the best response to induction chemotherapy between the F10 and M10 subgroups. These results may simply relate to the inclusion criteria of this study, which only comprised non-progressors after induction chemotherapy. However, in addition, the serum CEA level per se may reflect the tumor burden at a certain time point rather than a dynamic change in tumor volume. The mean serum CEA levels at pre- and post-induction were significantly different between the M1 stage subgroups (M1a vs. M1b + M1c 39.9 vs. 147.7, P = 0.034; 17.0 vs. 57.9, P = 0.17; data not shown). Therefore, serum CEA levels could be informative to tumor burden by monitoring the value at least before and after induction with Pem-Cis. Furthermore, decrement or normalization of serum CEA levels could provide valuable information of the efficacy and progression of pemetrexed treatment of NSCLC, in a complementary role to RECIST.

There were several limitations to this study. First, we could not analyze all of the information of enrolled patients because of the retrospective nature of this study, particularly data on biomarkers and adverse events. Evaluation of serum CEA levels and TS IHC was not performed in all patients, and data on adverse events were obtained from the patients’ medical records. Thus, a prospective study is warranted to investigate all possible factors in continuation maintenance treatment. Second, we investigated TS expression using archival formalin-fixed paraffin-embedded tissue in addition to fresh specimens. This could affect the intensity of IHC staining when merged with clinical data. Finally, the difference in patient numbers between subgroups, especially in the extreme responders, might have affected the statistical significance.

In conclusion, M1a stage and lower levels of TS expression are predictive factors for a long-term positive response to pemetrexed maintenance treatment. The normalization of serum CEA levels after induction chemotherapy could be an additional surrogate marker for response to long-term treatment, and serial monitoring of serum CEA levels from baseline at therapy initiation could be valuable.

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Disclosure
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References
1. Ferrara R, Mezquita L, Besse B. Progress in the management of advanced thoracic malignancies in 2017. J Thorac Oncol 2018; 13: 301–22.
2. Doroshow DB, Herbst RS. Treatment of advanced non-small cell lung cancer in 2018. JAMA Oncol 2018; 4: 569–70.
3. Scagliotti GV, Parikh P, von Pawel J et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced stage non-small-cell lung cancer. J Clin Oncol 2008; 26: 3543–51.
4. Yang CH, Simms L, Park K, Lee JS, Scagliotti G, Orlando M. Efficacy and safety of cisplatin/pemetrexed versus cisplatin/gemcitabine as first-line treatment in East Asian patients with advanced non-small cell lung cancer: Results of an exploratory subgroup analysis of a phase III trial. J Thorac Oncol 2010; 5: 688–95.
5. Paz-Ares L, de Marinis F, Dediu M et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): A double-blind, phase 3, randomised controlled trial. Lancet Oncol 2012; 13: 247–55.
6. Paz-Ares LG, de Marinis F, Dediu M et al. PARAMOUNT: Final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. J Clin Oncol 2013; 31: 2895–902.
7. Scagliotti GV, Gridelli C, de Marinis F et al. Efficacy and safety of maintenance pemetrexed in patients with advanced nonsquamous non-small cell lung cancer following pemetrexed plus cisplatin induction treatment: A cross-trial comparison of two phase III trials. Lung Cancer 2014; 85: 408–14.
8. Belani CP, Wu YL, Chen YM et al. Efficacy and safety of pemetrexed maintenance therapy versus best supportive care in patients from East Asia with advanced, nonsquamous non-small cell lung cancer: An exploratory subgroup analysis of a global, randomized, phase 3 clinical trial. J Thorac Oncol 2012; 7: 567–73.
9. Gronberg BH, Bremnes RM, Flotten O et al. Phase III study by the Norwegian Lung Cancer Study Group: Pemetrexed plus carboplatin compared with gemcitabine plus carboplatin as first-line chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol 2009; 27: 3217–24.
10. Park S, Kim HJ, Choi CM et al. Predictive factors for a long-term response duration in non-squamous cell lung cancer patients treated with pemetrexed. BMC Cancer 2016; 16: 417.
11. Solomon BJ, Mok T, Kim DW et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 2014; 371: 2167–77.
12. Park S, Park TS, Choi CM et al. Survival benefit of pemetrexed in lung adenocarcinoma patients with anaplastic lymphoma kinase gene rearrangements. Clin Lung Cancer 2015; 16: e83–9.
13. Shaw AT, Varghese AM, Solomon BJ et al. Pemetrexed-based chemotherapy in patients with advanced, ALK-positive non-small cell lung cancer. Ann Oncol 2013; 24: 59–66.
14. Sun JM, Han J, Ahn JS, Park K, Ahn MJ. Significance of thymidylate synthase and thyroid transcription factor 1 expression in patients with nonsquamous non-small cell lung cancer treated with pemetrexed-based chemotherapy. J Thorac Oncol 2011; 6: 1392–9.
15. Gronberg BH, Lund-Iversen M, Strom EH, Brustugun OT, Scott H. Associations between TS, TTF-1, FR-alpha, FPGS, and overall survival in patients with advanced non-small-cell lung cancer receiving pemetrexed plus carboplatin or gemcitabine plus carboplatin as first-line chemotherapy. J Thorac Oncol 2013; 8: 1255–64.
16. Sun JM, Ahn JS, Jung SH et al. Pemetrexed plus cisplatin versus gemcitabine plus cisplatin according to thymidylate synthase expression in nonsquamous non-small-cell lung cancer: A biomarker-stratified randomized phase II trial. J Clin Oncol 2015; 33: 2450–6.
17. Eisenhauer EA, Therasse P, Bogaerts J et al. New Response Evaluation Criteria in Solid Tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45: 228–47.
18. Grunnet M, Sorensen JB. Carcinoembryonic antigen (CEA) as tumor marker in lung cancer. Lung Cancer 2012; 76: 138–43.