Efficacy Differences of Pemetrexed by Histology in Pretreated Patients with Stage IIIB/IV Non-small Cell Lung Cancer

Review of Results from an Open-Label Randomized Phase II Study

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Introduction: Recent pivotal phase III studies in patients with advanced non-small cell lung cancer (NSCLC) consistently showed greater survival benefit of pemetrexed in patients with nonsquamous cell carcinoma histology (nonsquamous histology) compared with those with squamous cell carcinoma histology (squamous histology). To confirm the efficacy differences of pemetrexed by histologic type, we conducted an additional subgroup analysis of data from a Japanese randomized phase II study evaluating the efficacy and safety of pemetrexed 500 mg/m^2 (P500) and 1000 mg/m^2 (P1000) in patients with advanced NSCLC previously treated with chemotherapy. The efficacy and safety results of original phase II study have already been reported (Ohe et al., *Clin Cancer Res* 2008;14:4206–4212).

Methods: Objective response rates (ORRs), overall survival time, and progression-free survival time were analyzed by subgroup of histology, squamous, and nonsquamous, for the dose groups combined and separately.

Results: A total of 216 patients were evaluable for efficacy. One hundred sixty-eight patients had nonsquamous and 48 had squamous histology. ORRs were 20.8% and 2.1% ($p < 0.001$); median survival times (MST) were 16.0 and 8.5 months ($p < 0.001$); and median progression-free survival times (PFS) were 3.1 and 1.6 months ($p < 0.001$) for nonsquamous and squamous histology, respectively. In patients who were randomized to the P500 group, ORR were 23.5% and 0% ($p = 0.0062$); MST were 19.4 and 7.9 months ($p < 0.001$); and PFS were 3.1 and 1.4 months ($p < 0.001$) for nonsquamous and squamous histology, respectively. In patients who were randomized to the P1000 group, ORR were 18.1% and 4.0% ($p = 0.1113$); MST were 13.5 months and 8.6 months ($p = 0.0971$); and PFS were 3.1 and 1.7 months ($p = 0.0024$) for nonsquamous and squamous histology, respectively. There were no clinically relevant differences in the incidence of toxicities between histology groups.

Conclusions: This study showed the difference of pemetrexed efficacy by histologic type, and this result supports the treatment-by-histology effect observed in the past pivotal phase III studies. Higher dose of pemetrexed resulted in similar outcomes both in patients with nonsquamous histology and squamous histology. Pemetrexed is not as effective as alternative therapies for previously treated squamous histology; however, pemetrexed should be the key agent for the treatment of patients with nonsquamous histology.

Key Words: Pemetrexed, Non-small cell lung cancer, Nonsquamous, Squamous, Histology.

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Two-drug combinations of the third-generation agents (docetaxel, paclitaxel, gemcitabine, and vinorelbine) with a platinum compound have been considered the standard treatment option for advanced non-small cell lung cancer (NSCLC) based on several randomized studies.[1–3] Histology has not been consistently reported as prognostic or predictive for outcomes with cytotoxic cancer chemotherapy in advanced NSCLC, until publication of a large phase III study using cisplatin and pemetrexed.[4]

Pemetrexed is an inhibitor of thymidylate synthase, resulting in decreased thymidine necessary for pyrimidine synthesis, which is the primary mechanism of action.[5,6] Pemetrexed also inhibits dihydrofolate reductase and glycinamide ribonucleotide formyl transferase, the latter of which is a folate-dependent enzyme involved in purine synthesis. Unlike other classic antifolates, pemetrexed has a unique pyrrolopyrimidine nucleus and can inhibit multiple folate-dependent enzymes.

The phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced NSCLC demonstrated noninferiority of cisplatin plus pemetrexed to cisplatin plus gemcitabine in the overall study population, with significantly less febrile neutropenia, anemia, thrombocytopenia, and alopecia favor-

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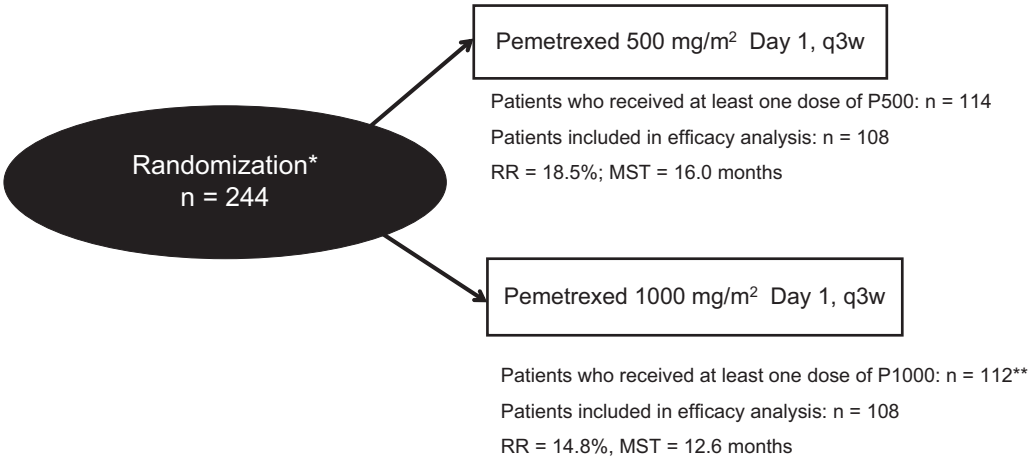

FIGURE 1. Trial design and efficacy data. From phase II randomized study.[8] NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; PS, performance status; q, every; w, weekly; *n*, number of patients; RR, response rate; MST, median survival time. *Patients: stage IIIB/IV NSCLC, 1 to 2 prior chemotherapeutic regimens, and ECOG PS 0 to 2; Stratified by: gender, ECOG PS, disease stage, platinum use, time for prechemotherapy, and study site. **One patient was excluded from statistical analysis because the data of this patient was not available.

ing cisplatin plus pemetrexed.[4] This study showed that overall survival was statistically superior for cisplatin plus pemetrexed in patients with nonsquamous histology. In contrast, survival was shorter for cisplatin plus pemetrexed compared with cisplatin plus gemcitabine in patients with squamous cell carcinoma. This was the first phase III study in NSCLC that prospectively demonstrated survival differences for chemotherapy based on histologic type.

In the subgroup analysis of the phase III study, which compared pemetrexed alone with docetaxel in patients with NSCLC previously treated with chemotherapy, also demonstrated that overall survival was significantly longer for pemetrexed versus docetaxel in patients with nonsquamous histology, whereas conversely, survival was shorter for pemetrexed compared with docetaxel in patients with squamous histology.[7]

On the basis of these phase III results, we conducted an additional subgroup analysis of data from a Japanese phase II study, which randomized previously treated patients with NSCLC to pemetrexed 500 mg/m^2 (P500) or 1000 mg/m^2 (P1000) to further examine efficacy differences for pemetrexed by histology. The efficacy and safety results of original phase II study have already been reported[8]; Figure 1 shows the trial design and efficacy data of this phase II study. Of the 216 patients evaluable for efficacy (108 in each arm), response rates were18.5% (90% confidence interval, 12.6–25.8%) and 14.8% (90% confidence interval, 9.5–21.6%), median survival times (MSTs) were 16.0 and 12.6 months, 1-year survival rates were 59.2% and 53.7%, and median progression-free survival were 3.0 and 2.5 months for the P500 and P1000, respectively. Drug-related toxicity was generally tolerable for both doses.

PATIENTS AND METHODS

Trial Design

We analyzed the data from the randomized, open-label, multicenter study[8] in which patients were registered through the central registration system. Two hundred forty-four patients with advanced NSCLC previously treated with chemotherapy at 28 medical institutions in Japan were registered between October 2004 and October 2005, and 226 patients were randomized to receive either pemetrexed 500 mg/m^2 (P500) or 1000 mg/m^2 (P1000) (Figure 1). The randomization was done by an independent registration center and was dynamically balanced for Eastern Cooperative Oncology Group performance status (PS), previous platinum chemotherapy, disease stage, gender, a time from prior chemotherapy to the enrollment, and hospital. Patients were balanced with respect to the study drug in each stratum for each prognostic factor using the minimization method. The primary end point was response rate, and the secondary end points included overall survival time, progression-free survival time, and incidence of toxicities.

The sample size was calculated to ensure that the response rate in each group exceeded 5%.[8] The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki after being approved by the institutional review board of individual hospitals. Primary results of this trial and further details regarding the study design and statistical analyses have been published previously.[8]

Patients and Treatment

Patients who satisfied all of the following criteria were included into the study[8]: age 20 to 75 years, performance status 0 to 2, stage III or IV diagnosed by images before the registration to this study, NSCLC confirmed by histology or cytology, at least one measurable tumor according to the Response Evaluation Criteria in Solid Tumor (RECIST criteria),[9] previously received one or two chemotherapy regimens for NSCLC, adequate organ function, life expectancy of at least 12 weeks, and written consent to participate in the study. Histologic subtypes outcome of NSCLC were examined in each institution.

Patients were randomly allocated to either pemetrexed 500 mg/m^2 (P500) arm or pemetrexed 1000 mg/m^2 (P1000) arm. Pemetrexed was administered as an intravenous, 10-minute infusion on day 1 of a 21-day cycle. Patients were instructed to take orally 1 g/d of a multivitamin containing 500 μg folic acid from at least 7 days before the day 1 of cycle 1 until 22 days after the last administration of pemetrexed. Vitamin B_{12} (1000 μg) was injected intramuscularly, at least 7 days before the day 1 of cycle 1 and repeated every 9 weeks until 22 days after the last administration of pemetrexed.

Assessments

The antitumor effect of pemetrexed was evaluated based on the RECIST criteria. Response rate represented the percentage of patients whose best overall response had been either complete response or partial response. Survival time was defined as the period from the registered date of first administration until the date of death regardless of the causality with pemetrexed. Progression-free survival time was defined as the period from the registered date of first administration until the day on which progressive disease was determined or the date of death regardless of the causality with pemetrexed. All adverse events were graded based on the Common Terminology Criteria for Adverse Events, version 3.0.

Statistical Analysis

Of 226 patients enrolled in the study, the efficacy analysis included 216 patients who satisfied all the inclusion criteria, did not meet any of the exclusion criteria, and received at least one dose of pemetrexed. The safety analysis included 225 patients who received at least one dose of pemetrexed.

Efficacy and safety results were analyzed by histology for the dose groups combined and separately. Response rates, disease control rates, overall survival time, and progression-free survival time were compared between the histologic types (nonsquamous and squamous histology) for the P500 and P1000 arms combined and separately. Differences of response rates were compared by using Fisher's exact test. A Kaplan-Meier method was used to estimate overall survival time and progression-free survival time. Differences of time-to-event distributions by histology were compared using a log-rank test. A Cox proportional hazard model was used for hazard ratio estimation (squamous/nonsquamous histology). Two-sided significance level of 5% was used in all tests. In the safety analysis, number of deaths, serious adverse events,

TABLE 1. Characteristics of Patients

	Nonsquamous			Squamous		
Variable	**P500**	**P1000**	**Total**	**P500**	**P1000**	**Total**
Patients who received at least 1 dose of pemetrexed, *n*	89	85	174	25	26	51
Gender, *n* (%)						
Female	40 (44.9)	36 (42.4)	76 (43.7)	2 (8.0)	4 (15.4)	6 (11.8)
Male	49 (55.1)	49 (57.6)	98 (56.3)	23 (92.0)	22 (84.6)	45 (88.2)
Age (yr)						
Median	60	62	61	67	64	65
Range	37–74	26–74	26–74	58–74	50–74	50–74
ECOG PS, *n* (%)						
0	34 (38.2)	29 (34.1)	63 (36.2)	11 (44.0)	8 (30.8)	19 (37.3)
1	50 (56.2)	51 (60.0)	101 (58.0)	13 (52.0)	17 (65.4)	30 (58.8)
2	5 (5.6)	5 (5.9)	10 (5.7)	1 (4.0)	1 (3.8)	2 (3.9)
Disease stage, *n* (%)						
III	15 (16.9)	16 (18.8)	31 (17.8)	7 (28.0)	8 (30.8)	15 (29.4)
IV	74 (83.1)	69 (81.2)	143 (82.2)	18 (72.0)	18 (69.2)	36 (70.6)
No. of prior chemotherapy, *n* (%)						
1	32 (36.0)	39 (45.9)	71 (40.8)	12 (48.0)	14 (53.8)	26 (51.0)
2	54 (60.7)	45 (52.9)	99 (56.9)	13 (52.0)	12 (46.2)	25 (49.0)
3	3 (3.4)	1 (1.2)	4 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)
Prior platinum, *n* (%)						
No	4 (4.5)	6 (7.1)	10 (5.7)	2 (8.0)	1 (3.8)	3 (5.9)
Yes	85 (95.5)	79 (92.9)	164 (94.3)	23 (92.0)	25 (96.2)	48 (94.1)
Interval from last prior chemotherapy, *n* (%)						
≥3 mo	31 (34.8)	34 (40.0)	65 (37.4)	11 (44.0)	11 (42.3)	22 (43.1)
<3 mo	58 (65.2)	51 (60.0)	109 (62.6)	14 (56.0)	15 (57.7)	29 (56.9)

n, number of patients; P500, pemetrexed 500 mg/m^2 arm; P1000, pemetrexed 1000 mg/m^2 arm; ECOG PS, Eastern Cooperative Oncology Group performance status.

grade 2 adverse events, and grade 3/4/5 adverse events were calculated separately for nonsquamous and squamous histology in each dose group.

RESULTS

Patient Characteristics

Patient characteristics are shown by histology and dose group (P500 or P1000) in Table 1. Total of 225 patients received pemetrexed 500 mg/m^2 or 1000 mg/m^2 at least once during the study. Baseline patient characteristics by histology were well balanced between the two dose groups.

TABLE 2. Summary of Efficacy Results by Histology

Variable	Nonsquamous (n = 168)	Squamous (n = 48)	p
Response rate (%)	20.8	2.1	<0.001[a]
Disease control rate (%)	57.1	29.2	<0.001[a]
Overall survival (median) (mo)	16.0	8.5	<0.001[b]
Progression-free survival (median) (mo)	3.1	1.6	<0.001[b]

[a] Fisher's exact test.
[b] Log-rank test.

Efficacy

Results of the efficacy analysis (response rate, disease control rate, overall survival time, and progression-free survival time) by histology for the dose groups combined are summarized in Table 2. Kaplan-Meier curves for overall survival time and progression-free survival time are shown in Figures 2*A*, *B*, respectively. Response rates in patients with nonsquamous and squamous histology were 20.8% (35/168) and 2.1% (1/48) ($p < 0.001$), and disease control rates in patients with nonsquamous and squamous histology were 57.1% (96/168) and 29.2% (14/48) ($p < 0.001$), respectively. MSTs in patients with nonsquamous and squamous histology were 16.0 and 8.5 months (hazard ratio, 2.11; log-rank test, $p < 0.001$), and median progression-free survival times were 3.1 and 1.6 months (hazard ratio, 2.19; log-rank test, $p < 0.001$), respectively.

Results of the efficacy analysis (response rate, overall survival time, and progression-free survival time) by histology for each dose group are summarized in Table 3. Kaplan-Meier curves for overall survival time and progression-free

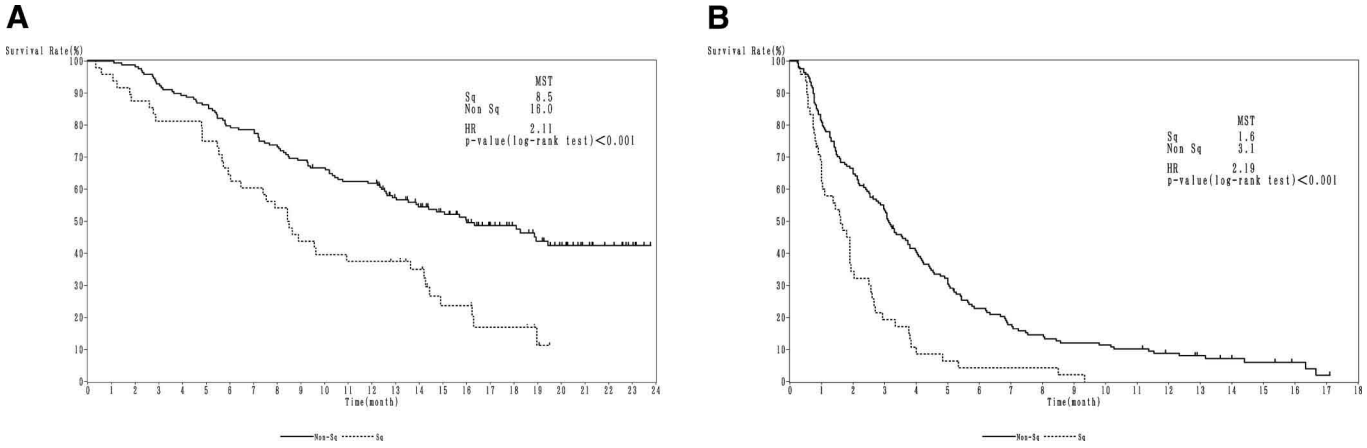

FIGURE 2. *A,* Kaplan-Meier curves for overall survival by histology. *B,* Kaplan-Meier curves for progression-free survival by histology. MST, median survival time.

TABLE 3. Summary for Efficacy Results by Dose and Histology

	P500			P1000		
	Nonsquamous (n = 85)	Squamous (n = 23)	p	Nonsquamous (n = 83)	Squamous (n = 25)	p
Response rate (%)	23.5	0.0	0.0062[a]	18.1	4.0	0.1113[a]
Disease control rate (%)	62.4	30.4	0.0088[a]	51.8	28.0	0.0419[a]
Overall survival (median) (mo)	19.4[b]	7.9	<0.001[c]	13.5	8.6	0.0971[c]
Progression-free survival (median) (mo)	3.1	1.4	<0.001[c]	3.1	1.7	0.0024[c]

Adjustment of multiplicity was not performed.
[a] Fisher's exact test.
[b] Survival rate was 50.03%.
[c] Log-rank test.
P500, pemetrexed 500 mg/m^2 arm; P1000, pemetrexed 1000 mg/m^2 arm.

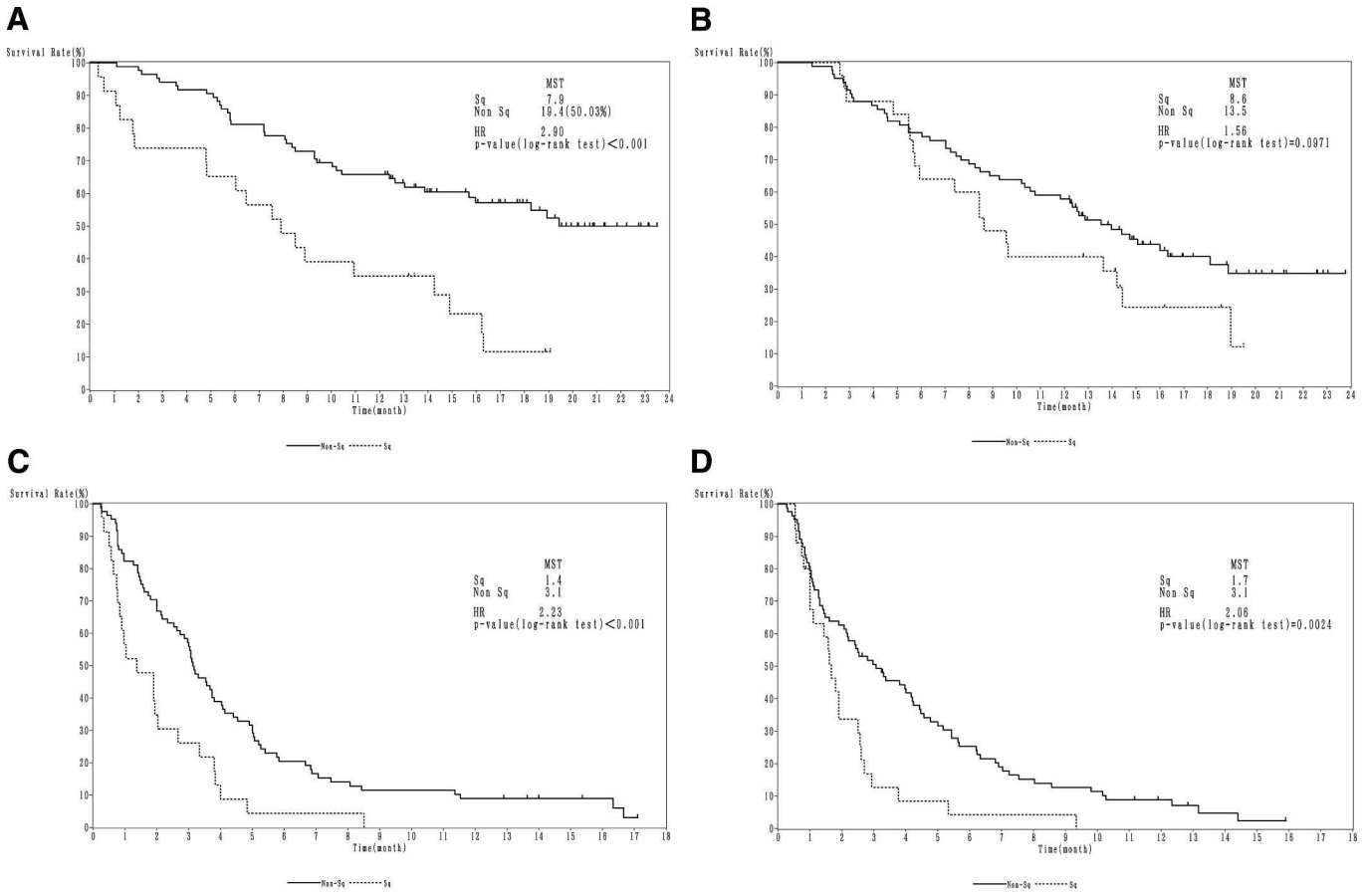

FIGURE 3. Kaplan-Meier curves for overall survival by dose and histology: (*A*) patients treated with pemetrexed 500 mg/m^2 and (*B*) patients treated with pemetrexed 1000 mg/m^2. Kaplan-Meier curves for progression-free survival by dose and histology: (*C*) patients treated with pemetrexed 500 mg/m^2 and (*D*) patients treated with pemetrexed 1000 mg/m^2. MST, median survival time.

survival time are shown in Figures 3*A–D*. Response rates of nonsquamous and squamous histology patients were 23.5% (20/85) and 0% (0/23) in P500 ($p = 0.0062$) and 18.1% (15/83) and 4.0% (1/25) in P1000 ($p = 0.1113$). Disease control rates of nonsquamous and squamous histology patients were 62.4% (53/85) and 30.4% (7/23) in P500 ($p = 0.0088$) and 51.8% (43/83) and 28.0% (7/25) in P1000 ($p = 0.0419$). In the P500 group, median overall survival time was 19.4 months in patients with nonsquamous histology (survival rate: 50.03%) and 7.9 months in patients with squamous histology patients (incidence of events: 50.00%) (hazard ratio, 2.90; log-rank test, $p < 0.001$). In the P1000 group, median overall survival time was 13.5 months in patients with nonsquamous histology and 8.6 months in patients with squamous histology (hazard ratio, 1.56; log-rank test, $p = 0.0971$). Median progression-free survival time was 3.1 months in patients with nonsquamous histology and 1.4 months in patients with squamous histology in the P500 group (hazard ratio, 2.23; log-rank test, $p < 0.001$). In the P1000 group, median progression-free survival time was 3.1 months in patients with nonsquamous histology and 1.7 months in patients with squamous histology (hazard ratio, 2.06; log-rank test, $p = 0.0024$).

Safety

The safety of pemetrexed 500 mg/m^2 and 1000 mg/m^2 has been reported by Ohe et al.[8] Major adverse events occurred in the study participants are shown by dose group (P500 and P1000) and histology in Table 4. Grade 3/4/5 pneumonitis regardless to causality with pemetrexed was observed in two nonsquamous and two squamous histology patients in the P500 group and one nonsquamous and two squamous histology patients in the P1000 group. Toxicities occurred in both dose groups were tolerable, and there were no clinically relevant differences in the incidence of toxicities by histology.

DISCUSSION

The results of subgroup analysis demonstrated efficacy differences of pemetrexed by histology in pretreated patients with advanced NSCLC. Objective response rate of pemetrexed was 20.8% in patients with nonsquamous histology and only 2.1% in squamous histology patients. Overall survival and progression-free survival were significantly better for patients with nonsquamous than squamous histology. MST of 16.0 months in nonsquamous histology patients is

TABLE 4. Major Hematologic and Nonhematologic Toxicity by Common Terminology Criteria for Adverse Events Version 3.0[a]

	P500				P1000			
	Nonsquamous (n = 89)		Squamous (n = 25)		Nonsquamous (n = 85)		Squamous (n = 26)	
	Grade 2	Grade 3/4/5	Grade 2	Grade 3/4/5	Grade 2	Grade 3/4/5	Grade 2	Grade 3/4/5
Leukopenia	36.0	13.5	20.0	20.0	40.0	27.1	34.6	3.8
Neutropenia	28.1	21.3	16.0	20.0	29.4	28.2	23.1	11.5
Lymphopenia	31.5	6.7	24.0	40.0	25.9	25.9	34.6	7.7
Anemia	19.1	5.6	28.0	20.0	40.0	8.2	19.2	15.4
Thrombocytopenia	0	0	0	0	8.2	0	7.7	3.8
Nausea	16.9	1.1	16.0	4.0	17.6	3.5	7.7	3.8
Vomiting	9.0	1.1	8.0	0	10.6	3.5	15.4	0
Anorexia	19.1	2.2	8.0	12.0	15.3	14.1	11.5	19.2
Fatigue	2.2	1.1	8.0	0	4.7	1.2	3.8	11.5
Diarrhea	3.4	1.1	0	0	3.5	2.4	3.8	0
Constipation	9.0	1.1	0	0	5.9	3.5	7.7	3.8
Rash	51.7	3.4	44.0	0	63.5	5.9	61.5	0
Alopecia	0	0	0	0	0	0	0	0
Pneumonitis	1.1	2.2	4.0	8.0	0	1.2	0	7.7
AST	27.0	6.7	4.0	12.0	29.4	5.9	11.5	0
ALT	20.2	19.1	8.0	12.0	35.3	9.4	23.1	3.8

The values are given in percentage.
[a] Major adverse events of grade 2 or grade 3/4/5 are shown irrespective of causal relationship with pemetrexed.
P500, pemetrexed 500 mg/m^2 arm; P1000, pemetrexed 1000 mg/m^2 arm; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

encouraging in this situation. The efficacy of pemetrexed for nonsquamous histology was shown in the recommended dose of 500 mg/m^2 and also in the higher dose of 1000 mg/m^2. Higher dose of pemetrexed resulted in similar outcomes both in patients with nonsquamous histology and squamous histology.

The difference in survival benefit of pemetrexed between the histologic types may in part be explained by a differential expression of thymidylate synthase, which is the primary mechanism of actions. In specimens from chemonaive patients with early-stage NSCLC, expression of thymidylate synthase was observed to be elevated in squamous histology compared with adenocarcinoma.[10] A preclinical study showed the overexpression of thymidylate synthase was associated with the decreased in vitro sensitivity of pemetrexed.[11] Translational studies are needed to evaluate biologic markers using clinical samples.

Pemetrexed was well tolerated in both the P500 and P1000 arms,[8] and also there were no clinically relevant differences in the toxicities between histologic groups. This is in contrast to vascular endothelial growth factor inhibitors, e.g., bevacizumab, which have an increased risk of life-threatening toxicities in patients with certain squamous cell lung tumors.

A randomized phase III trial designed to evaluate maintenance chemotherapy of pemetrexed versus placebo after platinum-based chemotherapy demonstrated that progression-free and overall survival were significantly longer with pemetrexed in patients with nonsquamous histology, whereas no treatment advantage was observed in patients with squamous histology.[12,13] This is the third phase III study to demonstrate efficacy differences by histology in the treatment of advanced NSCLC.

It has been regarded that two drug combinations of platinum agents with third generation agents have similar efficacy.[14,15] Gemcitabine-containing regimens showed significant longer progression-free survival than nongemcitabine-containing regimens in a meta-analysis.[16] Thus, cispaltin plus gemcitabine is one of the most active regimens for NSCLC. However, the randomized trial comparing cisplatin plus pemetrexed with cisplatin plus gemcitabine demonstrated statistically significant survival benefit favoring cisplatin plus pemetrexed in patients with nonsquamous histology. Considering the consistent results of other studies[4,17] using pemetrexed and favorable toxicity profile, cisplatin plus pemetrexed should be a reference regimen in future trials for patients with nonsquamous histology.

In conclusion, the results of subgroup analysis showed the difference of pemetrexed efficacy by histologic type, and this result supports the treatment-by-histology effect observed in the past pivotal phase III studies. Higher dose of pemetrexed resulted in similar outcomes both in patients with nonsquamous histology and squamous histology. Pemetrexed is not as effective as alternative therapies for previously treated squamous histology, however, pemetrexed should be the key agent for the treatment of patients with nonsquamous histology.

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