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The Differential Efficacy of Pemetrexed According to NSCLC Histology: A Review of Two Phase III Studies

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ABSTRACT

Background. Recent studies of pemetrexed have identified a predictive role for non-small cell lung cancer (NSCLC) histology. We further reviewed the differential efficacy of pemetrexed according to histology in two large, phase III NSCLC trials.

Methods. One study tested pemetrexed versus docetaxel in previously treated patients ($n = 571$) and the other tested cisplatin plus pemetrexed versus cisplatin plus gemcitabine in chemotherapy-naive patients ($n = 1,725$) with advanced NSCLC. Cox proportional hazard models were used to test for covariate-adjusted treatment-by-histology interactions (THIs) for overall survival (OS) and progression-free survival (PFS). For each histologic subgroup, the Kaplan–Meier method was used to estimate unadjusted within-arm medians, and Cox models were used to estimate covariate-adjusted between-arm hazard ratios (HRs).

Results. In both studies, treatment arms were well balanced for histology. THIs were statistically significant ($p < .005$) for both OS and PFS. Nonsquamous patients treated with pemetrexed-based therapy experienced longer survival than the comparators (HR, 0.78 and 0.84, respectively), whereas squamous patients had shorter survival (HR, 1.56 and 1.23, respectively). Whereas the efficacy of pemetrexed regimens differed according to histology, it did not differ for docetaxel or for cisplatin plus gemcitabine. Pemetrexed was well tolerated across histologic groups.

Conclusions. **The consistency of these results across studies confirms the predictive effect of histology for pemetrexed and the survival advantage for pemetrexed in patients with nonsquamous histology. These analyses suggest pemetrexed should not be rec-** **ommended for the treatment of squamous cell carcinoma, but, because of efficacy and safety advantages, pemetrexed may be preferable to other agents for treatment of patients with nonsquamous NSCLC.** *The Oncologist* **2009;14:253–263**

INTRODUCTION

Evaluation of new treatment strategies is ongoing to identify more effective treatment for patients with advanced non-small cell lung cancer (NSCLC). The identification of prognostic and predictive factors may enable tailored therapies for specific patient populations. Prognostic factors are associated with outcomes that are independent of treatment, whereas predictive factors are associated with, and predict, the clinical outcome of a specific treatment. Clinical prognostic factors that have been associated with survival in NSCLC include disease stage at the time of diagnosis, performance status (PS), recent weight loss (>10% of body weight), and sex [1–3].

Although histology has not consistently been associated with clinical outcomes in advanced NSCLC, it has recently emerged as a potential predictive factor [4–6]. Response rates (RRs) for epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are higher in patients with a histologic diagnosis of adenocarcinoma [6] as well as in females, Asians, and never-smokers [7, 8]. Recently, a predictive role for histology was reported in three pemetrexed studies in advanced NSCLC [4, 9, 10], which was further supported by phase II studies [11, 12].

Pemetrexed is an antifolate that inhibits multiple enzymes involved in purine and pyrimidine synthesis. Thymidylate synthase (TS) is the primary target of pemetrexed, and dihydrofolate reductase and glycinamide ribonucleotide formyl transferase are secondary targets [13, 14]. These targeted folate-dependent enzymes include pathways that, when amplified, are associated with reduced efficacy for conventional cytotoxic agents [15].

The first phase III study of pemetrexed in NSCLC established similar efficacy and a favorable safety profile for pemetrexed compared with docetaxel in the second-line setting [16]. In a retrospective analysis of this trial, significant associations were identified between histologic subtype and efficacy outcomes for pemetrexed [9]. A second phase III study of pemetrexed in NSCLC reported noninferior efficacy and better tolerability for cisplatin plus pemetrexed than for cisplatin plus gemcitabine in the frontline setting [4].

Because of the emerging evidence of a differential expression of TS between adenocarcinoma and squamous cell carcinoma [17], a prespecified subgroup analysis by histology was planned in the frontline study [4]. Additionally, this phase III study identified a significant treatment-by-histology interaction for pemetrexed [4]. As a result of this study, pemetrexed was recently approved in Europe, Canada, and the U.S. in combination with cisplatin for the frontline treatment of nonsquamous NSCLC.

We further analyzed the differential efficacy of pemetrexed according to histology in these two large, phase III trials [4, 16]. This paper presents the detailed findings of efficacy results for pemetrexed according to NSCLC histology.

PATIENTS AND METHODS

Patients

Details regarding the two studies have been published previously [4, 16]. The second-line study included 571 previously treated patients with advanced or metastatic NSCLC [16]. Key eligibility criteria included a clinical diagnosis of stage III or IV NSCLC, prior exposure to only one chemotherapy regimen for advanced disease, an Eastern Cooperative Oncology Group (ECOG) PS score [18] of 0–2, and adequate bone marrow reserve, renal function, and hepatic function. Exclusion criteria included prior docetaxel or pemetrexed treatment, grade 3 or 4 peripheral neuropathy, weight loss ≥10% during the 6 weeks before study entry, symptomatic brain metastases, an inability to interrupt nonsteroidal anti-inflammatory drugs, and uncontrolled pleural effusions.

The frontline study included 1,725 chemotherapy-naive patients with NSCLC [4]. Key eligibility criteria included histologically or cytologically confirmed stage IIIB or IV NSCLC with at least one measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) [19], an ECOG PS score of 0 or 1, and adequate bone marrow reserve and organ function. Prior radiation therapy was permitted if it had occurred at least 4 weeks prior to study enrollment and patients had recovered fully from its acute effects. Exclusion criteria included National Cancer Institute Common Toxicity Criteria (CTC) [20] grade ≥1 peripheral neuropathy, progressive brain metastases, uncontrolled third-space fluid retention before study entry,

Table 1. Pemetrexed versus docetaxel: baseline patient and disease characteristics for randomized patients by histologic type

Characteristic[a]	Pemetrexed (n = 283)				Docetaxel (n = 288)			
	Adeno-carcinoma (n = 158)	Large cell carcinoma (n = 18)	Other[b] (n = 29)	Squamous cell carcinoma (n = 78)	Adeno-carcinoma (n = 144)	Large cell carcinoma (n = 29)	Other[b] (n = 21)	Squamous cell carcinoma (n = 94)
Median age (yrs)	57.4	60.3	59.3	61.3	56.7	55.6	62.2	60.2
Female/male (%)	39/61	33/67	45/55	10/90	34/66	28/72	14/86	12/88
Stage III/IV (%)	18/82	22/78	17/83	42/58	20/80	24/76	24/76	34/66
ECOG PS score 0/1/2 (%)[c]	23/62/15	13/81/6	14/82/4	17/75/8	19/70/11	18/75/7	10/80/10	16/66/17
White/E. Asian (%)	72/18	78/6	59/24	74/10	66/24	79/4	76/10	70/13

[a]No smoking status data were collected.
[b]Other histology was histologic diagnosis that did not clearly qualify as adenocarcinoma, large cell carcinoma, or squamous cell carcinoma.
[c]Baseline ECOG PS data not available for some patients in each category.
Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

and an inability to interrupt aspirin and other nonsteroidal anti-inflammatory drugs or to take folic acid, vitamin B_{12}, and corticosteroids.

All patients signed written informed consent prior to treatment. The protocols were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and were approved by each participating institutional ethics review board.

Study Design and Treatment

Both studies assessed overall survival (OS) as the primary endpoint using a noninferiority design. Secondary objectives included comparisons of progression-free survival (PFS), time to progressive disease (TTP), time to treatment failure (TTF), tumor RR, duration of response, and toxicity.

Patients in the second-line study randomly received either pemetrexed (Alimta®; Eli Lilly and Company, Indianapolis, IN) at a dose of 500 mg/m² as a 10-minute i.v. infusion or docetaxel (Taxotere®; Sanofi-Aventis, Paris, France) at 75 mg/m² as a 1-hour i.v. infusion on day 1, every 21 days [16]. Treatment continued until disease progression, unacceptable toxicity, or a request for therapy discontinuation. Randomization factors included PS, prior platinum or paclitaxel treatment, the number of prior chemotherapy regimens, the time since last chemotherapy, the best response to last chemotherapy, disease stage, and baseline plasma homocysteine level.

Patients in the frontline study randomly received either cisplatin (Platinol®; Bristol-Myers Squibb, Princeton, NJ) at 75 mg/m² on day 1 plus gemcitabine (Gemzar®; Eli Lilly and Company, Indianapolis, IN) at 1,250 mg/m² on days 1

and 8 or cisplatin at 75 mg/m² plus pemetrexed at 500 mg/m² on day 1 [4]. Chemotherapy was repeated every 3 weeks for a maximum of six cycles. Random assignment was performed according to disease stage, PS score, history of brain metastases, gender, and pathologic diagnosis (histologic versus cytologic).

While on study, patients on the pemetrexed arm of both studies and on the cisplatin plus gemcitabine arm of the frontline study received folic acid and vitamin B_{12}, and patients on both arms of both studies received prophylactic dexamethasone. All patients underwent comprehensive baseline assessments including clinical laboratory tests and imaging studies. Patients also received follow-up assessments and monitoring at regular intervals. Toxicity evaluations were based on the CTC, version 2.0. Tumor measurements were assessed in the second-line study by Southwest Oncology Group criteria after every two cycles [16, 21] or in the frontline study by the RECIST after every other cycle [4, 19].

Statistical Analyses

For both studies, all patients who received at least one dose of the study drug were considered assessable for safety, whereas the efficacy analyses incorporated all randomized patients on an intent-to-treat basis unless otherwise noted.

The histologic subtypes of NSCLC were reported by investigators, and then grouped for statistical analysis into four main categories: adenocarcinoma, large cell carcinoma, squamous cell carcinoma, and other NSCLC/not otherwise specified (NOS). There was no central review of pathology. Treatment-by-histology analyses are also pre-

sented for the combined group of patients without squamous cell histology, that is, "nonsquamous," which includes patients with adenocarcinoma, large cell carcinoma, and other NSCLC/NOS.

Cox proportional hazard models were used to test for covariate-adjusted treatment-by-histology interactions for both OS and PFS. These models included a treatment term (pemetrexed arm versus control arm), a histology term (nonsquamous versus squamous), and an interaction term (nonsquamous pemetrexed arm versus all other), as well as terms for predefined baseline prognostic factors. Each treatment-by-histology interaction hazard ratio (HR) from these models is the treatment effect for nonsquamous patients divided by the treatment effect for squamous patients:

$$\frac{\text{HR (pemetrexed arm relative to control arm) for nonsquamous patients}}{\text{HR (pemetrexed arm relative to control arm) for squamous patients}}$$

An interaction HR <1.00 indicates that the benefit for pemetrexed relative to the control arm is greater in patients with nonsquamous histology than in those with squamous histology. In addition, for each histologic subgroup, the Kaplan–Meier method [22] was used to estimate unadjusted within-arm medians (with 95% confidence intervals [CIs]), and Cox models were used to estimate covariate-adjusted between-arm HRs with 95% CIs. A significant treatment-by-histology interaction indicates a differential treatment effect according to histology. The subgroup analysis clarifies how the treatment effect differs by histology.

RESULTS

Baseline Demographics
Tables 1 and 2 show the baseline patient and disease characteristics for the two studies by histologic subgroup. For the pemetrexed versus docetaxel study [16], numeric differences were observed between arms for the distribution of some baseline characteristics (Table 1). However, OS and PFS were analyzed with Cox models adjusted for prognostic cofactors. As a result, any imbalances in baseline characteristics were accounted for in the efficacy analyses. In the cisplatin plus pemetrexed versus cisplatin plus gemcitabine study [4], no clinically relevant differences between the baseline demographics by histology were observed (Table 2).

Efficacy

Second-Line Trial of Pemetrexed Versus Docetaxel
In the pemetrexed versus docetaxel study, significant treatment-by-histology interactions for both OS ($p = .001$) and

PFS ($p = .004$) indicated greater efficacy for nonsquamous patients treated with pemetrexed (Table 3). Nonsquamous patients had a longer OS time on pemetrexed than on docetaxel (HR, 0.78; 95% CI, 0.61–1.00; $p = .047$), whereas squamous patients had a shorter OS time on pemetrexed than on docetaxel (HR, 1.56; 95% CI, 1.08–2.26; $p = .018$). Similarly, nonsquamous patients had a numerically longer PFS time on pemetrexed than on docetaxel (HR, 0.82; 95% CI, 0.66–1.02; $p = .076$) as shown in Table 3. As with OS, squamous patients had a shorter PFS time on pemetrexed than on docetaxel (HR, 1.40; 95% CI, 1.01–1.96; $p = .046$). Whereas the efficacy of pemetrexed differed by histologic type, the efficacy of docetaxel did not.

Within the nonsquamous histologic subgroups shown in Table 4, the HRs for OS and PFS consistently numerically favored pemetrexed over docetaxel, reaching statistical significance for the limited number of patients with large cell carcinoma (OS HR, 0.27; 95% CI, 0.11–0.63; $p = .003$). For patients with adenocarcinoma or other NSCLC/NOS tumors, the HRs for OS and PFS were not statistically significantly different between the two arms (Table 4). Figure 1 shows the Kaplan–Meier OS curves for each of the histologic groups in Table 4. Figure 2 shows the Kaplan–Meier curve of OS for the nonsquamous patients in this study. Differences were also observed in the RR by histologic type. Higher RRs occurred in the pemetrexed arm than in the docetaxel arm in patients with adenocarcinoma (12.8% versus 9.9%) or large cell carcinoma (12.5% versus 3.7%), whereas RRs for patients with squamous cell carcinoma (2.8% versus 8.1%) or other NSCLC/NOS tumors (3.7% versus 10.0%) favored docetaxel (Table 4).

Frontline Trial of Cisplatin plus Pemetrexed Versus Cisplatin Plus Gemcitabine
Treatment-by-histology interactions for OS and PFS were also statistically significant (both $p = .002$) in the cisplatin plus pemetrexed versus cisplatin plus gemcitabine study, indicating that patients with nonsquamous histology who were treated with cisplatin plus pemetrexed had longer OS and PFS times than all other patients (Table 3). Nonsquamous patients had a longer OS time on cisplatin plus pemetrexed than on cisplatin plus gemcitabine (HR, 0.84; 95% CI, 0.74–0.96; $p = .011$), whereas squamous patients had a shorter OS time on cisplatin plus pemetrexed than on cisplatin plus gemcitabine (HR, 1.23; 95% CI, 1.00–1.51; $p = .050$). Similarly, nonsquamous patients showed a trend that was not significant for a longer PFS time on cisplatin plus pemetrexed than on cisplatin plus gemcitabine (HR, 0.95; 95% CI, 0.84–1.06; $p = .349$). As with OS, squamous patients had a shorter PFS time on cisplatin plus pemetrexed than on cisplatin plus gemcitabine (HR, 1.36; 95% CI,

Table 2. Cisplatin plus pemetrexed versus cisplatin plus gemcitabine: baseline patient and disease characteristics for randomized patients by histologic type

	Cisplatin plus pemetrexed (n = 862)				Cisplatin plus gemcitabine (n = 863)			
Characteristic	Adeno-carcinoma (n = 436)	Large cell carcinoma (n = 76)	Other[a] (n = 106)	Squamous cell carcinoma (n = 244)	Adeno-carcinoma (n = 411)	Large cell carcinoma (n = 77)	Other[a] (n = 146)	Squamous cell carcinoma (n = 229)
Median age (yrs)	60.8	60.4	60.2	62.8	59.3	61.8	60.9	63.3
Female/male (%)	37/63	30/70	32/68	15/85	35/66	26/74	34/66	21/80
Ever-/never-smoker (%)	67/21	79/9	75/12	82/7	67/20	84/1	73/15	82/7
Stage IIIB/IV (%)	20/80	21/79	21/79	32/68	21/79	20/81	30/71	28/72
ECOG PS score 0/1 (%)	35/65	33/67	38/61	36/64	38/62	33/68	38/61	31/69
White/E. Asian (%)	73/16	95/1	76/13	81/12	75/14	97/1	78/13	80/11

[a]Other histology was histologic diagnosis that did not clearly qualify as adenocarcinoma, large cell carcinoma, or squamous cell carcinoma.
Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 3. Treatment-by-histology interactions for overall survival and progression-free survival for the pemetrexed versus docetaxel and cisplatin plus pemetrexed versus cisplatin plus gemcitabine studies

	Pemetrexed versus docetaxel (n = 571)		Cisplatin plus pemetrexed versus cisplatin plus gemcitabine (n = 1,725)	
Efficacy parameter	Nonsquamous[a] (n = 399)	Squamous (n = 172)	Nonsquamous[a] (n = 1,252)	Squamous (n = 473)
OS adjusted HR[b] (95% CI)	0.78 (0.61–1.00)	1.56 (1.08–2.26)	0.84 (0.74–0.96)	1.23 (1.00–1.51)
Superiority p-value	.047	.018	.011	.050
Treatment-by-histology interaction test p-value[c]	.001		.002	
PFS adjusted HR[b] (95% CI)	0.82 (0.66–1.02)	1.40 (1.01–1.96)	0.95 (0.84–1.06)	1.36 (1.12–1.65)
Superiority p-value	.076	.046	.349	.002
Treatment-by-histology interaction test p-value[c]	.004		.002	

[a]Nonsquamous histology comprises adenocarcinoma, large cell carcinoma, and other histologies.
[b]HR <1.0 favors pemetrexed study arm; HR >1.0 favors comparator.
[c]Tests for statistically significant treatment-by-histology interactions were performed for PFS and OS using cofactor-adjusted Cox proportional hazards models.
Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

1.12–1.65; $p = .002$). The efficacy of cisplatin plus pemetrexed differed according to histologic type, whereas the efficacy of cisplatin plus gemcitabine did not.

Within the nonsquamous histologic subgroups shown in Table 4, the HRs for OS for both adenocarcinoma and large cell carcinoma significantly favored cisplatin plus pemetrexed over cisplatin plus gemcitabine (adenocarcinoma HR, 0.84; 95% CI, 0.71–0.99; $p = .033$; large cell carcinoma HR, 0.67; 95% CI, 0.48–0.96; $p = .027$). The HRs for PFS consistently trended in favor of cisplatin plus pemetrexed. For patients with other NSCLC/NOS tumors, OS

and PFS were not statistically significantly different between the two arms (Table 4). Figure 3 shows the Kaplan–Meier OS curves for each of the histologic subgroups in Table 4. Figure 2 also shows the Kaplan–Meier curve of OS for the nonsquamous patients in this study.

RRs for this study also varied by histologic type. Higher RRs occurred in the cisplatin plus pemetrexed arm than in the cisplatin plus gemcitabine arm in patients with adenocarcinoma (28.9% versus 21.7%) or other NSCLC/NOS tumors (28.3% versus 21.2%); higher RRs occurred in patients with squamous cell carcinoma (23.4% versus

Table 4. Summary of OS, PFS, and RR by histologic type for the pemetrexed versus docetaxel and cisplatin plus pemetrexed versus cisplatin plus gemcitabine studies

Histologic subgroup	Pemetrexed versus docetaxel (n = 571)		Cisplatin plus pemetrexed versus cisplatin plus gemcitabine (n = 1,725)	
	Pemetrexed	Docetaxel	Cisplatin plus pemetrexed	Cisplatin plus gemcitabine
Adenocarcinoma[a] (n)	158	144	436	411
Median OS (mos)	9.0	9.2	12.6	10.9
HR (95% CI)	0.92 (0.69–1.22)		0.84 (0.71–0.99)	
p-value	.551		.033	
Median PFS (mos)	3.5	3.5	5.5	5.0
HR (95% CI)	0.83 (0.65–1.06)		0.90 (0.78–1.03)	
p-value	.135		.125	
Response rate (%)[b]	12.8	9.9	28.9	21.7
Large cell carcinoma[a] (n)	18	29	76	77
Median OS (mos)	12.8	4.5	10.4	6.7
HR (95% CI)	0.27 (0.11–0.63)		0.67 (0.48–0.96)	
p-value	.003		.027	
Median PFS (mos)	2.9	1.3	4.5	4.2
HR (95% CI)	0.43 (0.20–0.94)		0.89 (0.65–1.24)	
p-value	.330		.499	
Response rate (%)[b]	12.5	3.7	27.6	27.3
Other NSCLC/NOS[a,c] (n)	29	21	106	146
Median OS (mos)	9.4	7.9	8.6	9.2
HR (95% CI)	0.57 (0.27–1.20)		1.08 (0.81–1.45)	
p-value	.141		.586	
Median PFS (mos)	1.8	1.9	4.5	5.6
HR (95% CI)	0.94 (0.49–1.80)		1.28 (0.99–1.67)	
p-value	.857		.064	
Response rate (%)[b]	3.7	10.0	28.3	21.2
Squamous cell carcinoma (n)	78	94	244	229
Median OS (mos)	6.2	7.4	9.4	10.8
HR (95% CI)	1.56 (1.08–2.26)		1.23 (1.00–1.51)	
p-value	.018		.050	
Median PFS (mos)	2.3	2.7	4.4	5.5
HR (95% CI)	1.40 (1.01–1.96)		1.36 (1.12–1.65)	
p-value	.046		.002	
Response rate (%)[b]	2.8	8.1	23.4	31.4
Nonsquamous (n)	205	194	618	634
Median OS (mos)	9.3	8.0	11.0	10.1
HR (95% CI)	0.78 (0.61–1.00)		0.84 (0.74–0.96)	
p-value	.048		.011	
Median PFS (mos)	3.1	3.0	5.26	4.96
HR (95% CI)	0.82 (0.66–1.02)		0.95 (0.84–1.06)	
p-value	.076		.349	
Response rate (%)[b]	11.5	9.0	28.6	22.2

[a]Nonsquamous histology comprises adenocarcinoma, large cell carcinoma, and other histologies.
[b]Tumor response data not available for some patients in each category.
[c]Other histology was a histologic diagnosis that did not clearly qualify as adenocarcinoma, large cell carcinoma, or squamous cell carcinoma.
Abbreviations: CI, confidence interval; HR, hazard ratio; NSCLC/NOS, non-small cell lung cancer not otherwise specified; OS, overall survival; PFS, progression-free survival; RR, response rate.
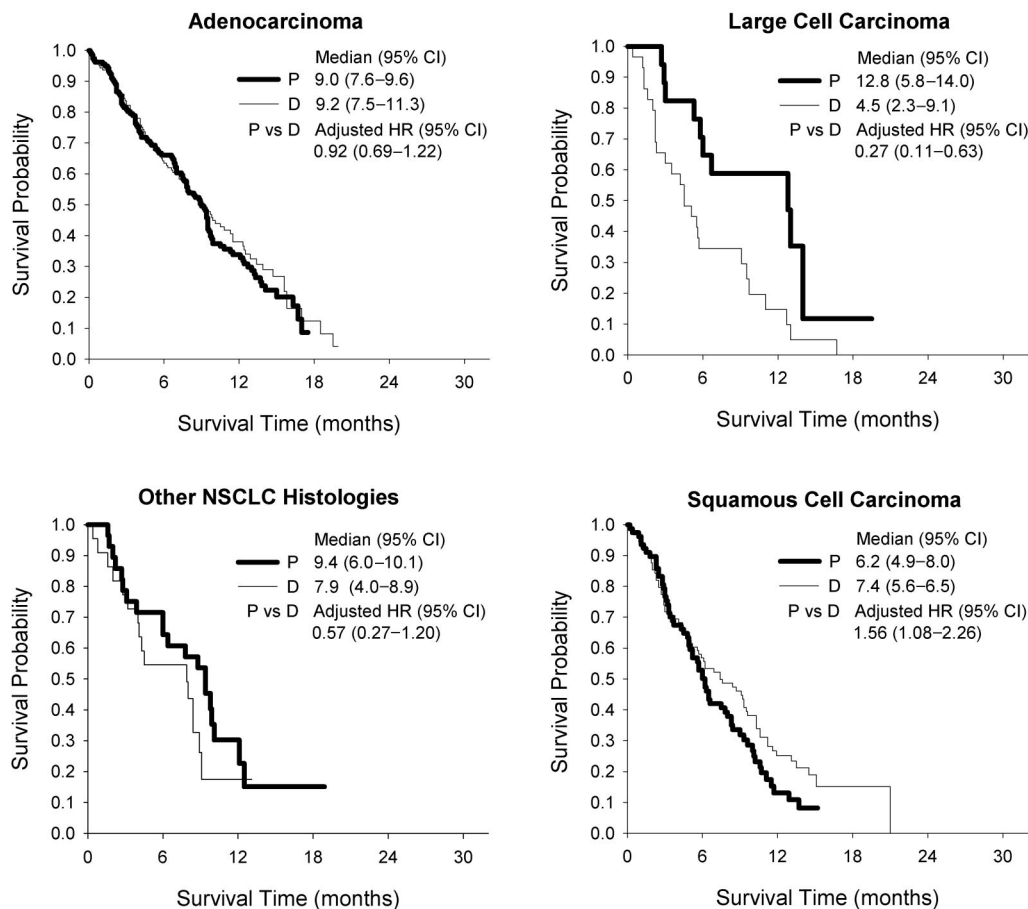
Figure 1. Kaplan–Meier overall survival curves for the pemetrexed versus docetaxel study for each of the histologic subgroups: adenocarcinoma, large cell carcinoma, squamous cell carcinoma, and other NSCLC/NOS histologies.

Abbreviations: CI, confidence interval; D, docetaxel; HR, hazard ratio; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; P, pemetrexed.
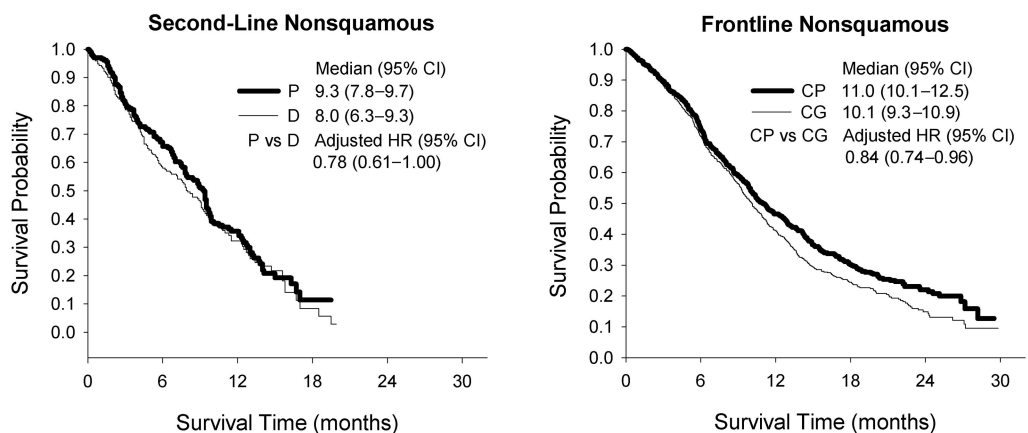
Figure 2. Kaplan–Meier overall survival curves for the nonsquamous patient groups in each of the two studies (the pemetrexed versus docetaxel study and the cisplatin plus pemetrexed versus cisplatin plus gemcitabine study), comprised of patients with adenocarcinoma, large cell carcinoma, and other histologies.

Abbreviations: CI, confidence interval; CG, cisplatin plus gemcitabine; CP, cisplatin plus pemetrexed; D, docetaxel; HR, hazard ratio; P, pemetrexed.
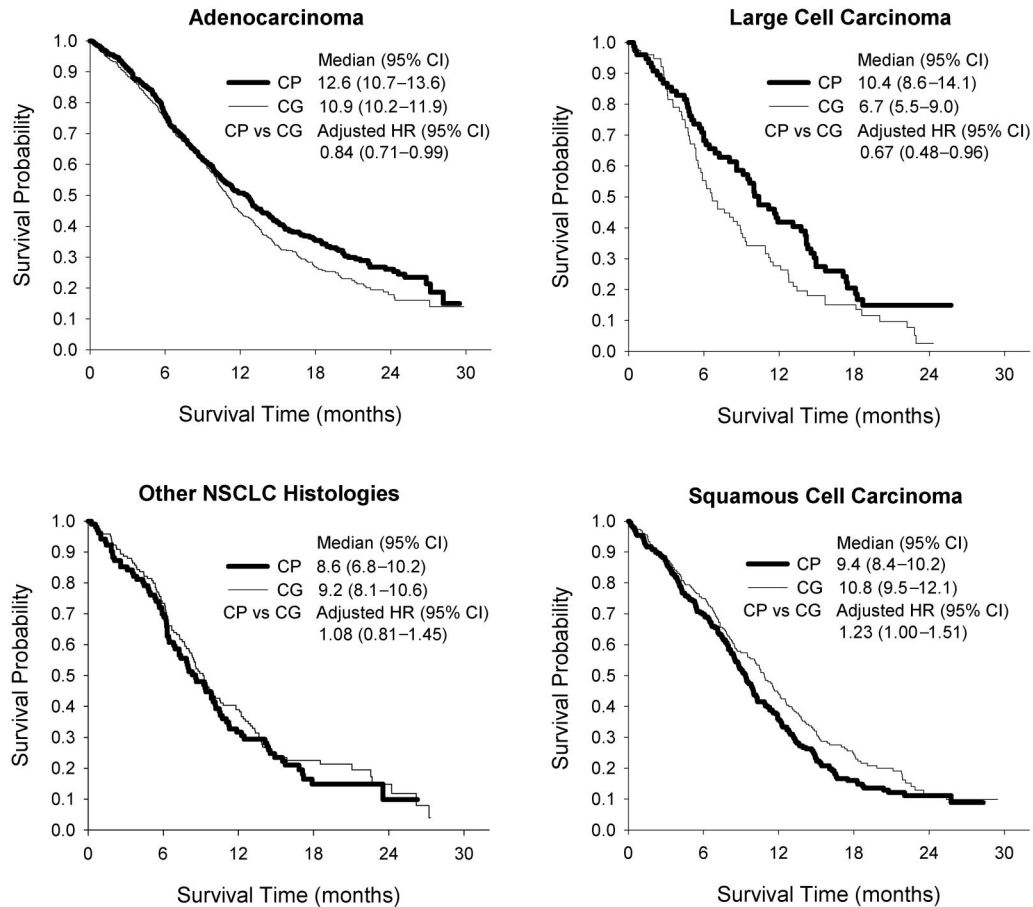
Figure 3. Kaplan–Meier overall survival curves for the cisplatin plus pemetrexed versus cisplatin plus gemcitabine study for each of the histologic subgroups: adenocarcinoma, large cell carcinoma, squamous cell carcinoma, and other NSCLC/NOS histologies.

Abbreviations: CI, confidence interval; CG, cisplatin plus gemcitabine; CP, cisplatin plus pemetrexed; HR, hazard ratio; NOS, not otherwise specified; NSCLC, non-small cell lung cancer.

31.4%) on cisplatin plus gemcitabine. For patients with large cell carcinoma, response rates were not statistically significantly different between the two arms (Table 4).

Safety

In both studies, pemetrexed was well tolerated and demonstrated a consistent safety profile [4, 16]. No significant differences were observed in the incidence of grade 3 or 4 toxicities by histologic type. Safety within the histologic subgroups was consistent with the overall results reported for the studies, and supported the favorable safety profile of pemetrexed regimens versus comparators. Additionally, in the frontline study [4], hospitalizations resulting from adverse events, transfusions, and the use of concomitant medications were also similar across histologic subtypes [23].

DISCUSSION

In our retrospective review of two large, randomized, phase III studies [4, 16], we detected a significant and consistent treatment-by-histology interaction for pemetrexed, thereby

confirming a predictive role for NSCLC histology. Although histology was not a randomization factor for either study, treatment arms of both studies were well balanced for histology, and in the frontline study, other baseline characteristics were balanced within histologic subgroups. In the smaller second-line study, there were imbalances in prognostic factors across histologic subgroups and treatment arms. Adjusted analyses were more appropriate in this context because they took these differences into account and therefore more accurately reflected the survival differences between treatment arms according to histology. Although the two studies had different NSCLC patient populations, with previously treated patients in the second-line study and chemotherapy-naive patients in the frontline study, in both studies the predictive effect of histology for pemetrexed showed that patients with nonsquamous histology who received pemetrexed therapy had a longer survival time than all other patients. No other chemotherapeutic agent has consistently shown differential efficacy according to NSCLC histology. Although a central pathology re-

view was not performed in either study, this is not common practice in large phase III trials and is logistically challenging.

Historically, clinicians have selected the most appropriate systemic chemotherapy for patients with advanced NSCLC based on factors such as age, PS score, and comorbidities in addition to the safety profile of the available treatment options. During the last decade, other potential factors have influenced treatment choice, including ethnicity, gender, smoking status, histology, and pharmacogenomic markers. In our review, the significantly better efficacy results consistently observed in the two studies of pemetrexed in nonsquamous patients are striking and have the potential to influence future clinical decisions.

Tumor response to EGFR TKIs has been associated with clinical factors, including female gender, adenocarcinoma and bronchiole-alveolar carcinoma, Asian ethnicity, and nonsmoking status, which may be related to the presence of *EGFR* mutations [24–27]. However, a significant interaction between tumor histology and survival benefit was not demonstrated in either of the large placebo-controlled trials of the EGFR TKIs gefitinib [28] and erlotinib [29]. A differential effect for bevacizumab therapy according to NSCLC histology has not been reported; however, toxicity concerns restrict bevacizumab use to patients with nonsquamous tumors [30].

Both of these large, phase III pemetrexed studies demonstrated a well-tolerated safety profile that is consistent with that of previous pemetrexed studies. Pemetrexed toxicities did not differ between squamous and nonsquamous patients. Although a differential efficacy effect according to histology was observed for pemetrexed-based regimens, the safety profile for pemetrexed was not affected.

Additional studies exploring the activity of pemetrexed in NSCLC have reported similar findings. A phase II study comparing the efficacy and safety of two doses of pemetrexed in Japanese patients with previously treated NSCLC also found significant differences in efficacy outcomes by histology. In that study, the median survival time of nonsquamous patients was longer than that of patients with squamous cell carcinoma (16.0 versus 9.3 months; $p = .003$) [12]. A recent analysis of pooled data from two phase II pemetrexed trials also showed histology effects for pemetrexed [11]. In another recent phase III study of pemetrexed versus placebo as maintenance therapy following frontline treatment, a prespecified analysis of efficacy by histology showed significant differences in RRs, PFS, and preliminary OS according to histologic subtype [10].

The consistency of the results across pemetrexed studies may have an underlying molecular basis. TS and S-phase kinase associated protein (Skp2) are two genes that are tran-

scriptionally regulated in the S phase of the cell cycle by the transcription factor E2F-1 [31, 32]. Preclinical data have indicated that overexpression of TS correlates with lower sensitivity to pemetrexed [15, 33, 34]. A recent study examined TS using specimens from chemotherapy-naive patients with early-stage adenocarcinoma or squamous cell carcinoma. TS expression was evaluated using real-time polymerase chain reaction (PCR) and immunohistochemistry (IHC). Baseline expression levels of the *TS* gene and TS protein were significantly higher in patients with squamous cell carcinoma than in those with adenocarcinoma ($p < .0001$) [17]. Like TS, elevated expression of Skp2 has been more commonly found in patients with squamous carcinoma than in those with adenocarcinoma [35].

In a companion pharmacogenomic study of the cisplatin plus gemcitabine versus cisplatin plus pemetrexed trial [36], low TS mRNA expression was associated with a longer TTP and TTF for cisplatin plus pemetrexed. Additionally, a high EGFR expression level was associated with a longer PFS time and TTP regardless of treatment. However, because of a limited number of samples, these findings were not statistically significant and must be considered hypothesis-generating.

In another recent study evaluating TS expression using real-time PCR and IHC, very high TS expression levels were detected in small cell lung cancer [37], a histologic type of lung cancer in which pemetrexed activity is limited [38–40].

Although a lower baseline TS expression level may be a plausible explanation for the higher activity of pemetrexed in adenocarcinoma, alternate molecular hypotheses should be considered. The hypothesis of a "worse molecular profile" [5] in the cisplatin plus gemcitabine arm cannot be completely excluded, although this seems unlikely given consistent results from several phase II and III pemetrexed studies. Another potential explanation may be the deletion of the housekeeping gene methylthioadenosine phosphorylase (*MTAP*), which is more frequently reported in adenocarcinoma. *MTAP* may be a potential molecular mechanism for higher cellular sensitivity to agents that reduce "de novo" purine synthesis (such as pemetrexed) [41]. However, this hypothesis is not entirely supported by preclinical data [42]. Finally, it is unclear if tumor biology may vary based on the patients' exposure to previous chemotherapy or other factors.

Further investigation and reporting of treatment outcomes according to NSCLC histology are warranted [6]. In particular, future studies evaluating pemetrexed activity should include histology as a randomization factor and in prespecified analyses, in addition to biomarker investigations. In an ongoing phase III trial, patients with stage II–III

completely resected NSCLC are being treated with either standard adjuvant chemotherapy or a tailored treatment determined by *TS* and *ERCC1* gene expression levels [International TAilored Chemotherapy Adjuvant (ITACA) trial].

Future studies exploring histology as a predictive factor should also assess the impact of smoking status and ethnicity. Because recent evidence suggests that these factors might have a significant prognostic impact [8, 28], any imbalances in these factors could have confounded the results in our studies. In the second-line pemetrexed study [16], data on smoking status were not collected for all patients, and so this factor could not be included in our multivariate analysis. In the cisplatin plus pemetrexed versus cisplatin plus gemcitabine study, smoking information was collected and no evidence of imbalance across treatment arms or histologies was seen; however, a significant prognostic impact was detected [4].

The impact of poststudy therapy on survival is difficult to evaluate because both studies permitted subsequent treatment at the discretion of the investigators. However, the percentage of patients receiving poststudy therapy was consistent with what has been previously reported in the literature; the selection of postdiscontinuation therapy did not appear to influence the overall survival conclusions in either study [4, 16, 43].

In this comprehensive review of two phase III NSCLC studies, we have provided evidence of a significant interaction between NSCLC histology and pemetrexed treatment effect, regardless of the control arm. A significant treatment-by-histology interaction for pemetrexed was also ob-served in a third phase III study comparing pemetrexed with placebo [10], thereby eliminating the potential that the treatment-by-histology effect is a function of the comparator drug rather than pemetrexed. The consistency of these results across studies confirms that the treatment advantage for pemetrexed in patients with nonsquamous histology is reproducible and valid. On the basis of these studies, tumor histology should be assessed carefully when selecting treatment options for patients with advanced NSCLC. These analyses suggest that pemetrexed should not be recommended for the treatment of squamous cell carcinoma, but because of the efficacy and safety advantages observed, pemetrexed may be preferable to other agents for the treatment of patients with nonsquamous NSCLC.

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