Treatment-by-Histology Interaction Analyses in Three Phase III Trials Show Superiority of Pemetrexed in Nonsquamous Non-small Cell Lung Cancer

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Introduction: Recently, histology has emerged as a predictive factor for pemetrexed efficacy in non-small cell lung cancer (NSCLC). These analyses evaluate whether the differential efficacy of pemetrexed by NSCLC histology is reproducible and consistent across three registration studies of different lines of therapy (first-line/second-line and maintenance settings).

Methods: The reported studies for patients with advanced NSCLC were pemetrexed versus docetaxel in previously treated patients (N = 571), cisplatin plus pemetrexed versus cisplatin plus gemcitabine in chemotherapy-naive patients (N = 1725), and maintenance pemetrexed plus best supportive care versus placebo plus best supportive care (N = 663). Cox models of overall survival (OS) and progression-free survival (PFS) were used to test for a significant treatment-by-histology interaction (THI). A significant THI indicates that the efficacy benefit for pemetrexed relative to the control arm is greater in patients with nonsquamous histology than in those with squamous histology. Subsequent Cox models were used to estimate hazard ratios for OS and PFS according to histology.

Results: Histology was well balanced between treatment arms in each study. Across all three studies, no clinically relevant differences were observed for the safety profile of pemetrexed among histologic groups. THIs were statistically significant in all three studies for OS (p = 0.001, 0.002, and 0.033, respectively) and PFS (p = 0.004, 0.002, and 0.036, respectively).

Conclusions: These analyses demonstrate a statistically significant interaction between treatment effect and NSCLC histology, indicating superior efficacy of pemetrexed in nonsquamous patients compared with other standard treatment options. Thus, histology is consistently predictive of the improved efficacy of pemetrexed in patients with nonsquamous NSCLC.

Key Words: Non-small cell lung cancer, Pemetrexed, Histology, Adenocarcinoma, Squamous cell carcinoma, Large cell carcinoma, Nonsquamous, Thymidylate synthase.

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Prognostic factors that have been associated with survival in non-small cell lung cancer (NSCLC) include disease stage at the time of diagnosis, performance status (PS), and recent weight loss (>10% of body weight). Although sex, tumor size, and histology have also been shown to influence outcomes, their impact has been marginal and without statistical significance.1–4 Even when combination chemotherapies including third-generation agents were considered, histology was not definitively prognostic.5,6

Recently, histology has emerged as a predictive factor for pemetrexed efficacy7–10 and a determinant of toxicity with bevacizumab for patients with advanced NSCLC.11 Historically, the association of histology with clinical outcomes has been inconsistent.9 A histologic diagnosis of adenocarcinoma and female sex, Asian ethnicity, and never-smoking status predicted higher response rates (RRs) for epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), but only a nonsmoking history was predictive of a differentially greater survival benefit.12–14 In advanced NSCLC, the predictive role of nonsquamous histology for the efficacy of pem-
Pemetrexed therapy has been reported in the retrospective analyses of two phase II studies\textsuperscript{15,16} and one phase III study\textsuperscript{17} and prospectively in two additional phase III studies\textsuperscript{7,18,19}. We further evaluated the differential efficacy of pemetrexed by histology in the second-line, first-line, and maintenance settings in the context of these three large phase III studies\textsuperscript{7,17–19}. These randomized studies were selected because all met regulatory criteria for approval of pemetrexed in different settings of disease treatment and were sufficiently robust to allow treatment-by-histology interaction (THI) analyses. The objective of these analyses was to evaluate the differential efficacy of pemetrexed by NSCLC histology and its reproducibility and consistency across the three studies in three different settings of pemetrexed therapy.

**PATIENTS AND METHODS**

**Patients**

Details regarding the three studies have been published previously\textsuperscript{7,17,19}. The second-line study had 571 previously treated patients with advanced or metastatic NSCLC, the first-line study had 1725 chemotherapy-naïve patients with NSCLC, and the maintenance study had 663 patients with NSCLC who had not progressed after four cycles of platinum-based chemotherapy\textsuperscript{7,17,19}.

All patients signed written informed consent before 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 Designs and Treatments**

The second- and first-line studies assessed overall survival (OS) as the primary end point using a noninferiority design, whereas the maintenance trial was designed to demonstrate superiority for the primary end point of progression-free survival (PFS). Secondary objectives (which varied depending on the study) included comparisons of PFS, OS, tumor RR, duration of response, and toxicity.

Patients in the second-line study randomly received either pemetrexed 500 mg/m\textsuperscript{2} as a 10-minute intravenous (IV) infusion or docetaxel (Taxotere, Sanofi-Aventis, France) 75 mg/m\textsuperscript{2} as a 1-hour IV infusion on day 1, every 21 days. Treatment continued until disease progression, unacceptable toxicity, or a request for therapy discontinuation.

Patients in the first-line study randomly received either cisplatin 75 mg/m\textsuperscript{2} plus pemetrexed (ALIMTA, Eli Lilly and Company, USA) 500 mg/m\textsuperscript{2} on day 1 or cisplatin 75 mg/m\textsuperscript{2} on day 1 plus gemcitabine (Gemzar, Eli Lilly and Company, USA) 1250 mg/m\textsuperscript{2} on days 1 and 8. Chemotherapy was repeated every 3 weeks for a maximum of six cycles.

Patients in the maintenance study randomly received, in a 2:1 ratio, pemetrexed 500 mg/m\textsuperscript{2} as a 10-minute IV infusion on day 1 plus best supportive care or placebo (0.9% sodium chloride) intravenously on day 1 plus best supportive care in 21-day cycles until disease progression. Patients and physicians were blinded to treatment.

During study treatment, all patients received supplementation with folic acid and vitamin B\textsubscript{12} (except in the second-line docetaxel arm) and all patients received prophylactic dexamethasone (administered according to the pemetrexed or docetaxel prescribing information).

Comprehensive baseline and follow-up assessments, including clinical laboratory tests and imaging studies, have been reported previously. Toxicity evaluations were based on Common Toxicity Criteria version 2.0\textsuperscript{17,19} or Common Terminology Criteria for Adverse Events version 3.0\textsuperscript{19}. Tumor measurements were assessed every two cycles in the second-line study using Southwest Oncology Group criteria\textsuperscript{17,20} and in the first-line and maintenance studies using Response Evaluation Criteria in Solid Tumors version 1.0\textsuperscript{7,19,21}.

NSCLC histology was reported by each investigator and grouped for statistical analyses into four types: adenocarcinoma, large cell carcinoma, squamous cell carcinoma, and other NSCLC/unclassified.

**Statistical Analyses**

All patients who received at least one dose of study drug were considered evaluable for safety analyses. All randomized patients, on an intent-to-treat basis, were included in efficacy analyses unless otherwise noted. For the purpose of these specific statistical analyses, results were dichotomized into two histologic groups: patients with squamous cell histology and patients with nonsquamous histology, which comprises adenocarcinoma, large cell carcinoma, and other NSCLC/unclassified. Histologic analyses were evaluated retrospectively in the second-line study and prospectively (for prespecified subset analyses) in the first-line and maintenance studies.

For all three studies, for the two histologic groups, the Kaplan-Meier method\textsuperscript{22} was used to estimate unadjusted within-arm medians (with 95% confidence intervals [CIs]), and Cox proportional hazard models were used to estimate covariate-adjusted between-arm hazard ratios (HRs) with 95% CIs. A THI analysis was also performed for all three studies, which is a test for interaction based on multivariate Cox models. A significant THI indicates a differential treatment effect according to histology. The histologic group analysis clarifies how the treatment effect differs by histology.

To test for covariate-adjusted THIs for both OS and PFS, the Cox 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), and terms for predefined baseline prognostic factors. The baseline prognostic factors included PS, disease stage, sex, and time since prior therapy for the second-line study; PS, disease stage, sex, and basis of diagnosis for the first-line study; and PS, induction response, East Asian ethnicity, sex, age, and nonsmoker status for the maintenance study. The interaction (THI) HR is the ratio of two HRs: the treatment effect (OS or PFS) for nonsquamous patients divided by the treatment effect (OS or PFS) for squamous patients:

\[
HR = \frac{HR_{\text{pemetrexed arm relative to control arm}}_{\text{nonsquamous patients}}}{HR_{\text{pemetrexed arm relative to control arm}}_{\text{squamous patients}}}\]

An interaction HR less than 1.0 indicates that the benefit for pemetrexed relative to the control arm is greater in patients with nonsquamous histology than in those with squamous histology.
TABLE 1. Baseline Patient and Disease Characteristics for Randomized Patients for Three Pemetrexed Studies

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Second-Line Pemetrexed vs. Docetaxel</th>
<th>First-Line Cisplatin + Pemetrexed vs. Cisplatin + Gemcitabine</th>
<th>Maintenance Pemetrexed vs. Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 283)</td>
<td>(n = 288)</td>
<td>(n = 862)</td>
</tr>
<tr>
<td>Median age (yr)</td>
<td>59</td>
<td>57</td>
<td>61</td>
</tr>
<tr>
<td>Female/Male (%)</td>
<td>31/69</td>
<td>25/75</td>
<td>30/70</td>
</tr>
<tr>
<td>Disease stage IIB/IV (%)</td>
<td>25/75</td>
<td>25/75</td>
<td>24/76</td>
</tr>
<tr>
<td>ECOG PS 0/1/2 (%)</td>
<td>NA</td>
<td>NA</td>
<td>35/65/—</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>72</td>
<td>67</td>
<td>72</td>
</tr>
<tr>
<td>Nonsquamous histology (%)</td>
<td>28</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>Squamous histology (%)</td>
<td>N/A</td>
<td>N/A</td>
<td>31/69</td>
</tr>
</tbody>
</table>

a Baseline ECOG PS data not available for some patients in each category.
b No smoking status data were collected.
c Number of patients per histologic type.
d Independently reviewed data.

TABLE 2. Treatment-by-Histology Interactions for Overall Survival and Progression-Free Survival for Three Pemetrexed Studies

<table>
<thead>
<tr>
<th>Efficacy Parameters</th>
<th>Second-Line Pemetrexed vs. Docetaxel (N = 571)</th>
<th>First-Line Cisplatin + Pemetrexed vs. Cisplatin + Gemcitabine (N = 1725)</th>
<th>Maintenance Pemetrexed vs. Placebo (N = 663)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall survival</td>
<td>Adjusted HR † (95% CI) 0.78 (0.61–1.00)</td>
<td>0.84 (0.74–0.96)</td>
<td>0.70 (0.56–0.88)</td>
</tr>
<tr>
<td></td>
<td>Superiority p</td>
<td>0.048</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Treatment-by-histology interaction test p ‡</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Progression-free survival</td>
<td>Adjusted HR † (95% CI) 0.82 (0.66–1.02)</td>
<td>0.95 (0.84–1.06)</td>
<td>0.47 (0.37–0.60)</td>
</tr>
<tr>
<td></td>
<td>Superiority p</td>
<td>0.076</td>
<td>0.349</td>
</tr>
<tr>
<td></td>
<td>Treatment-by-histology interaction test p ‡</td>
<td>0.004</td>
<td>0.002</td>
</tr>
</tbody>
</table>

a Nonsquamous histology comprises adenocarcinoma, large cell, 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 progression-free survival and overall survival using covariate-adjusted Cox proportional hazards models.
d Independently reviewed data.

RESULTS

Baseline Demographics

Patient and disease baseline characteristics were balanced between arms for the three studies, as shown in Table 1. Patient and disease characteristics within histologic groups were also balanced and have been published previously for the three studies.18,19

Efficacy

Second-Line Study of Pemetrexed Versus Docetaxel

In the second-line study, THIs for OS and PFS were statistically significant (OS, p = 0.001 and PFS, p = 0.004; Table 2). Nonsquamous patients treated with pemetrexed had significantly longer OS (HR = 0.78; 95% CI: 0.61–1.00; p = 0.048; Figure 1) than those treated with docetaxel (Table 3).

Squamous patients had shorter OS (HR = 1.56; 95% CI: 1.08–2.26; p = 0.018) and PFS (HR = 1.40; 95% CI: 1.01–1.96; p = 0.046) on pemetrexed compared with docetaxel. The efficacy of pemetrexed varied and favored nonsquamous histology, whereas the efficacy of docetaxel did not vary by histologic group.

In nonsquamous patients, numerically higher RRs occurred in those treated with pemetrexed (11.5%) compared with docetaxel (9.0%). In squamous cell carcinoma, patients treated with docetaxel had numerically higher RRs than those treated with pemetrexed (8.1% versus 2.8%; Table 3).

First-Line Study of Cisplatin Plus Pemetrexed Versus Cisplatin Plus Gemcitabine

In the first-line study, THIs for OS and PFS were also statistically significant (both p = 0.002), demonstrating a
differential treatment effect for pemetrexed by histology, with improved efficacy in patients with nonsquamous histology (Table 2). Nonsquamous patients treated with cisplatin plus pemetrexed had significantly longer OS (HR = 0.70; 95% CI: 0.56–0.88; p = 0.002; Figure 1) and PFS (HR = 0.47; 95% CI: 0.37–0.60; p < 0.0001) than those treated with placebo (Table 3). Squamous patients did not benefit from pemetrexed compared with placebo, showing no significant difference for OS (HR = 1.07; 95% CI: 0.77–1.50; p = 0.678) or PFS (HR = 1.03; 95% CI: 0.71–1.49; p = 0.896). PFS results were based on an analysis of independently reviewed data.

Independently reviewed RR data for this study also varied by histologic group. Significantly higher (p = 0.035) RRs occurred in the nonsquamous patients treated with pemetrexed (3.4%) compared with placebo (0.0%). In patients with squamous cell carcinoma, RRs were not significantly higher (3.1% versus 1.8%; p > 0.999; Table 3).

Probability
Although the three NSCLC studies targeted different therapeutic settings (first line, maintenance, and second line), they demonstrated a consistent treatment effect for pemetrexed regimens by histology for both OS and PFS. As such, the multiplicative rule of mathematics can be applied to determine and measure the probability of a given event (the pemetrexed treatment effect) occurring in these three independent settings. By multiplying each OS THI value, the chance of the histology effect being due to chance alone can be evaluated as follows: 0.001 x 0.002 x 0.033 = 0.000000066 or 1 in 15 million.

Postdiscontinuation Therapy
For all three studies, decisions regarding poststudy therapy were at the discretion of the individual investigators. Details regarding the types of agents administered have been published previously; docetaxel was the most commonly used chemotherapeutic agent.7,17,19 The use of postdiscontinuation therapy did not differ significantly between pemetrexed arms and comparator arms, with the exception of crossing over to postdiscontinuation pemetrexed; however, the cross-over rates were low (13–18%).7,19 The use of EGFR TKIs (erlotinib or gefitinib) also did not differ significantly between arms.7,17,19 The selection of postdiscontinuation therapies in the histologic groups was similar to that of the overall study groups.18,19

Safety
In all three studies, pemetrexed was well tolerated and demonstrated a consistent safety profile (Table 4).7,17,19 Toxicities were also analyzed by histologic group, and results have shown that the incidence of pemetrexed-related grade
Pemetrexed Studies

68 associate the differential treatment effect of pemetrexed with the at the discretion of the investigator has lead to a potential bias to effect observed for pemetrexed across the subgroups. baseline characteristics, there was a consistent treatment effect on OS in the nonsquamous population was analyzed by randomization factor. Even when the pemetrexed treatment arms, including histology, although histology was not a patient and treatment characteristics were balanced between confirm a treatment advantage for pemetrexed in patients with nonsquamous NSCLC. In all these studies, baseline Table 4. Percentage of Patients with Selected Grade 3/4 Drug-Related Toxicities for Three Pemetrexed Studies

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Second-Line Pemetrexed vs. Docetaxel (N = 571)</th>
<th>First-Line Cisplatin + Pemetrexed vs. Cisplatin + Gemcitabine (N = 1725)</th>
<th>Maintenance Pemetrexed vs. Placebo (N = 663)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pemetrexed (n = 265) vs. Docetaxel (n = 276)</td>
<td>Cisplatin + Pemetrexed (n = 839) vs. Cisplatin + Gemcitabine (n = 830)</td>
<td>Cisplatin (n = 441) vs. Placebo (n = 222)</td>
</tr>
<tr>
<td>Anemia</td>
<td>4.2 vs. 4.3</td>
<td>5.6 vs. 9.9</td>
<td>2.7 vs. 0.5</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>5.3 vs. 40.2</td>
<td>15.1 vs. 26.7</td>
<td>2.9 vs. 0.0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1.9 vs. 0.4</td>
<td>4.1 vs. 12.7</td>
<td>2.0 vs. 0.5</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>1.9 vs. 12.7</td>
<td>1.3 vs. 3.7</td>
<td>0.7 vs. 0.0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5.3 vs. 5.4</td>
<td>6.7 vs. 4.9</td>
<td>5.0 vs. 0.5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1.5 vs. 1.1</td>
<td>6.1 vs. 6.1</td>
<td>0.2 vs. 0.0</td>
</tr>
</tbody>
</table>

n, number of patients.

DISCUSSION

The THI analyses of three large phase III studies confirm a treatment advantage for pemetrexed in patients with nonsquamous NSCLC. In all these studies, baseline patient and treatment characteristics were balanced between arms, including histology, although histology was not a randomization factor. Even when the pemetrexed treatment effect on OS in the nonsquamous population was analyzed by baseline characteristics, there was a consistent treatment effect observed for pemetrexed across the subgroups. The fact that studies allowed postdiscontinuation therapy at the discretion of the investigator has lead to a potential bias to associate the differential treatment effect of pemetrexed with the poststudy therapy received, especially EGFR therapy (gefitinib or erlotinib). Nevertheless, for the three studies, except for pemetrexed, the selection of poststudy agents did not differ significantly between study arms. Moreover, the selection of therapies in the histologic groups reflected that of the overall study populations and there were no significant differences between arms in the use of gefitinib or erlotinib. The percentage of patients receiving postdiscontinuation therapy was consistent with that of other published studies and, in the three studies examined, the OS data do not seem to be influenced by the selection of poststudy therapy.

Other chemotherapeutic agents have not demonstrated a similar treatment effect by tumor histology. In addition, large placebo-controlled studies of EGFR TKIs (gefitinib and erlotinib) have not shown a significant interaction between survival benefit and tumor histology.

The differential activity of pemetrexed by histology was not observed in a phase III NSCLC study from Norway. 3/4 toxicities did not vary significantly by histologic group, and the safety profile reported for pemetrexed across the histologic groups was consistent with the results reported for the overall study populations.
first-line study, the assessment of quality of life was the primary end point, and 446 patients were randomly assigned to receive carboplatin plus pemetrexed or carboplatin plus gemcitabine. When compared with the other existing first-line study,7 however, the Norwegian study has several disparities including differences in sample size, treatment regimens (including the combination agent of carboplatin versus cisplatin), dosing, baseline patient and disease characteristics, standard of care in Norway, and statistical analyses.28 In addition, in the Norwegian study, the number of treatment cycles was limited to four, and the pemetrexed dose was automatically reduced by 25% for patients older than 75 years. The baseline patient and disease characteristics of the Norwegian study differed from those of the three studies we examined. Two of the three studies excluded patients with a PS of 2,7,19 whereas 11 to 12% of patients had PS 2 in the second-line study.17 The Norwegian trial, however, reported 22 to 23% of patients with a PS of 2. Additionally, because the Norwegian trial was not designed for subgroup analyses, the histology findings should be interpreted with caution, as acknowledged by the publication’s authors.7,19,28

The consistency of the pemetrexed treatment effect across these three studies suggests a possible underlying molecular basis for the effect, related to the mechanism of action of pemetrexed. Pemetrexed is an antifolate with multiple enzyme targets affecting purine and pyrimidine synthesis. The primary target of pemetrexed is thymidylate synthase (TS), and secondary targets are dihydrofolate reductase and glycaminamide ribonucleotide formyl transferase.29,30 In preclinical studies, reduced sensitivity to pemetrexed has been correlated with an overexpression of TS.31–33 A study of specimens from chemonaive patients with early-stage adenocarcinoma or squamous cell carcinoma evaluated TS using immunohistochemistry and real-time polymerase chain reaction. Patients with squamous cell carcinoma had significantly higher (p < 0.0001) baseline expression of both the TS gene and protein than patients with adenocarcinoma.34 Research on freshly explanted human tumor specimens showed that mRNA gene expression related to pemetrexed mechanism of action correlated with in vitro chemosensitivity to pemetrexed,35 and low levels of TS (as well as glycinamide ribonucleotide formyl transferase, dihydrofolate reductase, and MRP4) gene expression significantly correlated with chemosensitivity to pemetrexed.35 Prospective studies are needed to validate TS or other enzymes as a robust candidate biomarker for pemetrexed activity in NSCLC.

These results across three lines of therapy and in three separate studies demonstrate the consistency of the pemetrexed treatment effect for nonsquamous patients with NSCLC; however, the comparison of different patient populations in different lines of therapy might also be considered a limitation of these analyses. Future studies evaluating pemetrexed activity should include histology as a randomization factor and in prespecified analyses.

Comprehensive analyses of these three large, randomized, phase III NSCLC studies of three different lines of therapy (first line, maintenance, and second line) consistently demonstrate significant interactions between NSCLC histology and a pemetrexed treatment effect, regardless of the control arm. The analyses indicate the superior efficacy of pemetrexed in nonsquamous patients and a favorable safety profile compared with other standard treatment options. Thus, histology is predictive of the improved efficacy of pemetrexed in patients with nonsquamous NSCLC.

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REFERENCES


