Pemetrexed Disodium: A Novel Antifolate Clinically Active Against Multiple Solid Tumors

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ABSTRACT

Pemetrexed disodium (ALIMTA®, “pemetrexed”) is a novel, multi-targeted antifolate that has demonstrated promising clinical activity in a wide variety of solid tumors, including non-small cell lung, breast, mesothelioma, colorectal, pancreatic, gastric, bladder, cervix, and head and neck. Pemetrexed inhibits multiple folate-dependent enzymes involved in both purine and pyrimidine synthesis including thymidylate synthase, dihydrofolate reductase, glycaminamide ribonucleotide formyltransferase, and aminoimidazole carboxamide ribonucleotide formyltransferase. As a single agent, pemetrexed exhibits a moderate toxicity profile at a dose of 500 mg/m² by 10-minute infusion once every 21 days with myelosuppression being the dose-limiting toxicity. Folic acid added to the diet in preclinical studies reduced toxicities while maintaining antitumor activity. Based on this observation and clinical toxicities, folic acid and vitamin B₁₂ dietary supplementation has been recently introduced into all ongoing trials. Studies combining pemetrexed with other active chemotherapeutic agents demonstrate that these combination therapies may become important treatment regimens in a variety of cancer types. Currently, pemetrexed phase III trials are ongoing in mesothelioma and non-small cell lung cancer with future trials planned to explore this unique multitargeted antifolate. The Oncologist 2001;6:363-373

INTRODUCTION

Folate-dependent pathways are key targets in the development of effective anticancer agents. Unlike most other vital enzyme systems within the body, there is little redundancy in the folate-dependent systems that lead to DNA synthesis. Research into the development of antifolates as antitumor agents has been actively pursued since the 1950s and has led to the most successful antifolate to date, methotrexate (MTX). In 1992, a report by Taylor et al. [1] described the discovery of pemetrexed disodium ([ALIMTA®, “pemetrexed”], LY231514; Eli Lilly and Company; Indianapolis, IN), a multitargeted folate analogue that suppresses tumor growth by impeding both DNA synthesis and folate metabolism. By its nature, pemetrexed is a broadly acting agent and because of this, multiple clinical indications are currently being pursued. In phase II trials, pemetrexed has demonstrated single-agent activity in a variety of tumor types, including non-small cell lung [2-3], breast [4-5], colorectal [6-7], head and neck [8], gastric [9], bladder [10], cervix [11], and pancreas cancers [12]. Pemetrexed is currently in phase III studies for mesothelioma and non-small cell lung cancer (NSCLC).

CHEMICAL STRUCTURE AND MECHANISM OF ACTION

Pemetrexed is a novel pyrrolo[2,3-d]pyrimidine-based antifolate (Fig. 1). It gains entry to the cell via the reduced folate carrier. Once inside the cell, the antifolate is polyglutamated by folylpolyglutamate synthase, an enzyme for which it shows high affinity. Pemetrexed and its tri- and pentaglutamate derivatives demonstrate significant inhibitory activity for multiple enzyme systems (Table 1). Like 5-fluorouracil (5-FU) and raltitrexed, inhibition of thymidylate synthase (TS) is the primary mechanism of action [13, 14] (Fig. 2). TS, a folate-dependent enzyme, catalyzes the transformation of deoxyuridine monophosphate to deoxymethylidine monophosphate. Inhibition of TS results in a decrease in the available thymidine necessary for DNA synthesis leading to a subsequent decline in cellular proliferation, of particular significance in rapidly proliferating tumor cells [15, 16]. Pemetrexed and its...
polyglutamated derivatives also potently inhibit dihydrofolate reductase (DHFR), which produces tetrahydrofolate and is the primary target for MTX, and glycinamide ribonucleotide formyl transferase (GARFT), involved in de novo purine biosynthesis [17]. To a lesser extent, pemetrexed and its polyglutamated forms inhibit aminomimidazole carboxamide ribonucleotide formyltransferase (AICARFT). Like GARFT, this enzyme is involved in purine biosynthesis. The pentaglutamate derivative is the predominant intracellular form and is >60-fold more potent in its inhibition of TS than its antimetabolite parent. In fact, with the exception of DHFR, the tri- and pentaglutamate forms exhibit markedly higher antagonistic activity against folate-dependent enzymes than the parent compound [18]. Separate from the increased inhibitory activity polyglutamation bestows on pemetrexed, glutamation also increases cellular retention of the molecule, which translates into both a longer exposure time and increased intracellular concentrations of the drug.

**PRECLINICAL STUDIES**

Preclinical studies demonstrated that pemetrexed is cytotoxic against a number of cell lines including CCRF-CEM leukemia, GC3/C1 colon carcinoma, and HCT-8 ileocecal carcinoma cells [19, 20]. In CCRF-CEM cells, the inhibition was only partially reversed upon the addition of thymidine, suggesting the importance of the secondary sites of action for the activity of pemetrexed. Further, MCFTDX and H630RIO cells, which overexpress TS and are resistant to the TS inhibitors 5-FU and raltitrexed, were shown to be 261- and 1,289-fold less resistant to pemetrexed than to raltitrexed. These findings suggest the importance of secondary inhibitory pathways in the activity of pemetrexed and indicate that the agent may be useful in 5-FU- and raltitrexed-resistant tumors.

Teicher and coworkers [21] have investigated the therapeutic advantage of combining pemetrexed with other

![Figure 1. Structure of pemetrexed.](image1)

![Figure 2. Mechanism of action of pemetrexed. Key target enzymes include TS, DHFR, and GARFT. Pemetrexed disodium is a competitive inhibitor of these and other enzymes inducing disturbances in both pyrimidine and purine neosynthesis.](image2)

| Table 1. Inhibitory activity of pemetrexed (LY231514), MTX, and their polyglutamates against recombinant human (rHu)TS, rHuDHFR, recombinant murine (rm)GARFT, and rHuAICARFT |
|---------------------------------|-----------------|-----------------|-----------------|------------------|
| **Compound**                   | **Ki (means ± SE nM)** |
| LY231514                        | 109 ± 9         | 7.0 ± 1.9       | 9,300 ± 690     | 3,580            |
| LY231514-glu3                   | 1.6 ± 0.1       | 7.1 ± 1.6       | 380 ± 92        | 480              |
| LY231514-glu5                   | 1.3 ± 0.3       | 7.2 ± 0.4       | 65 ± 16         | 265              |
| MTX                             | 13,000          | 0.0034          | 80,000          | 143,000          |
| MTX-glu5                        | 47              | 0.0014          | 2,500           | 56               |

aKi values for LY231514 and its polyglutamates taken from [14].

bKi values for MTX and its polyglutamates against all enzymes except DHFR taken from [50]. The Ki values for MTX against DHFR taken from [51].
antitumor agents in human tumor xenografts (breast or NSCLC). When pemetrexed was combined with 5-FU or paclitaxel, a synergistic antitumor effect was achieved. Notably, the antitumor effect obtained with pemetrexed/5-FU was greater than that achieved with the combination of 5-FU and MTX. In a similar manner, when human tumor xenografts were incubated in the presence of combinations of pemetrexed with gemcitabine or one of the platinum-containing compounds (carboplatin, cisplatin, or oxaliplatin), these regimens produced additive or synergistic antitumor effects. Additive antitumor effects were seen when pemetrexed was combined with vinorelbine, cyclophosphamide, or doxorubicin. The effects on tumor suppression were additive when pemetrexed was given prior to fractionated radiation. Combined treatment resulted in a strong increase of efficacy with an enhancement ratio of 3.3 [22]. The success of combining pemetrexed with other anticancer agents or radiotherapy in human tumor xenografts suggested that exploration of these same combinations might prove clinically beneficial.

The mechanism by which natural folates protect in vitro cell cultures from the toxic effects of antifolates is generally believed to be the result of competition at the levels of transport into the cell, polyglutamation, or target interaction, either independently or in combination [23]. However, the results from the in vitro studies done using single cell types are not readily translated into the in vivo situation where multiple cell types are involved. Protection against toxicities without impairment of drug efficacy implies a differential response to the folate/drug combination between tumor cells and normal cells such that the outcome favors the survival of normal cells. Studies to address such a differential in folate requirement for various cell types are difficult. Clinical attempts to combine pemetrexed and folic acid have been designed on an empirical basis. However, preliminary data gathered are encouraging and support the notion that folate supplementation is beneficial and reduces toxicities.

**Clinical Pharmacology**

**Single-Agent Phase I Studies and Pharmacokinetic Parameters**

Given the schedule dependency observed in animal models, phase I studies were conducted exploring three treatment schedules: daily × 5 on a 3-week cycle [24]; weekly × 4 on a 6-week cycle [25]; and once every 21 days [26]. *McDonald et al.* [24] administered pemetrexed beginning at 0.2 mg/m² as a daily 10-minute infusion for the first 5 days of each 3-week cycle. The maximum tolerated dose on this schedule was 5.2 mg/m², with neutropenia being the dose-limiting toxicity. However, the inconvenience of repeated i.v. administration (and decreased patient compliance) combined with the less than optimal dose intensity achievable with this regimen made this dose and schedule of pemetrexed undesirable, especially in light of success of the once every 21 day cycle. *Rinaldi et al.* [25] investigated a schedule of pemetrexed in a weekly × 4 every 6 weeks cycle beginning at a dose of 10 mg/m². The maximum tolerated dose was 40 mg/m², with neutropenia again being the dose-limiting toxicity. This reversible neutropenia limited the escalation of the dose beyond 40 mg/m² and thus, limited the dose intensity achievable with this regimen. Another schedule, which was carried forward in all subsequent trials, was studied by *Rinaldi et al.* [26]. These investigators administered pemetrexed beginning at 50 mg/m² in a 10-minute infusion once every 3 weeks. The maximum tolerated dose was 700 mg/m², with the dose-limiting toxicities being neutropenia, thrombocytopenia, and cumulative fatigue. Based on this study, the recommended phase II dose for pemetrexed was 600 mg/m². Of the total 100 patients evaluated during phase I studies, six deaths were considered drug-related. Toxicities associated with these drug-related deaths included neutropenia, mucositis, diarrhea, and severe nausea and vomiting [24-26].

Pharmacokinetic parameters were investigated in two separate studies by *Rinaldi et al.* [26] and *Ouellet et al.* [27] (Table 2). Pemetrexed plasma concentration-time functions followed a two-compartment model. The apparent volume of distribution was 6.8 l/m², which is rather small and suggests that pemetrexed is primarily confined to the plasma and interstitial compartments. The relatively rapid clearance from the body may play a role in the small volume of distribution. More significantly, pemetrexed is a polar-charged compound which must use a transporter to gain access to the cell, and this inability to readily penetrate biomembranes probably is the major determining factor for the small volume of distribution. The peak plasma concentration at the recommended dose of 600 mg/m² was 137 µg/ml. Linear relationships exist between dose and peak plasma concentration and between dose and area under the curve (AUC). The half-life of pemetrexed was 3.0 hours and the clearance was 40 ml/min/m², with approximately 78% excreted unchanged in the urine in the first 24 hours. All pemetrexed studies exclude patients whose calculated creatinine clearance is below 45 ml/min (modified *Cockcroft* and *Gault* formula; corresponds to 60 ml/min using the standard *Cockcroft* and *Gault* formula). Additionally, patients whose creatinine clearance drops below 45 ml/min may not be retreated until their clearance rises above this threshold.

Oral bioavailability has not been evaluated since pemetrexed is intended for use by short-term i.v. infusion. However, oral absorption was evaluated in mice using 20 mg/kg i.v. and oral doses of 14C radiolabeled pemetrexed.
Results from mice indicate that the oral absorption is low with only 13% of an oral dose absorbed in mice.

**Single-Agent Phase II Studies**

**NSCLC**

Pemetrexed as a single agent has been investigated for antitumor activity in patients with advanced NSCLC who were either previously untreated [2, 3] or previously treated [28] (Table 3). Pemetrexed 500 or 600 mg/m² was administered as a 10-minute infusion once every 21 days. The lower dose of 500 mg/m² was instituted due to toxicities seen both in the study by Rusthoven et al. [3] and a colorectal study by Cripps et al. [6] conducted at the same institution. Results from the study by Rusthoven et al. [3] and one by Clarke et al. [2] were consistent with regard to end points, with an overall response rate of 23% [3] and 18% [2] (Table 3). Sixty-seven percent of patients with stage IIIb responded to therapy compared to 13% with stage IV [3]. Duration of response was 3.1 months in the study by Rusthoven et al. and 5.6 months in the study by Clarke et al. Overall survival in the two studies was similar, with 9.2 months and 9.8 months, respectively. In the study by Rusthoven et al., principal toxicities were grade 3/4 neutropenia (39% of patients) and grade 3 rash (39% of patients). In addition, 13% of patients experienced febrile neutropenia. Similarly, the study by Clarke et al. reported principal toxicities to be grade 3/4 neutropenia (45% of patients) and grade 3/4 rash (34% of patients). Later analysis of these data led to subsequent addition of prophylactic corticosteroids, which has reduced the severity and frequency of skin rash. These studies suggest that pemetrexed has clinically meaningful antitumor activity with moderate toxicity and is similar to other single agents used in the treatment of NSCLC. Further, these results indicate that combination studies are warranted.

A third single-agent study investigated the safety and efficacy of pemetrexed in NSCLC patients who had relapsed after either platinum- or non-platinum-based regimens [28]. Patients who had failed treatment with non-platinum agents (such as gemcitabine, vinorelbine, paclitaxel) achieved an objective response rate of 30% (including one complete response [CR]) compared to a response rate of only 13% for those who had previously failed platinum-based therapies. Principle grade 3/4 toxicities in this study were neutropenia (23% of cycles) and leukopenia (22%). The findings from this study suggest that second-line therapy with pemetrexed is feasible and carries promise.

**Breast Cancer**

Three studies investigating the safety and efficacy of pemetrexed as a salvage regimen in locally advanced or metastatic breast cancer have been reported [4, 5, 29]. In all of these studies, pemetrexed was administered without folic acid and vitamin B₁₂ supplementation. Pemetrexed at 500 or 600 mg/m² was administered as a 10-minute infusion once every 21 days. Lind et al. [4] reported the effect of pemetrexed when given to patients with either locally advanced or metastatic breast cancer, with 33 of 38 patients having recurrence after primary chemotherapy. An objective response rate of 31% was achieved, including 1 CR and duration of response was 8+ months. Primary grade 3/4 toxicities included neutropenia (50%), thrombocytopenia (15%), and rash (19%). In this mixed population, pemetrexed showed

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>n Patients</th>
<th>Volume of distribution (l/m²)</th>
<th>Maximum plasma concentration (µg/ml)</th>
<th>AUC (µg·h/ml)</th>
<th>Half-life (h)</th>
<th>Clearance (ml/min/m²)</th>
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</thead>
<tbody>
<tr>
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<td>1</td>
<td>7</td>
<td>12</td>
<td>22</td>
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<tr>
<td>75</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>14</td>
<td>1.5</td>
<td>92</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>6</td>
<td>27</td>
<td>39</td>
<td>2.4</td>
<td>42</td>
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<td>54</td>
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</tr>
<tr>
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<tr>
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<td>91</td>
<td>158</td>
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<td>4</td>
<td>7</td>
<td>121</td>
<td>231</td>
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<td>41</td>
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<tr>
<td>600*</td>
<td>20</td>
<td>7</td>
<td>137</td>
<td>265</td>
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<tr>
<td>700</td>
<td>5</td>
<td>6</td>
<td>175</td>
<td>397</td>
<td>3.7</td>
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<tr>
<td>Mean values</td>
<td>6.8</td>
<td>ND**</td>
<td>ND</td>
<td>ND</td>
<td>3.0</td>
<td>40</td>
</tr>
</tbody>
</table>

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*Recommended phase II dose level.

**Not determined**
Table 3. Results of single-agent phase II trials with pemetrexed administered on day 1 of a 21-day cycle

<table>
<thead>
<tr>
<th>Phase II trials</th>
<th>n Patients evaluable</th>
<th>Dose (mg/m²)</th>
<th>Objective response rate</th>
<th>Duration of response</th>
<th>Overall survival*</th>
<th>Principle toxicities†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSCLC</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Rusthoven [3]</td>
<td>30/33</td>
<td>500 (30 patients); 600 (3 patients)</td>
<td>23%</td>
<td>3.1 months (2.3-13.5 months)</td>
<td>9.2 months</td>
<td>G3/4 neutropenia (39%); Febrile neutropenia (13%); G3 rash (39%)</td>
</tr>
<tr>
<td>Clarke [2]</td>
<td>34/40</td>
<td>500</td>
<td>18%</td>
<td>5.6 months (4.6-14.1 months)</td>
<td>9.8 months</td>
<td>G3/4 neutropenia (45%); G3/4 rash (34%)</td>
</tr>
<tr>
<td>Postmus [28]</td>
<td>43</td>
<td>500</td>
<td>30% PT arm; 13% NP arm</td>
<td></td>
<td></td>
<td>G3/4 neutropenia (23%); G3/4 leukopenia (22%)</td>
</tr>
<tr>
<td><strong>Breast</strong></td>
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<tr>
<td>Lind [4]</td>
<td>36</td>
<td>600</td>
<td>31% (1 CR)</td>
<td>8+ months</td>
<td></td>
<td>G3/4 neutropenia (50%); G3/4 thrombocytopenia (15%); G3/4 rash (19%)</td>
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<tr>
<td>Theodoulou [29]</td>
<td>24</td>
<td>500</td>
<td>19%</td>
<td></td>
<td></td>
<td>G3/4 neutropenia (29%)</td>
</tr>
<tr>
<td>Spielmann [5]</td>
<td>69</td>
<td>600</td>
<td>29% AF; 19% AR; 28% T</td>
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<td></td>
<td>G3/4 neutropenia (7%)</td>
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<tr>
<td><strong>Colorectal</strong></td>
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<tr>
<td>Cripps [6]</td>
<td>29</td>
<td>600 (9 patients); 500 (23 patients)</td>
<td>17%</td>
<td>4.3 months (2.1-10.4 months)</td>
<td>15.1 months</td>
<td>G3/4 neutropenia (50%); G3/4 leukopenia (46%); G3/4 thrombocytopenia (13%)</td>
</tr>
<tr>
<td>John [7]</td>
<td>30/40</td>
<td>600</td>
<td>15%</td>
<td>9.1 months</td>
<td>16.2 months</td>
<td>G3/4 neutropenia (56%); G3/4 leukopenia (54%); G3/4 thrombocytopenia (18%)</td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Miller [12]</td>
<td>35/42</td>
<td>600</td>
<td>6% (1 CR)</td>
<td>12 months</td>
<td>6.5 months</td>
<td>G3/4 neutropenia (40%); G3/4 leukopenia (43%); G3/4 anemia (19%); G3/4 thrombocytopenia (17%)</td>
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<tr>
<td><strong>Cervix</strong></td>
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<tr>
<td><strong>Bladder</strong></td>
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<tr>
<td>Paz-Ares [10]</td>
<td>23</td>
<td>500 (17 patients); 600 (6 patients)</td>
<td>30%</td>
<td></td>
<td></td>
<td>G4 neutropenia (35%); G3/4 anemia (17%); G3/4 thrombocytopenia (9%)</td>
</tr>
<tr>
<td><strong>Head and neck</strong></td>
<td></td>
<td>500</td>
<td>33%</td>
<td></td>
<td></td>
<td>G3/4 neutropenia (43%); G3/4 anemia (12%); Febrile neutropenia (13% of cycles)</td>
</tr>
</tbody>
</table>

Abbreviations: PT = previously treated with platinum-based regimens; AF = anthracycline failures; NP = previously treated with nonplatinum-based regimens; T = taxane-refractory; AR = anthracycline refractory; CR = complete response; G = grade.

1Chemotherapy naïve patients.
2Patients who had received prior chemotherapy with or without platinum.
3Mixed population – no more than two prior chemotherapies.
4Patients who had received anthracyclines and taxanes.
5Patients who had received anthracyclines.
6Objective response rate, duration of response, and overall survival are based on n of patients evaluable for each study.
7Principle toxicities are based on n of patients evaluable for toxicity for each study.
promising antitumor activity with acceptable toxicity. Two other studies have investigated pemetrexed when administered to metastatic breast cancer patients previously treated with anthracyclines and/or taxanes. Theodoulou et al. [29] evaluated pemetrexed as a third-line therapy in metastatic breast cancer patients who had failed previous treatment with both taxanes and anthracyclines. In a preliminary report, 19% of patients achieved an objective response. The major adverse event was grade 3/4 neutropenia (29% of patients). Spielmann et al. [5] conducted a study with a similar patient population. All patients in this study had metastatic breast cancer and had previously received an anthracycline. A subset of patients (42%) had also received previous taxane therapy. For analysis, patients were divided into either anthracycline-refractory (progression ≤30 days after treatment) or anthracycline failures (progression >30 days after treatment). Anthracycline-failure patients achieved an objective response rate of 29% and anthracycline-refractory patients achieved an objective response rate of 19%. Of those patients who had also received a taxane in previous therapy, 28% responded to treatment with pemetrexed, regardless of whether they were anthracycline-refractory or anthracycline failure patients. Toxicity in the study was quite mild, with only 7% of patients experiencing grade 3/4 neutropenia. These studies support the conclusion that pemetrexed, as a single agent, is effective as a second- or third-line regimen in the treatment of locally advanced or metastatic breast cancer. As with NSCLC, further investigation of pemetrexed in combination with other agents appears to be warranted.

**Gastrointestinal Cancers**

**Colorectal Studies**

Clinical activity of pemetrexed (without folic acid and vitamin B12 supplementation) in metastatic colorectal carcinoma has been demonstrated in two multicenter trials performed by Cripps et al. [6] and John et al. [7]. The U.S. study used pemetrexed at 600 mg/m² throughout the trial, while the Canadian study reduced the starting dose of 600 mg/m² to 500 mg/m² after dose reductions were required in five of the first nine patients. Toxicities leading to these reductions included neutropenia, febrile neutropenia, mucositis, and rash. In the study by John et al., objective tumor responses were observed in 15% of patients (with 1 CR) and in 17% of patients (with 1 CR) in the study by Cripps et al. The median duration of response was markedly different with 4.3 months in the trial by Cripps et al. and 9.1 months in the trial by John et al. Overall survival was 15.1 months and 16.2 months, respectively. In the study by John et al., 56% of patients experienced grade 3/4 neutropenia, 54% grade 3/4 leukopenia, and 18% grade 3/4 thrombocytopenia. In the study by Cripps et al., of the nine patients who received a starting dose of 600 mg/m², five patients experienced grade 3/4 neutropenia, four patients experienced grade 3/4 leukopenia, and three patients experienced grade 3/4 thrombocytopenia. Hematologic toxicities were less frequent after the dose of pemetrexed was reduced to 500 mg/m². Of the 23 patients receiving this dose, 11 patients developed grade 3/4 neutropenia, seven patients developed grade 3/4 leukopenia, and one patient grade 3/4 thrombocytopenia. One patient died due to neutropenic sepsis. Additional trials investigated pemetrexed in patients with colorectal cancer refractory to 5-FU and 5-FU/irinotecan [30] with only minor responses reported. These studies demonstrated that pemetrexed has activity in metastatic colorectal cancer in a magnitude similar to that of other agents. Studies are under way which will investigate the clinical benefit of combining pemetrexed with other agents active in colorectal cancer, including irinotecan.

**Pancreatic Cancer**

Thus far, only one phase II study has investigated the clinical usefulness of pemetrexed in advanced pancreatic cancer [12]. Miller et al. administered pemetrexed 600 mg/m² to patients with unresectable or metastatic pancreatic cancer. While direct antitumor activity was modest (6% objective response rate), 1 CR lasted 16 months. Also, 40% of patients achieved disease stabilization. Median survival was 7 months, which compares well to the most frequently used chemotherapy with gemcitabine. Principal grade 3/4 toxicities included neutropenia (40%), leukopenia (43%), anemia (19%), and thrombocytopenia (17%). Based on the preliminary observation of activity in pancreatic cancer, combination regimens using pemetrexed and gemcitabine have been initiated and randomized phase III studies appear to be warranted.

**Gastric Cancer**

Celio et al. [9] have reported encouraging preliminary findings when pemetrexed is given to patients with gastric cancer. Several responses have been reported. Initially, pemetrexed 500 mg/m² was administered to six patients, as a 10-minute infusion every 21 days without folic acid and vitamin B12 supplementation. Each of these patients reported at least one grade 3/4 toxicity. Three of these patients died due to treatment-related side effects. Subsequently, seven patients have been given high-dose intermittent folic acid (5 mg daily, days -2 to +2) during pemetrexed treatment and no deaths or serious toxicities have been observed thus far. Although this is a limited series of patients, these data indicate that folic acid supplementation is associated with a decrease in serious side effects, while the antitumor activity
of pemetrexed appears to be preserved. This study is ongoing using the above folic acid dose schedule, and mature results are awaited before final conclusions can be determined.

Other Cancers

Cervical Cancer

Goedhals and van Wijk [11] presented the results of a phase II trial investigating the antitumor activity of pemetrexed (without folic acid and vitamin B₁₂ supplementation) in women with advanced cervical cancer. Initially, patients received pemetrexed 600 mg/m², but with evidence of high toxicity, the dosage was reduced to 500 mg/m² and folic acid supplementation was added. At present, only data for patients receiving 600 mg/m² are available for review. The overall response rate for these patients was 21%, with 71% of patients achieving stable disease. Of the patients with stable disease, 25% had unconfirmed partial responses. However, in this patient population, there was a high incidence of decreasing creatinine clearance that necessitated withdrawal from the study. Grade 3/4 hematologic toxicities included neutropenia (84%), leukopenia (62%), and anemia (35%). Grade 3/4 non-hematologic toxicities included vomiting (8%). Additionally, one death was related to study drug. The clinical benefit obtained with pemetrexed in advanced cervical cancer must be balanced against the compound's toxicity profile. If the reduction in dose and the supplementation with folic acid can moderate the frequency and intensity of adverse events observed here, then pemetrexed may be pursued as a single agent or in combination regimens against cervical cancer.

Bladder Cancer

Pac-Ares et al. [10] investigated pemetrexed (without folic acid and vitamin B₁₂ supplementation) in patients with advanced transitional cell carcinoma of the bladder. The initial starting dose of pemetrexed was 600 mg/m², but subsequently the dose was reduced to 500 mg/m² due to toxicities. Pemetrexed showed remarkable clinical activity with an objective response rate of 30%. Additionally, stable disease was achieved in 35% of patients. Main toxicities included grade 4 neutropenia (35%), grade 3/4 anemia (17%), and grade 3/4 thrombocytopenia (9%). Twenty-two percent of patients developed febrile neutropenia and two patients died from either renal failure or sepsis related to study drug. Pemetrexed thus appears to also be active in advanced bladder cancer.

Head and Neck Cancers

Pivot et al. [8] administered pemetrexed 500 mg/m² (without folic acid and vitamin B₁₂ supplementation) to patients with squamous cell carcinoma of the head and neck with a response rate of 33% and an equal percent achieving stable disease. Grade 3/4 hematologic toxicities were neutropenia (43%) and anemia (12%). Additionally, febrile neutropenia occurred in 13% of the cycles, with one patient death from neutropenic sepsis. These data suggest that pemetrexed is active as a single agent and that combination regimens that include pemetrexed may prove to be clinically beneficial in advanced head and neck cancers.

Phase I Combination Studies

Based on the activity of pemetrexed as a single agent in a number of tumor types, combination activity demonstrated in human tumor xenografts [21], and the unique mechanism of action of pemetrexed, regimens combining this antifolate with other active agents have been explored clinically. Phase I combination regimens investigated thus far have included pemetrexed with platinum-containing agents (cisplatin [31], carboplatin [32], oxaliplatin [33], and gemcitabine [34]). Additional combinations under investigation include pemetrexed with 5-FU, irinotecan, taxanes, and anthracyclines [35-37]. These phase I studies, like the single-agent phase I trials discussed above, determined feasible and alternative scheduling and dosing regimens. The recommended schedule and dosing for the combination of pemetrexed with cisplatin and gemcitabine have been determined. For pemetrexed/cisplatin, the recommended schedule was pemetrexed 500 mg/m² (without folic acid and vitamin B₁₂ supplementation) administered as a 10-minute infusion, followed by a 30-minute wash-out period, and then cisplatin 75 mg/m² administered over a 120-minute period given on day 1 of a 21-day cycle. Thöörmann et al. [31] observed 11 objective responses out of the 40 patients who were administered the day 1 dose and schedule. Responders included patients with head and neck cancer (1 CR and 2 partial responses [PR]), and PR in patients with colorectal cancer (1), mesothelioma (5), and NSCLC (1). Of particular interest, 5 PR were documented from 11 patients assessable with pleural malignant mesothelioma. Based upon these data, a randomized phase III study with pemetrexed/cisplatin versus cisplatin has been initiated.

Final results from the pemetrexed/cisplatin combination by Calvert et al. in patients with mesothelioma [32] are not available at the time of this review. However, in a preliminary report, 27 patients enrolled with malignant mesothelioma treated with pemetrexed/cisplatin (ranging from pemetrexed 400 mg/m², cisplatin AUC 4 to 500/6), have shown toxicities were generally manageable and responses have been noted in 10 out of the 20 evaluable patients to date. Final results of this phase I trial are awaited to define the recommended dose and schedule for this promising combination.

For the pemetrexed/gemcitabine combination, the recommended dose and schedule for future phase II studies...
was gemcitabine 1,250 mg/m² administered on days 1 and 8, with pemetrexed 500 mg/m² administered 90 minutes after gemcitabine on day 8 of a 21-day cycle. Out of 55 evaluable patients, Adjei et al. [34] documented 13 objective responses in a variety of tumor types including gall bladder (2), NSCLC (3), ovarian (2), mesothelioma (1), colorectal (3), breast cancer (1), and adenocarcinoma of unknown primary (1). A phase II study [38] is currently investigating the feasibility of combining pemetrexed with gemcitabine as first-line therapy for locally advanced or metastatic NSCLC using the recommended dose and schedule from the phase I trial.

**PHASE II COMBINATION STUDIES**

To date, there have been two reports of combination regimens with pemetrexed/cisplatin as front-line therapies in locally advanced or metastatic NSCLC [39-40] (Table 4). These studies by Manegold et al. [39] and Shepherd et al. [40] (performed without vitamin supplementation) used similar patient populations and produced similar results. Using the schedule and dosing recommended in the phase I trials, Manegold et al. reported an overall response rate of 39%, with 47% of patients having disease stabilization. Median duration of response was 10.4 months and overall survival was 10.9 months. Shepherd et al. reported an overall response rate of 43%, with one patient achieving a CR. Median duration of response was 5.8 months and overall survival was 7.3 months. Primary toxicities varied between the studies. Manegold et al. reported grade 3/4 toxicities of neutropenia (59%), thrombocytopenia (17%), and diarrhea (6%), while Shepherd et al. reported grade 3/4 toxicities of neutropenia (30%), anemia (20%), and diarrhea (10%). The latter trial also had a 3% incidence of febrile neutropenia. The combination regimen of pemetrexed with cisplatin thus appears to be quite active in NSCLC with a well-tolerated, convenient patient treatment regimen.

**SAFETY AND THE ADDITION OF FOLIC ACID AND VITAMIN B₁₂**

Thus far, 872 patients (without vitamin supplementation) who have been treated with either 500 or 600 mg/m² on the once every 21-day schedule in phase II trials are evaluable for a safety analysis. Grade 3 and 4 hematologic toxicities included neutropenia (23% and 27%, respectively) and thrombocytopenia (8% and 7%, respectively). The frequency of serious infections, as well as bleeding episodes, has been low (grade 4 infection of 2% and serious episodes of bleeding <1%). Grade 3 and 4 skin rash was experienced by 5% and 2% of patients, respectively. However, prophylactic dexamethasone has been reported to improve or prevent the rash in subsequent cycles. As seen in clinical studies of other antifolates, transient grade 3 and 4 elevations of liver transaminases were common, but not dose-limiting. There have been no cases of persistent transaminase elevation. Although pemetrexed has clinically significant activity in a number of tumor types, the high incidence of hematological toxicity as well as treatment-associated fatalities associated with this antifolate has, until recently, limited its prospects as a major antitumor agent. Studies with other antifolates inhibiting DHFR and TS have suggested that poor nutritional status contributes to the likelihood that a patient will experience severe toxicity when exposed to these drugs [41-44]. More specifically, these studies have investigated the relationship between folic acid and the toxicity of these agents and have concluded that the addition of folic acid significantly reduces toxicity while preserving the antitumor activity of the drug. In a study conducted by Morgan et al. [41], patients with rheumatoid arthritis who were given a combination of MTX and folic acid experienced less than half the toxicities as compared to those toxicities seen in the placebo group. Subsequently, a multivariate analysis involving 246 phase II patients with tumors of the colon, breast, pancreas,
and esophagus treated with pemetrexed was conducted to assess the relationship of vitamin deficiency markers, drug exposure, and prespecified baseline patient characteristics to toxicity following therapy with pemetrexed [45]. In this study, homocysteine was specifically selected because a high homocysteine level has been shown to correlate with low folate pools [46, 47]. These results demonstrated a statistically significant correlation between baseline plasma homocysteine concentrations and febrile neutropenia ($p < 0.0001$), grade 4 neutropenia ($p = 0.0191$), grade 4 thrombocytopenia ($p < 0.001$), and grade 3 or 4 diarrhea ($p < 0.0001$). Concurrent with this analysis, preclinical data supported the notion that oral folate supplementation markedly reduced toxicities in mice while maintaining antitumor efficacy [48]. Based on these and other preclinical findings, a phase I study combining pemetrexed and high-dose intermittent oral folic acid (5 mg on days –2 to day +2 of every cycle) has been initiated. While this trial is currently still ongoing, preliminary results indicate that the addition of folic acid ameliorates toxicities permitting dose escalations of pemetrexed up to at least 925 mg/m$^2$ in heavily pretreated patients [49]. As noted earlier, a recent report of an ongoing phase II trial that administered pemetrexed with or without oral folic acid (5 mg on days –2 to day +2 of every cycle) to patients with gastric cancer also indicated a marked difference in the safety profile following folic acid supplementation [9]. As a consequence, all patients who enroll in pemetrexed studies now receive folic acid (350-1,000 µg orally/day starting 1 week prior to the first drug administration) and vitamin B$_{12}$ (1,000 µg i.m. every 3 cycles) while they remain on study. Preliminary safety data suggest that these measures have considerably improved the safety profile of pemetrexed (Table 5). However, final data addressing both safety and efficacy using the lower doses of folic acid and vitamin B$_{12}$ are yet to mature.

### Future Perspectives

Early and preliminary evidence suggests that the addition of low-dose folic acid and vitamin B$_{12}$ decrease the frequency of severe clinical toxicities of pemetrexed and allows this agent to be applied clinically with a markedly improved safety margin. Because of the multiple aspects involved in the development of pemetrexed, future trials will not only be focused on randomized phase III trials. While phase III trials are under way in mesothelioma and NSCLC, additional trials will address the question of whether molecular markers in patients’ tumor tissue can be used to predict sensitivity or resistance to pemetrexed. In addition, the impact of pemetrexed on the expression pattern of its main target enzymes needs to be further explored in order to better understand secondary resistance to this agent. Additionally, the relative role of folic acid compared to vitamin B$_{12}$ should be scrutinized, whether there is an effect of these vitamins on antitumor efficacy, and whether vitamin supplementation may allow for a clinically meaningful dose escalation beyond 600 mg/m$^2$.

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