Biochemical Pharmacology of Pemetrexed

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Pemetrexed (Alimta) is a novel antitumor agent that inhibits the folate-dependent enzymes thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase. Pemetrexed has demonstrated activity in clinical trials in a variety of tumor types, including lung, breast, colon, mesothelioma, pancreatic, gastric, bladder, head and neck, and cervix. Pemetrexed is rapidly metabolized into active polyglutamate forms that are potent inhibitors of several tetrahydrofolate cofactor-requiring enzymes critical to the synthesis of purines and thymidine. Functionally, pemetrexed acts as a prodrug for its polyglutamate forms. Two different transporters are known to take extracellular folates, and some antifolates, into the cell. These are the reduced folate carrier and the folate receptor. One of the many attributes that make pemetrexed unique is that methodology has been developed to eliminate and control many of its associated clinical toxicities. Multivariate analyses demonstrated that pretreatment total plasma homocysteine levels significantly predicted severe thrombocytopenia and neutropenia, with or without associated grade 3/4 diarrhea, mucositis, or infection. Routine vitamin B12 and folic acid supplementation have resulted in decreased frequency/severity of toxicities associated with pemetrexed without affecting efficacy, making this novel antifolate a safe and efficacious anticancer agent.

Antimetabolites are active chemotherapeutic agents in the treatment of solid tumors and hematologic malignancies. The three main categories of antimetabolites that exist include folate antagonists (antifolates), purine analogs, and pyrimidine analogs. Antifolates were first used in the late 1940s with the discovery of aminopterin, and soon after, methotrexate.[1] Since these discoveries, folate-dependent pathways have been an area of interest in the development of new and effective anticancer agents. The antifolates interfere with the binding of natural folate cofactors to important biosynthetic enzymes, such as thymidylate synthase (TS), dihydrofolate reductase (DHFR), glycinamide ribonucleotide formyl transferase (GARFT), and aminopurine riboside carboxyltransferase (AICARFT). Inhibition of these enzymes results in impeded synthesis of nucleotides, which ultimately interferes with DNA and RNA synthesis.[2] Resistance to antimetabolites can occur by many mechanisms, including use of salvage pathways, gene amplification of the target enzymes, and reduced cell-membrane transport of the drug. Pemetrexed (Alimta) is approved by the US Food and Drug Administration (FDA) for use in combination with cisplatin for the treatment of malignant pleural mesothelioma and second-line non-small-cell lung cancer. It is also advanced in clinical development in other tumor types. It is an antifolate agent that inhibits at least three key folate-requiring enzymes involved in DNA synthesis: TS, DHFR, and GARFT. Pemetrexed was associated with sporadic toxicities in early clinical trials; however, methodology to eliminate and control many of these toxicities has been developed. This article will discuss the biochemical pharmacology of pemetrexed action and its modulation by physiological folates, the clinical significance of the multiple mechanisms of action, and the impact of folic acid and vitamin B12 supplementation on dosing, toxicity, and efficacy of pemetrexed. The unique biochemical pharmacology of pemetrexed probably contributes to its clinical activity, and in particular, to its broad spectrum of activity in various tumor types.
Folic acid, in the form of 5,10-methylene-tetrahydrofolic acid, is essential for the synthesis of precursors of purine nucleotides for DNA and RNA, and for the synthesis of thymidine nucleotide that is incorporated exclusively into DNA. Folic acid, in the fully reduced form, picks up one-carbon units from amino acids and transfers them into the synthetic pathways for the purines. Also, when deoxyuridine nucleotide is converted to thymidine nucleotide by the thymidylate synthase enzyme, the folate cofactor provides the carbon atom and is oxidized to dihydrofolate during the reaction. The various antifolates and the other commonly used drugs that interact with folate pathways are illustrated in Figure 1. DHFR is inhibited by methotrexate, TS is inhibited by fluorouracil (5-FU), and DHFR, TS, and GARFT are inhibited by pemetrexed.[2]

Pemetrexed is an analog of folic acid (Figure 2). Pemetrexed, as a single agent and in combination regimens, has demonstrated activity in clinical trials in a range of solid tumors, including mesothelioma,[3] lung,[4-8] breast,[9,10] colon,[11-13] pancreatic,[14,15] gastric,[16] and bladder, head and neck, and cervix.[17] The toxicities reported in clinical studies of pemetrexed include dose-limiting myelosuppression, rash, mucosal toxicities, elevations in transaminases, and asthenia.

**Mechanism of Action** Pemetrexed is a novel pyrrrol[2,3-d] pyrimidine-based antifolate antimetabolite, with multiple enzyme targets that are involved in both pyrimidine and purine syntheses. Pemetrexed was originally investigated as a thymidylate synthase inhibitor, but early data indicated that two other enzymes, DHFR and GARFT, were also inhibited by pemetrexed. Pemetrexed is rapidly metabolized into active polyglutamates derivatives. These are potent inhibitors of several tetrahydrofolate (THF) cofactor-requiring enzymes critical to the synthesis of purines and thymidine nucleotides. Most antifolates and all natural folates are converted intracellularly to polyglutamates by the enzyme folypolyglutamate synthase (FPGS). Pemetrexed is one of the most avid substrates for FPGS.[18] Polyglutamates of pemetrexed are more potent inhibitors of the two target enzymes, TS and GARFT, than are the monoglutamate forms. Also polyglutamates are retained in the cell longer due to their negative charges, thus increasing their potency due to a more prolonged inhibition of the target enzymes. The addition of pemetrexed to cells in vitro leads to rapid buildup of polyglutamates that result in the suppression of TS. These polyglutamates may also inhibit GARFT and thus purine syntheses.[18] There are two features of pemetrexed pharmacology that may contribute to its selective antitumor effect. First,
methylthiadenosine phosphorylase (MTAP) is a purine salvage pathway that is responsible for recycling purines within the cell. Cells that do not contain this pathway are more dependent on producing their own purines than those that utilize MTAP. MTAP is located on the 9P21 genome next to the P16 putative tumor suppressor gene. A mutation in the 9P21 region that leads to a deletion of P16 function is also likely to cause a loss of function of the adjacent MTAP gene, making the tumor cell more dependent on purine synthesis than its normal cellular counterpart. Because pemetrexed polyglutamates inhibit GARFT and this de novo purine synthesis, cells with MTAP deletions may be expected to be particularly sensitive to pemetrexed. In patients with non-small-cell lung cancer, approximately 38% of tumors have been reported to show MTAP deletion, regardless of histologic subtype, which may lead to enhanced action of pemetrexed in those patients. The second feature that may contribute to the selective antitumor efficacy of pemetrexed relates to the specific folic acid transporters that may target pemetrexed towards some malignant cell types. For example, the folate receptor has been shown to be overexpressed in some tumors and also to be capable of transporting pemetrexed into the tumor cell. This is discussed in more detail below. Pemetrexed thus possesses some characteristics that are more closely related to targeted agents than traditional agents, when the mechanism of action is examined in detail.

**Pharmacokinetics**

End product reversal studies performed in cell culture indicate that the growth inhibition induced by low concentrations of pemetrexed may be reversed by the addition of thymidine alone, implying that the effective target is TS. However, higher concentrations (> 0.1 μM) required in addition a source of purine to overcome the growth inhibition, implying that, at these higher concentrations, the inhibition of GARFT was also becoming significant.[19] Data derived from patients on numerous phase II trials of pemetrexed demonstrated that the pharmacokinetics of pemetrexed behaved in a highly predictable fashion with an initial/distribution half-life of 0.63 hours, and an effective terminal/elimination half-life of 2.73 hours. The volume of distribution at steady state was 16.51 L. In particular, the plasma level exceeded 0.1 μg/mL for 12 hours in all patients and for 24 hours in many. Pemetrexed is not highly protein bound in plasma so it appears that the levels achieved clinically exceed by a wide margin those necessary to cause inhibition of GARFT in cell culture.[20]

**Folate Transport**

The efficacy of antifolates will be affected by their transport into the cell. Two different transport systems are known that transport folates and some antifolates into cells. Following transport into the cell, conversion of the antifolate to its polyglutamate derivates both renders it more potent against the target enzymes and causes longer retention in the cell. The hydrolysis of antifolate polyglutamates that can efflux through the cell membrane may also be an important feature in some cell types. In all of these processes, there is the potential of natural folates to compete with antifolates, leading to the expectation that the nutritional status of the patient with respect to folic acid may be an important determinant of toxicity. One of the best known folate transporters is the reduced folate carrier that has a high capacity and transports tetrahydrofolates, methotrexate, raltitrexed (Tomudex), and pemetrexed. Although the reduced folate carrier has a high capacity, it has a relatively low affinity for most folates and antifolates and its role in the transport of folic acid at physiological levels is uncertain.

**Figure 2: Pemetrexed—Structure.**

The second receptor is a high-affinity glycosylphosphatidylinositol-anchored receptor for folate that works by binding the molecules on the cell surface and undergoing clustering to form structures called caveolae that are internalized.[21] Although this receptor, which is known as the folate receptor-alpha, has a high affinity, it has a much lower capacity than the reduced folate carrier. Until
recently, the folate receptor has not been considered to be important for drug action because of its low capacity; however, there is evidence to suggest that it may be an important factor in the action of some agents such as pemetrexed and CB 3717. In the case of mesothelioma in particular, there is evidence that the folate receptor-alpha, which potentially transports pemetrexed, is highly overexpressed.[22] Safety The safety profile is an important feature, as clinicians have reported safety problems and sporadic drug-related deaths with pemetrexed and some other antifolates. One of the many unique characteristics of pemetrexed is the methodology that has been developed to eliminate and/or control its associated toxicities. Some of the problems with toxicity occurred with pemetrexed in early studies that were conducted before the routine use of vitamin B$_{12}$ and folic acid supplementation was included in the study design.[10] Grade 4 neutropenia with grade 3/4 infection, grade 3/4 diarrhea, or grade 3/4 mucositis were some potentially life-threatening toxicities seen in these early studies. Also, in some cases, lethal complications were seen in these early studies, with a 4% incidence of drug-related deaths.[23]

<table>
<thead>
<tr>
<th>Table 1</th>
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<td><strong>Comparison of Single-Agent Phase II Data</strong></td>
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<table>
<thead>
<tr>
<th></th>
<th>Raltitrexed</th>
<th>Pemetrexed</th>
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<tr>
<td></td>
<td>Number of Eligible Pts</td>
<td>Response Rate</td>
</tr>
<tr>
<td>Colorectal (1st line)</td>
<td>173</td>
<td>26%[21]</td>
</tr>
<tr>
<td>Breast (failed hormonal therapy)</td>
<td>43</td>
<td>26%[25]</td>
</tr>
<tr>
<td>Pancreas (1st line)</td>
<td>42</td>
<td>5%[26]</td>
</tr>
<tr>
<td>NSCLC (1st line)</td>
<td>22</td>
<td>9%[27]</td>
</tr>
<tr>
<td>NSCLC (2nd line)</td>
<td>21</td>
<td>0%[29]</td>
</tr>
<tr>
<td>Ovarian (2nd line)</td>
<td>31</td>
<td>7%[31]</td>
</tr>
<tr>
<td>Gastric (2nd line/ 1st line)</td>
<td>33</td>
<td>0%[32]</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>33</td>
<td>0%[27] (minor responses only)</td>
</tr>
<tr>
<td>NSCLC (2nd line)</td>
<td>21</td>
<td>0%[27]</td>
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NSCLC = non–small-cell lung cancer; SCLC = small-cell lung cancer. Folic Acid and Vitamin B$_{12}$ Because pemetrexed is an antifolate, one might expect the patient's folic acid status to predict toxicity. However, measurement of either plasma or red cell folate did not provide information that allowed the patients who developed toxicity to be identified in advance.[23,13,24,25] The reasoning that folic acid levels are not very predictive for antifolate toxicities is that plasma or red cells may be associated with the fact that folic acid levels actually do not reflect the functional folate status of the patient. (These are the folate pathways that were involved in generating the precursors for cell replication for DNA synthesis.) The conversion of homocysteine to methionine is an important cellular process because methionine is involved in a number of methylation reactions. The enzyme methionine synthase is responsible for this conversion and used 5-methyltetrahydrofolate as a substrate. It is also a vitamin B$_{12}$-dependent enzyme. If the patient is mildly vitamin B$_{12}$- or folate-deficient, this enzyme will convert homocysteine to methionine more slowly, resulting in an elevation of the plasma homocysteine level. Thus, the
plasma homocysteine level is a sensitive surrogate marker for the folate status of the patient. **Rationale for Vitamin Supplementation** In the early phase II studies conducted prior to routine supplementation with vitamin B$_{12}$ and folic acid, the pretreatment homocysteine levels correlated very strongly with the neutropenia, thrombocytopenia, and diarrhea reported with pemetrexed administration. These data, coupled with information based on experience with lometrexol (a GARFT inhibitor), led to the collection of several vitamin deficiency markers from patients accrued on clinical trials during the early phase II clinical development of pemetrexed. Two multivariate analyses were conducted that demonstrated that pretreatment total plasma homocysteine (tHcy) levels significantly predicted severe thrombocytopenia and neutropenia, with or without associated grade 3/4 diarrhea, mucositis, or infection. During this period, a drug-related death rate of 7% was observed early in a phase III mesothelioma trial comparing pemetrexed plus cisplatin with cisplatin monotherapy. A decision was made in December 1999 that required all patients receiving pemetrexed be treated concomitantly with folic acid and vitamin B$_{12}$ to minimize the risk of severe toxicity. The rationale for administering it to all patients rather than selecting patients follows: although there is a very strong correlation of toxicity with elevated homocysteine levels that suggest that it is indicative of poor folate status for the patient, there is not a defined level or cutoff point that allows precise identification of individual patients. After this clinical practice was introduced, the percentage of toxic deaths decreased drastically. The percentage of hematologic grade 3 and 4 toxicity decreased to approximately 6%, grade 4 neutropenia to about 2%, and grade 4 thrombocytopenia disappeared. The use of routine vitamin B$_{12}$ and folate supplementation with pemetrexed thus makes pemetrexed an extraordinarily safe drug, without apparently interfering with its antitumor activity. **Clinical Activity of Pemetrexed** Does pemetrexed work better in various tumor types than other agents, including other antifolates? Based on clinical trial data, the only drug that one may compare presently with pemetrexed is raltitrexed, a water-soluble quinazoline antifolate that specifically inhibits the TS enzyme. Table 1 summarized response rates that have been reported for both drugs administered as single agents in clinical trials, albeit not by direct comparison in the study design. In a majority of the trials shown in Table 1, investigators reported higher response rates with pemetrexed than with raltitrexed. **Conclusion** Pemetrexed is a novel antifolate that differs from related, licensed, and experimental drugs both in cell membrane transport and intracellular loci. Pemetrexed, when administered with folic acid and vitamin B$_{12}$ supplementation, is safe, and has a very low level of subjective and objective toxicity. Pemetrexed possesses substantial anticancer activity in a range of solid tumors, and may be given in combination with a number of major anticancer agents. As approved by the FDA, pemetrexed in combination with cisplatin significantly prolonged survival in patients with mesothelioma; exploration of other indications is ongoing and has also led to the approval of the drug in second-line non-small-cell lung cancer. Vitamin B$_{12}$ and folic acid supplementation does not adversely affect, and might improve, activity in stomach cancer and mesothelioma. Pemetrexed demonstrated interesting activity in breast cancer; however, there have been some data suggesting that vitamin supplementation may reduce the response rate in breast cancer patients. Further investigation in various tumor types is warranted.

**Disclosures:** The authors have no significant financial interest or other relationship with the manufacturers of any products or providers of any service mentioned in this article.

**References:**

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