Review Article

The current and future role of the multitargeted antifolate pemetrexed in non–small cell lung cancer

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ABSTRACT Pemetrexed was approved for the treatment of relapsed or chemotherapy refractory non-small cell lung cancer patients, as it produced similar response and survival outcomes and less toxicity as compared to taxotere. Pemetrexed in combination with platinum analogs or with gemcitabine or vinorelbine, produce equivalent responses and overall survival results compared to combinations of platinum analogs with other drugs. The role of bevacizumab and the inhibitors of epithelial growth factor receptor also should be evaluated in selected patients with NSCLC treated with pemetrexed combinations.In this review, the mechanisms of action, clinical activity of the multitargeted antifolate pemetrexed for previously treated patients with NSCLC, is presented. Phase II and III single-agent pemetrexed studies, the combination of pemetrexed with other drugs and the future outlook of this compound in newer chemotherapy regimens is reviewed.

KeyWords: pemetrexed; non-small cell lung cancer

Introduction

Despite significant advances in the treatment of non-small cell lung cancer (NSCLC) over the past several years, the prognosis for patients with advanced disease is still very poor. NSCLC patients treated with standard regimens containing cisplatin or carboplatin have a median survival of 8 11 months for patients with Stage IV disease and 5 7 months for patients with relapsed disease. Newer agents such as the epithelial growth factor receptor (EGFR)-targeted lung cancer drugs, erlotinib and gefinitib, are effective in patients with activating EGFR mutations and have been approved for patients with locally advanced or metastatic NSCLC after chemotherapy failure. The addition of the vascular endothelial growth factor (VEGF) inhibitor, bevacizumab, to paclitaxel/ carboplatin recently was reported to improve both the response rate and the survival of patients with non-squamous NSCLC (from 10.2 to 12.5 months) (1).

In this review, the mechanisms of action, clinical activity of the multitargeted antifolate pemetrexed for previously treated patients with NSCLC, is presented. Phase II and III single-agent pemetrexed studies, the combination of pemetrexed with other drugs and the future outlook of this compound in newer chemotherapy regimens is reviewed.

Evolution of Antifolates as Chemotherapeutic Agents

The history of the antifolates as anticancer drugs dates back to the 1940s when Lederle Laboratories discovered the structure of the active bacterial growth promoting principal isolated from beef liver, now known as folic acid (2). In the process, closely related compounds were synthesized; some of which were found to interfere with folic acid dependent bacterial growth and negated the antianemic and growthpromoting effects of folic acid in chicks (3). One of the folic acid analogues was aminopterin (4-amino-folic acid).

At that time, there was no specific treatment for children with acute leukemia beyond supportive care. Based upon the observation by Lewisohn et al. at the Mt. Sinai Hospital in New York City that "folic acid concentrate" caused regression of breast cancer in mice (4), clinical investigators led by Sidney Farber at the Children's Hospital in Boston obtained folic acid polyglutamate conjugates from Lederle Laboratories in an attempt to reproduce these findings in children with cancer (5). To their surprise, the administration of folic acid exacerbated the disease in children with leukemia. This suggested that the proliferation of leukemia cells might be limited by the supply of the vitamin, folic acid, or, as learned later, its active tetrahydrofolate (THF) cofactor metabolites. Thus, Sidney Farber obtained folic acid antagonists from Y. SubbaRow et al.

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at Lederle Laboratories, and in a historic article in the New England Journal of Medicine in 1948 (6), Farber et al. reported that one of these agents, aminopterin, produced complete remissions in children with acute leukemia. At the time, a tent city grew on Boston Commons of families who had brought their children with leukemia to be treated with this miracle drug.

A year later, 2-amino-10-methyl-folic acid (amethopterin, methotrexate) was introduced (7), and because of its purported lesser toxicity, it replaced aminopterin in the clinics. Shortly thereafter, the antifolate action of methotrexate was shown to be due to its potent inhibition of dihydrofolate reductase (DHFR; ref. 8). Over the next three decades, there followed an intensive drug synthesis effort to identify a ''better'' methotrexate. However, methotrexate was simply a remarkable drug, and none of the hundreds of DHFR inhibitors made proved to be superior. Remarkably, methotrexate remains an important component of the treatment regimens for childhood acute lymphoblastic leukemia (9)and continues to be used for the treatment of a variety of neoplasms as well as rheumatoid arthritis (10) and psoriasis (11).

The understanding of the mechanism of methotrexate action and its selectivity was broadened when it was recognized in the mid-1970s that, like the physiologic folates, methotrexate was metabolized to polyglutamate derivatives in cells (12, 13), and that this metabolism permitted high levels of accumulation of active antifolate that markedly prolonged their retention in tumor cells (14 17), resulting in enhanced chemotherapeutic efficacy. Polyglutamation was also shown to be an important element in the selectivity of this agent. These derivatives accumulate to a much greater extent in susceptible tumor cells than in progenitor cells of the bone marrow or the intestinal tract (18 21). Hence, effects of the drug tend to be transient in the latter host cells as the monoglutamate level increases and decreases with the blood level, whereas methotrexate effects in tumor are sustained due to the accumulation and retention of the polyglutamate congeners. This is an inherent selective advantage for any antifolate that forms these derivatives in cells. This metabolic transformation also resulted in an altered spectrum of activity for methotrexate.

As a monoglutamate, methotrexate is a highly potent inhibitor of DHFR, but its polyglutamate derivatives are also inhibitory to 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase and thymidylate synthase (22 24). This insight led to an important new perspective on antifolate actions: the realization that the polyglutamate derivatives and not the parent drug could be the active agents (25). There followed a new focus on the identification of antifolates that achieved their major activities in their polyglutamate forms.

The key to the further development of antifolates came when efforts were directed to the development of inhibitors of THF cofactor dependent biosynthetic enzymes by avoiding the 2,4-diamino-pteridine pharmacophore that inevitably directly drug to the active site of DHFR. The Imperial Cancer Research Laboratories at Sutton, England and their collaborators at the then ICI Pharmaceutical branch, now AstraZeneca Pharmaceuticals, led a successful effort to improve the inhibition of thymidylate synthase shown by the 5,8-dideazafolates. The key intermediate in this development process was the remarkable compound 10-propar gyl-5, 8-dideazafolate or CB3717 (26).

This compound furnished proof-of-principle that antifolates in their polyglutamate forms could be potent inhibitors of THF cofac tor dependent enzymes, in this case, thymidylate synthase, and use ful chemotherapeutic agents. This ultimately led to the discovery and development of raltitrexed or Tomudex, which has been in clinical use in Europe and elsewhere but was never approved in the United States (27).

This review will describe the evolution of the development of the third antifolate (after aminopterin and methotrexate) to achieve approval for the treatment of cancer in the United States (peme trexed) and the several novel properties of this agent.

Origins of Pemetrexed

The synthesis of pemetrexed evolved from a collaboration between E.C. (Ted) Taylor at Princeton and a team of chemists at Eli Lilly led by Chuan (Joe) Shih as part of an effort to develop antifolate inhibitors of THF cofactor dependent enzymes. This drug discovery program stemmed from earlier collaborative development at Princeton, Yale, the University of Southern California, and Lilly of the prototypical inhibitor of de novo purine synthesis, 5,10-dideazatetrahydrofolate (lometrexol; 28). The structure of lometrexol had been suggested by G. Peter Beardsley to his former mentor Ted Taylor as a potential antifolate, initially thought to be a likely inhibitor of thymidylate synthase; to their surprise, it was not, but it was a potent inhibitor of tumor cell growth (29). Cell culture experiments (29, 30), then direct enzymology on isolated (30, 31) and recombinant (32) proteins in the laboratory of one of the authors, showed that lometrexol was the first potent antifolate inhibitory to purine synthesis due to its direct suppression of glycinamide ribonucleotide transferase (GARFT)activity. Because this was not a nucleotide analogue, it was considered not to pose a mutagenic nor carcinogenic threat. Subsequently, second- and third-generation GARFT inhibitor analogues were synthesized in an attempt to improve on the therapeutic and toxicologic profiles of lometrexol (33 37).

Lometrexol posed a problem; it was made by a complex 23-step process, and the product was a mixture of diastereomers about carbon 6. The decision was made to separate the diastereomers before clinical trials to meet Food and Drug Administration requirements. However, the fractional crystallization used to obtain a pure diastereomer proved to have low yield (30), and, ultimately, another approach was pursued: exploration of related compounds that would eliminate chirality at the 6-position. One strategy replaced the 5-deazapteridine ring of lometrexol with a pyrrolopyrimidine ring, resulting in LY231514 (38), later to be known as pemetrexed. The outcome was unexpected. Cell culture end-product protection

experiments and enzymology indicated that this compound was, primarily, a potent inhibitor of thymidylate synthase, although there was weaker inhibition at other enzyme targets (38, 39).

Mechanism of action

Pemetrexed, a pyrriol (2,3-d) pyrimidine antifolate (Figure 1) is unique among the folate antagonists in that it is a powerful inhibitor of not only thymidylate synthase (TS) but also dihydrofolate reductase (DHFR) and the purine synthetic enzyme, glycinamide ribonucleotide formyltransferase (GARFT) (Fig.2). Like methotrexate (MTX), pemetrexed enters cells primarily via the reduced folate carrier (RFC), a low affinity high capacity active transporter. An additional route of transport used by pemetrexed, but not MTX, is via the folate receptor (folate binding protein, FBP), a lowcapacity, high affinity transporter, highly expressed on some epithelial tumors, particularly on ovarian cancer and mesothelioma (40). It is possible that the activity of pemetrexed in this latter tumor is enhanced due to the high expression level of the FBP (vide infra). Once inside the cell, pemetrexed is an excellent substrate for the ubiquitous enzyme folylpolyglutamate synthetase (FPGS) and is converted to tri- and penta-glutamates. This conversion results in even more potent inhibition of TS and GARFT. The main target of pemetrexed is considered to be TS, although in a colorectal cancer cell line, resistant to specific TS inhibitor raltitrexed, due to TS amplification, pemetrexed is still active, indicating that inhibition of DHFR and of GARFT plays a role in its activity (41). At high pemetrexed concentrations, hypoxanthine in addition to thymidine is necessary to rescue tumor cell lines from cytotoxicity (42). These observations provide further evidence that inhibition of purine synthesis is an important mechanism of action in pemetrexed's cytotoxicity.

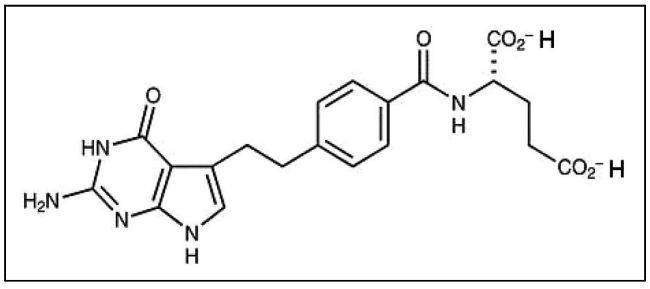

Fig.1 Structure of Pemetrexed. Pemetrexed is metabolized intracellularly to polyglutamates. Glutamate residues are added via linkage to the gamma–carboxyl and up to 5 or more glutamates may be added by folylpolyglutamyl synthetase (FPGS) enzyme.

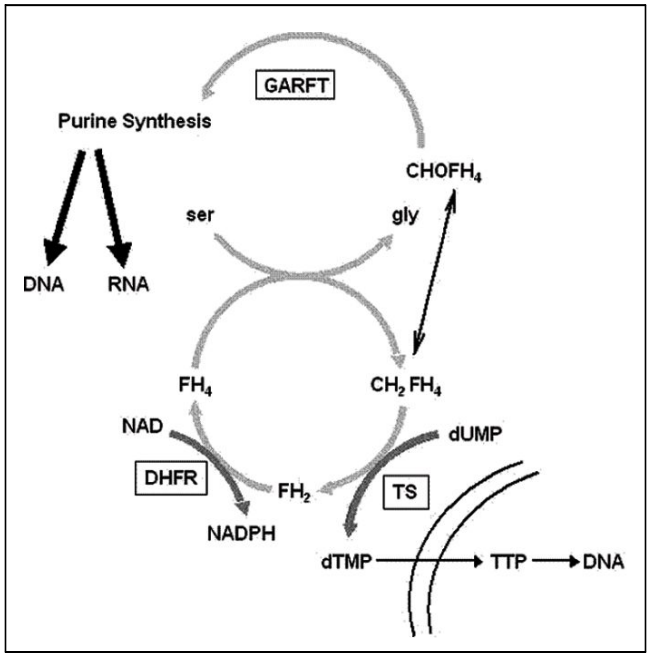

Fig.2 Sites of inhibition of DNA and RNA synthesis by Pemetrexed and its polyglutamates. TS: thymidylate synthase; DHFR: dihydrofolate reductase; GARFT: glycinamide ribonucleotide transformylase; dUMP: deoxyuridine monophosphate; TMP: thymidylate; FH2: dihydrofolate; FH4: tetrahydrofolate; CH2FH4: 5–10 mthylene tetrahydrofolate; CHOFH4: 10–formyl tetrahydrofolate.

Contrasting Methotrexate and Pemetrexed

Although pemetrexed has been viewed by some as a "super methotrexate," there are profound differences in the properties of these drugs. Methotrexate, as a monoglutamate, is a potent inhibitor of DHFR, and after a brief exposure to this drug, there is essentially complete suppression of this enzyme, the rapid conversion of THF cofactors to dihydrofolate resulting in THF cofactor depletion and cessation of THF-dependent biosynthetic processes due to this metabolic sequestration of folates as dihydrofolate (43). Methotrexate polyglutamate derivatives slowly build up in cells and have the potential for direct suppression of 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase and thymidylate synthase (22 24), but these effects are minor compared with the inhibition of DHFR and, following inhibition of DHFR by methotrexate, thymidylate synthase and purine synthesis have already ceased due to cofactor depletion. These polyglutamate derivatives become important during "leucovorin rescue" when they selectively suppress utilization of the added reduced folates in tumors in which they accumulate, but not in proliferative cells of the bone marrow and gut in which they accumulate to a far lesser extent (18 21, 44).

The metabolic sequelae of methotrexate inhibition of its primary target enzyme are also quite different from pemetrexed. When methotrexate inhibits the growth of tumor cells, cytosolic THF cofactors available as one-carbon donors accumulate as dihydrofolate and compete with drug for the target enzyme. Because of this and the marked excess of DHFR in almost all tumor cells over what is needed to optimally drive thymidylate synthesis, methotrexate has no effect on cellular proliferation until in excess of 95% of enzyme

is inhibited. As methotrexate blood levels decrease, cell growth resumes to uninhibited rates as ≥ 5% of DHFR becomes available for dihydrofolate reduction. Sustained inhibition of DHFR requires the accumulation of substantial levels of intracellular methotrexate polyglutamates, a slow process for this drug (43).

The situation for pemetrexed is quite different. When pemetrexed inhibits its target enzymes, there is no redistribution of folates within cells; the levels of folate substrates are not changed (45). In addition, inhibition of thymidylate synthase or of GARFT directly decreases the growth rate of tumor cells, as both seem to be limiting to DNA synthesis; that is, inhibition of 50% of either of these enzymes would decrease growth rates of tumors by 50%. Although the rate of pemetrexed transport into cells is controlled by the membrane transport processes, the key event seems to be the very rapid polyglutamation of pemetrexed that begins when the drug is present at very low concentrations in the cytosol due to the superior kinetic characteristics of pemetrexed with FPGS (Fig. 3; refs. 46, 47). The Km for pemetrexed is one hundredth that of methotrexate for this enzyme (47). Hence, cellular levels of pemetrexed polyglutamates sufficient to completely block thymidylate synthase activity rapidly accumulate. Suppression of GARFT comes later because it requires 50-fold higher concentrations of pemetrexed polyglutamates. Because suppression of thymidylate synthase blocks the formation of dihydrofolate, the requirement for DHFR is obviated (48); hence, any potential inhibitory effect of pemetrexed at the level of DHFR is superfluous. Neither amplification of DHFR nor loss of RFC function seems to be associated with acquired pemetrexed resistance in vitro; more common are mutations or down-regulation of FPGS (49, 50)

and increased thymidylate synthase expression (51, 52). Hence, although both methotrexate and pemetrexed result in suppression of THF-dependent reactions, the effects of methotrexate are achieved primarily by depletion of cellular THF cofactors, whereas

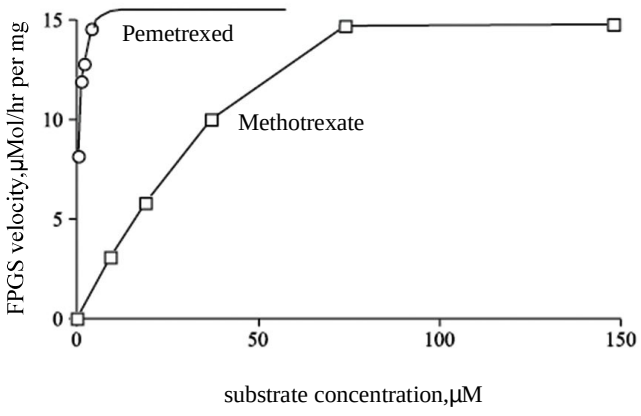

Fig.3 Comparison of pemetrexed and methotrexate polyglutamylation kinetics. Pemetrexed has a far lower Michaelis constant for human recombinant FPGS than does methotrexate. Experimental techniques are detailed in ref. (46)

pemetrexed acts by a direct block of THF cofactor dependent biosynthetic enzymes without an effect on the level of cellular THF cofactor pools. Likewise, the mechanisms by which tumor cells develop resistance to these agents are different. This suggests that acquired resistance to methotrexate in the clinical setting is unlikely to be associated with collateral resistance to pemetrexed and vice versa.

Contrasting Pemetrexed and Raltitrexed

Raltitrexed is an antifolate that has much in common with pemetrexed. Both agents have comparable FPGS activity (47), and their higher polyglutamate derivatives have comparable inhibition constants for thymidylate synthase (27, 46). Pemetrexed has an additional target at higher concentrations, and the paradigm has been that this should provide a therapeutic advantage. Yet, the IC50 for raltitrexed against in vitro tumors is one sixth that of pemetrexed (53), and the maximum tolerated dose for raltitrexed in humans is 2 orders of magnitude lower than that of pemetrexed (54). What can account for this marked difference in activity? Although raltitrexed seems to have only a slightly higher affinity for RFC (Ki f 2 Amol/L versus Ki f 5 Amol/L for pemetrexed; ref. 55), a direct measurement of raltitrexed transport has not been possible due to the unavailability of radiolabeled drug (55). However, there is evidence for a role for transport in the difference between these drugs. In wild-type HeLa cells growing in 5-formyltetrahydrofolate or folic acid, the IC50 for raltitrexed is one sixth to one fifteenth that of pemetrexed (56). However, in HeLa R5 cells that lack genomic RFC, the IC50 for raltitrexed is more than 10 times greater than that of pemetrexed in medium containing 5-formyltetrahydrofolate (56) and more than 4 times greater in folic acid medium (56, 57). This may be due to the much lower affinity of raltitrexed relative to pemetrexed for the PCFT that is the predominant pathway for uptake into cells under these conditions (54). In a variant of HeLa R5 cells, in which RFC is absent and the residual low-pH route is markedly diminished, the IC50 for these agents is identical during growth in folic acid (the cells grow poorly in 5-formytetrahydrofolate under these conditions; 1,000 F 60 for both; ref. 57). Hence, the differences in activity of raltitrexed relative to pemetrexed seems to be due, largely, to transport differences that involve RFC and the PCFT.

Contrasting 5–Fluorouracil and Pemetrexed

The mechanisms of action of 5-fluorouracil (5-FU) and its deoxynucleoside 5-fluorodeoxyuridine derivative have been studied for half a century. A tremendous amount of information is available on the disposition of these agents in cells, their biochemical effects, their mechanisms of cytotoxicity, and the spectrum and limitations of their clinical effects (59). 5-FU is converted intracellularly to its active metabolites 5-fluoro-dUMP (FdUMP)and 5-fluoro-UTP (59). These derivatives disrupt DNA synthesis by inhibition of thymidylate synthase (FdUMP) and RNA function by mis

incorproration of 5-fluoro-UTP into RNA species. Both mechanisms contribute to the effects of 5-FU, although, when 5-FU is used in combination with folinic acid (leucovorin), the mechanism is predominately inhibition of thymidylate synthase.

Both pemetrexed and 5-FU are thymidylate synthase inhibitors. However, their interactions with this enzyme are quite different. Thymidylate synthase has two substrate binding sites: one for a nucleotide (dUMP)and the other for a folate (5,10-methylenetetrahydrofolate polyglutamate). When thymidylate synthase is inhibited after treatment with an inhibitor, the de novo synthesis of thymidylate is suppressed, but accumulation of dUMP continues, often to millimolar concentrations (60 62). Although FdUMP can form a stable covalent bond with thymidylate synthase, the initial interaction of thymidylate synthase with FdUMP has an association constant equivalent to that of thymidylate synthase with dUMP (63). Hence, the accumulation of dUMP substantially, and sometimes completely, terminates inhibition of thymidylate synthase by FdUMP (63 65). The same accumulation of dUMP occurs after exposure of tumor cells to pemetrexed (90), but because the excess dUMP binds to a different site on the enzyme (66), it does not inhibit pemetrexed binding and, in fact, may enhance the binding of pemetrexed polyglutamates to thymidylate synthase.

Tumor cells are often deficient in cellular THF cofactor pools. The covalent complex formed between thymidylate synthase and FdUMP proceeds via chemistry that requires the presence of the THF cofactor (67). If cellular folates are low, the formation of ternary complex either is impeded or does not occur at all. Furthermore, high levels of THF cofactors in cells causes the persistence of cellular thymidylate synthase in the inactive ternary complex due to a kinetic trapping effect (68). Because of these considerations, 5-FU is coadministered with 5-formyltetrahydrofolate in the form of leucovorin (the calcium salt of the mixture of diastereomers about carbon 6). In contrast, low cellular THF cofactor levels enhance pemetrexed polyglutamation and enhance inhibition of its target enzymes. The case could be made, in general, that inhibition of a THF cofactor dependent enzyme by an analogue of the THF cofactor would be much a more efficient drug than an analogue of the nucleotide substrate because the nonfolate substrate will accumulate and block drug action, whereas the folate substrate will not.

Clinical Studies

Pemetrexed monotherapy for NSCLC

Three Phase II trials of single-agent pemetrexed were performed, two on previously untreated NSCLC patients (Table 1). A Phase II study reported by Rusthoven et al. (69) showed that the overall response rate was 23.3 percent, and the median time to progression was 3.1 months. The median survival time was 9.2 months and one-year survival rate was 25.3 percent. Due to early toxicity, the starting dose of pemetrexed for NSCLC patients was reduced from 600 mg/m2 to 500 mg/m2. Thirtynine percent of patients experienced Grade 3/4 hematological toxicity. Severe and most common non-hematological toxicity included skin rash (39 percent), lethargy (27 percent), nausea (12 percent), vomiting (9 percent), and diarrhea (9 percent). Nonhematological biochemical changes were mild. Of importance, this study was performed without vitamin supplementation.

Another Phase II single-agent pemetrexed trial recruited 45 patients with advanced, previously untreated NSCLC (Table 1) (70). Pemetrexed was administered continuously over 10 minutes at a median dose of 600 mg/m2, and the courses were repeated every 3 weeks up to 12 cycles. Nine (15.8 percent) patients achieved a partial response, and 27 (47 percent) patients had stable disease. Median duration of response was 4 months, the median time to disease progression and median survival were 4.4 and 7.2 months, respectively. The probability of one-year survival rate was 32 percent. The starting dose of pemetrexed was 600 mg/m2 in the trial, but 13 (22 percent) patients had dose reduction due to toxicity. The incidence of Grade 3/4 neutropenia was 41 percent but infection was uncommon, similar to the previous trial (69). The liver function abnormalities were clinically asymptomatic, self-limiting, and improved with continued treatment without dose reduction and/or delay. Again, vitamin supplementation was not given in this trial.

Smit et al. (71) performed a Phase II study on pemetrexed as second-line therapy for NSCLC. Eighty-two pretreated patients with advanced NSCLC were included. The agent was administered at 500 mg/m2 by 10-minute intravenous infusion once every 3 weeks. The overall response rate was 8.9 percent. Twenty-five (31.6 percent) patients achieved stable disease. Thirty (38.0 per

Table 1
Phase II single agent studies with Pemetrexed in patients with NSCLC

Studies	Number of patients	Pemetrexed mg/m2 (cycles)	DR	ORR	OS
Rusthoven et al, 1999	30 chemo-naive	600 d1/3wks (4)	3.1	23.3%	9.2
Clarke et al, 2002	59 chemo-naive	600 d1/3wks (4)	4.9	15.8%	7.2
Smit et al, 2003	44 platinum pretreated	500 d1/3wks (2)	1.6	4.5%	6.4
	35 nonplatinum pretreated	500 d1/3wks (2)	6.8	11.4%	4

DR, Duration of response (months); ORR, Objective response rate; OS, Overall survival (months)

cent) had progressive disease and 17 (21.5 percent) had no response. The median survival timewas 5.7 months. The probability of a patient surviving 6 months was estimated to be 48 percent. The median time to progression was 2 months. These Phase II studies revealed that pemetrexedwas an active drug in patients with NSCLC, and its activity in both untreated and treated patients was comparable to the activity of the other agents used for treating this disease.

A Phase III single agent trial

Based on these encouraging Phase II studies, a multinational Phase III trial compared docetaxel with pemetrexed as second line therapy for patients with NSCLC (72). Five hundred seventy-one patients were randomized to receive either: pemetrexed, 500 mg/m2 over 10 minutes, every 3 weeks plus supplementation with folic acid and vitamin B12; or docetaxel 75 mg/m2 over 60 minutes every 3 weeks until disease progression or drug toxicity were observed. Both agents had similar clinical outcomes with respect to their response rates (9.1 versus 8.8 percent), time to disease progression (2.9 months), and median survival time (8.3 versus 7.9 months). The group treated with docetaxel had more hematological toxicity compared to patients receiving pemetrexed, including more episodes of neutropenia (40.2 versus 5.3 percent), neutropenic fever (12.7 versus 1.9 percent) and infections (3.3 versus 0 percent), and hospitalizations due to febrile neutropenia (13.4 versus 1.5 percent), and more use of granulocyte colony-stimulating factor support (19.2 versus 1.6 percent). Supplementation with folic acid and vitamin B12 during pemetrexed therapy, therefore, reduced the incidence and severity of toxicities with no apparent effect on clinical activity. Based on this randomized trial, on August 19, 2004, pemetrexed received approval as monotherapy for the treatment of patients with locally advanced or metastatic NSCLC who had received prior chemotherapy (73).

Phase II combination studies with pemetrexed in NSCLC (Table 2)

In a Phase II trial, 37 (36 chemo-naive) patients with NSCLC received pemetrexed at a dose of 500 mg/m2 and vinorelbine at the dose of 30 mg/m2. Patients completed a median of 4 cycles (range 1 8). Assessable tumor response was 40 percent. Hematologic toxicities were neutropenia (65 percent) and febrile neutropenia (11 percent), while the most common non-hematologic toxicity was fatigue (74).

In two other front-line Phase II clinical trials (75, 76), the pemetrexed/cisplatin doublet was evaluated in 67 chemo-na?ve patients with advanced NSCLC (Stage IIB/IV). In both studies patients received pemetrexed at the dose of 500 mg/m2 followed by cisplatin at 75 mg/m2 on Day 1 of a 21-day cycle. For the European study (75), overall response rate, median survival time, median duration of response, and one-year survival rate were 39 percent, 10.9 months, 10.4 months, and 50 percent, respectively, while these parameters were as 45 percent, 8.9 months, 6.1 months, and 49 percent in the Canadian study (76). Grade 3/4 neutropenia as well as fatigue were the most important chemotherapy related toxicities in both studies (75, 76). Despite the activity shown, cisplatin-based

Table 2
Phase II combination studies with Pemetrexed in patients with NSCLC

Studies	No.	Dose mg/m2	DR	ORR	OS
Clarke et al, 2005	35[a]	Pemetrexed 500 d1/3wks Navelbine 30 d1 and 8/3 wks	4.6	40%	7.9
Manegold et al, 2000	36	Pemetrexed 500 d1/3wks Cisplatin 75 d1/3wks	10.4	39%	10.9
Sheperd et al, 2001	31	Pemetrexed 500 d1/3wks Cisplatin 75 d1/3wks	8.9	45%	8.9
Zinner et al, 2005	49[a]	Pemtrexed 500 d1/3wks Carboplatin AUC 6 d1/3wks	5.4	24%	13.5
Scagliotti, et al, 2005	41[a]	Pemtrexed 500 d1/3wks Oxaliplatin 120 d1/3wks	5.5	26.8%	10.5
	39[a]	Pemetrexed 500 d1/3wks Carboplatin AUC 6 d1/3wks	5.7	31.6%	10.5
Monerrat et al, 2004	58[a]	Pemetrexed 500 d1/3wks Gemcitabine 1250 d1 and 8/3wks	3.3	15.5%	10.1
Ma et al, 2005	59[a]	Pemetrexed 500 d1/3wks Gemcitabine 1250 d1 and 8/3wks	4.4	31%	11.4

No., No. of patients; DR, Duration of response (months); ORR, Objective response rate; OS, Overall survival (months); aPatients received folic acid and vitamin B12 supplementation.

regimens require extensive hydration before and after treatment to avoid severe toxicities. In order to reduce the problem of renal clearance of pemetrexed following cisplatin, the combination of pemetrexed and carboplatin was evaluated. In a Phase II clinical study (77), 50 patients with advanced or metastatic NSCLC were treated with carboplatin, AUC of 6, and pemetrexed (500 mg/m2) on Day 1. The median number of chemotherapy cycles administered was 6 (range 1 15). The most important hematologic toxicitywas neutropenia, followed by anemia. The non-hematologic toxicity included nausea and/or vomiting, and fatigue. The partial response rate was 24 percent and the median survival time was 13.5 months (77).

In another Phase II clinical trial patients with advanced NSCLC were randomly assigned to receive either pemetrexed 500 mg/m2 plus oxaliplatin 120 mg/m2 (PemOx) or pemetrexed plus carboplatin AUC6 (PemCb) (78). All drugs were given every 21 days for up to 6 cycles. Forty-one patients received PemOx and 39 received PemCb. Tumor response rate, median time to progression, one-year rate survival were 26.8 percent, 5.5 months and 49 percent for the PemOx group, and 31.6 percent, 5.7 months and 43.9 percent for the PemCb group. Median overall survival times were 10.5 months for both groups. Hematologic toxicities in both groups were Grade 3/4 neutropenia, Grade 3 thrombocytopenia, and Grade 3 anemia.

These studies demonstrated that carboplatin or oxaliplatin combinations have similar activity to the cisplatin combination with lower incidence of toxicities. A Phase II study of the pemetrexed/gemcitabine combination (79) was performed in sixty chemo-naive patients with inoperable NSCLC. Gemcitabine (1250 mg/m2) was given intravenously on Days 1 and 8, followed by pemetrexed (500 mg/m2) on Day 8. Of the 58 assessable patients, the overall response rate was 15.5 percent. Median overall survival was 10.1 months with a 1- and 2-year overall survival of 42.6 and 18.5 percent, respectively. Even though the response rate was low, the median survival time was better as compared to the platinum combinations without the platinumassociated toxicities (79).

More recently a randomized Phase II trial of 3 schedules of pemetrexed and gemcitabine was conducted in order to identify the optimal schedule for the combination of gemcitabine and pemetrexed as a front-line therapy for the patients with advanced NSCLC (80). One hundred fifty-two patients with advanced NSCLC were randomly assigned to 3 schedules of pemetrexed: 500 mg/m2 plus gemcitabine 1,250 mg/m2 on a 21-day cycle. In Schedule A, 59 patients received pemetrexed followed by gemcitabine on Days 1 and 8. In schedule B, 31 patients were treated with gemcitabine followed by pemetrexed on Day 1 and then gemcitabine on Day 8. In schedule C, in which 62 patients were enrolled, gemcitabine was administered on Day 1 and pemetrexed followed by gemcitabine on Day 8. Patients received a median of 5 (schedule A), 2 (schedule B) and 4 (schedule C) cycles of treatment. Sixty-six percent of the patients experienced Grade 3/4 neutropenia. Non-hematologic toxicities were dyspnea, fatigue, and transaminase elevation. Response rates were 31 percent in schedule A, and 6.5 percent and 16.1 percent in the schedules B and C, respectively. Schedule B was discontinued after interim analysis because of the poor response rate. Schedule A was less toxic than schedule C and met the protocol-defined efficacy criteria.

The future role of pemetrexed in the treatment of NSCLC

Recent advances in molecular biology are beginning to modify our traditional approach to the diagnosis and treatment of NSCLC. High-throughput DNA sequencing and gene expression profiling are used to identify molecular markers for the early detection of lung cancer, for prognosis, and for finding new targets (81). For example, prognostic genetic markers such as K-ras mutations, high retinoic acid receptor-β mRNA levels, low expression of interleukin-10 and collapsing response mediator protein 1 (CRMP-1 mRNA), overexpression of Cyclooxygenase-2 and interleukin-8 mRNA, and loss of heterozygosity (LOH) at chromosome segment 11p15.5 are associated with poor survival (82).

The knowledge of mechanisms of action of pemetrexed, and the understanding of the determinants of response- that include transport, the role of MTAP deletions, and the level of TS, may allow the clinician to select patients who are more likely to respond to this drug, either used alone or in combination. Studies need to be done to show that these measurements, either of message or protein, are of value in predicting response. The decrease in toxicity when pemetrexed treatment is supplemented with folic acid and vitamin B12, has allowed the use of full or near full dose of pemetrexed both in single agent treatment and in combination with full dose of the second agent. The pemetrexed/gemcitabine combination, given in the correct sequence could also be used to add a third drug with activity, for example, cisplatin, or the sequencing of this doublet with a platinum doublet with a taxane or vinorelbine. The role of bevacizumab or an EGFR-targeted drug given with the gemcitabine/pemetrexed combination also deserves exploration, as does strategies to protect the marrow using transfer of drug resistance genes.

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