Clinical studies of pemetrexed and gemcitabine combinations

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Pemetrexed (ALIMTA™) is a novel multitargeted antifolate that inhibits thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase. This agent is broadly active in a wide variety of solid tumors, including breast cancer, bladder cancer, mesothelioma, non-small-cell lung cancer, pancreatic cancer and ovarian cancer. Pemetrexed has also shown clinically relevant activity in combination with gemcitabine. This combination has been, and continues to be evaluated for the treatment of a number of malignancies, including non-small cell lung and ovarian cancer. A recently published randomized trial of different sequences has identified the sequence of pemetrexed on day 1 followed by gemcitabine on day 1 and gemcitabine on day 8, every 21 days as the most efficacious and least toxic sequence.

Key words: dihydrofolate reductase, glycinamide ribonucleotide formyltransferase, multitargeted antifolate, thymidylate synthase

introduction

Gemcitabine (2',2'-difluorodeoxycytidine, Gemzar®, Eli Lilly and Co., Indianapolis, IN) is a broadly active S phase-specific pyrimidine analog of deoxycytidine approved for the treatment of non-small-cell lung cancer (NSCLC) and pancreatic cancer. It is anabolized sequentially to the nucleoside mono-, di- and triphosphate intracellularly. The triphosphate, difluorodeoxycitidine triphosphate, is incorporated into DNA, resulting in chain termination. Moreover, the diphosphate derivative inhibits ribonucleotide reductase, thus depleting intracellular pools of dCTP for DNA synthesis [1].

Pemetrexed disodium (Alimta®, LY231514, MTA, Eli Lilly and Co., Indianapolis, IN), is a broadly active multitargeted folate analogue that suppresses tumor growth by inhibiting various folate-dependent enzymes, primarily thymidylate synthase (TS), dihydrofolate reductase (DHFR) and the purine biosynthetic enzyme, glycinamide ribonucleotide formyl transferase (GARFT) [2]. The key enzyme targets for pemetrexed are shown in Fig. 1. As can be seen in this figure, pemetrexed is analogous to methotrexate in inhibiting DHFR, and analogous to 5-fluorouracil and raltitrexed in inhibiting TS. However, pemetrexed additionally demonstrates inhibitory activity against GARFT, an enzyme which is not targeted by any current antineoplastic agent. Pemetrexed, by inhibiting other enzyme targets, promises to improve on the clinical activity of the classical TS inhibitors.

Pemetrexed demonstrated single-agent activity during phase II trials against a variety of tumor types, including non-small cell lung [3, 4], colorectal [5, 6] breast [7], pancreas [8], gastric [9], head and neck [10], bladder [11], and cervical cancers [12]. In phase III testing, pemetrexed combined with cisplatin was superior to cisplatin alone in pleural mesothelioma [13]. Pemetrexed gains entry to the cell via the reduced folate carrier and once localized, is an excellent substrate for folypolyglutamate synthase (FPGS). The pentaglutamate form of pemetrexed is the predominant intracellular form and is >60-fold more potent in its inhibition of TS than the monoglutamate [14]. The pharmacology and clinical activity of pemetrexed has been comprehensively reviewed [15].

pemetrexed in combination with Gemcitabine

A phase 1 combination of pemetrexed and gemcitabine was published several years ago. This combination was based on preclinical studies that demonstrated synergistic cytotoxicity when gemcitabine exposure preceded pemetrexed exposure in HCT-8 cultured human colon cancer cell lines [16]. Later reports, however, demonstrated synergistic cytotoxicity for the opposite sequence of pemetrexed exposure followed 24 h later by gemcitabine exposure in HT29 colon cancer cell lines and xenografts [17]. These data suggest that patterns of interaction with these two agents may be cell-line specific. The administration sequence used in the phase I study has been validated by promising clinical activity in a variety of tumor types in 13 out of 55 evaluable patients. The objective responses were documented in non-small cell lung cancer (3), cholangiocarcinoma (2), ovarian carcinoma (2), colorectal cancer (3), breast cancer (1), mesothelioma (1), and an adenocarcinoma of unknown primary site (1). The most
common toxicity in this study was neutropenia, which was dose-limiting. Other common toxicities included arthralgia, nausea, fatigue, fever, rash, and elevated hepatic transaminases. Gemcitabine had no effect on the disposition of pemetrexed. The recommended doses and sequence for Phase 2 studies were pemetrexed administered on days 1 and 8 with pemetrexed administered on day 8, 90 min after gemcitabine, at doses of gemcitabine 1250 mg/m² and pemetrexed 500 mg/m² [16]. These doses and schedule have been utilized in clinical trials in breast cancer, non-small cell lung cancer, pancreatic cancer, and mesothelioma, with an ongoing trial in ovarian cancer.

**pemetrexed in combination with Gemcitabine in breast cancer**

In the phase I study described above, 1 out of 3 heavily pretreated breast cancer patients with soft tissue disease achieved a durable partial response. In addition, both pemetrexed and gemcitabine have single-agent activity in breast cancer. Based on these factors, a phase II study of pemetrexed in combination with gemcitabine in metastatic breast cancer has been completed by the North Central Cancer Treatment Group. Eligible patients must have previously received an anthracycline and a taxane. These regimens could have been given in the adjuvant or metastatic setting, or a combination of both. Patients must not have received more than 1 chemotherapy regimen for metastatic disease (unless these were a taxane and an anthracycline). For example, a patient could receive cyclophosphamide, methotrexate and 5-fluorouracil for adjuvant therapy followed by an anthracycline and followed by a taxane for metastatic disease. Fifty-nine patients received a median of 5 cycles (range: 1–21) of treatment with gemcitabine 1250 mg/m² (IV; d1, 8) and pemetrexed 500 mg/m² (IV; d8) every 21 days. Patients were followed until death or a median of 17 months (range 13.4–28 months) among living patients.

One complete response (CR) and 13 partial responses (PR) for an overall response rate of 24% (95% CI: 16–39%) were documented in this population. Nine (15%; CI 5–32%) patients had stable disease (SD) for greater than 6 months. The median survival time was 10.3 months (95% CI 8.3–18.9 months) and the 1 year survival rate was 49% (95% CI 38–64%). The median time to progression was estimated to be 3.7 months (95% CI 2.3–5.3 months). The most common grade 3 or 4 toxicities were hematologic, and comprised neutropenia and thrombocytopenia in 83% and 27% of patients, respectively. Fourteen percent of patients experienced febrile neutropenia. Other common grade 3 or 4 non-hematologic toxicities included fatigue (17%), dyspnea (13%), rash (7%) and anorexia (5%). The investigators concluded that this combination of pemetrexed and gemcitabine is clinically active, with a clinical benefit rate (CR + PR + SD ≥ 6 months) of 39% in patients with metastatic breast cancer who have previously been treated with an anthracycline and a taxane [18].

**pemetrexed in combination with Gemcitabine in pancreatic cancer**

This combination was evaluated in a multicenter phase II trial in previously untreated patients with advanced pancreatic cancer. Patient characteristics: male 64%; median age 60 (range 34–79); Karnofsky PS 100 5%, 90 55%, 80 36%, 70 2%; stage IV 95%.

In a report of 42 patients 212 cycles were delivered (range 1–17, median 3). There were 6 partial responses (PR) (overall response rate 15%). Median survival was 6.5 months and 1-year survival was 29%. Median time to progression was 3.6 months. Of 33 eligible patients, 13% had a clinical benefit response. Grade 3/4 hematologic toxicities (% pts) neutropenia 81%, leukopenia 74%, anemia 14%, thrombocytopenia 26%, neutropenic fever 14%. Grade 3/4 nonhematologic toxicities: diarrhea 5%, fatigue 14%. It was concluded that the pemetrexed/gemcitabine combination was active in pancreatic cancer, with acceptable toxicity [19]. Based on these data, a 520 patient international randomized phase III trial of pemetrexed/gemcitabine versus gemcitabine was performed. However, while tumor response rate (14.8% versus 7.1%; P = 0.004) was significantly better on the combination arm, overall survival was similar (6.2 months on the combination, compared to 6.3 months on the control arm of single-agent gemcitabine; P = 0.8477). Progression-free survival was 3.9 months versus 3.3 months (P = 0.1109). Thus, the combination did not improve overall survival, compared to single-agent gemcitabine [20].

**pemetrexed in combination with Gemcitabine in non-small cell lung cancer**

The above combination has been tested in NSCLC in a number of studies that have been reviewed by Monnerat et al., in this issue [21].

In spite of the studies outlined above, two outstanding clinical issues remained in the use of the pemetrexed/gemcitabine combination. First, the optimal sequence of administration remained unsettled. Second, in the phase I study as well as most
early studies, there was a 90-min separation between the administration of both agents. The two studies described below were designed to address these questions.

**pemetrexed in combination with Gemcitabine: Drug Sequencing**

There is currently a discordance between the various in vivo and in vitro preclinical models regarding the optimal sequence of administration of pemetrexed and gemcitabine, [16, 17, 22–24]. In order to identify the optimal sequence of administration of these two agents, a randomized three-arm phase II study was undertaken to evaluate 3 different administration schedules of pemetrexed and gemcitabine in chemo-naïve patients with NSCLC.

Patients were randomly assigned to 3 different schedules of pemetrexed 500 mg/m² plus gemcitabine 1250 mg/m² on a 21-day cycle as follows: Schedule A, pemetrexed followed 90 min later by gemcitabine on day 1, gemcitabine on day 8; Schedule B, gemcitabine followed 90 min later by pemetrexed on day 1, gemcitabine on day 8; Schedule C, gemcitabine on day 1, pemetrexed followed 90 min later by gemcitabine on day 8. One hundred and fifty-two eligible patients (A 59, B 31, and C 62) received a median of 5 (A), 2 (B) and 4 (C) treatment cycles. There was a trend towards less severe toxicity on schedule A compared to schedule C (grade 3+ events: 86% vs. 94%, P value=0.19; grade 4+ events: 39% vs. 50%, P value=0.22). Schedule B was closed at interim analysis for inferior efficacy (< 3 confirmed responses in the first 19 patients). Schedule A, with a confirmed response rate of 31% (95% CI 19.2–43.9%) met the protocol defined efficacy criteria, whereas schedule C with a confirmed response rate of 16.1% (95% CI 8.0–27.7%) did not. The median survival and time to progression were 11.4 (A 11.4, B 10.3, C 11.8) and 4.4 (A 4.7, B 4.1, C 4.4) months respectively, with no observable difference between the arms. It was concluded that pemetrexed and gemcitabine administered as outlined for schedule A was the optimal schedule for this combination [25].

**pemetrexed in combination with Gemcitabine pharmacokinetics**

As clinical development of this combination proceeds, it has become important to determine whether these two agents can be administered in rapid sequence without the 90-min interval between them. Adjei and colleagues therefore undertook a study to characterize any pharmacokinetic interaction between these two agents and to compare the toxicity and efficacy of this combination administered in close succession with similar data from a previous phase I study in which administration of the drugs was separated by a 90-min interval [26].

Fourteen patients received 84 cycles of treatment. Gemcitabine 1250 mg/m² was administered on days 1 and 8 of each 21-day cycle, with pemetrexed 500 mg/m² on day 8 immediately following gemcitabine administration. Pharmacokinetic analyses of plasma gemcitabine and pemetrexed concentrations were performed. Neutropenia was the most common severe toxicity. Non-hematologic toxicities, which included nausea, vomiting, fatigue, diarrhea, rash, and elevated transaminases were of mild-to-moderate severity. No increased toxicity was observed with this schedule in comparison to the previous phase I schedule. There was no pharmacokinetic interaction between the two drugs. One partial response was documented in a patient with non-small-cell-lung cancer. Eight patients had disease stabilization for 5 or more cycles. This study demonstrated that gemcitabine immediately followed by pemetrexed is well-tolerated, and clinically active. This dosing scheme has been utilized in at least two trials of this combination in NSCLC [27, 28].

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**Figure 2.** Illustration of the similarities and differences between the cellular mechanism of action MTA and methotrexate as well as MTA and 5-FU or raltitrexed.
conclusions

In summary, the combination of pemetrexed and gemcitabine is clinically active. It appears an optimum sequence of administration has been identified in a clinical trial. In addition, it has become clear that the inconvenient 90-min separation between the administration of both drugs may not be required for efficacy. Clinical trials designed to test this combination with this new schedule and sequence are warranted.

disclosures

Dr Adjei has indicated no financial relationships with companies whose products are mentioned in this article.

references