REVIEW

Platinum and Pemetrexed Combination in Advanced Solid Tumors

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Abstract: Pemetrexed is a third generation multi-target agent able to inhibit at least three crucial enzymes involved in the folate pathway. Pemetrexed is indicated in combination with cisplatin as first line treatment of malignant pleural mesothelioma (MPM) and in mono-chemotherapy as second line treatment of advanced non-small-cell lung cancer (NSCLC). In 2008, the combination of pemetrexed plus cisplatin has gained approval in Europe and in USA for first line therapy limited to non squamous NSCLC patients. More recently, the outcome of a large phase III trial, aimed at evaluating pemetrexed as maintenance therapy, confirmed the efficacy of the drug in non squamous NSCLC patients. Recent data suggest tumour histotype and TS expression levels as the most promising predictors of pemetrexed sensitivity in NSCLC.

The aim of this paper is to review literature data about platinum and pemetrexed combination in advanced solid tumors, especially NSCLC and MPM.

Keywords: pemetrexed, cisplatin, chemotherapy, malignant pleural mesothelioma, non small cell lung cancer

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Introduction

In the last few decades, several efforts have been made to improve the outcome of patients affected by human malignancies and the efficacy achievable with chemotherapy seems to have reached a therapeutic plateau. However, among newer cytotoxic agents, pemetrexed (ALIMTA® Eli Lilly, Indianapolis, Indiana) gained much interest because of its peculiar mechanism of action. Pemetrexed is a multi-target agent that inhibits at least three crucial enzymes involved in the folate pathway: thymidylate synthase (TS), dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyl transferase (GARFT). TS and DHFR are enzymes involved in pyrimidine synthesis process, whereas GARFT is a folate-dependent enzyme involved in de novo purine biosyntesis.[1] Purines and pyrimidines are both involved in the DNA synthesis. The ability of pemetrexed to inhibit multiple enzymes confers a clinical advantage by increasing the spectrum of tumors with biochemical profiles potentially sensitive to the drug. Among these enzymes, TS is considered the primary target of pemetrexed. In fact, pemetrexed is only a weak inhibitor of GARFT and when TS is inhibited, oxidation of tetrahydrofolate is stopped, and thus DHFR activity is unnecessary.[2] Based on these findings, several trials have evaluated the interactions between pemetrexed and TS. Pre-clinical data have shown that TS mRNA expression levels were inversely correlated with pemetrexed activity in different tumor cells.[3] Recently, TS resulted to be differentially expressed among different histotypes of non-small-cell lung cancer (NSCLC): in particular TS is highly expressed in squamous cell carcinoma and in high grade carcinoma, suggesting a different sensitivity profile to the drug, based on histology.[4] The current recommended dosage for pemetrexed is 500 mg/mq intravenously every 3 weeks both alone and combined with other drugs.[5] Generally, pemetrexed is considered a well tolerated drug. Myelo-suppression was the predominant dose-limiting toxicity of pemetrexed reported in Phase I studies. Identification of the correlation between poor folate status and increased pemetrexed toxicity in a multivariate analysis led to the requirement of folic acid and vitamin B12 supplementation for patients in all pemetrexed studies, with a resulting noted decrease in pemetrexed toxicity.[6] The standard dose for oral folic acid is 300–1000 µg daily, while for vitamin B12 injection is 1000 µg every 9 weeks, beginning at least 1 week before pemetrexed administration and continuing until 3 weeks after the last administration. Moreover, the side effect of rush is reduced by the administration of dexamethasone (4 mg orally twice daily) for 3 days starting the day before pemetrexed infusion.

The pharmacokinetics of pemetrexed, when administered as a single agent once every 21 days, have been examined.[7] Pemetrexed is predominantly eliminated renally. Pharmacokinetic evaluations have shown that pemetrexed is ~80% protein bound, with rapid plasma distribution and elimination phases, and exhibits linear pharmacokinetic over a broad range of doses ($0.2–1,400$ mg/m^2). Pemetrexed is rapidly eliminated from the plasma by urinary excretion [half-life ($t1/2$) = 3.5 hours], with about 70% to 90% of the administered dose recovered unchanged in the urine within 24 hours. The steady-state volume of distribution of pemetrexed is small (16 L), suggesting limited tissue distribution.[8,9] Mild nephrotoxicity occurred in patients treated with multiple cycles of therapy. Concurrent nonsteroidal anti-inflammatory agents have been excluded from pemetrexed trials because they may decrease the renal clearance of pemetrexed.

Cisplatin is also renally eliminated and is highly protein bound (>90%). Most of the platinum derived from cisplatin is rapidly and irreversibly bound to plasma proteins. Whereas free platinum is rapidly eliminated from the plasma ($t1/2$ = 0.5 hour), $t1/2$ of total platinum is 5.4 days +/−1, reflecting the plasma protein binding.[10]

The combination of pemetrexed with cisplatin resulted feasible and effective. The pharmacokinetics of total platinum and pemetrexed were evaluated in patients with malignant pleural mesothelioma (MPM) using population pharmacokinetic methods and there was no significant influence of concomitant cisplatin administration on pemetrexed clearance or of concomitant pemetrexed administration on cisplatin clearance.[11] Moreover, the pha rmacokinetic of free platinum derived from cisplatin was not altered by co-administration with pemetrexed, and in agreement with this, no unexpected cisplatin-induced toxicities were observed when these drugs were combined.

Pemetrexed is currently approved in combination with cisplatin for first line treatment of MPM,[12] as a single agent for second line treatment of advanced NSCLC[5] and has recently been approved in Europe and in the United States for first line therapy in combination with cisplatin for non squamous NSCLC patients. Trials are also ongoing to test pemetrexed as single agent or in combination with other drugs in various solid tumors.[14,15] This paper reviews data from literature of platinum and pemetrexed combination in advanced solid tumors, especially MPM and NSCLC.

Malignant Pleural Mesothelioma
First line treatment

The only FDA-approved agent for MPM is pemetrexed. Pemetrexed may be more active in mesothelioma than in other cancers because of the presence in these cells of a high capacity cell membrane transporter which is highly specific for pemetrexed.[16] In particular, pemetrexed was shown to have activity as a single agent in a phase II trial in patients with MPM[17] and in phase I trials in combination with platinum analogs[18,19] (Table 1).

The phase II clinical trial evaluated the efficacy of pemetrexed (500 mg/mq) for the treatment of 64 MPM patients with a histological proven diagnosis, chemotherapy-naive measurable lesions, and adequate organ function. Most patients (43/64) also received folic acid and vitamin B12 supplementation to improve safety. As single agent, pemetrexed resulted in a moderate response rate (RR) (14.1%), with a median time to progression (TTP) of 4.7 months and a median overall survival (OS) of 10.7 months.[17] Seven of the nine responders were vitamin supplemented. The median (OS) was 13.0 months for supplemented patients and 8.0 months for non-supplemented patients. Vitamin-supplemented patients completed more cycles of therapy than non-supplemented patients (median, six versus two cycles, respectively). Grade 3/4 neutropenia (23.4%) and grade 3/4 leucopoenia (18.8%) were the most common laboratory toxicities. Fatigue and febrile neutropenia were the most commonly reported non-laboratory events (grade 3, 6.3%; grade 4, 0.0% each) and the incidence of these toxicities was generally lower in the vitamin-supplemented patients.

In a phase I trial designed to determine the maximum-tolerated dose (MTD), the dose-limiting toxicities (DLT), and the pharmacokinetics of pemetrexed combined with cisplatin, two patients had objective remissions of disease (one mesothelioma patient, one colon cancer patient).[18] The MTD was pemetrexed 600 mg/m^2 and cisplatin 75 mg/m^2. DLTs were neutropenic sepsis, diarrhea, and skin toxicity. In another phase I trial, the combination of pemetrexed plus carboplatin was found to be active and well tolerated in MPM patients, although no vitamin supplementation was administered.[19] The recommended dose of the combination for phase II studies was pemetrexed 500 mg/m^2 and carboplatin area under the plasma concentration-time curve (AUC) 5 mg/mL/min.

The use of pemetrexed in MPM patients was approved by the Food and Drug Administration (FDA) based on a single-blind, placebo-controlled, phase III study. In this trial, chemotherapy-naive

Table 1. First-line pemetrexed-based chemotherapy in MPM patients.

Authors	Phase	Regimen	No.	RR (%)	mTTP (mos)	mSv (mos)
Vogelzang[12]	III	CDDP	222	17.0	3.9	9.3
		Pem + CDDP	226	41.0	5.7	12.1
		Pem + CDDP*	168	45.5	6.1	13.3
Scagliotti[17]	II	Pem	64	14.1	4.7	10.7
Ceresoli[22]	II	Pem + CBDCA	102	18.6	6.5	12.7
Castagneto[23]	II	Pem + CBDCA	76	25.0	8.0	14.0
Santoro[25]	EAP	Pem + CDDP	745	26.3	7.0	63.1%°
		Pem + CBDCA	752	21.7	6.9	64.0%°

Abbreviations: Pem, pemetrexed; CDDP, cisplatin; CBDCA, carboplatin; EAP, retrospective data from Expanded Access Programs, MPM, malignant pleural mesothelioma; No, number of patients; RR, response rate; mTTP, median time to progression; mSv, median survival; mos, months; NR, not reported. *Only vitamin and dexamethasone supplemented patients; °1-year survival rate.

patients who were not eligible for curative surgery were randomized to pemetrexed 500 mg/m² and cisplatin 75 mg/m², or placebo and cisplatin 75 mg/m².[12] Both regimens were given intravenously every 21 days. A total of 456 patients were assigned: 226 received pemetrexed and cisplatin, 222 received cisplatin alone, and eight never received therapy. The RR for the combination was significantly greater than for single-agent cisplatin (41% vs. 17%; $P < 0.001$). Pemetrexed/cisplatin treated patients had a median OS of 12.1 months, compared with 9.3 months for patients treated with cisplatin alone ($P = 0.020$). The hazard ratio for death of patients in the pemetrexed/cisplatin arm versus those in the control arm was 0.77. Time to progression was also superior for patients treated with the combined chemotherapy (5.7 vs. 3.9 months, $P = 0.001$). In addition, treatment with this combination resulted in a significant improvement in pulmonary function, quality of life, and symptoms such as pain and dyspnea. After the first 117 patients enrolled in this study, all patients were supplemented with dietary doses of folate and vitamin B12. Vitamin supplementation improved RR (45.5% vs. 19.6%; $P < 0.001$) and survival (TTP 6.1 vs. 3.9 months, $P = 0.008$; OS 13.3 vs. 10 months, $P = 0.051$) in both treatment arms, and reduced the incidence of serious toxicity. In preclinical models, there is a very significant decrease in pemetrexed activity as the extracellular folate level increases above the physiologic range. This suggests that it may be appropriate to limit folate supplementation to no more than 400 µg, the amount found in a multivitamin, rather than the 1000 µg that is more frequently prescribed.[20]

Malignant pleural mesothelioma is a disease of the older patient, with a median age of onset of 74 years.[21] The typical non-haematological toxicity profile of cisplatin (gastro-intestinal, neurologic, and renal) is questionable in the context of a palliative treatment, especially for poor performance and elderly patients. Carboplatin has the potential advantages of having a better adverse effect profile and better ease of administration. In a phase I study in 25 patients with MPM, the combination of pemetrexed and carboplatin was active and well tolerated, with a reported RR of 32%.[19] Starting from these data, some combined schedules containing carboplatin, instead of cisplatin,

were tested in MPM patients in an attempt to reduce toxicity maintaining the same survival outcomes.[22,23]

In a phase II trial of 102 MPM patients treated with pemetrexed plus carboplatin, a similar time to progression (6.5 months) and overall survival (12.7 months) were observed as in the phase III trial of pemetrexed-cisplatin.[22] The toxicity profile seemed to be better in the pemetrexed-carboplatin trial than in the pemetrexed-cisplatin trial, especially considering the non-hematological toxicity. A 76-patient phase II study reported a time to progression of 8.0 months, a median survival of 14 months, and a response rate of 25% using the same regimen.[23] Moreover, no significant difference was observed in terms of overall disease control (60.4% vs. 66.9%, $P = 0.47$), TTP (7.2 vs. 7.5 months, $P = 0.42$) and survival (10.7 vs. 13.9 months, $P = 0.12$) between elderly patients compared with younger individuals in a retrospective analysis of pooled data from the two phase II trials of pemetrexed and carboplatin as first-line therapy.[24]

Data from the International Expanded Access Program (EAP) suggested an activity of both pemetrexed plus cisplatin and pemetrexed plus carboplatin in 1704 chemonaïve MPM patients not amenable to curative surgery, showing similar time to progressive disease and 1-year survival rates. In particular, the pemetrexed plus cisplatin group obtained a RR of 26.3% compared with 21.7% for the pemetrexed plus carboplatin group, with 1-year survival rates of 63.1% versus 64.0% and median TTP disease of 7 months versus 6.9 months.[25]

There are several unresolved questions regarding timing and duration of pemetrexed treatment. Some epithelial mesothelioma patients may have prolonged stable disease for months or even years without chemotherapy. (There is no agreement about) It is not known whether these patients should be treated at diagnosis, at symptom progression, or at radiographic progression. In a very small pilot study from the Royal Marsden Hospital, there was a trend toward a longer time to symptomatic progression and overall survival in those patients who received chemotherapy at diagnosis rather than at symptom progression;[26] however, these results need to be validated in a larger study with a more active chemotherapy regimen than the one employed in that study. We also do not know the optimum treatment length. Most patients receive

between 4 and 8 cycles of pemetrexed with cis- or carboplatin, few can tolerate more. Should they stop treatment at that point, or continue with single-agent pemetrexed? A small, non-randomized Dutch feasibility study of pemetrexed maintenance demonstrated that maintenance is well tolerated, and that responses can occur after six cycles of treatment.[27] The Cancer and Leukemia Group (CALGB) is currently designing a larger, randomized study to more definitively address this question.

Second line treatment

In the last years, pemetrexed has been extensively explored as second-line therapy or beyond in MPM patients not previously exposed to this agent (Table 2). Sørensen et al reported the results of a study in which data sets of treatment of two different cohorts were combined.[28] Thirty-nine patients previously treated with platinum-based regimens without pemetrexed were included. Twenty-eight patients received pemetrexed alone (in 3 cases as third-line treatment), whereas 11 patients received pemetrexed plus carboplatin. Treatment was generally well tolerated. Partial response (PR) rates were 21% and 18%, median TTP was 21 weeks and 32 weeks, and median survival was 42 weeks and 39 weeks with pemetrexed and pemetrexed/carboplatin, respectively. Jänne et al[29] reported the results of the use of pemetrexed alone or in combination with cisplatin within an Expanded Access Program in 187 patients who had received previous systemic chemotherapy. Patients were treated with pemetrexed alone (n = 91) or in combination with cisplatin (n = 96). Gemcitabine was the most common prior therapy used, followed by cisplatin,

carboplatin, paclitaxel and vinorelbine. Response data were available for 153 patients. The overall response rate was 32.5% for pemetrexed/cisplatin and 5.5% for pemetrexed alone; stable disease was achieved in 36.3% and 41.1% of patients, respectively. Median OS was 7.6 months in patients receiving combination therapy, and 4.1 months in those receiving single-agent pemetrexed. However, due to the limitations of the study design, no comparison can be done between treatment groups. In fact, patients receiving combination chemotherapy were younger and fitter at baseline, and had a higher response rate to first-line treatment. This is reflected by the increased number of treatment cycles administered to the combination therapy group. In another analysis from the Expanded Access Program database, the safety and efficacy data of MPM patients who were treated with single-agent pemetrexed were reported.[30] Of a total of 812 patients, 643 were evaluable for efficacy. The overall response rate for the pre-treated patients (n = 396) was 12.1%; median TTP was 4.9 months, and the median OS was not estimable due to high censoring. Hematological toxicity was mild in both groups, with neutropenia (<18%) as the main side effect.

The use of pemetrexed in this setting was further explored prospectively in a randomized, multi-center phase III study examining single-agent pemetrexed as second-line chemotherapy versus best supportive care (BSC).[31] Primary endpoint of the study was OS. Secondary endpoints included RR, TTP, progression-free survival (PFS), time to treatment failure (TTF), and toxicity. Patients with relapsed MPM after first-line chemotherapy were randomly assigned to receive pemetrexed 500 mg/m^2 plus best supportive

Table 2. Second-line chemotherapy in pemetrexed-naïve MPM patients.

Authors	Phase	Regimen	No.	RR (%)	mTTP (mos)	mSv (mos)
Sørensen[28]	II	Pem	28	21.0	4.9	9.8
		Pem + CBDCA	11	18.0	7.4	9.1
Jänne[29]	EAP	Pem	91	5.5	NR	4.1
		Pem + CDDP	96	32.5	NR	7.6
Taylor[30]	EAP	Pem	396	12.1	4.9	NR
Jassem[31]	III	Pem + BSC	123	18.7	3.6	8.4
		BSC	120	1.7	1.5	9.7

Abbreviations: EAP, retrospective data from Expanded Access Programs; b, randomized trial of pemetrexed vs. best supportive care (data reported for the pemetrexed arm only); MPM, malignant pleural mesothelioma; No, number of patients; RR, response rate; mTTP, median time to progression; mSv, median survival; mos, months; NR, not reported.

care (BSC) every 21 days or BSC alone. The study enrolled 243 patients. PR was achieved in 18.7% of patients receiving pemetrexed, and a disease control (Partial Response + Stable disease SD) was achieved in 59.3% and 19.2% of patients in pemetrexed and BSC arms, respectively ($P < 0.0001$). Median TTP was significantly improved in pemetrexed arm (3.8 vs. 1.5 months), as well as the other time-to-event measures. Chemotherapy was well tolerated, with expected mild (4% to 7%) grade 3 and 4 hematological toxicities. Use of post-discontinuation chemotherapy was significantly greater and earlier among BSC patients. Median OS time was not significantly different between the arms (8.4 months for patients treated with pemetrexed vs. 9.7 months for those receiving BSC only), possibly because of the significant imbalance in post-study chemotherapy (PSC). A trend towards a survival benefit was observed for patients who had responded to first-line therapy.[31]

In conclusion, in pemetrexed-naïve patients, data from a randomized trial versus BSC suggest the use of single agent pemetrexed as a standard second-line treatment. This line of evidence is supported also by the results of the Expanded Access Programs. In the growing population of pemetrexed-pretreated patients, there is no standard approach. In selected cases with a prolonged response to first-line pemetrexed-based chemotherapy, re-treatment with a pemetrexed-based regimen should be considered. When a trial is not available or patients are not eligible for an experimental approach, single agents vinorelbine or gemcitabine seem to be a reasonable option for palliation. However, second-line therapy in MPM remains an ideal field in which to test new chemotherapy agents as well as new therapeutic strategies.

Predictive factors of pemetrexed sensitivity in MPM

The combination of cisplatin/carboplatin and pemetrexed represents the standard of care in the first-line treatment of MPM. However, more than one third of patients do not respond to this schedule, receiving useless toxicity. Considering the toxicity profile of this platinum compound containing schedule and the poor performance status of the majority of MPM patients, due to their advanced age and to the usual

advanced disease extension at diagnosis, it represents not only an important medical problem but also an needless expenditure of economical and human resources. Unfortunately, up to now there are no many data about pemetrexed and/or cisplatin/carboplatin predictors of response in MPM patients. In a recent retrospective analysis, Righi et al investigated the correlation between baseline gene expression levels of TS and excision repair cross-complementation group 1 (ERCC1), evaluated by real-time polymerase chain reaction and by immunohistochemistry (using the H-score), in MPM patients treated with pemetrexed (P) based chemotherapy.[32] They observed that low TS protein levels are predictive of improved TTP and OS. In particular, a significant correlation between low TS protein expression and longer time to progression (TTP; 17.9 vs. 7.9 months; hazard ratio [HR], 2.05, 95% CI, 1.19 to 3.77; $P = 0.02$) or overall survival (OS; 30 vs. 16.7 months; HR, 2.38; 95% CI, 1.15 to 4.91; $P = 0.019$) was found when patients were divided according to median H-score. Conversely, they did not find a significant correlation between TS mRNA and outcome. In platinum-treated patients (n = 45), no correlation was found with survival according to ERCC1 median H-score, but patients in the lower tertile had a significantly shorter survival (HR, 3.06; 95% CI, 1.08 to 8.69; $P = 0.035$). In the multivariate analysis, TS protein levels were found as the only independent prognostic factor for both TTP (HR, 2.71; 95% CI, 1.13 to 6.49; $P = 0.02$) and OS (HR, 6.91; 95% CI, 1.90 to 25.07; $P = 0.003$). Another retrospective analysis correlated the immunohistochemical expression of ERCC1 and TS with the outcomes of 72 MPM patients treated with carboplatin/pemetrexed in first line setting.[33] Interestingly, the higher TS expression was associated with progressive disease (PD) (odds ratio 1.02; $P = 0.061$). In particular, the odds ratio of PD for patients with a TS expression $\geq$20% was 11.7 ($P = 0.003$), with a risk to progress of approximately 2 times higher than patients with a TS expression <20% (HR 1.90; $P = 0.014$). This trend was confirmed also for overall survival (HR 1.77; $P = 0.044$). On the basis of these results, TS expression may be considered as a potential predictor of response to the pemetrexed treatment in MPM patients. However, considering its significant correlation with response, PFS, and OS in a retrospective analysis, adequate

prospective studies are needed to confirm its possible predictive and/or prognostic role.

Non-Small-Cell Lung Cancer
Second line treatment

At the beginning Pemetrexed was investigate as single agent in previously treated metastatic NSCLC patients. In a phase II study conducted in 81 NSCLC patients who had progressed during or within 3 months after first line chemotherapy, pemetrexed demonstrated in an intent-to-treat population a response rate (RR) of 8.9% and a median overall survival (OS) of 5.7 months.[34] Based on these data, a phase III trial was conducted to compare pemetrexed to docetaxel, the standard second line chemotherapy.[5] In this study, 571 patients which had progressed after first line chemotherapy were randomized to pemetrexed (500 mg/m^2 on day 1 every 21 days) or docetaxel (75 mg/m^2 on day 1 every 21 days). All study population received folate and vitamin B12 supplementation, according to the results of Scagliotti's study showing that pemetrexed therapy without vitamin supplementation resulted in significantly higher haematological and non haematological toxicity.[17] No significant difference between pemetrexed and docetaxel was observed in terms of RR (9.1% versus 8.8%, $P = 0.10$) or in terms of survival (median OS 8.3 versus 7.9 months; 1-year survival = 29.7% in both groups; hazard ratio 0.99). Patients receiving docetaxel were more likely to have grade 3–4 neutropenia (40.2% versus 5.3%, $P < 0.01$), febrile neutropenia (12.7% versus 1.9%, $P < 0.01$), neutropenia with infections (3.3% versus 0%, $P = 0.04$), hospitalizations due to other drug related adverse events (10.5% vs. 6.4%; $P = 0.092$), use of granulocyte colony-stimulating factor support (19.2% vs. 2.6%, $P < 0.001$), and all grade alopecia (37.7% vs. 6.4%; $P < 0.001$) compared with patients receiving pemetrexed. Based on these results, pemetrexed was approved in 2004 by Food and Drug Administration (FDA) for second line treatment of advanced NSCLC.

The introduction of vitamin supplementation reduced the toxicity profile of pemetrexed, suggesting the administration of higher doses of pemetrexed. Two studies evaluated whether an increased dosage of pemetrexed resulted in an improvement of the efficacy.[35,36] Cullen et al conducted a randomized phase III study comparing standard and high dose pemetrexed as second line treatment of NSCLC.[35] A total of 588 patients with advanced or metastatic NSCLC progressed after platinum-based chemotherapy were randomized to receive pemetrexed 500 mg/m^2 every 21 days or pemetrexed 900 mg/m^2 every 21 days. No differences was observed in terms of RR (standard dose arm 7.1% versus high dose arm 4.3%, $P = 0.16$), as well as in terms of median OS (6.7 versus 6.9 months, HR = 1.01, 95% Confidence Interval (CI) 0.837–1.226) and progression free survival (PFS) (2.6 versus 2.8 months, HR 0.96, 95% CI 0.817–1.147). The incidence of drug-related grade 3–4 toxicity was <5% on both arms, but patients in the high dose arm had a higher frequency of most toxic effects and need for supportive care, transfusions and hospitalization.

Similarly, a Japanese phase II study compared pemetrexed 500 mg/m^2 on day 1 every 21 days to pemetrexed 1000 mg/mq on day 1 every 21 days, in pretreated metastatic NSCLC.[36] The primary objective was RR. A total of 216 patients were valuable for efficacy, showing no differences between the two arms in terms of RR (18.5% versus 14.8% 90% CI 9.5%–21.6%), median OS (16.0 versus 12.6 months), 1-year survival rates (59.2% versus 53.7%), and median PFS (3.0 and 2.5 months; 95% CI 2.8–6.1 months). Although toxicity in both arms was mild, it is to note that it was more frequent in the higher dose arm. Overall, the two trials demonstrated that higher dose of pemetrexed did not yield improved efficacy. Recently, 240 NSCLC patients relapsed after first line chemotherapy were randomized in a phase II trial to receive pemetrexed 500 mg/m^2 or pemetrexed 500 mg/m^2 plus carboplatin at area under curve (AUC) of 5.[37] The aim was to achieve a better outcome with the combined schedule and the primary endpoint was median time to progression (TTP). The combination arm achieved a longer median TTP (4.2 months versus 2.8 months; HR = 0.67; 95% CI 0.51–0.89, $P = 0.005$). However, median OS (7.6 months versus 8.0 months; HR 0.85; 95% CI 0.63–1.2; P = not significant) and overall RR (4% versus 9%) were not significantly different. The frequency of treatment–related toxicity exceeding grade 2 was inferior to 5% for all categories and was more frequently observed in the combination arm. Hematologic toxicity was mild and only rarely complicated by clinical sequelae. Hospitalization for

febrile neutropenia was 2% in both arms. The authors concluded that the combination of carboplatin and pemetrexed is effective and increases PFS. Table 3 shows the results of pemetrexed trials in metastatic NSCLC patients treated in second line setting.

First line treatment

Several trials evaluated pemetrexed in first line setting as single agent as well as in combination with other drugs. Two small phase II trials have investigated pemetrexed as a single agent in chemonaïve NSCLC patients.[38,39] In both trials the primary endpoint was RR. In the first study conducted in 59 patients, pemetrexed was administered at the dose of 600 mg/m^2 on day 1 every 21 days achieving a RR of 16%, a median OS of 7.2 months, and a 1-year survival rate of 32%.[38] Grade 3–4 neutropenia and skin rash were observed in 42% and in 31% of patients respectively.

In the second study, a total of 33 previously untreated NSCLC patients received pemetrexed at the dose of 600 mg/m^2 every 21 days.[39] The overall RR was 23.3%, the median OS was 9.2 months, and 1-year survival rate was 25.3%. The toxicity profile was similar to the previously mentioned study, showing grade 3–4 neutropenia as the predominant hematologic toxicity. It is to note that, at that time, vitamin supplementation and pre-medication with dexamethasone were not yet instituted which may explain the low safety profile observed in these trials.

More recently, Gridelli et al[40] conducted a randomized phase II study in unfit or elderly chemonaïve NSCLC patients exploring the role of pemetrexed as single agent versus pemetrexed/gemcitabine in sequential combination. Eighty-seven patients were randomized to receive single agent pemetrexed 500 mg/m^2 on day 1 every 21 days or pemetrexed at the same dose on cycles 1 and 2 followed by gemcitabine 1200 mg/mq on day 1 and 8 every 21 day on cycles 3 and 4. The patients treated in the sequential arm achieved a higher RR (11.6% versus 4.5%) and longer OS (5.4 versus 4.7 months) compared with patients treated in the single agent arm. Hematological and non hematological toxicity were mild in both arms. The study demonstrated a moderate activity and a good safety profile for metastatic NSCLC unsuitable for platinum based chemotherapy.

The encouraging single agent response rate observed in the phase II trials led to investigate combination chemotherapy with pemetrexed and platinum compounds. Several phase II studies were conducted to investigate the efficacy of pemetrexed in combination with cisplatin.[41,42] Shepherd et al enrolled 32 patients to receive pemetrexed 500 mg/m^2 on day 1 and cisplatin 75 mg/m^2 on day 1 every 3 weeks.[41] All patients received prophylactic dexamethasone, without vitamin supplementation. The overall RR was 45% with median OS of 8.9 months. A similar trial was conducted in 36 untreated NSCLC patients showing an overall RR of 39% and a median OS of 10.9 months.[42] The most common toxicity was haematological in both trials (grade 3–4 neutropenia in 33% and 28% of patients in the Shepherd and Manegold trials, respectively; grade 3–4 thrombocytopenia in 3% of patients in both trials) likely due to the absence of vitamin supplementation.

Table 3. Results of pemetrexed trials in metastatic NSCLC patients in second line setting.

Authors	Phase	Regimen	No.	RR (%)	mTTP (mos)	mSv (mos)
Hanna[5]	III	Pem	283	9.1	3.4	8.3
		Txt	288	8.8	3.5	7.9
Cullen[35]	III	Pem 500	295	7.1	NR	6.7
		Pem 900	293	4.3		6.9
Ohe[36]	II	Pem 500	108	18.5	NR	16
		Pem 1000	108	14.8		12.6
Smit[37]	II	Pem	121	4	2.8	7.6
		Pem + CBDCA	119	9	4.2	8.0

Abbreviations: Pem, pemetrexed; Txt, docetaxel; CBDCA, carboplatin RR, response rate; mTTP, median time to progression; mSv, median survival; mos, months; NR, not reported.

Due to the better toxicity profile and the greater ease of administration, carboplatin and oxaliplatin have been also tested combined with pemetrexed. Zinner et al conducted a phase II trial to evaluate the efficacy and the tolerability of pemetrexed 500 mg/m^2 combined with carboplatin AUC 6 every 3 weeks, with vitamin and prophylactic dexamethasone supplementation, in 50 chemonaïve NSCLC patients.[43] The combination achieved a RR of 24%, a median OS of 13.4 months and a 1-year survival rate of 56%. The most common grade 3–4 toxicities were haematological mainly consisting of neutropenia (26%). Similarly, another phase II trial explored the efficacy of the same combination (carboplatin AUC 6 on day 1 and pemetrexed 500 mg/m^2 day 1 every 21-days with vitamin and dexamethasone supplementation) in 50 untreated NSCLC patients showing an overall RR of 28% and an OS of 13.5 months.[44] Toxicities included grade 3–4 neutropenia (34%), anemia (10%), thrombocytopenia (4%), nausea/vomiting and diarrhoea (2%). Consisting with these data, a randomized phase II trial in 80 chemonaïve patients with locally advanced or metastatic NSCLC was done.[45] The patients were randomized to pemetrexed 500 mg/m^2 plus carboplatin AUC 6 on day 1 every 21 days, or to pemetrexed 500 mg/m^2 plus oxaliplatin 120 mg/m^2 on day 1 every 21 days. All patients received dexamethasone and vitamin supplementation. The primary endpoint was RR without any direct comparison between the two arms. Overall, objective RR (31.6% for pemetrexed/carboplatin arm; 26.8% for pemetexed/oxaliplatin arm) and median OS (10.5 months for both arms) were similar between the two groups. Haematological and non-haematological toxicity profile was slightly better in the oxaliplatin/pemetrexed arm compared to carboplatin/pemetrexed arm.

Two randomized phase III trials[45,46] compared platinum plus gemcitabine, one of the most widely used regimen in first line setting of NSCLC treatment, versus the newer and less toxic combination of platinum plus pemetrexed. In the first trial, 1725 chemonaïve NSCLC patients were randomized to cisplatin 75 mg/m^2 on day 1 plus gemcitabine 1250 mg/m^2 on day 1 and 8 every 21 days, versus cisplatin 75 mg/m^2 on day 1 plus pemetrexed 500 mg/m^2 on day 1 every 21 days.[45] All patients received vitamin and steroids supplementation. The primary endpoint was the comparison of OS between treatment arms according to a non-inferiority study design. The results showed no differences between the two arms in terms of survival. In fact, the median OS was 10.3 months in each group (HR = 0.94, 95% CI, 0.84–1.05). Progression free survival was also not inferior (5.1 versus 4.8 months, respectively). Objective RR was similar between the two arms (28.2% versus 30.6%, respectively). Interestingly, a pre-specified sub-group analysis for each of the three histological groups (large cell carcinoma, adenocarcinoma and squamous) demonstrated a significantly better survival in adenocarcinoma patients treated with cisplatin/pemetrexed (12.6 versus 10.9 months, respectively; HR = 0.84, 95% CI, 0.71 to 0.99, $P = 0.03$) compared with cisplatin/gemcitabine. Conversely, patients with squamous histology treated in the cisplatin/pemetrexed arm had a shorter OS compared with patients treated in the cisplatin/gemcitabine arm (9.4 versus 10.8 months; HR 1.23, 95% CI, 1.00 to 1.51, $P = 0.05$). Although both regimen were well tolerated, haematological and non-haematological safety profile favoured cisplatin/pemetrexed combination.

The second phase III trial was conducted in 446 previously untreated NSCLC patients randomly assigned to carboplatin AUC 5 on day 1 plus pemetrexed 500 mg/m^2 on day 1 every 3 weeks or carboplatin AUC 5 on day 1 plus gemcitabine 1000 mg/m^2 on day 1 and 8 every 3 weeks.[46] The primary endpoint was to demonstrate a better health-related quality of life (HRQoL) and toxicity profile in carboplatin/pemetrexed arm. No differences in HRQoL were seen between the two arms. Significantly fewer patients experienced grade 3–4 leucopoenia in carboplatin/pemetrexed arm (23% versus 46%, respectively, $P < 0.001$), neutropenia (40% versus 51%, respectively; $P = 0.024$), thrombocytopenia (29% versus 43%, respectively, $P < 0.001$) and need for transfusions of red blood cells (29% versus 43%, respectively, $P = 0.03$). The overall survival was similar in the two arms (7.3 for carboplatin/pemetrexed arm versus 7.0 months for carboplatin/gemcitabine arm; $P = 0.63$). However, this study did not reveal any association between histology and survival ($P = 0.77$). Table 4 shows the results of pemetrexed plus cis/carboplatin trials in metastatic NSCLC patients treated in second line setting.

Table 4. Results of pemetrexed and cis/carboplatin trials in metastatic NSCLC in first line setting.

Authors	Phase	Regimen	No.	RR (%)	mTTP (mos)	mSv (mos)
Shepherd[41]	II	Pem + CDDP	31	45.8	NR	8.9
Manegold[42]	II	Pem + CDDP	36	39	NR	10.9
Zinner[43]	II	Pem + CBDCA	50	24	5.4	13.5
Koshy[44]	II	Pem + CBDCA	50	28	4.9	13.5
Scagliotti[45]	II	Pem + CBDCA	39	31.6	5.7	10.5
		vs. Pem + Oxa	41	26.8	5.5	10.5
Scagliotti[13]	III	Pem + CDDP	862	30.6	4.8	10.3
		vs. Gem + CDDP	863	28.2	5.1	10.3
Gronberg[46]	III	Pem + CBDCA	219	NR	NR	7.3
		vs. Gem + CBDCA	217			7.0

Abbreviations: Pem, pemetrexed; CDDP, cisplatin; CBDCA, carboplatin; Oxa, oxaliplatin Gem, gemcitabine RR, response rate; mTTP, median time to progression; mSv, median survival; mos, months; NR, not reported.

Role of pemetrexed in maintenance therapy

Maintenance therapy is a prolonged therapy at the end of a specified number of cycles once disease stabilization or tumor response has been achieved, although a universal consensus on definition of this term has not been reached. Pemetrexed, due to its good toxicity profile and ease of administration, is an ideal drug to be tested in this setting.

A recently published, randomized double-blind phase III trial tested efficacy and safety of pemetrexed versus placebo in advanced/metastatic NSCLC patients who did not progressed after four cycles of first line platinum-based chemotherapy.[47] Patients were randomized with 2:1 ratio to pemetrexed 500 mg/m^2 on day 1 every 21 days plus best supportive care (BSC) or intravenous placebo plus BSC. Treatment was continued until disease progression or unacceptable toxicities. The primary endpoint was PFS. In the intention-to-treat population of 663 randomly assigned patients, the PFS was significantly higher with pemetrexed than with placebo (4.3 versus 2.6 months; HR 0.50, 95% CI 0.42–0.61; $P < 0.0001$) as well as disease control rate (49.1% and 28.9% respectively in pemetrexed and placebo arm). Median OS in the intent-to-treat population also improved significantly with pemetrexed compared to placebo (13.4 versus 10.6 months; HR 0.79, 95% CI 0.65–0.95; $P = 0.012$). Moreover, the treatment with pemetrexed was well tolerated: hematological and non-hematological toxicities, which occurred mainly in pemetrexed arm, were considered

mild. A pre-specified analysis of survival according to histology confirmed a longer PFS and OS for patients treated with pemetrexed limited to non-squamous histology.

Another study evaluated Pemetrexed in maintenance setting.[48] This was a phase II trial aiming to test efficacy and safety of pemetrexed, carboplatin and bevacizumab as first line therapy followed by maintenance pemetrexed and bevacizumab until disease progression or unacceptable toxicities. Fifty patients received pemetrexed 500 mg/m^2, carboplatin AUC 6 and bevacizumab 15 mg/kg every 3 weeks for six cycles. Forty-nine patients were assessable for response, achieving an overall response rate of 55%. The median PFS and OS were 7.8 and 14.1 months respectively. The median number of cycles was six (induction plus maintenance therapy), among 30 patients (60%) entered in maintenance phase, 9 (18%) completed ≥18 cycles. Grade 3–4 hematological toxicity was anemia (6%; 0), neutropenia (4%; 0), and thrombocytopenia (0; 8%). Major non-hematological toxicities included proteinuria (2%; 0), venous thrombosis (4%; 2%), arterial thrombosis (2%; 0) and unexpected diverticulitis (6%; 2%).

Taken together, these data suggest that maintenance therapy opens new perspectives for slowing disease progression after first line chemotherapy. Nevertheless, there are some concerns to be highlighted: in the Ciuleanu trial, about 50% of patients in the placebo arm never received additional therapy after progression disease and out of those who received a second line chemotherapy only 18% was treated with

pemetrexed. Moreover, this study was designed before the completion of the pivotal first line study of pemetrexed/cisplatin versus gemcitabine/cisplatin; therefore, before drawing definitive conclusions, safety and efficacy of maintenance pemetrexed should be assessed after first line treatment with pemetrexed containing regimens.

Predictive factors of pemetrexed sensitivity in NSCLC

Until some years ago, the choice of a specific treatment was mainly based on clinical factors such as age, performance status, co-morbidities, safety profile and availability of chemotherapy drugs. The identification of prognostic and predictive factors is an important tool to decide a customized therapy for a specific patient population. To this regard, it is important to specify that prognostic factors affect the outcome regardless of the treatment used, while predictive factors provide information on outcome with regard to a specific therapy.

Several retrospective analysis looking at predictive factors of sensitivity to pemetrexed have been conducted since pemetrexed was approved for second line therapy in NSCLC patients.[5] Retrospective analysis from pemetrexed versus docetxel trial[49] in previously treated NSCLC patients evaluated whether the efficacy was different according to histology. A Cox model of overall survival was used to test for a significant treatment by histology interaction and to estimate HR for OS and PFS.[50] The analysis demonstrated that non-squamous patients treated with pemetrexed had a statistically significant longer OS compared with those treated with docetaxel (9.3 versus 8 months respectively; HR 0.778, 95% CI, 0.607–0.997). Conversely, patients with squamous histology who received docetaxel experienced a statistically higher OS than those treated with pemetrexed (7.4 versus 6.2 months respectively; HR 1.563; 95% CI, 1.079–2.264). Interaction test by histology was also statistically significant ($P = 0.001$).

In a Japanese randomized phase II trial evaluating two different dosages of pemetrexed in pretreated patient, a Cox multiple regression analysis identified some good prognostic factors such as gender (female), good performance status (PS), early stage disease, longer intervals from prior che-

motherapy and histologic type (non-squamous cell carcinoma).[36] Of note, the median survival time of patients with non-squamous cell carcinoma was significantly longer compared with those with other histological subtypes (16.0 months versus 9.3 months; $P = 0.0026$). Smit et al[37] in a phase II trial comparing pemetrexed alone versus combination of pemetrexed plus carboplatin as second line therapy, investigated the presence of polymorphisms related to the enzymes involved in the folate pathway and their association with clinical outcome. A significant correlation between the methylenetetrahydrofolate reductase (MTHFR) C 677T homozygous mutation and an improved clinical outcome ($P = 0.03$) was found. However, there was not a significant correlation between genotypes and chemotherapy-related toxicity and between high and low TS expression genotypes and tumor histology (squamous versus non-squamous).

Recently, based on the results of a large randomized phase III trial, the combination of cisplatin plus pemetrexed gained approval in Europe and in the United States for first line therapy for non-squamous non small cell lung cancer patients.[13] This was the first phase III study in NSCLC showing, in a prospective way, differences in survival based on histology. Cox proportional models were used for treatment-by-histology interactions for OS and PFS ($P = 0.002$) indicating that patients with non-squamous histology who were treated with cisplatin plus pemetrexed had a longer OS and PFS than all other patients. Moreover, within non-squamous histological subgroups, adenocarcinoma and large cell carcinoma had a better outcome in terms of OS when treated with pemetrexed/cisplatin than gemcitabine/cisplatin combination (adenocarcinoma HR 0.84; 95% CI, 0.71–0.99, $P = 0.033$; large cell carcinoma HR 0.67; 95% CI, 0.48–0.96; $P = 0.027$).

These data have been confirmed by another randomized double-blind phase III trial comparing efficacy and safety of pemetrexed versus placebo in advanced/metastatic NSCLC patients non progressed after four cycles of platinum-based chemotherapy as maintenance therapy.[47] Pemetrexed again showed a statistically significant superior PFS (4.5 versus 2.6 months; HR 0.44; $P < 0.0001$) and OS (15.5 versus 10.3 months, HR 0.70, $P < 0.0001$) in non-squamous histology compared with squamous

histology (PFS 2.8 versus 2.6 months, HR 0.69, $P = 0.89$; OS 9.9 versus 10.8 months, HR 1.07, $P = 0.67$). Among patients with non-squamous disease, the improvement in both PFS and OS was significant for pemetrexed in adenocarcinoma and other NSCLC subgroups.

A potential explanation of these results could be related to different expression levels of TS in NSCLC histotypes. Preclinical studies have indicated that over-expression of TS correlate with reduced sensitivity to pemetrexed.[51,52] Consisting with these data, Ceppi et al observed higher expression of TS in squamous cell carcinoma compared with non-squamous histotypes ($P < 0.0001$), providing a rational explanation for better outcome among patients with adenocarcinoma treated with pemetrexed.[4] TS expression has been tested both at mRNA and protein levels in undifferentiated large cell carcinoma using desmocillin-3 (DSC-3) immunostaining as a marker of squamous cell differentiation[53,54] showing higher TS levels in DSC-3 positive compared to DSC-3 negative tumors ($P = 0.02$). Furthermore, Monica et al[55] reported a differential TS mRNA expression in two groups of large cell carcinoma (LCC). Specifically, non-neuroendocrine LCCs had different TS expression, based on their phenotypes: TS levels were higher in the group expressing the adenocarcinoma lineage markers, confirming again the role of histology.

Other Solid Tumors

Multiple Phase II clinical trials have demonstrated that pemetrexed has promising single-agent activity in many other solid tumors, including gastric, colon, pancreatic, breast cancers, bladder, head and neck, and cervical cancers.[56–59] Combination regimens consisting of pemetrexed and other chemotherapeutics or novel molecular-targeted agents are currently under investigation. The combination pemetrexed/cisplatin was evaluated in head and neck and gastric cancers.

Head and neck cancer

Cisplatin monotherapy is a standard of care in patients with advanced head and neck cancer (HNC). Single agent pemetrexed has shown promising activity in patients with locally advanced or metastatic HNC.[60] In a phase I study conducted in approximately 12 patients with advanced solid tumors, 3 patients with HNC had partial responses to combined treatment with cisplatin and pemetrexed.[61] An ongoing phase III study is evaluating pemetrexed in combination with cisplatin versus cisplatin alone in patients with recurrent/metastatic head and neck cancer [www.clinicaltrials.gov].

Gastric cancer

In a phase II trial, pemetrexed showed an overall RR of 21% in patients with advanced gastric cancer.[62] Pemetrexed in combination with cisplatin has shown additive or synergistic activity in gastric cancer cells and human tumor xenografts.[63] In a phase II study, 51 chemonaive patients with advanced gastric cancer received pemetrexed 500 mg/mq and cisplatin 75 mg/mq day 1, every 3 weeks plus vitamin supplementation.[64] Of the 50 patients evaluable for efficacy, 13 had partial response for an overall RR of 26% (95% CI, 14.6%–40.3%) and 15 (30%) had stable disease. Median TTP was 2.8 months (95% CI, 2.2–4.4 months), and median OS was 6.6 months (95% CI, 4.8–10.4 months). Of the 51 patients evaluable for safety, the most frequent NCI-CTC grade 3/4 toxicities were neutropenia in 49% of patients (25% of cycles) and anorexia in 10% of patients (4% of cycles). Recently, in a phase II study, the pemetrexed/oxaliplatin combination showed a response rate (36%; 16/44 patients; 4 CR and 12 PR), time to tumour progression (6.2 months), and survival (10.8 months) comparable to those achieved in studies using different 5-fluorouracil (5-FU)—oxaliplatin combinations, without the inconvenience of prolonged 5-FU schedules, in chemonaive patients with advanced gastric cancer.[65] In conclusion, the pemetrexed/cisplatin combination has a modest activity and acceptable toxicity profile in patients with advanced gastric cancer. There are not yet phase III trials ongoing or planning to evaluate the impact of this combination on survival in gastric cancer.

Conclusions

Pemetrexed is a multitargeted antifolate that has demonstrated antitumor activity, as a single agent and in combination with other chemotherapeutic agents, in various tumor types, especially MPM and NSCLC. Myelo-suppression was the predominant dose-limiting toxicity of pemetrexed. However, the addition of folic acid and vitamin B12 markedly improved

its safety, also when combined with cisplatin. The combination of pemetrexed with cisplatin resulted feasible and effective, without pharmacokinetic interferences. Actually, pemetrexed/cisplatin combination is currently approved for first line treatment of MPM and it has recently gained approval in Europe and in the United States for first line therapy for non squamous NSCLC patients. Moreover, the results obtained about the role of pemetrexed as a maintenance therapy in NSCLC open new perspectives for slowing disease progression after first line chemotherapy. Results in other solid tumors are still preliminary. The tumour histotype and TS expression levels seem to predict sensitivity to pemetrexed in NSCLC and the identification of predictors of response will be an interesting field of study to better define the cancer patient population that really benefits from this combination.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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