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Homocysteine and Methylmalonic Acid: Markers to Predict and Avoid Toxicity from Pemetrexed Therapy[1]

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Abstract

The purpose of this study was to identify predictive factors for severe toxicity caused by antifolate-chemotherapy using pemetrexed (ALIMTA, LY231514), as a model. Data on potential predictive factors for severe toxicity from pemetrexed were collected from 246 patients treated between 1995 and 1999. Multivariate stepwise regression methods were used to identify markers predictive of severe toxicity. Using a multiple logistic regression model allowed us to quantify the relative risk of developing toxicities and to generate a validated clinical hypothesis on ways to improve the safety profile of pemetrexed. Pretreatment total plasma homocysteine (tHcy) levels significantly predict severe thrombocytopenia and neutropenia with or without associated grade 3/4 diarrhea, mucositis, or infection. Pretreatment methylmalonic acid (MMA) levels significantly and independently predict grade 3/4 diarrhea and mucositis; however, these toxicities are still predicted by tHcy alone. Patients with elevated baseline levels of tHcy alone, or of both tHcy and MMA, were found to have a high risk of severe toxicity that led us to postulate that reducing tHcy would result in a reduction of severe toxicity with no harm to efficacy. This study points out for the first time the importance of pretreatment tHcy levels in predicting severe toxicity associated with an antifolate and sets the stage for a prospective clinical intervention to protect patients from pemetrexed-induced severe toxicity and possibly improve the drug's efficacy. Antifolates as a class have been associated with sporadic severe myelosuppression with gastrointestinal toxicity. Although infrequent, a combination of such toxicities can carry a high risk of mortality. This phenomenon had been unpredictable until now. Our work shows that by measuring tHcy, one can identify patients that are at risk of toxicity before treatment. Most importantly, decreasing homocysteine levels via vitamin supplementation leads to a better safety profile of pemetrexed and possibly to an improved efficacy.

Introduction

In 1998, it was estimated that 90% of new anticancer agents designed in laboratories around the world never make it into routine clinical use (1). Three main reasons were put forth for this sobering statistic: (*a*) high toxicity seen with new agents that carry serious safety concerns; (*b*) lack of efficacy of these agents; and (*c*) a disconnect between the work of preclinical bench scientists and bedside clinicians that often reflected a failure to ensure that clinical trial designs for new agents were based on the best-known mechanism of action of the agent.

Because most anticancer agents have a narrow therapeutic window, optimizing the chance that a treatment succeeds without causing undue harm to the patient is of paramount importance. Accurate information about both the new drug and the patient becomes critical. Interruption of development of a new drug or limitation of its effectiveness or wide use occurs when either severe toxicity or lack of efficacy is noted. It is not unusual that, when a new agent shows toxicity with limited antitumor activity, little effort is made to persistently look for ways to circumvent the toxicity with the possibility of improving efficacy. Toxicity or lack of efficacy could be related to a patient's individual clinical, demographic, or genetic profile. Ideally, the goal is to devise a simple, optimal dosing strategy for a new agent that incorporates what is known about its mechanism of action and about the patient characteristics. This paradigm is the subject of the present study. We discuss how, after serious safety concerns arose, predictive factors for severe toxicity associated with pemetrexed were identified, and how these factors led to the formulation of a clinical intervention to modulate the toxicity of this antifolate/anticancer agent while improving its efficacy. The results of this prospective clinical intervention are the subject of a separate upcoming publication.[3]

Antifolates represent one of the most extensively investigated classes of antineoplastic agents, with aminopterin initially demonstrating clinical activity more than 50 years ago (2). Methotrexate was developed shortly thereafter and, today, is a standard component of chemotherapeutic regimens effective for malignancies such as lymphoma, breast cancer, and head and neck cancer (3–6). The cytotoxic activity and subsequent effectiveness of antifolates can be associated

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[3] J. Rusthoven, C. Niyikiza, P. Bunn, *et al.* Reducing toxicity from pemetrexed therapy with folic acid and vitamin B_{12} supplementation, submitted for publication.

with substantial toxicity for some patients. Antifolates, as a class, have been associated with sporadic severe myelosuppression with gastrointestinal toxicity. Although infrequent, a combination of such toxicities, can carry a high risk of mortality. The inability to control these toxicities has led to the discontinuation of clinical development of some antifolates, such as CB3717, and has complicated the clinical development of others, such as lometrexol and raltitrexed (7–9). The ability to predict those patients that are at greater risk of developing severe toxicity would represent an important advantage in the use of these agents.

Lometrexol [LY249543 (disodium form); Lilly Research Laboratories, Tularik Inc., South San Francisco, CA), an antifolate GARFT[4] inhibitor was curtailed in its development by Lilly because of severe and cumulative toxicities. The onset of profound myelosuppression and/or mucositis, in most patients 6–8 weeks after dosing, prevented repeated administration of this anticancer agent in most studies. This led to additional studies in mice (10–11) that revealed that therapeutic efficacy and toxicity of lometrexol were highly dependent on dietary folic acid intake. A subsequent Phase I study showed that lometrexol toxicity could be modulated by folic acid supplementation and that the maximum tolerated dose could be substantially increased (8). Yet, pharmacokinetic studies conducted with 5 mg of folic acid supplementation suggested that folic acid was not acting by enhancing lometrexol plasma clearance (12). Despite a 5-year effort in a series of preclinical and clinical investigations, researchers were still unable to ascertain the mechanism responsible for the reduction in lometrexol toxicity. The lometrexol experience was useful when a second generation GARFT inhibitor (LY249543; Eli Lilly and Company), entered its clinical development with 5 mg of folic acid supplementation 2 days before, the day of, and 2 days after, as part of standard dosage of this anticancer agent. The development was also curtailed because of toxicity leaving some investigators to suspect that the folic acid supplementation regimen that was used was likely inadequate.

It is with this background on antifolate GARFT inhibitors that pemetrexed (ALIMTA, LY231514; Eli Lilly and Company, Indianapolis, IN) clinical development was undertaken. Pemetrexed is a multitargeted antifolate that has demonstrated broad-spectrum antitumor activity in the Phase II setting and is currently undergoing active clinical development (13). This new generation antifolate inhibits several key folate-requiring enzymes of the thymidine and purine biosynthetic pathways, in particular, thymidylate synthase, DHFR, and GARFT, by competing with reduced folate for binding sites (14). The consequent inhibition of intracellular folate metabolism leads to the inhibition of cell growth.

During the course of pemetrexed clinical development, myelosuppression emerged as the principal drug-related toxicity, with 50% of all patients experiencing grade 3/4 neutropenia (13). In particular, Grade 4 neutropenia with grade 3/4 infection, grade 3/4 diarrhea, or grade 3/4 mucositis became life threatening. These toxicities, occurring typically after two cycles of therapy, prompted a renewed aggressive clinical effort to search for ways to avoid them.

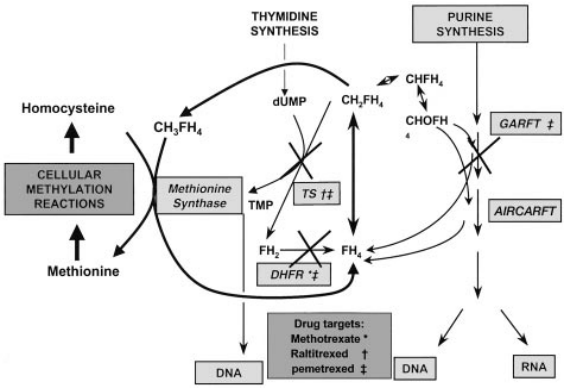

Fig. 1. Folate and homocysteine metabolism.

Given the relevance of folic acid to the toxicity profile previously witnessed with an antifolate such as lometrexol, it was reasonable to postulate that functional folate status could be a useful predictor of toxicity from treatment with pemetrexed. The significant reciprocal association of homocysteine to serum folate and RBC folate has been well established (15–16), and, thus, tHcy concentration may be used as a measure of functional folate status. Folates are required for the metabolism of tHcy, which is converted to methionine by the transfer of a methyl group from the cosubstrate 5-methytetrahydrofolate by methionine synthase, an enzyme that also requires the cofactor methylcobalamin (Vitamin B_{12}). Thus, under conditions of folate and/or cobalamin deficiency, tHcy concentrations rise (Refs. 17–18; see Fig. 1). Because the enzyme L-methylmalonyl CoA mutase is vitamin B_{12} dependent, a B_{12} deficiency will lead to an increase in MMA (19). MMA concentrations are, therefore, a useful tool in differentiating folate and cobalamin deficiency (17–18).

Because of previous observations on the impact of folic acid supplementation on the toxicity profile of lometrexol, a Phase I study of pemetrexed with 5 mg of folic acid 2 days before, the day of, and 2 days after, dosing was initiated. It was shown that the maximum tolerated dose could be substantially increased (20). Still unanswered was the question pertaining to the precise mechanism responsible for the reduction in lometrexol and pemetrexed toxicity when a patient receives folic acid supplementation. To find an answer, a programmatic change was made during the Phase II clinical development of pemetrexed to collect from all treated patients a number of vitamin deficiency markers. Included in the panel of markers were the folic acid and/or vitamins B_{12} deficiency markers, tHcy and MMA, and the vitamin B6 deficiency marker, cystathionine. This study provided initial answers. The objective was to identify one or more specific factors that might predict for pemetrexed-induced toxicity

[4] The abbreviations used are: GARFT, glycinamide ribonucleotide formyltransferase; DHFR, dihydrofolate reductase; MMA, methylmalonic acid; tHcy, total plasma homocysteine; PS, performance status; AP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; AIRCARFT, aminoimidazocarboxamide ribonucleotide formultransferase.

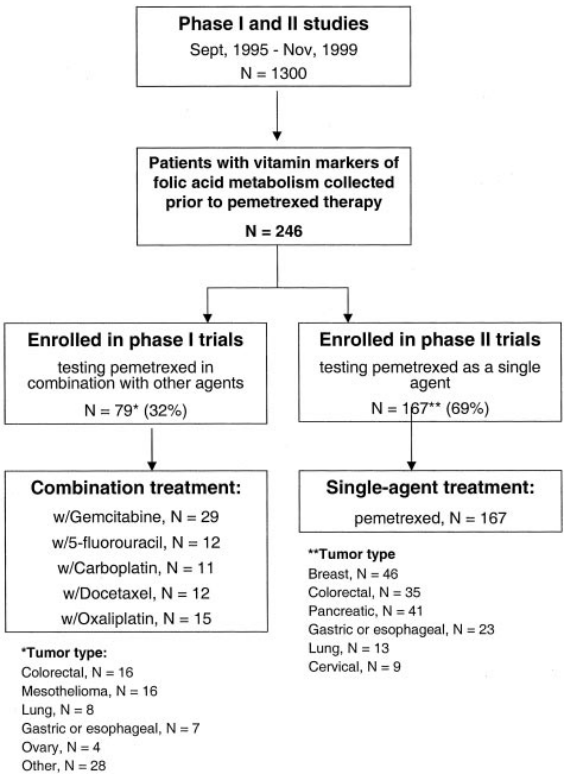

Fig. 2. Patient population description.

such as baseline patient characteristics, cumulative dose, and baseline levels of the vitamin deficiency markers tHcy, MMA, and cystathionine.

Patients and Methods

Patient Population. A total of 305 of the 1300 patients, treated in Phases I and II between September 1995 and November 1995, had the vitamin deficiency markers of tHcy, MMA, and cystathionine collected before and during pemetrexed therapy. Pemetrexed was developed with doses of 500 mg or 600 mg/m^2 every 21 days. Other dosing schedules were explored early in the Phase 1 program but were found not to be feasible for further development. To eliminate the complicating factor of folic acid supplementation on toxicity and the impact of doses not pursued, any patient who received folic acid supplementation at any point during therapy or who received any dosing regimen other than pemetrexed 500–600 mg/m^2, was removed from the analysis. This left a final sample size of 246 patients with data on vitamin deficiency markers (see Fig. 2).

Protocol and informed consent documents were approved by each site's Ethical Review Board before the enrollment of any patient. All of the patients were informed of the nature of the study, and all of the patients signed a written informed consent document before enrollment.

Data Collection and Statistical Analysis. Data from multiple, potentially predictive (or independent) variables were collected before pemetrexed treatment. These variables included age; gender; baseline PS; prior chemotherapy; tumor type; and pre-pemetrexed-treatment serum albumin, liver enzymes, AP, ALT, AST, platelet count, absolute neutrophil count, calculated area under the curve (AUC), and vitamin deficiency markers including tHcy, cystathionine, and MMA. Vitamin deficiency markers were measured over time before each cycle of treatment as long as the patient remained on study. Weekly laboratory studies included complete blood cell and differential WBC counts, serum creatinine, total bilirubin, ALT, AST, and AP. Vitamin deficiency markers were quantified using previously published methods (21). Normal ranges were determined previously using 50 blood donors (25 male, 25 female; ages, 18–65) at the Belle Bonfils Blood in Denver, Colorado. Whole blood was allowed to clot for 1 h at room temperature before serum was collected. Values were calculated as the mean $\pm$ 2 SDs after log normalization.

In the multivariate statistical search for predictive factors for toxicity, dependent outcome variables included the following worst-grade toxicities: (*a*) grade 4 neutropenia; (*b*) grade 4 thrombocytopenia; (*c*) grade 3 or 4 mucositis; (*d*) grade 3 or 4 diarrhea; (*e*) grade 4 neutropenia and grade 3 or 4 infection; and (*f*) grade 4 hematological toxicity or grade 3 or 4 nonhematological toxicity, where a patient experienced any or a combination of the above-listed toxicities. Toxicity was graded according to the National Cancer Institute common toxicity criteria (22).

To identify the most statistically significant predictive factor(s) for a given toxicity, multivariate stepwise regression methods were used whereby variables significant at the 0.25 level were entered into the model and those not significant at the 0.10 level were removed from the model. At each step, a test was performed to verify that the factors included in the model significantly impacted the toxicity of interest (23).

To assess the risk of developing severe hematological or nonhematological toxicities associated with the vitamin deficiency marker of tHcy, alone or with MMA, at study entry, a multiple logistic regression analysis was performed separately for tHcy and MMA while adjusting for the other independent factors (23). Quartiles were determined for each marker using baseline distribution of the marker levels. Ranges were defined using these quartiles to calculate the risk of toxicity for a given patient falling within a specific range. Odds ratios were also calculated as a measure of the extent to which the risk of severe toxicity was affected as baseline tHcy and MMA levels fell above or below the selected reference range.

Results

A total of 1063 courses of pemetrexed were administered. The number of courses per patient ranged from 1 to 17 cycles with a mean of 4 cycles. There were an equal number of males and females (Table 1). Age ranged from 25 to 90 years (mean, 57.8 years), and 25% of the patients were 65 years or older. Ninety percent of patients had a PS of 0 or 1.

Myelosuppression was the major toxicity encountered (Table 2). Grade 4 neutropenia was seen in ~32% of the patients, whereas the presence of grade 4 hematological or grade 3 or 4 nonhematological toxicity was observed in 37%

Table 1 Patient demographics and baseline folic acid, B_6, and B_{12} vitamin deficiency markers

Study	Age (no. of patients)		Gender (no. of patients)		BSA[a] (m^2) mean, range	PS (no. of patients)		tHcy (μmol/liter) mean, range	Cyst (μmol/liter) mean, range	MMA (nmol/liter) mean, range
	<65	≥65	Male	Female		0–1	2			
Phase I combination,[b] $n = 79$	61	18	51	28	1.89, 1.27–2.47	79	7	9.06, 3.6–27.4	376, 98–2481	206, 67–1065
Phase II										
Colorectal cancer, $n = 35$	24	11	22	13	1.90, 1.45–2.23	31	4	11.7, 6–22.5	224, 50–1303	280, 68–2170
Pancreas cancer, $n = 41$	26	15	23	18	1.85, 1.22–2.61	34	7	12.8, 4.7–132.4	235, 52–869	341, 29–8507
Esophageal and gastric cancer, $n = 23$	16	7	17	6	1.69, 1.26–2.22	21	2	12.6, 6.3–31.9	250, 87–718	262, 96–1192
Breast cancer, $n = 46$	42	4	0	46	1.77, 1.48–2.22	42	4	9.0, 4.3–20.5	396, 80–2234	173, 79–734
Cervical cancer, $n = 9$	8	1	0	9	1.61, 1.25–1.97	9	0	7.7, 5.4–10.5	155, 89–282	223, 78–482
NSCLC cancer, $n = 13$	9	4	10	3	1.84, 1.30–2.16	11	2	9.1, 3.7–15.4	533, 198–1921	179, 97–303
Total patients	186	60	123	123		224	22			
Mean values					1.84, 1.22–2.61			10.3, 3.5–132.4	309, 50–2481	237, 29–8507

[a] BSA, body surface area; Cyst, cystathionine; NSCLC, non-small cell lung cancer.
[b] Patients with different primary tumor types.

Table 2 Prevalence of selected toxicities in patients treated with pemetrexed ($n = 246$)

Toxicity	No. of patients	%
Grade 4 neutropenia	79	32
Grade 4 thrombocytopenia	20	8
Grade 3/4 mucositis	12	5
Grade 3/4 diarrhea	15	6
Any grade 4 hematological toxicity or grade 3/4 nonhematological toxicity	92	37
Grade 4 neutropenia + grade 3/4 mucositis	8	3
Grade 4 neutropenia + grade 3/4 diarrhea	8	3
Grade 4 neutropenia + grade 3/4 infection	6	2

of the patients. Grade 4 neutropenia coupled with grade 3 or 4 diarrhea was observed in 3% of patients.

Baseline tHcy and MMA were found to be highly correlated ($R^2 = 0.8870$). The analysis performed to further assess the impact of this correlation (both with and without MMA, as an independent variable) revealed an important interaction between these two vitamin deficiency markers with respect to pemetrexed induced toxicity. tHcy correlated significantly with severe hematological toxicity, as well as with severe diarrhea and severe neutropenia, whether or not MMA was included in the model (significance levels are shown in Table 3). Interestingly, when MMA was included as an independent variable in the model, it was significantly correlated with diarrhea and mucositis, whereas tHcy was not. However, when MMA was excluded from the analysis, the model selected tHcy as the main predictor for diarrhea and mucositis.

The prevalence of selected severe toxicities was found to increase as pretreatment tHcy and MMA levels increased (see Fig. 3). A χ^2 test for trend (24) indicated significantly increased prevalence of severe toxicities with increased pretreatment levels of tHcy (grade 4 neutropenia, $P = 0.0185$; grade 4 thrombocytopenia, $P = 0.0002$; and grade 4 neutropenia + grade 3/4 infection, $P = 0.0064$), and MMA (grade 4 neutropenia, $P < 0.0001$ and grade 4 neutropenia + grade 3/4 diarrhea, $P = 0.0005$). This trend was seen also with selected hematological and nonhematological toxicity (see Fig. 4). Statistically significant increases in prevalence of severe hematological or nonhematological toxicity with increasing pretreatment levels were observed for MMA ($P = 0.0001$), homocysteine ($P = 0.0011$), and for tHcy and MMA quartile intersections ($P = 0.0014$). The most dramatic increase in such toxicities was observed in patients with simultaneous elevations of both markers, in which 15 of 19 patients experienced severe toxicity.

Using quartile-defined ranges, we performed multiple logistic regression analyses, including the same independent variables reported in Table 3. These analyses were run separately for tHcy and MMA and are reported in Fig. 5. In addition, the relative risk for a patient whose pretreatment levels fell above the third quartile for both homocysteine (>11.5 μmol/liter) and MMA (>219.3 nmol/liter) was reported. The tHcy interquartile range of 7.4–11.5 μmol/liter in this study is similar to that considered a normal range in the cardiovascular literature (25–28). As a result, the interquartile range of 7.4–11.5 μmol/liter was used as the normal range for the purpose of relative risk assessment for toxicity (see Fig. 5*C*). Patients with pretreatment tHcy levels below 7.5 μmol/liter had an odds ratio of 0.7, a 30% reduction in the risk of developing a severe toxicity when compared with patients with normal baseline tHcy. This risk reduction was not found to be statistically significant ($P = 0.3672$). Patients with baseline tHcy levels above 11.5 μmol/liter had an odds ratio of 3.1, a 300% increase in the risk of developing a severe hematological or nonhematological toxicity when compared again to those patients with normal baseline tHcy. This increase in the risk was found to be highly statistically significant ($P = 0.0040$).

Using MMA interquartile range as reference in the analysis, we showed that patients with baseline MMA <119.0 nmol/liter had a statistically significant decrease in risk of severe toxicity, with an odds ratio of 0.3 when compared with that of patients with MMA in the reference range of 119.0–219.3 nmol/liter. Patients whose MMA was >219.3 nmol/liter had a borderline significant increase in risk, with an odds ratio of 2.2 when compared with the same reference range (Fig. 5*C*).

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