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ORIGINAL ARTICLE

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Weekly lometrexol with daily oral folic acid is appropriate for phase II evaluation

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Abstract *Purpose*: Lometrexol [(6*R*)-5,10-dideaza-5,6,7,8-tetrahydrofolate] is the prototype folate antimetabolite that targets the de novo purine synthesis pathway. Early phase I trials were confounded by cumulative myelosuppression that prevented repetitive administration. Subsequent preclinical and clinical studies suggested that coadministration of folic acid might favorably modulate lometrexol toxicity without eliminating potential antitumor activity. We set out to determine if concurrent folic acid would allow administration of lometrexol on a weekly schedule, and, if so, to identify an appropriate dose combination for phase II trials. Pharmacokinetic and metabolism studies were undertaken in an attempt to improve our understanding of lometrexol pharmacodynamics. *Methods*: Patients with advanced cancer received daily oral folic acid beginning 7 days before lometrexol and continuing for 7 days beyond the last lometrexol dose. Lometrexol was administered by short i.v. infusion weekly for 8 weeks. Scheduled lometrexol doses were omitted for toxicity of more than grade 2 present on the day of treatment, and dose-limiting toxicity was prospectively defined in terms of frequency of dose omission as well as the occurrence of severe toxic events. Plasma and whole blood total lometrexol contents (lometrexol plus lometrexol polyglutamates) were measured in samples taken just prior to each lometrexol dose. *Results*: A total of 18 patients were treated at five lometrexol dose levels. The maximum tolerated dose was identified by frequent dose omission due to thrombocytopenia and mucositis. The recommended phase II dose combination is lometrexol 10.4 mg/m^2 per week i.v. with folic acid 3 mg/m^2 per day orally. One patient with melanoma experienced a partial response, and three patients, two with melanoma and one with renal cell carcinoma, experienced stable disease. Lometrexol was not detectable in any predose plasma sample tested. The total red blood cell content of lometrexol increased over several weeks and then appeared to plateau. *Conclusions*: Weekly administration of lometrexol is feasible and well-tolerated when coadministered with daily oral folic acid. The nature of the interaction between natural folates and lometrexol that renders this schedule feasible remains unclear. A definition of dose-limiting toxicity that incorporated attention to dose omissions allowed efficient identification of a recommended phase II dose that reflects the maximum feasible dose intensity for a weekly schedule. Lometrexol is a promising, anticancer agent.

Key words Lometrexol · (6*R*)-5,10-dideaza-5,6,7,8-tetrahydrofolate · Folate antimetabolite · Phase I trial

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Introduction

Lometrexol [(6*R*)-5,10-dideaza-5,6,7,8-tetrahydrofolate] is a folate analog candidate anticancer agent of considerable interest for three reasons: it is the prototype selective inhibitor of glycinamide ribonucleotide formyltransferase (GARFT), a folate-dependent enzyme of the de novo purine synthesis pathway [4]; it exhibits a broad range of antitumor activity in murine and human tumor xenograft models [18, 23]; and it has shown sporadic activity against a variety of human cancers in phase I trials [11, 16, 17, 25].

Yet, despite initiation of phase I trials in 1988, lometrexol has yet to enter phase II, largely due to the characteristics of toxicities seen in phase I. In early phase I trials toxicities typically increased in severity and duration with continuing treatment so that administration of more than two or three courses on schedule was impossible [14, 16]. These cumulative toxicities, typically myelosuppression and mucositis, occurred at doses that were unexpectedly low in comparison with murine toxicity studies. As standard laboratory mouse chow contains high levels of folic acid, it was suspected that cumulative toxicity in humans at unexpectedly low doses was related in some manner to differences in folate status between the human subjects and the murine models. Further murine experimentation demonstrated that coadministration of folic acid with lometrexol ameliorates toxicity without necessarily ablating antitumor activity [7]. These observations led to a series of phase I trials of lometrexol administered with folic acid or leucovorin [11, 16, 17, 25]. Nevertheless, a suitable schedule and dose for phase II evaluation has remained elusive.

In order to address this problem, we initiated a phase I trial of lometrexol administered weekly as an intravenous (i.v.) bolus with continuous daily oral folic acid supplementation. We considered it likely that the dose-limiting phenomenon might be inability to adhere to a weekly administration schedule due to mild toxicity, sufficient to require dose omission, rather than severe toxic events. Accordingly, the study was designed to identify, if possible, a dose combination that could be administered over a prolonged period of time without dose-limiting cumulative toxicity and incorporated a prospectively defined criterion for dose omission as a dose-limiting phenomenon.

Materials and methods

Materials

Lometrexol was provided by the Cancer Therapy Evaluation Program of the National Cancer Institute. A large single lot of folic acid 1-mg tablets was purchased yearly from commercial sources during each year of the study.

Patients

Patient eligibility criteria specified: adults with histologically or cytologically confirmed solid tumor malignancy or lymphoma for which there was no reasonable prospect of benefit from any conventional therapy if administered at the time of enrollment; a Zubrod performance status of less than or equal to 2; a life expectancy of more than 16 weeks; at least 4 weeks from prior chemotherapy and radiation therapy; no planned concurrent chemotherapy, anticancer hormonal therapy, or radiation therapy; and acceptable bone marrow (leukocytes $3.7–15.0 \times 10^3/\mu l$, platelets $130–500 \times 10^3/\mu l$, hemoglobin >10.0 g/dl), renal (creatinine <1.5 dl), and liver (AST normal or, in the case of metastatic disease, <2.5 times the upper limit of normal, prothrombin time normal, total bilirubin <1.5 mg/dl) status. Pregnant or nursing women and patients with a continuing requirement for allopurinol treatment, myocardial infarction within the past 12 months or unstable cardiac disease, gastrointestinal disease with significant malabsorption, or other serious complicating medical conditions were not eligible. Patients gave informed consent and agreed to practice a medically acceptable form of contraception.

Study design and patient treatment

Patients received folic acid orally daily beginning 7 days before the first scheduled dose of lometrexol and continuing for 7 days beyond the last administered dose of lometrexol. Lometrexol was administered by short (<2 min) i.v. infusion weekly.

Weekly patient evaluations included an interval history, physical examination, complete blood cell count, reticulocyte count, chemistry panel (creatinine, conjugated and unconjugated bilirubin, and AST), prothrombin time, and urinalysis. The first required tumor evaluation followed the eighth dose of lometrexol. Patients experiencing a response or stable disease were eligible for continuation therapy. Toxicities observed during continuation therapy were not considered in the evaluation of dose-limiting toxicity. All adverse events were identified, and adverse events considered to be possibly, probably, or definitely related to lometrexol were scored according to the NCI Common Toxicity Criteria. Weekly doses were omitted for platelets $<100 \times 10^3/\mu l$ or any toxicity more than grade 2 (except hemoglobin, local alopecia, and weight loss). Dose-limiting toxicity was defined as any grade 3 or greater nonhematological toxicity (except infection, local, and weight loss), grade 3 or greater thrombocytopenia, grade 4 leukopenia, omission of more than three of the first nine scheduled doses due to toxicity (ability to administer dose 9 was evaluated even if it had been determined that treatment would be discontinued due to tumor progression), and transfusion of more than two units of packed red blood cells in excess of documented cumulative phlebotomy subsequent to initiation of folic acid. Patients were enrolled in cohorts of not less than three and not more than five. A dose combination associated with dose-limiting toxicity in three patients was defined as excessively toxic.

Starting doses were folic acid 3 mg/m^2 and lometrexol 5 mg/m^2. The dose escalation scheme called for escalation of the lometrexol dose in successive patient cohorts through four or five dose escalations (initially 60% increments; upon observation of toxicity more than grade 1, 30% increments). Up to a total of eight patients were to be treated at the recommended phase II dose. Appropriate supportive care was to be provided, including red blood cell transfusion for symptomatic anemia or hemoglobin <8 g/dl, but hematopoietic growth factors were not to be used during the first 8 weeks of lometrexol therapy. The study was conducted according to appropriate approvals obtained from the human subjects research committees of the participating institutions. During the course of the study, an independent audit of selected patient records was performed by the Massey Cancer Center.

Pharmacokinetic and pharmacodynamic studies

Blood samples were drawn weekly just prior to lometrexol dosing and prior to ingestion of that day's folic acid. Samples were collected in standard EDTA Vacutainer tubes. Within 30 min, a 0.5-ml aliquot was diluted into a freshly prepared ascorbic acid solution (5 mg in 4.5 ml sterile water), and the resulting suspension was incubated at 37 °C for 30 min and then stored at −20 °C. The plasma fraction of the remaining sample was isolated by centrifugation, and 1.0-ml aliquots were placed in vials containing ascorbic acid 1 mg, and stored at −20 °C. Processed samples were shipped on dry ice to the City of Hope National Medical Center for analysis. Selected samples were analyzed for lometrexol with a high-performance liquid chromatography assay with electrochemical detection as previously described [20]. As incubation of lometrexol polyglutamates in the presence of endogenous serum conjugase reduces the drug to its monoglutamate form, the assay detects total lometrexol content (parent drug and polyglutamate forms). Differences between plasma and whole blood lometrexol levels were presumed to represent red blood cell lometrexol. The lower limit of detection was approximately 5 ng/ml (10 n*M*).

Results

From 22 February 1995 through 16 December 1996, 18 patients were treated (Table 1)[1]. Patient characteristics, primary tumor sites, and prior therapies were typical for subjects enrolled in phase I trials (Tables 1 and 2).

All patients received folic acid 3 mg/m^2 beginning 7 days prior to the first lometrexol dose and ending 7 days following the last lometrexol dose (Table 3). The first 16 patients were treated at four lometrexol dose levels ranging from 5 to 13.5 mg/m^2 (Tables 3 and 4). At level 2, one patient experienced dose-limiting anemia and one patient experienced dose-limiting dose omissions due to stomatitis, but in three other patients treatment was well tolerated, and dose escalation was resumed. At level 3, no patients experienced dose-limiting toxicity. At level 4, three of four patients experienced dose-limiting toxicity, apparent as frequent dose omissions due to stomatitis (one patient) or thrombocytopenia (two patients, one of whom had grade 3 thrombocytopenia).

Two patients were enrolled at a fifth "intermediate" lometrexol dose level defined as treatment initiation at 13.5 mg/m^2 with the contingency to reduce the dose to 10.4 mg/m^2 following the first dose omission. One patient received nine of nine scheduled doses, but a second received only three of five scheduled doses prior to observation of progression of disease. As neither of these patients actually received a dose at 10.4 mg/m^2, levels 4 and 5 were combined for the calculation of dose intensity (Table 4).

Although the prescribed lometrexol dose increased 30% from level 3 (10.4 mg/m^2) to level 4 + 5 (13.5 mg/m^2), dose intensity increased only slightly from 9.2 to 9.4 mg/m^2 per week due to a marked increase in dose omissions that were almost exclusively due to grade 2 toxicities. One patient treated at these doses required dose omission every third week, but patterns of dose omission in other patients were quite varied (Fig. 1).

Platelet counts and reticulocyte percentages tended to fall gradually over the course of several weeks following initiation of lometrexol, but usually rebounded rapidly following omission of one or two lometrexol doses (Fig. 2). Other toxicities (grade 2 or less) observed during the first 9 weeks of treatment included fatigue, diarrhea, elevated AST, nausea, vomiting, leukopenia/neutropenia, and constipation. A high frequency of stable or responding disease permitted observation of a number of patients on treatment beyond an initial 9 weeks, and, in general, therapy continued to be well tolerated. Two patients received more than 4 months of treatment. Both of these patients developed anemia requiring transfusion. One showed a response to erythropoietin while continuing lometrexol until progression of disease.

[1] One patient was enrolled but not treated due to problems with venous access

Table 1 Patient characteristics

Gender (number of patients)	
Men	14
Women	4
Age (years)	
Median	54
Range	29–70
Race/ethnicity (number of patients)	
European-American	10
African-American	5
Asian-American	2
Hispanic	1
Performance status (number of patients)	
0	6
1	11
2	1

It had been hypothesized that cumulative lometrexol toxicity was due to a prolonged terminal elimination phase and increasing lometrexol plasma levels with repetitive dosing [22]. In order to address this hypothesis, we obtained plasma samples for pharmacokinetic analysis immediately before each lometrexol dose. Lometrexol was not detected in any sample tested (data not shown).

It also had been hypothesized that cumulative toxicity was due to accumulation of lometrexol and lometrexol polyglutamates in target tissues or in non-target tissues with subsequent redistribution to target tissues (the latter phenomenon might account for a delayed terminal elimination phase and cumulative pharmacokinetics) [15]. As mature red blood cells lack folylpolyglutamate synthetase [3] and therefore do not actively accumulate folate polyglutamates, we studied red blood cell total lometrexol content as a surrogate for bone marrow stem cell lometrexol content. Red blood cell total lometrexol content gradually increased during the first 8 weeks of treatment and then appeared to plateau (Fig. 2).

Table 2 Tumor types and prior therapy

	No. of patients
Primary site	
Colon	4
Melanoma	3
Stomach	3
Sarcoma	2
Lung	2
Adrenal gland	1
Kidney	1
Pancreas	1
Rectum	1
Prior therapy	
Surgery + chemo	7
Surgery + chemo + radio	7
Chemo + surgery + immuno	2
Chemo + immuno + surgery + radio	1
Chemo + radio	1

Table 3 Dose escalation and dose-limiting events

Dose level	Lometrexol (mg/m^2)	Folic acid (mg/m^2)	Patients treated	Patients with		
				Dose-limiting dose omissions	Grade ≥3 toxicity	Dose-limiting toxicity (total)
1	5	3	3	0	0	0
2	8	3	5	1	1	2[a]
3	10.4	3	4	0	0	0
4	13.5	3	4	3	1	4[b]
5	13.5/10.4	3	2	0	0	0

[a] One patient with dose-limiting dose omissions due to stomatitis; one patient with anemia requiring transfusion (grade 3)
[b] Three patients with dose-limiting dose omissions due to stomatitis (one) and thrombocytopenia (two); one patient with grade 3 thrombocytopenia

Table 4 Adherence to schedule (see Methods for definitions)

Dose level	Lometrexol (mg/m^2)	Patients	Doses delivered					Lometrexol dose intensity (mg/m^2/week)[a]
			9/9	8/9	7/9	6/9[b]	≤ 5/9[b]	
1	5	3	3	0	0	0	0	5.0
2	8	5[c]	1	1	1	1	0	6.7
3	10.4	4	1	2	1	0	0	9.2
4 + 5	13.5	6[d]	1	0	1	1	1	9.4

[a] Patients with progression of disease prior to 9th week included in calculation of lometrexol dose intensity but not in tabular summary of doses delivered
[b] Dose-limiting toxicity by definition
[c] One patient with progression of disease prior to the scheduled ninth dose
[d] Two patients with progression of disease prior to the scheduled ninth dose; one of these had missed four of eight doses prior to identification of progression of disease and therefore qualified as dose-limiting dose omission

One patient experienced a partial response (Table 5). This patient had melanoma of the nasal mucosa with persistent local and regional involvement despite prior surgery, radiation, cryotherapy, and chemohormonal therapy. At the time of enrollment he had a small, palpable tumor nodule of the neck and evaluable-only disease of the nasal cavity and sinuses associated with chronic scarring and acute and chronic inflammation. Under treatment the nasal and sinus disease was stable and the neck nodule resolved completely. The progression-free interval was 5.5 months, following which a new neck nodule developed. Three other patients, one with mucosal melanoma, one with cutaneous melanoma, and one with renal cell carcinoma, experienced stable disease and received continuation therapy. The remaining 13 patients experienced progression of disease within the first 9 weeks.

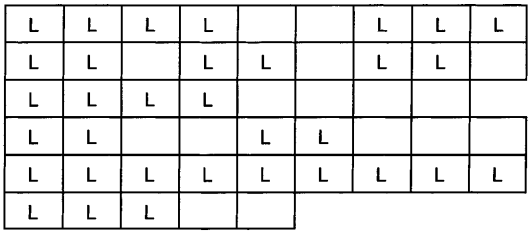

Fig. 1 Patterns of dose omission with lometrexol 13.5/folic acid 3 mg/m^2 per week for up to 9 weeks of observation. Patients were evaluated for treatment weekly. An *L* indicates lometrexol dosing or absence of toxicity that would mandate dose omission. An *empty square* indicates dose omission for toxicity. Fewer than nine squares are shown when cessation of treatment due to progression of disease or other circumstances prevented further assessment

Discussion

Our recommended phase II dose combination is lometrexol 10.4 mg/m^2 with folic acid 3 mg/m^2 per day. With this schedule and dose combination, toxicities rarely were severe (grade 3 or greater) and routinely resolved rapidly upon interruption of dosing. This contrasts with the experience of Ray et al., who showed that lometrexol 3–6 mg/m^2 weekly for 3 weeks every 5 weeks without concurrent folic acid supplementation was associated with severe, cumulative toxicity [16]. We conclude that daily oral folic acid modulates the toxicities of lometrexol administered weekly in a manner that renders frequent administration of a maximum tolerated lometrexol dose feasible and safe.

There are two other complete reports of phase I studies that convey recommended schedules and doses for phase II evaluation. Laohavinij et al. conducted a phase I trial in which lometrexol was administered as an i.v. bolus every 4 weeks (subsequently, every 3 weeks) with folic acid 5 mg administered orally daily for 1 week preceding and following each dose [11]. The recommended phase II dose was lometrexol 170 mg/m^2.

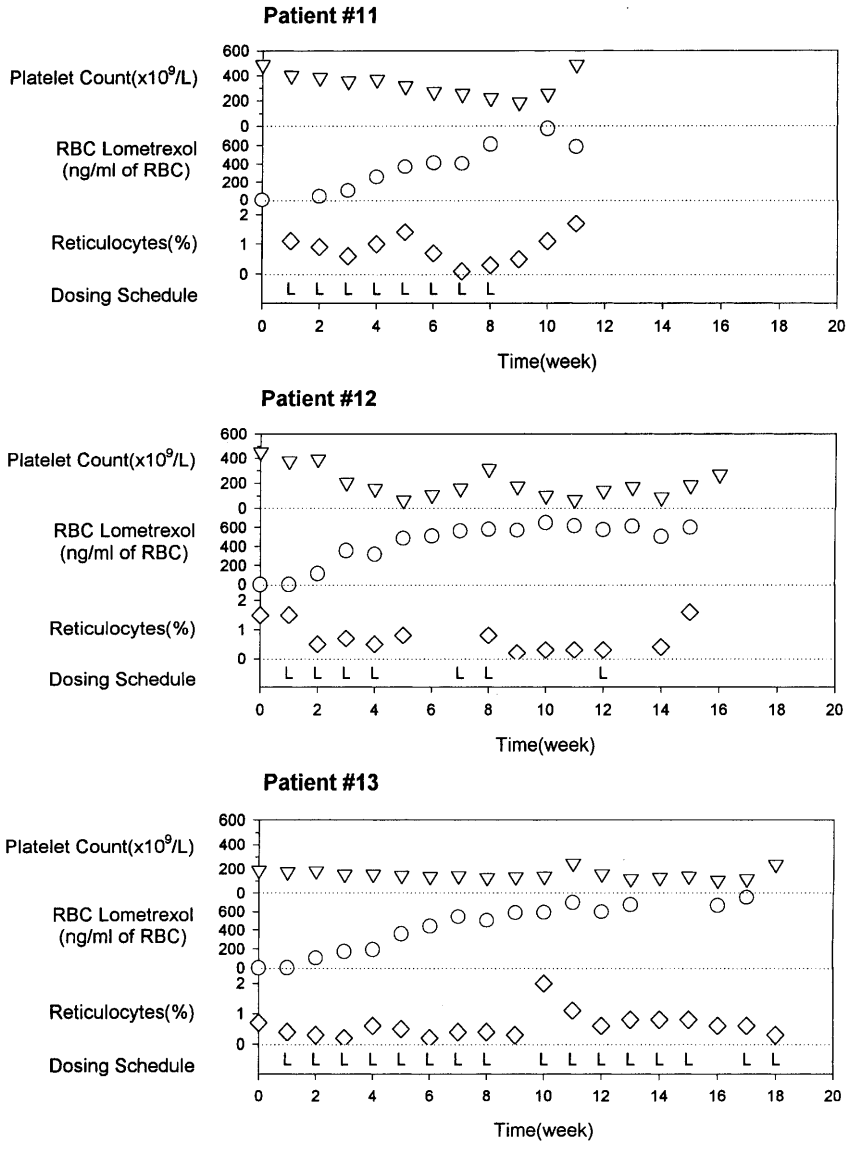

Fig. 2 Lometrexol dosing, platelet counts, reticulocyte percentages, and red blood cell (RBC) total lometrexol content in three patients treated at level 3, lometrexol 10.4 mg/m^2 per week

Comparison with our study indicates that an every-3-weeks schedule allows for administration of almost sixfold more lometrexol per unit time than a weekly schedule. Sessa et al. reported a complex, multiphasic phase I study involving lometrexol without and with delayed leucovorin administration [17]. The recommended phase II schedule and dose combination was lometrexol 60 mg/m^2 every 4 weeks with leucovorin 15 mg orally four times daily days 5–7. Anemia was the dose-limiting toxicity, and one partial response was observed. This schedule is somewhat more dose intense than that proposed by us.

We believe that the schedule and dose combination we have identified represent the most promising for phase II evaluation. First, there are several reasons to prefer lometrexol administration weekly to every 3 or 4 weeks: (1) previous clinical experience with antimetabolites as a class in general indicates greater activity with more frequent administration, (2) preclinical studies have suggested that non-lethally injured cells recover from lometrexol exposure after about 6 days [19], and (3) preclinical tumor model studies have suggested greater lometrexol anticancer activity with more frequent administration [12]. Second, in the case of the study of Laohavinij et al., and as those authors comment, although the dose combination they identified showed some therapeutic activity, it may not represent

Table 5 Disease responses

	Other	Melanoma	Sarcoma[a]	Kidney
Progression of disease	12	0	1	0
Stable disease	0	2	0	1
Partial response	0	1	0	0

[a] One patient not evaluable

the best lometrexol dose for this schedule and folic acid dose, as the trial was ended before dose-limiting toxicity had been identified [11].

As our study was initiated, more complete studies of the effects of folate supplementation upon lometrexol toxicity and antitumor activity in mice have been reported [2]. These have demonstrated a 1000-fold difference in toxic lometrexol dose levels at low and high levels of folic acid intake. Further, they have shown that (1) at low levels of folic acid intake, lometrexol has an extremely narrow therapeutic window that permits only minimal therapeutic effects, (2) at high levels of folic acid intake, lometrexol is non-toxic and non-therapeutic, (3) at intermediate levels of folic acid intake, lometrexol has a therapeutic window that spans an approximately tenfold dose range, and (4) within that intermediate folic acid dose range, lometrexol antitumor activity increases with increasing lometrexol dose until limited by toxicity. A reasonable inference is that, within the intermediate folic acid dose range, there is an array of therapeutic folic acid/(maximum tolerated) lometrexol dose combinations. Clinical data available to date do not conflict with any of these findings. The preclinical data do not address a further subtlety of potential clinical relevance. The presumed array of folic acid/(maximum tolerated) lometrexol dose combinations might be equivalent therapeutically, or, as suggested by the absence of therapeutic activity at very high levels of folic acid intake, within this array there may be a best (most therapeutic) combination. If the latter is the case, the best clinical combination could be identified only in disease activity (phase II) studies.

A preliminary study by Cole et al. [6] perhaps identifies the lower limit for effective folic acid supplementation. In this study of lometrexol weekly ×3 with daily oral folic acid beginning 1 day before, three dose combinations of lometrexol and folic acid, respectively, were studied: 4 mg/m^2 and 1 mg; 5 mg/m^2 and 1 mg; and 5 mg/m^2 and 2 mg. Whereas significant toxicity was observed with the first two dose combinations, only minimal toxicity was seen with the third. This suggests that the lower threshold for effective folic acid supplementation is in the dose range of 1–2 mg/day (total dose). These doses may be compared with the recommended daily intake of 400 μg for persons without unusual demands and estimates of total body stores of 5–20 mg. In order to precisely control folic acid supplementation, we identified all medications, prescription and otherwise, that patients were taking and eliminated all but dietary folate and folic acid as prescribed by protocol. The non-dietary intakes observed generally were less than 1 mg/day.

Other approaches to folate supplementation can be ineffective for modulation of lometrexol toxicity. Muggia et al., for example, conducted a phase I trial to test whether administration of folic acid i.v. immediately before lometrexol might improve tolerance and prevent cumulative toxicity [13]. Lometrexol 15 to 30 mg/m^2 every 3 weeks was administered with folic acid 5 mg i.v. 1 h before lometrexol, or folic acid 25 mg/m^2 3 h before lometrexol. Neither folic acid regimen prevented cumulative lometrexol toxicity.

It has been hypothesized that cumulative lometrexol toxicity is due to accumulation of lometrexol polyglutamates in stem cells. The gradual increase in red blood cell total lometrexol observed in our study is consistent with this hypothesis. The correlation between change in red blood cell lometrexol content and changes in reticulocyte percentage and platelets was poor (Fig. 2), but this is perhaps to be expected as polyglutamate formation presumably occurs in bone marrow progenitors, while polyglutamates persist within the red blood cell for the duration of its life. Thus, red blood cell lometrexol content presumably represents an average of bone marrow progenitor lometrexol contents over the time interval of release of the circulating red blood cell pool. Similar accumulation of methotrexate in red blood cells has been reported [8–10]. Further, there may be significant differences among erythroid, granulocyte-macrophage, and megakaryocyte stem cells in lometrexol accumulation, its cytotoxic effects, and their modulation by folic acid. Red blood cell total lometrexol content has been measured within the context of a phase I trial of lometrexol and a folic acid regimen that was not effective in modulating lometrexol cumulative toxicity [7]. Dose-limiting anemia was frequent in this study and infrequent in our study, yet the quantity and kinetics of red blood cell lometrexol accumulation were quite similar [21]. This suggests that folic acid protection of erythroid stem cells is mediated through a mechanism other than alteration of lometrexol accumulation.

It also has been hypothesized that cumulative toxicity is due to accumulation of lometrexol polyglutamates in non-target tissues such as the liver with delayed release and redistribution to target tissues such as bone marrow and gut. This suggestion is an extrapolation from preclinical studies in which mice fed a standard laboratory (high folate) diet demonstrated no significant accumulation of radiolabelled lometrexol in liver, whereas mice fed a folate-deficient diet demonstrated marked accumulation of lometrexol in liver [15]. There are no clinical observations that directly address this hypothesis. From this hypothesis, however, one might suggest that clinically significant toxicity would become apparent as redistribution of lometrexol or metabolites became apparent as a prolonged terminal elimination phase. This suggestion is reasonable as current lometrexol detection methodologies allow detection of total lometrexol in the 10–40 n*M* range, which overlaps with the concentration range for median cytotoxic effects against tumor cell lines in tissue culture [5, 20]. Nevertheless, we did not observe measurable plasma total lometrexol levels in samples obtained immediately prior to lometrexol dosing following several weeks of lometrexol at maximum tolerated doses or, in individual patients, at times of lometrexol toxicity.

It also does not appear that the effectiveness of folic acid in modulating lometrexol toxicity is due to a change

in the early phases of lometrexol pharmacokinetics. Wedge et al. saw no difference in lometrexol pharmacokinetics in patients receiving 45 mg/m^2 every 4 weeks with or without effective folic acid supplementation [24]. Thus, the cumulative nature of lometrexol toxicity in humans and its modulation by folic acid remains incompletely understood.

We anticipated that dose omissions due to mild toxicities might be frequent and might effectively limit drug administration, and we incorporated into our trial design an empiric, prospective definition of dose-limiting toxicity in terms of dose omission. This appears to have been successful, as an increase in dose prescribed from level 3 to level 4 + 5 yielded no substantial increase in dose intensity. Although not without precedent (see, for example, reference 1), it is our impression that this approach is unusual. We suspect that broader use of this approach might clarify endpoints and result in exposure of fewer phase I subjects to severe toxicities at unrealistically high dose levels (levels at which dose omission already is dose-limiting). It is conceivable, further, that assessment of dose intensity might be used to justify more rapid dose escalation in the early phases of similar phase I trials.

We observed a bona fide partial response in a patient with melanoma, and a number of other patients experienced stable disease for at least 2 months. This experience is consistent with prior phase I studies in which sporadic antitumor activity was perhaps the norm with responses observed in breast, head and neck, non-small-cell lung, and ovarian cancers, and soft tissue sarcoma, as well as minimal responses in colon cancer. As has been commented upon by others, activity in phase I has been a hallmark of agents destined to demonstrate meaningful clinical activity in subsequent studies. By this criterion, lometrexol is a promising agent indeed. The results of this study may also be relevant to the clinical development of second and third generation selective GARFT inhibitors that recently have entered clinical trials. It seems quite likely that a new class of antitumor agents will emerge from among these candidates.

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References

1. Akerley W, Glantz M, Choy H, Rege V, Sambandam S, Joseph P, Yee L, Rodrigues B, Wingate P, Leone L (1998) Phase I trial of weekly paclitaxel in advanced lung cancer. J Clin Oncol 16: 153
2. Alati T, Worzalla J, Shih C, Bewley JR, Lewis S, Moran RG, Grindey G (1996) Augmentation of the therapeutic activity of lometrexol [(6R)-5,10-dideazatetrahydrofolate] by oral folic acid. Cancer Res 56: 2331
3. Barredo J, Moran RG (1992) Determinants of antifolate cytotoxicity: folylpolyglutamate synthetase activity during cellular proliferation and development. Mol Pharmacol 42: 687
4. Beardsley GP, Moroson BA, Taylor EC, Moran RG (1989) A new folate antimetabolite, 5,10-dideaza-5,6,7,8-tetrahydrofolate, is a potent inhibitor of de novo purine synthesis. J Biol Chem 264: 328
5. Boschelli DH, Webber S, Whiteley JM, Oronsky AL, Kerwar SS (1988) Synthesis and biological properties of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid. Arch Biochem Biophys 265: 43
6. Cole JT, Gralla RJ, Karcinal CG, Rivera NP (1992) Lometrexol (DDATHF): phase I trial of a weekly schedule of this new antifolate. Proc Am Assoc Cancer Res 33: 2468
7. Grindey GB, Alati T, Shih C (1991) Reversal of the toxicity but not the antitumor activity of lometrexol by folic acid. Proc Am Assoc Cancer Res 32: 324
8. Kamen BA, Holcenberg JS, Turo K, Whitehead VM (1984) Methotrexate and folate content of erythrocytes in patients receiving oral vs intramuscular therapy with methotrexate. J Pediatr 104: 131
9. Krakower GR, Kamen BA (1984) The reticulocyte rat: a model for analysis of methotrexate polyglutamate dynamics in situ. J Pharmacol Exp Ther 231: 43
10. Kremer JM, Galivan J, Streckfuss A, Kamen B (1986) Methotrexate metabolism analysis in blood and liver of rheumatoid arthritis patients. Arthritis Rheum 29: 832
11. Laohavinij S, Wedge SR, Lind MJ, Bailey N, Humphreys A, Proctor M, Chapman F, Simmons D, Oakley A, Robson L, Gumbrell L, Taylor GA, Thomas HD, Boddy AV, Newell DR, Calvert AH (1996) Phase I clinical study of the antipurine antifolate lometrexol (DDATHF) given with oral folic acid. Invest New Drugs 14: 325
12. Mendelsohn LG, Shih C, Schultz RM, Worzalla JF (1996) Biochemistry and pharmacology of glycinamide ribonucleotide formyltransferase inhibitors: LY309887 and lometrexol. Invest New Drugs 14: 287
13. Muggia FM, Synold TW, Newman EM, Jeffers S, Leichman LP, Doroshow JH, Johnson K, Groshen S (1996) Failure of pretreatment with intravenous folic acid to alter the cumulative hematologic toxicity of lometrexol. J Natl Cancer Inst 88: 1495
14. Nelson R, Butler F, Dugan W Jr, Davis-Land C, Stone M, Dyke R (1990) Phase I clinical trial of LY264618 (dideazatetrahydrofolic acid; DDATHF). Proc Am Soc Clin Oncol 9: 76
15. Pohland RC, Alati R, Lantz RJ, Grindey GB (1994) Whole-body autoradiographic disposition and plasma pharmacokinetics of 5,10-dideazatetrahydrofolic acid in mice fed folic acid-deficient or regular diets. J Pharm Sci 83: 1396
16. Ray MS, Muggia FM, Leichman CG, Grunberg SM, Nelson RL, Dyke RW (1993) Phase I study of (6R)-5,10-dideazatetrahydrofolate: a folate antimetabolite inhibitory to de novo purine synthesis. J Natl Cancer Inst 85: 1154
17. Sessa C, Jong J de, D'Incalci M, Hatty S, Pagani O, Cavilli F (1996) Phase I study of the antipurine antifolate lometrexol (DDATHF) with folinic acid rescue. Clin Cancer Res 2: 1123
18. Shih C, Grindey GB, Houghton PJ, Houghton JA (1998) In vivo antitumor activity of 5,10-dideazatetrahydrofolic acid (DDATHF) and its diastereomeric isomers. Proc Am Assoc Cancer Res 29: 283
19. Smith SG, Lehman NL, Moran RG (1993) Cytotoxicity of antifolate inhibitors of thymidylate and purine synthesis to WiDr colonic carcinoma cells. Cancer Res 53: 5697
20. Synold TW, Xi B, Newman EM, Muggia FM, Doroshow JH (1996) Simple and sensitive method for the quantitative analysis of lometrexol in plasma using high-performance liquid chromatography with electrochemical detection. J Chromatogr B Biomed Sci Appl 683: 245
21. Synold TW, Newman EM, Carroll M, Muggia FM, Groshen S, Johnson K, Doroshow JH (1998) Cellular but not plasma

pharmacokinetics of lometrexol correlate with the occurrence of cumulative hematological toxicity. Clin Cancer Res 4: 2349
22. Taber LD, O'Brien P, Bowsher RR, Sportsman JR (1991) Competitive particle concentration fluorescence immunoassay for measuring 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (lometrexol) in serum. Clin Chem 37: 254
23. Taylor EC (1990) New pathways from pteridines. Design and synthesis of a new class of potent and selective antitumor agents. J Heterocycl Chem 27: 1
24. Wedge SR, Laohavinij S, Taylor G, Boddy A, Calvert AH, Newell DR (1995) Clinical pharmacokinetics of the antipurine antifolate (6R)-5,10-dideaza-5,6,7,8-tetrahydrofolic acid (lometrexol) administered with an oral folic acid supplement. Clin Cancer Res 1: 1479
25. Young CW, Currie VE, Muindi JF, Saltz LB, Pisters KMW, Esposito AJ, Dyke RW (1992) Improved clinical tolerance of lometrexol with oral folic acid. Proc Am Assoc Cancer Res 33: 406