A Population Pharmacokinetic and Pharmacodynamic Evaluation of Pralatrexate in Patients With Relapsed or Refractory Non-Hodgkin's or Hodgkin's Lymphoma

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In a pralatrexate phase I study, patients displayed a high incidence of mucositis of grades 3 and 4. Preliminary evaluations of the pharmacokinetics of the drug and its association with mucositis suggested that pralatrexate exposure (area under the concentration–time curve (AUC)) could be controlled with body size (e.g., weight or body surface area)–based dosing and that pretreatment with folic acid and vitamin B_{12} might diminish the incidence and severity of mucositis. The study was amended, with revised dosing and vitamin B_{12} administration. Data from 47 patients were evaluated using NONMEM. Weight and methylmalonic acid (MMA) level were predictive of pharmacokinetic (PK) variability. AUC and MMA level were positively correlated with the risk of developing mucositis. A lower AUC schedule with vitamin B_{12} pretreatment may control mucositis without compromising efficacy. The covariates identified in this study are comparable with other antifolate analogs. The application of modeling was a critical step in the development of pralatrexate, yielding important suggestions for dose, scheduling, and pretreatment modifications.

T-cell lymphomas (TCLs) represent a heterogeneous group of diseases that are notably more difficult to treat than their B-cell lymphoma counterparts.[1,2] Pralatrexate is a 10-deazaminopoterin folate analog that was designed to have high affinity for the reduced folate carrier,[3–7] an oncofetal protein that is preferentially overexpressed in fetal and malignant tissues and is known to be upregulated by a variety of oncogenes.[6,7] Because of this restricted expression, the reduced folate carrier represents a novel therapeutic target with the potential for a better therapeutic window than other antifolate analogs.

Pralatrexate has marked activity in patients with chemotherapy-resistant TCL, producing a complete or durable response in ~40–50% of patients.[6–8] Research experience with non-small-cell lung cancer established mucositis as the dose-limiting toxicity (DLT) at pralatrexate dosages of 135–150 mg/m^2 every other week.[9,10] The initial incidence of mucositis in lymphoma patients was considerably higher than that seen in the non-small-cell lung cancer study. Population pharmacokinetic (PK) and pharmacodynamic (PD) evaluations of the preliminary data suggested that pralatrexate exposure and nutritional covariates (homocysteine and methylmalonic acid (MMA)) could predict the risk of developing mucositis and that pretreatment of patients with folic acid and vitamin B_{12} could reduce the risk of developing mucositis. The study was amended to a weekly dosing schedule with a requirement for normalized homocysteine and MMA levels, or a 10-day course of folic acid/vitamin B_{12} repletion. These collective data were then used with the objective of developing a population PK/PD model to describe the PK profile of pralatrexate in patients with lymphoma and the impact of the nutritional and PK determinants on the risk of developing mucositis (PD).

RESULTS

Pharmacokinetics

The final PK model was a three-compartment model, parameterized for clearance (CL), volumes of distribution of the central (V_1),

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peripheral (V_2), and deep peripheral (V_3) compartments, and intercompartmental CLs between the central and peripheral compartments (Q_2) and the central and deep peripheral compartments (Q_3). Interindividual variability (IIV) was estimated for all structural parameters with a full variance–covariance matrix. A proportional and additive residual error model was employed. There was an effect of baseline MMA level on CL, as well as an effect of baseline body size (e.g., weight or body surface area), which was included as an allometric function. The PK parameters, associated asymptotic SEs, and the 95% bootstrap confidence intervals are presented in **Table 1**. In general, the parameters are well estimated with low SEs. The bootstrapped confidence intervals are reasonably narrow and do not include 0 for any of the structural parameters. The estimates of Bayesian shrinkage suggested that only Q_2 had notable shrinkage (0.247).

The results of the visual predictive check performed on the final population PK model are presented in **Figure 1** stratified by dose: patients receiving ≤50 and >50 mg/m^2. These figures are presented as concentration vs. time after administration of the dose. Individual predictions were used to represent concentrations reported as below the limit of quantitation (N = 58). For both dose groups, the observed concentrations are centered about the median simulated line up to 48 h; however, after incorporation of predicted below the limit of quantitation concentrations, the bias was seen to be marginal.

Pharmacodynamics

A box-and-whisker plot of the individual pralatrexate area under the concentration–time curve (AUC) values and individual baseline mean MMA values by grade of mucositis is shown in **Figure 2a,b**. Although they are variable, the median AUC and MMA values generally increase with increasing grades of toxicity, thereby suggesting that both covariates are predictors of mucositis. Frequency histograms of the most severe grade of mucositis per patient, stratified by vitamin pretreatment status, were constructed (**Figure 2c**). Because the number of patients receiving vitamin pretreatment was much higher (n = 36) than that of those who were not (n = 11), the adverse event (AE) frequencies were normalized by the total number of patients in each group. **Figure 2c** shows that there was a shift toward less severe grades of mucositis with vitamin pretreatment; this would also make it more difficult to see trends in **Figure 2a,b**. It should be noted that the original data used to identify this trend were also included in the database.

The best model for mucositis identified MMA level and pralatrexate AUC as explanatory variables that predict toxicity. The parameters obtained from the logistic regression along with associated 95% confidence intervals are presented in **Table 2**. Individual patient exposures to pralatrexate as measured by AUC and MMA level were found to be a significant predictor of the probability of developing gastrointestinal toxicity. No other covariates were identified in this evaluation as being predictive of the grade of mucositis that will develop. The IIV associated with the best model is high, and this is probably due to the very small number of subjects in the database. The confidence intervals for the effect of pralatrexate AUC and MMA level do not include zero, suggesting that these covariates are valid.

DISCUSSION

Pralatrexate is a promising new drug for the treatment of lymphoma, particularly of TCL. The TCLs are a complex and challenging set of diseases with few agreed-on treatment alternatives and virtually no consensus regarding frontline or beyond-treatment scenarios. The results of a recent phase II study[11,12] have demonstrated several important aspects regarding the clinical development of pralatrexate. An important initial finding

Table 1 Parameter estimates, associated SEs, and nonparametric bootstrap 95% confidence intervals for final pharmacokinetic model

Parameter (units)		Population mean (SE[a]) (95% CI) (mean of BS)	%CV interindividual variance (SE[a]) (95% CI) (mean of BS)
CL (l/h)	θ_1	13.5 (13.8) (10.4–16.8) (13.6)	
Effect of MMA level	θ_7	−0.261 (27.5) (−0.553–0.0031) (−0.202)	50.0 (46.8) (39.0–87.0) (43.7)
Effect of weight		0.75 Fixed	
V_1 (l)	θ_2	7.31 (20.5) (5.51–9.87) (7.19)	40.1 (77.0) (22.0–69.0) (20.1)
Effect of weight		1 Fixed	
Q_2 (l/h)	θ_3	18.3 (19.0) (11.3–29.7) (17.1)	28.1 (22.4) (14.0–95.0) (27.7)
Effect of weight		0.75 Fixed	
V_2 (l)	θ_4	11.4 (10.4) (9.84–16.1) (11.5)	35.8 (59.1) (15.0–53.0) (38.2)
Effect of weight		1 Fixed	
Q_3 (l/h)	θ_5	2.41 (20.0) (1.36–3.81) (2.70)	65.0 (54.1) (31.0–99.0) (66.3)
Effect of weight		0.75 Fixed	
V_3 (l)	θ_6	22.4 (19.0) (19.0–48.5) (26.0)	67.6 (70.5) (24.0–95.0) (71.1)
Effect of weight		1 Fixed	
CCV residual error (as %CV)		28.4 (4.4) (22.0–34.0) (32.7)	
ADD residual error (as SD)		1.43 (49.6) (0.02–1.42) (0.29)	

$CL = \theta_1 \times (\text{weight}/70)^{0.75} \times (\text{MMA}/190)^{\theta_7}$; $V_1 = \theta_2 \times (\text{weight}/70)$; $Q_2 = \theta_3 \times (\text{weight}/70)^{0.75}$; $V_2 = \theta_4 \times (\text{weight}/70)$; $Q_3 = \theta_5 \times (\text{weight}/70)^{0.75}$; $V_3 = \theta_6 \times (\text{weight}/70)$.

ADD, additive; BS, bootstrap; CCV, proportional; CI, confidence interval; CL, clearance; CV, coefficient of variation; MMA, methylmalonic acid.

[a]SE given as %CV.

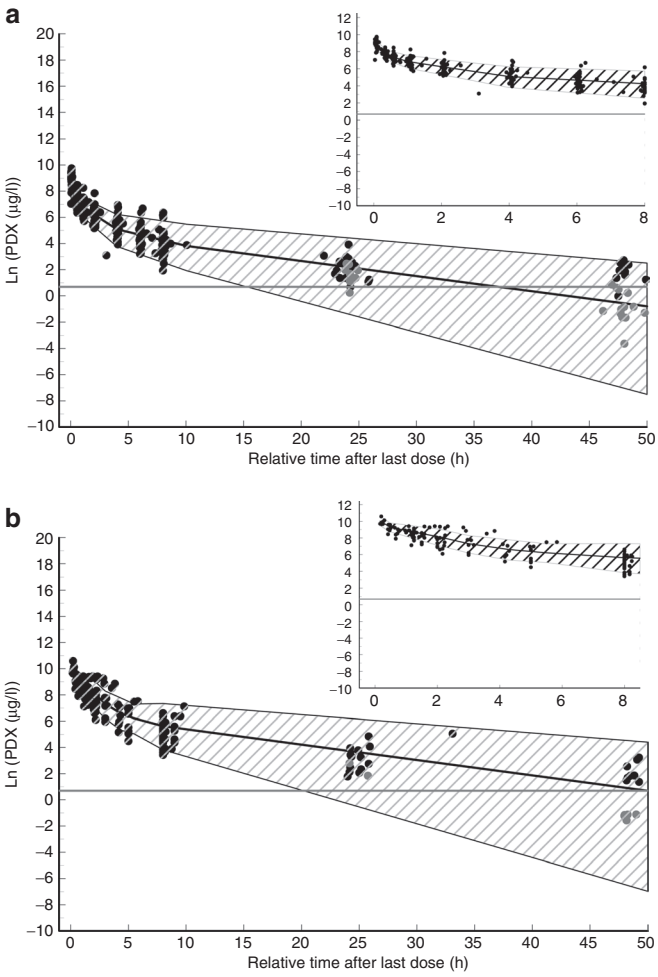

Figure 1 Visual predictive check for pharmacokinetic model. (**a**) Patients receiving up to 50 mg/m^2 pralatrexate. (**b**) Patients receiving >50 mg/m^2. The filled black circles are the observed data, the filled gray circles are the concentrations reported to be below the limit of quantitation (individual model-based predictions), the horizontal line is the assay limit of quantitation, the shaded areas are the 95% prediction intervals, and the solid line is the median of the simulated concentrations. The inset (upper right) represents the first 8 h after administration of the dose, to facilitate visualization of the early time points.

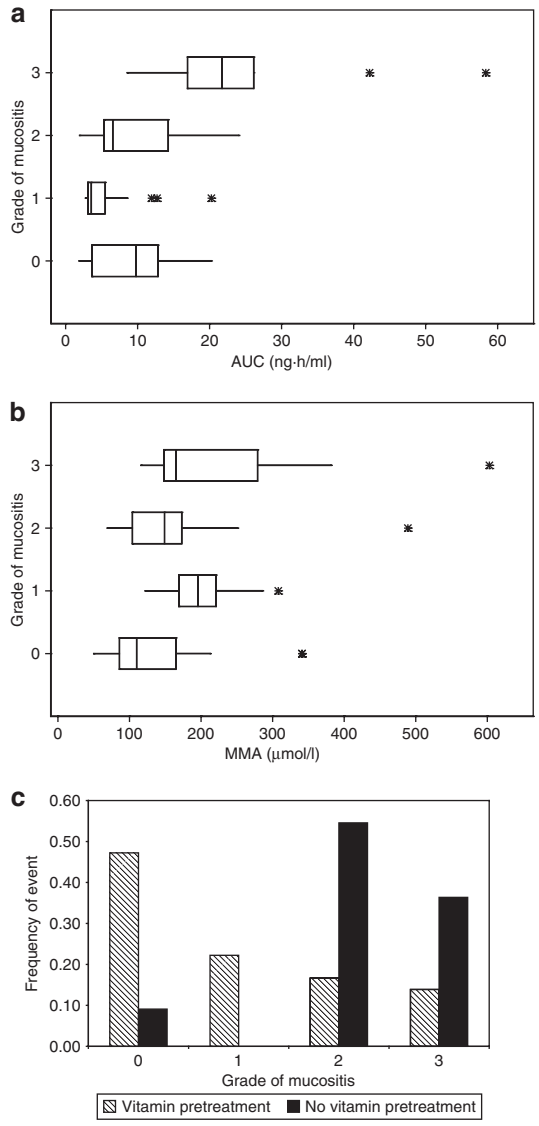

Figure 2 Graphical evaluation of relationship between methylmalonic acid (MMA) pralatrexate exposure and mucositis. (**a**) Horizontal box-and-whisker plot of individual pralatrexate area under the concentration–time curve (AUC) values by grade of mucositis. (**b**) Horizontal box-and-whisker plot of individual baseline MMA levels by grade of mucositis. The boxes represent the distribution of individual values from the lower 25th percentile to the upper 75th percentile, the solid line in the middle of the box is the median value, the lower and upper whiskers represent the 5th and 95th percentiles, and other symbols represent outliers. (**c**) Frequency of events of most severe grade of mucositis by vitamin pretreatment status. The filled black histograms represent the normalized frequency of events for patients without vitamin pretreatment, and the striped bars represent the normalized frequency of adverse events for patients with vitamin pretreatment.

was that patients with lymphoma experienced a significantly higher risk of developing mucositis as compared with patients with non-small-cell lung cancer. Preclinical murine xenograft studies suggested comparable to superior efficacy with more frequent dosing schedules. Also, preliminary PK/PD modeling suggested that patients with high MMA levels were more likely to experience mucositis; this finding led to studies involving the evaluation of alternative dosing and scheduling (D.R. Mould and O.A. O'Connor, unpublished data).

The PKs of pralatrexate were best described using a linear, three-compartment model. Weight and MMA level were found to be predictive of PK variability: the addition of these covariates reduced IIV in CL by 11%; in V_2 by 4.8%; and in Q_2 by 45.71%. Over the range of weights in this database (44–116 kg), pralatrexate CL would be expected to change from 9.5 to 19.7 l/h, assuming a constant MMA value of 190 µmol/l. This twofold change in CL supports the decision to fix the dose on the basis of body surface area, which follows the allometric trend for CL. Over the range of MMA levels in the database (60–600 µmol/l), CL would be expected to decrease from 18.2 to 10 l/h—a 1.8-fold decrease. The identified covariates were reasonable; folate deficiency decreases the oxidative activity of the liver,[13] and body weight is commonly identified as a covariate for highly metabolized drugs.

Table 2 Parameter estimates, associated SEs, and nonparametric bootstrap 95% confidence intervals for the final pharmacodynamic model

Parameter		Estimate (SE) (95% CI) (mean of BS)
B_0	θ_1	6.36 (29.4) (3.38–13.4) (6.98)
B_1	θ_2	1.67 (36.3) (0.761–4.33) (1.87)
B_2	θ_3	5.04 (29.6) (2.88–11.1) (5.64)
Effect of AUC	θ_4	0.435 (42.1) (0.195–1.23) (0.490)
Effect of MMA level	θ_5	2.35 (24.7) (0.313–4.72) (2.41)
Interindividual variability	η_Y	4.38 (66.1) (2.24–13.5) (4.42)

$(P(Y \le 0|\eta)) = \theta_1 - \text{AUC} \times \theta_4 - (\text{MMA}/190) \times \theta_5 + \eta_Y$; $(P(Y \le 1|\eta)) = \theta_2 - \text{AUC} \times \theta_4 - (\text{MMA}/190) \times \theta_5 + \eta_Y$; $(P(Y \le 2|\eta)) = \theta_3 - \text{AUC} \times \theta_4 - (\text{MMA}/190) \times \theta_5 + \eta_Y$.

AUC, area under the concentration–time curve; BS, bootstrap; CI, confidence interval; MMA, methylmalonic acid.

Table 3 Baseline demographic values ($n = 47$ patients)

Demographic	Mean (SD)	Range
Age (years)	54.2 (15.4)	23–80
Weight (kg)	75.3 (18.3)	44–116
BSA (m^2)	1.85 (0.25)	1.41–2.34
CrCL (ml/min)	81.6 (29.7)	31.8–153
HCY (μmol/l)	9.65 (4.21)	4.6–33
B_{12}	901 (496)	279–1,923
MMA level (μmol/l)	188 (103)	61–603
Received vitamins	Yes = 36; no = 11	
Gender	Males = 19; females = 28	

BSA, body surface area; CrCL, creatinine clearance; HCY, homocysteine; MMA, methylmalonic acid.

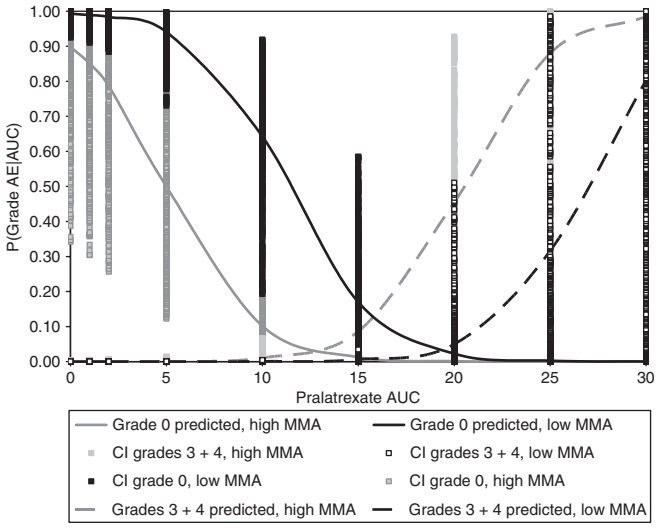

Figure 3 Probability values of grades 0 and 4 mucositis as a function of pralatrexate area under the concentration–time curve (AUC) for patients with low MMA levels (MMA = 100) and high MMA levels (MMA = 300). The solid gray line represents grade 0 toxicity for patients with high MMA levels, the solid black line represents grade 0 toxicity for patients with low MMA levels, the broken gray line represents grades 3 and 4 toxicity for patients with high MMA levels, and the broken black line represents grades 3 and 4 toxicity for patients with low MMA levels. 90% Confidence intervals (CIs) for each probability curve are presented as square symbols. MMA, methylmalonic acid.

Pralatrexate exposure and MMA level were identified as predictors of developing mucositis. The effect of elevated MMA level on the probability of developing mucositis resulted in an alternative dose strategy of vitamin pretreatment. It is known that folate deficiency contributes to impaired DNA synthesis, especially thymidylate synthesis. Gastrointestinal toxicity resulting from folate deficiency is probably attributable to impaired turnover of normal epithelium, which typically exhibits a high rate of proliferation owing to the continual shedding of the mucosa. No other covariates were identified in this evaluation.

The PK profile of pralatrexate was generally consistent with that of other antifolate agents that exhibit multiphasic PK behavior. In addition, the covariates identified as being associated with pralatrexate were also generally consistent with those found to be associated with other structurally similar antineoplastic agents. A population PK evaluation of pemetrexed (Alimta)[14] used a two-compartment model; however, the structural parameters were similar to those identified for pralatrexate. The PKs of pemetrexed were found to be influenced by creatinine CL (CrCL), body weight, alanine transferase level, and folate deficiency. Also, raltitrexed (Tomudex) PK parameters were similar to those of pralatrexate, and the disposition was found to be influenced by weight, CrCL, and albumin level.[15] Finally, the PKs of methotrexate were well described using a two-compartment model and are influenced by body weight.[16] Although CrCL was not identified in this evaluation, there is evidence that pralatrexate undergoes renal elimination through active transport.[17] Multiple covariate functions were tested, and the distribution of CrCL values was found to be normal with good representation across the entire CrCL range, as shown in **Table 3**. The failure to identify CrCL as a covariate may be a consequence of the small sample size.

Figure 3 shows a graphical representation of the mucositis probability curves (grades 0 and 3) as a function of pralatrexate AUC for patients with low and high MMA levels. For patients with high MMA levels, the probability curve for toxicity is substantially shifted to the left for both grades of toxicity, indicating that at any pralatrexate AUC value, the probability of developing grade 3 or 4 mucositis is substantially higher for patients with high MMA levels. This result supports the recommendation that patients should be given folate and vitamin B_{12} treatment prior to initiating therapy with pralatrexate. Furthermore, given that vitamin pretreatment normalizes MMA level, the need to adjust doses on the basis of this covariate becomes irrelevant.

Although the small sample size limits a robust logistic regression evaluation, there is some evidence to support the relevance of the covariates identified in this evaluation. The data presented in **Figure 2b** suggest that pretreatment with vitamins results in a shift toward lower grades of gastrointestinal toxicity. Furthermore, the covariates identified in this evaluation were consistent with findings for other antifolate agents. For example, Ortiz *et al.*,[18] in a study involving rheumatoid arthritis patients taking methotrexate, reported on the efficacy of folic acid supplementation in reducing or

abrogating gastrointestinal toxicity. Although their patient population was different from the one in this study, it is worth noting that similar trends have been reported with the use of vitamin supplements in cancer patients as well.[19–22]

The results of this evaluation strongly support individualized dosing of pralatrexate on the basis of body surface area and that pretreatment with folate and vitamin B_{12} may be essential for alleviating the gastrointestinal toxicity caused by pralatrexate. The application of model-based evaluation was an important component in dose selection for this drug and has facilitated its further development. These data have supported the initiation of a large, international, registration-directed study of pralatrexate for the treatment of patients with relapsed or refractory TCL. Additional PK and PD data are being obtained from this study. These findings are based on data from a limited number of subjects, and therefore all the covariates identified in this evaluation warrant further evaluation.

In conclusion, a population PK/PD model was developed for pralatrexate. This model supports dosing on the basis of body surface area so as to reduce variability in patient exposure. The model identified pralatrexate AUC and MMA level as predictors of the grade of mucositis that could develop and suggested pretreatment with folate and vitamin B_{12} to reduce the risk of patients developing mucositis.

METHODS

Patient demographics and study design. Details of the study design and the method of conducting the study have been presented elsewhere.[8,12] Briefly, only patients having histologically confirmed non-Hodgkin's lymphoma—as defined by the World Health Organization/Revised European–American Lymphoma classification—or Hodgkin's disease were enrolled. Patients were required to meet standard eligibility criteria, and all patients were required to indicate their informed consent by signing an institutional review board–approved consent form. The study was a single-center, single-agent phase "II-I-II" study, conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983).

For phase II, dosing began at 135 mg/m^2. Patients who tolerated this dose were eligible for dose escalation by 15 mg/m^2 until toxicity developed. During the phase II study, dose escalation involved a modified Fibonacci schema starting at 30 mg/m^2 weekly for 3 of 4 weeks. Three new patients per cohort were treated and observed for ≥7 weeks before opening the next cohort. At each new dose, patients were enrolled until the first DLT, defined as: (i) grade 3 or 4 nonhematologic toxicity (excluding alopecia and infusion-site reactions), (ii) nausea, vomiting, or diarrhea persisting for >10 days of dosing and uncontrolled by aggressive treatments, (iii) grade 3 febrile neutropenia or grade 4 neutropenia that precludes administration of the next dose, (iv) grade 4 thrombocytopenia, (v) any toxicity not defined as a DLT that lasts 3 weeks, and (vi) any patient who misses more than one-third of the doses in cycle 1 secondary to failure to meet eligibility criteria. Dose escalation was as follows: 2X, 3X, 4X, and so on, with no upper limit, where X = 15 mg/m^2. If any one patient experienced a DLT, three additional patients were added. If two out of six patients experienced a DLT during the first cycle, this dose level was declared to be the maximum administered dose, and the previous dose level was declared the maximum tolerated dose. Once the maximum administered dose was defined, there was no further dose escalation. An additional 20–30 patients were allowed to be treated at the weekly maximum tolerated dose.

Dose modifications were based on the grade of mucositis observed. If patients failed to meet the criteria for retreatment, it was delayed by 1 week. Dose escalation was allowed after the patient had received two cycles of pralatrexate with no evidence of toxic side effects. Dose modifications related to the development of mucositis were as follows. (i) If no toxicity was observed after two cycles, the dose was escalated to 150 mg/m^2 for two cycles. If no mucositis was noted after these two additional cycles, escalation in 15 mg/m^2 increments was allowed indefinitely. (ii) Patients with grade 1 or 2 mucositis during cycle 1 or 2 received folate (5 mg orally q.d. beginning 3 days prior to pralatrexate and continuing on the day of and the day after pralatrexate) and vitamin B_{12} (1,000 μg orally q.d. or 100 μg i.m. every 8–9 weeks) in the third cycle. Continued mucositis of grade 1 or 2 could lead to dose reduction at the discretion of the investigator. The dose was reduced to 100 mg/m^2 for patients experiencing grade 3 or 4 toxicity. If a patient had no mucositis (grade 0) while on folic acid, the dose was escalated, and if grade 4 mucositis developed subsequently, the patient was removed from the study. (iii) Patients who developed grade 3 mucositis received folate/B_{12} supplementation, and the dose of pralatrexate was reduced to 100 mg/m^2. If they experienced no mucositis while on folate/B_{12} at 100 mg/m^2, the dose of pralatrexate was escalated to 135 mg/m^2. If these patients subsequently developed mucositis of grade 2 or 3, the dose was reduced to 100 mg/m^2. If they developed a grade 1 mucositis after vitamin repletion and dose reduction, they were maintained at the lower dose level and continued on vitamins. (iv) Patients who developed grade 4 mucositis were given vitamin supplementation and were removed from the study.

Patients who developed symptoms of toxicity unrelated to mucositis were assessed according to the following criteria. Patients who developed grade 2 or 3 toxicity were treated with a repeat cycle of pralatrexate at 120 mg/m^2. If grade 2 or 3 toxicity persisted, dose reduction to 100 mg/m^2 was allowed. Continued grade 2 or 3 toxicity after the second reduction resulted in removal of the patient from the study. Patients who developed grade 4 toxicity were removed from the study.

Determination of pralatrexate concentration. Plasma samples were analyzed for pralatrexate concentration using a sensitive dihydrofolate reductase base assay similar to that used for the quantitation of methotrexate.[23] This assay was validated with pralatrexate. The lower limit of detection was 0.5 ng/ml, with low interday variability.

Population PK/PD data sets. The doses evaluated in this study ranged from 30 to 254 mg/m^2 administered as an intravenous infusion, initially administered over 1–2 h in the first seven patients, but over 2–20 min in all subsequent patients. Dose modifications were allowed for all patients, including dose escalation and reduction in the original every-other-week phase II component; thereafter, only dose reductions were allowed once the maximum tolerated dose was defined on the weekly schedule in the phase I study. Dense plasma concentrations were obtained during the first cycle of treatment, with only trough (e.g., at 48 h after the dose) concentrations being collected afterward. After PK data were merged, there were a total of 462 pralatrexate exposure determinations from 47 patients.

Baseline covariate values were available for this evaluation. CrCL was estimated using the Cockroft and Gault formula,[24] which was capped at 150 ml/min.[25] Body surface area was estimated using the equation of DuBois and DuBois.[26] Baseline demographic information is presented in **Table 3**.

The relationship between pralatrexate exposure and the development of mucositis was evaluated. Once the PK model was developed, AUC values were calculated using individual CL estimates. Mucositis was graded using the National Cancer Institute Common Toxicity Criteria scale, version 2.0. Patients were evaluated for up to 15 cycles of pralatrexate treatment. In total, there were 128 PD observations of mucositis from 47 patients: 73 observations involving no mucositis, 18 observations of grade 1 events, 27 observations of grade 2 events, 9 observations of grade 3 events, and 1 observation of a grade 4 event (this last event was combined with the grade 3 events for analysis).

Population PK modeling. Pralatrexate PKs were analyzed using nonlinear mixed-effects modeling as implemented in the computer program NONMEM (version V, level 1.1; GloboMax, Hanover, MD).[27–31] A log-transform-both-sides approach was used. The YLO option was tested but did not affect model parameters. Standard model-building approaches were employed for this analysis.[32,33] For all models, the reduction in the objective function, reduction in the magnitude of IIV, and graphical improvement in diagnostic plots were assessed. Preference was given to models that converged successfully and produced SEs of the parameter estimates. The first-order conditional estimation method was used for all evaluations.

The modeling procedure first identified a model that adequately described the observed concentration data; subsequently, stochastic models were explored. IIV was described using an exponential model. Interoccasion variability was also evaluated.

A combined additive and proportional model was used to describe residual variability.

$$\mathrm{Ln}\left(C_{ij}\right)=\mathrm{Ln}\left(\hat{C}_{ij}\right)+\sqrt{\varepsilon_{ij}^{\ 2}+\frac{\varepsilon_{ij}^{\ 2}}{\hat{C}_{ij}^{\ 2}}}, \tag{1}$$

where C_{ij} is the jth plasma concentration measured in the ith individual, $\hat{C}_{ij}$ is the individual model-predicted plasma concentration, and ε_{ij_1} and ε_{ij_2} are the proportional and additive components, respectively.

Once the base model was established, models evaluating the effects of normalized covariates on relevant PK parameters were explored. Covariate selection was based on the previous criteria as well as on the criterion of the covariate being clinically relevant (e.g., causing a >20% change in the parameter over the range of covariates in the database). Single covariate models identified at the commonly used P value of ≤0.005 were pooled into a full model, and backward elimination at $P < 0.001$ was conducted.

Continuous covariates were modeled as:

$$\mathrm{TVP}=P_{\mathrm{pop}}\cdot\prod_{k=1}^{m}\left(\frac{\mathrm{cov}_k}{\mathrm{reference}}\right)^{\theta_k}, \tag{2}$$

where TVP represents the model-predicted PK parameter (e.g., CL) for the "typical" individual with covariate value(s) cov_k, P_{pop} represents the intercept value for the parameter TVP, cov_k represents the individual value for that covariate, reference represents a normalization value, and θ_k represents the power term. For CrCL, several alternative functions (e.g., linear) were also tested. Because body weight can affect more than one parameter simultaneously, an allometric scale function[34,35] of the form given below was also tested to evaluate the effect of body size. Although this was not a nested model, the function was selected on the basis of previously described improvements in similar models (e.g., improvements in diagnostic plots, reduction in IIV).

$$\begin{aligned}\mathrm{TVP}_{\mathrm{clearance}}&=P_{\mathrm{pop}}\times\left(\frac{\mathrm{Wt}}{\mathrm{median}}\right)^{0.75}\\ \mathrm{TVP}_{\mathrm{volume}}&=P_{\mathrm{pop}}\times\left(\frac{\mathrm{Wt}}{\mathrm{median}}\right).\end{aligned} \tag{3}$$

For binary covariates (e.g., gender), the models were parameterized:

$$\mathrm{TVP}=P_{\mathrm{pop}}\cdot\prod_{k=1}^{m}\left(\theta_k^{\mathrm{cov}_k}\right), \tag{4}$$

where cov_k is either 0 (for the standard or reference patient) or 1 for the comparative patient, and θ_k represents a scale factor for the influence of that covariate.

For this analysis, the reference patient was a Caucasian man, 55 years old, weighing 70 kg, with a body surface area of 1.8 m^2. The patient had a CrCL of 80 ml/min, a total homocysteine level of 10 µmol/l, and an MMA level of 190 µmol/l.

The population PK model was evaluated though nonparametric bootstrap analysis[36] and a visual predictive check.[37] In addition, shrinkage estimates were calculated using the method described by Karlsson.[38] Shrinkage occurs when there are too few data from each individual, resulting in individual parameter estimates approaching the population mean. Shrinkage can adversely affect individual estimates of exposure, such as AUC.

Population PD modeling. The PD evaluation was performed using logistic regression for ordinal data.[39–41] The base model was used to investigate and substantiate the relationship between the reported grade of mucositis and pralatrexate AUC. Given that grade 4 mucositis was infrequent (only one event), the analysis combined grades 3 and 4. The demographics for this database were consistent with those listed in **Table 3**.

Individual AUC values were calculated as AUC = dose/CL, based on each patient's administered dose for each cycle of treatment and individual estimate of CL from the final population PK model. Evaluation of PK interoccasion variability suggested that random between-occasion variability in CL was low (~ 2%), and therefore this was not considered in the calculation of AUC values.

The probability that an AE grade Y is less than or equal to an AE of grade m (where m ranges from 0 to 3, because grades 3 and 4 were combined) is given by the function described below:

$$h\{P(Y\le m|\eta)\}=f_{\mathrm{pretreat}}-f_{\mathrm{covariate}}+\eta_Y, \tag{5}$$

where $h\{P(Y\le m|\eta)\}$ is the log odds of a specific grade of AE occurring, f_{pretreat} is the cumulative log odds of an AE occurring in the absence of covariate factors, $f_{\mathrm{covariate}}$ is the function describing the effect of a particular covariate such as AUC, and η_Y is the IIV in the log odds of the probability of experiencing an AE of a specific grade.

The preliminary descriptions of the AE data did not include a term for drug exposure. Afterward, the effects of drug exposure, followed by covariates such as age, were evaluated.

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CONFLICT OF INTEREST

D.R.M., K.S., and S.D. were paid consultants of Allos Therapeutics through Projections Research, Inc. The other authors declared no conflict of interest.

1. Gisselbrecht, C. *et al.* Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). *Blood* **92**, 76–82 (1998).
2. Rüdiger, T. *et al.* Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's Lymphoma Classification Project. *Ann. Oncol.* **13**, 140–149 (2002).
3. Wang, E.S., O'Connor, O.A., She, Y., Zelenetz, A.D., Sirotnak, F.M. & Moore, M.A. Activity of a novel anti-folate (pralatrexate, 10-propargyl 10-deazaaminopterin) against human lymphoma is superior to methotrexate and correlates with tumor RFC-1 gene expression. *Leuk. Lymphoma* **44**, 1027–1035 (2003).
4. Sirotnak, F.M., DeGraw, J.I., Schmid, F.A., Goutas, L.J. & Moccio, D.M. New folate analogs of the 10-deaza-aminopterin series. Further evidence for markedly increased antitumor efficacy compared with methotrexate in ascitic and solid murine tumor models. *Cancer Chemother. Pharmacol.* **12**, 26–30 (1984).
5. Schmid, F.A., Sirotnak, F.M., Otter, G.M. & DeGraw, J.I. New folate analogs of the 10-deaza-aminopterin series: markedly increased antitumor activity of the 10-ethyl analog compared to the parent compound and methotrexate against some human tumor xenografts in nude mice. *Cancer Treat Rep* **69**, 551–553 (1985).

6. O'Connor, O.A. Developing new drugs for the treatment of lymphoma. *Eur. J. Haematol. Suppl.* 150–158 (2005).
7. O'Connor, O.A. Pralatrexate: an emerging new agent with activity in T-cell lymphomas. *Curr. Opin. Oncol.* **18**, 591–597 (2006).
8. O'Connor, O.A. *et al.* Pralatrexate, a novel class of antifol with high affinity for the reduced folate carrier-type 1, produces marked complete and durable remissions in a diversity of chemotherapy refractory cases of T-cell lymphoma. *Br. J. Haematol.* **139**, 425–428 (2007).
9. Krug, L.M. *et al.* 10-Propargyl-10-deazaaminopterin: an antifolate with activity in patients with previously treated non-small cell lung cancer. *Clin. Cancer Res.* **6**, 3493–3498 (2000).
10. Krug, L.M. *et al.* Phase I and pharmacokinetic study of 10-propargyl-10-deazaaminopterin, a new antifolate. *Clin. Cancer Res.* **9**, 2072–2078 (2003).
11. O'Connor, O.A. *et al.* A phase "2-1-2" study of two different doses and schedules of pralatrexate, a high affinity substrate for the reduced folate carrier (RFC-1), in patients with relapsed or refractory lymphoma reveals marked activity in T-cell malignancies (abstract C85). Proceedings of AACR-NCI-EORTC International Conference Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications, 22–26 October 2007, 283.
12. O'Connor, O.A. *et al.* A phase "2-1-2" study of two different doses and schedules of pralatrexate, a high affinity substrate for the reduced folate carrier (RFC-1), in patients with relapsed or refractory lymphoma reveals marked activity in T-cell malignancies. *J. Clin. Oncol.* (in press).
13. Williams, J.N. Jr.,Monson W.J., Sreenivisan, A., Dietrich, L.S., Harper, A.E. & Elvehjem, C.A. *et al.* Effects of a vitamin B12 deficiency on liver enzymes in the rat. *J. Biol. Chem.* **202**, 151–156 (1953).
14. Ouellet, D., Periclou, A.P., Johnson, R.D., Woodworth, J.R. & Lalonde, R.L. Population pharmacokinetics of pemetrexed disodium (ALIMTA) in patients with cancer. *Cancer Chemother. Pharmacol.* **46**, 227–234 (2000).
15. Blair, E.Y., Rivory, L.P., Clarke, S.J. & McLachlan A.J. Population pharmacokinetics of raltitrexed in patients with advanced solid tumours. *Br. J. Clin. Pharmacol.* **57**, 416–426 (2004).
16. Aumente, D., Buelga, D.S., Lukas, J.C., Gomez, P., Torres, A. & García, M.J. Population pharmacokinetics of high-dose methotrexate in children with acute lymphoblastic leukaemia. *Clin. Pharmacokinet.* **45**, 1227–1238 (2006).
17. Fury, M.G. *et al.* A phase I clinical pharmacologic study of pralatrexate in combination with probenecid in adults with advanced solid tumors. *Cancer Chemother. Pharmacol.* **57**, 671–677 (2006).
18. Ortiz, Z., Shea, B., Suarez-Almazor, M.E., Moher, D., Wells, G.A. & Tugwell, P. The efficacy of folic acid and folinic acid in reducing methotrexate induced gastrointestinal toxicity in rheumatoid arthritis. A metaanalysis of randomised controlled trials. *J. Rheumatol.* **25**, 36–43 (1998).
19. Branda, R.F., Naud, S.J., Brooks, E.M., Chen, Z. & Muss, H. Effect of vitamin B12, folate, and dietary supplements on breast carcinoma chemotherapy-induced mucositis and neutropenia. *Cancer* **101**, 1058–1064 (2004).
20. Malempati, S. *et al.* Phase I trial and pharmacokinetic study of pemetrexed in children with refractory solid tumors: the Children's Oncology Group. *J. Clin. Oncol.* **25**, 1505–1511 (2007).
21. Fossella, F.V. & Gatzemeir, U. Phase I trials of pemetrexed. *Semin. Oncol.* **29** (2 suppl. 5),8–16 (2002).
22. Niyikiza, C. *et al.* Homocysteine and methylmalonic acid: markers to predict and avoid toxicity from pemetrexed therapy. *Mol. Cancer Ther.* **1**, 545–552 (2002).
23. Kinahan, J.J. *et al.* Fluorometric analysis of 10-deazaaminopterin, 10-ethyl-10-deazaaminopterin and known metabolites. *Ann. Biochem.* **150**, 203–213 (1985).
24. Cockroft, D.W. & Gault, M.H. Prediction of creatinine clearance from serum creatinine. *Nephron* **16**, 31–41 (1976).
25. Kirkpatrick, C.M., Duffull, S.B. & Begg, E.J. Pharmacokinetics of gentamicin in 957 patients with varying renal function dosed once daily. *Br. J. Clin. Pharmacol.* **47**, 637–643 (1999).
26. DuBois, D. & DuBois, E.F. A formula to estimate the approximate surface area if height and weight are known. *Arch. Intern. Med.* **17**, 863–871 (1916).
27. Boeckmann, A.J., Beal, S.L. & Sheiner, L.B. NONMEM Users Guide: Parts I–VIII (University of California, San Francisco, San Francisco, CA, 1998).
28. Sheiner, L.B. & Beal, S.L. Bayesian individualization of pharmacokinetics: implementation and comparison with non-Bayesian methods. *J. Pharm. Sci.* **71**, 1344–1348 (1982).
29. Sheiner, L.B., Rosenberg, B. & Marathe, V.V. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. *J. Pharmacokinet. Biopharm.* **5**, 445–479 (1977).
30. Sheiner, L.B., Beal, S., Rosenberg, G.B. & Marathe, V.V. Forecasting individual pharmacokinetics. *Clin. Pharmacol. Ther.* **36**, 294–305 (1979).
31. Beal, S.L. & Sheiner, L.B. The NONMEM system. *Am. Stat.* **34**, 118–119 (1980).
32. Mandema, J.W., Verotta, D. & Sheiner, L.B. Building population pharmacokinetic-pharmacodynamic models. *J. Pharmacokinet. Biopharm.* **20**, 511–528 (1992).
33. Wade, J.R., Beal, S.L. & Sambol, N.C. Interaction between structural, statistical, and covariate models in population pharmacokinetic analysis. *J. Pharmacokinet. Biopharm.* **22**, 165–177 (1994).
34. Anderson, B.J., McKee, A.D. & Holford, N.H. Size, myths and the clinical pharmacokinetics of analgesia in paediatric subjects. *Clin. Pharmacokinet.* **33**, 313–327 (1997).
35. Holford, N.H. A size standard for pharmacokinetics. *Clin. Pharmacokinet.* **30**, 329–332 (1996).
36. Yafune, A. & Ishiguro, M. Bootstrap approach for constructing confidence intervals for population pharmacokinetic parameters. I: A use of bootstrap standard error. *Stat. Med.* **18**, 581–599 (1999).
37. Yano, Y., Beal, S.L. & Sheiner, L.B. Evaluating pharmacokinetic/pharmacodynamic models using the posterior predictive check. *J. Pharmacokinet. Biopharm.* **28**, 171–192 (2001).
38. Karlsson, M.O. Model-building diagnostics. DIA Meeting, Philadelphia, PA, M5–6 December 2005.
39. Sheiner, L.B,. Beal, S.L. & Dunne, A. Analysis of nonrandomly censored ordered categorical longitudinal data from analgesic trials. *J. Am. Stat. Assoc.* **92**, 1235–1255 (1997).
40. Mccullugh, P. Regression models for ordinal data. *J. R. Stat. Soc. Ser B* **42**, 109–142 (1980).
41. Mandema, J.W. & Stanski, D.R. Population pharmacodynamic model for keterolac analgesia. *Clin. Pharmacol. Ther.* **60**, 619–635 (1996).