Cancer Chemother Pharmacol (2006) 57: 401–411
DOI 10.1007/s00280-005-0036-1

ORIGINAL ARTICLE

Jane E Latz · Ajai Chaudhary · Atalanta Ghosh
Robert D Johnson

# Population pharmacokinetic analysis of ten phase II clinical trials of pemetrexed in cancer patients

Received: 22 December 2004 / Accepted: 17 April 2005 / Published online: 2 December 2005
© Springer-Verlag 2005

**Abstract** *Purpose*: The objectives of these population pharmacokinetic analyses were to (1) assess the overall disposition of pemetrexed, (2) characterize between-patient and within-patient variability and identify influential covariates with respect to pemetrexed pharmacokinetics; and, (3) provide individual empirical Bayesian estimates of pharmacokinetic parameters for use in a subsequent pharmacokinetic/pharmacodynamic evaluation of neutropenia following pemetrexed administration. *Patients and methods*: Data from 287 patients who received 441 cycles without folic acid or vitamin $B_{12}$ supplementation during participation in one of ten phase II cancer trials were evaluated by population pharmacokinetic analysis using NONMEM. Starting doses were 500 or 600 mg pemetrexed per $m^2$ body surface area, administered as 10-min intravenous infusions every 21 days (1 cycle). The model was developed using data from eight of the ten studies. Predictive performance was evaluated using data from the other two studies. *Results*: The population pharmacokinetics of pemetrexed administered as a 10-min intravenous infusion are well characterized by a two-compartment model. Typical values of total systemic clearance, central volume of distribution, distributional clearance, and peripheral volume of distribution were 91.6 ml/min, 12.9 l, 14.4 ml/min, and 3.38 l, respectively. Based on these parameter estimates, the terminal elimination half-life of pemetrexed was approximately 3.5 h. Renal function was identified as a covariate with respect to total systemic clearance, and body surface area as a covariate with respect to the central volume of distribution. *Conclusion*: Total systemic exposure (AUC) for a given dose of pemetrexed increases as renal function decreases. Since pharmacodynamic analyses have shown that AUC and not $C_{max}$ is the primary determinant of neutropenic response to pemetrexed, this suggests that dose adjustments based on renal function, rather than body surface area, might be considered for pemetrexed.

**Keywords** Cancer · Pemetrexed · Population pharmacokinetics · NONMEM · Clearance · Renal elimination · Alimta

Robert D Johnson was an employee of Eli Lilly and Company, Indianapolis, USA, at the time this work was completed

J. E Latz (✉) · A. Chaudhary · A. Ghosh
Global Pharmacokinetics, Pharmacodynamics, and Trial Simulation, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285 USA
E-mail: latz_jane_e@lilly.com
Tel.: +1-317-2761336
Fax: +1-317-2767040

R. D Johnson
Proctor & Gamble Pharmaceuticals, Cincinnati, OH, USA

## Introduction

Pemetrexed is a novel anticancer agent that has recently been approved in combination with cisplatin for treatment of malignant pleural mesothelioma [46] and as single-agent therapy for second-line treatment of non-small cell lung cancer [17]. As a cytotoxic agent, the primary mechanism of action of pemetrexed is inhibition of the enzyme thymidylate synthase (TS) [43, 16], which decreases the thymidine available for DNA synthesis [15, 39]. Pemetrexed also inhibits dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyl transferase (GARFT), a folate-dependent enzyme that is involved in purine synthesis [43]. Pemetrexed is clinically active in a range of solid tumors, including mesothelioma, nonsmall cell lung, bladder, head and neck, breast, cervical, colorectal, pancreatic, and gastric cancers [1, 27, 38, 36, 29, 30, 44, 14, 9, 23, 3].

The pharmacokinetics of pemetrexed following single-agent administration as a 10-min intravenous infusion were evaluated in three phase I dose-escalation studies [34, 35, 21]. Pemetrexed had a small steady-state volume of distribution ($V_{ss} \sim 15$ l), was eliminated rapidly from plasma (effective terminal elimination half-life of 2–5 h at doses ranging from 525 mg/$m^2$ to

700 mg/m$^2$) primarily by urinary excretion of unchanged drug (70–90% of dose), and exhibited dose-proportional increases in plasma concentrations.

A more extensive evaluation of the pharmacokinetics of pemetrexed was performed using population pharmacokinetic techniques with plasma concentration-time data from 287 patients that participated in 10 phase II cancer trials. The studies were prospectively designed to include sparse blood-sampling strategies. After establishing the model and confirming its predictive ability, individual empirical Bayesian estimates of clearance based on the model were used to explore the impact of dosing regimens (body surface area [BSA]-based, fixed, and renal function-based) on pemetrexed total systemic exposure. Individual estimates of pharmacokinetic parameters were also incorporated into a subsequent pharmacokinetic/pharmacodynamic evaluation characterizing the time course of neutropenia following pemetrexed administration [18].

## Methods

### Clinical studies and biological sampling

Ten phase II studies of single-agent pemetrexed in individual tumor types (Table 1) were included in the population pharmacokinetic analyses. The primary objective for each of these nonrandomized, single-arm studies without controls was to determine the response rate in each tumor type for patients treated with pemetrexed. The trials were approved by the relevant ethics committee at the participating medical institutions and sponsored by Eli Lilly and Company. All participants gave written informed consent, and studies were conducted in accordance with the ethical principles of the most recent version of the Declaration of Helsinki.

Eligibility criteria included: male and nonpregnant, nonlactating female patients; 18 years of age or older; histologically or cytologically confirmed carcinoma with bidimensionally measurable lesions with clearly defined margins as assessed by medical photograph, computerized tomography, magnetic resonance imaging, other imaging scan, or palpation; estimated life expectancy of at least 12 weeks; and a performance status of 0–2 on the Eastern Cooperative Oncology Group/World Heath Organization scale.

Each study was prospectively designed to include sparse blood-sampling strategies. Three to five samples per patient were collected at cycles 1 and 3. Sampling times were randomly selected from designated collection intervals (0–2, 2–6, 6–12, and 12–36 h) relative to the start of pemetrexed infusion. The collection intervals were selected based on the full pharmacokinetic profile obtained from a phase I study [35] to provide an adequate characterization of the pharmacokinetic profile after intravenous administration.

### Doses and formulation

Patients were dosed based on BSA and were initially assigned to receive pemetrexed 500 or 600 mg/m$^2$ as a 10-min intravenous infusion every 21 days (1 cycle). Patients did not receive folic acid or vitamin $B_{12}$ supplementation. Dose adjustments (i.e., reductions) at the start of subsequent courses of therapy were based on nadir counts or maximal nonhematologic toxicity from the preceding cycle of therapy.

### Measurement methods

Two assays were used to quantitate the plasma pemetrexed concentrations used in these analyses. Samples from four of the studies were analyzed using a high-performance liquid chromatographic (HPLC) method with ultraviolet detection as previously described [34]. An assay based on HPLC coupled to tandem mass spectrometry with electrospray ionization was used for the remaining six studies [7]. The plasma sample (0.5 ml) and internal standard (ISTD: [$^2H_4$]pemetrexed) were precipitated with 7% perchloric acid. The samples were centrifuged for 5 min at 21,000 RCF. A sufficient

**Table 1** Summary of data contributed to the population pharmacokinetic analyses by study

| | Total patients (F/M) | Total treatment cycles |
|---|---|---|
| **Studies included in the index dataset** | | |
| Colorectal | 39 (15F, 24M) | 68 |
| Pancreatic | 35 (16F, 19M) | 53 |
| Breast | 25 (25F, 0M) | 36 |
| Esophageal | 11 (4F, 7M) | 18 |
| Renal | 27 (6F, 21M) | 39 |
| Head and neck | 26 (3F, 23M) | 43 |
| Bladder | 14 (0F, 14M) | 15 |
| Cervical | 32 (32F, 0M) | 52 |
| Index dataset total | 209 (101F, 108M) | 324 |
| **Studies included in the validation dataset** | | |
| NSCLC(1) | 55 (19F, 36M) | 87 |
| NSCLC(2) | 23 (8F, 15M) | 30 |
| Validation dataset total | 78 (27F, 51M) | 117 |
| **Total of index and validation datasets** | 287 (128F, 159M) | 441 |

*F* female; *M* male; *NSCLC* nonsmall cell lung cancer

amount of supernatant was filtered through a 0.22 μm filter and transferred to an autosampler vial. The sample was chromatographed under reverse phase conditions on an YMC Basic™ C8 column using a gradient system with water and acetonitrile containing 0.2% formic acid. Results were calculated using a weighted linear regression of the standard curves [1/(concentration)$^2$]. The validated standard curve was 10–2,000 ng/ml. The interassay precision for the low-range assay was ≤ 10.3% and the interassay accuracy ranged from −3.4% to 10.0%. A second method was validated with an analytical range from 1,000 ng/ml to 200,000 ng/ml. Except for plasma volume (0.1 ml), details of the second method are identical to the aforementioned low-range assay. The interassay precision for the high-range assay was ≤ 9.6%, and the interassay accuracy ranged from −2.8% to 0.3%. Pemetrexed (for concentrations up to 200,000 ng/ml) was stable in human plasma for at least 26 months when stored at either approximately −20°C or approximately −70°C.

Pemetrexed concentrations for approximately 6% of the samples were reported as below the quantifiable limit (BQL) of the assay. Therefore, BQLs were excluded from the population pharmacokinetic analyses. The majority of these results (> 70%) were 14–21 days after dosing.

Values for creatinine clearance (CrCL; standard Cockcroft-Gault formula [$CrCL_{cg.std}$] and lean body mass [LBM] formula [$CrCL_{cg.lbm}$]) and BSA were calculated using standard formulae [8, 40].

## Data analysis

The analyses included data from 287 patients (441 cycles) enrolled in 10 studies as summarized in Table 1. A population pharmacokinetic model was developed using an index dataset from eight of the ten studies. In order to verify that the model adequately described data beyond that used for model development, the predictive ability of the final model was then evaluated using a validation dataset comprised of data from the two remaining studies. All analyses were performed using the nonlinear mixed-effect modeling program, NONMEM (version V) with PREDPP (version V) using first-order conditional estimation (FOCE) with interaction ($\sigma$–$\Omega$ interaction) [4, 42, 45].

## Pharmacostatistical Base Model Development

An open two-compartment model with constant rate input (intravenous infusion) and parameterized in terms of clearance (CL), central volume of distribution ($V_1$), intercompartmental clearance ($Q$), and peripheral volume of distribution ($V_2$) was selected to describe the pharmacokinetics of pemetrexed. The selection of this model was based on results from phase I studies where extensive serial blood sampling was performed [35] and on an earlier analysis [28] of interim data using a portion of the patients in the current dataset.

A series of two-compartment pharmacostatistical models were systematically evaluated to identify the model that best described the data. Between-patient variability was modeled using an exponential error structure (log-normal distribution). The following models were tested: between-patient variability on CL; between-patient variability on CL and $V_1$; between-patient variability on CL, $V_1$, and $V_2$; and between-patient variability on CL, $V_1$, $V_2$, and $Q$. For each of the between-patient variability models tested, three residual error models (additive, proportional, and combined additive and proportional) were evaluated.

## Covariate identification

Patient factors evaluated as potential covariates were specified a priori and included the following continuous variables: age, alanine transaminase, albumin, alkaline phosphatase, aspartate transaminase, body mass index, BSA, body weight, CrCL, dose, serum creatinine, total bilirubin, and total protein. Categorical variables evaluated as potential covariates included: alcohol use, assay method, ethnic origin, vitamin deficiency marker status, gender, smoking status, and cycle. Each potential covariate was tested for relationships with CL, $V_1$, and $V_2$. Continuous covariates were tested using linear additive, proportional, and power models. For linear and proportional models, potential covariates were normalized by their population median; for example, linear models were coded $P = \theta_1 + \theta_2 \bullet$ COV, where $P$ is the individual's estimate of the parameter (e.g., CL, $V_1$, or $V_2$), $\theta_1$ represents the typical value of the parameter, $\theta_2$ represents the effect of the covariate, and COV is the ratio of the individual's covariate value to the population median value.

Since pemetrexed is eliminated primarily by renal excretion of unchanged drug, a strong relationship between CL and $CrCL_{cg.std}$ was observed (change in the minimum value of the objective function [ΔMOF] with inclusion of $CrCL_{cg.std}$ as a covariate = −95.065). Therefore, all other potential covariates were tested in combination with $CrCL_{cg.std}$.

Potentially significant covariates were identified, based on maximum likelihood criterion, as those which, when added on a single parameter to the model containing $CrCL_{cg.std}$ as a covariate relative to CL, resulted in a decrease in the NONMEM objective function of 3.84 points or more ($P$ value ≤ 0.05 based on $\chi^2$ distribution with one degree of freedom).

## Final model development

A full model was developed using established covariate search and model development strategies. Potential covariates were added to the model sequentially, based on

the change in objective function for the individual covariate in combination with $CrCL_{cg.std}$; those covariates that reduced the objective function the greatest amount when added in combination with $CrCL_{cg.std}$ were added to the model first. Potential covariates that did not result in a decrease in the objective function of 3.84 points or more on sequential addition to the model were removed from the analysis. Once a full model was established, the process was then reversed, with each potential covariate being removed individually from the full model. Covariates retained in the final model were those that resulted in a statistically significant increase in MOF (≥10.8 points for 1 degree of freedom, $P < 0.001$), when removed from the full model.

### Model evaluation

Objective function mapping [41, 20], leverage analysis [20, 12], and an assessment of predictive ability based on prediction errors [22] were used to evaluate the robustness of the final population pharmacokinetic model.

### Evaluation of pemetrexed pharmacokinetics by cancer type

Once the predictive ability of the final model was confirmed, cancer type was evaluated as a covariate on the established pharmacokinetic model. Although alterations in the pharmacokinetics of pemetrexed by cancer type might be statistically significant, results were only considered clinically relevant as follows: CL >20% change; $V_1$ or $V_2$ >40% change. Therefore, although statistically significant, a result was not considered clinically relevant unless the 95% confidence interval contained 0.8 or 1.2 for CL or 0.6 or 1.4 for $V_1$ or $V_2$. The criteria for clinical relevance were based on AUC (not $C_{max}$ or $t_{1/2}$) having been identified a primary determinant of neutropenic response [35].

## Results

### Patient characteristics

Population pharmacokinetic evaluations included 287 patients. As indicated in Table 2, patient characteristics were similar between the index and validation datasets.

### Plasma concentration and doses

The index dataset was comprised of 1,184 concentrations from 209 patients (324 cycles) or about 75% of the available data, and the validation dataset was comprised of 412 concentrations from 78 patients (117 cycles). The clinical trials were conducted using BSA-based dosing, which yielded a dose range of 126 mg/m$^2$ to 838 mg/m$^2$ (mean 561 mg/m$^2$). Pharmacokinetic modeling was based on absolute dose with a corresponding dose range of 244 mg to 1,494 mg (mean 990 mg). The distribution of doses was similar between the two datasets.

The distribution of blood sample collection times relative to the beginning of pemetrexed infusion was sufficient to characterize the disposition of pemetrexed. The overall profile was consistent with phase I data where plasma concentrations of pemetrexed declined over three orders of magnitude within 48 h after the start of infusion (Fig. 1).

**Table 2** Summary of patient characteristics

| Patient characteristic | Index dataset ($n = 209$) | | | Validation dataset ($n = 78$) | | |
|---|---|---|---|---|---|---|
| | Mean (%CV) | Median | Range | Mean (%CV) | Median | Range |
| Age (years) | 57.3 (19) | 57.3 | 26.3–79.1 | 60.6 (16) | 62.3 | 36.6–80.2 |
| BSA (m$^2$) | 1.76 (14) | 1.74 | 1.26–2.50 | 1.78 (12) | 1.77 | 1.28–2.35 |
| Weight (kg) | 68.3 (25) | 66.0 | 34.0–138 | 69.3 (23) | 69.0 | 36.0–127 |
| $CrCL_{cg.std}$ (ml/min) | 96.9 (32) | 92.6 | 44.3–225 | 92.8 (27) | 94.9 | 40.7–162 |
| Gender (%) | | | | | | |
| Female | | 48 | | | 35 | |
| Male | | 52 | | | 65 | |
| Smoking (%) | | | | | | |
| Yes | | 31 | | | 31 | |
| No | | 69 | | | 40 | |
| Unknown | | | | | 30 | |
| Alcohol consumption (%) | | | | | | |
| Yes | | 24 | | | 24 | |
| No | | 73 | | | 46 | |
| Unknown | | 3 | | | 30 | |
| Ethnic origin (%) | | | | | | |
| Caucasian | | 77 | | | 76 | |
| African descent | | 17 | | | 4 | |
| Asian | | 1 | | | 4 | |
| Hispanic | | 1 | | | 0 | |
| Undefined | | 5 | | | 17 | |

*BSA* body surface area; *$CrCL_{cg.std}$* standard Cockcroft-Gault creatinine clearance

## Base model

A two-compartment model parameterized in terms of CL, $V_1$, $V_2$, and $Q$ was determined to be an appropriate base model. The model also incorporated proportional between-patient variability for CL, $V_1$, and $V_2$, and a proportional residual error term.

## Covariate identification

A linear model ($CL = 43.0 + 47.2 \bullet CrCL_{cg.std}/92.6$, Table 3) described the relationship between pemetrexed CL and $CrCL_{cg.std}$ (Fig. 2). Predicted pemetrexed CL decreased from 90.2 ml/min to 61.3 ml/min as $CrCL_{cg.std}$ decreased from 92.6 ml/min (population median) to 36.1 ml/min (population minimum). Thus, a 63% decrease in CrCL produced approximately a 32% decrease in pemetrexed CL, which corresponded to a 45% increase in overall systemic exposure (AUC).

For pemetrexed clinical trials, enrollment criteria relative to renal function have been based on an estimate of $CrCL_{cg.std}$ incorporating LBM. The estimate of CrCL using the LBM formula did not offer significant advantage over the standard Cockcroft-Gault method when evaluated in the model. Additionally, the LBM formula is a more complex calculation. Therefore, it was not explored in further analyses.

Of the indices of body size (weight, BSA, and body mass index) evaluated as covariates, BSA was the strongest predictor of $V_1$. A power model ($V_1 = 6.13 \bullet BSA^{1.32}$, Table 3) described the relationship between $V_1$ and BSA over a BSA range from 1.21 $m^2$ to 2.50 $m^2$ (Fig. 2), with $V_1$ increasing as BSA increased. Because BSA is a predictor of $V_1$ and not CL, the effect of BSA is reflected in peak pemetrexed concentration and not on total systemic exposure to the drug (AUC).

Gender, age, ethnic origin, smoking status, and alcohol consumption were also examined as potential covariates. None of these factors met the criteria for inclusion in the final model ($\Delta MOF < 10.828$ points). After examining available vitamin deficiency marker concentrations (653 observations from 115 patients), five patients were categorized as folate-deficient based on published criteria (homocysteine $> 13.9$ μM, cystathionine $> 342$ μ$M$, and methylmalonic acid between 73 μM

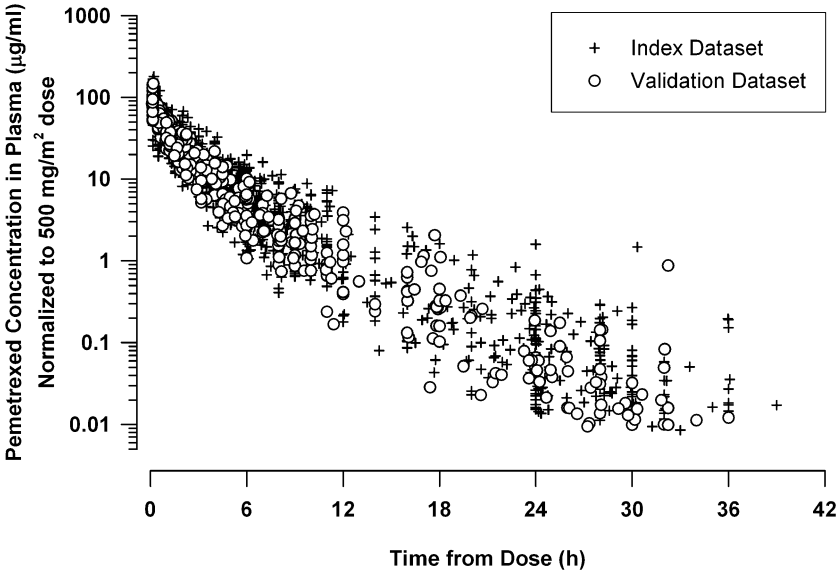


**Fig. 1** Pemetrexed concentration in plasma versus time from start of infusion (all doses, normalized to 500 mg/$m^2$)

**Table 3** Pharmacokinetic and covariate parameters in final pemetrexed population model

| Parameter description | Population estimate (%SEE) | Between-patient variability (%SEE) |
|---|---|---|
| Clearance (CL) | | |
| TVCL, base parameter for CL (ml/min) | 43.0 (16.6) | 19.3% (14.1) |
| $\Theta_1$, parameter for effect of $CrCL_{cg.std}$ on CL (ml/min)[a] | 47.2 (14.8) | |
| Central volume of distribution ($V_1$) | | |
| TVV1, base parameter for $V_1$ (l) | 6.13 (9.04) | 16.6% (29.3) |
| $\Theta_2$, parameter for effect of BSA on $V_1$[b] | 1.32 (11.6) | |
| Intercompartmental clearance ($Q$) | | |
| Parameter for $Q$ (ml/min) | 14.5 (17.6) | – |
| Peripheral volume of distribution ($V_2$) | | |
| Parameter for $V_2$ (l) | 3.38 (10.9) | 24.5% (24.6) |
| Residual error (proportional) | 28.4% (8.22) | |

Method: FOCE with interaction
*SEE* standard error of the estimate; *TVCL* typical value of CL; *TVV1* typical value of $V_1$
[a] $CL = TVCL + \Theta_1 \bullet CrCL_{cg.std}/92.6$ where 92.6 is the median baseline $CrCL_{cg.std}$
[b] $V_1 = TVV1 \bullet BSA^{\Theta_2}$

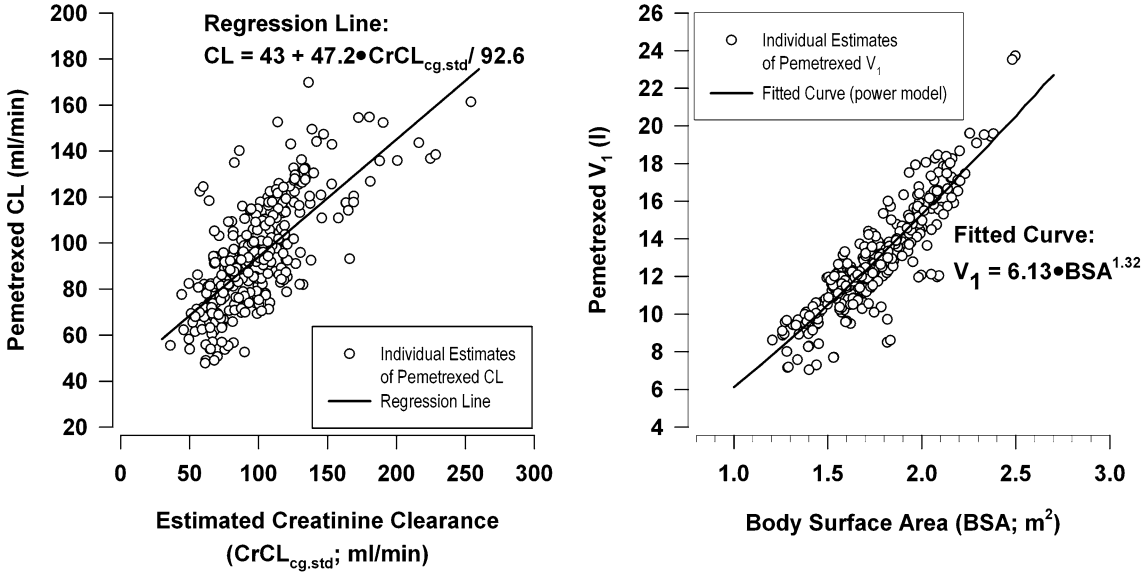


**Fig. 2** Relationship between estimated creatinine clearance ($CrCL_{cg.std}$) and pemetrexed clearance (CL) (*left panel*) and body surface area (BSA) and pemetrexed central volume of distribution ($V_1$) (*right panel*)

and 271 μM) [2], each for a single cycle. Pemetrexed CL was estimated to be 33% lower in these patients than in those with normal folate status. Since pemetrexed will be co-administered with folic acid and vitamin $B_{12}$, thus preventing folate deficiency, the effect was not retained in the final model.

These analyses also demonstrated the pharmacokinetics of pemetrexed to be dose-independent (i.e., linear) and time-invariant.

## Final model

The final population pharmacokinetic model is shown in Table 3. Between-patient variability was 19.3% (14.1 % standard error of the estimate [%SEE]) in CL, 16.6% (29.3 %SEE) in $V_1$, and 24.5 % (24.6 %SEE) in $V_2$, and residual error was 28.4% (8.22 %SEE). The addition of $CrCL_{cg.std}$ and BSA to the basic pharmacokinetic model decreased between-patient variability in CL from 25.8% to 19.3%, in $V_1$ from 24.0% to 16.6%, and in $V_2$ from 26.0% to 24.5%. Thus, renal function (i.e., $CrCL_{cg.std}$) explained approximately 25% of the between-patient variability in CL, and body size (i.e., BSA) explained approximately 30% of the between-patient variability in $V_1$. Proportional residual variability was essentially unchanged relative to the base model.

## Model evaluation

Objective function mapping, leverage analysis, and an evaluation of the predictive ability of the model supported the validity of the model to describe the pharmacokinetics of pemetrexed in this patient population. The predictive ability of the model is demonstrated in Fig. 3 by: agreement between predicted and observed concentrations; the distribution of weighted residuals; and the consistency in the goodness-of-fit plots between the index and validation datasets. The predictive ability of the model was further confirmed quantitatively by the minimal (<10%) difference in mean prediction error (MPE) (1.33 for the index dataset versus 1.44 for the validation dataset) and mean relative error (MRE) (0.112 for the index dataset versus 0.102 for the validation dataset) between the datasets. This indicates that the overall performance of the model is similar for the two populations.

Comparison of individual predicted and observed concentrations (Fig. 3) indicates that concentrations greater than ~75 μg/ml are underpredicted by the model. Comparison of prediction errors for concentrations ≥75 μg/ml relative to those <75 μg/ml (Table 4) indicated that performance of the model is less than optimal for the higher concentrations, but it also suggested that the overall predictive performance of the model was not highly impacted by the concentrations ≥75 μg/ml since they represent only a small (5%) portion of the data evaluated. Further, the agreement between individual predicted and observed concentrations indicates that individual empirical Bayesian estimates of pharmacokinetic parameters based on the model are suitable for use in subsequent pharmacokinetic/pharmacodynamic modeling efforts.

## Evaluation of pemetrexed pharmacokinetics by cancer type

There were no statistically significant (ΔMOF <10.8) alterations in pemetrexed CL by cancer type. Although patients in one of the two NSCLC studies included in these analyses had smaller $V_1$ (30% lower; 95% confi-

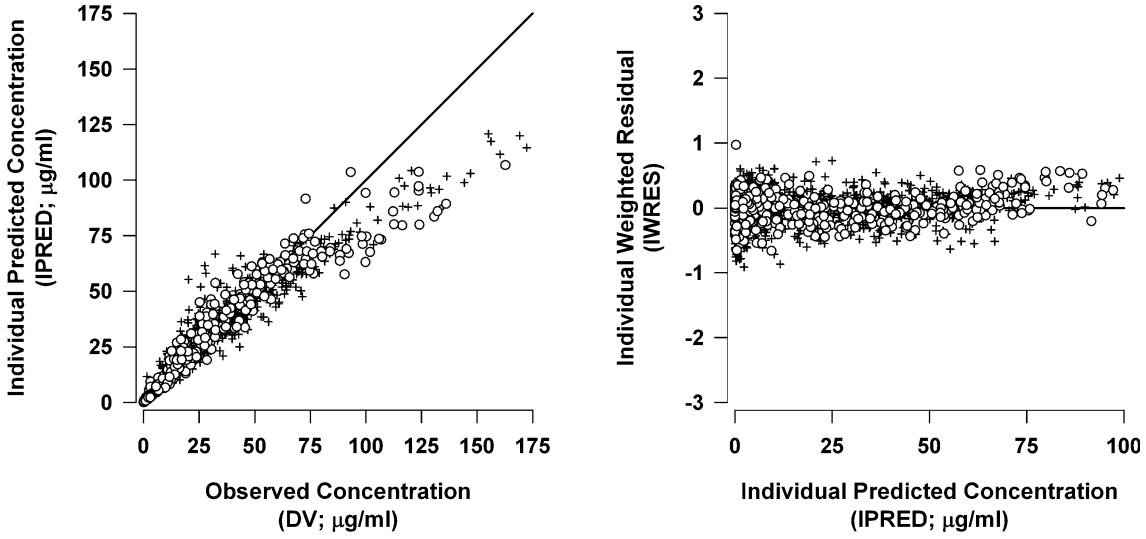


**Fig. 3** Individual predicted concentrations and weighted residuals for final pemetrexed model developed from the index dataset (*black symbols*) and for final pemetrexed model applied to the validation dataset (*white symbols*)

dence interval, 0.683–0.831), the result was not considered clinically relevant. And similarly, a smaller $V_2$ (20% lower; 95% confidence interval, 0.684–0.892) for breast cancer patients was not considered clinically relevant. This analysis indicated that there were no overt differences in pemetrexed pharmacokinetics by cancer type. Therefore, the model adequately describes the pharmacokinetics of pemetrexed for patients with malignant pleural mesothelioma, breast, cervical, esophageal, head and neck, gastric, pancreatic, colorectal, renal, bladder, and nonsmall cell lung cancer.

### Application of results

Individual empirical Bayesian estimates of CL from the final population pharmacokinetic model and the corresponding measures of $CrCL_{cg.std}$ and BSA were used to examine different dosing paradigms. Figure 4 shows the predicted exposure for three dosing strategies for the patients included in the pharmacokinetic analyses. Lack of systematic pattern in predicted exposures (Panel C) represents the desired outcome that overall exposure is independent of body size or renal function. Figure 4, Panel A shows predicted exposure based on fixed-dose administration. The fixed-dose strategy results in smaller patients having higher exposure to pemetrexed on average and, conversely, some of the larger patients having lower exposure (Panel A, left). The increase in pemetrexed exposure with decreasing renal function is also evident (Panel A, right). BSA-based dosing (Fig. 4, Panel B), the prevailing clinical practice for oncolytic agents [10, 13, 37, 31, 32], the intent of which is to attain similar drug exposure regardless of patient size, actually overcorrects and results in larger patients having higher exposure to pemetrexed (Panel B, left). The increase in pemetrexed exposure with decreasing renal function is uncorrected by this practice, however (Panel B, right). A renal function-based dosing strategy (Fig. 4, Panel C), similar to the target-AUC dosing approach used for carboplatin [6], eliminates patterns in pemetrexed exposure by BSA (Panel C, left) and by CrCL (Panel C, right) and may offer additional control of systemic exposure for pemetrexed as indicated by the decreased variability represented in the plot. A renal function-based dosing strategy enables >90% of patients to be within 25% of a desired AUC, whereas a BSA-based or fixed-dose regimen results in <75% of patients being within 25% of a desired AUC.

## Discussion

The pharmacokinetics of pemetrexed were adequately characterized by an open two-compartment model with constant rate infusion. The typical value of total systemic CL was 91.6 ml/min; $V_1$ was 12.9 l; $Q$ was 14.4 ml/min; and $V_2$ was 3.38 l. Based on these parameter estimates, the terminal elimination half-life of pemetrexed was approximately 3.5 h. The range of systemic CL estimates that represents approximately 95% of all patients in the study population was 53.2 to 146 ml/min (31.2–81.3 ml/min/$m^2$). These results are consistent with those obtained from phase I studies [35, 33]. The final population pharmacokinetic model adequately describes the individual plasma concentration data in both the index and validation datasets.

Pemetrexed is eliminated primarily by renal excretion of unchanged drug [35, 24]. The population pharmacokinetic analysis demonstrated a strong relationship ($CL = 43.0 + 47.2 \bullet CrCL_{cg.std}/92.6$) between CL and $CrCL_{cg.std}$, with $CrCL_{cg.std}$ values ranging from 36.1 to 254 ml/min. This population represents patients that would be considered mild to moderately renally im-

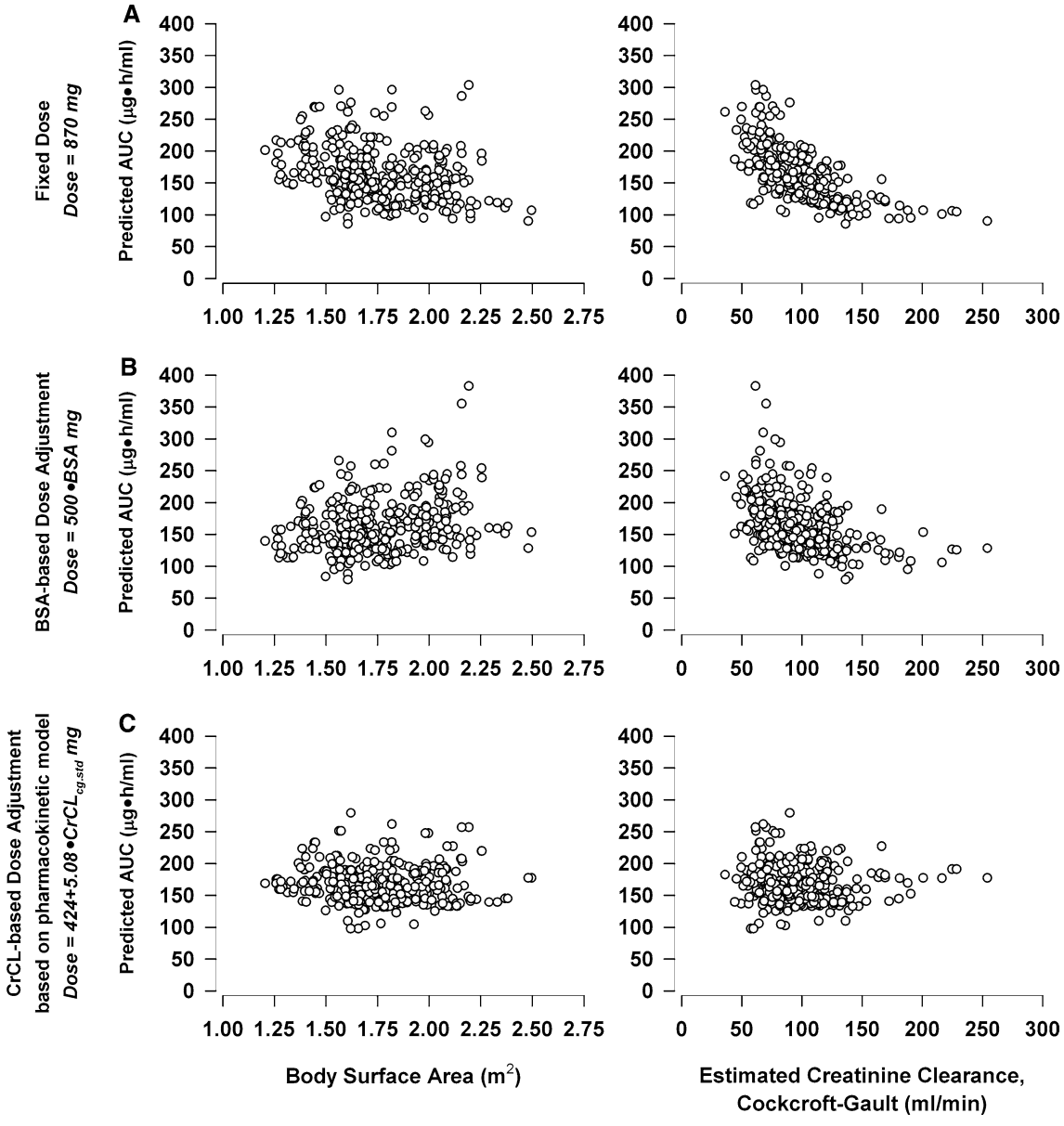


**Fig. 4** Patterns in pemetrexed exposure (AUC) by body surface area or renal function for each of three dosing strategies. **a** illustrates variability in overall exposure for a fixed dose; smaller patients and those with reduced renal function have higher pemetrexed exposure. **b** illustrates variability in overall exposure for body surface area (BSA)-based dosing; larger patients and those with reduced renal function have higher pemetrexed exposure; BSA-based dosing increases the variability in exposure since clearance correlates with renal function and BSA offers no further explanatory value. **c** illustrates variability in overall exposure where a constant AUC is targeted and the dose is calculated based on renal function; there is no trend in exposure due to body size or renal function

paired ($CrCL_{cg.std} < 80$ ml/min) and patients with normal renal function ($CrCL_{cg.std} \geq 80$ ml/min). The strong correlation of CL with $CrCL_{cg.std}$ is entirely consistent with the elimination pathway of pemetrexed. On further examination, the pattern of CL versus $CrCL_{cg.std}$ (Fig. 2) suggests that the model describing this relationship might be further refined. The results suggest that a sigmoid model or split-line model may better describe the relationship between $CrCL_{cg.std}$ and pemetrexed CL. Since individual empirical Bayesian estimates of clearance take into account unexplained interpatient variability, those estimates from the current model were appropriate for use in predicting exposures based on dosing regimen and for subsequent use in pharmacodynamic modeling.

Protein-binding studies have shown pemetrexed to be approximately 81% bound to plasma proteins [11]. Only the unbound fraction is expected to be filtered. Therefore, the product of the fraction unbound ($f_u$) and glomerular filtration rate (GFR; [$f_u \bullet$ GFR; $0.19 \bullet 120 = 23$ ml/min]) represents the filtration capacity of the kidney to eliminate pemetrexed. The population esti-

mate of CL from the final model with $CrCL_{cg.std}$ set to the population median (90.2 ml/min) is approximately four times that of filtration. Consistent with phase I results [35] based on the combination of plasma and urine data, active tubular secretion appears to contribute significantly to the renal elimination of pemetrexed.

Except for folate deficiency, no additional relationships were identified between CL and the other potential covariates examined. Based on very limited data ($n = 4$), pemetrexed CL appeared to be lower in patients who were folate-deficient. If real, this result further supports the clinical decision to administer pemetrexed with vitamins to correct folate deficiencies [5, 25, 26]. Alternatively, the association between folate-deficiency and decreased pemetrexed CL may indicate other underlying physiological issues [2]. Since measurement of vitamin deficiency markers to determine folate status is not a routine practice and pemetrexed will be co-administered with folic acid and vitamin $B_{12}$ supplementation, thereby ensuring satisfactory folate status, this effect was not retained in the final model. Not retaining folate deficiency in the model assumes that vitamin supplementation corrects whatever is the cause of decreased pemetrexed CL in these patients. However, it is also possible that vitamin supplementation will not correct the cause of decreased pemetrexed CL and these patients, although no longer folate deficient, will still have decreased CL.

Consistent with phase I data [35, 33], pemetrexed demonstrated a relatively small volume of distribution ($V_{ss} = 16.3$ l), suggesting that the compound has limited tissue distribution. Of this 16.3 l, approximately 80% was associated with $V_1$. A relationship between $V_1$ and body size (ie, BSA) was shown to be the only significant predictor of $V_1$ ($V_1 = 6.13 \bullet BSA^{1.32}$). Since $V_1$ probably represents a combination of total blood volume and well-perfused organs, it is reasonable that larger individuals have greater blood volume and organ size and thus a larger volume of distribution. The relationship identified between $V_1$ and BSA was consistent with this assumption. No relationships were established between $V_2$ and the potential covariates investigated.

An earlier analysis [28] on interim data from four of the ten studies included in the current analyses was conducted early in the development of pemetrexed to support a multivariate safety analysis [25]. The previous analyses included 632 plasma concentrations from 103 patients, or approximately half of the current index dataset used for model development. The earlier model, developed using the first order (FO) and FOCE estimation methods, used slightly more inclusive criteria for retaining covariates in the final model (increase in MOF of ≥7.879 points for one degree of freedom, $P < 0.005$). The resultant model included effects of body weight, estimated CrCL (Cockcroft and Gault method based on LBM), alanine transaminase, and a folate status descriptor on CL; gender and body weight on $V_1$; and BSA and albumin on $V_2$. Residual variability was previously modeled using a combined additive/proportional error structure. The previous model was used to guide model development in the current analyses, and all covariates included in the previous model were examined during the current analyses.

Interpatient variability in CL was similar for the two models, while estimates of interpatient variability in $V_1$ and $V_2$ from the earlier model were slightly smaller than those from the current model. Residual error was also slightly smaller in the earlier model. Although the two models differ in their complexity and in the interpatient variability and residual error terms, their predictive abilities, when applied to validation data not used to develop either model, were similar (Table 4). Thus, although the earlier model had less unexplained variability due to the more inclusive covariate retention criteria, which was reflected in a low MPE (0.383) and a low MRE (0.0404) relative to its model development dataset, the model's predictive ability was not retained when the model was applied to data that was not used for model development. Further investigation of the prediction errors also indicated that, while the earlier model has the better predictive performance at concentrations ≥75 μg/ml, the situation is reversed at concentrations <75 μg/ml, and the current model has the better predictive ability (Table 4).

The current model, based on twice as much data as the earlier model and developed using a more robust estimation method (FOCE with interaction), is a simpler model, performs more consistently than the earlier model, and is more consistent with the known elimination pathway of pemetrexed.

There were no clinically relevant alterations in pemetrexed CL, $V_1$, or $V_2$ attributable to cancer type. Therefore, differences in response rate by cancer type are based on sensitivity of the tumor to pemetrexed and are not attributable to differences in the pharmacokinetics of pemetrexed. The established population pharmacokinetic model adequately describes pemetrexed pharmacokinetics for patients with malignant pleural mesothelioma, breast, cervical, esophageal, head and neck, gastric, pancreatic, colorectal, renal, bladder, and nonsmall cell lung cancer.

The practice of BSA-based dosing for drugs where BSA is not the main predictor of CL (and therefore AUC) is being increasingly questioned [10, 13, 37, 31,

**Table 4** Comparison of the predictive abilities of two pemetrexed population pharmacokinetic models

| | Current model | Earlier model |
|---|---|---|
| Overall (all concentrations) | | |
| MPE | 1.44 | −1.73 |
| MRE | 0.102 | −0.143 |
| Concentrations ≥75 μg/ml | | |
| MPE | 38.7 | 17.6 |
| MRE | 0.833 | 0.582 |
| Concentrations <75 μg/ml | | |
| MPE | −0.717 | −2.27 |
| MRE | −0.106 | −0.270 |

32]. The results of the population pharmacokinetic analyses suggest that dose adjustments based on renal function, rather than BSA, might be considered for pemetrexed. The need for dose adjustments based on considerations other than BSA takes into account body size, renal function, and position within the therapeutic window. For example, a large individual with low renal function that is being dosed near the maximum tolerated dose would benefit from a dose adjustment based on renal function to lessen the probability of a high systemic exposure that would increase the probability of toxicity. Likewise, a small individual (low BSA) with high renal function that is being dosed near the lower end of the therapeutic window would benefit from a dose adjustment based on renal function to increase the probability that their exposure is adequate to obtain an efficacious response. Additional analyses are planned to provide further information toward the consideration of alternative dosing strategies for pemetrexed. The relationships between exposure (overall exposure and peak concentration) and toxicity were further explored during subsequent pharmacodynamic analyses [18] examining the time course of neutropenia following administration of pemetrexed.

In conclusion, population pharmacokinetic analyses showed pemetrexed CL, and therefore total systemic exposure (i.e., AUC), correlates with renal function, while $V_1$ correlates with BSA. Since pharmacodynamic analyses have shown that AUC and not $C_{max}$ is a primary determinant of neutropenic response [35, 18, 19], this suggests that dose adjustments based on renal function, rather than BSA, might be considered for pemetrexed.

**Acknowledgements** The authors wish to thank Dinesh Dealwis (scientific advisor), Mary Brandes Dugan and Pete Fairfield (scientific writing and editorial support), and David B Radtke (operations and project management) for their assistance with the conduct of these analyses and preparation of this manuscript.

## References

1. Adjei AA (2003) Pemetrexed (Alimta): a novel multitargeted antifolate agent. Expert Rev Anticancer Ther 3:145–156
2. Allen RH, Stabler SP, Savage DG, Lindenbaum J (1993) Metabolic abnormalities in cobalamin (vitamin $B_{12}$) and folate deficiency. FASEB J 7:1344–1353
3. Bajetta E, Celio L, Buzzoni R et al (2003) Phase II study of pemetrexed disodium (Alimta) administered with oral folic acid in patients with advanced gastric cancer. Ann Oncol 14:1543–1548
4. Beal SL, Sheiner LB (1992) NONMEM users guide. NONMEM project group, University of California, San Francisco
5. Bunn P, Paoletti P, Niyikiza C et al (2001) Vitamin $B_{12}$ and folate reduce toxicity of ALIMTA (pemetrexed disodium, LY231514, MTA), a novel antifolate/antimetabolite. Proc Am Soc Clin Oncol 20:76a
6. Calvert AH, Newell DR, Gumbrell LA et al (1989) Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 7:1748–1756
7. Chaudhary AK, Schannen V, Knadler MP, Lantz R, Le Lacheur RM (1999) Analysis of LY231514 by LC/MS/MS. Proceedings 47th ASMS conference on Mass Spectrometry Allied Topics, Dallas, TX
8. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41
9. Cripps C, Burnell M, Jolivet J et al (1999) Phase II study of first-line LY231514 (multitargeted antifolate) in patients with locally advanced or metastatic colorectal cancer: an NCIC Clinical Trials Group study. Ann Oncol 10:1175–1179
10. Egorin MJ (2003) Horseshoes, hand grenades, and body-surface area-based dosing: aiming for a target. J Clin Oncol 21:182–183
11. Eli Lilly and Company (2004) Package insert: Alimta, pemetrexed for injection
12. Ette EI (1997) Stability and performance of a population pharmacokinetic model. J Clin Pharmacol 37:486–495
13. Felici A, Verweij J, Sparreboom A (2002) Dosing strategies for anticancer drugs: the good, the bad, and body-surface area. Eur J Cancer 38:1677–1684
14. Goedhals L,van Wijk AL (1998) MTA (LY231514) in advanced carcinoma of the cervix. Ann Oncol 9(suppl 4):70
15. Grem JL (1990) Fluorinated pyrimidines. In: Chabner BA, Collins JM (eds) Cancer chemotherapy: principles and practice. Lippincott, Philadelphia, pp 180–224
16. Grindey GB, Shih C, Barnett CJ et al (1992) LY231514, a novel pyrrolopyrimidine antifolate that inhibits thymidylate synthase (TS). Proc Am Assoc Cancer Res 33:411
17. Hanna N, Shepherd FA, Fossella FV et al (2004) Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small cell lung cancer previously treated with chemotherapy. J Clin Oncol 22:1589–1597
18. Latz JE, Karlsson MO, Rusthoven JJ, Ghosh A, Johnson RD (2006) A semimechanistic-physiologic population pharmacokinetic/pharmacodynamic model for neutropenia following pemetrexed therapy. Cancer Chemother Pharmacol. DOI 10.1007/s00280-005-0077-5
19. Latz JE, Rusthoven JJ, Karlsson MO, Ghosh A, Johnson RD (2006) Clinical application of a semimechanistic-physiologic population PK/PD model for neutropenia following pemetrexed therapy. Cancer Chemother Pharmacol. DOI 10.1007/s00280-005-0035-2
20. Mandema JW, Verotta D, Sheiner LB (1992) Building population pharmacokinetic-pharmacodynamic models. I. Models for covariate effects. J Pharmacokinet Biopharm 20:511–528
21. McDonald AC, Vasey PA, Adams L et al (1998) A phase I and pharmacokinetic study of LY231514, the multitargeted antifolate. Clin Cancer Res 4:605–610
22. Mentre F, Ebelin ME (1997) Validation of population pharmacokinetic/pharmacodynamic analyses: review of proposed approaches. In: Balant LP, Aarons L (eds) The Population Approach: Measuring and Managing Variability in Response, Concentration and Dose. Commission of the European Communities, Brussels, pp 147–160
23. Miller KD, Picus J, Blanke C et al (2000) Phase II study of the multitargeted antifolate LY231514 (ALIMTA, MTA, pemetrexed disodium) in patients with advanced pancreatic cancer. Ann Oncol 11:101–103
24. Mita AC, Sweeney CJ, Baker SD et al (2005) A phase I and pharmacokinetic study of pemetrexed administered every 3 weeks to advanced cancer patients with normal and impaired renal function [manuscript in press]
25. Niyikiza C, Baker SD, Seitz DE et al (2002) Homocysteine and methylmalonic acid: markers to predict and avoid toxicity from pemetrexed therapy. Mol Cancer Ther 1:545–552
26. Niyikiza C, Hanauske AR, Rusthoven JJ et al (2002) Pemetrexed safety and dosing strategy. Semin Oncol 29(6 Suppl 18):24–29
27. O'Dwyer PJ, Nelson K, Thornton DE (1999) Overview of phase II trials of MTA in solid tumors. Semin Oncol 26:99–104

28. Ouellet D, Periclou AP, Johnson RD, Woodworth JR, Lalonde RL (2000) Population pharmacokinetics of pemetrexed disodium (ALIMTA) in patients with cancer. Cancer Chemother Pharmacol 46:227–234
29. Paz-Ares L, Tabernero J, Moyano A et al (1999) Significant activity of the multitargeted antifolate MTA (LY231514) in advanced transitional cell carcinoma (TCC) of the bladder: results of a phase II trial. Eur J Cancer 35(suppl 2):S81
30. Pivot X, Raymond E, Laguerre B et al (2001) Pemetrexed disodium in recurrent locally advanced or metastatic squamous cell carcinoma of the head and neck. Br J Cancer 85:649–655
31. Ratain MJ (1998) Body-surface area as a basis for dosing of anticancer agents: science, myth, or habit? J Clin Oncol 16:2297–2298
32. Reilly JJ, Workman P (1993) Normalisation of anticancer drug dosage using body weight and surface area: is it worthwhile? A review of theoretical and practical considerations. Cancer Chemother Pharmacol 32:411–418
33. Rinaldi DA (1999) Overview of phase I trials of multitargeted antifolate (MTA, LY231514). Semin Oncol 26(2 Suppl 6):82–88
34. Rinaldi DA, Burris HA, Dorr FA et al (1995) Initial phase I evaluation of the novel thymidylate synthase inhibitor LY231514, using the modified continual reassessment method for dose escalation. J Clin Oncol 13:2842–2850
35. Rinaldi DA, Kuhn JG, Burris HA et al (1999) A phase I evaluation of multitargeted antifolate (MTA, LY231514), administered every 21 days, utilizing the modified continual reassessment method for dose escalation. Cancer Chemother Pharmacol 44:372–380
36. Rusthoven JJ, Eisenhauer E, Butts C et al (1999) Multitargeted antifolate LY231514 as first-line chemotherapy for patients with advanced non-small cell lung cancer: a phase II study. National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 17:1194–1199
37. Sawyer M, Ratain MJ (2001) Body surface area as a determinant of pharmacokinetics and drug dosing. Invest New Drugs 19:171–177
38. Scagliotti GV, Shin DM, Kindler HL et al (2003) Phase II study of pemetrexed with and without folic acid and vitamin $B_{12}$ as front-line therapy in malignant pleural mesothelioma. J Clin Oncol 21:1556–1561
39. Schilsky RL (1992) Antimetabolites. In: Perry MC (ed) The Chemotherapy Source Book. Williams & Wilkins, Baltimore, pp 301–317
40. Shargel L, Yu ABC (1985) Applied biopharmaceutics, 2nd ed. Appleton-Century-Crofts, Norwalk, pp 310–312
41. Sheiner LB (1986) Analysis of pharmacokinetic data using parametric models. III. Hypothesis tests and confidence intervals. J Pharmacokinet Biopharm 14:539–555
42. Sheiner LB, Steimer JL (2000) Pharmacokinetic/pharmacodynamic modeling in drug development. Annu Rev Pharmacol Toxicol 40:67–95
43. Shih C, Gosset L, Gates S et al (1996) LY231514 and its polyglutamates exhibit potent inhibition against both human dihydrofolate reductase (DHFR) and thymidylate synthase (TS): multiple folate enzyme inhibition. Ann Oncol 7(Suppl 1):85
44. Spielmann M, Martin M, Namer M, duBois A, Unger C, Dodwell DJ (2001) Activity of pemetrexed (ALIMTA, multitargeted antifolate, LY231514) in metastatic breast cancer patients previously treated with an anthracycline and a taxane: an interim analysis. Clin Breast Cancer 2:47–51
45. Sun H, Fadiran EO, Jones CD et al (1999) Population pharmacokinetics. A regulatory perspective. Clin Pharmacokinet 37:41–58
46. Vogelzang NJ, Rusthoven JJ, Symanowski J et al (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 21:2636–2644