

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
22-468

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

NDA 22-468 Folutyn

Clin Pharm Division Director Memo

Concurred with primary review dated 9-3-09.

NDA 22-468 Folutyn

Clin Pharm Team Leader Memo

Concurred with primary review dated 9-3-09.

Clinical Pharmacology and Biopharmaceutics NDA Review

Brand name: Folutyn

Generic name: pralatrexate

Type of dosage form and strength(s): solution for parenteral administration contained in single-use vials at a concentration of 20 mg/mL

Indication(s): the Applicant's proposed indication is, "Treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL)."

NDA number, type: NDA 22-468, 1P

Applicant name: Allos Therapeutics, Inc.

Submission date (letter date): 23-Mar-2009 N 000

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1. Executive Summary

Folotyn (pralatrexate) is a anti-neoplastic folate analog that exerts activity via the inhibition of dihydrofolate reductase (DHFR). This submission is the original NDA for pralatrexate.

A single clinical study, PDX-008, is the primary basis for establishing efficacy in the NDA. Study PDX-008 is a single arm Phase 2 study that used a single regimen: 30 mg/m² administered intravenously once weekly for six weeks on a seven week cycle. The primary endpoint for **efficacy was response rate and the applicant's response rate was 27%** by independent central review.

Exposure-response analysis showed a relationship between concentration and adverse responses (mucositis and thrombocytopenia), but no significant relationship between concentration and efficacy could be observed.

Pralatrexate is not a substrate, inhibitor or inducer of CYP enzymes nor is it a substrate or inhibitor of the P-glycoprotein transporter. A mass balance study has not been conducted, but is planned. A post-marketing requirement to complete the mass balance study, and, contingent on the results, possibly perform a study in patients with hepatic impairment, is recommended.

No specific population studies were conducted. A covariate search of the population pharmacokinetics data reveals that clearance correlates with renal function. A post-marketing requirement to perform a study in patients with renal impairment to include patients with severe renal impairment is recommended.

Urinary excretion of pralatrexate was virtually absent in three of the 41 subjects with urine data (0-3% of dose recovered for both stereo-isomers), and these subjects experienced severe adverse events. We recommend a post-marketing commitment to perform *in vitro* experiments to learn if transporters are involved in the elimination of pralatrexate.

1.1. Recommendations

This NDA is acceptable from the clinical pharmacology and biopharmaceutics perspective.

1.2. Identify recommended post-marketing requirements if the NDA is judged approvable

We recommend two post-marketing requirements:

1. a clinical study in patients with renal impairment to include patients with severe renal impairment, and
2. completion of the planned mass balance study, and contingent on FDA judgment of the mass balance results, a study in patients with hepatic impairment.

1.3. Identify recommended post-marketing commitments if the NDA is judged approvable

We recommend a post-marketing commitment to perform *in vitro* experiments to learn if transporters are involved in the elimination of pralatrexate.

1.4 Comments to the Applicant

(b) (4)

1.5 Summary of Clinical Pharmacology and Biopharmaceutics Findings

Folotyn (pralatrexate) is a anti-neoplastic folate analog that exerts activity via the inhibition of dihydrofolate reductase (DHFR). It is a racemate and the stereo-isomers are thought to be equal in anti-neoplastic activity. The indication sought is the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL), a disease that occurs in approximately 9,500 patients per year in the United States and has been granted orphan status.

The single efficacy and safety study in PTCL included sparse sampling for pharmacokinetics. A relationship between concentration and adverse events (mucositis and thrombocytopenia) was discerned, while no significant relationship between concentration and efficacy could be observed.

Pharmacokinetics data was acquired exclusively in patients, as the drug is cytotoxic. Stereo-selective pharmacokinetics were present: the PDX-10b isomer of pralatrexate showed approximately double the exposure than the PDX-10a isomer. Terminal elimination half-lives, however, are more similar: PDX-10a = 18 hours, PDX-10b = 12 hours. The biologic reason for the stereo-selective pharmacokinetics is unknown. The ratio of the isomers appears constant; there is no evidence of inter-conversion.

The formulation is a simple aqueous solution and is administered by rapid injection into an already present line (IV push). Pralatrexate is not a substrate, inhibitor or inducer of CYP enzymes nor is it a substrate or inhibitor of the P-glycoprotein transporter. A mass balance study has not been conducted, but is planned. Approximately one-third of the drug is excreted in urine as parent; the elimination and excretion of the drug have not been further characterized. A post-marketing commitment to complete the mass balance study, and, contingent on the results, possibly perform a study in patients with hepatic impairment, is recommended.

No specific population studies were conducted. A covariate search of the population pharmacokinetics data reveals that clearance correlates with renal function. However, the magnitude of the effect is not pronounced; the model predicts that a patient on the border of severe renal impairment (creatinine clearance = 30 mL/min) would have a reduction in pralatrexate clearance of approximately 25%. These data should be interpreted cautiously, as a

study in patients with renal impairment has not been conducted. A post-marketing commitment to perform a study in patients with renal impairment to include patients with severe renal impairment is recommended

Urinary excretion of pralatrexate was virtually absent in three of the 41 subjects with urine data (0-3% of dose recovered for both stereo-isomers), and these subjects experienced severe adverse events. We recommend a post-marketing commitment to perform *in vitro* experiments to learn if transporters are involved in the elimination of pralatrexate. If the *in vitro* results are positive, further studies to identify patients that may have reduced elimination and risk for toxicity due to drug interactions or genetic polymorphisms may be warranted.

2. Question-Based Review

2.1. General attributes of the drug

- 2.1.1. What pertinent regulatory background or history contributes to the current assessment of the clinical pharmacology and biopharmaceutics of this drug?

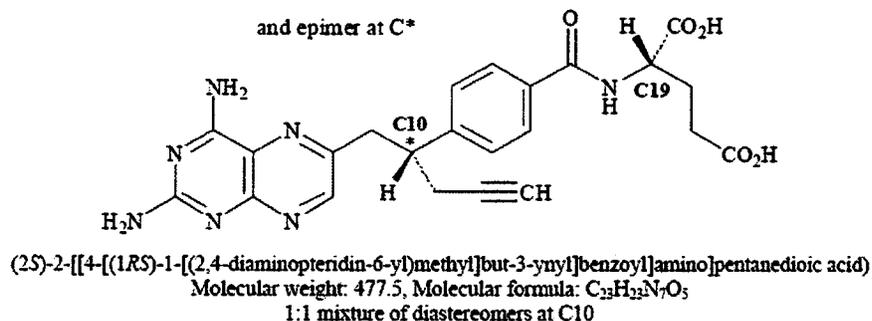
There is no unusual regulatory history for this application. There are no approved agents for treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL). PTCL occurs in approximately 9,500 patients per year in the United States (Vose JM et al., Journal of Clinical Oncology, 26: 4124-30, 2008) and pralatrexate for PTCL has been granted orphan status. The clinical trial that is the primary evidence of efficacy and safety in PTCL, Study PDX-008, was the topic of an FDA Special Protocol Assessment (SPA). An SPA agreement between the sponsor and the FDA was reached, but the Agency specified that what constitutes an acceptable clinical response rate and duration of response would be NDA review issues.

- 2.1.2. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Pralatrexate has the chemical name (2*S*)-2-[[4-[(1*R*)-1-[(2, 4-diaminopteridin-6-yl)methyl]but-3-ynyl]benzoyl]amino]pentanedioic acid. The molecular formula is C₂₃H₂₃N₇O₅ and the molecular weight is 477.48 g/mol. The structural formula is shown in **FDA Figure 1**.

FDA Figure 1. Folutyn Chemical Structure (Applicant's Figure 2.7.1.1 from page 4 of Section 2.7.1)

Figure 2.7.1.1. Pralatrexate Chemical Structure with C10 and C19 Chiral Centers Identified



The drug substance is manufactured so that it contains an approximately equal racemic mixture of the *R*- and *S*-configurations at the C10 chiral center, and $\geq 98\%$ of the *S*-isomer at the C19 chiral center. That is, there are four isomers, two of which are present only in very low amounts. The two C10 diastereomers are referred to as:

PDX-10a [*S*-diastereomer]

Chemical name: (2*S*)-2-[[4-[(1*S*)-1-[(2,4-diaminopteridin-6-yl)methyl]but-3-ynyl]benzoyl]amino]pentanedioic acid)

PDX-10b [*R*-diastereomer]

Chemical name: (2*S*)-2-[[4-[(1*R*)-1-[(2,4-diaminopteridin-6-yl)methyl]but-3-ynyl]benzoyl]amino]pentanedioic acid)

Folutyn (Pralatrexate Injection) is supplied as a preservative-free, sterile, isotonic, non-pyrogenic, clear yellow aqueous parenteral solution contained in a single-use, 2-mL size, clear glass vial (Type I) for IV administration. Each 1 mL of solution contains 20 mg of pralatrexate, 0.6% sodium chloride (NaCl) to achieve an isotonic solution (280-300 mOsm), and sufficient sodium hydroxide (NaOH), and hydrochloric acid (HCl) if needed, to maintain the pH at 7.5-8.5. Folutyn is supplied as either 20 mg (1 mL) or 40 mg (2 mL) single-use vials at a concentration of 20 mg/mL.

Folutyn is to be administered intravenously over 30 seconds to 5 minutes via the side port of a free flowing 0.9% Sodium Chloride Injection, USP IV line.

2.1.3. What are the proposed mechanism(s) of action and therapeutic indication(s)?

The **Mechanism of Action** and INDICATIONS AND USAGE sections of the proposed package insert are reproduced (indented, below).

2.1.4. What are the proposed dosage(s) and route(s) of administration?

The recommended dose is 30 mg/m² administered intravenous over (b) seconds to 5 minutes via the side port of a free flowing 0.9% Sodium Chloride Injection, USP IV line (IV push) once weekly for 6 weeks in 7-week cycles until progressive disease or unacceptable toxicity occurs. The proposed package insert includes three tables for dose modifications due to mucositis, hematologic toxicities, and all other treatment-related toxicities, respectively.

2.2. General clinical pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The dose for Study PDX-008 (PTCL patients) was selected based upon Study PDX-02-078 (patients with lymphoproliferative malignancies including but not limited to PTCL or TCL). In the initial version of Study 02078 the starting dose of pralatrexate was 135 mg/m² given every 2 weeks with intra-patient dose escalation. A higher than anticipated incidence of Grade 3 or 4 stomatitis occurred at this dose in patients with homocysteine (Hcy) and methylmalonic acid (MMA) concentrations greater than 10 µmol/L and 200 nmol/L, respectively. The protocol was amended to require folic acid and vitamin B12 supplementation to normalize Hcy and MMA levels and to determine if vitamin supplementation would allow patients to tolerate higher doses of study drug. In addition, many patients with palpable disease experienced marked reductions in their disease by day 7, which grew back to baseline levels by day 15 (i.e., cytokinetic failures).

Although no reference for the historical data are given, the applicant states, “**Because of the** observation of cytokinetic failures, the more frequent weekly schedule was adopted, which is concordant with well-established theories regarding the important PK parameters and dosing schedules known to be critical in the use of antimetabolites (ie, AUC exposures are more important than larger maximum concentrations [C_{max}]).”. **The study was amended to become a** Phase 1/2 study with an interpatient dose escalation scheme starting at 30 mg/m² weekly for 3 weeks of a 4-week cycle with subsequent increases in number of consecutive doses and dose amount. When dose-limiting toxicities (DLTs) occurred at the dose of 45 mg/m² for 6 weeks of a 7-week cycle, the maximum tolerated dose (MTD) was determined to be 30 mg/m²/week for 6 weeks on a 7-week cycle.

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

The primary efficacy endpoint was response rate, which was assessed based on central review of imaging and clinical data according to the International Workshop Criteria (IWC) developed by the National Cancer Institute (NCI)-sponsored International Working Group.

No biomarkers for safety or efficacy were assessed in the efficacy/safety and pharmacokinetic studies.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

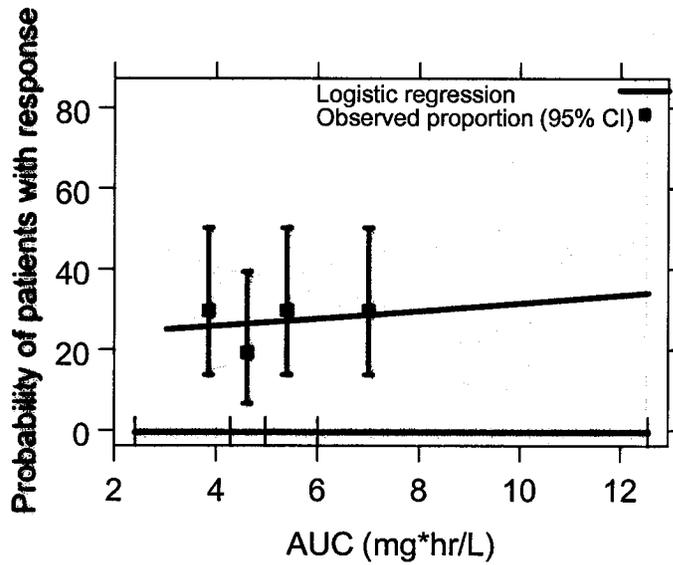
No metabolites have been identified. No mass balance study was performed. As discussed in Section 2.4.2, pralatrexate does not appear to be subject to significant phase I or II liver metabolism. Approximately one-third of the dose was excreted as parent drug in urine in the first 24 hours post-dosing.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for *efficacy*? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

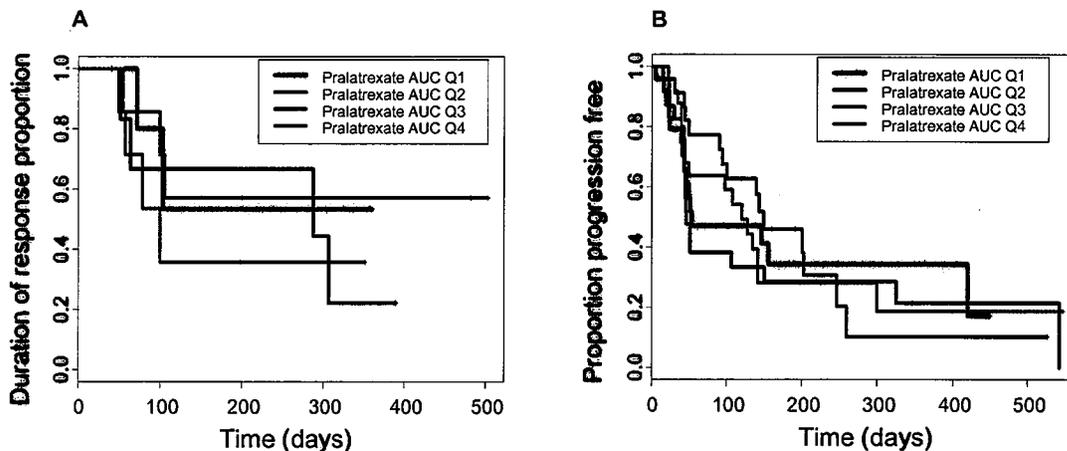
Attempts to correlate concentrations and efficacy in cancer patients were not made by the applicant. The FDA pharmacometrics reviewer found no evidence of an exposure-response relationship for effectiveness in PDX-008 (pivotal efficacy trial) following 30 mg/m² pralatrexate (the only regimen investigated in the study).

A logistic regression was performed to assess the exposure-response relationship for effectiveness based on response rate (primary efficacy endpoint). No relationship is observed as evidenced by a nearly flat mean logistic prediction in **FDA Figure 2**.



FDA Figure 2. The probability of patients with response-AUC profile for pralatrexate. Solid black symbols represent the observed percentage of patients responding to treatment in each AUC quartile. The vertical black bars represent the 95% confidence interval. The solid line represents the mean logistic regression prediction. The shaded area represents the 95% confidence interval of the prediction. The exposure range in each AUC quartile is denoted by the horizontal black line.

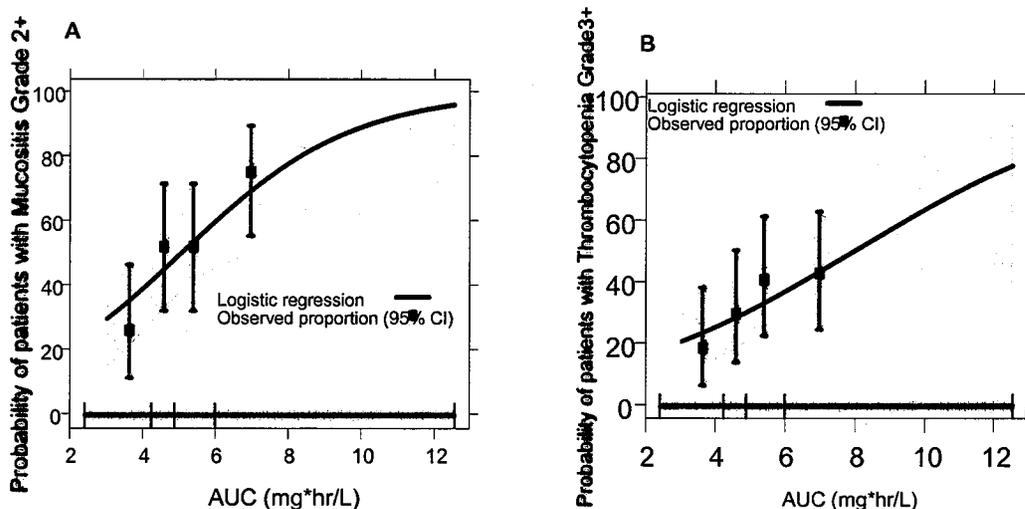
Kaplan-Meier analysis was performed to assess the exposure-response relationship for effectiveness based on duration of response and progression free survival (secondary efficacy endpoints). The duration of response and progression free survival curves of patients in different AUC-quartile groups overlapped (FDA Figure 3.), **thus indicating a lack of an exposure-response relationship.**



FDA Figure 3.: Kaplan-Meier plots for A) duration of response and B) progression free survival for treatment groups. Q1, Q2, Q3 and Q4 are AUC quartiles following 30 mg/m² pralatrexate.

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for *safety*? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.

There is evidence of exposure-response relationship for mucositis and thrombocytopenia in PDX-008 following 30 mg/m² pralatrexate. Logistic regression models were used to explore the relationship between exposure and treatment emergent AE's. The various AEs explored for relationship with the pralatrexate exposure are mucositis grade 2+, thrombocytopenia grade 3+, and neutropenia grade 3+ because the applicant has recommended dose adjustments for these AEs in the label. AUC was found to be a predictor for mucositis and thrombocytopenia (FDA Figure 4.) with p-value <0.05.



FDA Figure 4. The probability of patients with various adverse events A) Mucositis grade 2+, B) Thrombocytopenia grade 3+. Solid black symbols represent the observed proportion of patients experiencing AEs in each AUC quartile. The vertical black bars represent the 95% confidence interval. The solid line represents the mean logistic regression prediction. The shaded area represents the 95% confidence interval of the prediction. The exposure range in each AUC quartile is denoted by the horizontal black line.

Study PDX-008 was a single-dose regimen study. An attempt to determine if a lower dose would have been safer, and equi-effective, was not made by the applicant.

2.2.4.3 Does this drug prolong the QT or QTc interval? *(You must answer this question, unless this is addressed in the question above.)*

The QT information in the submission was reviewed by the QT Interdisciplinary Review Team (the QT-IRT). Sections 1.1 **OVERALL SUMMARY OF FINDINGS** and 1.2 **QT-IRT COMMENTS** from the QT-IRT review are reproduced.

1.1 OVERALL SUMMARY OF FINDINGS

This was a Phase 1, non-randomized, open-label, two-center study designed to determine the maximal tolerated dose (MTD) of pralatrexate. Triplicate ECGs at pre-specified time points were collected in 14 patients who received pralatrexate at doses of 190 or 230 mg/m² administered every 2 weeks over 3-5 minutes or over 1 hour in three treatment cohorts. When data from all cohorts were combined, the upper bound of the two-sided 90% CI for QTcF change from Pre-injection was <10 ms. No patient exhibited a QTcF interval >500 msec. No major changes in HR, PR interval, or QRS interval duration were noted.

1.2 QT-IRT COMMENTS

- Because the doses studied in this trial are at-least 6-fold greater than the proposed therapeutic dose for PTCL (30 mg/m²), 14 subjects (pooled dose analysis) are adequate to rule out large direct effects (>20 ms) on the QT interval.

- [REDACTED] (b) (4)

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

See Section 2.1.1 for details on how the regimen was selected. The ability of lower doses to produce equi- or near equi- effect with reduced toxicity is unknown.

The proposed dose reductions for mucositis and thrombocytopenia are reasonable since an exposure-response relationship for safety was identified (see Section 2.2.4.2). The exposure-response relationships for adverse events suggests that decreasing the dose from 30 to 20 mg/m² would reduce the probability of patients experiencing mucositis grade 2+ and thrombocytopenia grade 3+ from 47.9% to 32% and 30.1% to 21.8 %, respectively (FDA Table 1.). Furthermore, the lack of an exposure-response relationship for effectiveness (response, duration of response, and progression free survival) following 30 mg/m² suggests that reducing the dose is not likely to affect the effectiveness of pralatrexate (FDA Figures 2. and 3. presented in Section 2.2.4.1.).

FDA Table 1. Reviewer’s Logistic Regression Results for Dose Reduction

Dose (mg/m ²)	Median AUC (mg*hr/L)	Probability of patients with Grade 2+ Mucositis (%)	Probability of patients with Grade 3+ Thrombocytopenia (%)
30	4.88	47.9	30.1
20	3.29	32	21.8

2.2.5 What are the PK characteristics of the drug and its major metabolite?

2.2.5.1 What are the single dose and multiple dose PK parameters?

FDA Table 2. presents the pharmacokinetic data for 10 PTCL patients densely sampled after their first dose. There are no multiple dose data available (the drug was never administered on a schedule that resulted in accumulation). Data from repeated single doses will be presented in Section 2.2.5.9.

FDA Table 2. Single dose pharmacokinetics in PDX-008 (30 mg/m² IV push to PTCL patients)

PDX-10a					
	Cmax (ng/mL)	AUC (ng/mL*min)	Cl (mL/min)	VdSS (L)	terminal t1/2 (h)
mean	2478	93900	417	105	18
%CV	68	55	62	75	120
n	10	10	10	10	10
PDX-10b					
	Cmax (ng/mL)	AUC (ng/mL*min)	Cl (mL/min)	VdSS (L)	terminal t1/2 (h)
mean	3337	173954	191	37	12
%CV	41	41	38	53	62
n	10	10	10	10	10

PDX-10b has approximately 2-fold higher systemic exposures than PDX-10a; both clearance and VdSS are lower for PDX-10b. The biological cause for the observed stereo-selectivity is unknown.

Pharmacokinetic data for pralatrexate given as a 60-minute infusion, rather than over < 5 minutes, is available from 10 patients (5 patients receiving 190 mg/m² and 5 receiving 230 mg/m²) studied in a parallel group (not a cross-over) study, Study PDX-007. On average, the 60-minute infusion resulted in a 22 – 45% increase in clearance (FDA Table 3.). Because the small sample sizes are small, and the data were acquired at supra-clinical doses (190 and 230 mg/m², the clinical dose is 30 mg/m²), it is not possible to draw firm conclusions from these data.

FDA Table 3. Pharmacokinetics of Folotyn following < 5 minute injection and 60 minute infusion (Applicant's Table 2f)

Nominal Dose [mg/m ²]	PDX-10a		PDX-10a	
	Injection	Infusion	Injection	Infusion
150	843 n=1		358 n=1	
190	475 (29%) n=3	701 (72%) n=5	226 (48%) n=3	277 (62%) n=5
230	328 (54%) n=5	474 (45%) n=5	155 (52%) n=5	219 (55%) n=5
270	439 (39%) n=16		224 (34%) n=16	
325	705 (77%) n=3		243 (52%) n=3	

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

Pralatrexate is cytotoxic; studies in healthy subjects were not performed.

2.2.5.3 What are the characteristics of drug absorption?

Pralatrexate has been administered only parenterally (intravenously).

2.2.5.4 What are the characteristics of drug distribution? (*Include protein binding.*)

Steady-state volume of distribution was 105 L (PDX10a, *S*-diastereomer) and 37 L (PDX10b, *R*-diastereomer). *In vitro* studies indicate that pralatrexate is approximately 67% bound to plasma proteins. *In vitro* pralatrexate binding was not displaced (test pralatrexate concentration of 17 µM approximated *in vivo* C_{max} of 13 µM) by tested reference drugs (FDA Table 4.), nor did pralatrexate (at 160 M, approximately 12-fold *in vivo* C_{max}) displace tested reference drugs (FDA Table 5.).

FDA Table 4. Displacement of pralatrexate protein binding by reference compounds (Applicant's Table 2.7.2.3.)

Study: PDX-K-07043-U		Test Article: Pralatrexate				
Study Title: Equilibrium dialysis						
Type of Study	In vitro assessment of drug displacement from human plasma proteins					
Method	Equilibrium dialysis					
Plasma protein binding site	Albumin Site I		Albumin Site II	Albumin Site III	α1-acid glycoprotein and albumin (minor)	α1-acid glycoprotein, albumin, and lipoproteins
Drug used to displace pralatrexate	Phenytoin	Warfarin	Ceftriaxone	Digoxin	Disopyramide	Propranolol
Maximum Change in % bound pralatrexate (Control = 66.7%)	-0.3	-2.6	-11	-8.5	-7.9	-10

¹⁴C = carbon-14, µg = microgram, mL = milliliter

FDA Table 5. Displacement of reference compound protein binding by pralatrexate (Applicant's Table 2.7.2.4.)

Study: PDX-K-07049-U			Test Article: Pralatrexate			
Study Title: Evaluation of the Ability of PDX for Displacement of Bound Reference Compounds from Human Plasma Proteins Using an Ultrafiltration Assay						
Plasma protein binding site	Albumin Site I		Albumin Site II	Albumin Site III	α 1-acid glycoprotein and albumin (minor)	α 1-acid glycoprotein, albumin, and lipoproteins
Drug bound to plasma protein	Phenytoin	Warfarin	Ibuprofen	Digoxin	Disopyramide	Propranolol
Change in % bound after incubation with 160 μ g/mL pralatrexate	-2.0	0.1	0.1	-1.0	-1.2	0.1

μ g = microgram, mL = milliliter

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

A mass balance study was not performed. Seventy-two hour urine collection resulted in approximately one-third of the dose being recovered as parent drug in urine.

2.2.5.6 What are the characteristics of drug metabolism?

In data acquired from *in vitro* systems using human hepatic biomaterials, it appears that pralatrexate is not metabolized.

In both human hepatocytes and human liver microsomes pralatrexate, at 10 and 100 μ M initial concentrations, and incubations of up to two hours, the maximal observed disappearance of pralatrexate was 15% (FDA Table 6.). These experiments included negative controls (pralatrexate in the absence of liver preparation) and positive controls (the substrate benzyresorufin).

FDA Table 6. *In vitro* pralatrexate stability as a function of system, initial concentration, and incubation time

Values are % of initial concentration remaining relative to a negative control				
	0 minutes	30 minutes	60 minutes	120 minutes
microsomes, 10 uM initial concentration	116	109	80	106
microsomes, 100 uM initial concentration	98	95	93	86
hepatocytes, 10 uM initial concentration	116	116	105	98
hepatocytes, 100 uM initial concentration	106	99	107	93

Pralatrexate was also metabolically stable in human liver S9 fractions (**FDA Table 7.**). The positive control for CYPs, testosterone, was not stable (> 80% disappearance in 60 minutes), nor was the positive control for glucuronidation, 7-hydroxycoumarin (> 97% disappearance in 60 minutes).

FDA Table 7. Percentage of remaining pralatrexate (PDX) in human liver S9 fractions (Applicant's Table 2. from page 10 of report PDX-K-08061-U)

Time (min)	Remaining PDX (% to T=0 min)	RSTD (%)
0	100	3.9
15	94.4	0.8
30	97.9	4.3
60	96.2	1.4

RSTD: Relative Standard Deviation

The metabolic stability of pralatrexate was further confirmed in a CYP450 phenotyping study using human liver microsomes and recombinantly expressed human metabolic enzymes. The results are shown in **FDA Table 8.**

FDA Table 8. Reaction phenotyping of pralatrexate (PDX) by chemical inhibitors (Applicant's Table 1. from page 4 of report PDX-K-08062-U)

CYP Isoform	Standard Inhibitor	Inhibitor Conc. (µM)	Pre-incubation (15 min.)	Conc. at 60 min (µM, n=3)		% Remaining of Negative Control
				Mean	S.D.	
Negative Control	Without Inhibitor, without NADPH	N/A	No	1.34	0.03	100
Positive Control 1	Without Inhibitor, with NADPH	N/A	Yes	1.60	0.16	119
1A2	Furafylline	15	Yes	1.40	0.10	104
2A6	8-Methoxypsoralen	1	Yes	1.48	0.13	110
Positive Control 2	Without Inhibitor, with NADPH	N/A	No	1.39	0.02	104
2B6	Thio-TEPA	50	No	1.24	0.09	93
2C8	Quercetin	30	No	1.31	0.10	98
2C9	Sulfaphenazole	10	No	1.40	0.03	104
2C19	(+)-N-3-benzylirivanol	5	No	1.31	0.06	98
2D6	Quinidine	1	No	1.33	0.08	99
2E1	4-Methylpyrazole	5	No	1.41	0.08	105
3A4	Ketoconazole	5	No	1.39	0.02	104

In all cases pralatrexate was found to be refractory to metabolism and thus pralatrexate does not appear to be subject to significant human CYP-450 liver metabolism.

2.2.5.7 What are the characteristics of drug excretion?

In clinical studies PDX-008 and PDX-007 the mean percent of unchanged pralatrexate (PDX-10a and PDX-10b) excreted in urine ranged from 25%-38%. Neither collection of drug in feces nor characterization of metabolites in urine was performed. A mass balance study with ¹⁴C pralatrexate in 4 to 6 oncology patients was to be initiated in March/April 2009.

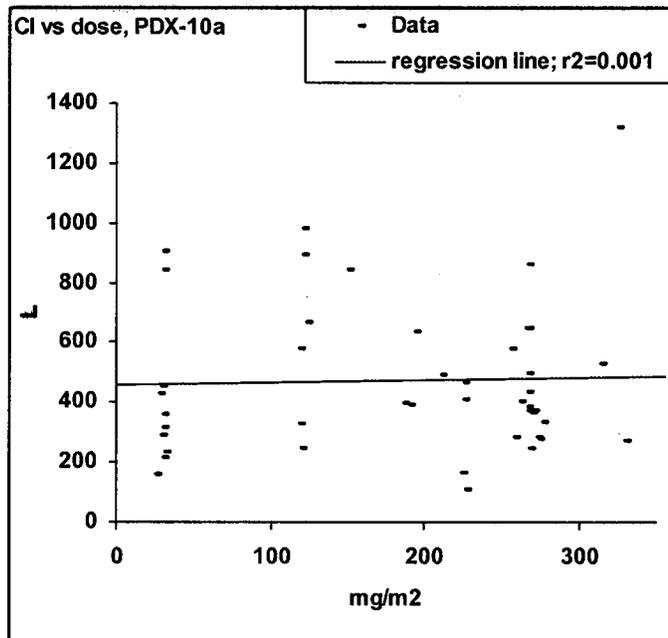
2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Using the combined data for all studies with rapid IV administration, dense sampling, non-compartmental analysis, and isomer-selective analytical methods (Studies PDX-008, PDX-007, and PDX-99-083), there appears to be linearity (lack of change in parameters across doses) of both clearance and VdSS for both isomers (FDA Figures 5a.-5d.). This result is consistent with the applicant's cross-study conclusions derived from review of the non-compartmental analyses. However, both the FDA pharmacometrics review and the applicant's population pharmacokinetics analysis, which include sparse sampling data, find that clearance was lower in Study PDX-008 (30 mg/m² dose) than with higher doses. It should be noted that, if any non-

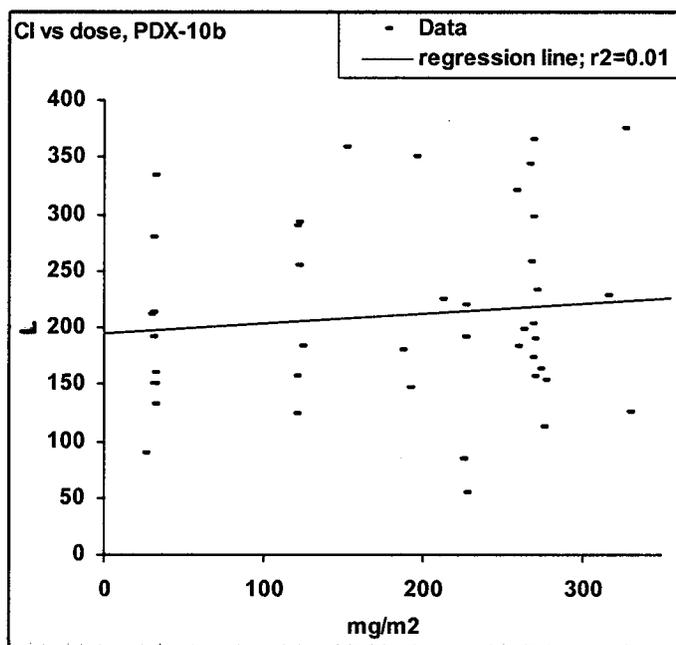
linearity is truly present, it would lead to exposure being less than dose proportional. The applicant makes the following comments (indented) regarding the issue of the sparse sampling data showing apparent non-linearity.

“Predictive check results confirm that the 10 patients with full PK sampling profiles in study PDX-008 had similar pralatrexate CL to those patients enrolled in the other two studies. Additional plots of CL estimates stratified by study and profile type further supported these findings. These results raise questions [sic] on the suitability of the sparse sampling design to characterize pralatrexate CL and on the conduct of the study for sparse PK patients (eg, incorrect record of dosing times).”

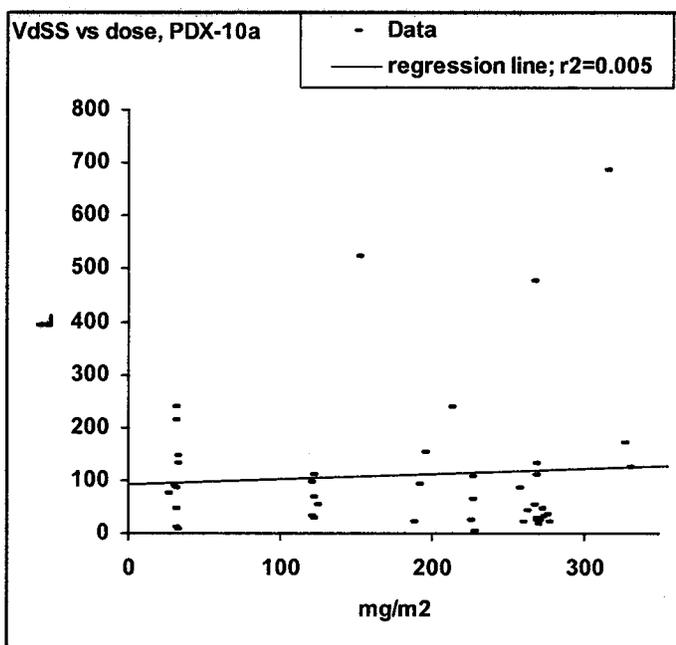
FDA Figure 5a. Linearity of Clearance of PDX-10a



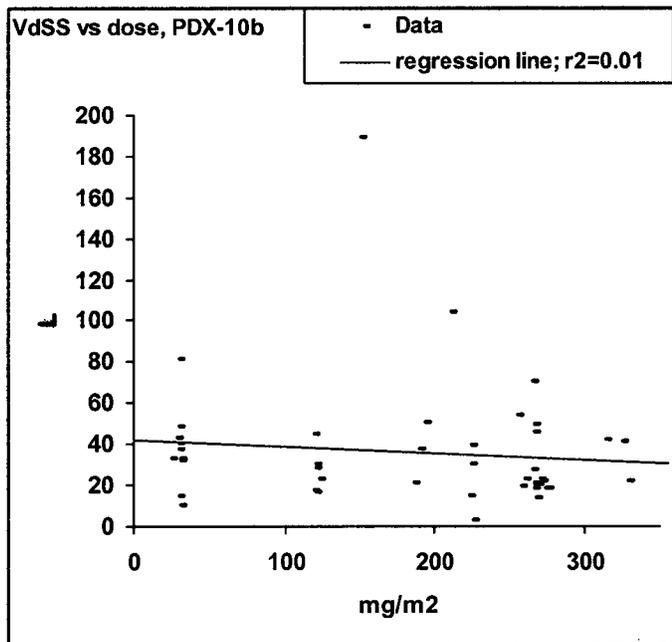
FDA Figure 5b. Linearity of Clearance of PDX-10b.



FDA Figure 5c. Linearity of VdSS of PDX-10a.



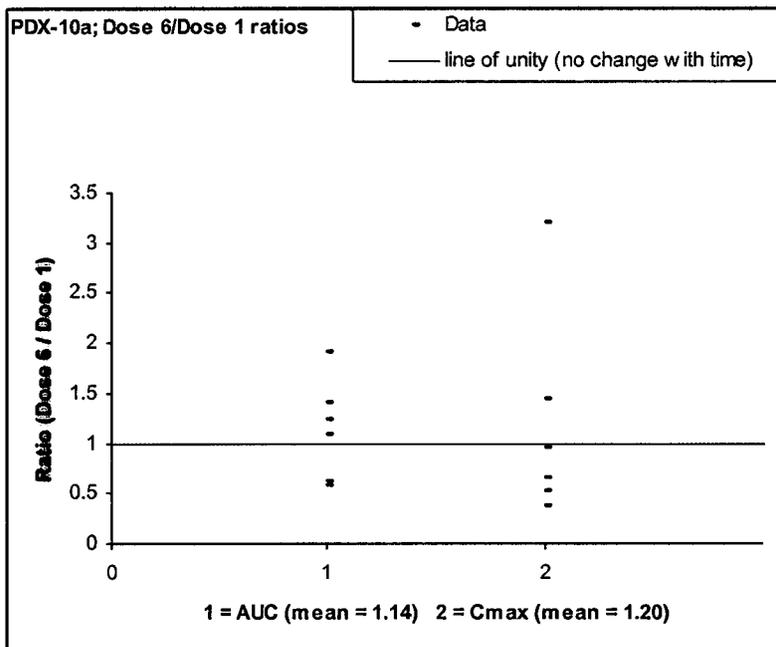
FDA Figure 5d. Linearity of VdSS of PDX-10b.



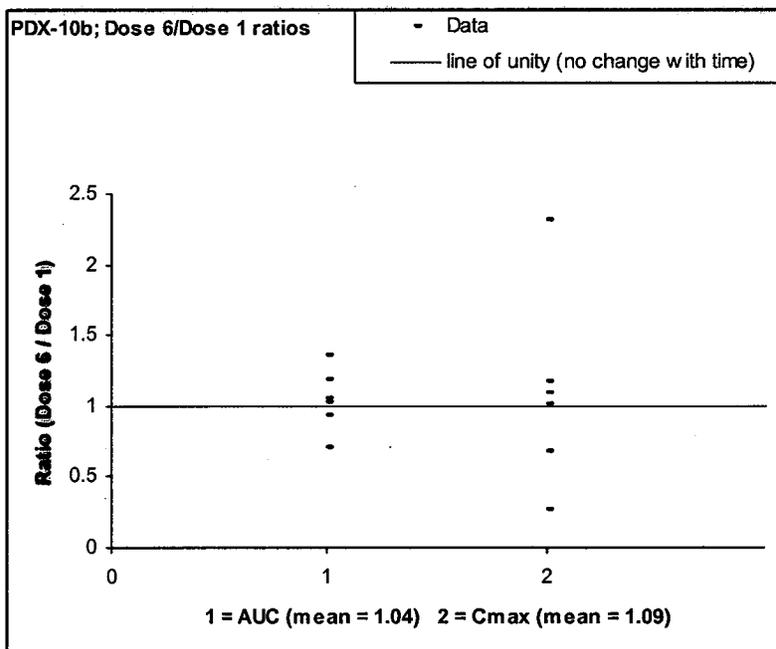
2.2.5.9 How do the PK parameters change with time following chronic dosing?
(This may include time to steady-state; single dose prediction of multiple dose PK;
accumulation ratio.)

In Study PDX-008 six patients were densely sampled for pharmacokinetics on doses 1 and 6 of cycle 1. FDA Figures 6a. and 6b. show the ratios of the AUC and Cmax values for each stereoisomer across the two administrations six weeks apart. There are no apparent differences in AUC or Cmax for either isomer across the two administrations. The FDA pharmacometrics review reached the same conclusion.

FDA Figure 6a. Pharmacokinetics comparison of Dose 6 to Dose 1 for PDX-10a



FDA Figure 6b. Pharmacokinetics comparison of Dose 6 to Dose 1 for PDX-10b



2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

FDA Table 2. presents the pharmacokinetic data for 10 PTCL patients densely sampled after their first dose and includes inter-subject variability (%CV).

FDA Table 2. Single dose pharmacokinetics in PDX-008 (30 mg/m² IV push to PTCL patients)

PDX-10a					
	Cmax (ng/mL)	AUC (ng/mL*min)	Cl (mL/min)	VdSS (L)	terminal t1/2 (h)
mean	2478	93900	417	105	18
%CV	68	55	62	75	120
n	10	10	10	10	10
PDX-10b					
	Cmax (ng/mL)	AUC (ng/mL*min)	Cl (mL/min)	VdSS (L)	terminal t1/2 (h)
mean	3337	173954	191	37	12
%CV	41	41	38	53	62
n	10	10	10	10	10

Intra-subject variability was measured only in the context of doses separated by weeks; **FDA Figures 6a.** and **6b.**, present these data graphically.

As will be discussed in Section 2.3, differences in age and renal function may be a minor cause of the observed variabilities. The major causes of the variabilities are unknown.

2.3. Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

No studies in specific populations were performed. There appears to be a minor effect of renal function on pharmacokinetics. Currently, the limited data predicts that the impact on efficacy and safety in patients with mild to moderate renal function is likely to be slight.

2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations (examples shown below), what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

2.3.2.1 Elderly

The population pharmacokinetic database included patients aged 21 to 85 years. After accounting for renal function, neither the applicant's nor the FDA's analysis of the population pharmacokinetic data identified age as a relevant covariate. No dosage regimen adjustments are recommended for age.

2.3.2.2 Pediatric patients. Also, what is the status of pediatric studies and/or any pediatric plan for study?

No pediatric patients have been studied. The applicant has not communicated any plans to study pediatric patients.

Since pralatrexate was granted orphan designation for the treatment of relapsed or refractory PTCL, pediatric data are not required for the application and a waiver is not needed.

2.3.2.2 Gender

The population pharmacokinetic database consisted of 94 males and 60 females. Neither the applicant's nor the FDA's analysis of the population pharmacokinetic data identified age as a relevant covariate. No dosage regimen adjustments are recommended for age.

2.3.2.4 Race

The population pharmacokinetic database consisted of 117 White, 19 Black, 9 Hispanic, 7 Asian, 1 Middle Eastern, and 1 unknown. **Neither the applicant's nor the FDA's analysis of the population pharmacokinetic data identified race as a relevant covariate.** The applicant states that, **"Several random effects parameters showed some differences with race, but the limited number of patients representing race categories other than White make interpretation of these differences difficult."**

The study population consisted of 94 males and 60 females with ages ranging from 21 to 85 years and weights ranging from 42.9 to 158 kg. The distribution of races in this study population was predominantly white: 117 White, 19 Black, 9 Hispanic, 7 Asian, 1 Middle Eastern, and 1 unknown.

2.3.2.5 Renal impairment

A study in patients with renal impairment was not performed.

Both the applicant's and the FDA's population pharmacokinetics analyses concluded that a slight trend is observed between creatinine clearance (CRCL) and CL for patients with mild and moderate renal impairment (**FDA Figure 7**).

(b) (4)



FDA Figure 7: Clearance vs. Creatinine Clearance for A) PDX-10a and B) PDX-10b in Study PDX-008.

At the extreme low value of CRCL observed in this study (29.5 mL/min), the estimated clearances for PDX-10a and PDX-10b were reduced 19% and 23% compared to patients with normal renal function (CRCL > 79 mL/min).

No dose adjustments are recommended for mild renal impairment but the increase in exposure in severe renal impaired patients is unknown and a post-marketing commitment to study such patients is recommended.

2.3.2.6 Hepatic impairment

A study in patients with hepatic impairment was not performed.

Neither the applicants nor the FDA's population pharmacokinetics analysis identified total bilirubin as a significant covariate. Due to inclusion/exclusion criteria there was a limited range of hepatic function variability in the data set: only eight patients had values above 1 mg/dL, of these only two had values above 1.3 mg/dL.

The applicant plans to perform a mass balance study. We recommend that completion of such a study be a post-marketing commitment and that the results from that study be used to inform on the need to perform a study in patients with hepatic impairment.

2.3.2.7 What pharmacogenetics information is there in the application and is it important or not?

Although optional collection of pharmacogenomic information was included in the protocol for Study PDX-008, little data (six patients) was actually collected. The pharmacogenomics reviewer examined the dataset and determined that it was too limited to allow for analysis. The applicant performed no analysis of the pharmacogenomic data.

2.3.2.8 What pregnancy and lactation use information is there in the application?

There is no pregnancy and lactation use information in the application.

2.3.2.9 Are there other human factors that are important to understanding the drug's efficacy and safety?

No other intrinsic factors known to be important to efficacy and safety have been identified.

An exploratory analysis of heterogeneity across a number of factors was conducted by the pharmacogenomics reviewer. A potential signal was identified regarding urinary excretion; three of the 41 subjects with urine data had very little recovery of pralatrexate (0-3% of dose recovered for both stereo-isomers) in the urine, and these subjects experienced severe adverse events. We recommend a post-marketing commitment to perform *in vitro* experiments to learn if transporters are involved in the elimination of pralatrexate. If the *in vitro* results are positive, further study may be needed to identify patients that may have reduced elimination and risk for toxicity due to drug interactions at the transporter or genetic polymorphisms of the transporter.

2.4. Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?

2.4.1.1 Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.

With the exception of drug-drug interaction data acquired with probenecid, no studies were conducted to assess correlations between extrinsic factors and the pharmacokinetics of pralatrexate.

2.4.2 Drug-drug interactions

2.4.2.1 Is there an *in vitro* basis to suspect *in vivo* drug-drug interactions?

No. Section 2.2.5.6 discusses the inability of CYP P450 enzymes to metabolize pralatrexate, Section 2.4.2.3 discusses the inability of pralatrexate to inhibit CYP P450 enzymes and Section 2.4.2.4 discusses the inability of pralatrexate to act a substrate or inhibitor of p-glycoprotein.

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

Section 2.2.5.6 presents data that pralatrexate is not a CYP substrate.

There are no data indicating that metabolism is influenced by genetics, but we recommend a post-marketing commitment to explore if transporters might account for heterogeneity in renal excretion (see section 2.3.2.9).

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

The ability of pralatrexate to inhibit CYP enzymes was investigated in studies using positive controls performed in both human liver microsomes (FDA Table 9.) and cDNA-Expressed CYP450 Isoforms (FDA Table 10.). In interpreting these data it is useful to know that *in vivo* Cmax for the pralatrexate (sum of both stereo-isomers) is approximately 13 μ M.

FDA Table 9. Pralatrexate as an inhibitor of CYP-450 in human liver microsomes (Applicant's Table 2.7.2.6.)

Study: PDX-K-07032-U		Test Article: Pralatrexate						
Study Title: Evaluation of P450 Inhibition Potential of Pralatrexate (PDX) in Human Liver Microsomes								
% CYP450 inhibition after 1h incubation at 37°C								
CYP450 isozyme	1A2	2A6	2C8	2C9	2C19	2D6	2E1	3A4
20 μ M Pralatrexate	0	21	ND	0	29	0	5	ND
50 μ M Pralatrexate	8	23	ND	0	> 50	0	32	ND
100 μ M Pralatrexate	0	23	ND	0	> 50	0	28	ND

CYP450 = cytochrome P450, h = hour, °C = degrees Celcius, μ M = microMolar, ND = not determined

FDA Table 10. Pralatrexate as an inhibitor in recombinant human CYP-450 enzymes (Applicant's Table 2.7.2.7.)

Study: PDX-K-07043-U		Test Article: Pralatrexate						
Study Title: Drug-Drug Interaction Study: Evaluation of CYP450 Inhibition Potential of Pralatrexate (PDX) in cDNA-Expressed CYP450 Isoforms								
% CYP450 inhibition after incubation at 37°C according manufacturer recommended protocol								
CYP450 isozyme	1A2	2A6	2C8	2C9	2C19	2D6	2E1	3A4
100 µM Pralatrexate	0	ND	0	0	53	ND	ND	13

PDX = pralatrexate, CYP450 = cytochrome P450, cDNA = complementary deoxyribonucleic acid, °C = degrees Celcius, µM = microMolar, ND = not determined

Based on these data, experiments to determine I/Ki for the inhibition of CYP2C19 were performed in human liver microsomes. In experiments using positive controls, pralatrexate inhibited CYP2C19 by either a mixed or a non-competitive model, with Ki values of 1142 µM (545.3 µg/mL) or 2063 µM (985.0 µg/mL), respectively. These result in I/Ki values of 0.01 and 0.006, respectively.

Using the criteria of the FDA drug interaction guidance, the potential for significant *in vivo* drug interactions due to the CYP inhibiting activity of pralatrexate is “remote.”

The induction potential of pralatrexate was evaluated in experiments using positive controls performed in human hepatocytes (FDA Table 11.). At 50 uM (a concentration approximately 4-fold Cmax), it appeared that induction did not occur.

FDA Table 11. Assessment of *in vitro* CYP-450 induction by pralatrexate (Applicant's Table 2.7.2.8)

Study: PDX-K-08060-U		Test Article: Pralatrexate		
Study Title: Induction Assessment of CYP1A2, 3A4, and 2C19 by PDX in Fresh Human Hepatocytes				
CYP450 activity in % of control after 72h incubation of human hepatocytes with pralatrexate				
CYP450 isozyme	1A2	2C19	3A4	
50 µM Pralatrexate	108	94	93	

PDX = pralatrexate, CYP or CYP450 = cytochrome P450, h = hour, µM = micromolar

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

In MDR1-MDCK cells pralatrexate at concentrations up to 500 uM (*in vivo* Cmax = 13 µM) appeared to not be a substrate of P-gp (FDA Table 12.), while the positive control digoxin was transported. In a CACO-2 system, 500 µM pralatrexate inhibited digoxin transport < 50%, while

cyclosporine and ketoconazole were both effective (FDA Table 12.). It appears that pralatrexate is neither significantly transported by P-gp nor significantly inhibits P-gp.

FDA Table 12. Assessment of pralatrexate as an *in vitro* substrate and inhibitor of P-gp (Applicant's Table 2.7.2.9.)

Study Number: PDX-K-08059-U		Test Article: Pralatrexate	
Study Title: Assessment of PDX as a Substrate and an Inhibitor of P-glycoproteins in Different Cell Systems			
Test System and Method: MDR1-MDCK and Caco-2 cell permeability analysis by LC/MS/MS			
MDR1-MDCK permeability	A to B P_{app} ($\times 10^{-6}$ cm/s)	B to A P_{app} ($\times 10^{-6}$ cm/s)	Efflux Ratio
PDX (5 μ M)	0.06	0.08	ND
PDX (50 μ M)	0.08	0.12	1.5
PDX (500 μ M)	0.07	0.12	1.6
Digoxin (10 μ M)	0.218	8.26	37.8
Caco-2: digoxin permeability			
Digoxin (10 μ M)	0.84	14.4	17.1
Digoxin (10 μ M) + PDX (500 μ M)	1.23	13.0	10.6
Digoxin (10 μ M) + CsA	3.74	4.06	1.1
Digoxin (10 μ M) + ketoconazole	4.26	3.74	0.9

PDX = pralatrexate, μ M = micromolar, MDR1 = multi-drug resistance protein 1, A = apical, B = basolateral, P_{app} = apparent permeability, cm = centimeter, s = second, CsA = cyclosporine A, ND = not determined

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

None have been described, but see review section 2.3.2.9 regarding the finding of an exploratory analysis that could be explained by heterogeneity of an unknown transporter.

2.4.2.6 Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

Folotyn is administered as mono-therapy. However, patients are supplemented with vitamin B₁₂ 1 mg IM q 8-10 weeks and folic acid 1.0-1.25 mg PO QD. The interaction potential between the supplements and pralatrexate has not been evaluated.

2.4.2.7 What other co-medications are likely to be administered to the target patient population?

In Study PDX-008 the most frequently reported concomitant medications were categorized as stomatological preparations (n = 72, 65%), drugs for peptic ulcer and gastro-esophageal reflux disease (GERD) (n = 62, 56%), opioids (n = 58, 52%), antiemetics and antinauseants (n = 56, 50%), and analgesics and antipyretics (n = 53, 48%).

2.4.2.8 Are there any *in vivo* drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

No formal clinical assessments of PK drug-drug interactions between pralatrexate and other drugs have been conducted. Approximately one-third of the pralatrexate dose administered to patients is excreted unchanged in urine. It has been shown that co-administration of the uricosuric drug probenecid decreases renal tubular excretion of methotrexate, resulting in increases in plasma concentrations of methotrexate. A Phase 1 clinical study (PDX-01-014) was performed to study the effect of probenecid co-administration on pralatrexate PK. The bioanalytical method used in this PK study was an unvalidated HPLC method that did not distinguish between the two pralatrexate diastereomers.

PDX-01-014 was an open-label, single-center, dose-escalation study to determine the maximum tolerated dose (MTD) for IV pralatrexate and probenecid given in combination every 2 weeks to cancer patients. Pralatrexate was administered by direct IV injection through the side arm of a freely flowing IV line. The initial starting dose was 40 mg/m² pralatrexate IV given in combination with 70 mg/m² probenecid every 2 weeks in 4-week cycles. Probenecid was injected intravenously 10 minutes prior to pralatrexate administration. The dose of pralatrexate was fixed at 40 mg/m² and the probenecid dose was escalated to 140 and 233 mg/m², the latter providing the MTD for the combination. Patients did not receive vitamin B12 or folic acid supplementation in this study.

With the first dose (day 1), blood samples were obtained at the following times: pre-injection; end of injection (0 time); 5, 10, 20, 30, and 60 minutes after injection; and 2, 3, 4.5, 6, 8, 24, 30, and 48 hours after injection. On the second dose day (day 15), samples were obtained for the same times as day 1, except for pre-injection. The pharmacokinetic results are presented in **FDA Table 13**.

FDA Table 13. Effect of probenecid dose on pralatrexate (total pralatrexate = PDX-10a + PDX 10b) pharmacokinetics

Dose Cohort (mg/m ² pralatrexate / mg/m ² probenecid)	n	AUC (ng/mL * min)	Cmax (ng/mL)
40/70	3	81667	4900
40/140	9	90000	4700
40/233	5	103333	6700

Interpretation of these data is difficult, as there is no control group (pralatrexate in the absence of probenecid), the number of patients studied is small, and an un-validated, non-stereo-selective bioanalytical assay was used. It does appear that there is a trend toward a dose-dependent increase in pralatrexate exposure with increasing dose of probenecid.

2.4.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

No non-clinical pharmacodynamic studies have been performed to specifically evaluate possible interactions of pralatrexate with other drugs that may be co-administered. There is no known mechanistic basis for pharmacodynamic drug interactions.

2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

Completion of a mass balance study to identify excretion route and metabolites is an unresolved issue. We recommend a post-marketing commitment to acquire such data and allow an informed determination of the need for a study in patients with hepatic impairment.

2.4.2 What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

The clinical activity of pralatrexate doses lower than 30 mg/m² is largely unknown. Thus, it is possible that a lower dose could provide less toxicity while retaining efficacy. This could be considered an insignificant omission, as maximum tolerated dosing is the norm for cytotoxic chemotherapy.

2.5. General Biopharmaceutics

2.5.1 Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

Pralatrexate is administered parenterally (intravenously).

2.5.2 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial formulation?

The to-be-marketed formulation was used in the pivotal clinical trial.

2.5.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

The effect of food was not studied. Pralatrexate is administered parenterally (intravenously).

2.5.4 When would a fed BE study be appropriate and was one conducted?

Such a study would not be appropriate and was not conducted.

2.5.5 How do the dissolution conditions and specifications ensure *in vivo* performance and quality of the product?

Pralatrexate is administered as a solution parenterally (intravenously).

2.5.6 If different strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to-be-marketed product?

Two compositionally proportional solution formulations of 20 mg/mL are being marketed for parenteral (intravenous) administration.

2.5.7 If the NDA is for a modified release formulation of an approved immediate product without supportive safety and efficacy studies, what dosing regimen changes are necessary, if any, in the presence or absence of PK-PD relationship?

The NDA is not for a modified release formulation of an approved immediate release product.

2.5.8 If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either *in vitro* or *in vivo* data to evaluate BE?

Unapproved products or altered approved products were not used as active controls

2.5.9 What other significant, unresolved issues related to *in vitro* dissolution or *in vivo* BA and BE need to be addressed?

There are no unresolved issues related to *in vitro* dissolution or *in vivo* BA and BE.

2.5 Analytical section

- 2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?
- 2.6.2 Which metabolites have been selected for analysis and why?
- 2.6.3 For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

In all studies, only total parent PDX-10a and PDX-10b were measured. Protein binding in human plasma was approximately 67%. Measurement of total drug appears appropriate.

- 2.6.4 What bioanalytical methods are used to assess concentrations?
 - 2.6.4.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?
 - 2.6.4.2 What are the lower and upper limits of quantification (LLOQ/ULOQ)?
 - 2.6.4.3 What are the accuracy, precision, and selectivity at these limits?
 - 2.6.4.4 What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?
 - 2.6.4.5 What is the QC sample plan?

The bioanalytical method involves extraction of PDX-10a and PDX-10b from the matrix utilizing (b) (4) (b) (4) for separation and detection. The (b) (4) extracts are injected on a chiral high-performance liquid chromatography (HPLC) column for quantitation of each diastereomer by liquid chromatography-tandem mass spectrometry (LC/MS/MS). The lower limit of quantitation (LLOQ) for both diastereomers in plasma and urine matrices was 0.5 ng/mL.

To determine specificity, human plasma and urine from six donors and urine from four donors was assayed with and without addition of internal standard. No significant interferences were noted at the retention times of either enantiomer in any of the specificity determination samples, or at the retention time of the (b) (4) internal standard in any of the samples that had not received internal standard.

Stability evaluations included the stability of analytes in both human matrices through three freeze thaw cycles, stability in both matrices at room temperature for 24 hr, and the stability in injection extracts held at room temperature for 5 days. In all cases stability was observed: (relative standard deviation, %RSD) and accuracy (relative error, %RE), were within 15%.

Duplicate quality control (QC) pools at 3 concentrations and a set of quantitation standards extracted out of the matrix being analyzed were assayed with each sample setup for pralatrexate diastereomer quantitation. Data from in-run quality control samples are summarized in FDA

Table 14. The Reviewer finds the analytical methods of sufficient quality to allow for interpretation of the studies performed and thus construction of the package insert.

FDA Table 14. Intrabatch/interbatch validation results for PDX-10a and PDX 10-b (Applicant's Tables 3 – 6 from pages 34-37 of report PDX-K-05014-U)

TABLE 3 PDX-10a Intrabatch/Interbatch Validation Results for Human Plasma

	HQC 750 ng/mL	MQC 75 ng/mL	LQC 1.5 ng/mL		LLQC 0.5 ng/mL		
			All Results	Outliers Excluded	All Results	Outliers Excluded	
INTRABATCH 1	793	95.6	4.98	4.98*	0.34	0.34	
	856	73.2	1.62	1.62	0.42	0.42	
	842	71.5	1.47	1.47	0.40	0.40	
	816	73.4	1.47	1.47	0.52	0.52	
	871	89.6	1.61	1.61	0.92	0.92*	
	MEAN	836	80.7	2.23	1.54	0.52	0.42
	RSD (%)	3.7	14	69	5.4	45	18
RE (%)	11	8	49	2.7	4.0	-16	
INTRABATCH 2	689	79.9	1.49	1.49	0.64	0.64	
	764	75.0	1.38	1.38	0.60	0.60	
	916	89.1	1.63	1.63	0.56	0.56	
	819	60.1	1.48	1.48	0.63	0.63	
	796	73.7	1.32	1.32	0.47	0.47	
	MEAN	797	75.6	1.46	1.46	0.58	0.58
	RSD (%)	10	14	8.1	8.1	12	12
RE (%)	6.3	0.8	-2.7	-2.7	16	16	
INTRABATCH 3	768	78.6	1.53	1.53	0.58	0.58	
	835	84.2	1.64	1.64	0.57	0.57	
	795	77.2	1.35	1.35	0.55	0.55	
	612	67.4	1.46	1.46	0.59	0.59	
	717	70.3	1.49	1.49	0.58	0.58	
	MEAN	745	75.5	1.49	1.49	0.57	0.57
	RSD (%)	12	8.9	7.1	7.1	2.7	2.7
RE (%)	-0.7	0.7	-0.7	-0.7	14	14	
INTERBATCH	MEAN	793	77	1.73	1.50	0.56	0.53
	RSD (%)	10	12	52	6.8	24	17
	RE (%)	5.7	2.7	15	0	12	6.0

* Excluded from calculation of statistics based on ADC outlier test.

FDA Table 14. Intrabatch/interbatch validation results for PDX-10a and PDX 10-b (Applicant's Tables 3 – 6 from pages 34-37 of report PDX-K-05014-U)

TABLE 4 PDX-10b Intrabatch/Interbatch Validation Results for Human Plasma

	HQC 750 ng/mL	MQC 75 ng/mL	LQC 1.5 ng/mL		LLQC 0.5 ng/mL		
			All Results	Outliers Excluded	All Results	Outliers Excluded	
INTRABATCH 1	814	97.9	5.18	5.18*	0.41	0.41	
	879	78.4	1.66	1.66	0.50	0.50	
	808	74.7	1.50	1.50	0.42	0.42	
	823	77.1	1.50	1.50	0.57	0.57	
	853	97.6	1.57	1.57	0.99	0.99*	
	MEAN	835	85.1	2.28	1.56	0.58	0.48
	RSD (%)	3.6	14	71	4.9	41	16
	RE (%)	11	13	52	4.0	16	-4.0
INTRABATCH 2	678	69.5	1.58	1.58	0.67	0.67	
	706	70.4	1.26	1.26	0.49	0.49	
	805	79.8	1.57	1.57	0.71	0.71	
	820	79.6	1.54	1.54	0.49	0.49	
	688	67.4	1.23	1.23	0.50	0.50	
	MEAN	739	73.3	1.44	1.44	0.57	0.57
	RSD (%)	9.2	8.1	12	12	19	19
	RE (%)	-1.5	-2.3	-4.0	-4.0	14	14
INTRABATCH 3	778	77.1	1.45	1.45	0.57	0.57	
	844	81.5	1.48	1.48	0.54	0.54	
	806	77.6	1.42	1.42	0.58	0.58	
	626	68.1	1.38	1.38	0.55	0.55	
	739	74.5	1.43	1.43	0.54	0.54	
	MEAN	759	75.8	1.43	1.43	0.56	0.56
	RSD (%)	11	6.5	2.6	2.6	3.2	3.2
	RE (%)	1.2	1.1	-4.7	-4.7	12	12
INTERBATCH	MEAN	778	78	1.72	1.47	0.57	0.54
	RSD (%)	9.4	12	56	8.2	25	15
	RE (%)	3.7	4.0	15	-2.0	14	8.0

* Excluded from calculation of statistics based on ADC outlier test.

FDA Table 14. Intrabatch/interbatch validation results for PDX-10a and PDX 10-b (Applicant's Tables 3 – 6 from pages 34-37 of report PDX-K-05014-U)

TABLE 5 PDX-10a Intrabatch/Interbatch Validation Results for Human Urine

	HQC	MQC	LQC	LLQC	
	750 ng/mL	75 ng/mL	1.5 ng/mL	0.5 ng/mL	
INTRABATCH 1	796	87.5	1.42	0.63	
	729	80.6	1.58	0.60	
	803	84.7	1.66	0.56	
	662	81.5	1.85	0.66	
	719	79.2	1.93	0.51	
	MEAN	742	82.7	1.69	0.59
	RSD (%)	7.9	4.1	12	10
RE (%)	-1.1	10	13	18	
INTRABATCH 2	842	65.6	1.62	0.49	
	734	70.8	1.26	0.53	
	790	81.3	1.31	0.52	
	686	65.6	1.33	0.40	
	627	60.9	1.50	0.49	
	MEAN	736	68.8	1.40	0.49
	RSD (%)	11	11	11	10
RE (%)	-1.9	-8.3	-6.7	-2.0	
INTRABATCH 3	830	75.8	1.51	0.50	
	782	73.3	1.49	0.47	
	843	70.7	1.44	0.46	
	794	73.0	1.45	0.50	
	706	72.1	1.48	0.45	
	MEAN	791	73.0	1.47	0.48
	RSD (%)	6.8	2.6	2.0	4.8
RE (%)	5.5	-2.7	-2.0	-4.0	
INTERBATCH	MEAN	756	75	1.52	0.52
	RSD (%)	8.9	10	12	13
	RE (%)	0.8	0	1.3	4.0

FDA Table 14. Intrabatch/interbatch validation results for PDX-10a and PDX 10-b (Applicant's Tables 3 – 6 from pages 34-37 of report PDX-K-05014-U)

TABLE 6 PDX-10b Intrabatch/Interbatch Validation Results for Human Urine

	HQC	MQC	LQC	LLQC	
	750 ng/mL	75 ng/mL	1.5 ng/mL	0.5 ng/mL	
INTRABATCH 1	827	92.0	1.27	0.55	
	766	84.0	1.48	0.54	
	804	81.5	1.63	0.52	
	700	83.4	1.69	0.51	
	759	82.8	1.74	0.51	
	MEAN	771	84.7	1.56	0.53
	RSD (%)	6.3	4.9	12	3.4
RE (%)	2.8	13	4.0	6.0	
INTRABATCH 2	956	63.1	1.26	0.47	
	789	70.2	1.30	0.53	
	862	84.9	1.30	0.46	
	774	67.7	1.38	0.43	
	702	61.1	1.41	0.46	
	MEAN	817	69.4	1.33	0.47
	RSD (%)	12	14	4.7	7.8
RE (%)	8.9	-7.5	-11	-6.0	
INTRABATCH 3	829	73.0	1.45	0.47	
	806	74.5	1.39	0.55	
	814	75.3	1.38	0.46	
	797	71.5	1.42	0.53	
	763	71.9	1.41	0.52	
	MEAN	802	73.2	1.41	0.51
	RSD (%)	3.1	2.2	1.9	7.7
RE (%)	6.9	-2.4	-6.0	2.0	
INTERBATCH	MEAN	797	76	1.43	0.50
	RSD (%)	7.8	12	10	7.7
	RE (%)	6.3	1.3	-4.7	0

3 Pages Withheld as
Trade Secret/
Confidential b(4)

4 *Appendices*

4.1 *Proposed Package Insert (Original)*

4.2 *Pharmacometrics review*

4.3 *Pharmacogenomics Review*

4.4 *Cover Sheet and OCPB Filing/Review Form*

4.1 *Proposed Package Insert (Original)*

15 Pages Withheld as
Trade Secret/
Confidential b(4)

4.2 *Pharmacometrics Review*

**OFFICE OF CLINICAL PHARMACOLOGY:
PHARMACOMETRIC REVIEW**

Application Number	22468
Submission Number (Date)	March 23, 2009
Compound	Folotyn (Pralatrexate 30 mg/m ²)
Clinical Division	DDOP
Primary PM Reviewer	Anshu Marathe, Ph.D.
Secondary PM Reviewer	Christoffer W. Tornoe, Ph.D.

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1 SUMMARY OF FINDINGS

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 Is there evidence of exposure-response for effectiveness?

No, there is no evidence of exposure-response relationship for effectiveness in PDX-008 (pivotal efficacy trial) following 30 mg/m² pralatrexate. A logistic regression was performed to assess the exposure-response relationship for effectiveness based on response rate (primary efficacy endpoint). Lack of relationship is observed by a nearly flat mean logistic prediction in Figure 1.

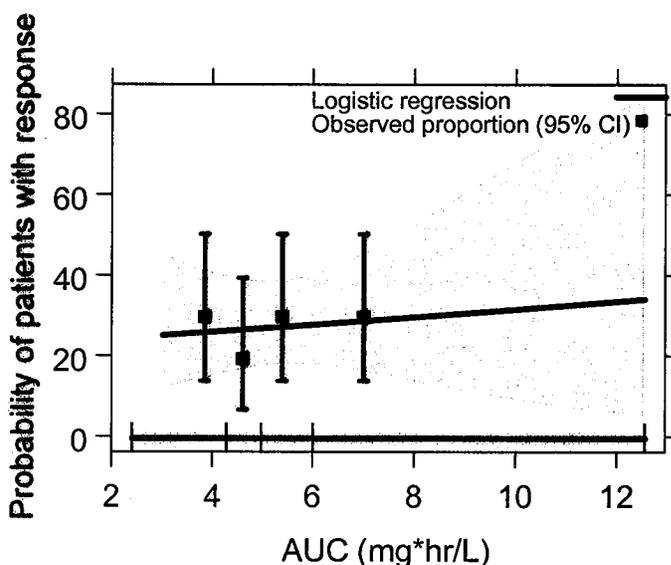


Figure 1: The probability of patients with response-AUC profile for Pralatrexate. Solid black symbols represent the observed percentage of patients responding to treatment in each AUC quartile. The vertical black bars represent the 95% confidence interval. The solid red line represents the mean logistic regression prediction. The shaded area represents the 95% confidence interval of the prediction. The exposure range in each AUC quartile is denoted by the horizontal black line.

Kaplan-Meier analysis was performed to assess the exposure-response relationship for effectiveness based on duration of response and progression free survival (secondary efficacy endpoints). The duration of response and progression free survival curves of patients in different AUC-quartile groups overlapped in Figure 2, thus indicating lack of **exposure-response relationship**. A likely reason for not observing exposure-response relationship is that this was a single arm trial with only one dose that did not result in a wide range of exposures.

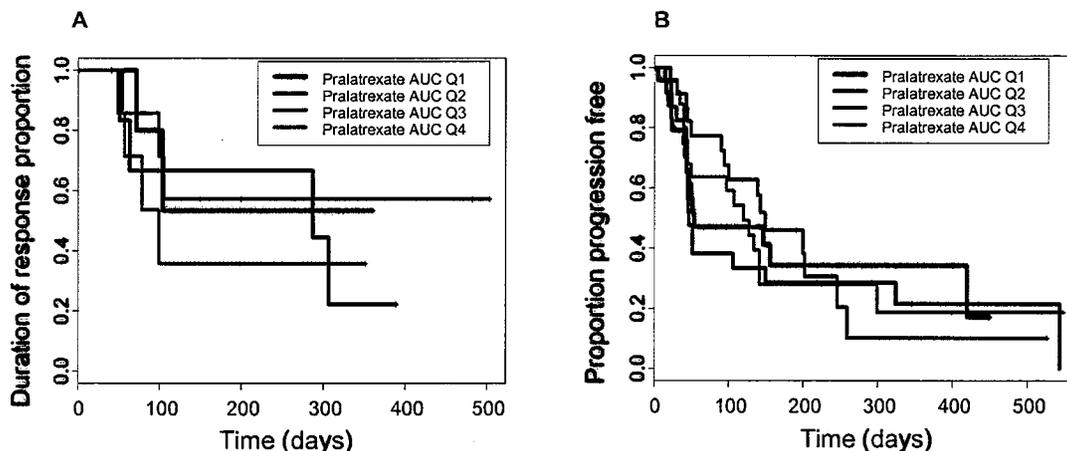


Figure 2: Kaplan-Meier plots for A) duration of response and B) progression free survival for treatment groups. Q1, Q2, Q3 and Q4 are AUC quartiles following 30 mg/m² pralatrexate.

1.1.2 Is there evidence of exposure-response for safety?

Yes, there is evidence of exposure-response relationship for mucositis and thrombocytopenia in PDX-008 following 30 mg/m² pralatrexate. Logistic regression models were used to explore the relationship between exposure and treatment emergent AE's. The various AEs explored for relationship with the pralatrexate exposure are mucositis grade 2+, thrombocytopenia grade 3+, and neutropenia grade 3+ because sponsor has recommended dose adjustments for these AEs in the label. AUC was found to be a predictor for mucositis and thrombocytopenia (Figure 3) with p-value <0.05.

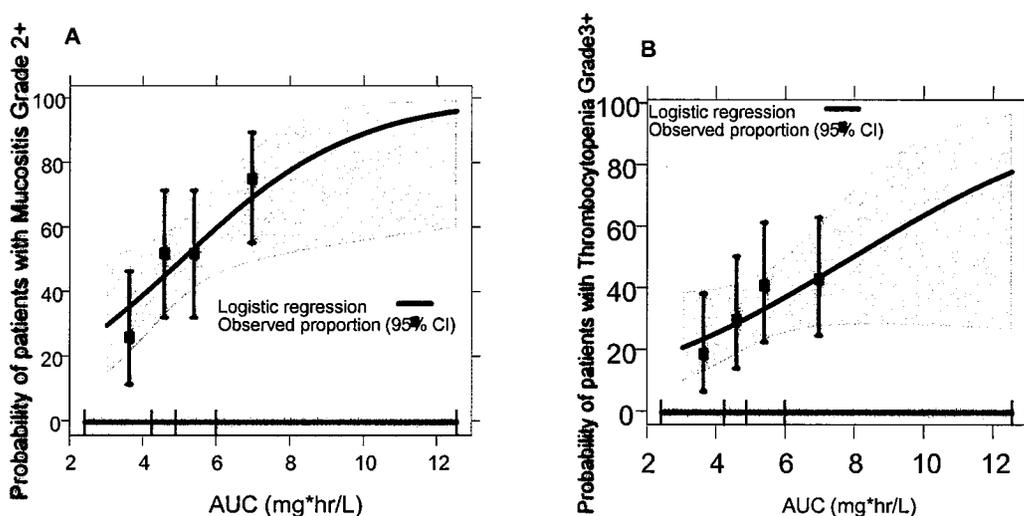


Figure 3: The probability of patients with various adverse events A) Mucositis grade 2+ B) Thrombocytopenia grade 3+. Solid black symbols represent the observed proportion of patients experiencing AEs in each AUC quartile. The vertical black bars represent the 95% confidence interval. The solid red line represents the mean logistic regression prediction. The shaded area represents the 95% confidence interval of the prediction. The exposure range in each AUC quartile is denoted by the horizontal black line.

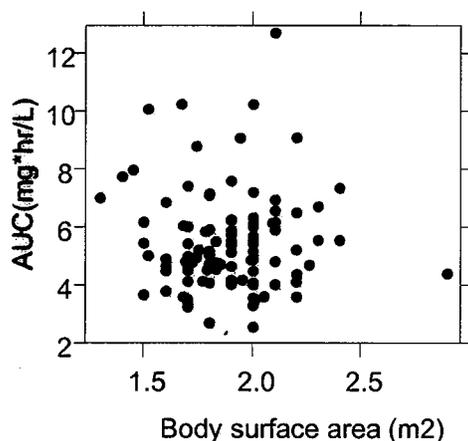
1.1.3 Is the proposed dose adjustment for patients with adverse events appropriate?

Yes, dose reduction for mucositis and thrombocytopenia are reasonable since an exposure-response relationship for safety was identified (see section 3). The exposure-response relationship for adverse events suggests that decreasing the dose from 30 to 20 mg/m² would reduce the probability of patients experiencing mucositis grade 2+ and thrombocytopenia grade 3+ from 47.9% to 32% and 30.1% to 21.8 %, respectively (Figure 3 and Table 8). Furthermore, the lack of an exposure-response relationship for effectiveness (response, duration of response, and progression free survival) following 30 mg/m² suggests that reducing the dose is not likely to affect the effectiveness of pralatrexate (Figure 1 and Figure 2).

1.1.4 Is the proposed dose of 30 mg/m² adequate to obtain similar exposures across patients?

Yes, body surface area (BSA)-based dosing, normalizes the AUC values across patients (Figure 4). No other PK covariates were found to be clinically significant.

A



B

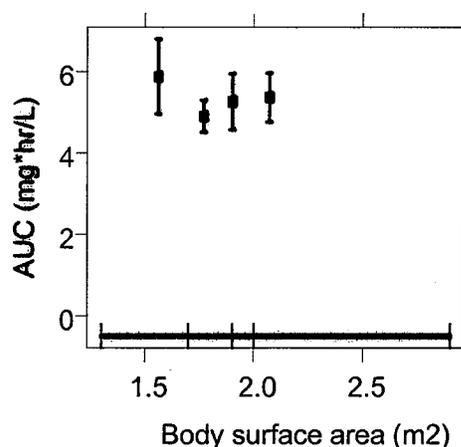


Figure 4: A) Scatter plot of AUC vs. body surface area (BSA). B) Quartile plot of AUC vs. BSA. Solid black symbols represent the AUC in each BSA quartile. The BSA range is denoted by the horizontal black line.

A slight trend is observed between creatinine clearance (CRCL) and clearance (CL) for patients in the pivotal trial with mild and moderate renal impairment (Figure 5). No dose adjustments are necessary for mild renal impairment but the increase in exposure in moderate/severe renal impaired patients is unknown and needs to be explored.

(b) (4)

Figure 5: Clearance vs. Creatinine Clearance for A) PDX-10a and B) PDX-10b in the pivotal trial.

1.2 Recommendations

Division of Pharmacometrics finds the NDA acceptable from a clinical pharmacology perspective.

1.3 Label Statements

The following are the labeling recommendations relevant to clinical pharmacology for NDA 22468. The ~~red strikeout font~~ is used to show the proposed text to be deleted and underline blue font to show text to be included or comments communicated to the sponsor.

(b) (4)



2 RESULTS OF SPONSOR'S ANALYSIS

The sponsor conducted a population PK analysis to estimate the population PK parameters including typical values, inter-individual variation, inter-occasion variation and residual variability for pralatrexate diastereomers. The effect of individual-specific covariates that are predictive of unexplained random variability in PK was also examined.

2.1 Methods

Data from a total of 154 patients from three studies were used in this analysis. A description of the different studies used, is provided in Table 1. In PDX-008, ten patients had complete plasma sampling on C1D1 and 6 of these patients also had sampling on C1D6 (N = 5) or C2D6 (N = 1). The remainder of the patients had limited plasma sampling on C1D1 and C1D6. Full PK samples were obtained from all patients on C1D1 in PDX-007. Full PK samples were obtained on day 1 and day 15 from 6 patients in PDX-99-083.

Table 1: Analysis Data Sets.

Study	Dose	No of patients	Indication
PDX-008 (pivotal)	30 mg/m ² /week for 6 weeks in 7 week cycle as an IV push over 3-5 minutes	109	Relapsed or refractory PTCL
PDX-007	150 to 325 mg/m ² every 2 weeks as an IV push over 3-5 minutes or as a 60-minute IV infusion	39	NSCLC
PDX-99-083	120 mg/m ² every 2 weeks as an IV push followed by Docetaxel (35 mg/m ² as a 60 minute infusion)	6	Advanced solid tumors

2.2 Conclusions

- The population PK for both S and R diastereomers of pralatrexate was described by a three compartment model.
- The full model included WT as a covariate on V2, V3, Q2 and Q3. IBW was included as a covariate on V1 and CL, Study, CRCL (for values < 80) and gender were included as covariates on CL.
- The typical estimates of pralatrexate S-diastereomer PK model parameters for the reference covariate effects (Male, 70 kg, Studies PDX-007, PDX-99-083) were 35.0 L/hr, and 11.0 L for clearance (CL) and Volume of central compartment (V1). Pralatrexate R-diastereomer PK model parameters were estimated to be 17.2 L/h and 8.89 L.

Reviewer's comments on Sponsor's Population PK Analysis:

Sponsor's population PK analysis is generally adequate and acceptable. The covariates that were identified in the final model are likely not to be significant as the inclusion of all the covariates resulted in the reduction in the objective function value (OFV) from 12267 to 12209.5 from the sponsor's base model to final model for the S diastereomers. Similarly for the R diastereomer, the objective function was reduced from 13500.05 to 13478.91. The inter-individual variability on clearance (CL) was reduced from 42.4% to 28.6% for S diastereomer and from 36.7% to 25.8% for the R diastereomer. Inclusion of WT and IBW caused an increase in the OFV (see Table 7 in Sponsor's Population PK report). Also no clear trends were identified between the PK parameters obtained from the base model and WT/IBW/gender in reviewer's analysis (see section 3.3). A slight trend was observed between CL and CRCL for patients with moderate and mild renal impairment (see reviewer's analysis). Since 31% (S-diastereomer) and 38% (R-diastereomer) of the drug is cleared renally, it is likely that a stronger relation would exist between CL and CRCL for patients with severe renal impairment.

3 RESULTS OF REVIEWER'S ANALYSIS

3.1 Objectives

The reviewer's analysis objectives are:

1. To determine the exposure-response relationship for effectiveness and safety of pralatrexate.
2. To use the results of objective (1) to establish whether proposed dose adjustment for adverse events is adequate.
3. To explore whether the proposed dose of 30mg/m² is adequate to obtain similar exposures across patients.

3.2 Methods

3.2.1 Data Sets

Data sets used are summarized in Table 2.

Table 2: Analysis Data Sets.

Study Number	Name	Link to EDR
PDX-008 PDX-007 PDX-99-083	plasmaa2.xpt plasmab2.xpt	\\Cdsesub1\evsprod\NDA022468\0000\m5\datasets\population-pk-report\analysis
PDX-008	resp.xpt	\\Cdsesub1\evsprod\NDA022468\0000\m5\datasets\pdx008\analysis
PDX-008	adverse.xpt	\\Cdsesub1\evsprod\NDA022468\0000\m5\datasets\pdx008\analysis

3.2.2 Software

SAS, R, S-PLUS, NONMEM were used for the reviewer's analyses.

3.3 Results

3.3.1 Population Pharmacokinetic Analysis

Similar to sponsor's population PK findings, a three-compartment disposition model with first-order elimination was found adequate to describe the pralatrexate concentration-time profile following a dose of 30 mg/m², administered as an IV push of 3-5 minutes. The parameter estimates of the base model are provided in Table 9 in Section 4: Appendix A. The parameters obtained were similar to the sponsor. No significant trend was observed between volume of central compartment (V1) or clearance (CL) with IBW and CL with gender (see Figure 6 and Figure 7). The CL of subjects in the pivotal study, PDX-008 was lower than the subjects in PDX-007 and PDX-99-083 (Figure 6 and Figure 7).

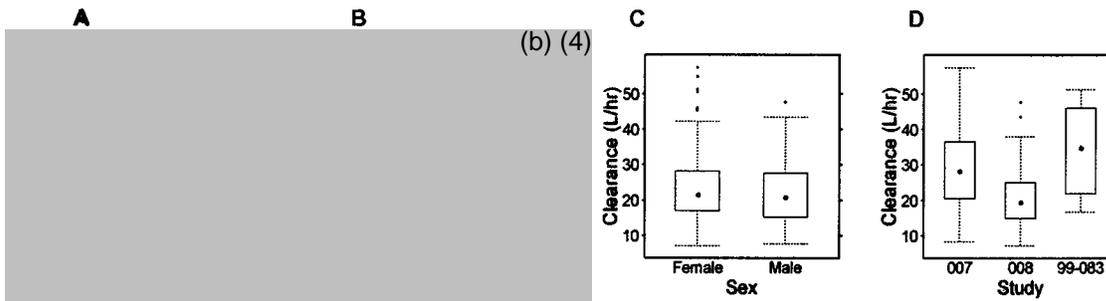


Figure 6: Demographic covariate-PK parameter relationships for Pralatrexate (S-diastereomer) A) Central volume (V1) vs. Ideal body weight (IBW) B) Clearance (CL) vs. IBW C) CL vs. SEX and D) CL vs. Study

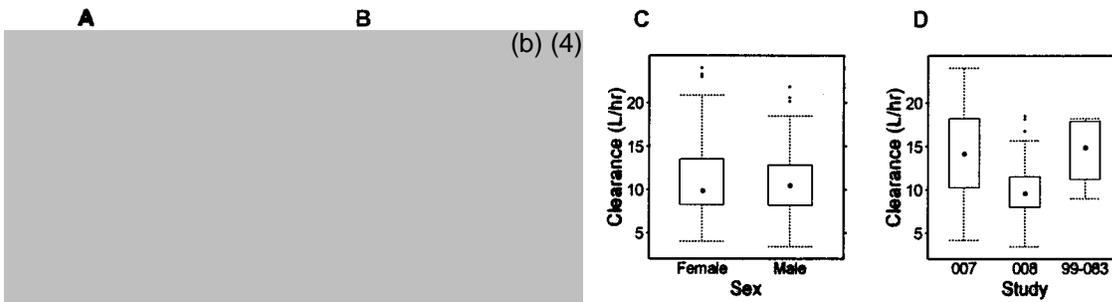


Figure 7: Demographic covariate-PK parameter relationships for Pralatrexate (R-diastereomer) A) V1 vs. Ideal body weight (IBW) B) Clearance (CL) vs. IBW C) CL vs. SEX and D) CL vs. Study (STUD)

A slight trend is observed between creatinine clearance (CRCL) and CL for patients in the pivotal trial with mild and moderate renal impairment (Figure 5). This implies that a stronger relation is likely to exist for patients with severe renal impairment and thus needs to be further explored by the sponsor. **Based on the observed trend, the sponsor's piecewise linear model for CL with CRCL as the covariate is acceptable**

Overall, the sponsor's population PK analysis was found to be acceptable. The full model with covariates was used to calculate the AUCs for exposure-response analyses and the parameters of the full model are provided in Table 10 in Section 4: Appendix A.

Boxplots of dose versus clearance again show that the subjects in the pivotal trial with a dose of 30mg/m² had lower clearance than the subjects in the supporting trials with higher doses ranging from 120-325 mg/m² (Figure 8). The clearance values for higher doses (120-325 mg/m²) were similar. The volume of the central compartment remained similar across doses.

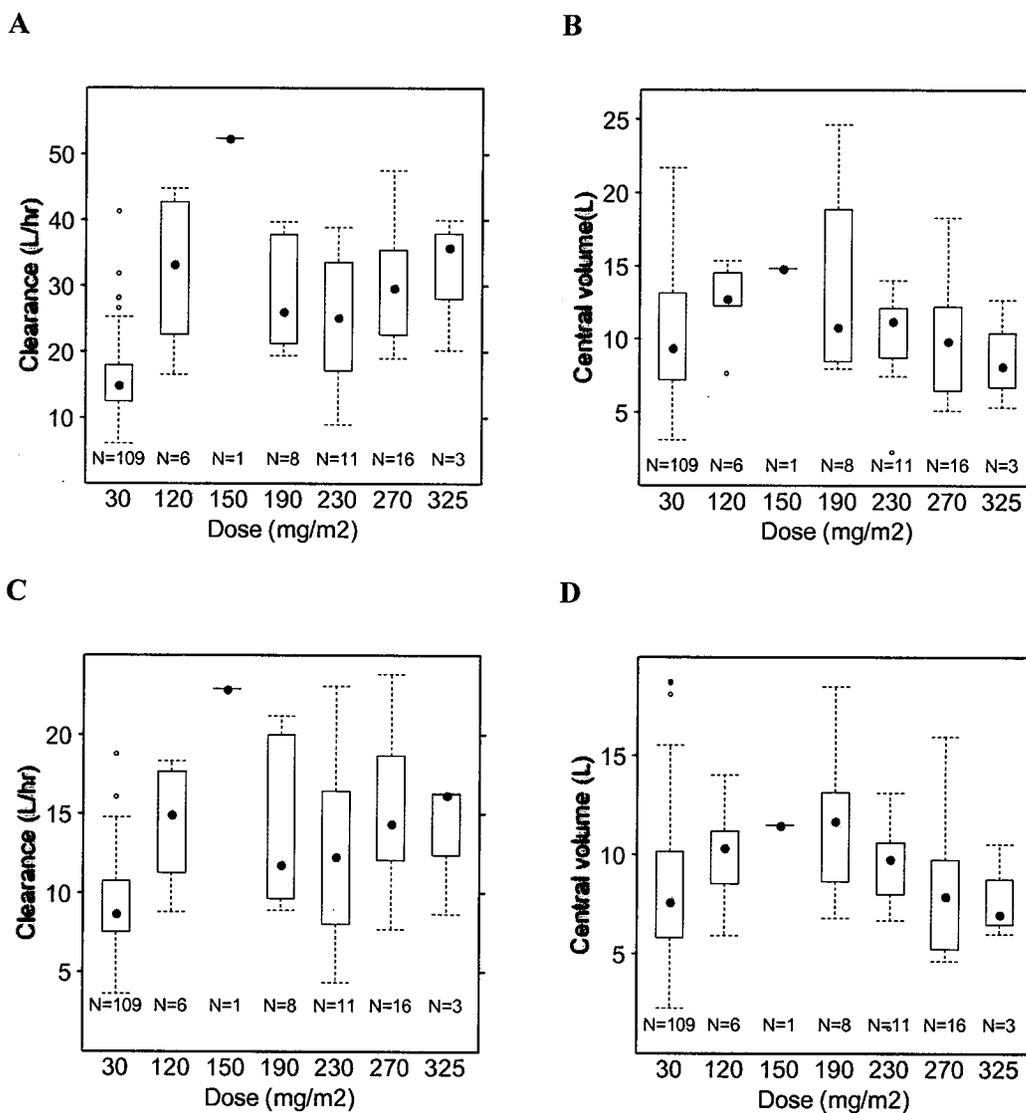


Figure 8: A) Clearance vs. Dose and B) Central volume vs. Dose for Pralatrexate (S-diastereomer) C) Clearance vs. Dose and D) Central volume vs. Dose for Pralatrexate (R-diastereomer)

Boxplots of the interoccasion variability on clearance and volume show that there is no systematic trend in the change between day 1 and day 6 evidenced by median of zero (Figure 9).

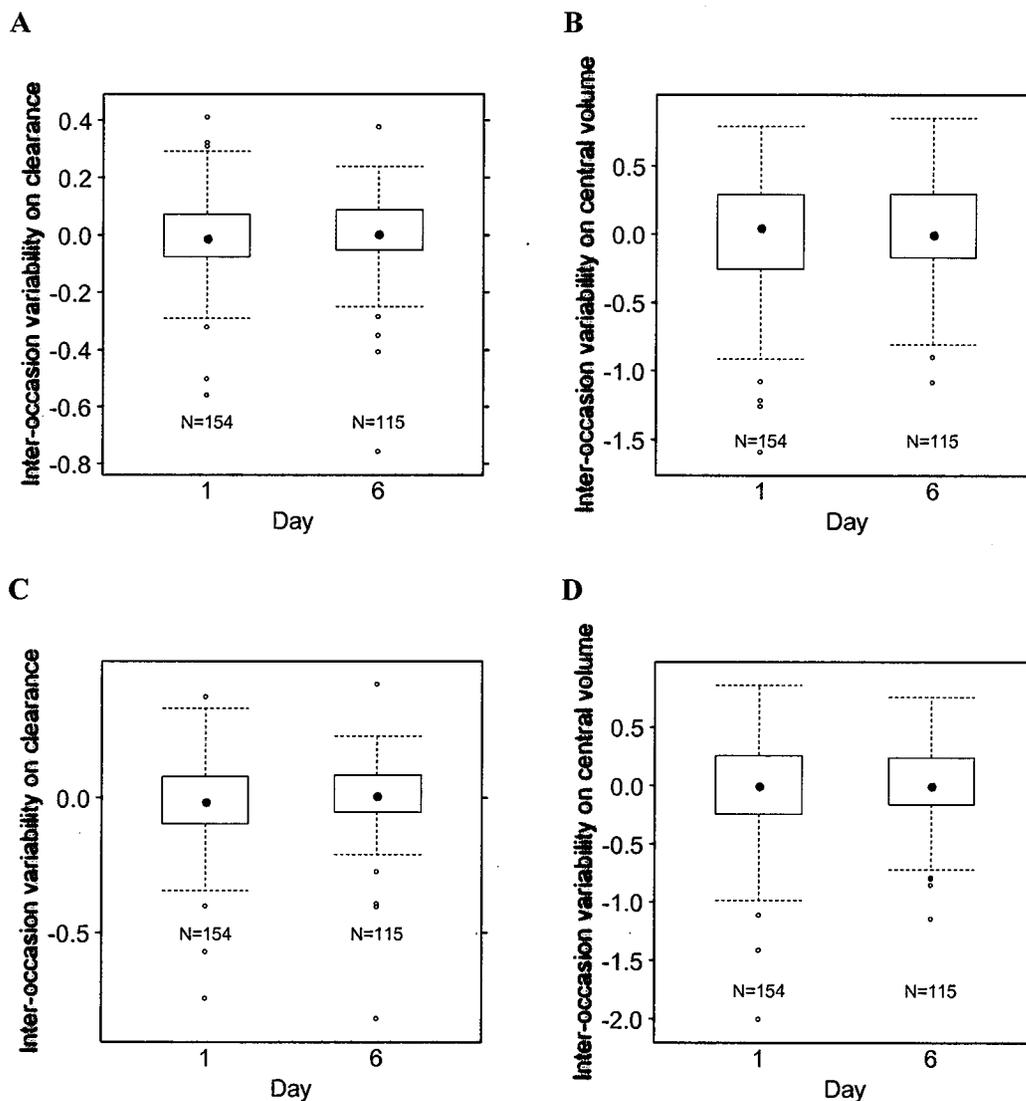


Figure 9: A) Inter-occasion variability on clearance vs. Day and B) Inter-occasion variability on volume vs. Day for Pralatrexate (S-diastereomer) C) Inter-occasion variability on clearance vs. Day and D) Inter-occasion variability on volume vs. Day for Pralatrexate (R-diastereomer)

3.3.2 Exposure-Response Analysis for Efficacy

The primary endpoint in the pivotal study was response rate, which was assessed based on central review of imaging and clinical data according to the International Workshop Criteria (IWC) developed by the National Cancer Institute (NCI)-sponsored International Working Group. Response rate was defined as the number of responders (complete

response [CR] + complete response unconfirmed [CRu] + partial response [PR]) divided by the number of evaluable patients. The sponsor's data for response rate from PDX-008 is provided in Table 3.

Table 3: Summary of Best Response.

		Efficacy Analysis Set (N=109)		
		n	(%)	(95% CI)
Best Response per Central Review - IWC	CR+CRu+PR	29	(27)	(19, 36)
	CR	7	(6)	
	CRu	2	(2)	
	PR	20	(18)	
	SD	24	(22)	
	PD	40	(37)	
	UE	2	(2)	
	Missing: off-treatment in cycle 1	14	(13)	

(Source: Sponsor's Summary of Clinical Efficacy, Table 2.7.3.5, Pg 25)

An exposure-response model for effectiveness was developed correlating the individual's AUC obtained from the reviewer's population PK analysis utilizing the full covariate model (Table 10). Predicted AUCs were utilized because only 10 patients out of 107 patients with PK in the efficacy data set had full PK sampling. Rest had sparse sampled PK. A linear logistic regression was used to correlate response (CR+CRu+PR) with AUC. Modeling results are presented in Figure 1 and Table 4. Lack of relationship is observed by a nearly flat mean logistic prediction with a p-value = 0.721 and an odds ratio that included 1. Data from 107 subjects were used in this analysis.

Table 4: Reviewer's Logistic Regression Analysis Parameter Estimates.

Parameter	Estimate	P-value	Odds Ratio (95% CI)	N
intercept	-1.23	-	-	107
slope	0.0454	0.721	1.05 (0.817-1.34)	107

The secondary endpoints in this study were duration of response, progression free survival (PFS), and overall survival. Duration of response was measured from first day of documented response until progression (PD) or death. PFS was measure from treatment day 1 until event. Kaplan-Meier analysis was performed to evaluate the duration of response and progression free survival curves among patients in different AUC-quartile groups. Figure 2 show that the duration of response and progression free survival curves of patients in different AUC-quartile groups overlapped, suggesting lack of exposure-response relationship. A likely reason for not observing exposure-response relationship is that this was a single arm trial with only one dose that did not result in a wide range of exposures.

3.3.3 Exposure-Response Analysis for Safety

The most common treatment related adverse events experienced by patients in the pivotal study were mucositis, thrombocytopenia, nausea and fatigue. Dose modifications for mucositis grade 2+, thrombocytopenia grade 3 +, neutropenia grade 3+ and any other toxicity grade 3+ has been recommended by the sponsor and is provided in Table 5. Table 6 shows the number of patients experiencing mucositis, thrombocytopenia and neutropenia.

Table 5: Dose Modification for AE's.

Mucositis Grade ^a on Day of Treatment	Action	Dose upon recovery to ≤Grade 1
Grade 2	Omit dose	Continue prior dose
Grade 2 recurrence	Omit dose	20 mg/m ²
Grade 3	Omit dose	20 mg/m ²
Grade 4	Stop therapy	

Blood Count on Day of Treatment	Duration of Toxicity	Action	Dose upon restart
Platelet < 50,000/μL	1 week	Omit dose	Continue prior dose
	2 weeks	Omit dose	20 mg/m ²
	3 weeks	Stop therapy	
ANC 500-1,000/μL and no fever	1 week	Omit dose	Continue prior dose
ANC 500-1,000/μL with fever or ANC <500/μL	1 week	Omit dose, give cytokine support	Continue prior dose with cytokine support
	2 weeks or recurrence	Omit dose, give cytokine support	20 mg/m ² with cytokine support
	3 weeks or 2nd recurrence	Stop therapy	

Toxicity Grade ^a on Day of Treatment	Action	Dose upon recovery to ≤Grade 2
Grade 3	Omit dose	20mg/m ²
Grade 4	Stop therapy	

(Source: Sponsor's Label)

Table 6: Adverse Events Occurring in ≥ 10% of Patients.

AE	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Total n (%)
Mucositis	22 (20)	33 (30)	19 (17)	4 (4)	78 (70)
Thrombocytopenia	1 (1)	8 (7)	15 (14)	21 (19)	45 (41)
Neutropenia	0 (0)	5 (5)	14 (13)	8 (7)	27 (24)

(Source: Sponsor's Summary of Clinical Safety, Table 2.7.4.4, Pg 38)

Logistic regression models were used to explore the relationship between exposure and treatment emergent AE's specifically mucositis, thrombocytopenia, and neutropenia as the sponsor has recommended dose adjustments for these in the label.

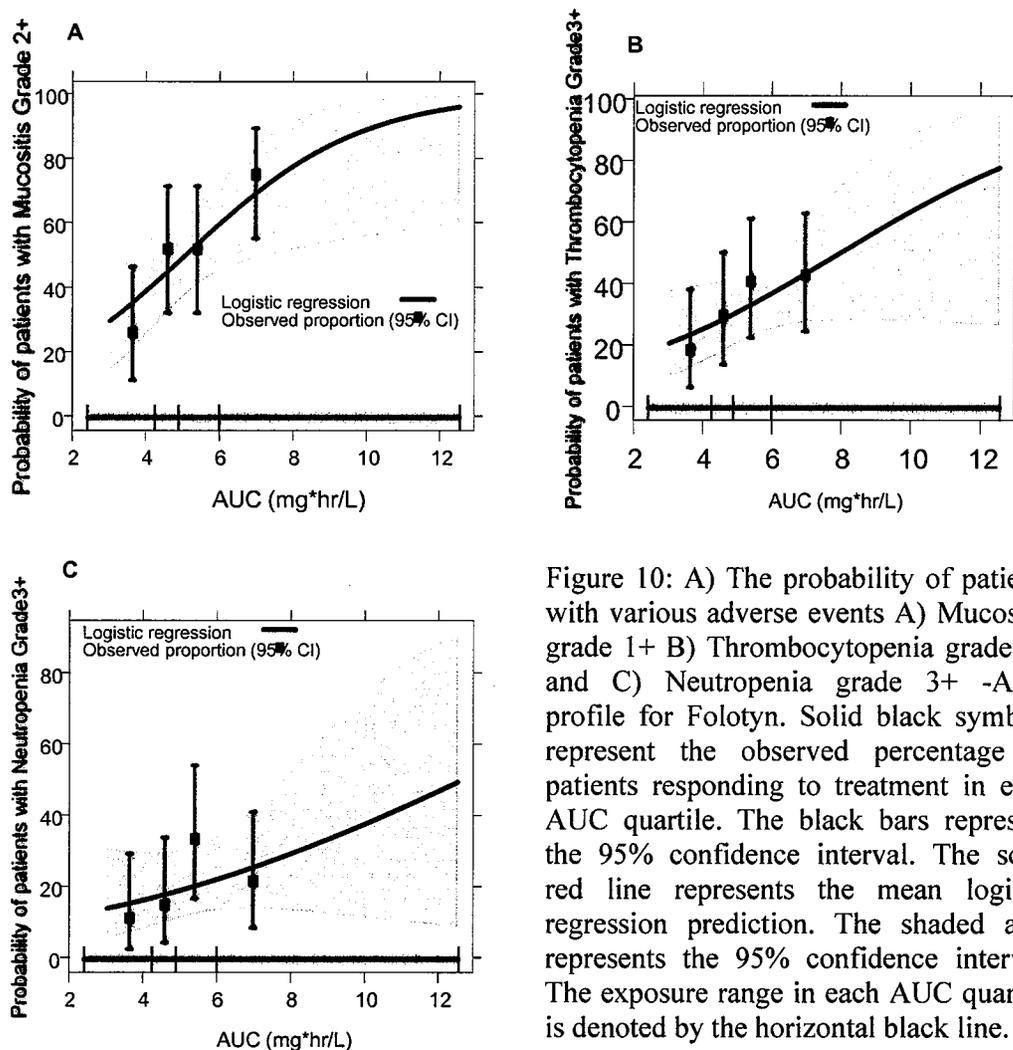


Figure 10: A) The probability of patients with various adverse events A) Mucositis grade 1+ B) Thrombocytopenia grade 3+ and C) Neutropenia grade 3+ -AUC profile for Folutyn. Solid black symbols represent the observed percentage of patients responding to treatment in each AUC quartile. The black bars represent the 95% confidence interval. The solid red line represents the mean logistic regression prediction. The shaded area represents the 95% confidence interval. The exposure range in each AUC quartile is denoted by the horizontal black line.

Table 7: Reviewer's Logistic Regression Analysis Parameter Estimates.

Adverse Event	Intercept Estimate	Slope Estimate	P-value	Odds Ratio (95% CI)	N
Mucositis Grade 2+	-2.15	0.424	.0013	1.53 (1.14-2.04)	109
Thrombocytopenia Grade 3+	-2.18	0.273	.0243	1.31 (1.03-1.68)	109
Neutropenia Grade 3+	-2.41	0.19	0.155	1.21 (0.934-1.56)	109

An increase in the probability of patients with these adverse events with AUC was observed (Figure 10). AUC significantly affected the probability of patients with grade 2+ mucositis ($p = 0.0013$) and thrombocytopenia grade 3+ ($p = 0.0243$). The odds ratio for these cases also excluded 1 (Table 7). No significant affect of AUC was observed on the probability of patients with grade 3+ neutropenia ($p=0.155$). The exposure-response relationship for adverse events suggests that decreasing the dose would reduce the **probability of experiencing AE's**. The **probability** of patients with grade 2+ mucositis and grade 3+ thrombocytopenia for median AUC values (exposures) obtained after administration of 30 and 20 mg/m² doses are shown in Table 8. The probability of patients experiencing mucositis grade 2+ and thrombocytopenia grade 3+ reduces from 47.9% to 32% and 30.1% to 21.8 respectively upon dose reduction. Since dose reduction is not likely to affect efficacy (Figure 1 and Figure 2), reducing the dose from 30 to 20 mg/m² due to toxicities appears acceptable.

Table 8: Reviewer's Logistic Regression Results for Dose Reduction

Dose (mg/m²)	Median AUC (mg*hr/L)	Probability of patients with Grade 2+ Mucositis (%)	Probability of patients with Grade 3+ Thrombocytopenia (%)
30	4.88	47.9	30.1
20	3.29	32	21.8

4 APPENDIX A: REVIEWER'S POPULATION PK ANALYSIS

Table 9: Reviewer's Base Pralatrexate PK Model Parameter Estimates.

Parameter	Unit	Population parameters for Pralatrexate (S-diastereomer)		Population parameters for Pralatrexate (R-diastereomer)	
		Estimate	%RSE	Estimate	%RSE
<u>Fixed-Effects Parameters</u>					
V1	[L]	10.1	4.43	7.82	4.35
V2	[L]	16.3	4.48	8.32	9.95
V3	[L]	51.4	8.72	13.4	9.25
CL	[L/hr]	22.5	5.16	10.8	5.03
Q2	[L/hr]	5.51	12.9	6.12	4.89
Q3	[L/hr]	1.53	15.2	0.661	10.1
<u>Inter-Individual Variability</u>					
ω_{v1}	[CV%]	-	-	-	-
ω_{v2}	[CV%]	72.9	26.3	57.9	20.7
ω_{v3}	[CV%]	-	-	56	30.6
ω_{CL}	[CV%]	40.1	23.4	35.8	20.8
ω_{Q2}	[CV%]	84.0	24.9	-	-
ω_{Q3}	[CV%]	84.1	35.3	62.0	30.4
<u>Inter-Occasion Variability</u>					
κ_{v1}	[CV%]	65.1	13.9	48.7	14.4
κ_{CL}	[CV%]	28.9	17.0	22.1	15.9
<u>Residual Variability</u>					
Proportional error	[CV%]	28.3	5.47	25.6	5.85
Additive error	[ng/mL]	0.815	9.13	0.694	30.5

Table 10: Reviewer's Final Pralatrexate PK Model Parameter Estimates.

Parameter	Unit	Population parameters for Pralatrexate (S-diastereomer)		Population parameters for Pralatrexate (R-diastereomer)	
		Estimate	%RSE	Estimate	%RSE
<u>Fixed-Effects Parameters</u>					
V1	[L]	10.9	4.54	8.89	4.15
V2	[L]	9.41	7.45	6.79	9.59
V3	[L]	50.5	11.1	12.6	8.49
CL	[L/hr]	34.4	7.7	17.2	7.03
Q2	[L/hr]	6.98	6.33	5.53	5.35
Q3	[L/hr]	1.37	12.0	0.601	9.77
<u>Covariate-relationships for CL</u>					
CRCL slope	[L/hr/ml/min]	0.117	36.6	0.0778	26.6
Reduction in clearance for Study PDX -008	[-]	0.571	8.44	0.682	6.72
Male clearance relative to female	[-]	0.889	8.1	0.871	6.74
<u>Inter-Individual Variability</u>					
ω_{v1}	[CV%]	-	-	-	-
ω_{v2}	[CV%]	44.2	27.3	52.2	18.2
ω_{v3}	[CV%]	59.7	31.9	54.1	29.1
ω_{CL}	[CV%]	28.5	25.8	25.8	28.7
ω_{Q2}	[CV%]	-	-	-	-
ω_{Q3}	[CV%]	70.1	37.2	60.6	31.6
<u>Inter-Occasion Variability</u>					
κ_{v1}	[CV%]	51.2	16.5	47.4	14.2
κ_{CL}	[CV%]	22.5	17.8	23.0	15.3
<u>Residual Variability</u>					
Proportional error	[CV%]	34.4	6.42	25.8	5.85
Additive error	[ng/mL]	0.425	49.0	0.65	30.1

4.3 *Pharmacogenomics Review*

CLINICAL PHARMACOLOGY GENOMICS GROUP REVIEW

NDA Number	22,468
Submission Type	Priority
Applicant Name	Allos
Submission Date	23 Mar 2009
Brand Name	Folotyn
Generic Name	Pralatrexate
Proposed Indication	Peripheral T-cell lymphoma
Genomics Reviewer	Mike Pacanowski, Pharm.D., M.P.H.
Team Leader	Issam Zineh, Pharm.D., M.P.H.

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1. BACKGROUND

Pralatrexate is an antineoplastic folate analog that acts by inhibiting dihydrofolate reductase (DHFR) activity similar to methotrexate. The sponsor reports pralatrexate to have greater affinity for the reduced folate carrier (RFC) and undergo greater polyglutamylation, resulting in more efficient uptake and cellular accumulation than methotrexate (PMID: 19221750). The proposed indication for pralatrexate in the current submission is for the treatment of relapsed/refractory peripheral T-cell lymphoma (PTCL).

PTCL is uncommon and clinically heterogeneous, accounting for **10-15% of non-Hodgkin's lymphomas**. The prognosis is generally poor and varies according to the PTCL subtype; in one study, 5-year survival rates for PTCL (unspecified) were approximately 35%.(Savage, PMID 15367405) Prognosis is assessed based on age, performance status, stage, serum LDH, and number of extranodal sites of involvement according on International Prognostic Index criteria **for non-Hodgkin's lymphoma.(Shipp, PMID 8141877)** No drugs are approved for the treatment of relapsed/refractory PTCL.

The purpose of this review is to evaluate 1) heterogeneity in pralatrexate pharmacokinetics (PK) and/or response, 2) the sponsor's gene expression study, and 3) other potential predictors of pralatrexate response.

2. NDA CONTENT RELATED TO GENOMICS

Approval for this indication is being sought on the basis of a single efficacy and safety study. The clinical pharmacology database is comprised of a subset of patients in the pivotal study, as well as subjects being treated with pralatrexate for other malignancies (e.g., non-small cell lung cancer).

Pralatrexate efficacy and safety in relapsed or refractory PTCL was evaluated in a phase 2, single arm, non-randomized, open-label, multi-center, international study (PDX-008). Pralatrexate was administered concurrently with vitamin B12 and folic acid supplementation. The primary efficacy endpoint was response rate; secondary efficacy endpoints included duration of response, progression-free survival (PFS), and overall survival (OS). Ten subjects were available for dense PK sampling after the first dose. The sponsor submitted a six-gene expression profile for six subjects enrolled in this study. DNA was not collected in PDX-008 according to the protocol.

An ongoing study that included 24 patients with PTCL provides supportive evidence for efficacy in PTCL (PDX-02-078). Pralatrexate is also being studied for the treatment of relapsed/refractory cutaneous T-cell lymphoma (PDX-010), non-small cell lung cancer (multiple studies), transitional cell carcinoma of the urinary bladder (PDX-011, mesothelioma (PDX-01-076), and other advanced malignancies (multiple studies).

3. KEY QUESTIONS AND SUMMARY OF GENOMICS FINDINGS

3.1. Are pralatrexate PK and/or responses heterogeneous?

Yes. Substantial heterogeneity exists in the C_{max}, AUC, and clearance of pralatrexate. Three of 41 subjects with urine data (from studies PDX-008, PDX-007) had very little recovery of pralatrexate in the urine, and all of these subjects experienced severe adverse events. The response rate was 27%; approximately 37% of the exposed subjects had progressive disease (PD) as their best response.

The PK database for pralatrexate includes 44 subjects from two phase 1/2 studies (PDX-007 – non-small cell lung cancer [n=38], PDX-99-083 – advanced solid tumors treated with docetaxel [n=6]) and 10 subjects from PDX-008. Sparse sampling was available for an additional 100 subjects in PDX-008.

In PDX-008, after 20 or 30 mg/m² IV push over 3-5 minutes, CL_{tot}, V_{dss} and terminal half-life (t_{1/2term}) for R-pralatrexate (PDX) showed mean values (coefficient of variation (CV)) of 417 mL/min (62%), 105 L (75%) and 1,078 min (120%), while CL_{tot}, V_{dss} and terminal t_{1/2} for S-PDX showed mean values (CV) of 191 mL/min (38%), 37 L (53%) and 714 min (62%). For AUC_{0-∞}, plasma exposures to R-PDX ranged from 32,702 ng/ml-min to 195,078 ng/ml-min; terminal t_{1/2} ranged from 2.6 to 72 hours. One of 8 subjects with urine data had virtually no R-PDX collected in urine post-dose (Subject 93, CrCl 84 ml/min). Similar findings were observed for S-PDX. This subject missed >5 doses over the study period, and eventually developed dehydration and a catheter site infection, and later oral candidiasis, both of which required hospitalization. AUC_{0-∞} and Cl_{tot} did not appear to be extreme in this subject. A failure of the assay was not ruled out.

Table 1. PK data for subjects with dense PK sampling in Study PDX-008

PDX-10a																																																																													
Patient No	Cycle/ Dose	Dosing Regimen			Plasma PK										Urine PK																																																														
		Racemic Dose [mg/m ²]	Isomeric Dose [mg]	T _{inf} [min]	C _{max} [ng/mL]	t _{max} [min]	AUC _{0-∞} [ng/mL*min]	AUC _{0-24h} [%]	CL _{tot} [mL/min]	V _{dss} [L]	MRT _{sys} [min]	t _{1/2term} [min]	U _{0-24h} [mg]	f _e [%]	CL _{ren} [mL/min]	CL _{nonren} [mL/min]	t _{1/2term} [min]																																																												
(b) (4)																																																																													
<table border="1"> <thead> <tr> <th colspan="2">using actual doses</th> <th colspan="5">using actual times</th> <th colspan="8">using nominal times</th> </tr> <tr> <th>mean</th> <th>COV</th> <th>4</th> <th>2478</th> <th>9</th> <th>93000</th> <th>2.4%</th> <th>417</th> <th>105</th> <th>288</th> <th>1078</th> <th>9.0</th> <th>31%</th> <th>119</th> <th>251</th> <th>718</th> </tr> <tr> <td></td> <td></td> <td>24%</td> <td>68%</td> <td>100%</td> <td>55%</td> <td>87%</td> <td>62%</td> <td>75%</td> <td>85%</td> <td>120%</td> <td>49%</td> <td>47%</td> <td>68%</td> <td>60%</td> <td>34%</td> </tr> <tr> <td>n</td> <td></td> <td>10</td> <td>10</td> <td>10</td> <td>10</td> <td>10</td> <td>10</td> <td>10</td> <td>10</td> <td>10</td> <td>8</td> <td>8</td> <td>8</td> <td>8</td> <td>7</td> </tr> </thead> </table>															using actual doses		using actual times					using nominal times								mean	COV	4	2478	9	93000	2.4%	417	105	288	1078	9.0	31%	119	251	718			24%	68%	100%	55%	87%	62%	75%	85%	120%	49%	47%	68%	60%	34%	n		10	10	10	10	10	10	10	10	10	8	8	8	8	7
using actual doses		using actual times					using nominal times																																																																						
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n		10	10	10	10	10	10	10	10	10	8	8	8	8	7																																																														
(b) (4)																																																																													

Source: Sponsor's Integrated PK Report

Similar variability was observed in PDX-007 (n=38). After 270 mg/m², two subjects (Subjects 21 and 23) out of 33 with urine data who had relatively normal renal function (CrCl of 130 and 75 mL/min) showed urinary recovery of <2% for R-PDX and <3% for S-PDX, despite ordinary urine volumes. Subject 23 developed severe mucositis requiring hospitalization 6 days after the first dose. AUC_{0-∞} and Cl_{tot} did not appear to be extreme in these subjects. A failure of the

assay was not ruled out.

It is noted that probenecid resulted in a dose-dependent increase in pralatrexate exposure and prolonged half-life in a phase 1 study (PDX-01-014).

In PDX-008, the response rate to pralatrexate was 27% (95%CI 19-36%) based on the International Workshop Criteria (IWC). Complete responses (CRs) were apparent in 7 subjects, and 40 subjects had progressive disease (PD). Investigator-reported response rates were higher at 39% (95%CI 29-48%), due to higher reporting of CRs. Response rates provided by the sponsor according to IWC review are shown in the following table. The median PFS was 3.8 months (95%CI 1.8-5.1) and the median OS was 14.5 months (95%CI 10.6-indeterminate). **The sponsor's analysis was confirmed.**

Table 2. Response Analysis per Independent Central Review (IWC)

Evaluable Subjects (n=109)		
Best Response	n (%)	(95% CI)
CR+CRu+PR	29 (27)	(19, 36)
CR/CRu	9 (6)	
PR	20 (18)	
SD	24 (22)	
PD	40 (37)	
Not Evaluated ^a	16 (15)	
Duration of Response	Median	Range
CR+CRu+PR	287 days (9.4 months)	1-503 days

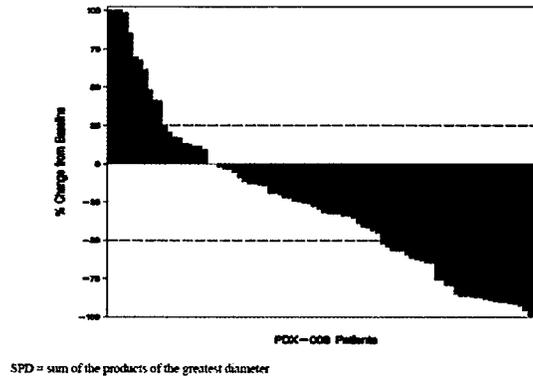
^a14 subjects went off treatment in cycle 1; 2 subjects were unevaluable for response by IWC due to insufficient materials provided to central review.

n = number
 CI = confidence interval
 CR = Complete Response
 CRu = Complete Response/unconfirmed, PR = Partial Response
 SD = Stable Disease
 PD = Progressive Disease

Source: Sponsor's proposed label

Individual tumor responses are illustrated in the following waterfall plot, as provided by the sponsor (analysis not verified by reviewer). **Based on the sponsor's analysis, 66 out of 88 patients (75%) had decrease in SPD from baseline, while 20 patients had an increase (23%).**

Figure 1. Maximum decrease in sum of the products of the greatest diameters (SPD) by subject in PDX-008



Overall, progressive disease was the best response in approximately one-third of the patients. Grade 3 or 4 toxicities of mucositis, fatigue, anemia, neutropenia, and thrombocytopenia were common; AEs resulted in a 23% discontinuation rate.

3.2. What is the impact of pralatrexate on tumor gene expression?

Tumor gene expression was profiled in 6 subjects in Study PDX-008. Due to the small sample size, no robust inferences regarding gene expression as a marker of pralatrexate can be drawn from this study.

Tumor tissue from biopsy was collected for gene expression studies in Study PDX-008. The goal of this investigation was to identify predictors of pralatrexate response. A separate consent was required for this analysis. Tumor samples were to be obtained at screening or when the subject developed PD. The gene expression study focused on components of the folate metabolism pathway as follows: RFC-1, dihydrofolate reductase (DHFR), foylpolylglutamate synthase (FPGS), thymidylate synthase (TS), glycinamide ribonucleotide formyltransferase (GARFT), and gamma-glutamyl hydrolase (GGH). Relative expression was determined by quantitative PCR at a central laboratory using the Δ Ct method with β -actin as the internal control.

Only 6 subjects consented to gene expression studies. Three patients were not included in the analysis. Three samples were obtained from skin and three from lymph nodes. All sample collection dates preceded the study consent date (at most delayed by 7 months) and may not be reflective of the patients' actual pretreatment baseline.

The profile for each gene is shown in the following table.

Table 3. Gene expression profiles in PDX-008

Gene	Tissue	-(Delta Delta CT)	2 ⁻ (-Delta Delta CT)	(95% CI)
DHFR	D. Colon	-0.56	0.68	(0.36, 1.28)
	Lung	1.07	2.10	(1.11, 3.97)
	A. Liver/St. Universal	-2.62	0.16	(0.08, 0.32)
FPGS	D. Colon	0.77	1.70	(1.07, 2.69)
	Lung	1.10	2.15	(1.36, 3.40)
	A. Liver/St. Universal	0.56	1.47	(0.92, 2.34)
GARFT	D. Colon	-1.11	0.46	(0.35, 0.61)
	Lung	-0.62	0.65	(0.50, 0.85)
	A. Liver/St. Universal	-2.49	0.18	(0.14, 0.23)
GGH	D. Colon	-0.71	0.61	(0.41, 0.92)
	Lung	-0.57	0.68	(0.45, 1.02)
	A. Liver/St. Universal	-4.82	0.04	(0.02, 0.08)
RFC1	D. Colon	-1.64	0.32	(0.26, 0.39)
	Lung	-0.56	0.68	(0.55, 0.84)
	A. Liver/St. Universal	-2.46	0.18	(0.12, 0.27)
TS	D. Colon	-1.55	0.34	(0.20, 0.59)
	Lung	1.88	3.67	(2.11, 6.40)
	A. Liver/St. Universal	-0.17	0.89	(0.34, 2.31)

3.3. What biomarkers or clinical factors predict responses to pralatrexate or similar compounds (e.g., methotrexate)?

Pralatrexate is eliminated as unchanged drug in the urine (approximately 1/3). The sponsor should characterize the transporters involved in the elimination of pralatrexate to identify patients that may have reduced elimination and risk for toxicity due to drug interactions or genetic polymorphisms. Many genomic/proteomic factors may modulate the pharmacodynamics and antitumor activity of pralatrexate, although this remains exploratory and biological specimens to discover predictive biomarkers in the sponsor's database are not available.

Predictors of pralatrexate response may have clinical utility based on the high rate of disease progression and toxicity following pralatrexate treatment (the latter of which appears to be concentration dependent; see pharmacometrics review) and the absence of detectable drug in urine in three out of 48 subjects with dense PK data in studies PDX-007 and PDX-008.

Pharmacokinetic biomarkers

Pralatrexate does not undergo significant Phase I metabolism (i.e., CYP1A2, 2A6, 2B6, 2C9, 2C0, 2C19, 2D6, 2E1, 3A4; Study PDX-K-08062) or Phase II metabolism (Study PDX-K-08061), and it is not a high-affinity substrate for Pgp-mediated transport (Study PDX-K-08059). Methotrexate and pralatrexate are actively transported into cells via the reduced folate carrier (RFC1, *SLC19A1*), although the literature is not consistent with regard to a single functional *SLC19A1* polymorphism that affects methotrexate disposition or response. (Davidesen, PMID 18989161) Based on the structural similarity with methotrexate and evidence that methotrexate is transported via OAT1, OAT3, OAT4, and ABCC transporters (Takeda, PMID 12130730), and

that probenecid alters pralatrexate transport in vitro similar to methotrexate (Khokhar, PMID 11595715; Sirotnak, PMID 10999764), transporters other than P-gp may play a significant role in pralatrexate elimination.

Pharmacodynamic biomarkers

Intracellularly, methotrexate undergoes polyglutamylation, which is mediated by FPGS and GGH. DHFR, the drug target, and MTHFR, a critical component of folate metabolism, are also plausible candidates for pralatrexate response. The impact of genetic variations in these genes on methotrexate response/ toxicity has been studied in the setting of rheumatoid arthritis, but the database for human cancers is limited. The aforementioned markers and other potential candidates in the methotrexate response pathway are shown in Figure 2. Specific pharmacogenetic studies of pralatrexate have not been conducted.

Figure 2. Methotrexate response pathway
Copyright Material



Source: from pharmgkb.org

4. COMMENTS

Pralatrexate exposure and response are variable. Three of 41 subjects with urine data demonstrated extreme differences in the urinary excretion of pralatrexate (<3%), highlighting a potential pharmacogenetic issue.

Gene expression profiles were obtained from 6 consenting subjects in PDX-008. These data are insufficient to conduct any meaningful analysis related to pralatrexate response. The sponsor did not specify whether they collected and stored DNA in the protocols.

Pralatrexate does not appear to be metabolized by polymorphic drug metabolizing enzymes and is not a substrate for P-gp; it is not known whether pralatrexate is a substrate for other transporters. No other adequately validated markers of antifolate response have been described to date.

8/31/2009
NDA 22,468

5. RECOMMENDATION(S)

As a post-marketing commitment, the sponsor should characterize pralatrexate transporters in vitro to assess the potential for drug-drug or pharmacogenetic interactions.

Michael A. Pacanowski, Pharm.D., M.P.H.
Primary Reviewer, Genomics Group
Office of Clinical Pharmacology

Date

Issam Zineh, Pharm.D., M.P.H.
Associate Director for Genomics, Genomics Group
Office of Clinical Pharmacology

Date

4.4 *Cover Sheet and OCPB Filing/Review Form*

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission				
	Information		Information	
NDA Number	22-468	Brand Name	Folotyn	
DCP Division (I, II, III, IV, V)	V	Generic Name	pralatrexate	
Medical Division	DDOP	Drug Class	folate analog; dihydrofolate reductase (DHFR) inhibitor	
OCP Reviewer	Gene Williams	Indication(s)	for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL)	
OCP Team Leader	Brian Booth	Dosage Form	solution in a preservative free single use vial for parenteral administration	
		Dosing Regimen	30 mg/m ² once weekly over (b) min for 6 weeks on a 7 week cycle	
Date of Submission	March 23, 2009	Route of Administration	intravenous	
Estimated Due Date of OCP Review	July 30, 2009	Sponsor	Allos	
PDUFA Due Date	September 23, 2009	Priority Classification	1P	
Division Due Date	August 15, 2009			
Clinical Pharmacology Information				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments if any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:	X	1		
Blood/plasma ratio:	X	1		
Plasma protein binding:	X	3		
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:	X	1		
multiple dose:	X	4		
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	1		

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
geriatrics:				
renal impairment:				
hepatic impairment:				
pediatrics:				
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:	X	1		
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
QTC studies:	X	1		
In-Vitro Release BE				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		14		6 categories of in vitro (protein binding + stability/substrate + inhibitor + inducer + pGP + RBC partition); 8 in vivo (6 studies + 1 QT analysis + 1 popPK analysis)

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			x	

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

2	Has the applicant provided metabolism and drug-drug interaction information?	x			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	x			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	x			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?		x		some analysis for undesired effects
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?		x		
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x			
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?		x		

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE? yes

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Gene Williams

Reviewing Clinical Pharmacologist Date

Brian Booth

Team Leader/Supervisor Date

CC:
HFD-150 (CSO – M Vialpando; MTL – V Maher; MO – Q Ryan)
HFD-860 (Reviewer – G Williams; ATL/DDD - B Booth; DD - A Rahman)

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22468	ORIG-1	ALLOS THERAPEUTICS INC	FOLOTYN

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Interdisciplinary Review Team for QT Studies Consultation:

QT Study Review

NDA	22-468
Brand Name	Folotyn
Generic Name	Pralatrexate
Sponsor	Allos Therapeutics
Indication	Treatment of Patients with relapsed or refractory peripheral T-cell lymphoma (PTCL)
Dosage Form	Intravenous (IV) push
Drug Class	Cancer chemotherapy-folate analog
Therapeutic Dosing Regimen	The recommended dose is 30 mg/m ² administered as an intravenous (IV) push once weekly for 6 weeks in 7-week cycles until progressive disease or unacceptable toxicity.
Duration of Therapeutic Use	Chronic
Maximum Tolerated Dose	The MTD, as defined by the original protocol DLT criteria for the Phase 1 dose escalation clinical study of pralatrexate (PDX-007) administered IV over 3-5 minutes every 2 weeks with vitamin supplementation, is 270 mg/m ² . However, dose reductions to 230 and 190 mg/m ² administered IV over 3-5 minutes or over 1 hour have been explored to evaluate for enhanced tolerability.
Submission Number and Date	N000, 23 March 2009
Clinical Division	DDOP/HFD 150

1 SUMMARY

1.1 OVERALL SUMMARY OF FINDINGS

This was a Phase 1, non-randomized, open-label, two-center study designed to determine the maximal tolerated dose (MTD) of pralatrexate. Triplicate ECGs at pre-specified time points were collected in 14 patients who received pralatrexate at doses of 190 or 230 mg/m² administered every 2 weeks over 3-5 minutes or over 1 hour in three treatment cohorts. When data from all cohorts were combined, the upper bound of the two-sided 90% CI for QTcF change from Pre-injection was <10 ms. No patient exhibited a QTcF interval >500 msec. No major changes in HR, PR interval, or QRS interval duration were noted.

1.2 QT-IRT COMMENTS

- Because the doses studied in this trial are at-least 6-fold greater than the proposed therapeutic dose for PTCL (30 mg/m²), 14 subjects (pooled dose analysis) are adequate to rule out large direct effects (>20 ms) on the QT interval.

- [REDACTED] (b) (4)

2 PROPOSED LABEL

The sponsor has not included any labeling statements describing QT effects of pralatrexate.

3 BACKGROUND

Pralatrexate is an antineoplastic folate analog under clinical development for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL).

3.1 MARKET APPROVAL STATUS

Pralatrexate is not approved for marketing in any country.

3.2 PRECLINICAL INFORMATION

Source: Pharmacology Written Summary: eCTD 2.6.2

“The hERG channel was stably expressed in a sub-clone (CHO-K1/hERG) of the CHO-K1 cell line. Experiments were performed on CHO-K1/hERG cells because hERG-transfected cells allow for studying direct interactions between test substances and human K⁺ currents implicated in cardiac re-polarization. The control substance E-4031 was tested on 3 individual cells at a concentration of 3 μM, a concentration known to inhibit hERG K⁺ currents. The effect was measured on the maximum amplitude of the tail currents. This parameter was determined from current traces obtained from voltage-clamped CHO-K1/hERG cells, using patch-clamp techniques in the whole-cell configuration.

“The results of the study show that at the concentrations of 0.8, 2, and 4 mg/mL, pralatrexate inhibits hERG K⁺ currents by 37, 54, and 54%, respectively. At 0.4 mg/mL, pralatrexate had no effect on hERG K⁺ currents. Although hERG K⁺ current inhibition was observed with the high concentrations of pralatrexate in this in vitro test, these concentrations are far in excess of those observed clinically. In the Phase 2 clinical study PDX-008, the highest observed C_{max} of the combined pralatrexate diastereomers was ~22 μg/mL

“Purkinje fibers were isolated from hearts of anesthetized dogs, placed in a tissue bath perfused with gassed (95% oxygen [O₂]-5% carbon dioxide [CO₂]) Tyrode’s solution (4.9-5.0 mL/min) at 35-38°C, and electrically paced at 1.0 Hertz (Hz) using bipolar silver electrodes. Impalements were made with 3 M of potassium chloride (KCl)-filled glass microelectrodes to monitor membrane potential. After

1 hour of equilibration, fibers were exposed to nominal concentrations of 0.4, 0.8, or 2.0 mg/ml pralatrexate for at least 25 minutes, followed by dl-sotalol (50 μ M) as a positive control reference agent. Electrophysiological effects of treatments on the fibers were recorded at pacing frequencies of 1.0 and 0.5 Hz. Action Potential parameters measured included: resting membrane potential (RMP), overshoot (OS), action potential amplitude (APA), maximum rate of depolarization of the action potential upstroke (V_{max}), and action potential duration at 30, 60, and 90% (APD30, APD60, APD90) repolarization.

“Pralatrexate, at target concentrations of 0.4, 0.8, and 2.0 mg/mL (0.34, 0.67, and 1.66 mg/mL, according to the concentration analysis results) did not induce any statistically significant or biologically relevant (> 15%) changes in RMP, OS, APA, V_{max} , APD30, APD60, or APD90 at stimulation frequencies of 1.0 Hz or 0.5 Hz. The positive control agent, dl-sotalol, prolonged APD60 and APD90, indicating the responsiveness of the preparation to APD-prolonging agents.

“The results of the study indicate that pralatrexate, at 0.34, 0.67, and 1.66 mg/mL per the concentration analysis results, does not have any statistically significant or biologically relevant effects on canine Purkinje fiber action potential parameters.

“Pralatrexate was given to male and female Beagle dogs by slow bolus IV injection at doses of 0.1, 0.3 and 0.7 mg/kg for 2 or 6 cycles (1 cycle consists of 6 weekly doses followed by 1 drug-free week). A 9-lead electrocardiogram (ECG) was recorded in all animals at predose, at interim study days 81-83, at end of study (study day 282), and after a 4-week recovery period (study day 309). Measurements and evaluations included electrocardiographic durations, amplitudes, intervals and the long ECG rhythm strip. At interim study days 81-83 one female dosed at 0.3 mg/kg had a slightly prolonged PR interval of 0.17 seconds (normal range is 0.06-0.14 seconds). This slight PR prolongation has no biological significance. In all other animals the electrocardiographic parameters were within normal limits. At the end of study (study day 282) and after the 4-week recovery period (study day 309) all electrocardiographic parameters were found to be within normal limits. There were no biologically significant pralatrexate-related prolongations of the QTc interval in any animal. In conclusion, in this study, pralatrexate caused no biologically significant electrocardiographic effects.”

3.3 PREVIOUS CLINICAL EXPERIENCE

Source: Summary of Clinical safety eCTD 2.7.4

“Clinical experience with pralatrexate has involved the administration of this chemotherapeutic agent to nearly 400 cancer patients over the past 11 years. There were 111 patients in the PDX-008 registrational study in patients with relapsed or refractory PTCL who received at least 1 dose of pralatrexate of 30 mg/m² weekly for 6 of 7 weeks; 71 patients were treated with pralatrexate in single-agent supportive studies in lymphoproliferative malignancies (PDX-02-078, PDX-010) at various doses and with various dose regimens; 42 patients were

treated with pralatrexate in single-agent supportive studies in non-small cell lung cancer (NSCLC) (PDX-007, PDX-012) at various doses and with various dose regimens; 17 patients were treated with pralatrexate in combination with another cytotoxic chemotherapeutic agent (gemcitabine) in a study of lymphoproliferative malignancies (PDX-009); and 153 patients were treated with various doses and dose regimens of pralatrexate in a series of contributive studies in solid tumors as single-agent or combination therapies (PDX-97-006, PDX-99-053, PDX-99-083, PDX-01-014 and PDX-01-076).

“In PDX-008, 7 of 8 deaths (88%) were due to progressive disease (PD). Patient 048, died of cardiopulmonary arrest considered possibly related to pralatrexate. Approximately 2 weeks before his death, the patient was hospitalized for SAE of Grade 3 mucosal inflammation and febrile neutropenia (both considered related to pralatrexate treatment), which never resolved before his death. He died approximately 3 weeks after his last dose of pralatrexate (he was on study for 96 days). The investigator could not rule out a causal relationship to pralatrexate and assessed the event as possibly related to pralatrexate. Similarly, in the other patient populations, the largest proportion of patients died due to PD (5 of 12, 42%).

“Screening ECGs were performed as a clinical safety measure prior to dosing with pralatrexate in several studies (including lymphoma studies PDX-008 and PDX-009). However, no ECGs were performed post-dose in these studies, unless clinically indicated. Therefore, there is no comparison of QT/QTc effects of pralatrexate available for these trials.”

Reviewer’s Comments: There are no reports of sudden cardiac death, seizures or significant ventricular arrhythmias but clinical experience is limited.

3.4 CLINICAL PHARMACOLOGY

Appendix 6.1 summarizes the key features of pralatrexate’s clinical pharmacology.

4 SPONSOR’S SUBMISSION

4.1 OVERVIEW

The QT-IRT reviewed the protocol under IND 52604 and provided comments on June 17, 2008 and November 26, 2008. The sponsor submitted the cardiac safety report for PDX-007 including electronic datasets and waveforms to the ECG warehouse. Cardiac safety report for the sub-group of evaluable patients with triplicate ECGs and PK sampling has been submitted.

4.2 QT STUDY

4.2.1 Title

“A Phase 1 Open-label Study of (RS)-10-Propargyl-10-Deazaaminopterin [PDX] with Vitamin B₁₂ and Folic Acid Supplementation in Patients with Previously-treated Advanced Non-small Cell Lung Cancer (NSCLC).”

4.2.2 Protocol Number

PDX 007

4.2.3 Study Dates

Initiated Jan 13, 2005 and still ongoing,

4.2.4 Study Description

4.2.4.1 Design

PDX-007 was a Phase 1, non-randomized, open-label, two-center study designed to determine the maximal tolerated dose (MTD) of pralatrexate when administered concurrently with Vitamin B₁₂ and folic acid supplementation to patients with previously-treated Stage IIIB (pleural or pericardial disease) or Stage-IV NSCLC.

Patients received 1 cycle of pralatrexate with vitamin supplementation for dose determination. One cycle (4 weeks) consisted of 2 doses of pralatrexate given every other week with safety follow-up at the end of treatment. Dose delays of up to 2 weeks were allowed in Cycle 1 for evaluation of adverse events (AEs). Subsequent cycles were administered at the discretion of the Investigator.

Pralatrexate administration was allowed until a patient experienced any of the following:

- Development of progressive disease
- Development of clinically significant treatment-related AEs
- Development of toxicities resulting in more than 1 dose reduction of pralatrexate
- Initiation of subsequent systemic therapy for NSCLC
- Development of an intercurrent illness, condition, or procedural complication that may have interfered with the patient’s participation
- Patient withdrawal of consent
- Investigator decision
- Sponsor decision

4.2.5 Treatment Regimen

4.2.5.1 Treatment Arms

Four study cohorts were included to assess the impact of pralatrexate on cardiac repolarization. The cohorts were based on the dose and the rate of drug administration.

1. 230 mg/m² over 3-5 minutes (n=5)
2. 230 mg/m² over one hour (n=5)

3. 190 mg/m² over 3-5 minutes (n=2)
4. 190 mg/m² over one hour (n=5)

As a measure to mitigate the development of adverse effects of pralatrexate (e.g., mucositis), patients received vitamin supplementation starting at least 7 days prior to Cycle 1 Dose 1 of pralatrexate administration (study day -7) and continued throughout the study until discontinuation of pralatrexate. Vitamin supplementation consisted of 1 mg Vitamin B12 injected intramuscular every 8-10 weeks and 1 mg folic acid by mouth once daily.

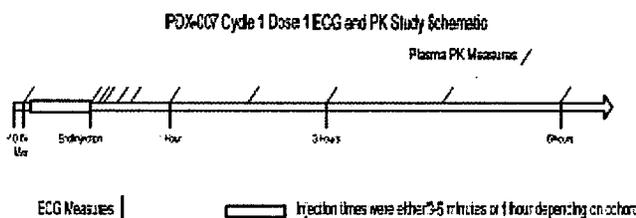
The 190 mg/m² IV push cohort did not have digital ECG data acquired and these patients were therefore not included in the electrocardiographic analysis. Patients received a significantly higher dose of pralatrexate than in the peripheral T-cell lymphoma indication (150 to 325 mg/m² vs. 30 mg/m²). As this drug is intended for use in a terminal oncology population, provision of placebo is typically considered unethical.

Reviewer's Comment: The sponsor used doses (190-230 mg/m²) that were higher than the therapeutic dose of 30 mg/m² administered as an intravenous (IV)-push for the peripheral T-cell lymphoma which is acceptable. However, there were only 5 subjects with a higher dose of 230 mg/m² with the same route of administration (IV push) as the therapeutic dose. Thus, the data in this study is very limited.

4.2.5.2 ECG and PK Assessments

A 12-lead ECG was performed at screening, 2 triplicate ECGs at baseline (just prior to pralatrexate injection), and then triplicate ECGs were obtained at the end of infusion and 1, 3, and 6 hours post-infusion in conjunction with pralatrexate plasma PK collections. In addition, single 12-lead ECGs were obtained pre-injection and within 30 minutes post-injection for the first dose of each odd-numbered cycle thereafter.

The sponsor's schematic below summarizes the ECG and PK acquisition times for the Cycle 1 Dose 1 treatment. In addition, serum plasma potassium concentrations were evaluated at pre-injection for consideration as potential covariates of QT interval changes.



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Plasma PK data were collected 5 minutes prior to injection, at end-injection, and then 5, 10, 20, and 30 minutes after injection, and at 1, 2, 3, 4.5 and 6 hours after injection. When ECG collection times corresponded with PK collection times, sites were instructed to record the ECG immediately prior to the blood draw in order to avoid changes in heart rate that could impact the QTc intervals post-phlebotomy.

Reviewer's Comment: ECG measurements were not collected frequently to monitor the effects of pralatrexate over a 24-hour interval. Since the PK samples were collected only for 6 hours post-dose, the study design was inadequate to capture any delayed effects of pralatrexate.

4.2.5.3 Baseline

Pre-dose baseline was used.

4.2.6 ECG Collection

ECGs were acquired in the resting supine position using General Electric (GE) MAC 5000 and 5500 machines with a sampling rate of 500 Hz at 2 study sites (Memorial Sloan Kettering and (b) (4)). All ECGs were transferred to the central ECG Core Lab. For each planned ECG acquisition, 3 10-second ECG strips were taken 1 minute apart, and 3 complexes were subsequently measured per strip.

A single cardiologist blinded to patient identifier, pralatrexate dose, and time, performed QT-interval measurements and interpretations. Generally, QT intervals were measured consistently using the same lead within the set of ECGs for each individual patient. Semi-automated calipers with manual over-read was used.

For this study, all ECGs for the 230-mg/m² cohorts were read at the ECG Core Lab on the same day by the same cardiologist, thus limiting temporal reader drift within these 2 cohorts. Similarly, ECGs for the 190-mg/m² cohort were read in a single batch except for 2 patients who had paper ECGs and were excluded from the analysis data set.

4.2.7 Sponsor's Results

4.2.7.1 Study Subjects

Seventeen adult patients with NSCLC were evaluated. Two patients were excluded from ECG analyses as they had only non-digital (paper) ECGs, and one patient with digital ECGs was excluded for ventricular paced rhythm, leaving 14 patients available for ECG and PK analyses among 3 cohorts.

4.2.7.2 Sponsor's Statistical Analyses

4.2.7.2.1 Primary Analysis

The sponsor's tables (Table 1, Table 2, Table 3) below represent the aforementioned change from pre-injection QTcF intervals by time, cohort, dose, and overall. A slight tendency for the QTcF value to rise immediately following injection and to fall at later time points post-injection was evident for all 3 evaluable cohorts. However, the fall to pre-injection values occurred more quickly in the 190-mg/m² cohort than in the 230-mg/m² cohorts. Also, a tendency for a slightly higher mean change from pre-injection was seen in the 3-5 minute injection regimen (6.1 ms) compared to the 1-hour regimen (1.8 ms) within the 230-mg/m² cohorts. Cohorts and times were not compared statistically. These temporal changes in QTcF for individual cohorts are visualized in Figure 1.

When data for all cohorts were combined the 2-sided 90% CIs for the mean change from Pre-injection QTcF intervals at End-injection, and at 1, 3, and 6 hours post-injection were (4.9 [2.0, 7.8], 4.2 [0.9, 7.5], 1.5 [-1.3, 4.4], and 0.8 [-1.6, 3.2] respectively).

Table 1: Change from Pre-injection QTcF Interval by time 230 mg/m² cohorts

Time	Statistic	PDX 230 mg/m ² over 3-5 min	PDX 230 mg/m ² over 1 hour	Combined 230 mg/m ² Dose
End-injection				
	n	5	5	10
	Mean (Std)	6.1 (7.20)	1.9 (7.02)	4.0 (6.97)
	90% Confidence Interval	(-3.6, 12.7)	(-4.3, 8.5)	(-3.1, 9.0)
	Median	4.2	1.4	2.4
	Range (Min, Max)	(-2.9, 15.3)	(-7.5, 12.0)	(-7.6, 15.3)
1 Hr Post-injection				
	n	5	5	10
	Mean (Std)	7.8 (5.84)	3.3 (5.97)	5.9 (7.15)
	90% Confidence Interval	(3.0, 12.6)	(-4.5, 12.5)	(1.7, 13.0)
	Median	9.1	1.4	6.4
	Range (Min, Max)	(0.2, 13.4)	(-4.4, 18.0)	(-4.4, 18.2)
3 Hrs Post-injection				
	n	5	5	10
	Mean (Std)	3.8 (5.44)	2.7 (5.91)	3.3 (5.96)
	90% Confidence Interval	(-2.3, 10.0)	(-2.9, 8.4)	(-2.1, 5.7)
	Median	6.0	1.7	2.3
	Range (Min, Max)	(-5.5, 11.5)	(-2.5, 12.6)	(-5.5, 12.5)
Date: 20JAN2009 Source: S05_eqg_rev7.sas Dataset: chg_tct.sas700af Page 1 of 2				
Time	Statistic	PDX 230 mg/m ² over 3-5 min	PDX 230 mg/m ² over 1 hour	Combined 230 mg/m ² Dose
6 Hrs Post-injection				
	n	4	4	8
	Mean (Std)	1.9 (5.24)	2.3 (5.38)	2.1 (4.93)
	90% Confidence Interval	(-4.3, 8.1)	(-4.0, 6.6)	(-1.2, 5.4)
	Median	3.7	4.3	3.7
	Range (Min, Max)	(-5.8, 5.9)	(-5.3, 6.5)	(-5.8, 6.6)
Date: 20JAN2009 Source: S05_eqg_rev7.sas Dataset: chg_tct.sas700af Page 2 of 2				

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Table 2: Change from Pre-injection QTcF Interval by time 190 mg/m² cohort

Time	Statistic	PDX 190 mg/m ² over 1 hour
End-injection	n	4
	Mean (Std)	7.4 (2.34)
	90% Confidence Interval	(4.6, 10.1)
	Median	8.1
	Range (Min, Max)	(4.1, 9.2)
1 Hr Post-injection	n	4
	Mean (Std)	0.3 (5.00)
	90% Confidence Interval	(-5.9, 5.9)
	Median	-1.3
	Range (Min, Max)	(-3.9, 6.6)
3 hrs Post-injection	n	4
	Mean (Std)	-2.6 (3.83)
	90% Confidence Interval	(-7.3, 1.7)
	Median	-4.4
	Range (Min, Max)	(-6.2, 2.9)
Date: 20JAN2009 Source: S05_ecg_rev7.sas Dataset: crq_txt.sas7bdat Page 1 of 2		

Time	Statistic	PDX 190 mg/m ² over 1 hour
6 Hrs Post-injection	n	4
	Mean (Std)	-1.6 (3.15)
	90% Confidence Interval	(-5.5, 2.3)
	Median	-2.4
	Range (Min, Max)	(-6.4, 0.2)
Date: 20JAN2009 Source: S05_ecg_rev7.sas Dataset: crq_txt.sas7bdat Page 2 of 2		

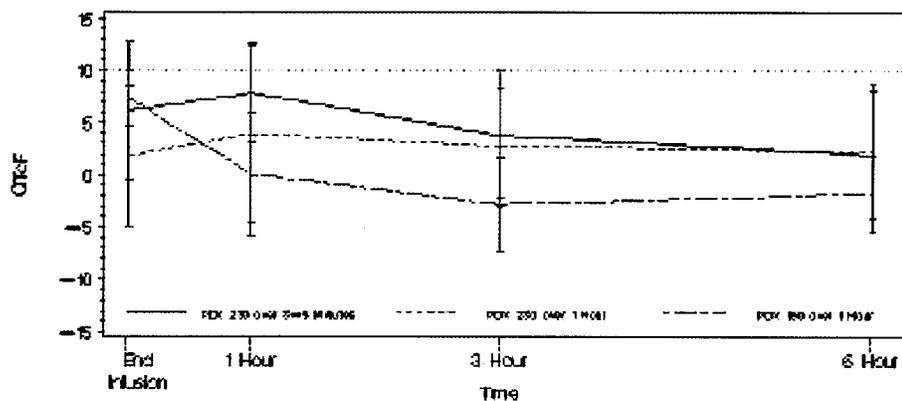
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Table 3: Change from Pre-injection QTcF Interval by Time, Dose and Overall

Time	Statistic	Combined 230 mg/m ² Dose	150 mg/m ² Dose	Overall
End-injection				
	n	10	4	14
	Mean (Std)	4.0 (5.97)	7.4 (2.34)	4.9 (6.12)
	90% Confidence Interval	(-0.1, 8.0)	(4.6, 10.1)	(2.0, 7.8)
	Median	2.4	8.1	4.1
	Range (Min, Max)	(-7.6, 15.9)	(4.1, 9.2)	(-7.6, 15.9)
1 Hr Post-injection				
	n	10	4	14
	Mean (Std)	5.9 (7.15)	0.0 (5.00)	4.2 (6.98)
	90% Confidence Interval	(1.7, 10.0)	(-5.9, 5.9)	(0.9, 7.5)
	Median	6.4	-1.3	3.5
	Range (Min, Max)	(-4.4, 18.3)	(-3.9, 6.6)	(-4.4, 18.3)
3 Hrs Post-injection				
	n	10	4	14
	Mean (Std)	3.3 (5.36)	-2.6 (3.82)	1.3 (5.33)
	90% Confidence Interval	(-0.1, 6.7)	(-7.3, 1.7)	(-1.3, 4.4)
	Median	2.3	-4.4	1.3
	Range (Min, Max)	(-5.5, 12.6)	(-5.2, 2.9)	(-5.5, 12.6)
Date: 20JAN2009 Source: S05_ecg_rev7.sas Dataset: chg_qtcf.sas700at Page 1 of 2				
Time	Statistic	Combined 230 mg/m ² Dose	150 mg/m ² Dose	Overall
6 Hrs Post-injection				
	n	8	4	12
	Mean (Std)	2.1 (4.93)	-1.6 (3.15)	2.8 (4.56)
	90% Confidence Interval	(-1.2, 5.4)	(-5.5, 2.3)	(-1.6, 3.2)
	Median	3.7	-3.4	1.3
	Range (Min, Max)	(-5.8, 6.6)	(-6.4, 0.2)	(-5.4, 6.6)
Date: 20JAN2009 Source: S05_ecg_rev7.sas Dataset: chg_qtcf.sas700at Page 2 of 2				

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Figure 1: Mean and 90% Confidence Intervals of Change from Pre-injection QTcF (ms) by Treatment Group



Program: S12_interaction_plots.sas
 Date: 29JAN2009
 Datasets: mean_pt1.sas7dat

4.2.7.2.2 Categorical Analysis

As shown below, no subject experienced an absolute QTcF over 500 ms or a change in QTcF over 60 ms.

Table 4: Categorical Summary of Maximum Post-injection QTC F, QTC B and QT Intervals (ms) by Dose and Overall

	Category	Combined 250 mg/m ² Dose n=10 n (%)	100 mg/m ² Dose n=4 n (%)	Overall n=14 n (%)
Maximum QTcF Interval	450 or less	10 (100%)	3 (75%)	13 (93%)
	451 to 480	0 (0%)	1 (25%)	1 (7%)
	481 to 510	0 (0%)	0 (0%)	0 (0%)
	greater than 510	0 (0%)	0 (0%)	0 (0%)
Maximum QTcB Interval	450 or less	10 (100%)	3 (75%)	13 (93%)
	451 to 480	0 (0%)	1 (25%)	1 (7%)
	481 to 510	0 (0%)	0 (0%)	0 (0%)
	greater than 510	0 (0%)	0 (0%)	0 (0%)
Maximum QT Interval	450 or less	10 (100%)	4 (100%)	14 (100%)
	451 to 480	0 (0%)	0 (0%)	0 (0%)
	481 to 510	0 (0%)	0 (0%)	0 (0%)
	greater than 510	0 (0%)	0 (0%)	0 (0%)

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4.2.7.2.3 Additional Analyses

Minimal change in heart rate from pre-injection was observed in each of the cohorts. The overall mean change in heart rate from pre-injection was -5.4 bpm at end-injection, -4.1 bpm at 1 hour post-injection, -3.2 bpm at 3 hours post-injection, and -0.3 bpm at 6 hours post-injection.

A negligible change in PR interval from pre-injection was observed in each of the cohorts. The overall mean change in PR interval from pre-injection was 3.6 ms at end-

injection, 4.8 ms at 1 hour post-injection, 3.4 ms at 3 hours post-injection, and 2.3 ms at 6 hours post-injection.

Minimal change in QRS interval from pre-injection was observed in each of the cohorts. The overall mean change in QRS interval from pre-injection was 1.9 ms at end-injection, 0.9 ms at 1 hour post-injection, 1.9 ms at 3 hours post-injection, and -0.3 ms at 6 hours post-injection.

Table 5: Change from Pre-injection Heart Rate (beats per minute) by Time, Dose and Overall

Time	Statistic	Combined 250 mg/m ² Dose	100 mg/m ² Dose	Overall
End-Injection	n	10	5	15
	Mean (Std)	-7.3 (6.14)	-2.3 (6.44)	-4.4 (6.44)
	90% Confidence Interval	(-10.5, -3.4)	(-9.4, 2.9)	(-8.3, -2.5)
	Median	-6.3	-0.2	-5.8
	Range (Min, Max)	(-17.7, 1.0)	(-12.7, 2.3)	(-17.7, 2.3)
1 Hr Post-Injection	n	10	5	15
	Mean (Std)	-6.3 (5.14)	-3.1 (3.52)	-4.1 (5.37)
	90% Confidence Interval	(-9.3, -3.3)	(-9.0, 2.7)	(-6.5, -1.7)
	Median	-6.1	0.8	-3.7
	Range (Min, Max)	(-13.5, 2.7)	(-3.7, 2.8)	(-13.5, 2.3)
3 Hrs Post-Injection	n	10	5	15
	Mean (Std)	-6.3 (4.97)	2.3 (4.74)	-3.2 (6.17)
	90% Confidence Interval	(-8.9, -3.2)	(-2.2, 6.9)	(-6.0, -0.4)
	Median	-6.0	4.0	-4.9
	Range (Min, Max)	(-11.5, 3.7)	(-6.0, 5.9)	(-11.5, 5.3)

Date: 26JAN2009
Source: S08_eqd_rev7.sas
Dataset: ch2_int.sas7cdat
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Table 6: Change from Pre-injection PR Interval (ms) by Time, Dose and Overall

Time	Statistic	Combined 250 mg/m ² Dose	100 mg/m ² Dose	Overall
End-Injection	n	10	4	14
	Mean (Std)	5.5 (7.35)	-1.3 (9.07)	3.6 (7.53)
	90% Confidence Interval	(1.5, 9.3)	(-10.9, 8.2)	(-2.2, 7.3)
	Median	6.0	3.0	4.3
	Range (Min, Max)	(-5.7, 17.3)	(-12.0, 6.7)	(-12.0, 17.3)
1 Hr Post-Injection	n	10	4	14
	Mean (Std)	6.5 (7.89)	3.7 (4.93)	4.8 (7.49)
	90% Confidence Interval	(1.9, 11.2)	(-2.1, 5.9)	(1.3, 8.4)
	Median	7.3	1.3	5.3
	Range (Min, Max)	(-9.0, 17.3)	(-8.2, 9.3)	(-9.0, 17.3)
3 Hrs Post-Injection	n	10	4	14
	Mean (Std)	5.9 (8.33)	-2.9 (4.69)	3.4 (8.23)
	90% Confidence Interval	(1.3, 10.5)	(-8.4, 2.7)	(-2.5, 7.3)
	Median	7.7	-4.3	4.0
	Range (Min, Max)	(-9.0, 19.3)	(-8.7, 4.0)	(-9.0, 19.3)

Date: 26JAN2009
Source: S08_eqd_rev7.sas
Dataset: mean_int.sas7cdat
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Time	Statistic	Combined 250 mg/m ² Dose	100 mg/m ² Dose	Overall
6 Hrs Post-injection	n	9	4	12
	Mean (Std)	5.7 (4.57)	-4.3 (5.70)	2.3 (6.95)
	95% Confidence Interval	(2.6, 8.9)	(-11.0, 2.4)	(-1.2, 9.9)
	Median	6.0	-4.7	3.0
	Range (Min, Max)	(-1.3, 12.0)	(-10.7, 2.7)	(-10.7, 12.0)
Date: 26.JAN.2009 Source: S08_ecg_rev7.sas Dataset: mean_txt.sas7cdat Page 2 of 2				

Table 7: Change from Pre-injection QRS Interval (ms) by Time, Dose and Overall

Time	Statistic	Combined 250 mg/m ² Dose	100 mg/m ² Dose	Overall
Pre-injection	n	10	4	14
	Mean (Std)	2.0 (3.55)	1.8 (2.53)	1.9 (3.32)
	95% Confidence Interval	(-0.3, 4.3)	(-1.5, 4.9)	(0.3, 3.6)
	Median	3.5	2.3	3.3
	Range (Min, Max)	(-7.3, 9.3)	(-1.3, 4.0)	(-7.3, 9.3)
1 Hr Post-injection	n	10	4	14
	Mean (Std)	0.8 (3.71)	1.2 (4.53)	0.9 (3.81)
	95% Confidence Interval	(-1.4, 2.9)	(-4.3, 6.5)	(-0.9, 2.7)
	Median	1.5	2.3	1.5
	Range (Min, Max)	(-6.0, 6.7)	(-9.3, 5.3)	(-6.0, 5.7)
3 Hrs Post-injection	n	10	4	14
	Mean (Std)	2.5 (4.05)	0.6 (1.94)	1.9 (3.61)
	95% Confidence Interval	(0.2, 4.9)	(-1.7, 2.7)	(0.2, 3.7)
	Median	2.3	0.3	1.5
	Range (Min, Max)	(-2.7, 10.7)	(-1.3, 2.7)	(-2.7, 10.7)
Date: 26.JAN.2009 Source: S08_ecg_rev7.sas Dataset: mean_txt.sas7cdat Page 1 of 2				

Time	Statistic	Combined 250 mg/m ² Dose	100 mg/m ² Dose	Overall
5 Hrs Post-injection	n	9	4	12
	Mean (Std)	-0.9 (2.99)	0.8 (4.53)	-0.3 (3.47)
	95% Confidence Interval	(-2.5, 1.1)	(-4.5, 6.2)	(-2.1, 1.5)
	Median	-2.1	1.7	-1.7
	Range (Min, Max)	(-4.0, 4.7)	(-9.3, 5.3)	(-9.3, 5.3)
Date: 26.JAN.2009 Source: S08_ecg_rev7.sas Dataset: mean_txt.sas7cdat Page 2 of 2				

4.2.7.3 Safety Analysis

Subject 043 (52 yr old female) was hospitalized 56 days after initiating treatment with pralatrexate. She received the last dose of study drug (115mg/m²) 13 days prior to death which was assessed as due to disease progression and unrelated to pralatrexate. No other information is available in the Medwatch report.

Subjects 031, 033, 038 and 039 discontinued due to the AEs of mucositis, DVT and periorbital edema. This was coded as an SAE for subjects 031 and 033.

Subject 039 experienced a syncopal episode in cycle 3 which resulted in study drug being held, last available ECG per the CRF was 2 weeks earlier and was normal with QT < 400 ms. He discontinued from the study due to disease progression 2 weeks later.

The abnormal ECG tracings included one patient (045) with electronic ventricular pacemaker, one (046) with chronic right bundle branch block, and a third (029) with a non-specific intraventricular conduction delay.

Reviewer's Comments: These tracings were reviewed in the ECG warehouse.

From Pre-injection to Post-injection, there were no patients who developed pathological U waves or T wave changes suggestive of abnormal cardiac repolarization. No new conduction delays or atrioventricular (AV) blocks were noted

4.2.7.4 Clinical Pharmacology

4.2.7.4.1 Pharmacokinetic Analysis

The mean plasma concentration-time profiles for PDX-10a (S-diastereomer) for 230 mg/m² administered as an IV push over 3-5 minutes, 230 mg/m² administered as an IV infusion over 1 hour and 190 mg/m² administered as an IV infusion over 1 hour are illustrated in Figure 2, Figure 3 and Figure 4. Similar concentration-time profiles for PDX-10b (R-diastereomer) are provided in Figure 5, Figure 6 and Figure 7.

Figure 2: Sponsor's Mean Plasma PDX-10a (S-diastereomer) concentration-time profiles for 230 mg/m² over 3-5 minutes

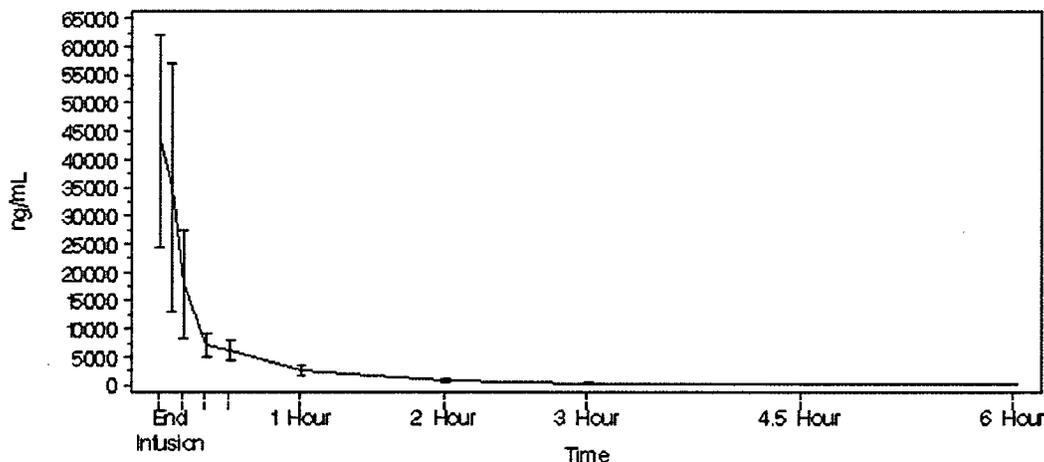


Figure 3: Sponsor's Mean Plasma PDX-10a (S-diastereomer) concentration-time profiles for 230 mg/m² over 1 hour

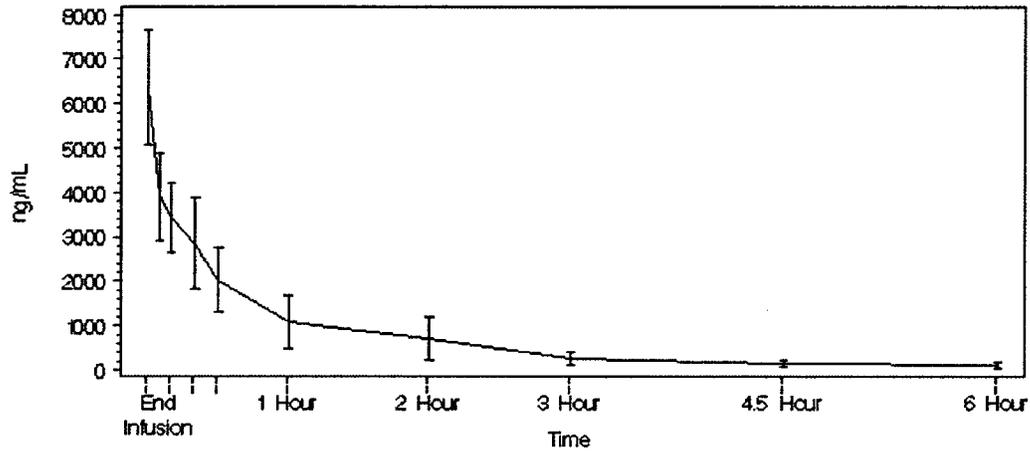


Figure 4: Sponsor's Mean Plasma PDX-10a (S-diastereomer) concentration-time profiles for 190 mg/m² over 1 hour

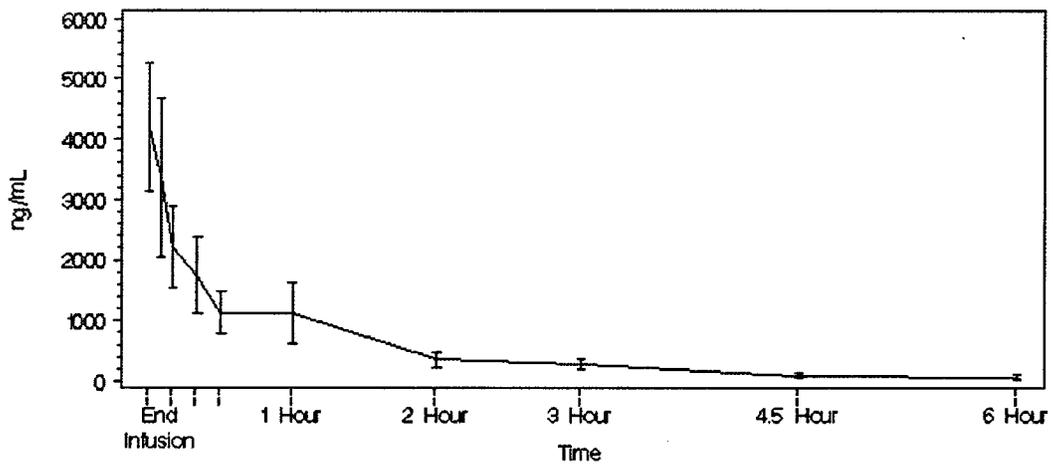


Figure 5: Sponsor's Mean Plasma PDX-10b (R-diastereomer) concentration-time profiles for 230 mg/m² over 3-5 minutes

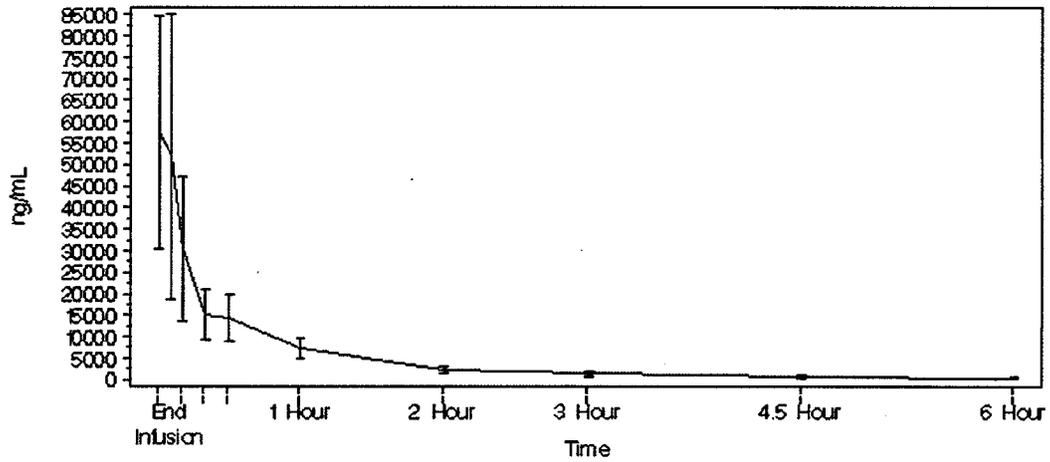


Figure 6: Sponsor's Mean Plasma PDX-10b (R-diastereomer) concentration-time profiles for 230 mg/m² over 1 hour

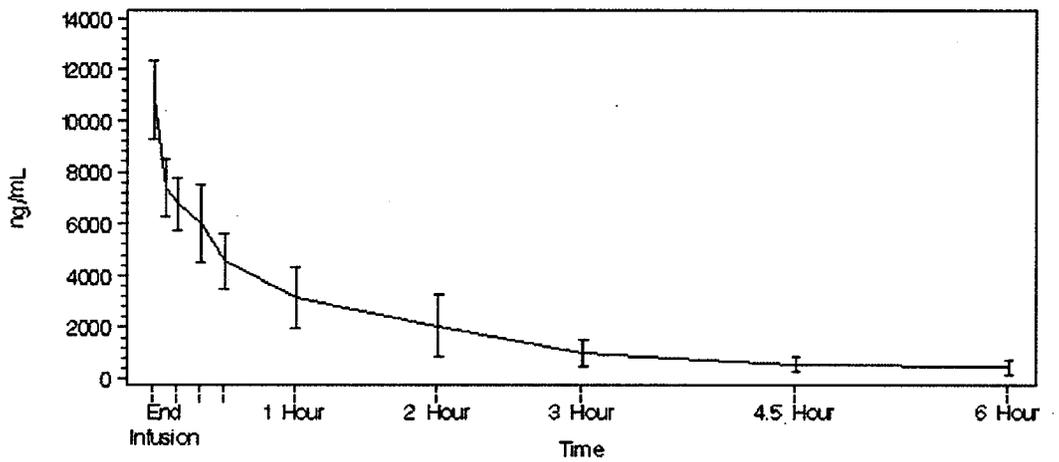
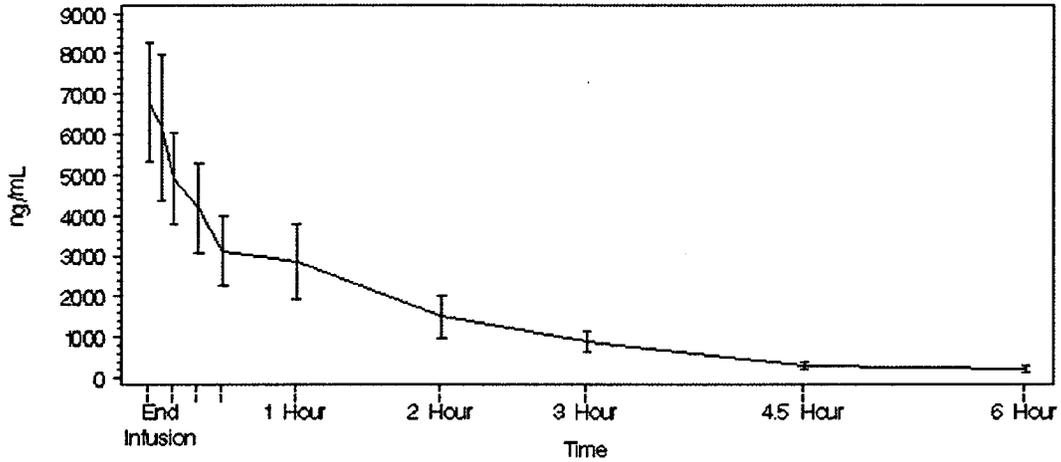


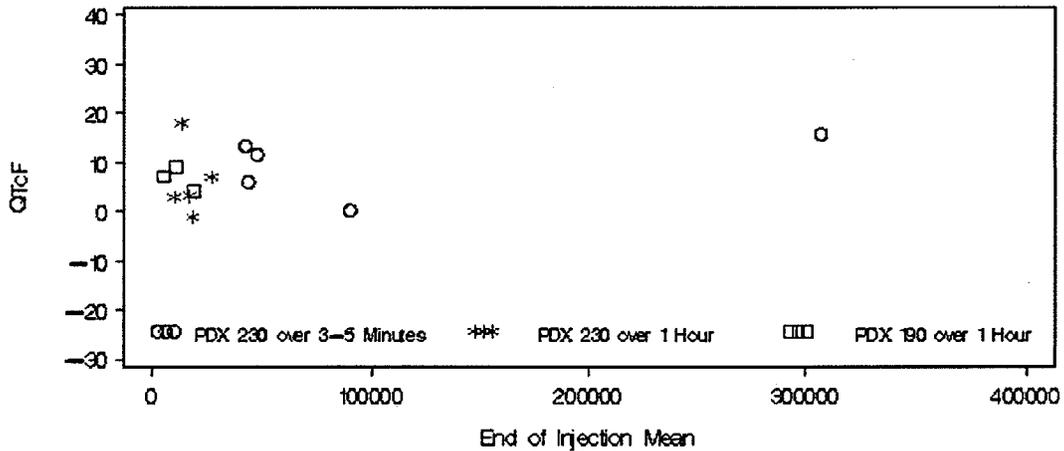
Figure 7: Sponsor's Mean Plasma PDX-10b (R-diastereomer) concentration-time profiles for 190 mg/m² over 1 hour



4.2.7.4.2 Exposure-Response Analysis

Scatter plots of the maximum post-injection change in QTcF by C_{max} is shown in Figure 8. Overall no relationship between maximum change in QTcF and C_{max} is observed.

Figure 8: Sponsor's Maximum Change from Pre-injection QTcF by C_{max} for PDX10a and PDX10b



Reviewer's Analysis: A plot of QTcF vs. drug concentrations is presented in Figure 9. An exposure-response analysis was performed and across the studied concentration range, there appeared to be no visual increase in QT duration

5 REVIEWERS' ASSESSMENT

5.1 CLINICAL PHARMACOLOGY ASSESSMENTS

The mean drug concentration-time profile is illustrated in Figure 2- Figure 7 in section 4.2.7.4.1

The relationship between QTcF and pralatrexate concentrations is visualized in Figure 9 with no evident exposure-response relationship.

Figure 9 : QTcF vs. Pralatrexate concentration



5.2 CLINICAL ASSESSMENTS

5.2.1 Safety Assessments

There are no reports of sudden cardiac death, seizures or significant ventricular arrhythmias in this sub-study. One subject experienced syncope but no ECG is available immediate to the event.

5.2.2 ECG Acquisition and Interpretation

Waveforms from the ECG warehouse were reviewed. According to ECG warehouse statistics over 91% of the ECGs were annotated in the primary lead (II), with less than 2% of ECGs reported to have significant QT bias (these belonged to the subject with the pacemaker and were excluded from the analysis), according to the automated algorithm. Overall ECG acquisition and interpretation in this study appears acceptable and comparable to ECGs from other QT evaluations conducted in patients with comorbidities.

5.2.3 PR and QRS Interpretation

The sponsor reports no clinically relevant effects on the PR and QRS intervals.

6 APPENDIX

6.1 HIGHLIGHTS OF CLINICAL PHARMACOLOGY

HIGHLIGHTS OF CLINICAL PHARMACOLOGY PRALATREXATE

Therapeutic dose	<p>In the Phase 1 dose escalation study (PDX-007) in NSCLC for which the ECG analysis was done, nominal doses ranged from 150-325 mg/m² IV administered once every 2 weeks with vitamin supplementation (folic acid 1 mg orally daily, vitamin B₁₂ 1 mg IM every 8-10 weeks).</p> <p>These therapeutic doses are in contrast to the nominal doses being administered in the Phase 2 study (PDX-008) in PTCL at 20-30 mg/m² weekly for 6 of 7 weeks.</p>	
Maximum tolerated dose	<p>The MTD, as defined by the original protocol DLT criteria for the Phase 1 dose escalation clinical study of pralatrexate (PDX-007) administered IV over 3-5 minutes every 2 weeks with vitamin supplementation, is 270 mg/m². However, dose reductions to 230 and 190 mg/m² administered IV over 3-5 minutes or over 1 hour have been explored to evaluate for enhanced tolerability.</p>	
Principal adverse events	<p>The most common AEs (all grades in all studies) seen with pralatrexate are: stomatitis/mucosal inflammation, fatigue, nausea, epistaxis, dyspnea, cough, anemia/low hemoglobin, constipation, edema/peripheral edema, diarrhea, pyrexia, vomiting, alopecia, peripheral sensory neuropathy, thrombocytopenia, pain, neutropenia, exfoliative rash and dermatoses.</p>	
Maximum dose tested*	Single Dose	<p>Actual dose of 329 mg/m² (1 patient), administered IV once with vitamin supplementation (folic acid 1 mg orally daily, vitamin B₁₂ 1 mg IM every 8-10 weeks).</p>
	Multiple Dose	<p>Nominal doses of 325 mg/m² administered IV once every 2 weeks in a 4 week cycle (2 patients). The cycles are repeated until development of PD, treatment-related AEs, toxicities, alternative therapy, other illness or patient/sponsor/investigator decision to stop.</p> <p>Doses of this cytotoxic drug (pralatrexate) are not administered to achieve steady state.</p>
Exposures Achieved at Maximum Tested Dose*	Single Dose	<p>C_{max}: 66,155 ng/mL and AUC: 2,167,199 ng/mL*min (mean data from patients [n = 3] dosed IV with nominal pralatrexate doses of 325 mg/m²)</p>
	Multiple Dose	<p>C_{max} is same as single dose (no drug accumulation has been observed). The next highest nominal dose tested was 270 mg/m² (6 patients with PK data). Data for this dose; mean C_{max} (%CV) = 64,050 ng/mL (41%), mean AUC (%CV) = 1,792,341 ng/mL*min (32%).</p>

Range of linear pharmacokinetics	Nominal dose range: 150-325mg/m ² , administered IV once every 2 weeks. The dose range of linear PK can be extended to nominal dose ranges of 30-325 mg/m ² when integrating the PK analysis across three Phase 1/2 studies (PDX-008, PDX-007, and PDX-99-083).	
Accumulation at steady state	No accumulation on weekly or once every other week schedules. The pulse dose nature of the administration of this cytotoxic chemotherapy is not intended to produce steady state PK.	
Metabolites	No metabolites have been identified in in vitro studies	
Absorption	Absolute/Relative Bioavailability	NA for this IV administered chemotherapeutic agent
	Time to peak maximum concentration (T_{max})	<ul style="list-style-type: none"> • End of infusion depending on administration time (3-5 minutes or 1 hour) • Median (range) for metabolites: NA
Distribution^a	Volume of distribution at steady state (V_{d,ss})^a	Mean V _{d,ss} (%CV): 75 L (108%)
	% bound	Mean (SD): 67% (1.0) ^b
Elimination	Route	<ul style="list-style-type: none"> • From results of a non-clinical mass balance study in rats (n = 4), the principal route of elimination is fecal, with approximately 39.90-70.81% excreted by this route. Pralatrexate is also eliminated by the renal (22.57-32.53%) and respiratory routes (2.68-17.33%)^c. Total recovery of radiolabel in this study was 91.42-96.97%. • In clinical PK studies, approximately 33% of the administered pralatrexate is excreted unchanged in urine.
	Terminal half life (t_{1/2})^a	<ul style="list-style-type: none"> • Mean (%CV) for parent: ~10 hours (54%) • Mean (%CV) for metabolites: NA
	Total clearance (CL_{tot})^a	CL _{tot} Mean (%CV): 361 mL/min (52%)

Intrinsic Factors	Age	No studies with pralatrexate were performed in patients below 18 years of age. The average age of the clinical PK population was approximately 60 (range 24-77) years. Covariate analysis revealed that CL_{crea}^{CG} and age ($p = 0.06$) were the only significant covariates, however, this accounted for only approximately 10% of the observed population variability. Since age is a factor in the estimation of CL_{crea}^{CG} , both covariate effects likely reflect the underlying reduction in pralatrexate CL_{tot} , resulting from the physiological age-related decline in renal function.
	Sex	There were no significant gender differences in PK parameters.
	Race	Covariate analysis did not reveal race as a significant covariate.
	Hepatic & Renal Impairment	No studies investigating pralatrexate in patients with renal or hepatic impairment have been performed. Due to the significant contribution of renal excretion to the overall clearance of pralatrexate, caution is advised when administering pralatrexate to patients with renal impairment.
Extrinsic Factors	Drug interactions^d	Drug-drug interaction studies using pralatrexate and a variety of reference plasma protein binding compounds did not show significant drug displacement interactions. Pralatrexate was not significantly metabolized by CYP450 isozymes, nor was it found to significantly inhibit or induce CYP450 isozymes, therefore it is unlikely that pralatrexate administration would affect metabolism of other drugs as a result of CYP450 interaction. Pralatrexate was not a substrate for P-gp, nor did it inhibit P-gp function, and therefore it is unlikely that pralatrexate will affect excretion of other drugs through an interaction with P-gp. Similar to what has been reported for methotrexate, co-treatment with probenecid increased doses of probenecid resulting in delayed clearance of pralatrexate and a commensurate increase in exposure.
	Food Effects	NA for this IV administered chemotherapeutic agent

Expected High Clinical Exposure Scenario	For the weekly or every 2 week administration of this chemotherapeutic agent, there is no accumulation of the drug. Therefore, the highest clinical exposure is expected to be directly correlated with the magnitude of the individual dose administration.
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^aPharmacokinetic data from Phase I Clinical Study PDX-007 (see Section 5.3.3.2); C_{max} and AUC values are sum of the values for the individual pralatrexate diastereomers (PDX-10a and PDX-10b) and V_{d_{ss}}, t_{1/2}, and CL_{tot} are the means of the values observed for PDX-10a and PDX-10b from PK data of patients dosed at nominal doses from 150-325 mg/m².

^bData from study PDX-K-07043-U; Equilibrium Dialysis.

^cData from studies PDX-K-07052-R and PDX-K-07053-R. Expiration of radiolabeled ¹⁴C in the form of CO₂ is likely due to incorporation of the radiolabel in the carboxylic acid side chain of the drug substance.

^dSee Section 2.7.2 for details

- | | | |
|---|--|-------------------------------------|
| NSCLC = non-small cell lung cancer | ECG = electrocardiogram | IV = intravenous |
| IM = intramuscular | PTCL = peripheral T-cell lymphoma | |
| MTD = maximum tolerated dose | DLT = dose-limiting toxicity | mg = milligram |
| m ² = square meter | AE = adverse event | PD = progressive disease |
| C _{max} = maximum concentration | AUC = area under the curve | PK = pharmacokinetics |
| %CV = coefficient of variance | ng = nanogram | mL = milliliter |
| min = minute | NA = not applicable | |
| T _{max} = time to maximum concentration | V _{d_{ss}} = volume of distribution at steady state | |
| SD = standard deviation | t _{1/2} = terminal half life | CL _{tot} = total clearance |
| CL _{crea} ^{CG} = creatinine clearance, as calculated by the Cockcroft-Gault formula | | p = probability value |
| CYP450 = cytochrome P450 isozyme | P-gp = P-glycoprotein | |

6.2 TABLE OF STUDY ASSESSMENTS

Treatment Period	Screening	Study Treatment Initiation		PDX Cycle 1			Cycle 1 Follow-up Visit ²	Subsequent Cycles of PDX	Final Safety Follow-up ³
		14 Days Pretreatment	Vitamin Dosing (Day -7)	PDX Dose 1 (Day 1)	24, 48, 72 Hours Post-end PDX	7 Days Post Dose 1			
Eligibility Criteria/Informed Consent/Privacy Authorization	X								
Medical/Surgical History	X								
Document Histopathology	X								
Document Staging of NSCLC	X								
Record Prior Treatments for NSCLC	X								
Record Medications	X	X	X		X	X	X	X	X
Record Baseline Symptoms		X							
Record AEs/Attribution		X ^{1,2}	X ¹	X ^{1,2}	X ^{1,2}	X ^{1,2}	X ^{1,2}	X ^{1,2}	X ^{1,2}
KPS Assessment	X								
Physical Examination		X				X	X		
Vital Signs: HR, RR, BP, Temperature		X	X ³			X ³	X		
Record Height in cm		X							
Record Weight in kg		X	X			X			
Calculate BSA		X							
Vitamin B ₁₂ Administration ¹		X	X		X	X	X	X	
Folic Acid Administration ²		X	X	X	X	X	X	X	
Folic Acid Drug Accountability and Resupply if Necessary			X		X	X	X	X ⁴	X ⁴
PDX Administration ¹			X			X		X	
Contrast-enhanced CT Scan of the Chest & Upper Abdomen ¹	X								
12-Lead ECG	X ¹		X ⁵					X ¹	

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Treatment Period	Screening	Study Treatment Initiation		PDX Cycle 1				Cycle 1 Follow-up Visit ⁶	Subsequent Cycles of PDX	Final Safety Follow-up ⁷
		Vitamin Dosing (Day -7)	PDX Dose 1 (Day 1)	24, 48, 72 Hours Post-end PDX	0 Days Post Dose 1	Dose 2	7 Days Post Dose 2			
Hematology ³	X		X		X	X	X	X	X	X
Chemistry ⁴	X		X			X		X	X	X
RBC Folate		X ⁴								
Homocysteine and MMA		X ⁴	X					X		
Serum hCG Pregnancy Test	X ³									
Urinalysis	X							X		
Blood for PDX PK ⁸			X	X		X				
Urine for PDX PK ⁹			X	X						
Tumor tissue for Correlative Pathologic Studies			X ²⁰							

¹Vitamin B₁₂, 1 mg IM q 8-10 weeks, initiated no later than day -7 and continuing until PDX is discontinued. See protocol Section 7.1 for details.
²Folic acid, 1 mg by mouth daily, initiated no later than day -7 and continuing until PDX is discontinued. ³Administer PDX IV push over 3-5 minutes or 1 hour depending on cohort. ⁴Within 28 days of day -7. ⁵See protocol Section 12.0 for specific laboratory tests. ⁶See protocol Section 12.2.1 for blood PK collection time points. ⁷See protocol Section 12.2.2 for urine PK collection time points. ⁸Visit should occur 2 weeks ±3 days after the last dose of PDX in cycle 1 and prior to further PDX administration. ⁹Should occur at least 30 days after the last dose of PDX. ¹⁰Record AEs that are study procedure-related, if applicable. ¹¹Record all AEs and attribution. ¹²Record all AEs and attribution through 30 days after the last study treatment. From 31 days after the last study treatment, only record AEs that are related to PDX and/or vitamins. ¹³Vital signs: Recorded pre-injection and 1 hour (+/- 15 mins) post-PDX injection. ¹⁴If patient will not be continuing PDX administration, collect remaining folic acid from the patient following accountability verification. ¹⁵Single ECG. ¹⁶Triplicate ECGs at end-PDX injection and, 60 minutes, 3 hours, and 6 hours post-end-injection. ¹⁷Single ECGs pre-injection and within 30 minutes post-end-injection. ¹⁸Within 5 days prior to day -7. ¹⁹Within 14 days of projected treatment initiation for women of childbearing potential. ²⁰Send tumor tissue, when available, to the central laboratory within 6 weeks of enrollment.

Source: From the protocol for PDX 007

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22468	ORIG 1	ALLOS THERAPEUTICS INC	FOLOTYN

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Anshu Marathe
08/26/2009

SUCHITRA M BALAKRISHNAN
08/26/2009

CHRISTINE E GARNETT
08/27/2009

NORMAN L STOCKBRIDGE
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