CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-468

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Folotyn (pralatrexate)

Date: September 14, 2009
To: File for NDA 22-468
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review provided by Dr. McGuinn and supervisory concurrence provided by Dr. Verbois. I concur with their conclusions that Folotyn may be approved. Labeling has not been reviewed.

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

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/s/

JOHN K LEIGHTON
09/14/2009

MEMORANDUM

Date: September 3, 2009
From: S. Leigh Verbois, Ph.D.
Supervisory Pharmacologist
Division of Drug Oncology Products
To: File for NDA #22-468
Pralatrexate (FOLOTYN)
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies that investigated the pharmacology and toxicology of pralatrexate provided to support NDA 22-468 for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma were reviewed in detail by W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T. The supporting information included studies of intravenously administered pralatrexate that investigated the drug's pharmacology, pharmacokinetics and ADME, safety pharmacology, general toxicology (rat and dog), genetic toxicity (*in vivo* and *in vitro*), and reproductive toxicity in both rats and rabbits. The studies cited in the review by Dr. McGuinn consist primarily of original research conducted by the applicant.

The pharmacology studies submitted to the NDA demonstrate that pralatrexate is a folate analogue metabolic inhibitor which competitively inhibits the reduced folate carrier 1 protein and dihydrofolate reductase. Dose limiting toxicity, necrosis or apoptosis in rapidly dividing cells, were consistent with the mechanism of action. Pralatrexate did not cause mutations in the Ames assay or the Chinese hamster ovary cell chromosome aberration assay. However, these assays do not reliably predict genotoxicity for this class of compounds. Neither methotrexate nor pemetrexed are positive for genotoxicity in these assays. In contrast pralatrexate was also negative in the mouse micronucleus assay, whereas both methotrexate and pemetrexed are genotoxic in this assay. Given the structural and mechanistic similarity of pralatrexate and methotrexate, these results are scientifically puzzling but the data provided by the sponsor was valid and supports approval of the drug product. The carcinogenic potential of pralatrexate was not assessed but studies are not necessary for the proposed indication.

Like other folate analogue metabolic inhibitors, pralatrexate is a highly toxic to the developing embryo or fetus causing resorption, pre- and post implantation loss and decreased weight. Although teratogenesis was not detected in embryofetal development studies in rats and rabbits, teratogenic effects of folate depletion are well established. However, the potential benefits from the use of the FOLOTYN in pregnant women in this patient population may outweigh the potential risk to the developing fetus thereby resulting in a Pregnancy Category D for this patient population.

Recommendations: I concur with Dr. McGuinn's conclusion that pharmacology and toxicology data support the approval of NDA 22-468, FOLOTYN. There are no outstanding nonclinical issues related to the approval of FOLOTYN for the proposed indication.

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

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/s/

SANDI L VERBOIS
09/08/2009

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

Pharmacology and Toxicology Review and Evaluation

NDA Number:	22-468
Serial Number:	000
Date Received by Center:	March 23, 2009
Product:	FOLOTYN™ (pralatrexate injection)
Intended Clinical Population:	patients with relapsed or refractory peripheral T-cell lymphoma (PTCL)
Sponsor:	Allos Therapeutics, Inc.
Documents Reviewed:	\\CDSESUB1\EVSPROD\NDA022468\022468.enx
Review Division:	Division of Oncology Drug Products (HFD-150)
Pharm/Tox Reviewer:	W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Pharm/Tox Supervisor:	S. Leigh Verbois, Ph. D.
Division Director:	Robert Justice, M.D.
Project Manager:	Milinda Vialpando

Date of review submission to Division File System (DFS): September 4, 2009

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Executive Summary

I. Recommendations

Recommendation on approvability

There are no toxicology issues that would prevent approval of pralatrexate for use in the proposed indication.

Recommendation for nonclinical studies

None

Recommendations on labeling

Any needed labeling changes will be described in a subsequent review.

II. Summary of nonclinical findings

Brief overview of nonclinical findings

Pralatrexate inhibits the enzyme dihydrofolate reductase, folate transport and possibly folate polyglutamation (by competition) to affect folate depletion and diminish the production of biological compounds the synthesis of which requires the addition of a reduced methyl group. Its mechanism is almost identical to that of the approved anti-cancer drugs, methotrexate and pemetrexed, the only demonstrated difference being that it has a somewhat higher affinity for the reduced folate transporter. The inhibition of folate systems and the depletion of folate results in decreased concentrations of thymidylate and certain purines the synthesis of which is down-stream of the production of dihydrofolate and tetrahydrofolate. This decrease in the concentrations of purines and a pyrimidine necessary for DNA synthesis leads to errors in DNA replication which brings about necrosis or apoptosis in rapidly dividing cells such as some cancer cells and normal cells of the gastric mucosa and bone marrow. Death can result from the destruction of the gastrointestinal epithelium and can occur rapidly at high doses. The toxic dose response curve is very steep, there being less than a factor of two between a relatively non-toxic dose and a fatal dose. Oral or IV folic acid or methyl-folate are effective antidotes, but these vitamins also diminish the effect of the drug on cancer cells. Repeat dosing results in reversible anemia and leukopenia. In the clinical studies submitted for registration, patients took supplemental folate. Supplemental folate was also given to dogs in the pivotal repeat dose study. There were some indications of hepatic toxicity in dogs. Rats have relatively high concentrations of thymidine in plasma, and are thus insensitive to anti-folate toxicity. They are not a useful model for anti-folate drugs.

The Ames test for mutagenicity was negative but this is a finding that is well established for this drug class. The CHO chromosomal aberration test was negative but this test is sensitive to compounds that directly interact with DNA. It is relatively insensitive to the inhibition of folate pathways as the reserves of thymidine are probably adequate for the single cycle of reproduction observed in the test. The mouse micronucleus test was negative. This finding is unusual as methotrexate and other anti-folates are usually strongly mutagenic or clastogenic in this assay and others.

Like methotrexate, pralatrexate is a very toxic to the developing embryo or fetus causing resorption, pre- and post implantation loss and decreased weight. Mammals developing in the womb are exquisitely sensitive to folate depletion. The pregnancy category of methotrexate is X due to extensive evidence that it causes malformations and spontaneous loss in humans even at doses much lower than those used to treat cancer (e.g. treatment of psoriasis). The pregnancy category of pralatrexate is D because of the lack of clinical evidence of embryofetal toxicity in humans. The potential benefits from the use of the pralatrexate in pregnant women in this patient population may outweigh the potential risk to the developing fetus.

Pralatrexate did not inhibit the action potential of dog Purkinje cells *in vitro*, nor did it inhibit the potassium current in the hERG assay. The doses used in studies of electrocardiographic parameters in dogs were too low to be informative.

The distribution and elimination of pralatrexate is complex. Plasma concentration verses time curves show three distinct phases, a rapid distribution phase, a slow partition phase and a very slow terminal elimination phase. The slow partition phase may represent enterohepatic recirculation but most likely represents redistribution into some compartment. The terminal elimination half-life is 17 hours in humans and about 5 hours in dogs.

In most cases, AUC and C_{max} increased linearly with dose and clearance was relatively invariant with dose. C_{max} at toxic or near toxic doses was in the 5 to 10 μM range. Clearance in dogs was about 10 mL/min/kg; in humans it was 5.3 mL/min/kg for humans). In humans about a third of a given dose is recovered in the urine in dogs about one fifth. Fecal elimination was not adequately determined. Little pralatrexate crosses the blood brain barrier (BBB). Plasma binding is about 67% and little of the drug crosses into RBCs.

Pooled human microsomes or S9 fraction do not appear to metabolize pralatrexate; neither do human hepatocytes *in vitro*. Metabolites were not observed in plasma *in vivo*. Inhibition of human cytochrome P450 2C19 only occurred at concentrations unlikely to be achieved clinically. Pralatrexate did not induce an increase in the activity of cytochromes P450 1A2, 3A4 or 2C19 when incubated with fresh human hepatocytes.

Nonclinical safety issues relevant to clinical use

Pralatrexate may inhibit dihydrofolate reductase irreversibly by the formation of a covalent bond at the active site. If so, normal intracellular concentrations of dihydrofolate reductase could only be regained by *de novo* synthesis. The concomitant use of anticancer drugs that interfere with protein biosynthesis at the level of DNA transcription, RNA translation, or protein post-translational modification may cause a significant increase in toxicity or a change in the spectrum of expected toxicities.

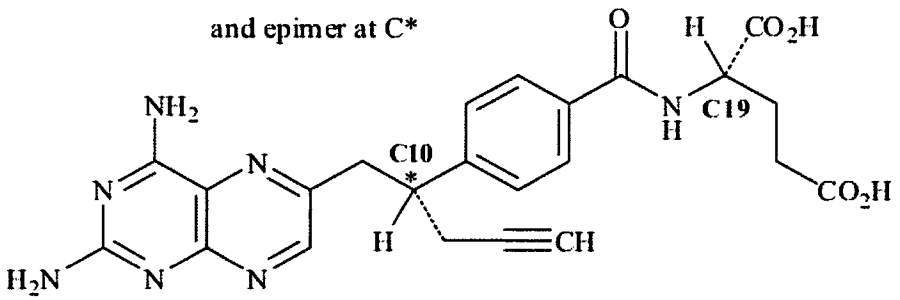
Pralatrexate is a potent abortifacient.

While pralatrexate was not genotoxic in the standard battery of tests, methotrexate and other antifolates that work by the same mechanism are. Further testing with other assays will probably demonstrate that pralatrexate is genotoxic.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	22-468
Review number:	1
Sequence number:	000
Letter Date	March 23, 2009
Type of submission:	E
Information to sponsor:	No
Sponsor:	Allos Therapeutics, Inc.
Intended Clinical Population:	Patients with relapsed or refractory peripheral T-cell lymphoma (PTCL)
Sponsor:	Allos Therapeutics, Inc.
Documents Reviewed:	\\CDSESUB1\EVSPROD\NDA022468\022468.enx
Manufacturer of drug substance:	(b) (4)
Reviewer name:	W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Division name:	Division of Oncology Drug Products (HFD-150)
HFD #:	150
Review completed:	July 20, 2009
Drug:	
Trade name:	FOLOTYN™
Generic name:	pralatrexate injection
Code name:	PDX, PDX-10a for the S diastereomer and PDX-10b R diastereomer
Chemical name:	Approximately 1 to 1 ratio of S diastereomer and R diastereomer (2S)-2-[[4-[(1S)-1-[(2,4-diaminopteridin-6-yl)methyl]but-3-ynyl]benzoyl]amino]pentanedioic acid) (2S)-2-[[4-[(1R)-1-[(2,4-diaminopteridin-6-yl)methyl]but-3-ynyl]benzoyl]amino]pentanedioic acid)
CAS registry number:	146164-95-1 Molecular formula: $C_{23}H_{23}N_7O_5$
Molecular weight:	477.48 g/mole



Structure:

Related IND: IND 52,604

Drug class: Folate analogue metabolic inhibitor

Intended clinical population: Relapsed or refractory peripheral T-cell lymphoma

Clinical formulation:

Component	Function	Quality Standard	Quantity		
			Per mL	Per 1 mL/2 mL Vial	Per 2 mL/2 mL Vial
Pralatrexate	Active	In-house	20 mg	20 mg	40 mg
Sodium chloride	Tonicity adjuster	USP	(b) (4)		
Sodium hydroxide	pH adjuster	NF			
Hydrochloric acid	pH adjuster	NF			
(b) (4)					

QS = quantity sufficient
USP = United States Pharmacopeia
NF = National Formulary

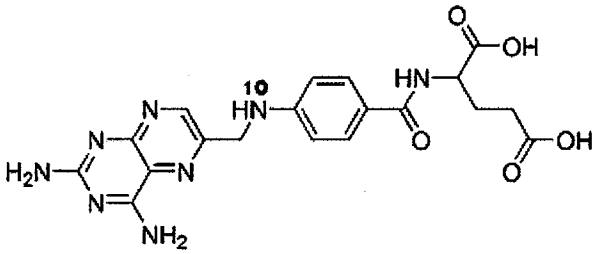
Route: IV injection (push)
Clinical Dose: 30 mg/m^2
Clinical Schedule: once weekly for 6 weeks in 7-week cycles until progressive disease or unacceptable toxicity

Patients in the clinical studies were treated with concomitant vitamin supplementation specified as follows:

Patients should take low-dose (1.0-1.25 mg) oral folic acid on a daily basis. Folic acid should be initiated during the 10-day period preceding the first dose of pralatrexate, and dosing should continue during the full course of therapy and for 30 days after the last dose of pralatrexate. Patients should also receive a vitamin B_{12} (1 mg) intramuscular injection no more than 10 weeks prior to the first dose of pralatrexate and every 8-10 weeks thereafter. Subsequent vitamin B_{12} injections may be given the same day as treatment with pralatrexate.

Introduction

In 1926, George Minot and William Murphy noticed that a diet containing relatively high amounts of rich iron containing foods, particularly liver, effectively reversed pernicious anemia (JAMA 1926, 87:470-476). In the early 1930s, Margret Balfour made Dr. Lucy Willis of Great Britain aware of the alarmingly high incidence of anemia associate with pregnancy in India (Bastian H, 2007, Lucy Wills (1888-1964): The life and research of an adventurous independent woman, The James Lind Library). In a series of animal experiments, Willis determined that a factor in marmite, a yeast extract, reversed the symptoms of anemia of pregnancy. She extended this work to human patients (L Willis, 1931, British Medical Journal 1:1059-1064 and L Willis, 1933, Lancet 1:1283-1286). In 1941, H. K. Mitchell, E. E. Snell and R. J. Williams at the University of Texas, using a Streptococcus Lactis R model, isolated "an acid nutrilitic with interesting physiological properties" by extracting four tons of spinach. They showed that this acid stimulated bacterial growth as a function of increasing dose. They called this nutrilitic folic acid (vitamin B_9), and suggested that it had 'vitamin like properties." In 1943, Robert Stokstad determined the structure of folic acid (see, Physiological Reviews, 1948, 28(1):51). In 1945, R. B. Angier *et al* described the synthesis of this molecule. Shortly after World War II, Sidney Farber of Harvard Medical School noticed that treatment of patients with ALL with folic acid exacerbated the disease. In collaboration, Farber had Harriett Kilte and chemists of Lederle Laboratories Farber synthesize a number of folate analogues. In 1948, Farber and others reported that one of these drugs, aminopterin, caused remission of AML in children (N. Engl. J. Med, 1948, 238:787-793).



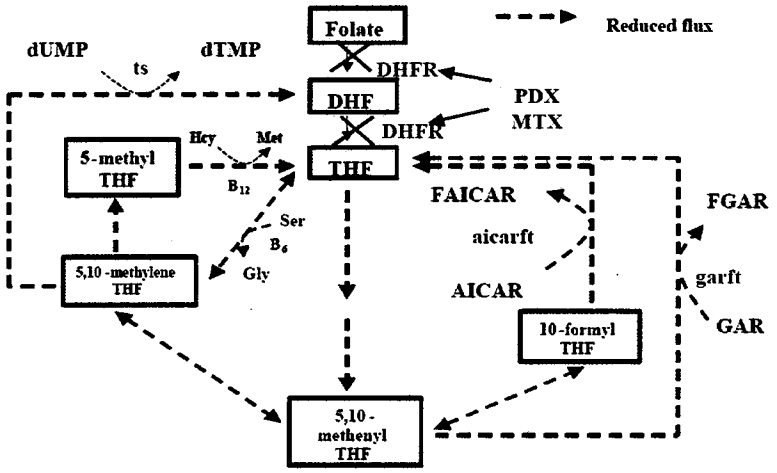
The structure of aminopterin

Lederle Laboratories marketed aminopterin in the United States from 1953 to 1964 for the treatment of leukemia. The drug was gradually superseded by methotrexate, an analog of aminopterin with a better therapeutic index.

Using NADPH as a source of electrons, the enzyme, dihydrofolate reductase (DHFR), reduces folic acid at the pyrazine portion of the pteridine ring to form dihydrofolate (DHF). In a similar reaction this same enzyme further reduces dihydrofolate to tetrahydrofolate (THF) again donating electrons to the pteridine ring resulting in further reduction of the pyrazine portion of the heterocycle. Serine hydroxymethyltransferase converts serine to glycine and in the process transfers a methyl group to THF to form N^5, N^{10}-methyene-tetrahydrofolate (mTHF), which then through the agency of thymidylate synthase transfers the methyl group to deoxyuridine monophosphate (dUMP) to form deoxythymidine monophosphate (dTMP). THF is also reduced to N^5, N^{10} methenyl-THF and subsequently to N^{10}-formyl-THF which is involved in the synthesis of purines. While many of these processes simply cycle THF (*vide infra*), thereby conserving available folate, the formation of thymidylate converts THF to DHF which cannot then be reduced back to THF in the presence of DHFR inhibitors. THF is also converted to 5-methyl-THF in a reaction that is considered irreversible. Thus, all available folate gradually accumulates in stagnant unusable pools in the presence of inhibitors of DHFR. As normal DNA breaks and

errors accumulate, no thymidylate is available to make needed repairs; single strand breaks and fragments accumulate, and the cell dies from necrosis or apoptosis. Cells in division make errors in the synthesis of new DNA that render them non-viable. Further, folate analogs can also inhibit the transport of folate into the cell and its retention within the cell through the mechanism of polyglutamation. Thus, in the synthesis of aminopterin, the simple replacement of the hydroxyl group on the pteridine ring with an amino group transforms a vitamin into a potent toxin. See J.J. McGuire (*Current Pharmaceutical Design,* 2003, **9**, 2593-2613) for a review of these mechanisms. The following schematic drawing from the sponsor's submission presents some of these mechanisms visually.

Figure 2.4.2.1. Folic Acid Metabolism and Dihydrofolate Reductase Inhibition



DHF = dihydrofolate, DHFR = dihydrofolate reductase, THF = tetrahydrofolate, PDX = pralatrexate, MTX = methotrexate, ts = thymidylate synthase, dUMP = deoxyuridine monophosphate, dTMP = deoxythymidine monophosphate, B_6 = vitamin B_6, B_{12} = vitamin B_{12}, Hcy = homocysteine, Met = methionine, Ser = serine, Gly = glycine, AICAR = 5-aminoimidazole-4-carboxamide 1-ß-D-ribofuranoside, FAICAR = 5-formamidoimidazole-4-carboxamide 1-ß-D-ribofuranoside, aicarft = AICAR formyl transferase, GAR = glycinamide ribonucleotide, FGAR = formyl GAR, garft = glycinamide ribonucleotide formyltransferase

In 1993, J. I. DeGraw *et al.* (*J. Med. Chem.* 1993, **36,** 2228-2231) described the original synthesis of pralatrexate and five other aminopterin derivatives. Pralatrexate differs from aminopterin in that the nitrogen in the 10 position is replaced by a methyl group which is further substituted with a propargyl group (propyl alkyne). These investigators showed that pralatrexate inhibits dihydrofolate reductase with a k_i of 18 ± 4 pM; the k_i of methotrexate is 5.8 ± 1 pM so it is a more potent inhibitor. But surprisingly the k_i for transport influx of folate was 0.45 ± 0.1 µM while that of methotrexate is 4.2 ± 5 µM, almost ten fold higher. This advantage was reflected in the inhibition of the growth of mouse lymphoma cells *in vitro* where the IC_{50} for pralatrexate was 2 nM while that for methotrexate was 9.5 nM. Pralatrexate was also more effective than methotrexate at delaying tumor growth as the following graph from the report shows.

Disclaimer:

I constructed all tabular and graphical information unless cited otherwise. I have excerpted portions of the sponsor's submission within the review. Dr. Chang Ahn reviewed the initial IND submission for IND 52,604; his review is appended to the end of this review. The studies he reviewed were done by investigators at Memorial Sloan Kettering Cancer Center, the sponsor of the initial IND. The current sponsor did not submit these studies to the NDA.

Studies reviewed within this submission: See Table of Contents

Studies not reviewed within this submission:

(b) (4)

(b) (4)

REVIEW

2.6.2 Pharmacology

2.6.2.1 Summary

Mechanism of Action

Pralatrexate inhibits the enzyme dihydrofolate reductase, folate transport and folate polyglutamation (by competition) to affect folate depletion and dramatic decreases in available intracellular thymidylate. This results in necrosis or apoptosis of rapidly dividing cells such as some cancer cells and normal cells of the gastric mucosa and bone marrow.

Primary Pharmacodynamics

Pralatrexate inhibited the growth of a broad variety of human cancer cell types at very low concentrations (GI_{50} < 0.1 μM) after 48 hours exposure in the standard NCI *in vitro* assay. In human hepatocytes in culture, pralatrexate caused relatively little cytotoxicity at concentrations as high as 200 μM (4 hour treatment) as measured by the ability of the cells to retain ATP. In other standard human tumor cell lines including HLaC, MDA-MB-231, MDA-MB-435, A549 and MV522 pralatrexate inhibited cell growth viability at mean concentrations between 12.8 and 65.1 nM (IC_{50} values). These concentrations are lower than those required by cisplatin and on the same order as those required by docetaxel and paclitaxel but with somewhat less variability. The compound inhibits other cell lines, MB-231, MDA-MB-435, NCI-H460, and SK-BR3 at mean concentrations between 6.7 and 54 nM. (b) (4) kills cells at concentrations only above 1 μM in this assay, thus it is considerably less toxic than pralatrexate in this assay.

Safety Pharmacology

Pralatrexate at concentrations of 0, 0.71, 1.40 or 3.48 mM, caused dose dependant decreases in V_{max} (about 11% at the highest concentration) and increases in action potential duration at 30, 60 and 90% repolarization (about 35%, 12% and 9% respectively, at the highest concentration) compared to controls in isolated dog Purkinje fibers. Nevertheless, these changes were not statistically significant and the concentrations of the drug are well above those that would be physiologically relevant.

Pralatrexate inhibited the hERG current in transfected CHO cells at concentrations of 0.4 mg/mL (2%, 0.84 mM), 0.8 mg/mL (37%, 1.7 mM), 2 mg/mL (54%, 4.2 mM) and 4 mg/mL (54%, 8.4 mM). The results for the highest three concentrations were statistically significant. These concentrations are well above those that would be pharmacologically relevant.

In conscious telemetered dogs, pralatrexate at doses up to 0.7 mg/kg caused no toxicologically significant changes in blood pressure (systolic, diastolic, and mean arterial pressure), heart rate, body temperature, electrocardiographic parameters (heart rate, PR, QRS, RR, and QT/QTc), respiratory function (respiratory rate, blood oxygen saturation, and end tidal CO_2). Nevertheless, this dose is only half that given clinically on a mg/m^2 basis.

A single IV dose of pralatrexate of 25 mg/kg (150 mg/m^2) caused no change in the neurobehavioral parameters or the functional ability of male and female rats but the doses in this study were too low to provide useful information about the neurotoxicity of pralatrexate.

2.6.2.2 Primary Pharmacodynamics

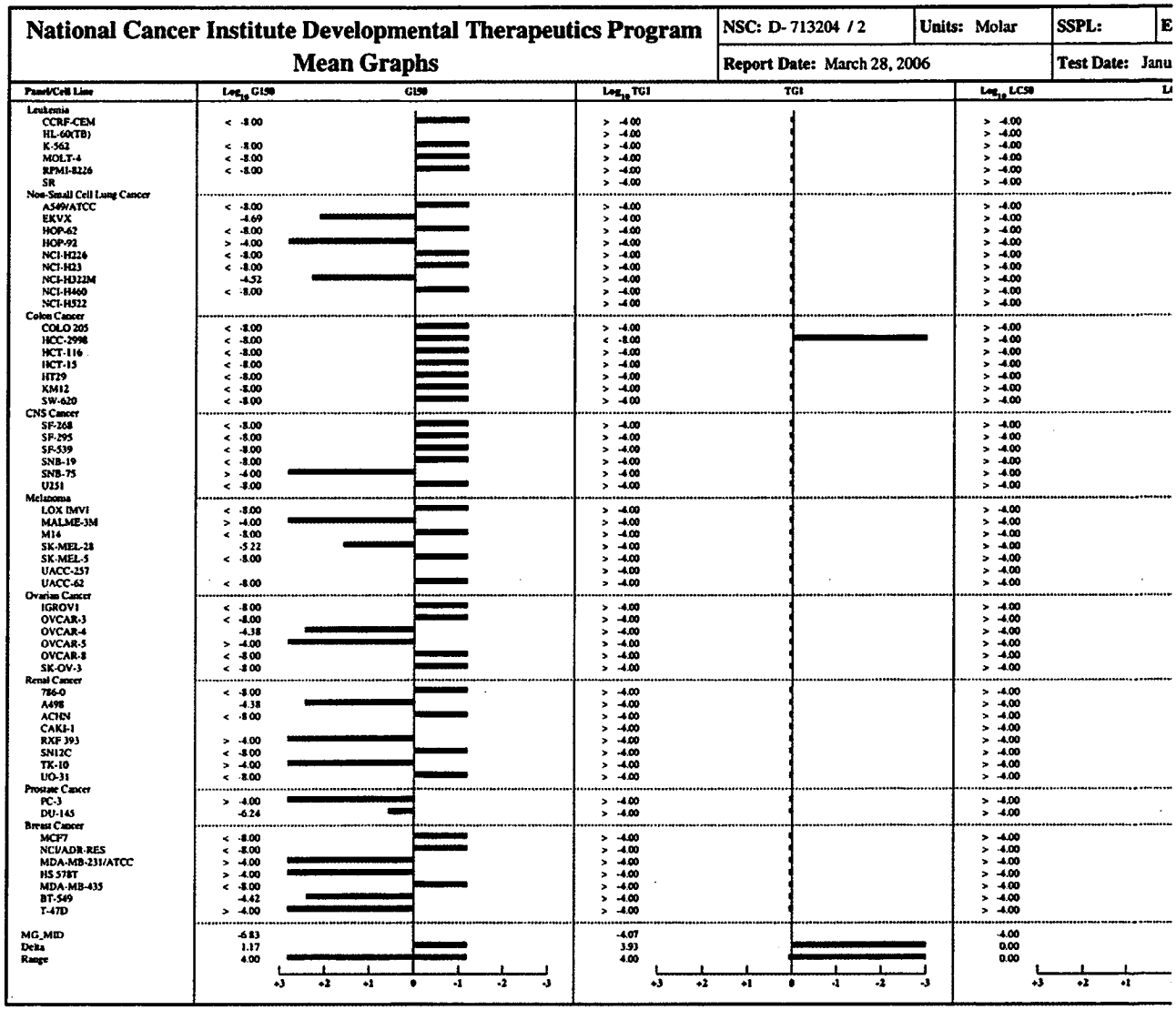
1) Growth Inhibitory Activity of Pralatrexate in the NCI-60 Cancer Cell Panel

Major Findings

Pralatrexate inhibited the growth of a broad variety of human cancer cell types at very low concentrations ($GI_{50} < 0.1$ μM) after 48 hours exposure.

Study Number	Sponsor PDX-P-06069-U, Laboratory 0601NS95-2
File name	PDX-K-06069u--nonclinical-data.pdf
Laboratory	NCI, Rockville, MD
Study Date	November, 2005
GLP	No
Audited	No
Drug	Pralatrexate, Lot 00510105, purity 98.5%
Methods	
Cells	Human tumor cell lines of the NCI cancer screening panel
Concentrations	At least five concentrations per cell line
Medium	RPMI 1640 medium with 5% FBS and 2 mM L-glutamine
Solvent	DMSO
Treatment time	48 hours
Assay	Cell viability

Table 4.1
Results of Screening of Pralatrexate in the NCI 60-Cancer Cell Panel



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2) Cytotoxicity of test article in human hepatocytes

Major findings

Pralatrexate caused relatively little cytotoxicity to human hepatocytes in culture at concentrations as high as 200 μM (4 hour treatment) as measured by the ability of the cells to retain ATP. As these cells are relatively quiescent, one would not expect toxicity with such a short exposure.

Study Number	Sponsor PDX-K-06020-U, Laboratory 1400-110105
File name	PDX-K-06020-U—nonclinical-data.pdf
Laboratory	(b) (4)
Study Date	November, 2005
GLP	No
Audited	No
Drug	Pralatrexate, Lot 00510105, purity 98.5%
Methods	
Cells	Cryopreserved human hepatocytes, 250,000 cells per mL (in the well)
Concentrations	0, 3.12, 6.25, 12.5, 25, 50, 100, 200 µM (triplicate assays)
Medium	DMEM-F12 medium
Solvent	PBS
Treatment times	1, 2, or 4 hours
Assay	Intracellular ATP, chemiluminescence
Positive control	Cadmium chloride

Results

The following table from the study report shows the relative viability of the hepatocytes in culture calculated as follows:

Relative Viability (%) = [ATP (treatment»]/[ATP (solvent-control)] x 100%;

	Treatment Time					
	1-hr		2-hr		4-hr	
uM	mean	sd	mean	sd	mean	sd
0	100.00	31.97	100.00	13.65	100.00	10.58
3.125	121.87	27.65	107.49	7.27	110.07	13.99
6.25	123.23	40.06	106.39	20.22	97.80	8.80
12.5	134.82	8.87	101.66	12.67	87.23	8.77
25	115.89	18.74	101.23	8.14	91.93	11.95
50	108.16	31.45	72.35	7.50	94.31	9.13
100	123.58	18.82	76.74	14.33	91.37	6.10
200	102.51	7.05	71.98	17.78	89.37	9.73

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3) Evaluation of Growth Inhibitory Activity of the Allos Agent PDX versus Several Human Tumor Cell Lines (*s/c*)

Major findings

The following table from the study report shows that pralatrexate inhibits cell growth at mean concentrations between 12.8 and 65.1 nM (IC_{50} values). These concentrations are lower than those required by cisplatin and on the same order as those required by docetaxel and paclitaxel but with somewhat less variability. The experiment did not compare methotrexate.

Results and Discussion

Table 1: Growth inhibitory activity (IC_{50}) of PDX and standard agents

Cell Line		PDX (nM)	Cisplatin (μM)	Paclitaxel (nM)	Docetaxel (nM)
HLaC	Exp 1	28.1	29.6	17.6	6.1
	Exp 2	23.5	31.8	26.2	7.0
	Mean IC_{50}	**25.8**	**30.7**	**21.9**	**6.6**
MDA-MB-231	Exp 1	16.0	10.1	53.3	6.8
	Exp 2	20.0	11.4	90.8	9.6
	Mean IC_{50}	**18.0**	**10.8**	**72.1**	**8.2**
MDA-MB-435	Exp 1	26.7	8.1	8.9	4.9
	Exp 2	21.4	7.8	9.9	5.3
	Mean IC_{50}	**24.1**	**8.0**	**9.4**	**5.1**
A549	Exp 1	59.5	22.4	107.7	33.3
	Exp 2	70.7	22.4	109.6	13.9
	Mean IC_{50}	**65.1**	**22.4**	**108.7**	**23.6**
MV522	Exp 1	14.4	35.9	104.3	118.2
	Exp 2	11.2	25.1	100.4	118.3
	Mean IC_{50}	**12.8**	**30.5**	**102.4**	**118.3**

Study Number	Sponsor PDX-K-04004-U,
File name	PDX-K-04004-U—nonclinical-data.pdf
Laboratory	(b) (4)
Study Date	May 2004
GLP	No
Audited	No
Drug	Pralatrexate, Lot and purity not stated and other approved cancer drugs (see above)
Methods	
Cells	Human cancer cell lines – HLaC (head and neck), MDA-MB-231(breast) MDA-MB-435 (breast), A549 and MV522 (NSCLC)
Concentrations	10 pM to 10 μM
Medium	RPMI with 10% FBS
Solvent	PBS
Exposure time	Three hours, 72 hour recovery
Assay	MTS colorimetric assay for viable cells

4) *In Vitro* Activity of PDX and (b) (4) in Five Cancer Cell Lines

Major finding

The following table from the study report shows that A549 cells are resistant to pralatrexate toxicity. These cells are polarized and may not take up pralatrexate. The compound inhibits other cell lines in this experiment at mean concentrations between 6.7 and 54 nM. (b) kills cells at concentrations only above 1 μM in this assay, thus it is considerably less toxic than pralatrexate in this assay.

Table 4.1 Growth Inhibitory Activity of PDX and (b) (4) in Human Cancer Cell Lines

Cell Line	Pralatrexate IC_{50}[a] in nM	(b) (4) IC_{50}[a] in nM
A549	>10,000	>10,000
NCI-H460	6.7	>10,000
MDA-MB231	25.0	1,980
MDA-MB435	35.1	1,377
SK-BR3	54.3	>10,000

[a] IC_{50} = medial growth inhibitory concentration

Study Number	Sponsor PDX-K-08068-U,
File name	PDX-K-08068-U—nonclinical-data.pdf
Laboratory	(b) (4)
Study Date	May 2008
GLP	No
Audited	No
Drug	Pralatrexate, Lot and purity not stated and other approved cancer drugs (see above)
Methods	
Cells	Human cancer cell lines – MDA-MB231 (human breast cancer), MDA-MB435 (human breast cancer), NCI-H460 (human lung cancer), A549 (human lung cancer), SK-BR3 (human breast cancer)
Concentrations	10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0.003 μM
Medium	Not Specified
Exposure time	Three hours, 72 hour recovery
Assay	MTS colorimetric assay for viable cells

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5) *In Vivo* Evaluation of PDX as a Single Agent in the NCI-H460 Human Non-Small Cell Lung Tumor Xenograft Model in Athymic Nu/Nu Mice

Major finding

In this experiment, only taxotere treatment slowed tumor growth appreciably over an 18 day course. Treatment with 2 mg/kg pralatrexate was somewhat more effective than treatment with 1 mg/kg with pralatrexate or treatment with methotrexate.

Study number	Sponsor – PDX-T-06045-m
Filename	PDX-T-06045m—nonclinical-data.pdf
Laboratory	(b) (4)
Date	November 2005
GLP	No
Audited	No
Drug	Pralatrexate, Lot 57000, 98.7% pure
Method	
Species	Female nude mice (nu/nu, (b) (4)
Implanted tumor	NCI-H460, Human non-small cell lung tumor line
N	9 per group

Doses	Group 1 – Control Group 2 – Pralatrexate – 1 mg/kg Group 3 – Pralatrexate – 2 mg/kg Group 4 – Pemetrexed – 150 mg/kg Group 5 – Methotrexate – 1 mg/kg Group 6 – Methotrexate – 2 mg/kg Group 7 – taxotere – 12.5 mg/kg
Route	IP fro groups 1 through 6, IV for group 7
Schedule	Daily for five days per week for two weeks in groups 1 through 6 Daily for two days for three cycles (not otherwise specified) for group 7
Body weight	Daily
Assessment	Change in Tumor volume, tumor necrosis
Necropsy	Day 18

Results

Mortality and Body Weight — The following table from the study report shows the changes in body weight and mortality in each of the study groups

Table 2: Single Agent Animal Weight Data (Day 15)

				% WEIGHT	NADIR		DRUG DEATHS	
GROUP	DOSE	ROUTE	SCHEDULE	CHANGE	% CHANGE	DAY	TOTAL	DAY (#)
0.9% Saline	---	IP	QDx5x2	3.7%	---	---	0	---
PDX (pralatrexate)	1 mg/kg	IP	QDx5x2	-3.7%	-3.7%	15	0	---
PDX (pralatrexate)	2 mg/kg	IP	QDx5x2	-15.8%	-15.8%	15	1	15
Alimta (pemetrexed)	150 mg/kg	IP	QDx5x2	-3.0%	-3.0%	15	0*	
Methotrexate	1 mg/kg	IP	QDx5x2	2.6%	-1.6%	11	1	15
Methotrexate	2 mg/kg	IP	QDx5x2	-6.5%	-6.5%	15	1	15
Taxotere (docetaxel)	12.5 mg/kg	IV	Q2Dx3	-26.3%	-26.3%	15	1	13

N=9/GRP ON DAY 1

Tumor volume — The following table from the study report shows drug related changes in tumor volume.

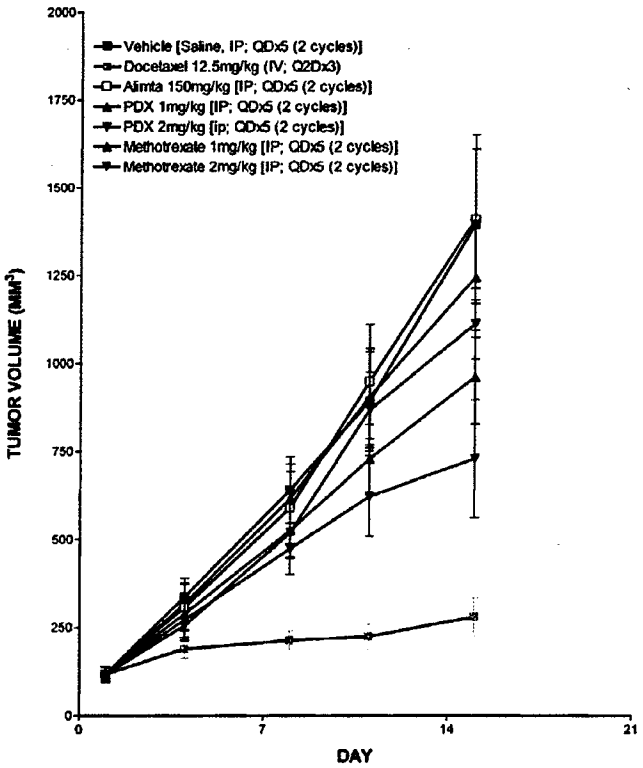
Table 3: Single Agent Tumor Volume Data (Day 15)

GROUP	DOSE	ROUTE	SCHEDULE	FINAL MEAN VOLUME ± SEM	%TGI	%T/C	#PR/CR	%PR
0.9% Saline	—	IP	QDx5x2	1396.5 ± 215.8	—	—	0/0	—
PDX (pralatrexate)	1 mg/kg	IP	QDx5x2	962.8 ± 133.4	34%	66%	0/0	—
PDX (pralatrexate)	2 mg/kg	IP	QDx5x2	730.7 ± 167.6	52%	48%	0/0	—
Alimta (pemetrexed)	150 mg/kg	IP	QDx5x2	1411.7 ± 240.5	—	—	0/0	—
Methotrexate	1 mg/kg	IP	QDx5x2	1245.5 ± 170.0	12%	88%	0/0	—
Methotrexate	2 mg/kg	IP	QDx5x2	1113.8 ± 53.2	22%	78%	0/0	—
Taxotere (docetaxel)	12.5 mg/kg	IV	Q2Dx3	208.4 ± 54.8	88%	12%	0/0	—

N=9/GRP ON DAY 1

The following graph from the study report presents these results as a function of time over the course of the experiment.

Figure 3
Mean Group Tumor Volume
PDX vs. H460 Human Lung Tumor Xenograft Model



Mechanism of action:

1) **DeGraw *et al.* (J. Med. Chem. 1993, 36, 2228-2231). Synthesis and antitumor activity of 10-propargyl-10-deazaaminopterin. J. I., J. Med. Chem. 1993, 36, 2228-2231. See Introduction above.**

2) **F.M. Sirotnak. J.I. DeGraw· W.T. Colwell· J.R. Piper, A new analogue of 10-deazaaminopterin with markedly enhanced curative effects against human tumor zenografts in mice, *Cancer Chemother Pharmacol* (1998) 42: 313-318**

This work follows on that of DeGraw *et al.* (*vide supra*). The authors compared three well established anti-folate drugs to PDX, aminopterin (AMT), methotrexate, and edatrexate (EDX) for differences in binding properties and cytotoxicity. The figure from the paper shows that these drugs differ in there structure only by the substituted alkyl group at the 10-carbon.

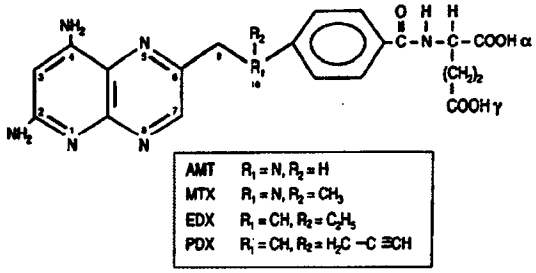


Fig. 1 Structural differences among the various folate analogues utilized in these studies

The investigators determined the kinetic properties for three different reactions for each of the four anti-folates in separate assays, the inhibition of DHFR (isolated enzyme), activity for the one-carbon, reduced folate transport (here folate influx into CCRF-CEM cells in vitro) and the activity of each as a substrate for folylpolyglutamate synthetase (FPGS, as a cell free extract) and provide the results in the following table.

Table 1 Biochemical and cellular pharmacokinetic properties of various folate analogues in CCRF-CEM cells. The values shown are means ± SE from three to five separate experiments. Experimental details are provided in the text

Compound	DHFR inhibition[a] K_i (p*M*)	Influx K_m (μ*M*)	Influx V_{max} (pmol/min/mg protein)	V_{max}/K_m	FPGS activity K_m (μ*M*)	FPGS activity V_{max} (pmol/h/mg protein)	V_{max}/K_m
AMT	4.9 ± 1	1.2 ± 0.2	3.6 ± 1	3.0	5.8 ± 1	117 ± 18	20.2
MTX	5.4 ± 2	4.8 ± 1	4.1 ± 1.2	0.9	32.2 ± 5	70 ± 10	2.2
EDX	5.8 ± 1	1.1 ± 0.1	3.9 ± 0.9	3.5	6.3 ± 1	65 ± 9	10.3
PDX	13.4 ± 3	0.3 ± 0.1	3.8 ± 1.3	12.6	5.9 ± 1	137 ± 26	23.2

[a] Reaction carried out at pH 6.9

The investigators seem here to have assumed a simple Michaels-Menten model for each of these reactions which is unlikely to accurately describe the reaction in any of the three. This is known not to be the case with methotrexate where there are two separate binding processes for methotrexate that involve two different conformations of the enzyme DHFR, a slow and fast binding phase (P. T. Ravi Rajagopalan *et al. PNAS.* October 15, 2002, **99**(21);13481–13486). Also, this assumption will provide an acceptable comparison if each of the four drugs work by the same mechanism; this is unknown as the kinetics of pralatrexate have not been adequately studied. Nevertheless, here again the effective inhibition of DHFR by pralatrexate is at a higher concentration than that of any of the other anti-folates, but the K_m for influx is the lowest

concentration among the four. The work also demonstrates that pralatrexate is polyglutamated at about the same rate and concentration as aminopterin and at a higher rate and lower concentration than methotrexate.

Drug activity related to proposed indication:

The submission included several studies demonstrating the destruction of cancer cells *in vitro* or the decrease in the size of tumor explants as a function of exposure to pralatrexate. I have not reviewed all of these studies as the results are usually not predictive of clinical efficacy.

2.6.2.3 Secondary pharmacodynamics

Not studied

2.6.2.4 Safety pharmacology

1) Effect of (RS)-1 O-Propargyl-l O-deazaaminopterine (PDX) on Action Potential Parameters in Dog Isolated Purkinje Fibers

Major findings

Pralatrexate at concentrations of 0, 0.34, 0.67 or 1.66 mg/mL (0, 0.71, 1.40 or 3.48 mM), caused dose dependant decreases in V_{max} (about 11% at the highest concentration) and increases in action potential duration at 30, 60 and 90% repolarization (about 35%, 12% and 9% respectively, at the highest concentration) compared to controls. These changes were not statistically significant and the concentrations of the drug are well above those that would be physiologically relevant. These concentrations did not affect resting membrane potential, overshoot or action potential amplitude. The positive control, dl-sotalol, gave a positive result.

Study number	Sponsor – PDX-T-04005-D, Laboratory – 1263DAS2.001
Filename	PDX-T-04005-D—nonclinical-data.pdf
Laboratory	(b) (4)
Date	November 2003
GLP	Yes
Audited	Yes
Drug	Pralatrexate, Lot 57000, 98.7% pure
Method	
Species	Beagle dogs
N	4
Tissue	Isolated perfused cardiac Purkinje fibers
Doses	0, 0.4, 0.8 or 2.0 mg/mL (0, 8.4, 1.68 or 4.2 mM) nominal 0, 0.34, 0.67 or 1.66 (0, 0.71, 1.40 or 3.48 mM) assayed Sequentially perfused, 25 minute equilibration
Perfusate	Tyrode's solution (5.9 to 5.0 mL/min)
Electrical pace	0.5 and 1 Hz
Parameters	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 and APD90) repolarization.

Positive control d,l-sotalol (end of each experiment)

Table 1
Effect of (RS)-10-Propargyl-10-deazaaminopterine (PDX) on Action Potential Parameters in Dog Isolated Purkinje Fibers
(b) (4)Study Number: 1263DA52.001

1.0 Hz

Treatment	n	RMP (mV)	OS (mV)	APA (mV)	V_{max} (mV/ms)	APD_{30} (ms)	APD_{60} (ms)	APD_{60} (% Change)#	APD_{90} (ms)	APD_{90} (% Change)#
Control	4	-92.5± 2.59	32.5± 1.09	125.0± 3.29	606.9± 27.17	67.6± 39.55	280.0± 24.27	0.0± 0.00	331.9± 25.97	0.0± 0.00
PDX 0.4 mg/ml	4	-93.0± 2.94	34.3± 1.68	127.4± 2.22	603.0± 61.31	82.3± 39.72	305.2± 45.70	8.6± 7.58	354.0± 43.89	6.5± 6.65
PDX 0.8 mg/ml	4	-93.9± 2.95	33.5± 0.51	127.4± 2.66	606.5± 19.98	76.7± 27.77	306.7± 43.25	9.3± 7.54	356.1± 45.10	7.2± 7.80
PDX 2.0 mg/ml	4	-93.2± 2.68	30.7± 5.45	123.9± 5.94	541.4± 87.86	91.4± 30.23	312.7± 39.80	11.4± 4.87	363.5± 49.18	9.3± 7.53
dl-Sotalol 50 µM	4	-91.8± 4.32	32.1± 2.77	124.0± 5.97	579.0± 149.28	75.8± 46.96	383.5± 22.45	23.7± 12.94	462.4± 60.06	27.6± 9.95*

Data are expressed as mean ± standard deviation
RMP = Resting Membrane Potential
OS = Overshoot
APA = Action Potential Amplitude
V_{max} = Maximum Rate of Depolarization of the Action Potential Upstroke
#Percentage changes for test article and dl-sotalol were calculated from control and PDX (2.0 mg/ml) values, respectively.

APD_{30} = Action Potential Duration at 30% repolarization
APD_{60} = Action Potential Duration at 60% repolarization
APD_{90} = Action Potential Duration at 90% repolarization
* Statistically significant ($P<0.05$) change when compared to th PDX (2.0 mg/ml) group. ANOVA followed by Tukey Multiple Comparison Test

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2) Effects of PDX on hERG K^+-Currents in CHO-K1 Cells

Major findings

Pralatrexate inhibited the hERG current in transfected CHO cells at concentrations of 0.4 (2%, 0.84 mM), 0.8 mg/mL (37%, 1.7 mM), 2 mg/mL (54%, 4.2 mM) and 4 mg/mL (54%, 8.4 mM). The results for the highest three concentrations were statistically significant. Nevertheless, these concentrations are well above those that would be pharmacologically relevant. The positive control gave a positive result at a concentration of 3 µM.

Study number	Sponsor – PDX-T-05018-H, Laboratory – 1767/THI/03
Filename	PDX-T-05018-H—nonclinical-data.pdf
Laboratory	(b) (4)
Date	September 2003
GLP	No
Audited	No
Drug	Pralatrexate, Lot 57000, 98.7% pure
Method	
Cells	CHO-Kl cells (Chinese hamster ovary cells transfected wit the human ether-a-go-go gene)

N	At least 5 tests per concentration (11 total cells used, 3 for control)
Doses	0, 0.4, 0.8, 2 or 4 mg/mL (0, 0.838, 1.68, 4.19, or 8.38 mM)
Positive control	E-4031(N-[4-[[1-[2-(6-Methyl-2-pyridinyl)ethyl]-4-piperidinyl] carbonyl]phenyl]methanesulfonamide dihydrochloride), 3 μM

Results

Table 1: Effect of PDX on tail currents, expressed as percentage of vehicle current and percentage inhibition of vehicle current.

		I_{tail}				
		0.4 mg/mL	0.8 mg/mL	2 mg/mL	4 mg/mL	Wash
PDX	Cell 1	99	53	-	-	-
	Cell 2	-	-	59	70	72
	Cell 3	103	60	-	-	-
	Cell 4	-	-	49	31	36
	Cell 5	100	79	33	31	37
	Cell 6	97	-	-	-	-
	Cell 7	-	76	-	-	-
	Cell 8	-	-	45	-	-
	Cell 9	-	-	-	55	79
	Cell 10	91	49	-	-	-
	Cell 11	-	-	41	44	52
% of vehicle current	Mean ± SEM	98 ± 2	63 ± 6	46 ± 4	46 ± 7	55 ± 9
% inhibition		2	37	54	54	45

- : Not Determined

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3) Pralatrexate (PDX): A Cardiovascular Safety Pharmacology and Respiratory Function Study in Beagle Dogs

Major findings

Treatment with pralatrexate at doses up to 0.7 mg/kg caused no toxicologically significant changes in blood pressure (systolic, diastolic, and mean arterial pressure), heart rate, body temperature, electrocardiographic parameters (heart rate, PR, QRS, RR, and QT/QTc), respiratory function (respiratory rate, blood oxygen saturation, and end tidal CO_2).

The doses in this study caused only minor and incidental gastrointestinal toxicity. The highest dose is only half that given clinically on a mg/m^2 basis. Because the doses were too low this study provides little useful information about the potential for cardio-respiratory toxicity of pralatrexate.

Study no:	Sponsor – PDX-T-07036-D; Laboratory – 1128-05866
File name:	pdxt07036d—nonclinical-data.pdf
Laboratory:	(b) (4)
Study Date:	October 2006
GLP:	Yes
Audited:	Yes
Drug:	Pralatrexate Injection, Lot 132I0606, Purity 98.14 %
Method	

Species	Beagle dogs, conscious with surgically implanted telemetry devices
N	Four males (10 to 11 months, 13 to 13.9 kg) Four females (9 to 12 months, 9.1 to 9.9 kg)
Doses	0, (SD 1 and 29) 0.1 mg/kg or 2 mg/m^2 (SD 8) 0.5 mg/kg or 10 mg/m^2 (SD 15) 0.7 mg/kg or 14 mg/m^2 (SD 22 and 36)
Schedule	Single doses on days 1, 8, 15, 22, 29 and 36
Route	IV, cephalic vein
Cage Side	Observed at least twice daily
Clinical Obs.	Prior to each dose
Post Dose Obs.	Three hours following each dose
Body weight	Prior to the administration of each dose and SD 37
Cardiac parameters	Heart rate, body temperature, systolic and diastolic blood pressure, mean arterial pressure (MAP), and ECG waveform were recorded from approximately 30 minutes (± 5 minutes) prior to dosing and continuously through 8 hours (± 30 minutes) post-dose and 22-24 hours (± 15 minutes) post-dose following dosing on SD 1, 8, 15, and 22
Respiratory	Blood oxygen saturation (SpO_2) and end-tidal CO2 ($ETCO_2$) were measured by capnogragh and respiration rate was measured manually prior to dosing (60 minutes ± 10 minutes), and at 30 minutes ± 5 minutes, 60 minutes ± 10 minutes, 90 minutes ± 15 minutes, 120 minutes ± 15 minutes, 4 hours ± 15 minutes, and 24 hours ± 15 minutes post-dose on SD 29 and SD 36

Results

Mortality	None
Clinical Observations	The incidence of diarrhea, emesis, soft feces increased with time (hence exposure)
Body Weight	Males and females lost weight while on study but the change was less than 5%
Body Temperature	No toxicologically significant effects
Heart Rate	No toxicologically significant effects
Blood Pressure	No toxicologically significant effects
ECG	No toxicologically significant effects
Respiratory function	No toxicologically significant effects

4) Pralatrexate (PDX): Single Intravenous-Dose Neurobehavioral Study in Sprague-Dawley Rats

Major findings

A single IV dose of pralatrexate of 25 mg/kg (150 mg/m^2) caused no change in the neurobehavioral parameters or the functional ability of male and female rats. The doses in this study were too low to provide useful information about the toxicity of pralatrexate.

Study no:	Sponsor – PDX-T-07037-R; Laboratory – 1128-05865
File name:	pdxt07037r—nonclinical-data.pdf
Laboratory:	(b) (4)
Study Date:	October 2006
GLP:	Yes (except for stability analysis)
Audited:	Yes
Drug:	Pralatrexate Injection, Lot 132I0606, Purity 98.14 %
Method	
Species	Sprague Dawley Rats
N	16 per sex per dose group 8 per sex per dose for Functional Observational Battery (FOB) 8 per sex per dose for Locomotor Activity (LMA)test
Doses	0, 5, 10, or 25 mg/kg (0, 30, 60, or 150 mg/m^2)
Schedule	Single dose
Route	IV tail vein
Dose Volume	1.27 mL/kg
Cage side	At least twice a day
Clinical Obs	24 hours before and after dosing
FOB	24 ± 2 hours prior to dosing (SD -1), 5 ± 2 minutes post dose (C_{max}) on SD 1 and 24 ± 2 hours post-dose (SD 2)
LMA	24 ± 2 hours prior to dosing (SD -1), 5 ± 2 minutes post dose (C_{max}) on SD 1 and 24 ± 2 hours post-dose (SD 2)
Histopathology	Not done

Results

Mortality	None
Clinical Observations	None
Body Weight	No effects
FOB	No toxicologically significant changes
LMA	No toxicologically significant changes

2.6.2.5 Pharmacodynamic drug interactions

Not Studied

2.6.3 Pharmacology Tabulated Summary

Study Title	Study Number	GLP	Test Syste	Dosing	Doses	Result
Growth Inhibitory Activity of Pralatrexate in the NCI-60 Cancer Cell Panel	PDX-P-06069-U	No	Human car	in vitro	< 0.1 µM	**Pralatrexate inhibited the variety of human cancer**
Cytotoxicity of test article in human hepatocytes	PDX-K-06020-U	No	Human hep	in vitro	up to 200µM	not toxic
Evaluation of Growth Inhibitory Activity of the Allos Agent PDX versus Several Human Tumor Cell Lines	PDX-K-04004-U	No	Human car	in vitro	12.8 to 65.1 nM	**Pralatrexate inhibited the** cancer cell types
In Vitro Activity of PDX and 4-OH PDX in Five Cancer Cell Lines	PDX-K-08068-U	No	Human car	in vitro	1 to 10 µM	Polarized cells may be m **pralatrexate than other ce**
In Vivo Evaluation of PDX as a Single Agent in the NCI-H460 Human Non-Small Cell Lung Tumor Xenograft Model in Athymic Nu/Nu Mice	PDX-T-06045-m	No	Mice	IP	1 to 2 mg/kg	**Pralatrexate caused a mi** growth
Effect of (RS)-1 O-Propargyl-l O-deazaaminopterine (PDX) on Action Potential Parameters in Dog Isolated Purkinje Fibers	PDX-T-04005-D	Yes	Dog	in vitro	0.7 to 3.5 mM	**No effect on purkinje acti physiologically relavant c**
Effects of PDX on hERG K+-Currents in CHO-K1 Cells	PDX-T-05018-H	No	CHO cells	in vitro	0.84 to 8.4 mM	**Pralatrexate did not inhibi at physiologically relavant**
Pralatrexate (PDX): A Cardiovascular Safety Pharmacology and Respiratory Function Study in Beagle Dogs	PDX-T-07036-D	Yes	Dog	IV	to 14 mg/m^2	**Pralatrexate caused no c parameters at low doses**
Pralatrexate (PDX): Single Intravenous-Dose Neurobehavioral Study in Sprague-Dawley Rats	PDX-T-07037-R	Yes	Rats	IV	to 150 mg/m^2	**Pralatrexate caused no c neurobehavioral paramet** doses

(b) (4)

2.6.4 Pharmacokinetics and Toxicokinetics

2.6.4.1 Brief summary

In males rats given doses of 30, 60 or 150 mg/m^2, elimination from plasma was triphasic with a rapid distribution phase (less than 20 minutes), a comparatively rapid elimination or further distribution, and a very long terminal elimination phase with a half life of 307 to 783 minutes for pralatrexate-10a and 151 to 1267 minutes for 10b. In females, the terminal elimination half-life values represent the shorter phase in males that follows initial distribution. Values for this phase in females are about 50 minutes on average. AUC and C_{max} increased linearly with dose and were dose proportional. Elimination in the urine appeared biphasic and the urine AUC did not increase linearly with dose suggesting the saturation of an elimination process. Renal excretion was less then 5% of total clearance in all cases. The volume of distribution at steady state indicates significant tissues distribution and increases with dose beyond 10 mg/kg, suggesting saturation of plasma protein binding. Clearance values in females are about two-fold than those in males.

In a study of bioavailability, a single IV bolus dose of 5 mg/kg (30 mg/m^2) in rats resulted in maximum concentrations of racemic pralatrexate 10a and 10b above 12 μM each. Elimination from plasma was similar to that described in the experiment above. Clearance was much greater than renal flow suggesting large extrahepatic clearance or irreversible tissue binding. In females, the elimination curve seen after intraduodenal or oral dosing suggests flip-flop kinetics where the reservoir in the GI is pumped into the circulation at a rate somewhat slower than elimination. That is absorption is rate limiting. The profiles for ID and PO are identical and the pattern is the same for PDX-10a and 10b. In males elimination after an ID or PO dose is somewhat different from that of females but consistent between PDX-10a and 10b. Factors that could cause the difference between PO and ID administration include pH differences between the duodenum and stomach or differences in the folate pumping mechanisms between the two tissues. In all cases, bioavailability is somewhat low, about 26% for PO and 18% ID administration for PDX-10a and about 19% for PO and 11% ID administration for PDX-10b (means both sexes).

In dogs given a single IV injection of 6, 20 or 60 mg/m^2, the C_{max} and AUC of pralatrexate 10a and 10b enantiomers increase linearly with increasing dose and are dose proportional. C_{max} for PDX-10a at the high dose of 60 mg/m^2 was 5.2 μM for males and 6.6 μM for females. C_{max} for PDX-10b at this dose was 4.0 μM for males and 4.4 for females. Thus, the combined concentration of both enantiomers after this dose exceeded 10 μM. Total clearance of PDX-10a was consistently about half the value of the total clearance of 10b resulting in consistently greater values for C_{max} and AUC of 10a. Clearance was consistently greater in males (range 0.08 to 0.12 L/min for 10a) compared to females (range 0.06 to 0.08 L/min for 10a) resulting in greater values for AUC and C_{max} in females. The ratio of non-renal to renal clearance is about 4 at the low dose (6 mg/m^2) but increases to 20 or more at the mid dose of 20 mg/m^2 implying saturation of the renal elimination mechanism. Non-renal clearance values are less than hepatic flow. Volume of distribution exceeds 20 L implying significant extravascular distribution. As with rats, elimination is visually triphasic with a rapid distribution phase, a slower elimination phase, and a very slow terminal elimination phase with a half-life that increases with increasing dose, again consistent with a saturable elimination. Urinary elimination is biphasic consistent with the two elimination phases demonstrated in plasma.

Little pralatrexate crosses the blood brain barrier (BBB). Plasma binding is about 67% and little of the drug crosses into RBCs.

Pooled human microsomes or S9 fraction do not appear to metabolize pralatrexate; neither do human hepatocytes *in vitro*. But in human liver microsomes, pralatrexate caused concentration dependant inhibition of the activity of cytochrome P450 2C19. Activity decreased to 71% at a concentration of 20 μM and was below detection at 50 μM or greater. The compound did not cause dose dependant inhibition of cytochromes P450 1A2, 2A6, 2C9, 2D6 or 2E1. Pralatrexate did not induce an increase in the activity of cytochromes P450 1A2, 3A4 or 2C19 when incubated with fresh human hepatocytes. The results for the experiment with 2C19 are somewhat ambiguous since pralatrexate inhibits the activity of this enzyme in human microsomes.

Mass balance determined by radiolabeled experiments provided values for urine and fecal elimination that were inconsistent with those determined by HPLC/MS/MS analysis of the parent compound. The values for elimination were much higher than those obtained from HPLC analysis. The radiolabel on pralatrexate used in the mass balance experiments was on the carboxylic acid moiety of the glutamic acid side chain, the most labile portion of the molecule so total recovery and percentages remaining in tissues do not represent the actual elimination of the folate analog backbone of the molecule.

2.6.4.2 Methods of Analysis

1) Validation of a Method for the Determination of PDX Enantiomers in Mouse Plasma

Major findings

The method was sensitive and specific for both enantiomers. The LOQ was 0.5 ng/mL for each enantiomer, as determined by acceptable accuracy and precision for QC pools prepared at this concentration (accuracy was required to be within ± 20% of theoretical and reproducibility was required to be within ± 20% RSD). The analysis was linear over the range of 0.5 to 1,000 ng/mL with r^2 values greater than 0.99.

Study number	PDX-K-08057-M
File name	pdxk08057m—nonclinical-data.pdf
Laboratory	(b) (4)
Study Date	September 2005
GLP	Yes
Audited	Yes
Drug	(10a)-10-Propargyl-10-deazaaminopterin (PDX-10a-isomer), Lot: 57349 Optical Purity (by HPLC): 98.8% 10b)-10-Propargyl-10-deazaaminopterin (PDX-10b-isomer), Lot: 57352 Optical Purity (by HPLC): 98.2%
Method	
Tissue	Mouse plasma
Concentrations	0 to 10000 ng/mL
Internal standard	(b) (4)
Extraction	(b) (4)
Mobile phase	700 mL of ethanol, 300 mL of methanol, and 0.5 mL of triethylamine
Analysis	HPLC
Detection	LC/MS/MS System: Sciex API 3000

All of the analytical validations were done in the same way as the one reviewed above thee included

2) **Validation of a Method for the Determination of PDX Enantiomers in Mouse Plasma** Study – PDX-K-08057-M

3) **Validation of a Method for the Determination of PDX Enantiomers in Rat Plasma and Urine** PDX-K-05012-R

4) **Validation of a Method for the Determination of PDX Enantiomers in Rabbit Plasma** PDX-K-08056-B

5) **Validation of a Method for the Determination of PDX Enantiomers in canine Plasma and Urine** PDX-K-05011-D

6) **Validation of an LC/MS/MS Method for the Quantitation of (b) (4) Canine Plasma** PDX-K-08070-D

The following table summarizes the results of these validation studies:

					LLOQ both enantiomers		Linear Range				
Study Number	Study	Tissue	Method	Matrix Effect	ng/mL	nM	Lower ng/mL	Upper ng/mL	Lower nM	Upper nM	R^2
PDX-K-08057-M	Validation of a Method for the Determination of PDX Enantiomers in Mouse Plasma	Plasma	HPLC/MS/MS	No	0.5	1.05	0.5	1000	1.0	2094.3	> 0.99
PDX-K-05012-R	Validation of a Method for the Determination of PDX Enantiomers in Rat Plasma and Urine	Plasma	HPLC/MS/MS	No	1	2.09	1	1000	2.1	2094.3	> 0.99
		Urine	HPLC/MS/MS	No	0.5	1.05	0.5	1000	1.0	2094.3	> 0.99
PDX-K-08056-B	Validation of a Method for the Determination of PDX Enantiomers in Rabbit Plasma	Plasma	HPLC/MS/MS	No	0.5	1.05	0.5	1000	1.0	2094.3	> 0.99
PDX-K-05011-D	Validation of a Method for the Determination of PDX Enantiomersin canine Plasma and Urine	Plasma	HPLC/MS/MS	No	0.5	1.05	0.5	1000	1.0	2094.3	> 0.99
		Urine	HPLC/MS/MS	No	0.5	1.05	0.5	1000	1.0	2094.3	> 0.99
PDX-K-08070-D	Validation of an LC/MS/MS Method for the Quantitation of (b) (4) in Canine Plasma	Plasma	HPLC/MS/MS	no	1	2.09	1	1000	2.1	2094.3	> 0.99

2.6.4.3 Absorption

1) **Bioavailability of Pralatrexate in Male and Female Sprague-Dawley Rats Following Intravenous, Oral Gavage, or Direct Duodenal Administration**

Major findings

A single IV bolus dose of 5 mg/kg (30 mg/m^2) in rats results in maximum concentrations of racemic pralatrexate 10a and 10b above 12 μM each. Elimination is visually triphasic with a rapid distribution phase, a slow elimination phase (or longer distribution phase) and a slow terminal elimination. The elimination curves suggest the possibility of redistribution or enterohepatic recirculation. Clearance is greater in males than in females and the clearance of

PDX-10a is greater than that of 10b thus the AUC values for PDX-10b are higher than 10a. Clearance is much greater than renal flow suggesting large extrahepatic clearance of irreversible tissue binding. The volume of distribution is greater than total body volume.

The elimination curves after oral or intraduodenal dosing suggest the possibility of flip-flop kinetics where absorption is slower than elimination. The absorption processes is probably limited by folate transporters.

Study number	PDX-K-07046-R
Filename	PDXk07046-R—nonclinical-data.pdf
Laboratory	(b) (4)
Study date	August 2007, September 2007
GLP	No
Audited	No
Drug	Pralatrexate, Lot 174I0706, 99.5% pure
Method	
Animals	Sprague-Dawley Rats (243.7 to 264.2 g females, 267.1 to 306.9 males)
Dose	5 mg/kg (30 mg/m^2) Nominally 2.5 mg/kg pralatrexate-10a and 2.5 mg/kg 10b Actual dose calculated from mean weight – 0.64 g females, 0.71 g males
N	12 per sex per treatment group
Route	Group 1 – single intravenous dose Group 2 – oral gavage dose Group 3 – direct duodenal dose (duodenal catheters implanted)
Dose volume	5 mL/kg
Solvent	Buffered saline
Sampling	5, 10, 20, 30, and 45 minutes post-dose, and 1, 2, 4, 12, 24, 36, and 48 hours post-dose, three rats per sex per time point
Histopathology	Not done
Analysis	HPLC
Parameter Analysis	Noncompartmental
Internal standard	(b) (4)

The initial study done in August was aborted "[b]ecause plasma volumes were less than required, the study was repeated using the original protocol."

Results

Mortality	One Group 3 female died during blood collection, the investigators did not consider the death drug related
Clinical Signs	None
Body Weight	Slight body weight loss over the first 24 hours possibly due to blood sampling
Pharmacokinetics	
Estimated LOQ	0.2 ng/mL = 0.42 nM

The following graphs from the study report show the decline in pralatrexate-10a and 10b in the plasma of female rats as a function of time when pralatrexate is given as an IV bolus, PO bolus or intraduodenal bolus.

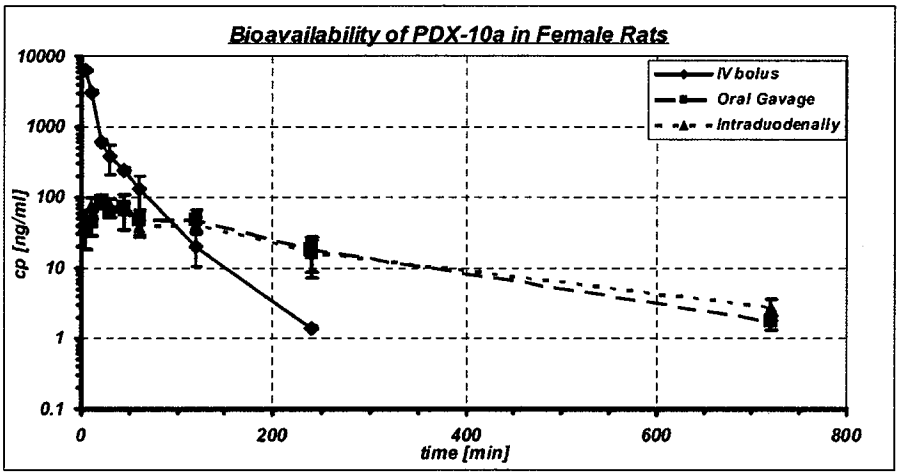

Figure 1a: Plasma concentration-time profiles of PDX-10a after I.V., P.O. and I.D. administration in female rats

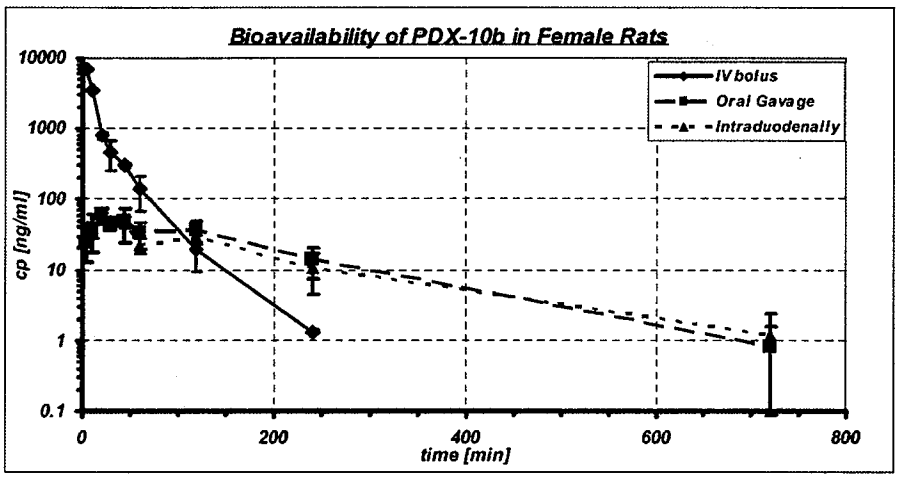

Figure 1b: Plasma concentration-time profiles of PDX-10b after I.V., P.O. and I.D. administration in female rats

The following graphs from the study report show the decline in pralatrexate-10a and 10b in the plasma of male rats as a function of time when pralatrexate is given as an IV bolus, PO bolus or intraduodenal bolus.

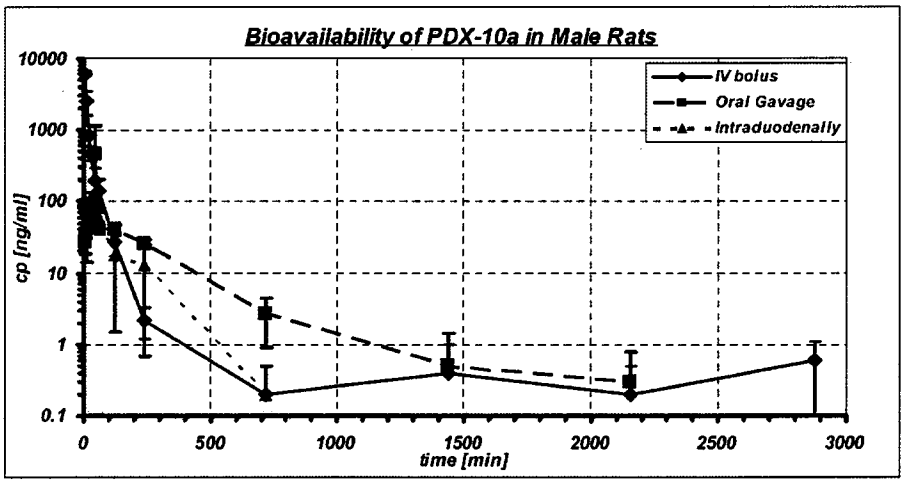

Figure 2a: Plasma concentration-time profiles of PDX-10a after I.V., P.O. and I.D. administration in male rats

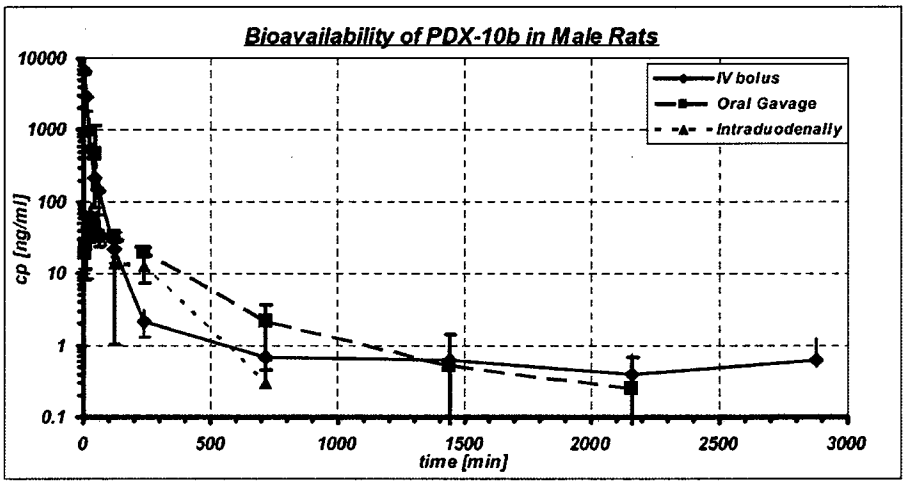

Figure 1d: Plasma concentration-time profiles of PDX-10b after I.V., P.O. and I.D. administration in male rats

Estimated Pharmacokinetic Parameters for Pralatrexate-10a

Females												
Route	Dose	C_{max}	t_{max}	AUC_{trap}	AUC_{∞}	$t_{1/2\ terminal}$	CL_{total}	MRT	V_{dss}	V_{dpss}	F	MAT
	mg	μM	min	μM*min	μM*min	min	mL/min	min	L	L	%	min
IV bolus	0.64	13.04	5	157.89	158.00	26.7	8.4	18	0.15	0.33		
P.O.	0.64	0.18	20	32.02	32.70	132.2		168.7			0.2	150.7
I.D.	0.64	0.19	20	30.53	31.88	164.9		191.2			0.2	173.1
Males												
	Dose	C_{max}	t_{max}	AUC_{trap}	AUC_{∞}	$t_{1/2\ terminal}$	CL_{total}	MRT	V_{dss}	V_{dpss}	F	MAT
Route	mg	μM	min	μM*min	μM*min	min	mL/min	min	L	L	%	min
IV bolus	0.71	12.83	5	159.17	160.42	688.1	9.3	69.5	0.65	9.23	0	
P.O.	0.71	0.98	45	50.36	50.77	454.2		215.5			0.32	146
I.D.	0.71	0.21	30	24.25	24.30	88.5		122.1			0.15	52.6
Mean Males and Females												
	Dose	C_{max}	t_{max}	AUC_{trap}	AUC_{∞}	$t_{1/2\ terminal}$	CL_{total}	MRT	V_{dss}	V_{dpss}	F	MAT
Route	mg	μM	min	μM*min	μM*min	min	mL/min	min	L	L	%	min
IV bolus	2.5	12.93	5	158.53	159.21	357.4	8.9	43.8	0.4	4.78		
P.O.	2.5	0.58	33	41.19	41.74	293.2		192.1			0.26	148.3
I.D.	2.5	0.20	25	27.39	28.09	126.7		156.6			0.18	112.9

Estimated Pharmacokinetic Parameters for Pralatrexate-10b

Females												
Route	Dose	C_{max}	t_{max}	AUC_{trap}	AUC_{∞}	$t_{1/2\ terminal}$	CL_{total}	MRT	V_{dss}	V_{dpss}	F	MAT
	mg	μM	min	μM*min	μM*min	min	mL/min	min	L	L	%	min
IV bolus	0.64	14.21	5	179.33	179.44	27.2	7.4	17.9	0.13	0.29	0	
P.O.	0.64	0.13	20	23.58	23.88	118		160.3			0.13	142.3
I.D.	0.64	0.11	20	19.71	20.24	145		170.9			0.11	153
Males												
	Dose	C_{max}	t_{max}	AUC_{trap}	AUC_{∞}	$t_{1/2\ terminal}$	CL_{total}	MRT	V_{dss}	V_{dpss}	F	MAT
Route	mg	μM	min	μM*min	μM*min	min	mL/min	min	L	L	%	min
IV bolus	0.71	13.71	5	175.91	178.89	1603.4	8.3	131.1	1.09	19.3	0	
P.O.	0.71	0.96	45	42.46	42.81	467.4		212.8			0.24	81.7
I.D.	0.71	0.13	30	19.08	19.17	102.4		143			0.11	11.9
Mean Males and Females												
	Dose	C_{max}	t_{max}	AUC_{trap}	AUC_{∞}	$t_{1/2\ terminal}$	CL_{total}	MRT	V_{dss}	V_{dpss}	F	MAT
Route	mg	μM	min	μM*min	μM*min	min	mL/min	min	L	L	%	min
IV bolus	2.5	13.96	5	177.62	179.16	815.3	7.9	74.5	0.61	9.79	0	
P.O.	2.5	0.55	33	33.02	33.34	292.7		186.5			0.19	112
I.D.	2.5	0.12	25	19.39	19.71	123.7		157			0.11	82.5

A single IV bolus dose of 5 mg/kg (30 mg/m^2) in rats results in maximum concentrations of racemic pralatrexate 10a and 10b above 12 μM each or a total pralatrexate maximum of over 24 μM. Elimination is visually probably triphasic with a rapid distribution phase, a slow elimination phase (or longer distribution phase) and a slow terminal elimination. The slow terminal elimination was not observable in females because concentrations dropped below the detection limit by the 240 min measurement, $t_{1/2}$ measurements are unreliable. In males there was a clear protracted elimination which may represent redistribution back into the plasma from some compartment or possibly enterohepatic redistribution. In males this terminal phase (at barely detectable concentrations) had a half-life of 688 minutes for PDX-10a and 1603 minutes for PDX-10b. Clearance is greater in males than in females and the clearance of PDX-10a is greater than that of 10b thus the AUC values for PDX-10b are higher than 10a. Clearance is much greater than renal flow suggesting large extrahepatic clearance of irreversible tissue binding. Volume of distribution is probably unreliable in females because the concentration drops below

detection so rapidly. Volume of distribution in males is large suggesting extensive tissue distribution.

In females, for both ID and PO administration, T_{max} occurs within the first fifty minutes after dosing. In females, this is followed by a plateau over the next hour that suggests a steady state between absorption processes and elimination or redistribution processes. This is followed by a long exponential elimination phase not seen after IV administration. This is probably due to flip-flop kinetics where the reservoir in the GI is pumped into the circulation at a rate somewhat slower than elimination. That is absorption is rate limiting. The profiles for ID and PO are identical and the pattern is the same for PDX-10a and 10b.

In males elimination after an ID or PO dose is somewhat different from that of females but consistent between PDX-10a and 10b. T_{max} is reached rapidly, within 50 minutes, and again this is followed by a plateau out to about 240 minutes. After ID administration, this plateau is followed by a single exponential decrease that drops below detection after 720 minutes. After PO administration, the plateau is followed by a long biphasic elimination process that once again suggests flip-flop kinetics. Factors that could cause the difference between PO and ID administration include pH differences between the duodenum and stomach or differences in the pumping mechanisms between the two tissues. In all cases, bioavailability is somewhat low, about 26% for PO and 18% ID administration for PDX-10a and about 19% for PO and 11% ID administration for PDX-10b (means both sexes).

The investigators suggested "[a] possible explanation of the limited bioavailability for PDX may be pre-systemic hepatic metabolism, in light of the high value for CL_{tot}, exceeding hepatic blood flow, which is likely to indicate high hepatic extraction. However, as suggested above, the CL_{tot} values are probably overestimating the true clearance since PDX accumulates in intracellular compartments, which return the drug only slowly into plasma, acting as a deep, virtually irreversible deep compartment. Thus, it is not impossible that PDX may actually be a low hepatic extraction ratio drug, and the limited absorption from the GI tract may be due to the effect of efflux transporters in the GI epithelium rather than hepatic (or GI) first-pass metabolism."

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2) PDX (10-propargyl-10-deazaaminopterin): Pharmacokinetic Study of PDX Following Intravenous Administration to Male and Female Sprague Dawley Rats

Major findings

A single intravenous dose of racemic pralatrexate to male and female Sprague Dawley rats at doses of 5, 10, and 25 mg/kg had no effect on mortality, clinical observations, or body weights. In males, elimination from plasma was triphasic by visual inspection with a rapid distribution phase (less than 20 minutes), a comparatively rapid elimination or further distribution, and a very long elimination phase with a half life of 307 to 783 minutes for pralatrexate-10a and 151 to 1267 minutes for 10b. The large variability in these values results from the fact that the values were close to the LLOQ. This long elimination phase did not occur in females, elimination was essentially complete within 200 minutes and the terminal elimination half-life values represent the shorter phase in males that follows initial distribution. Values for this phase in females are about 50 minutes on average. This second phase probably represents distribution into a compartment or enterohepatic recirculation. Plasma AUC increased linearly with dose and the slope of the dose normalized AUC vs dose curve was approximately 0 indication direct dose proportionality. C_{max} increased likewise and both values were greater for males than females. AUC and C_{max} were for both enantiomers were about the same.

The concentrations in the urine in the urine were approximately equal at all time points for the low and mid dose but increased significantly with the high dose suggesting the involvement of a second elimination process. Elimination in the urine appeared biphasic.

Renal excretion was less then 5% of total clearance in all cases. The volume of distribution at steady state indicates significant tissues distribution and increases with dose beyond 10 mg/kg, suggesting saturation of plasma protein binding. Clearance values are about two-fold higher in females than in males. This difference in clearance explains the lack of the third elimination phase in females.

Study number	PDX-K-05010-R, Laboratory – 02958
Filename	PDXK05010-R—nonclinical-data.pdf
Laboratory	(b) (4)
Study date	September 2004
GLP	No
Audited	No
Drug	Pralatrexate, Lot 05010304, 100.1 % pure
Method	
Animals	Sprague Dawley Rats, 8 weeks old males 265.9 - 297.1 g, females 187.2 - 243.2 g
Dose	5, 10 or 25 mg/kg (30, 60 or 150 mg/m^2)
N	12 per sex per dose group
Schedule	Single dose
Route	IV lateral tail vein, 5 minute
Solvent	Buffered saline
Blood Sampling	Pre dose, 5, 10, 20, 30, and 45 minutes, and 1, 2, 4, 12, 24, 36, and 48 hours post dose, three animals per time point
Analysis	HPLC, non-compartmental analysis of the data
Internal Standard	(b) (4)
Histopathology	Not done

Results	
Mortality	Two deaths due to blood collection
Clinical signs	none
Body weight	No effect
Pharmacokinetics	

The following graphs from the study report demonstrate the elimination of pralatrexate-10a and 10b from rat plasma.

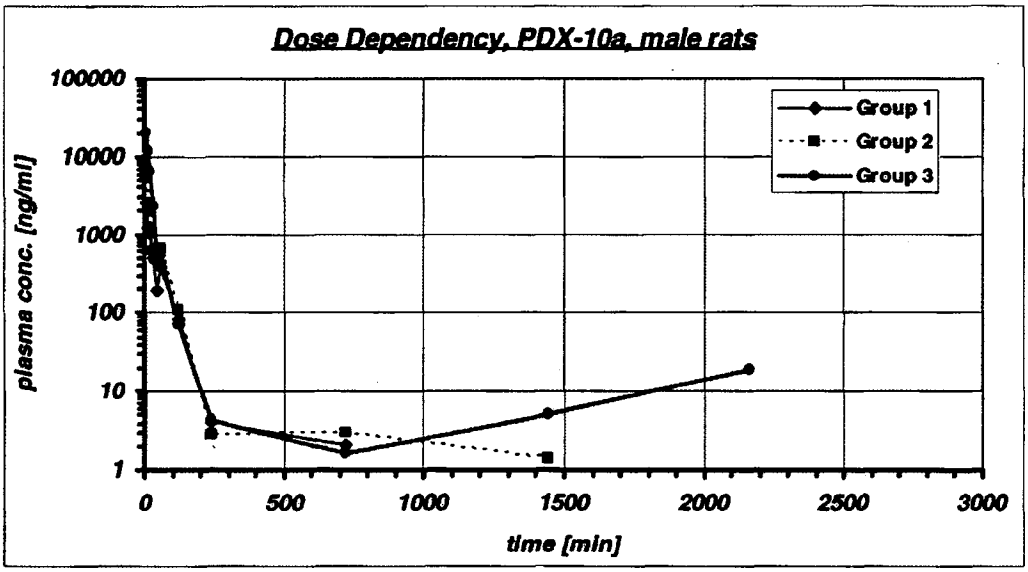


Figure 1a: Plasma concentration-time profile of PDX-10a in male rats

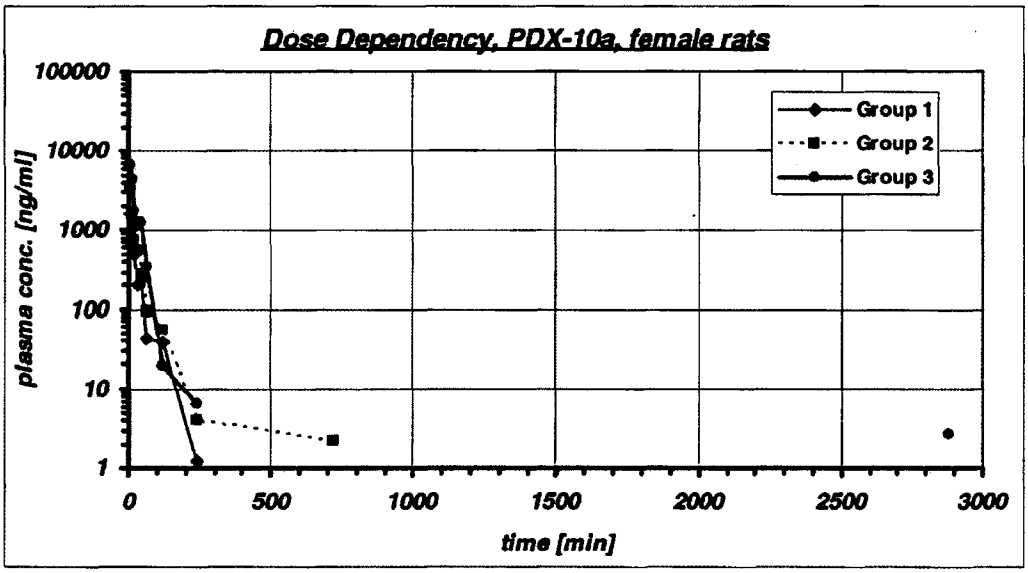


Figure 1b: Plasma concentration-time profile of PDX-10a in female rats

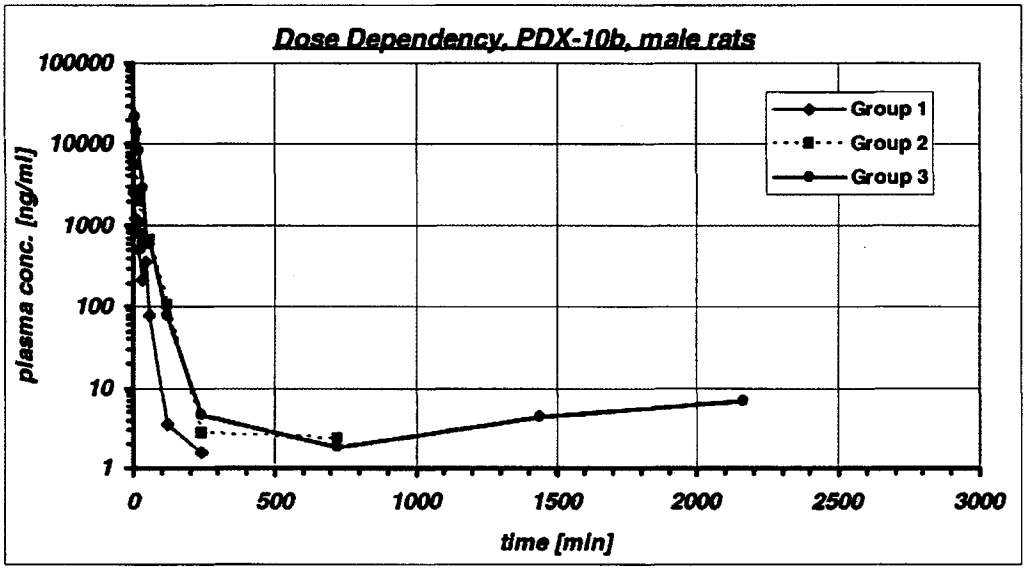


Figure 3a: Plasma concentration-time profile of PDX-10b in male rats

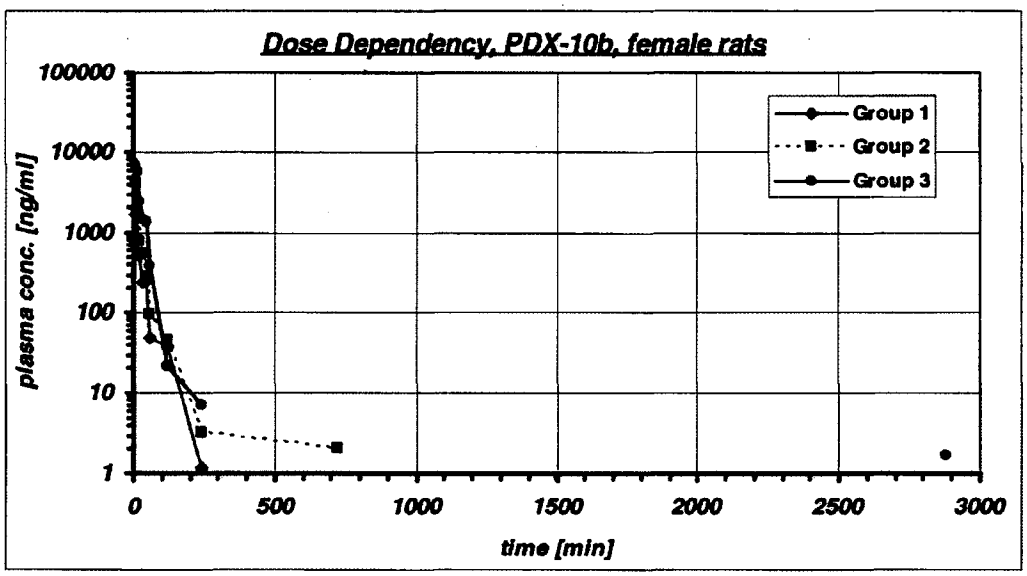
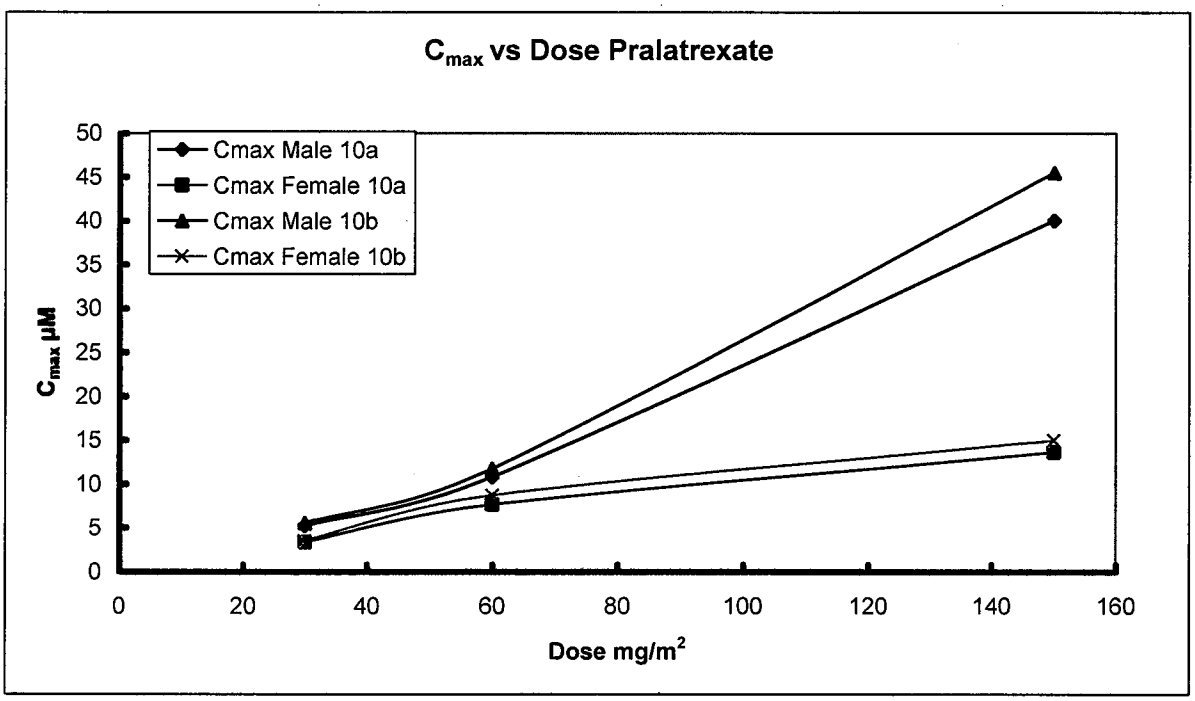


Figure 3b: Plasma concentration-time profile of PDX-10b in female rats

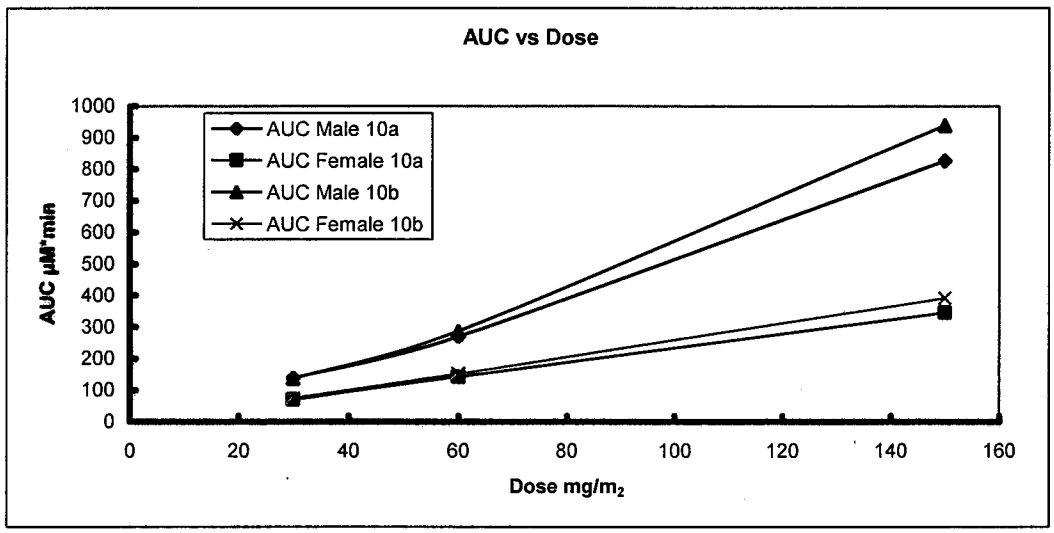
The following table shows the results of this experiment for pralatrexate-10a and 10b.

Analysis for PDX-10a													
units	Dose	Dose	C_{max}	AUC	Cl_{tot}	$t_{1/2}$	MRT_{sys}	V_{dss}	V_{dpss}	f_e	$t_{1/2}^{urine}$	Cl_{renal}	$Cl_{nonrenal}$
Sex	mg/kg	mg/m^2	µM	µM*min	L/min	min	min	L	L	%	min	L/min	L/min
male	5	30	5.24	138	0.0095	463	75	0.7	6.4	2.8%	876	0.26	9.19
male	10	60	10.83	271	0.0097	307	54	0.5	4.2	3.1%	911	0.3	9.4
male	25	150	40.09	826	0.0079	783	246	2	9	3.5%	1061	0.28	7.62
female	5	30	3.38	71	0.0148	29	29	0.5	0.6	4.4%	1350	0.65	14.1
female	10	60	7.65	142	0.0148	132	38	0.6	2.8	3.0%	846	0.44	14.31
female	25	150	13.63	345	0.0152	1262	250	3.8	27.6	4.5%	888	0.68	14.47
Analysis for PDX-10b													
	Dose	Dose	C_{max}	AUC	Cl_{tot}	$t_{1/2}$	MRT_{sys}	V_{dss}	V_{dpss}	f_e	$t_{1/2}^{urine}$	Cl_{renal}	$Cl_{nonrenal}$
Sex	mg/kg	mg/m^2	µM	µM*min	L/min	min	min	L	L	%	min	L/min	L/min
male	5	30	5.54	138	0.0095	435	69	0.7	6	2.8%	917	0.27	9.23
male	10	60	11.74	286	0.0092	151	42	0.4	2	3.1%	969	0.28	8.87
male	25	150	45.55	938	0.007	1267	154	1.1	12.8	3.8%	890	0.26	6.69
female	5	30	3.52	76	0.0139	28	28	0.4	5.5	4.6%	1148	0.64	13.21
female	10	60	8.72	153	0.0137	35	35	0.5	3.4	3.0%	1047	0.41	13.29
female	25	150	14.97	392	0.0134	126	126	1.7	17.9	4.5%	892	0.6	12.75

The following graph shows that C_{max} increases approximately linearly with dose but is much higher in males than in females.



The following graph shows that AUC increases approximately linearly with dose but is much higher in males than in females.



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3) PDX (10-propargyl-10-deazaaminopterin): Pharmacokinetic Study of PDX Following Intravenous Administration to Male and Female Beagle Dogs

Major findings

When given as a single IV injection the C_{max} and AUC of pralatrexate 10a and 10b enantiomers increase linearly with increasing dose. The slope of the dose normalized AUC vs dose curve is essentially zero indicating dose proportionality. C_{max} for PDX-10a at the high dose of 60 mg/m^2 was 5.2 µM for males and 6.6 µM for females. C_{max} for PDX-10b at this dose was 4.0 µM for males and 4.4 for females implying that the combined concentration of both enantiomers after this dose exceeded 10 µM. Total clearance of PDX-10a was consistently about half the value of the total clearance of 10b resulting in consistently greater values for C_{max} and AUC of 10a with a ratio of 10a to 10b of about 1.5. Clearance was consistently greater in males (range 0.08 to 0.12 L/min for 10a) compared to females (range 0.06 to 0.08 L/min for 10a) resulting in consistently greater values for AUC and C_{max} in females. The ratio of non-renal to renal clearance is about 4 at the low dose (6 mg/m^2) but increases to 20 or more at the mid dose of 20 mg/m^2 implying saturation of the renal elimination mechanism. Non-renal clearance values are less than hepatic flow. Volume of distribution exceeds 20 L implying significant extravascular distribution. Elimination is visually triphasic with a rapid distribution phase, a slower elimination phase, a rapid elimination phase and a very slow terminal elimination phase with a half-life that increases with increasing dose, again consistent with a saturable elimination. Urinary elimination is biphasic consistent with the two elimination phases demonstrated in plasma.

Study number	PDX-K-05009-D, Laboratory – 02959
Filename	PDXK05009-D—nonclinical-data.pdf
Laboratory	(b) (4)
Study date	September 2005
GLP	No

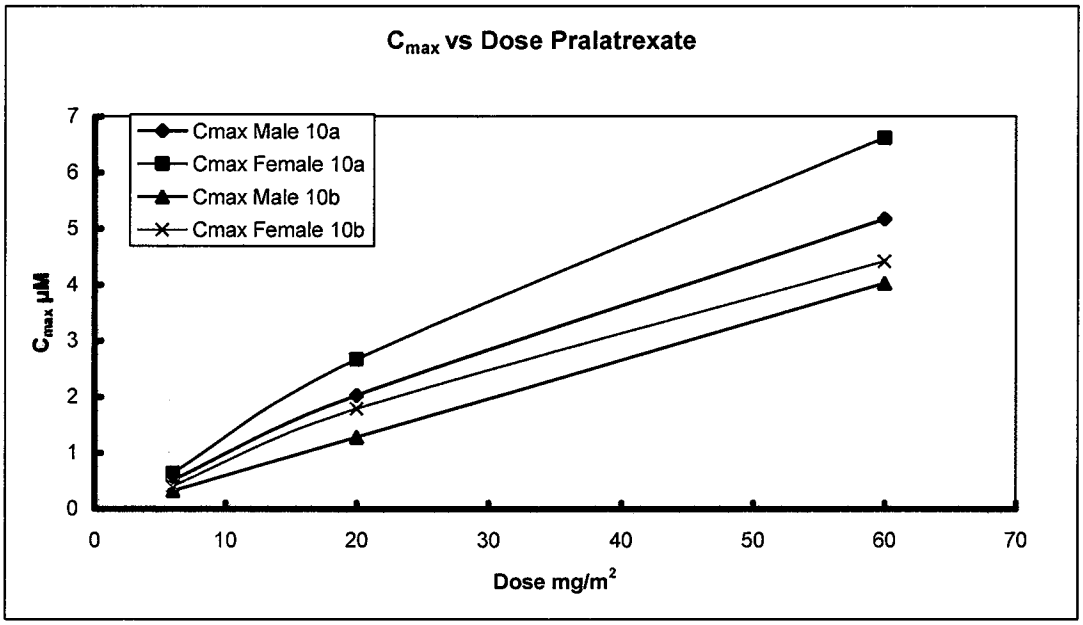
Audited	Yes
Drug	Pralatrexate, Lot 05010304, 100.1 % pure
Method	
Animals	Beagle dogs, 7 to 9 months old
Dose	0.3, 1 or 3 mg/kg (6, 20 or 60 mg/m^2)
N	3 per sex per dose group
Schedule	Single dose
Route	IV
Solvent	Buffered saline
Blood Sampling	Pre dose, 5, 10, 20, 30, and 45 minutes, and 1, 2, 4, 12, 24, 36, and 48 hours post dose, blood drawn from the jugular vein
Urine Sampling	Every 12 hours post dosing to 48 hours
Analysis	HPLC, non-compartmental analysis of the data
Internal Standard	(b) (4)
Histopathology	Not done

Results	
Mortality	None
Clinical signs	Dose-dependent increase in the frequency of emesis and fecal abnormalities including diarrhea, and discolored, mucoid, and soft feces in males and females
Body weight	Dose-dependent decrease in body weight gain was observed in both males and females which was probably secondary to GI toxicity
Pharmacokinetics	

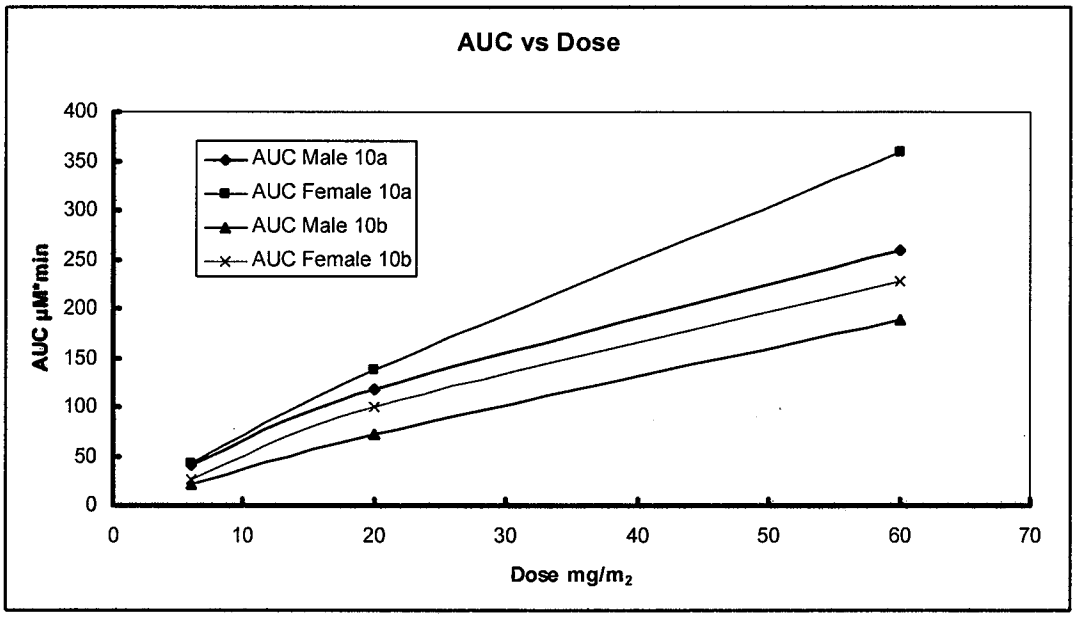
The following table shows the results of this experiment.

Analysis for PDX-10a													
units	mg/kg	mg/m^2	µM	µM*min	L/min	min	min	L	L	%	min	L/min	L/min
Sex	Dose	Dose	C_{max}	AUC	Cl_{tot}	$t_{1/2}$	MRT_{sys}	Vd_{ss}	Vd_{pss}	f_e	$t_{1/2}^{urine}$	Cl_{renal}	$Cl_{nonrenal}$
male	0.3	6	0.52	42	0.079	317	321	22	32	19.8%	959	0.016	0.063
male	1	20	2.03	117	0.107	378	213	24	58	6.3%	443	0.005	0.102
male	3	60	5.17	259	0.122	701	325	38	122	3.2%	695	0.004	0.118
female	0.3	6	0.65	43	0.059	636	350	20	52	21.7%	627	0.013	0.046
female	1	20	2.67	137	0.062	443	267	17	41	8.3%	1063	0.005	0.057
female	3	60	6.62	359	0.076	720	258	20	76	6.1%	539	0.004	0.072
Analysis for PDX-10b													
Sex	mg/kg	mg/m^2	µM	µM*min	L/min	min	min	L	L	%	min	L/min	L/min
	Dose	Dose	C_{max}	AUC	Cl_{tot}	$t_{1/2}$	MRT_{sys}	Vd_{ss}	Vd_{pss}	f_e	$t_{1/2}^{urine}$	Cl_{renal}	$Cl_{nonrenal}$
male	0.3	6	0.33	22	0.153	254	253	33	47	18.0%	1061	0.028	0.125
male	1	20	1.28	72	0.244	191	143	34	72	4.8%	474	0.009	0.235
male	3	60	4.03	189	0.166	1281	324	54	303	2.4%	667	0.004	0.162
female	0.3	6	0.42	26	0.098	253	196	18	35	18.9%	645	0.018	0.079
female	1	20	1.79	101	0.085	456	272	24	58	6.3%	1531	0.005	0.08
female	3	60	4.43	228	0.118	823	267	32	134	4.5%	575	0.005	0.113

The following graph shows that the increase in C_{max} with increasing dose is linear over this range of doses.



The following graph shows that AUC increases linearly with increasing dose. The slope of the dose normalized AUC vs dose ranges from -0.0002 to -0.047 or essentially 0 (Excel Linest) indicating dose proportionality.



The following two graphs from the study report show the mean plasma elimination curves for males and female dogs for pralatrexate-10a, the curves for pralatrexate-10b are similar. The

graphs suggest that the elimination of pralatrexate is triphasic with a rapid distribution phase, and a long and slow elimination phases.

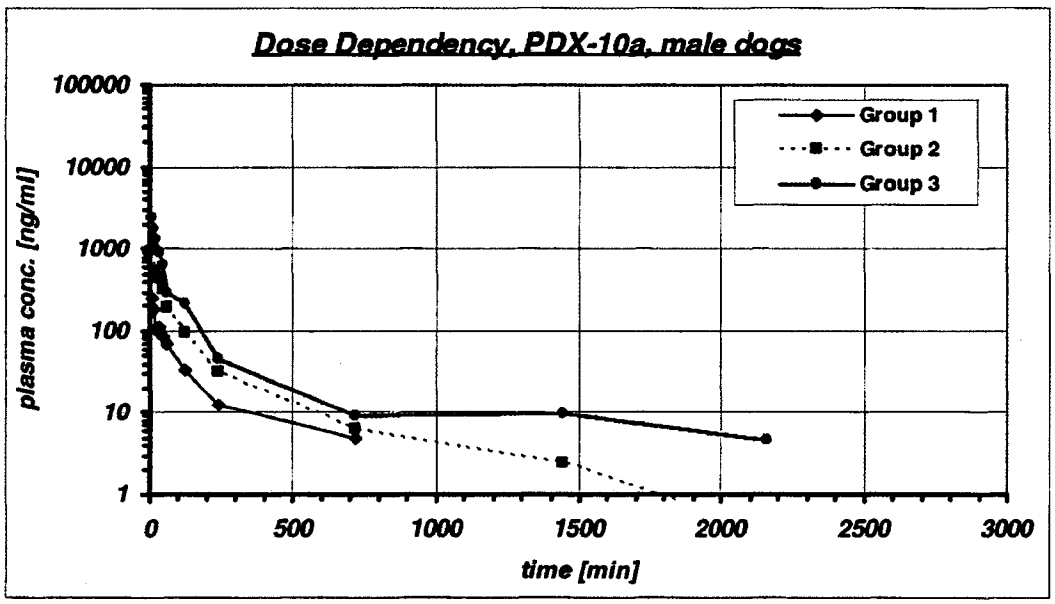


Figure 1a: Plasma concentration-time profile of PDX-10a in male dogs

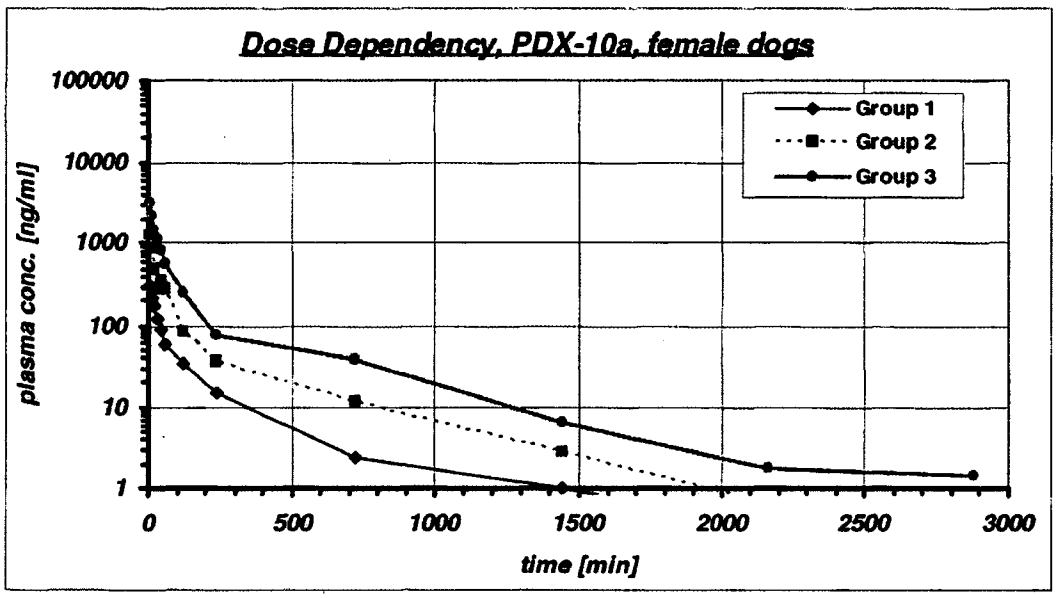


Figure 1b: Plasma concentration-time profile of PDX-10a in female dogs

The following graphs from the study report show that urine output was biphasic consistent with the biphasic elimination from urine. Urinary excretion increased with increasing dose.

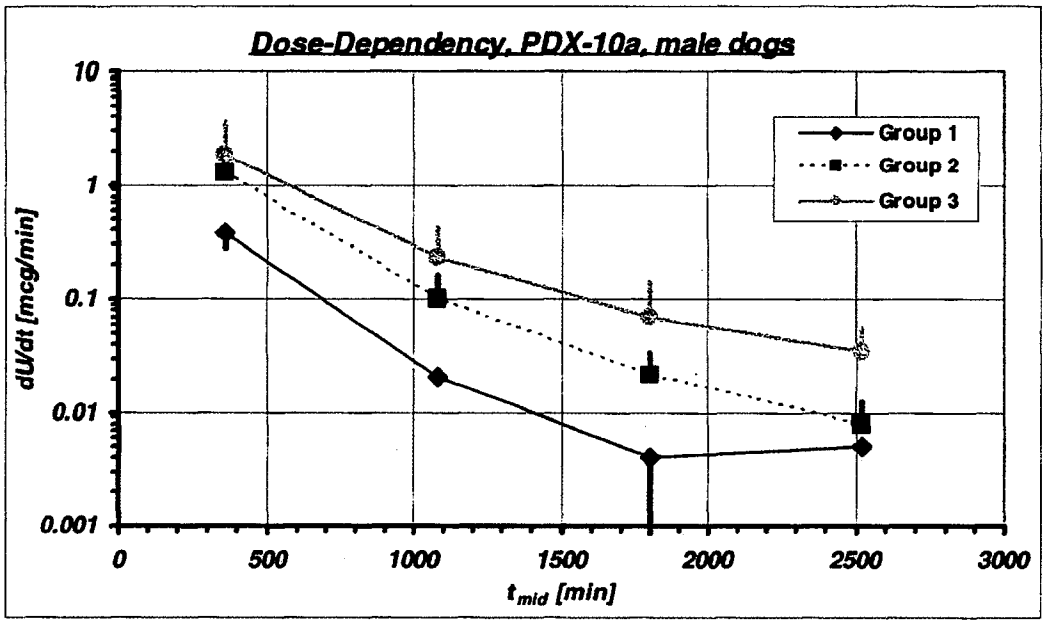


Figure 2a: Urinary excretion rate-time profile for PDX-10a in male dogs

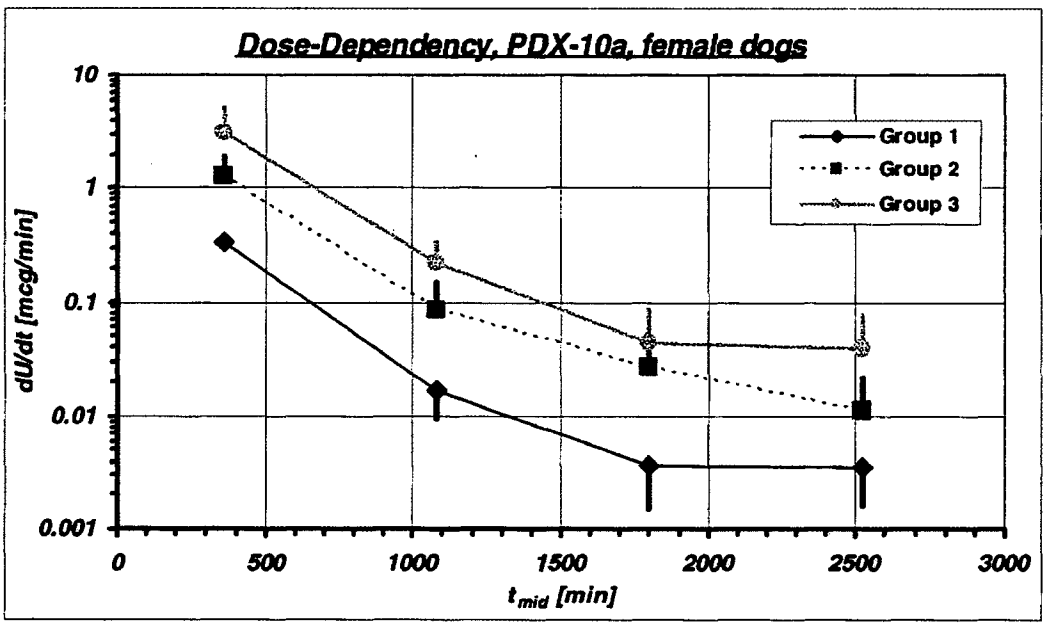


Figure 2b: Urinary excretion rate-time profile for PDX-10a in female dogs

4) Pralatrexate (PDX): A Nine-Month Intravenous Toxicity and Toxicokinetic Study in Beagle Dogs with a Four-Week Recovery Interval

Major findings:

In this study, investigators gave multiple doses of racemic pralatrexate to male and female dogs and then measured the pharmacokinetics of the elimination of the drug in plasma at study days 1, 85 and 281. On day one, both male and female dogs eliminated both enantiomers in three phases, a rapid distribution phase, a slower elimination or second distribution phase, and a terminal elimination phase. The initial distribution is difficult to resolve on the time scale of the observations of this study. The second phase has a half-life of between 30 to 60 minutes (estimated visually) and the half-life for the terminal elimination phase is between about 200 and 300 minutes.

AUC increases linearly with the highest values on day. This is probably because after the initial three weekly doses caused unacceptable toxicity, the dogs diet was supplemented with folate and vitamin B_{12}. The effect of this supplementation is evidenced by low AUC values on day 85 and 281. Thus, this experiment cannot rule out the possibility of accumulation, but it appears unlikely and if it does occur it is small in magnitude.

C_{max} and AUC values for pralatrexate-10b are lower than those of 10a consistent with higher values for total clearance for pralatrexate-10b. Volume of distribution is large, consistent with the possibility that in the second phase of the elimination curve the drug partitions into a compartment from a compartment on the order of total body weight or larger from which it is then released only slowly.

Study no: Sponsor – PDX-T-07054-D; Laboratory – 1128-04503
File name: pdxt0754d—nonclinical-data.pdf
Laboratory: (b) (4)
Study Date: September 2006
GLP: Yes
Audited: Yes
Drug: Pralatrexate Injection, Lot 132I0606, Purity 98.14 %

Methods:

Species: Beagle Dog, males 7.1 – 10.9 kg, females 5.1 – 8.8 kg
Age at first dose 4-5 months

Doses: The following table from the study report shows the doses and N

Text Table 4: Study Design

Group	Treatment	Nominal Dose (mg/kg)	Nominal Dose Concentration (mg/mL)	Number of Animals					
				Interim Phase		Main Phase		Recovery Phase	
				Male	Female	Male	Female	Male	Female
1	PBS	0	0	5	5	5	5	2	2
2	PDX	0.1	0.1	5	5	5	5	None	None
3	PDX	0.3	0.3	5	5	5	5	None	None
4	PDX	0.7	0.7	5	5	5	5	2	2

Note: Due to a communications error, the stock concentration was initially thought to be 19.8 mg/mL and this value was used for the first cycle of dosing. The difference in actual nominal dose was negligible.

Treatment Course: One IV dose weekly for six weeks followed by 1 week dose free interval
Schedule: Interim phase – 2 courses; Main Phase – 7 courses

Formulation: PBS
Dose volume: 1 mL/kg
Toxicokinetics: The following table from the study report shows the schedule for TK sampling. Nonparametric analysis.

Text Table 8: Blood Collection Information for Toxicokinetics

Collection Day	Time Points
Study Day 1/2	Predose, 5 and 30 minutes, and 1, 2, 4, 12, and 24 hours postdose
Study Day 85/86	Predose, 30 minutes, and 2, 4, 12, and 24 hours postdose
Study Day 183/184	Predose, 30 minutes, and 2, 4, 12, and 24 hours postdose
Study Day 281/282	Predose, 30 minutes, and 2, 4, 12, and 24 hours postdose

Necropsy: On sd 87 (interim-necropsy animals), sd 283 (terminal-necropsy animals), sd 309 (recovery-necropsy animals)

Results
For the toxicology results of this study see the toxicology section below.

The following tables show the estimated pharmacokinetic parameters for male and female dogs for pralatrexate-10a and 10b at the three different time points of this study.

Female Dogs Pralatrexate 10a

Day	Dose mg/kg	Dose mg/m^2	C_{max} µM	AUC_{∞} µM*min	AUC ratio relative to day 1	Cl_{total} mL/min	$t_{1/2}$ min	MRTsys min	Vd_{ss} L	Vd_{pss} L
1	0.1	2	0.19	18.4	1	0.041	167	168	7	10
85	0.1	2	0.13	15.1	0.81	0.053	80	119	6.4	6.2
281	0.1	2	0.12	18.2	1	0.048	349	356	13.5	19.3
1	0.3	6	0.88	60.9	1	0.038	245	195	7.2	12.9
85	0.3	6	0.26	37.5	0.63	0.061	301	0	11.7	24.1
281	0.3	6	0.29	38.6	0.68	0.058	544	0	28.5	42.2
1	0.7	14	1.47	101.7	1	0.055	294	253	12.9	23.1
85	0.7	14	0.37	44.1	0.47	0.117	371	175	20.3	61.4
281	0.7	14	0.70	85.9	0.91	0.060	327	291	17.5	28.3
Average						0.059	298	173	14	25
sd						0.023	131	121	7	17

Female dogs Pralatrexate 10b

Day	Dose mg/kg	Dose mg/m^2	C_{max} µM	AUC_{∞} µM*min	AUC ratio relative to day 1	Cl_{total} mL/min	$t_{1/2}$ min	MRTsys min	Vd_{ss} L	Vd_{pss} L
1	0.1	2	0.14	11.2	1	0.067	166	150	10.2	10
85	0.1	2	0.10	10.1	0.896012	0.076	73	108	8.6	8.3
281	0.1	2	0.09	12.7	1.13101	0.066	341	327	18.3	27.4
1	0.3	6	0.64	39.8	1	0.057	214	163	9.2	17.2
85	0.3	6	0.25	36.6	0.919402	0.064	241	184	11.3	20.7
281	0.3	6	0.19	24.4	0.612969	0.092	419	364	34.4	56.2
1	0.7	14	1.21	74.1	1	0.073	279	232	15.9	29.3
85	0.7	14	0.37	39.4	0.532634	0.134	140	114	14.8	25.2
281	0.7	14	0.53	64.9	0.876393	0.081	312	285	24	37.1
Average						0.079	243	214	16	26
sd						0.023	108	93	8	15

Male dogs Pralatrexate 10a

Day	Dose mg/kg	Dose mg/m^2	C_{max} µM	AUC_{∞} µM*min	AUC ratio relative to day 1	Cl_{total} mL/min	$t_{1/2}$ min	MRTsys min	Vd_{ss} L	Vd_{pss} L
1	0.1	2	0.35	23.3	1	0.039	247	211	8	13.6
85	0.1	2	0.16	16.6	0.73	0.056	60	91	5.1	4.7
281	0.1	2	0.19	27.5	1.22	0.033	147	171	5.8	7.3
1	0.3	6	0.74	47.4	1	0.059	280	195	11.7	22.9
85	0.3	6	0.37	46.2	1.02	0.060	143	129	7.8	13.2
281	0.3	6	0.53	73.5	1.6	0.041	263	188	8	15.5
1	0.7	14	2.86	131.2	1	0.050	288	154	7.9	21.5
85	0.7	14	0.79	101.7	0.85	0.066	269	194	12.8	25.1
281	0.7	14	1.05	119.3	0.93	0.058	284	243	15.2	24.4
Average						0.051	220	175	9	16
sd						0.011	82	45	3	7

Male dogs Pralatrexate 10b

Day	Dose mg/kg	Dose mg/m^2	C_{max} µM	AUC_{∞} µM*min	AUC ratio relative to day 1	Cl_{total} mL/min	$t_{1/2}$ min	MRTsys min	Vd_{ss} L	Vd_{pss} L
1	0.1	2	0.29	15.1	1	0.060	214	166	9.9	18.8
85	0.1	2	0.10	11.0	0.75	0.084	60	92	7.7	7.3
281	0.1	2	0.12	16.4	1.11	0.055	127	153	8.4	10.1
1	0.3	6	0.58	33.6	1	0.083	193	156	13.3	23
85	0.3	6	0.31	40.7	1.27	0.067	96	122	8.1	9.2
281	0.3	6	0.32	48.8	1.5	0.058	253	204	12.2	21.7
1	0.7	14	1.97	85.9	1	0.075	264	135	10.1	28.7
85	0.7	14	0.80	93.7	1.17	0.074	215	165	12.4	22.1
281	0.7	14	0.44	66.5	0.77	0.095	313	296	28.6	43.6
Average						0.072	193	165	12	21
sd						0.014	83	58	6	11

The following graphs from the study report show the elimination of pralatrexate-10a and 10b from plasma as a function of time on study day 1.

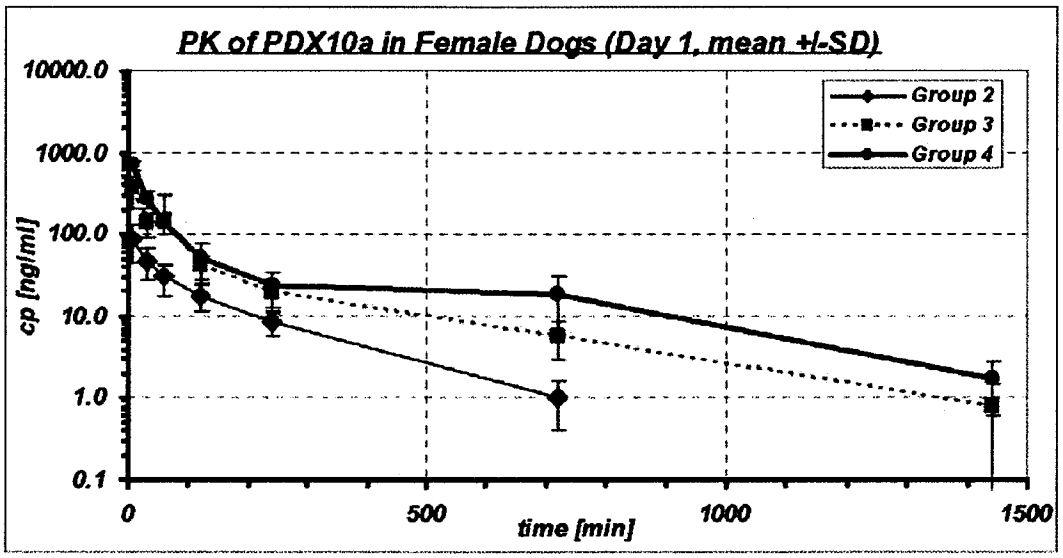


Figure 1a: Plasma concentration-time profiles of PDX-10a by group/dose (2: 0.1 mg/kg, 3: 0.3 mg/kg and 4: 0.7 mg/kg) on Day 1 in female dogs

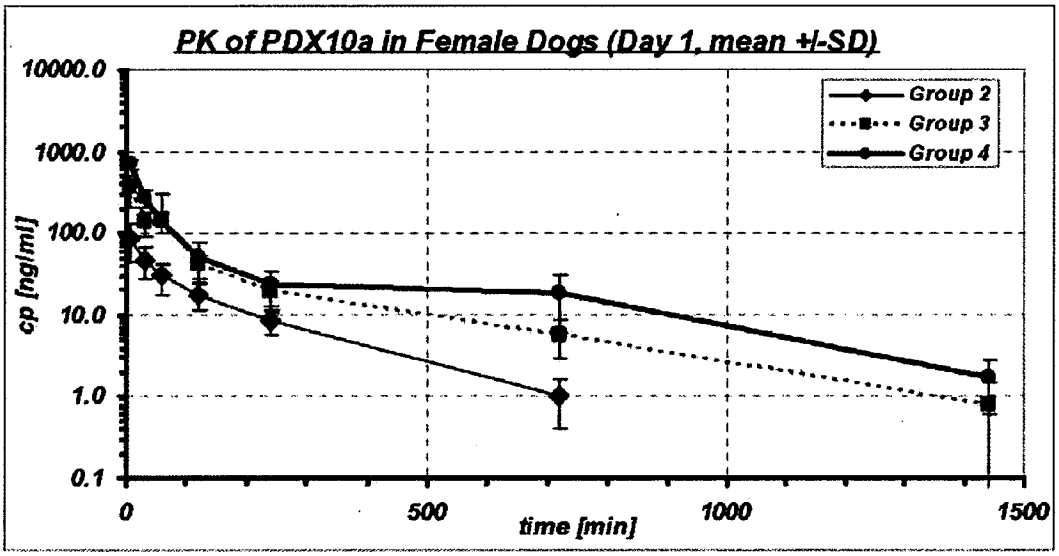


Figure 1a: Plasma concentration-time profiles of PDX-10a by group/dose (2: 0.1 mg/kg, 3: 0.3 mg/kg and 4: 0.7 mg/kg) on Day 1 in female dogs

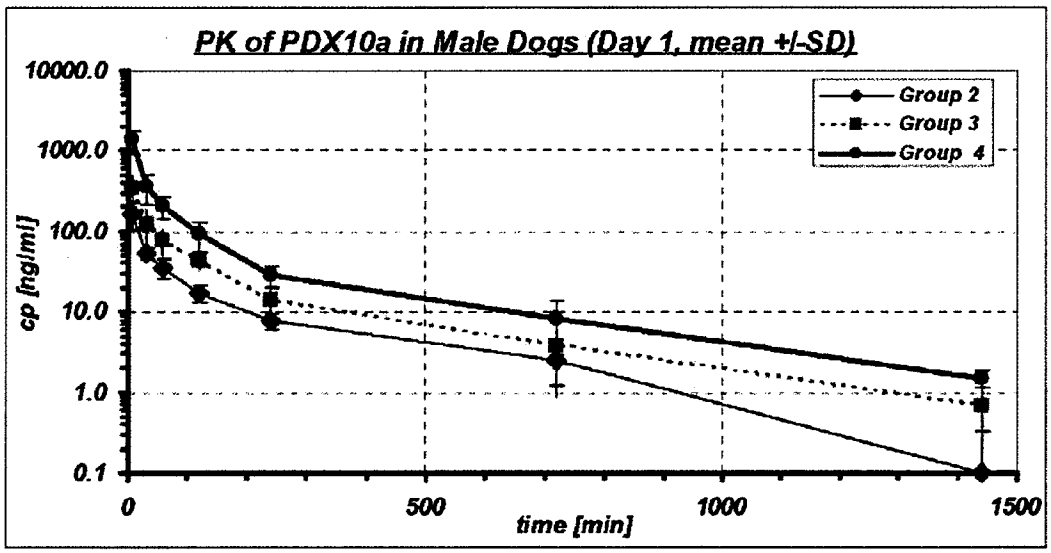


Figure 2a: Plasma concentration-time profiles of PDX-10a by group/dose (2: 0.1 mg/kg, 3: 0.3 mg/kg and 4: 0.7 mg/kg) on Day 1 in male dogs

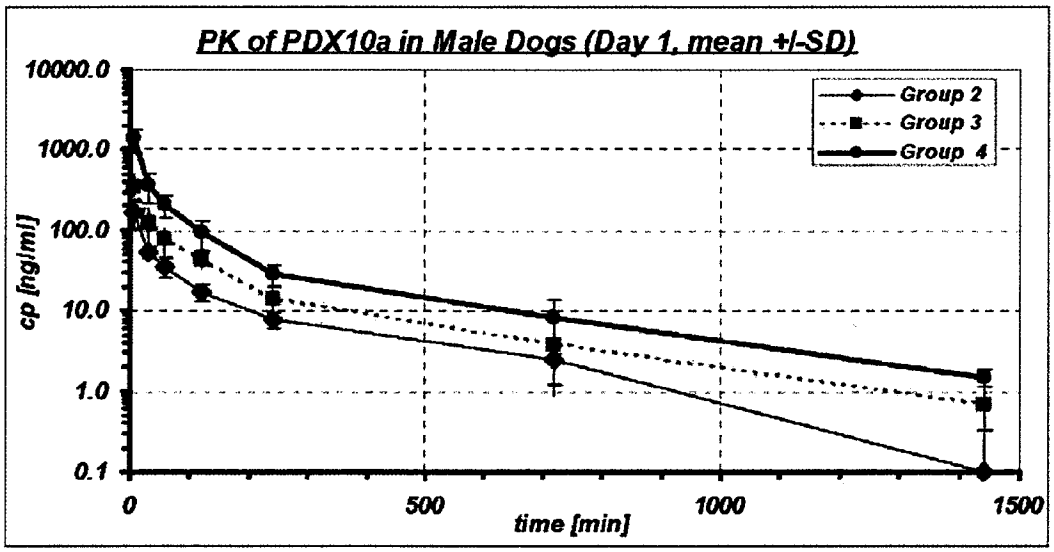
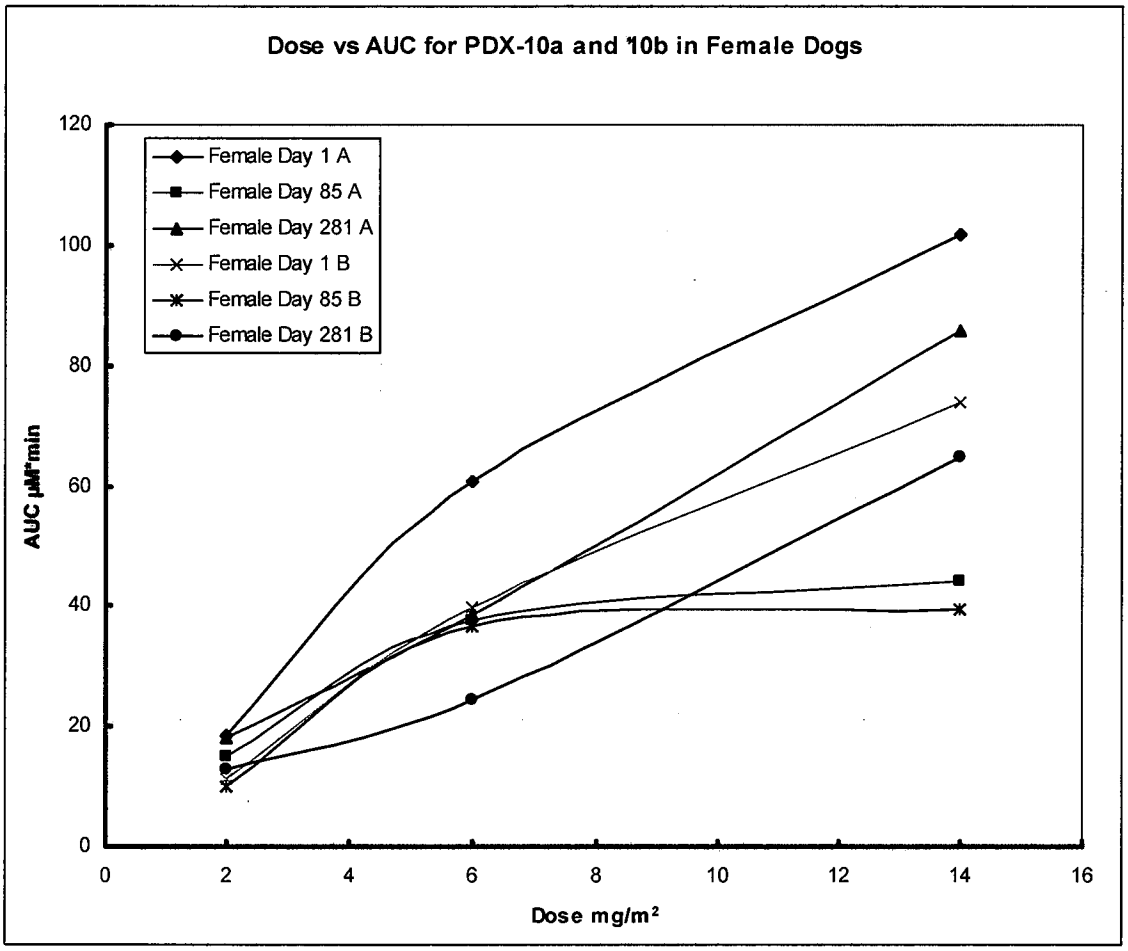
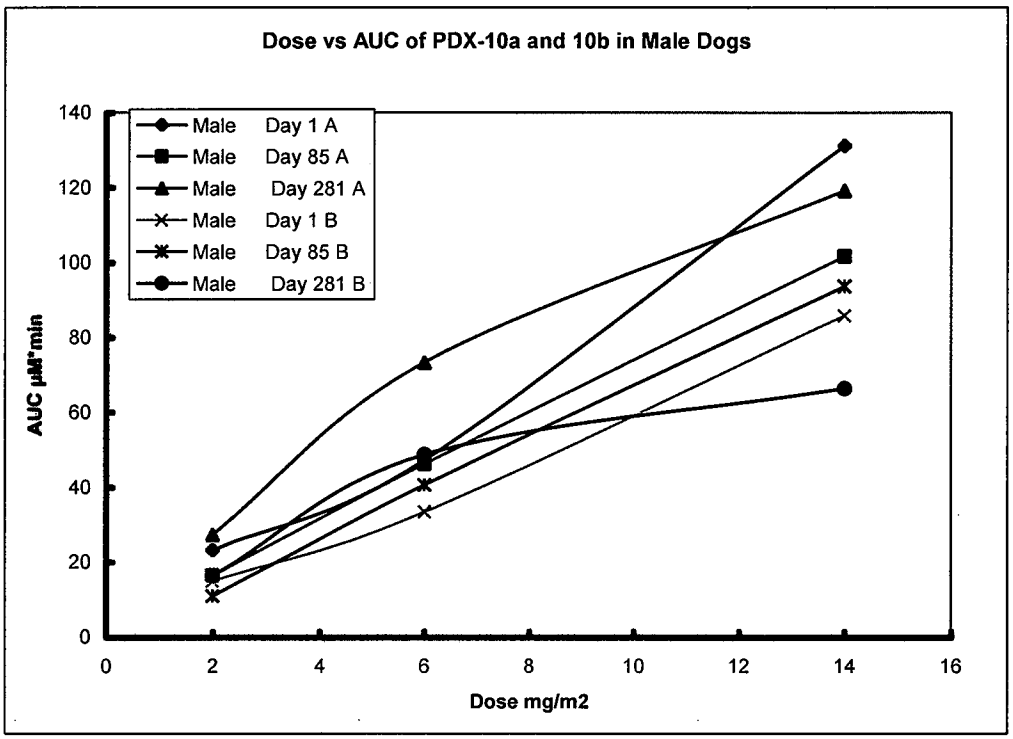


Figure 2a: Plasma concentration-time profiles of PDX-10a by group/dose (2: 0.1 mg/kg, 3: 0.3 mg/kg and 4: 0.7 mg/kg) on Day 1 in male dogs

The following graphs show the increase of AUC with time for pralatrexate-10a and 10b in male and female dogs.



The parameters for day 85 in the above graph are underestimated due to sparse sampling.



2.6.4.4 Distribution

1) Evaluation of the Brain Penetration Potential of Methotrexate and 10-Propargyl-10-Deazaaminopterin (PDX) via *In Situ* Rat Brain Perfusion

Major findings

The dose-normalized uptake rate of the positive control, antipyrine, in brain tissue was at least 40-fold greater than those of pralatrexate or methotrexate (MTX). The ability of methotrexate and pralatrexate to enter brain tissue from a circulating perfusate is comparable to that of the negative control, atenolol. This experiment suggests that pralatrexate will not readily cross the BBB.

Study number	PDX-P-03001-R
Filename	PDX-P-03001-R—nonclinical-data.pdf
Laboratory	(b) (4)
Study date	April 2003
GLP	No
Audited	No
Drug	Pralatrexate, lot not specified Methotrexate, lot not specified
Method	
Animals	Rats (breed not specified)

Doses	Methotrexate – 21 mg/mL (46.21 mM, 44.9 mM assay) Pralatrexate – 21 mg/mL (43.98 mM target, 34.8 mM assay) In both cases this dose is targeted to be equivalent to 600 mg/kg IV
N	4 for PDX, 2 for methotrexate
Perfusion buffer	Krebs-ringer's, pH 7.4, 20 mL/min
Perfusion time	30 sec with 30 sec washout
Positive control	Antipyrine 5 μM target, 4.1 μM assay
Negative control	Atenolol 50 μM target, 36 μM assay
Tissue extraction	Acetonitrile
Analysis	HPLC with LC/MS/MS detection

The following table shows the results of this experiment. The data is normalized for relative perfusion concentration by dividing the perfusion concentration of the test article by that of Atenolol (negative control) for each set of animals. The uptake rate is then divided by this normalization factor.

Table 7. Uptake rates of PDX, MTX, and antipyrine, normalized to the concentration of atenolol

Compound	*Uptake Rate (pmol/mg protein/sec) U.R.*	*Perfusate concentration (μM)*	*Normalization Factor*	*Normalized uptake rate (pmol/mg protein/sec)*
PDX	**34.77±8.85**	**34800**	***U.R./966.67***	**0.036±0.009**
MTX	**30.72**	**44900**	***U.R./1247.22***	**0.024**
Atenolol/PDX[1]	**0.049±0.01**	**36**	**1**	**0.049±0.010**
Atenolol/MTX[2]	**0.052**	**36**	**1**	**0.052±0.026**
Antipyrine/PDX[3]	**0.165±0.037**	**4.1**	***U.R. x 8.78***	**1.209±0.224**
Antipyrine/MTX[4]	**0.191**	**4.1**	***U.R. x 8.78***	**1.681±0.246**

1 Atenolol when co-administered with PDX

2 Atenolol when co-administered with MTX

3 Antipyrine when co-administered with PDX

4 Antipyrine when co-administered with MTX

2) Equilibrium Dialysis

Major findings

Plasma binding was about 63% at a concentration of 0.5 μM.

Study no:	Sponsor – PDX-k-06029-U; Laboratory – 6ALLOPIRI
File name:	pdxk06029u—legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	November 2006
GLP:	No
Audited:	No
Drug:	^{14}C-Pralatrexate, 28.4 mCi/mmol, not otherwise characterized
Methods:	
Tissue:	Human plasma
Concentration	70 and 17 μM
	2.7 to 2.7, 9.0 to 9.4, 27.2 to 27.8 mg/mL assay
N	3 repetitions per concentration
System	Dialysis
Vehicle	0.9% saline
Analysis	Scintillation counting

---

3) Equilibrium Dialysis

Major findings

About 67% of available [^{14}C]-PDX is bound to human plasma *in vitro* as measured by equilibrium dialysis. Relative to the total concentration, less than 3% was displaced from site I on albumin; 5-11% was displaced from site II on albumin, 9% from site III on albumin, and 8-10% from α1-acid glycoprotein. A small fraction of pralatrexate may be displaced from lipoproteins.

Study no:	Sponsor – PDX-k-07043-U; Laboratory – 7ALLOP2R2
File name:	pdxk070.43u—legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	June 2007
GLP:	No
Audited:	No
Drug:	^{14}C-Pralatrexate, 28.4 mCi/mmol, not otherwise characterized
Methods:	
Tissue:	Human plasma
Concentration	0.5 μCi/mL, 0.5 mL
N	3 repetitions per concentration
System	Dialysis with displacement by compounds known to bind plasma
Vehicle	0.9% saline
Analysis	Scintillation counting

---

4) *In Vitro* Study of Binding and Partitioning of Pralatrexate into Human Red Blood Cells

Major findings

In this study, investigators determined the *in vitro* accumulation of pralatrexate in erythrocytes. They partitioned pralatrexate into RBCs comparing concentrations with a plasma control. With this method, after 2 hours of incubation at 37 C, there was no significant partitioning of pralatrexate into human RBCs. Methotrexate was used as a comparative control. Consistent with previous literature reports (cited in the study) methotrexate concentrations within RBCs were very low after similar incubation.

Study no:	Sponsor – PDX-k-08067-U;
File name:	pdxk08067u—legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	October 2008
GLP:	Yes
Audited:	Yes
Drug:	Pralatrexate, Lot 09070074
Methods:	
Tissue:	Human plasma, blood and RBCs
Concentration	5, 25 and 100 µg/mL
N	3 repetitions per concentration
System	Dialysis with displacement by compounds known to bind plasma
Vehicle	0.9% saline
Positive control	Methotrexate
Assay	Fluorescence polarization immunoassay, LLOQ – 0.02 µM
Analysis	HPLC MS/MS, internal standard (b) (4)

2.6.4.5 Metabolism

1) Identifying Metabolites of the Customer's Test Compound in Pooled Human Liver Microsomal Incubates Using LC-MS/MS

Major findings

This method detected no metabolites of pralatrexate generated by of human liver microsomes.

Study no:	Sponsor – PDX-K-06028-U; Laboratory – 6ALLOP2R2
File name:	pdxK06028U —legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	December 2006
GLP:	No
Audited:	No
Drug:	Pralatrexate
Methods:	

Species:	Human, 16 donors mixed
Tissue	Microsomal Protein (plus NADPH) 0.5 mg/mL
Concentration	100 μM
Analysis	HPLC-MS or MS-MS
Positive control	Testosterone (substrate for cytochrome P450 3A4)

---

2) Evaluation of Metabolic Stability of Pralatrexate (PDX) in Human Hepatocytes and Human Liver Microsomes

Major findings

In this experiment, investigators incubated pralatrexate with human liver microsomes or human liver hepatocytes at concentrations of 10 or 100 μM. They sampled the incubation matrix at three time points, 30, 60, and 120 minutes for liver microsomes and 60, 120, and 240 minutes for hepatocytes. The assayed concentrations of pralatrexate remained within 15% of that of incubation controls (no hepatic metabolic system) throughout the experiment for both preparations. The results suggest human liver tissues do not extensively metabolize pralatrexate *in vitro*. The metabolic positive control, benzyresorufin, was metabolized to resorufin by the liver microsomes and hepatocytes, therefore demonstrating that the experimental conditions were adequate.

Study no:	Sponsor – PDX-K-07030-U; Laboratory – 1100-110105
File name:	pdxK07030U—legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	February 2006
GLP:	Yes
Audited:	Yes
Drug:	Pralatrexate, Lot 005I0105, 98.5% pure
Methods:	
Tissue	Human liver hepatocytes and human liver microsomes
Concentration	10 and 100 μM
Analysis	HPLC-MS or MS-MS
Positive control	Benzyresorufin (BzRez)

---

3) Metabolic Profiling of PDX in Pooled Human S9 Fractions

Major findings

The concentration of pralatrexate did not change within the typical accuracy of the LC-MS/MS method (± 15%) during 60 minutes of incubation with pooled, mixed gender human S9 fraction. PDX appeared to be metabolically stable in human S9 fractions. The positive control confirmed the validity of the assay.

Study no:	Sponsor – PDX-K-08061-U; Laboratory – 8ALLOP1R1
File name:	pdxK08061U —legacy-report.pdf

Laboratory:	(b) (4)
Study Date:	May 2008
GLP:	No
Audited:	Draft
Drug:	Pralatrexate, Lot not stated
Methods:	
Tissue	Human S9 fraction, pooled 50 donors
Concentration	2 μM
Analysis	HPLC-MS or MS-MS
Positive control	Testosterone and 7-hydroxycoumarin

---

4) Cytochrome P450 Phenotyping of PDX using chemical inhibitors and cDNA Expressed Supersomes

Major finding

Investigators in this study characterized pralatrexate metabolism using chemical inhibitors and reaction phenotyping with cDNA expressed human recombinant cytochrome P450 enzymes. Both methods showed that pralatrexate was stable to the metabolic effects of cytochromes P450 1A2, 2A6, 2B6, 2C8, 2C19, 2D6, 2E1, 3A4 and to monoamine oxidases, MAO-A and MAO-B.

Study no:	Sponsor – PDX-K-08062-U
File name:	pdxK08062U —legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	June 2008
GLP:	No
Audited:	Yes
Drug:	Pralatrexate, Lot not stated
Methods:	
Tissue	Human liver microsomes Baculovirus expression systems containing human CYP cDNA
Concentration	1 μM
Analysis	HPLC-MS or MS-MS

---

5) Evaluation of P450 inhibition potential of pralatrexate (PDX) in human liver microsomes

Major finding

Pralatrexate caused concentration dependant inhibition of the activity of cytochrome P450 2C19 in human liver microsomes as evidenced by a decrease in the hydroxylation of s-mephenytoin. Activity decreased to 71% at a concentration of 20 μM and was below detection at 50 μM or greater. Slight inhibition of the function of 2E1 and 2A6 (less than 30% decrease from the activity of control) was not concentration dependant. The compound did not inhibit the

activity of cytochromes P450 1A2, 2C9, 2D6 as measured by the metabolism of standard test substrates with appropriate positive controls.

Study no:	Sponsor – PDX-K-07032-U, Laboratory – 2200-031406
File name:	pdxK07032U —legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	April 2006
GLP:	Yes
Audited:	Yes
Drug:	Pralatrexate, Lot not stated, 98.5% pure
Methods:	
Tissue	Human liver microsomes with regenerating system
Concentration	40, 100 and 200 μM
Incubation	60 min

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6) Induction Assessment of CYP1A2, 3A4, and 2C19 by PDX in Fresh Human Hepatocytes

Major finding

Pralatrexate did not induce an increase in the activity of cytochromes P450 1A2, 3A4 or 2C19 when incubated with fresh human hepatocytes. Concentrations of 2, 10 or 50 μM were not cytotoxic. The results for the experiment with 2C19 are somewhat ambiguous since pralatrexate inhibits the activity of this enzyme in human microsomes (*vide supra*).

Study Number PDX-K-08060-U

2.6.4.6 Excretion

1) Mass Balance (Including CO_2 Capture) of ^{14}C-PDX Administered Intravenously in Male and Female Sprague Dawley Rats

Major finding

After a single IV dose of radiolabeled pralatrexate, male rats excreted about 47% of radiolabel in the feces, females about 66%. Male rats excreted about 31% of the radiolabel in the urine, females about 23%. Less than 2% of the radiolabel remained in the carcass. The total mean mass balance recovery, including expired air (CO_2), cage wash and carcass, was about 93% for males and 96% for females. Nevertheless, the radiolabel on pralatrexate was on the carboxylic acid moiety of the glutamic acid side chain, the most labile portion of the molecule. The results of this experiment probably do not provide reliable estimates of the relative excretion of the pralatrexate parent.

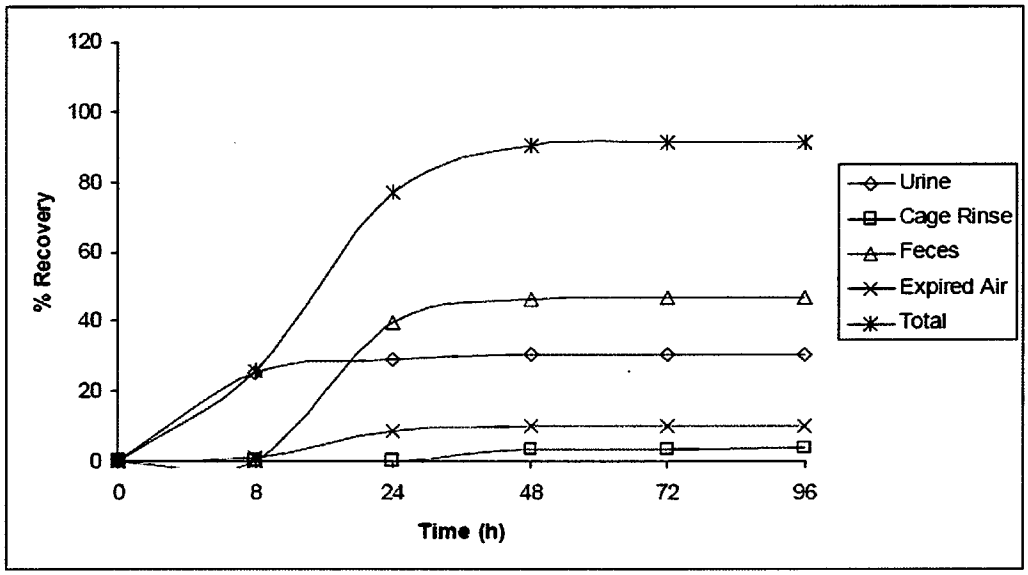
Study no:	Sponsor – PDX-K-07053-R, Laboratory – (b) 08-019
File name:	pdxK07053r —legacy-report.pdf

Laboratory:	(b) (4)
Study Date:	June 2008
GLP:	Yes
Audited:	Yes
Drug:	Pralatrexate, Lot not specified, purity greater than 98% ^{14}C-Pralatrexate, Lot not specified, purity greater than 97%, Specific activity 0.112 mCi/mg, 4.4 mg/mL
Methods:	
Species	Sprague Dawley rats, 7 to 8 weeks
Dose	10 mg/kg
N	Two rats per sex
Route	IV
Schedule	Single dose
Sample times	0 to 96 hours post dose
Vehicle	Normal saline
Excreta	Urine, feces and expired air
Analysis	scintillation counting

Results

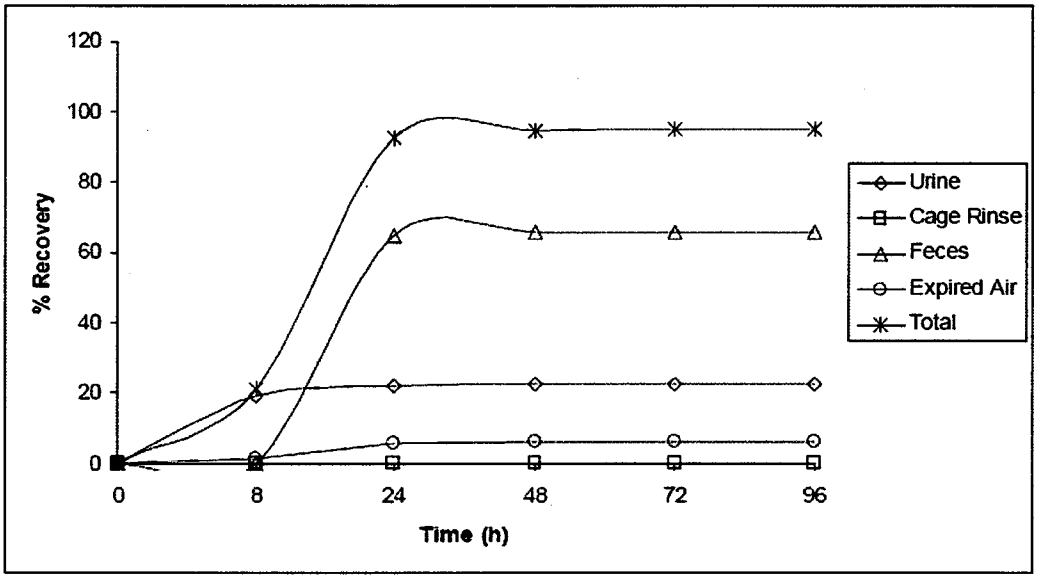
Mortality	All rats survived to scheduled necropsy
Clinical observations	None
Drug recovery	The following graphs from the study report present the results of this study

Figure 1. Mean Cumulative Excretion of ^{14}C-PDX Derived Radioactivity in Excreta of Male Sprague Dawley Rats (n = 2/route) After a Single 10-mg/kg Intravenous Dose Over a 96 Hour Period



Expired air reflects mean cumulative excretion of ^{14}C-PDX derived radioactivity from trap 1 only. Since trap 2 had negligible amount of radioactivity, it was not included in the above graph

Figure 2. Mean Cumulative Excretion of ^{14}C-PDX Derived Radioactivity in Excreta of Female Sprague Dawley Rats (n = 2/route) After a Single 10-mg/kg Intravenous Dose Over a 96 Hour Period



Expired air reflects mean cumulative excretion of ^{14}C-PDX derived radioactivity from trap 1 only. Since trap 2 had negligible amount of radioactivity, it was not included in the above graph

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2) Mass Balance and Tissue Distribution of ^{14}C-PDX Administered Intravenously in Male and Female Sprague Dawley Rats

Major finding

After a single IV dose of radiolabeled pralatrexate, male rats excreted 24 ± 3% of the radiolabel in the urine, females 20 ± 3%. Males excreted 44 ± 9% in feces, females 58 ± 4% in feces. Less than 5% was recovered in the cage wash. Less than 1% was recovered in the carcass. Expired CO2 was not monitored. After 168 hours, radioactivity was detectable in liver, lung, kidney and skin but at only at 0.11% (female liver) or less. There was no radioactivity in ileum, colon, bone marrow, brain, or skeletal muscle. Nevertheless, the radiolabel on pralatrexate was on the carboxylic acid moiety of the glutamic acid side chain, the most labile portion of the molecule. This study probably does not provide reliable information about the excretion and tissue distribution of pralatrexate.

Study no:	Sponsor – PDX-K-07052-R, Laboratory - (b) 07-067
File name:	pdxK07053r —legacy-report.pdf
Laboratory:	(b) (4)
Study Date:	August 2007
GLP:	Yes
Audited:	Yes

Drug:	Pralatrexate, Lot not specified, purity greater than 98% ^{14}C-Pralatrexate, Lot not specified, purity greater than 97%, Specific activity 0.112 mCi/mg, 4.4 mg/mL
Methods:	
Species	Sprague Dawley rats, 7 - 8 weeks, males, 205–212 g, females: 171–185 g
Dose	10 mg/kg, 1 mL/kg
N	Four rats per sex
Route	IV
Schedule	Single dose
Sample times	0 to 168 hours post dose
Vehicle	Normal saline
Tissues	Brain, liver, lung, ileum (section), colon (section), skeletal muscle, kidneys, skin, bone marrow (femur), remainder of carcass
Excreta	Urine, feces, cage wash
Analysis	scintillation counting
Necropsy	168 hours

Results

Mortality	All rats survived to scheduled necropsy
Clinical signs	None
Excreta Distribution	The following two tables from the study report show the time course for cumulative recovery from urine, cage rinse, feces and total radioactivity for male and female rats.

Table 6. Percent of Dose Recovered from ^{14}C-PDX-Derived Radioactivity in Tissues and Excreta of Male Sprague Dawley Rats after a Single Intravenous Administration over a 168 Hour Period

Sample	Percent of Dose					
	Animal Number				Mean	SD
	1M	2M	3M	4M		
Liver	0.13	0.21	0.16	0.30	0.20	0.07
Ileum	0.00	0.00	0.00	0.00	—	—
Colon	0.00	0.00	0.00	0.00	0.00	0.00
Bone Marrow	0.00[a]	0.00[a]	0.00[a]	0.00[a]	—	—
Brain	0.00	0.00	0.00	0.01	0.00	0.01
Skeletal Muscle	0.00[a]	0.00[a]	0.00[a]	0.00[a]	—	—
Lung	0.00	0.01	0.00	0.01	0.01	0.01
Kidney	0.03	0.04	0.03	0.05	0.04	0.01
Skin	0.01[a]	0.01[a]	0.01[a]	0.02[a]	0.01	0.01
Total tissues	**0.17**	**0.27**	**0.20**	**0.39**	**0.26**	**0.10**
Carcass	0.47	0.72	0.67	1.30	0.79	0.36
Total tissues including carcass	**0.64**	**0.99**	**0.87**	**1.69**	**1.05**	**0.45**
Urine	20.45	24.48	24.05	27.65	24.16	2.95
Cage rinse	3.24	4.63	4.67	2.68	3.81	1.00
Feces	54.48	45.04	45.23	32.17	44.23	9.17
Total excreta	**78.17**	**74.15**	**73.95**	**62.50**	**72.19**	**6.75**
Total tissues and excreta	**78.81**	**75.14**	**74.82**	**64.19**	**73.24**	**6.30**

[a] The values listed above are percent recovery/g of tissue. This is based on the portion of the tissue taken and not on the whole tissue.

The data was computer-generated and rounded appropriately for inclusion in the study file and report, hence the use of reported values to calculate subsequent parameters will, in some instances, yield minor variations.

— indicates not applicable.

Table 7. Percent of Dose Recovered from ^{14}C-PDX-Derived Radioactivity in Tissues and Excreta of Female Sprague Dawley Rats after a Single Intravenous Administration over a 168 Hour Period

Sample	Percent of Dose					
	Animal Number				Mean	SD
	1F	2F	3F	4F		
Liver	0.09	0.12	0.11	0.13	0.11	0.02
Ileum	0.00	0.00	0.00	0.00	—	—
Colon	0.00	0.00	0.00	0.00	—	—
Bone Marrow	0.00[a]	0.00[a]	0.00[a]	0.00[a]	—	—
Brain	0.00	0.00	0.00	0.00	—	—
Skeletal Muscle	0.00[a]	0.00[a]	0.00[a]	0.00[a]	—	—
Lung	0.00	0.00	0.00	0.00	—	—
Kidney	0.02	0.03	0.02	0.03	0.03	0.01
Skin	0.00[a]	0.00[a]	0.01[a]	0.00[a]	—	0.01
Total tissues	**0.11**	**0.15**	**0.14**	**0.16**	**0.14**	**0.02**
Carcass	0.08	0.13	0.30	0.26	0.19	0.10
Total tissues including carcass	**0.19**	**0.28**	**0.44**	**0.42**	**0.33**	**0.12**
Urine	16.99	22.46	20.35	22.40	20.55	2.57
Cage rinse	8.32	4.25	3.65	5.41	5.41	2.07
Feces	61.79	59.97	56.48	53.96	58.05	3.51
Total excreta	**87.10**	**86.68**	**80.48**	**81.77**	**84.01**	**3.37**
Total tissues and excreta	**87.29**	**86.96**	**80.92**	**82.19**	**84.34**	**3.26**

[a] The values listed above are percent recovery/g of tissue. This is based on the portion of the tissue taken and not on the whole tissue.

The data was computer-generated and rounded appropriately for inclusion in the study file and report, hence the use of reported values to calculate subsequent parameters will, in some instances, yield minor variations.

— indicates not applicable.

2.6.4.7 Pharmacokinetic drug interactions

Not studied

Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

See above

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pralatrexate 10a

		Folate Supplimentation	Day	Dose mg/kg	Dose mg/m^2	C_{max} µM	AUC_{∞} µM*min	Cl_{total} L/min	$t_{1/2}$ min	MRT_{sys} min	Vd_{ss} L	Vd_{ss} L/kg	Cl_{tot} mL/min/kg
	Human	Yes	1	29.2*	30	5.19	196.7	0.418	1078.5	288.1	104.6	1.3	5.3
PDX-T-07054	Female Dogs	Yes	85	0.7	14	0.37	44.1	0.117	371	175	20.3	2.5	14.7
PDX-T-07054	Male Dogs	Yes	85	0.7	14	0.79	101.7	0.066	269	194	12.8	1.6	8.2
PDX-T-07054	Female Dogs	No	1	0.7	14	1.47	101.7	0.055	294	253	12.9	1.6	6.9
PDX-T-07054	Male Dogs	No	1	0.7	14	2.86	131.2	0.050	288	154	7.9	1.0	6.3
PDX-K-05009	Female Dogs	No	1	1	20	2.67	137.5	0.062	443	267	17	2.1	7.8
PDX-K-05009	Male Dogs	No	1	1	20	2.03	117.4	0.107	378	213	24	3.0	13.4
PDX-K-05009	Female Dogs	No	1	3	60	6.62	359.5	0.076	720	258	20	2.5	9.5
PDX-K-05009	Male Dogs	No	1	3	60	5.17	259.3	0.122	701	325	38	4.8	15.3
PDX-K-05010	Female Rat	No	1	5	30	3.38	70.9	0.015	29	29	0.5	1.7	49.3
PDX-K-05010	Male Rat	No	1	5	30	5.24	138.2	0.010	463	75	0.7	2.3	31.7

Pralatrexate 10b

		Folate Supplimentation	Day	Dose mg/kg	Dose mg/m^2	C_{max} µM	AUC_{∞} µM*min	Cl_{total} L/min	$t_{1/2}$ min	MRT_{sys} min	Vd_{ss} L	Vd_{ss} L/kg	Cl_{tot} mL/min/kg
PDX-008	Human	Yes	1	29.2*	30	6.99	364.3	0.191	714.1	211.7	37.2	1.3	5.3
PDX-T-07054	Female Dogs	Yes	85	0.7	14	0.37	39.4	0.134	140	114	14.8	1.9	16.7
PDX-T-07054	Male Dogs	Yes	85	0.7	14	0.80	93.7	0.074	215	165	12.4	1.6	9.3
PDX-T-07054	Female Dogs	No	1	0.7	14	1.21	74.1	0.073	279	232	15.9	2.0	9.2
PDX-T-07054	Male Dogs	No	1	0.7	14	1.97	85.9	0.075	264	135	10.1	1.3	9.4
PDX-K-05009	Female Dogs	No	1	1	20	1.79	100.6	0.085	456	272	24	3.0	10.6
PDX-K-05009	Male Dogs	No	1	1	20	1.28	72.4	0.244	191	143	34	4.3	30.5
PDX-K-05009	Female Dogs	No	1	3	60	4.43	227.8	0.118	823	267	32	4.0	14.8
PDX-K-05009	Male Dogs	No	1	3	60	4.03	189.1	0.166	1281	324	54	6.8	20.8
PDX-K-05010	Female Rat	No	1	5	30	3.52	75.6	0.014	28	28	0.4	1.3	46.3
PDX-K-05010	Male Rat	No	1	5	30	5.54	137.5	0.010	435	69	0.7	2.3	31.7

* This dose in humans is the dose of each enantiomer (assumed to be 50% of total drug) in milligrams.

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2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Toxicology

Three weekly doses over a four week course with two courses at a dose of 75 mg/kg (450 mg/m^2) caused 80% mortality female rats. Fifty mg/kg (300 mg/m^2) was lethal to 60% of female rats. Clinical signs preceding associated with dosing included diarrhea, hunched posture, rough hair coat, and thin appearance in males and hunched posture, rough hair coat and languidness in females. Both males and females lost weight as a function of dose. This study did not include histopathology.

Doses of as high as 25 mg/kg (150 mg/m^2) given once weekly for six weeks with one week of no dosing (seven week course) for four courses did not cause excess mortality in rats. Nor was dosing associated with any clinical toxicities but it did cause a dose dependant decrease in body weight gain and weight loss in the high dose group. The high and mid dose (10 mg/kg, 60 mg/m^2) caused spleenic enlargement that correlated with splenic hematopoiesis. Dosing also caused bone marrow hypocellularity and hepatic hematopoiesis. The doses in this study were too low to completely demonstrate the toxicity of pralatrexate in rats.

Dogs cannot tolerate single IV doses of 60 mg/m^2 or greater. Death is preceded by profound gastrointestinal symptoms including diarrhea and hematemesis. These symptoms were apparent within three days after dosing. Gastrointestinal toxicity is the most likely cause of death after single large doses of pralatrexate.

In dogs, three weekly doses of pralatrexate given over two four week courses caused unacceptable mortality at a dose of 3 mg/kg (60 mg/m^2). Again the primary toxicity manifested as gastrointestinal symptoms including emesis, abnormal feces, and dehydration and decreased food consumption. On a more intense dosing schedule (six weekly doses over a seven week course for two courses) dogs could not tolerate 1 mg/kg (20 mg/m^2), when the investigators lowered the dose to 14 mg/m^2, dogs survived but were quite ill again with gastrointestinal symptoms. Other toxicities included an increase in absolute reticulocytes and reduction in thymic size and weight.

Weekly IV doses of pralatrexate of 0.7 mg/kg (14 mg/m^2) caused unacceptable mortality in dogs by week 3 in a study with a planned schedule of six weekly doses over a seven week course for seven courses. The dogs that died or were moribund showed signs of marked to severe damage to the gastrointestinal tract and the bone marrow. The investigators then provided dietary supplementation with canned food and with folate and B_{12} for the high dose dogs there by preventing further mortality but altered the spectrum and severity of the toxicities induced. This treatment also altered the pharmacokinetics of the drug. After supplementation, dose dependant damage to the gastrointestinal tract was only mild to moderate while there was no further damage to the bone marrow. A decrease in absolute heart weight and the heart-to-brain weight ratio and the lack of complete resolution of these decreases during recovery suggest the possibility of long term cardiac toxicity.

Genotoxicity

Pralatrexate caused no increase in the incidence of reverse mutation in the *Salmonella Typhimurium* or *Escherichia coli* strains with and without metabolic activation.

In vitro in mammalian cells, pralatrexate was cytotoxic at concentrations above 10 μM. In the absence of metabolic activation, the IC_{50} was 1.4 μM for CHO cells in culture based on

relative mitotic index. The addition of S9 increased the IC_{50} to 4.3 μM. The compound did not cause an increase in chromosomal aberrations at concentrations between 0.5 and 2.1 μM in the absence or presence of S9 (3 hour exposure). After exposure for 18 hours to concentrations between 0.0063 and 0.027 μM, pralatrexate caused no apparent increase in the percentage of cells with aberrations.

In the mouse micronucleus test, the IC_{50} for the decrease in percent of polychromatic erythrocytes (PCEs) was about 9 mg/m^2 in both males and females. Doses above 15 mg/m^2 (as high 1188 mg/m^2) caused almost complete myelosuppression but only minor systemic toxicity evidenced by mild weight loss over 72 hours. Doses up to 9 mg/m^2 did not cause an increase in micronucleated PCE in either males or females.

Reproductive Toxicology

In range finding studies, doses of 36 mg/m^2 in rabbits or 0.6 mg/m^2 in rats caused total litter resorption in most animals. Thus, the high doses in both non-range-finding reproductive toxicology studies were relatively low compared to the human dose, 0.06 mg/kg/day in rats (0.36 mg/m^2/day or about 1.2% of the single clinical dose on a mg/m^2 basis) and 1 mg/kg/day in rabbits (12 mg/m^2/day or about 40% of the single clinical dose). These doses caused only minor systemic maternal toxicity manifested as small decreases in weight gain (8% or less relative to controls). Nevertheless, these doses did severely reduce fetal viability. Three high dose rabbits (of 25) aborted their litters by gestation day 25 and 8 had totally resorbed litters at necropsy. Both species showed an increase in total resorptions (late and early in the rat and early in the rabbit). There were also dose dependant increases in percent post implantation loss and total post implantation loss that were statistically significant in the high dose groups. The mean number of live fetuses decreased with increasing dose. Mean fetal weight parameters decreased with increasing dose; these decreases were significant in the HD groups.

In neither rats nor rabbits was there an increase in morphological defects among fetuses treated with pralatrexate compared to controls but in both cases the number of fetuses was significantly diminished (severely in the rabbit).

Special Toxicology Studies

Intradermal injection of pralatrexate at a dose of 20 mg/kg (120 mg/m^2) caused minimal light pink swelling in some rats at the injection site by day 8 after the injection. The reaction was somewhat more severe in females. A single perivascular injection (saphenous vein) of 40 mg/kg (240 mg/m^2) caused no changes at the injection site.

In New Zealand white rabbits doses of 3.3 mg/kg (40 mg/m^2) pralatrexate and 5.8 mg/kg (70 mg/m^2) probenecid (IV ear vein) on days 1 and 14 caused no clinical signs of toxicity. There was no significant change in the liver enzyme levels.

Dogs dosed intravenously with doses 3, 10 or 30 mg/kg (60, 200 or 600 mg/m^2) of (b) (4) weekly for six weeks all vomited or showed signs of nausea shortly after dosing. This toxicity was subjectively worse in males than females. The time of onset suggests that this is an acute neurotoxicity. Other signs of toxicity included ataxia, pale gums, labored breathing, lethargic and unresponsiveness. Kidney-to-body weight ratios were higher in HD males. Grossly, the kidneys of one dog were enlarged. Microscopically all HD dogs had some degree of "subtle cortical tubular dilation and chronic inflammation." Three of four 30-mg/kg males had tubular mineralization present in the kidney cortex. This damage was less severe or absent in recovery animals suggesting that it was reversible. Thus the spectrum of toxicities of (b) (4) is distinct from that of pralatrexate.

2.6.6.2 Single-dose toxicity

1) Intravenous Dose Range Study of PDX in Beagle Dogs

Major findings:

By day three after the dose, all dogs receiving a single of dose (3, 6 or 9 mg/kg or 60, 120 or 180 mg/m^2) had developed bloody diarrhea. Dogs in LD and HD groups had developed hematemesis. By study day four, the male MD dogs also developed hematemesis. All of the dogs in groups 2 and 4 were considered moribund by day 4 and were killed humanly. The female in group 3 died on study day 5 and the male was killed humanly on day 6.

Dogs cannot tolerate single doses of 3 mg/kg (60 mg/m^2) or greater. The primary cause of death appears from the clinical evidence of diarrhea and hematemesis to be profound gastrointestinal toxicity of rapid onset.

Study no:	Sponsor – PDX-T-05008-D; Laboratory – 03099 (1128-123)
File name:	pdxt05008D.pdf
Laboratory:	(b) (4)
Study Date:	June 2005
GLP:	No statement, this study was presented only as an Executive Summary
Audited:	Yes
Drug:	Pralatrexate Injection, Lot 101105043, purity 94.5 % Described as "forced degradation; 20 mg/mL" The major impurity was (b) (4) comprising about (b)
Methods:	
Species:	Beagle Dog
N	1 per group per sex (eight dogs total)
Doses:	0, 3, 6 or 9 mg/kg (0, 60, 120 or 180 mg/m^2) nominal doses Corrected for impurities – 0, 2.8, 5.6 or 8.5 mg/kg
Treatment Course:	Single dose
Formulation:	PBS
Dose volume:	not stated

2.6.6.3 Repeat-dose toxicity

1) Intravenous Dose Range Study of PDX in Sprague Dawley Rats

Major findings:

Three weekly doses over a four week course with two courses at a dose of 75 mg/kg (450 mg/m^2) caused excess mortality in female rats (four of five). The mid dose of 50 mg/kg (300 mg/m^2) was lethal to three of five female rats. Dosing was associated with diarrhea, hunched posture, rough hair coat, and thin appearance in males and hunched posture, rough hair coat and languidness. Dosing caused a dose dependant decreases in body weight gain in all dosed animals

and a decrease in food consumption in females. There were dose dependent decreases in red cell parameters in LD and MD females (low survival in HD) and in MD and HD males.

While this study establishes that a dose of 300 mg/m^2 is too high for a longer term study in rats. The lethal toxicity is probably gastrointestinal damage.

Study no: Sponsor – PDX-T-05013-R; Laboratory – 02957
File name: pdxt05013r—nonclinical-data.pdf
Laboratory: (b) (4)
Study Date: May 2004
GLP: No
Audited: No
Drug: Pralatrexate Injection, Lot 101I0504, Purity 99 %
Methods:
Species: Male and female Sprague-Dawley rats, 9 weeks old
Doses: The following table from the study report shows the doses and N

Text Table 4: Study Design

Group	Treatment	Test Article Dosage (mg/kg)	Test Article Concentration (mg/mL)	Dose Route	Males		Females	
					N	Animal Numbers	N	Animal Numbers
1	PBS	0	0	Intravenous	5	10373-10377	5	10378-10382
2	PDX	25	20	Intravenous	5	10383-10387	5	10388-10392
3	PDX	50	20	Intravenous	5	10393-10397	5	10398-10402
4	PDX	75	20	Intravenous	5	10403-10407	5	10408-10412

N = number of animals per group

0, 150, 300 or 450 mg/m^2

Treatment course: One IV dose weekly for three weeks followed by 1 week dose free interval
Number of courses: Two
Formulation: PBS
Route IV tail vein
Cage side: Twice daily
Clinical signs: Daily
Body weights: Twice weekly
Food consumption: Weekly
Hematology: SD 22, 29 and 50
Clinical chemistry: SD 22, 29 and 50
Necropsy: SD 57
Histopathology: Not done

Results

Mortality
One control male dead SD 29
One MD male dead SD 43
One MD male moribund SD 39
Three MD females and four HD females dead between SD 12 and 50
One LD moribund SD 18

Clinical Signs	In males HD males, diarrhea, hunched posture, rough hair coat, and thin appearance In females, thin appearance at MD and HD, hunched posture, and languidness in all dose groups, rough hair coat and few feces at 75 mg/kg.
Body Weight	Significant dose dependant decreases in body weight gain in all dosed animals.
Food Consumption	Decreased in MD and HD females
Clinical Chemistry	In the single surviving HD female rat were suggestive of a mild metabolic acidosis secondary to damage of the renal tubular epithelium (decreased CO_2) Some indication of decreased protein synthesis
Hematology	Dose dependent decreases in red cell parameters in LD and MD females (low survival in HD) and in MD and HD males Dose dependent decrease in platelet volume

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2) Pralatrexate (PDX): A Six-Month Intravenous Toxicity and Toxicokinetic Study in Sprague Dawley Rats with a Two-Week Recovery Interval

Major findings:

Doses as high as 25 mg/kg (150 mg/m^2) given once weekly for six weeks with one week of no dosing (seven week course) for four courses did not cause excess mortality in rats. Nor was dosing associated with any clinical toxicities but it did cause a dose dependant decrease in body weight gain. The high and mid dose (10 mg/kg) dose caused spleenic enlargement that correlated with splenic hematopoiesis. Dosing also caused bone marrow hypocellularity and hepatic hematopoiesis. The doses in this study were to low to completely demonstrate the toxicity of pralatrexate in rats.

Study no:	Sponsor – PDX-T-07034-R; Laboratory – 1128-04504
File name:	pdxt0734tr—nonclinical-data.pdf
Laboratory:	(b) (4)
Study Date:	July 2006
GLP:	Yes
Audited:	Yes
Drug:	Pralatrexate Injection, Lot 132I0606, Purity 98.14 %
Methods:	
Species:	Male and female Sprague Dawley rats
Doses:	The following table from the study report shows the doses and N

Text Table 4: Study Design

Group	Treatment	Pralatrexate Dosage (mg/kg)	Pralatrexate Concentration (mg/mL)	Number of Animals					
				Main Phase		Recovery Phase		Toxicokinetic	
				Male	Female	Male	Female	Male	Female
1	PBS	0	0	20	20	5	5	0	0
2	PDX	5	3.92	20	20	0	0	9	9
3	PDX	10	7.84	20	20	0	0	9	9
4	PDX	25	19.6	20	20	5	5	9	9

Doses: 0, 30, 60 or 150 mg/m^2
Treatment Course: Once weekly dosing for six weeks followed by one week dose-free interval (seven week course)
Number of Courses: Two or four courses
Formulation: PBS
Dose volume: 1.27 mL/kg
Route: IV lateral tail vein
Cage side: Twice daily
Clinical signs: Weekly
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Prior to necropsy
Hematology: Prior to necropsy
Clinical chemistry: Prior to necropsy
Toxicokinetics: The following table from the study report shows the parameters for the toxicokinetic study

Text Table 8: Toxicokinetics

Animals/Sex/Group	First 3/Sex/Group	Second 3/Sex/Group	Third 3/Sex/Group
Target Collection Time: SD 1-2	5 minutes, 1 hour, and 8 hours postdose	15 minutes, 2 hours, and 12 hours postdose	Males: 30 minutes, 4 hours, and 24 hours postdose Females: 1.5 hours, 5 hours, and 25 hours postdose
Target Collection Time: SD 85-86 SD 183-184	Predose, 30 minutes, 4 hours, and 24 hours postdose	5 minutes, 1 hour, and 8 hours postdose	15 minutes, 2 hours, and 12 hours postdose
Collection Site	jugular vein		
Volume Collected	approximately 1 mL per time point		
Tubes Used	2-mL capacity, containing sodium heparin		

Note: Predose samples on SD 1 were collected from 3 extra animals/sex found acceptable for study use but not assigned.

Necropsy: Day 92 (after two cycles)
Day 190 (after four cycles)
Day 211 (recovery animals – four weeks after the final dose in cycle 4)
Gross pathology: Major organs – adequate battery
Histopathology: Adequate battery

Results:

Mortality: One HD male (two cycle group) moribund day 30 excessive weight loss due to a technical error that limited food access.
One control male (4 cycle group) found dead day 151 with no observable lesions prior to death and no visible lesions at necropsy.
One control female (4 cycle group) moribund day 176 tail abrasions that

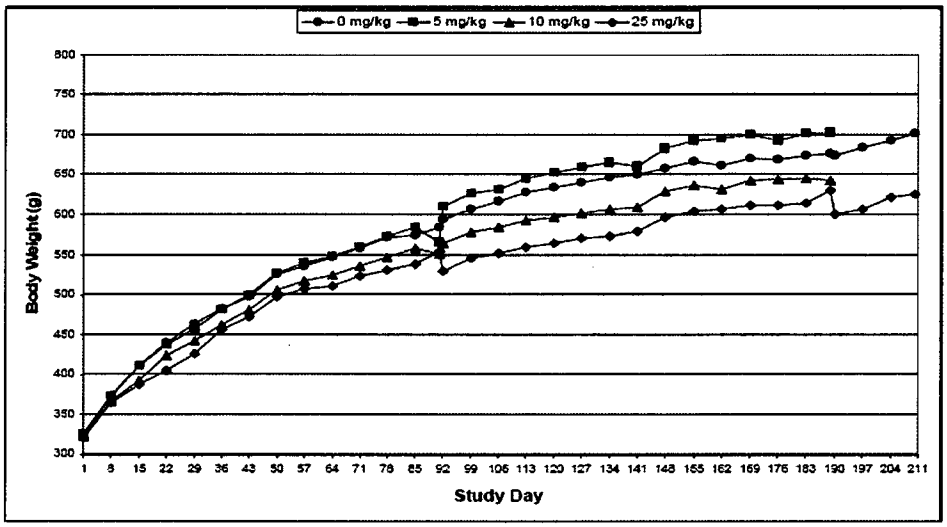
prevented further dosing
One HD males (TK group) found dead day 139 had no abnormalities recorded prior to death.
One HD males (TK group) found dead day 183 mild facial edema, moderate edema and gray discoloration of the right hind paw, and also had general observations of being hunched, pale, thin, squinting, and having a rough hair coat.
One MD female (TK group) moribund day 85 probably due to trauma (hind limb paralysis with possible spinal injury) during blood collection

The two deaths in the HD TK males are possibly dose related but these animals were not necropsied so this cannot be determined.

Clinical observations: Incidental and not toxicologically significant other than those listed above.

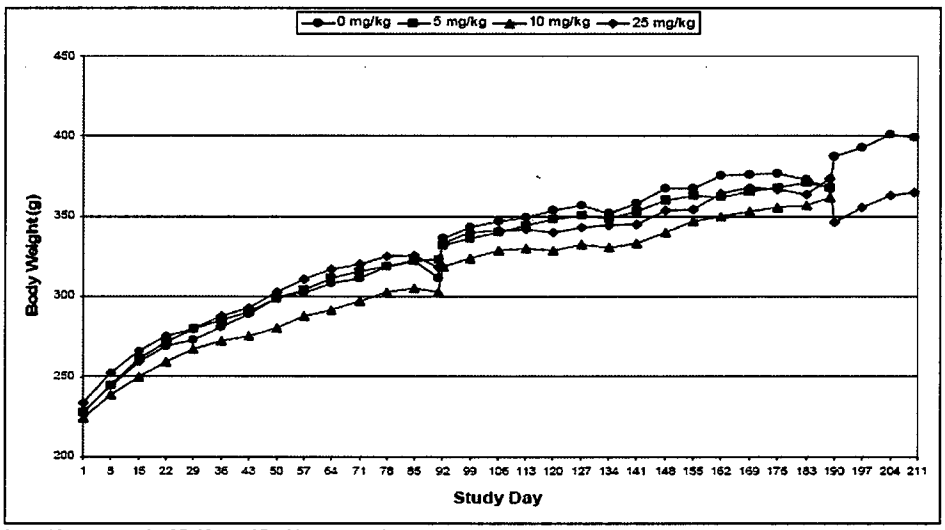
Body Weight: The following two graphs from the study report show that weight gain decreased with increasing dose throughout most of the study. The effect was more prominent in males than females.

Figure 1: Mean Body Weights - Males



Note: Mean values for SD 92 and SD 190 include unfasted body weights only

Figure 2: Mean Body Weights - Females



Note: Mean values for SD 92 and SD 190 include unfasted body weights only

Food Consumption: Mildly diminished in dosed males, the difference reached significance in HD and MD males at various points in the study. Females were relatively unaffected.

Ophthalmology: One control female and four females treated with 25 mg/kg pralatrexate were observed with erosion of the cornea on SD 209 (terminal kill). While possibly dose related this difference did not reach statistical significance by Fisher's Exact test ($p = 0.18$)

Hematology After two treatment cycles, treatment caused dose dependant decreases in erythrocyte counts, hemoglobin, and hematocrit of male and female rats.

	Dose-dependent increases were observed in mean corpuscular volume, absolute reticulocyte counts, and red cell distribution widths. Mean corpuscular hemoglobin increased in HD animals. These changes persisted after four treatment cycles, but mean corpuscular hemoglobin increased. MD and HD animals had slower mean and individual prothrombin time after four treatment cycles. This was probably due to decreased synthesis of coagulation proteins from the liver.
Clinical Chemistry	No toxicologically significant effects
Gross Pathology	Enlarged spleens were noted in two MD males and three HD males
Organ weights	Increased spleen weights in HD males and MD and HD females at interim necropsy, increased in MD and HD males and HD females at terminal necropsy Decreased testes weights, not completely recovered
Histopathology	In HD males and females there was hepatic and splenic hematopoiesis and hypocellularity of the bone marrow. The incidence and magnitude of these findings was actually greater at the interim in male rats. In terminal-necropsied animals, the frequency and magnitude of extramedullary hematopoiesis in the liver was similar between males and females, whereas females had slightly more frequent and marked extramedullary hematopoiesis in the spleen. These toxicities showed signs of recovery.

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3) PDX (10-propargyl-10-deazaaminopterin): 8-Week Intravenous Dose-Range Toxicity Study in Male and Female Beagle Dogs

Major findings:

Three weekly doses of pralatrexate given over two four week courses caused unacceptable mortality at a dose of 3 mg/kg (60 mg/m^2). The cause of death and most of the clinical signs in surviving dogs was gastrointestinal toxicity (emesis, abnormal feces, dehydration and decreased food consumption) that did not manifest as obvious gross damage. The study did not distinguish any differences between pralatrexate supplied by MSKCC and that supplied by Allos but the study was underpowered so any conclusions concerning the equivalence of the two drug products are observational and inconclusive.

Study no:	Sponsor – PDX-T-05015-D; Laboratory – 03597
File name:	pdxt05015d—nonclinical-data.pdf
Laboratory:	(b) (4)
Study Date:	May 2004
GLP:	No
Audited:	No
Drug:	Pralatrexate Injection, Lot 010804 from MSKCC Pralatrexate Injection, Lot 05010304 Purity not stated, investigators assumed 100%

Methods:

Species: Beagle Dog, males 8.3 to 13.5 kg, females 6.7 to 10.3 kg
Age at first dose at least six months

Doses: The following table from the study report shows the doses and N

Text Table 4: Study Design

Group	Treatment	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Males		Females	
					N	Animal Number	N	Animal Number
1	PBS	0	0	0.3	1	10336	1	10337
2	PDX (Allos)	0.3	20	0.015	1	10338	1	10339
3	PDX (Allos)	1	20	0.05	1	10340	1	10341
4	PDX (Allos)	3	20	0.15	1	10342	1	10343
5	PDX (MSK)	0.3	10	0.03	1	10344	1	10345
6	PDX (MSK)	1	10	0.1	1	10346	1	10347
7	PDX (MSK)	3	10	0.3	1	10348	1	10349

N = number of animals
Allos – Allos Therapeutics, Inc.
MSK – Memorial Sloan-Kettering

Treatment Course: One IV dose weekly for three weeks followed by 1 week dose free interval
Schedule: Two courses
Formulation: PBS
Cage side: Twice daily
Clinical signs: Daily
Body weights: Non-fasted body weights were taken on SD – 1, 3, 7, 10, 14, 17,21,24,28, 31,35,38,42,45,49,52, and 56. A fasted body weight was taken on SD 57
Food consumption: Daily – a statement in the results section says that the diet of these animals was "highly supplemented" but this supplementation is not mentioned in the methods section.
Hematology: SD 1 (before dosing); SD 22 (beginning week four of cycle one); SD 29 (before second-cycle dosing); SD 50 (beginning week four of cycle two); and SD 57
Clinical chemistry: SD 1 (before dosing); SD 22 (beginning week four of cycle one); SD 29 (before second-cycle dosing); SD 50 (beginning week four of cycle two); and SD 57
Necropsy SD 57
Histopathology Not done

Results

Mortality
Group 7 male (3 mg/kg MSKCC) moribund SD 40
Group 4 female (3 mg/kg Allos) moribund SD 40
These animals were languid and hypoactive and visibly thin due to decreased food consumption

Clinical Observations Gastrointestinal toxicities including emesis, abnormal feces (e.g. soft, mucoid, or diarrhea), and discolored feces in both sexes. Dehydration at doses of 1 mg/kg or greater

Body weight Both formulations caused weight loss or decreased body weight gain in dosed males and females. Weight loss was severe in the moribund animals.

Food consumption Food consumption dropped dramatically following each dose; decreased food consumption lasted for a period of two to three days in both sexes

Clinical Chem	No toxicologically significant changes
Hematology	No toxicologically significant changes
Gross pathology	No toxicologically significant changes

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4) Pralatrexate (PDX): Intravenous Dose-Range Finding Toxicity Study in Beagle Dogs

Major findings

Pralatrexate given as six weekly doses over a seven week course for two courses caused unacceptable mortality at a dose of 1 mg/kg (20 mg/m^2). Animals survived when the dose was reduced to 0.7 mg/kg (14 mg/m^2), but one female required B_{12} and folate supplementation. Gastrointestinal toxicities manifest clinically as emesis, soft feces, diarrhea, thin appearance, decreased electrolytes, decreased body weight and decreased food consumption. Other toxicities included an increase in absolute reticulocytes and reduction in thymic size and weight.

Study no:	Sponsor – PDX-T-07035-D; Laboratory – 1128-05083
File name:	pdxt07035d—nonclinical-data.pdf
Laboratory:	(b) (4)
Study Date:	May 2006
GLP:	No
Audited:	No
Drug:	Pralatrexate Injection, Lot 061I0206, Purity 98.9 %
Methods:	
Species:	Beagle Dog, males 7.3 – 8.7 kg, females 6.2 – 8.0 kg Age at first dose about 6 months
Doses:	The following table from the study report shows the doses and N

Text Table 4: Group Assignment and Doses

Group	Treatment	Target Dose Level (mg/kg/week)	Dose Concentration (mg/mL)	Nominal Dose Volume (mL/kg)	Animal Numbers	
					Males	Females
1	PBS	0	0	1	13479-13480	13481-13482
2	PDX	0.1	0.1	1	13483-13484	13485-13456
3	PDX	0.3	0.3	1	13487-13488	13489-13490
4	PDX	1.0 (SD 1, 8, 15) 0.7 (SD 22, 29) 0.7 (SD 50-85)	1 (SD 1, 8, 15) 0.7 (SD 22, 29) 0.7 (SD 50-85)	1 (SD 1, 8, 15) 0.7 (SD 22, 29) 1 (SD 50-85)	13491-13492 13989[a]	13493-13494 13990[ab]

a - Added to study due to early mortality in the high-dose group.
b - Supplemented with vitamin B_{12} and folic acid in Weeks 10-15 (SD 64 to 99).

The methods of this study including schedule were similar to those in the study that follows except that this study did not collect histopathology data.

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5) Pralatrexate (PDX): A Nine-Month Intravenous Toxicity and Toxicokinetic Study in Beagle Dogs with a Four-Week Recovery Interval

Major findings:

Weekly IV doses of pralatrexate of 0.7 mg/kg (14 mg/m^2) caused unacceptable mortality in dogs by week 3. The dogs that died or were moribund showed signs of marked to severe damage to the gastrointestinal tract and the bone marrow. Dietary supplementation with canned food and supplementation with folate and B_{12} for the high dose dogs prevented further mortality but altered the spectrum and severity of the toxicities induced. After supplementation, dose dependant damage to the gastrointestinal tract was only mild to moderate while there was no further damage to the bone marrow. Most decreases in organ weight at the end of dosing in dogs receiving pralatrexate were consistent with decreased body weight gain and decreased food consumption. Nevertheless, the decrease in absolute heart weight and the heart-to-brain weight ratio and the lack of complete resolution of this decrease during recovery suggest the possibility of a cardiac toxicity.

Study no: Sponsor – PDX-T-07054-D; Laboratory – 1128-04503
File name: pdxt0754d—nonclinical-data.pdf
Laboratory: (b) (4)
Study Date: September 2006
GLP: Yes
Audited: Yes
Drug: Pralatrexate Injection, Lot 132I0606, Purity 98.14 %

Methods:

Species: Beagle Dog, males 7.1 – 10.9 kg, females 5.1 – 8.8 kg
Age at first dose 4-5 months

Doses: The following table from the study report shows the doses and N

Text Table 4: Study Design

Group	Treatment	Nominal Dose (mg/kg)	Nominal Dose Concentration (mg/mL)	Number of Animals					
				Interim Phase		Main Phase		Recovery Phase	
				Male	Female	Male	Female	Male	Female
1	PBS	0	0	5	5	5	5	2	2
2	PDX	0.1	0.1	5	5	5	5	None	None
3	PDX	0.3	0.3	5	5	5	5	None	None
4	PDX	0.7	0.7	5	5	5	5	2	2

Note: Due to a communications error, the stock concentration was initially thought to be 19.8 mg/mL and this value was used for the first cycle of dosing. The difference in actual nominal dose was negligible.

Treatment Course: One IV dose weekly for six weeks followed by 1 week dose free interval
Schedule: Interim phase – 2 courses; Main Phase – 7 courses
Formulation: PBS
Dose volume: 1 mL/kg
Cage side: Twice daily
Clinical signs: Weekly
Body weights: Weekly
Food consumption: Daily (subjective)
Ophthalmoscopy: At Necropsy

EKG: Prior to first dose and prior to scheduled termination. Parameters measured included heart rate, PR, QRS, QT intervals and heart rate correction of the QT interval (QTC). RR was calculated from the heart rate. The protocol does not specify the time of the ECG recording relative to the time of dosing.
Hematology: Pre-dose, sd 87, sd 185, sd 283, sd 309
Clinical chemistry: Pre-dose, sd 87, sd 185, sd 283, sd 309
Toxicokinetics: The following table from the study report shows the schedule for TK sampling. Nonparametric analysis.

Text Table 8: Blood Collection Information for Toxicokinetics

Collection Day	Time Points
Study Day 1/2	Predose, 5 and 30 minutes, and 1, 2, 4, 12, and 24 hours postdose
Study Day 85/86	Predose, 30 minutes, and 2, 4, 12, and 24 hours postdose
Study Day 183/184	Predose, 30 minutes, and 2, 4, 12, and 24 hours postdose
Study Day 281/282	Predose, 30 minutes, and 2, 4, 12, and 24 hours postdose

Necropsy: On sd 87 (interim-necropsy animals), sd 283 (terminal-necropsy animals), sd 309 (recovery-necropsy animals)
Gross pathology: Major organs – adequate battery
Histopathology: Adequate battery

Protocol Deviations:

Due to adverse clinical signs, including death and early termination, body weight losses, and poor appetite, all 0.7 mg/kg animals were supplemented with vitamin B_{12} (0.5 mg/animal/week) and folic acid (5 mg/animal/day) beginning during Study Week 4. Thus the study mimics clinical treatment.

Results:

Mortality:
1) One HD female was found dead on day 19, there were no macroscopic findings. The femoral and sternal marrow was significantly hypocellular. There was significant microscopic damage in the GI tract including epithelial necrosis probably sufficient to cause the animals demise. There was marked atrophy of the thymus and the sternal and femoral marrow was markedly hypocellular.

2) One HD female was moribund on day 19. This dog had dark discoloration of the stomach and duodenum. The stomach discoloration correlated histologically with mild, multifocal, congestion of the lamina propria; the duodenal discoloration correlated histologically with minimal, multifocal congestion of the lamina propria. There was mild to moderate epithelial necrosis in the GI tract and marked atrophy of the thymus. The sternal and femoral marrow was markedly hypocellular.

3) One HD female moribund on day 283 (this dog was originally scheduled for recovery, but included in the pathology report as a normally terminated animal).

4) One HD male (#14143) was moribund on day 11; another dog was substituted into the study. The moribund dog had red fluid in the cecum and colon; mild, patchy red discoloration of the jejunum accompanied by moderate, diffuse, thick corrugations; and gelatinous fluid

accumulation in the ventral neck region. There were no histopathological correlates for the red fluid in the cecum and colon or the gelatinous fluid accumulation in the ventral neck. The macroscopic observations in the jejunum correlated histologically with moderate, diffuse epithelial and crypt necrosis. There was moderate atrophy of the salivary gland and moderate to marked atrophy of the thymus. BUN and creatinine were elevated and Na^+, K^+ and Cl^- were lower, consistent with hemo-concentration and electrolyte loss associated with diarrhea or emesis.

5) One HD male (#14151) moribund on day 88 (scheduled for 7 cycles) had red, diffuse discoloration of the duodenum and colon. There was no histological correlate for the duodenal discoloration; the colonic discoloration correlated histologically as mild, multifocal congestion of the lamina propria. There was microscopic evidence of mild to moderate epithelial necrosis in the GI tract and moderate atrophy of the salivary gland and moderate to marked atrophy of the thymus. The sternal and femoral marrow was moderately hypocellular.

Signs preceding death:	Prior to death emesis, diarrhea, mucoid feces, no feces, discolored feces, anorexia,
Clinical Signs:	Diarrhea most treated all treated animals
Surviving dogs:	Changes in fecal consistency and color Animals appeared somewhat emaciated in association with weight loss, HD males, LD, MD and HD females Anorexia Emesis The incidence of all these signs increased with dose and time on study
Body Weight	The following charts from the study report show that male and female dogs gained less weight than controls as a function of dose. HD animals received canned dog food as a supplement to their diet and still gained the least amount of weight. MD dogs received occasional supplementation and showed signs of weight recovery after day 100. The weight loss in males and Control females toward the end of the

dosing period is some what paradoxical.

Figure 1: Mean Body Weights – Male

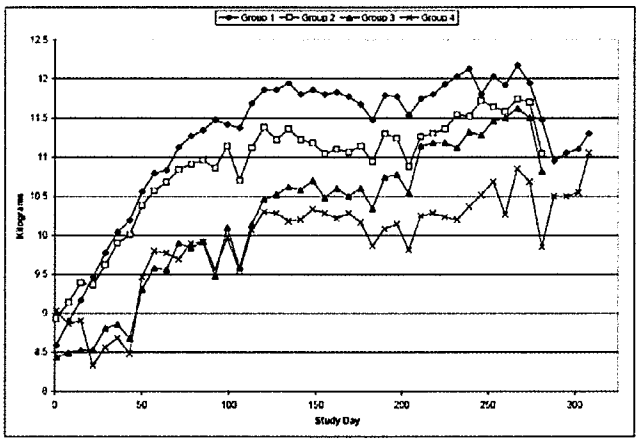
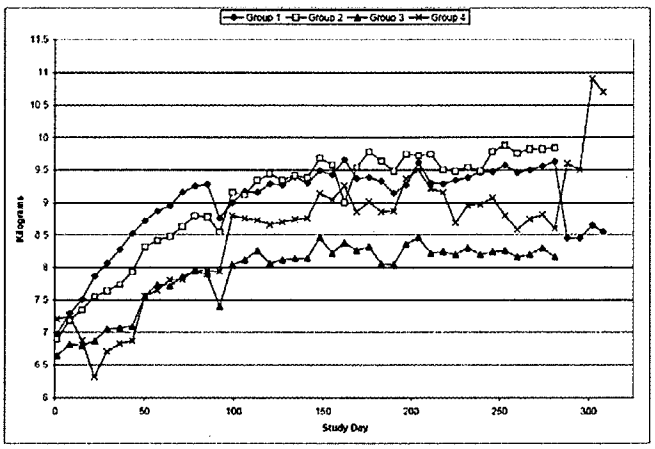


Figure 2: Mean Body Weights - Female



Food Cons.: There was a dose dependant decrease in food consumption. The nadir was usually 24 to 72 hours after dosing

Ophthalmology: No toxicologically significant findings

ECG: No toxicologically significant findings

Clinical Chemistry and Hematology:

Day 87 Male	Control Value	Percent Change from Control 2 mg/m^2	6 mg/m^2	14 mg/m^2
Creatinine	0.85	-2.4%	-9.4%	**-10.6%**
Ca^{++}	10.87	-0.1%	**-3.1%**	**-3.6%**
WBC	11.64	-13.5%	-1.8%	**-23.2%**
Lymphocyte	3.41	**-16.7%**	**-48.4%**	**-53.1%**
Eosinophils	0.29	10.3%	**-44.8%**	**-58.6%**
Basophils	0.06	**-83.3%**	**-100.0%**	**-100.0%**
platelets	2.95	0.3%	18.6%	**26.8%**
APTT	22.13	1.0%	111.5%	28.3%
fibrinogen	205	21.0%	**41.7%**	**34.1%**

Day 87		Percent Change from Control		
Females	Control Value	2 mg/m^2	6 mg/m^2	14 mg/m^2
Creatinine	0.86	**-11.6%**	**-11.6%**	**-17.4%**
Glucose	84.8	-4.5%	-9.7%	**-10.5%**
Ca^{++}	10.75	-1.4%	**-3.3%**	**-5.1%**
Albumin	2.88	-2.8%	-6.2%	-9.7%
Globulin	2.18	4.6%	**6.4%**	5.5%
A/G ratio	1.33	-7.5%	**-12.0%**	**-14.3%**
AST	37.25	10.9%	14.6%	**23.5%**
AlkPhos	101.6	-4.8%	-20.4%	-16.7%
RBC	6.75	**-7.7%**	**-10.8%**	**-9.9%**
Hbg	15.2	**-5.5%**	**-8.3%**	**-8.8%**
Hct	43.9	**-5.6%**	**-7.4%**	**-7.6%**
Neutrophils*	6.41	-14.2%	-2.8%	24.8%
Lymphocyte	3.77	**-25.5%**	**-43.0%**	**-48.8%**
Monocytes	0.55	-7.3%	20.0%	41.8%
Eosinophils	0.23	26.1%	0.0%	-26.1%
Basophils	0.06	**-83.3%**	**-100.0%**	**-83.3%**
platelets	310	5.2%	16.5%	**27.4%**
APTT	38.29	-31.4%	-12.4%	-16.4%
fibrinogen	145	**37.9%**	**46.2%**	**77.2%**
Prothrombin time	7.94	9.9%	**-5.9%**	-4.7%

* One HD animal had a very high neutrophil count of 16.3

Day 283	Percent Change from Control				
Male	Control Value	2 mg/m^2	6 mg/m^2	14 mg/m^2	Comment
Albumin	2.81	1.1%	-4.6%	**-9.3%**	recovered
A/G ratio	1.06	-4.7%	-6.6%	-10.4%	rebound to higher value
ALT	64.7	-24.9%	36.9%	13.8%	recovered
RBC	6.45	-3.7%	-8.1%	**-10.1%**	partial recovery
Hbg	15.43	-5.8%	-8.5%	**-10.4%**	recovered
Hct	44.4	-5.3%	-8.3%	**-10.3%**	partial recovery
WBC	10.99	-12.4%	-18.0%	**-32.9%**	partial recovery
Neutrophils	7.02	-12.7%	-13.0%	**-31.9%**	partial recovery
Lymphocyte	2.79	-7.9%	-25.4%	-34.8%	partial recovery
Eosinophils	0.54	-29.6%	-44.4%	-37.0%	continued to decrease
Basophils	0.05	**-80.0%**	**-80.0%**	**-80.0%**	recovered
platelets	316	-2.2%	3.8%	24.7%	decreased during recovery
APTT	14.26	26.9%	26.2%	0.5%	Increased to normal
fibrinogen	257	33.5%	56.4%	23.7%	rebound to lower value
Prothrombin time	7.41	4.7%	25.5%	11.3%	recovered

Day 283 Female	Control Value	Percent Change from Control 2 mg/m^2	6 mg/m^2	14 mg/m^2	Comment
Creatinine	0.89	-5.6%	-14.6%	**-16.9%**	remained low*
Ca^{++}	10.56	-2.8%	-1.7%	**-5.0%**	recovered
Albumin	2.97	-6.4%	-4.4%	**-13.5%**	rebound to higher value
A/G ratio	1.17	-8.5%	-12.8%	-17.1%	recovered
AST	31.43	34.9%	**58.4%**	**51.8%**	recovered
ALT	47.57	-5.8%	4.7%	349.9%	2 animals with very high values
RBC	6.72	-6.5%	-10.1%	**-11.8%**	rebound to higher value
Hbg	15.7	-3.2%	-7.4%	-9.0%	rebound to higher value
Hct	44.86	-3.0%	-7.0%	-7.8%	rebound to higher value
Neutrophils	6	13.2%	6.3%	22.2%	recovered
Lymphocyte	2.55	11.4%	-19.2%	**-39.2%**	recovered
Monocytes	0.52	-5.8%	1.9%	-25.0%	partial recovery
Eosinophils	0.36	22.2%	-22.2%	-41.7%	partial recovery
Basophils	0.06	-83.3%	-66.7%	-83.3%	partial recovery
platelets	338	3.0%	11.5%	26.0%	decreased during recovery
APTT	42.13	-31.1%	-63.2%	-46.5%	elevated
fibrinogen	227.7	17.1%	**39.9%**	**51.5%**	recovered
Prothrombin time	7.57	1.7%	-2.2%	1.7%	normal

Organ Weights

Organ Weight Male	Control Value	Percent Change from Control 2 mg/m^2	6 mg/m^2	14 mg/m^2
brain	82.22	-3.6%	4.5%	-5.7%
Heart	102.6	**-12.5%**	**-14.3%**	**-21.6%**
Heart/Brain ratio	1.25	-9.2%	-18.0%	-16.9%
Kidney	55.6	-8.6%	-15.6%	-15.1%
Kidney/Brain ratio	0.68	-5.2%	-19.3%	-9.9%
Spleen	30.5	-7.9%	-11.5%	-14.1%
Testes	21.4	0.0%	3.3%	-22.9%
Female				
Brain	79.549	-6.4%	-7.2%	-11.8%
Heart	86.8	-10.8%	-16.2%	-16.0%
Heart/Brain ratio	1.09	-4.8%	-9.7%	-4.8%
Kidney	44.4	-1.1%	-10.1%	-9.7%
Kidney/Brain ratio	0.56	5.6%	-3.2%	2.4%
Spleen	24.4	11.5%	16.4%	-0.8%
Thymus	8.3	8.4%	-13.3%	-14.9%
Thyroid	1.2	-26.0%	-12.5%	-23.3%
Uterus	11.2	-53.6%	-51.8%	-17.9%
Uterus/Brain ratio	0.14	-50.4%	-48.0%	-6.9%
Ovaries	1.42	-32.8%	-30.8%	-28.0%
Ovaries/Brain ratio	0.017851	-28.2%	-25.4%	-18.4%

Gross Pathology: At interim necropsy (SD 87), PDX administration was associated with lesions in the intestinal tract, mesenteric lymph node, and the injection site. Red discoloration of the duodenum, jejunum, ileum, cecum, colon, and/or rectum was observed in one, four, and four animals in the 0.1, 0.3, and 0.7 mg/kg groups, respectively. Discoloration of the mesenteric lymph nodes was observed in one, two, and three animals in the 0.1, 0.3, and 0.7 mg/kg groups, respectively. Red discoloration of the injection site was observed in female animals only (two, one, and one in the 0.1, 0.3, and 0.7 mg/kg groups, respectively).

At the terminal necropsy (SD 283), gross findings associated with PDX

administration were not as apparent as at the interim necropsy.

At the recovery necropsy (SD 309), there were no macroscopic lesions associated with previous administration of PDX in the Group 4 animals.

Histopathology

Histopathology Interim Kill Day 87	male				female			
	Control	2 mg/m^2	6 mg/m^2	14 mg/m^2	Control	2 mg/m^2	6 mg/m^2	14 mg/m^2
Cecum								
congestion; lamina propria; multifocal, mimimal							1/5	1/3
Colon								
congestion; lamina propria; multifocal								
minimal							2/5	
Duodenum								
congestion; lamina propria; multifocal								
minimal			1/5	1/5			2/5	
necrosis; epithelial; diffuse								
minimal			2/5	1/5				
mild				3/5				
dilation; crypt; multifocal								
minimal		2/5	1/5	3/5	1/5		2/5	
mild								2/3
Ileum								
congestion; lamina propria; multifocal								
minimal				1/5			1/5	
necrosis; epithelial; diffuse								
minimal							2/5	1/3
mild				3/5				2/3
Jejunum								
congestion; lamina propria; locally extensive								
minimal			1/5	1/5		1/5		1/3
necrosis; epithelial; diffuse								
minimal							4/5	
mild				2/5				3/3
moderate				2/5				
Mesenteric Lymph Node								
congestion; medullary; locally extensive								
mimimal		1/5					1/5	
mild			1/5	2/5			1/5	2/5
Rectum								
congestion; lamina propria; multifocal								
minimal							2/5	

Histopathology Terminal Kill Day 283	male				female			
	Control	2 mg/m^2	6 mg/m^2	14 mg/m^2	Control	2 mg/m^2	6 mg/m^2	14 mg/m^2
Cecum								
congestion; lamina propria; multifocal								
minimal				1/4				
necrosis; epithelial; crypt; multifocal								
minimal							1/5	
dilation; crypt; multifocal								
minimal							1/5	
Colon								
congestion; lamina propria; multifocal								
minimal			1/5					
necrosis; epithelial; crypt; multifocal								
minimal							1/4	
dilation; crypt; multifocal								
minimal							1/4	
Duodenum								
congestion; lamina propria; multifocal								
minimal			1/5					
necrosis; epithelial; diffuse								
minimal								1/5
necrosis; epithelial; crypt; focal								
minimal			1/5		1/5		1/4	
necrosis; epithelial; crypt; multifocal								
minimal	1/5		3/5			2/5	1/4	
dilation; crypt;focal								
minimal			1/5				1/4	
dilation; crypt; multifocal								
minimal	1/5	1/5	3/5		1/5	2/5	1/4	
villus fusion; chronic; diffuse								
minimal			2/5				4/4	
mild			3/5					
moderate				**4/4**				**5/5**
Ileum								
congestion; lamina propria; multifocal								
minimal			1/5		2/5			1/5
villus fusion; chronic; diffuse								
minimal			2/5				2/4	
mild			3/5				2/4	
moderate				**4/4**				**5/5**
Jejunum								
congestion; lamina propria; locally extensive								
minimal	1/5							1/5
necrosis; epithelial;crypt; diffuse								
minimal				1/4				1/5
necrosis; epithelial; crypt; focal								
minimal							1/4	
necrosis; epithelial; crypt; multifocal								
minimal							1/4	
villus fusion; chronic; diffuse								
minimal			2/5				3/4	
mild			3/5				1/4	
moderate				**4/4**				**5/5**
Mesenteric Lymph Node								
congestion; medullary; locally extensive								
mimimal			1/5					
mild				2/4				
Thymus								
atrophy; diffuse								
mild								1/5
moderate								2/5

The high dose in this study, 14 mg/m^2, was severely toxic and resulted in early mortality in both males and females. The deaths of these animals were probably the result of damage to the gastrointestinal tract seen both gross examination and microscopically and evidenced by anorexia prior to death. Damage was more severe in the upper GI than in the ileum. Animals also showed signs of thymic atrophy and significant hypocellularity of the bone marrow. Supplementation canned food, folic acid and vitamin B_{12} in study week 4 for high dose dogs profoundly changed the toxic dose response of this study. So much so, that the study would not

be predictive of expected toxicities if patients were not also provided with similar vitamin supplementation during the clinical course of treatment.

Male dogs showed a moderate dose related leukopenia on both SD 87 and 283 that partially recovered by day 303. This leukopenia manifested broadly as diminished lymphocyte, eosinophil and basophil counts suggesting marrow hypoplasia but microscopically the marrow was within normal limits. Male dogs also showed a mild decrease in red cell parameters consistent with marrow hypoplasia. MCH was unchanged so decrease in Hct was due to loss of RBCs. In contrast to the males, females showed a mild leukocytosis in the high dose group. This increase in white cells in females was probably the result of a generalized inflammatory response. This is also suggested by the increase globulin and fibrinogen and the accompanying decrease in APTT in females. Decreases in red cell parameters on day 283 were similar to those seen in males on day 87. These minor sex differences in the spectrum of toxicity are possibly due to differences in metabolism

Platelets in mid and HD males were higher than control values on day 283 but these values in HD males were significantly less than predose values (Students T, $p = 0.04$ one tailed test) as were the recovery values ($p < 0.001$). Platelets in HD animals at recovery were significantly lower than platelets in HD animals at day 283, thus platelets declined throughout dosing and continued to decline over the course of the recovery period. Platelets in other groups also declined over the course of dosing, including controls, but the control values increased somewhat (not statistically significant) during recovery.

Serum calcium decreased in all groups including the control group as the study progressed. The difference between values relative to predose concentrations was significant on days 87, 185 and 283 ($p < 0.01$, 1 way ANOVA, my analysis). A similar ANOVA of controls before dosing verses controls on day 283 and HD males on day 283 shows that both are different from predose controls ($p < 0.001$). The sponsor's analysis demonstrates a difference between treated animals and controls on days 87 and 185. This difference reaches significance in HD animals at both these observation times ($p < 0.05$).

The small decrease in serum creatinine and alkaline phosphatase is probably due to protein malnutrition. In HD females two individuals had severely elevated serum ALT concentrations on day 283 suggesting the possibility of some liver damage. There was no microscopic damage in the liver of these animals or any others.

Several organs (brain, kidney and spleen of males and females, the thymus and thyroid of females and the testes) weighed less than normal at the terminal kill (day 283) but returned to normal upon recovery. The recovery in females is difficult to assess as there was only one dog remaining alive. Most of this decrease in organ weight can be attributed to the decreased body weight; organ-to-body weight and organ to brain ratios were not significantly changed. Nevertheless, the weight of the heart in male and female dogs did decrease with increasing dose to as much as 22% less than controls by day 283. The heart to brain ratio was also decreased on day 283 and remained low at recovery. This finding is probably toxicologically significant despite the lack microscopic evidence of toxicity in the heart.

Microscopic organ damage was confined primarily to the gastrointestinal tract and increased in severity and incidence with increasing dose. The most severe damage manifested as moderate diffuse chronic fusion of the villi in the duodenum and ileum. This damage correlated with significant decreases in weight gain in dosed dogs. The damage would probably have been much worse without the folate, B_{12} and nutritional supplementation. There was some damage to the thymus in females and the mesenteric lymph node in males.

Treatment caused no microscopic damage to the reproductive organs of either sex. The testes in recovery males were of normal weight.
