CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-462

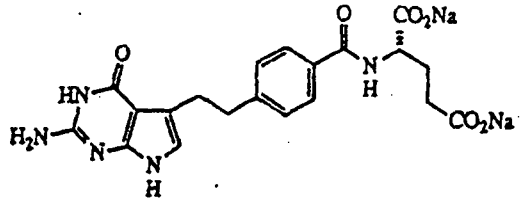
Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA:	21-462
Review number:	1
Sequence number/date/type of submission:	000/10/25/02/NDA
Information to sponsor:	Yes(x), No( )
Sponsor:	Eli Lilly and Company. Indianapolis, IN 46285
Manufacturer for drug product:	Eli Lilly and Company. Indianapolis, IN 46285
Reviewer name:	Doo Y. Lee Ham, Ph. D.
Division name:	Division Oncology Drug Products
HFD #:	HFD-150
Review completion date:	August 14, 2003

Drug:

Trade name:	ALIMTA (Pemetrexed for Injection)
Generic name:	Pemetrexed disodium (MTA, LY231514)
Code name:	LY231514
CAS number:	137281-23-3
Chemical name:	N-[4-[2-(2-Amino-4,7-dihydro-4-oxo-1H-pyrrolo [2,3-d] pyrimidin-5-yl) ethyl] benzoyl]-L-glutamic acid disodium salt
Molecular formula:	$C_{20}H_{19}N_5O_6 \cdot 2Na$
Molecular weight:	579. 49
Master file #:	Not provided
Structure:	



Relevant IND: IND 40,061 (LY231514)

Drug Class: A Thymidylate synthase inhibitor

Indication: Malignant pleural mesothelioma

Clinical Formulation: Alimta (Pemetrexed Disodium for Injection) 500 mg/vial is supplied as a powder for reconstitution for intravenous infusion.

Route of Administration: Intravenous Infusion

Proposed use: Alimta (Premetrexed Disodium) is a folate antagonist proposed for the treatment of malignant pleural mesothelioma in combination with cisplatin. The recommended dose of ALIMTA is 500 mg/m2 administered as an intravenous rapid infusion over 10 minutes once every 21 days followed approximately 30 minutes later by a 2 hr infusion of 75 mg/m2 cisplatin.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability:

The non-clinical studies adequately support the use of LY231514 (ALIMTA), by the intravenous infusion for the treatment of malignant pleural mesothelioma.

The pharmacology information in the application do support the applicant's claims for the mechanism of action.

B. Recommendation for Nonclinical Studies:

No further toxicology information is necessary to support the indication of malignant pleural mesothelioma.

II. Summary of Non-clinical Findings

A. Brief Overview of Non-clinical Findings

LY231514 (ALIMTA®; MTA; pemetrexed disodium) is a novel pyrrolopyrimidine antifolate antimetabolite and a specific inhibitor of thymidylate synthase (TS). It exerts its antifolate antineoplastic activity by disrupting crucial folate-dependent metabolic processes that are essential for cell replication. Many studies have shown that LY231514 requires intracellular polyglutamation for its cytotoxic effect and these polyglutamates potently inhibit several key folate-requiring enzymes, including thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT).

LY231514 has shown its antitumor activity as a single agent against NSCLC, head and neck, colon, and breast cancer. LY231514 has been evaluated in combination with other chemotherapeutic agents. In particular, studies with mesothelioma ell lines (NCI-2052 and MSTO-211H) showed greater effects when LY231514 was combined concurrently with cisplatin.

In a single dose studies, LY231514 demonstrated low toxicity in both mice (MLD=>4722 mg/m2) and rats (MLD=>8718 mg/m2) but higher toxicity in dogs (MLD=>2000 mg/m2). Death was preceded by hunched posture, ataxia, piloerection, decreased food intake, and clonic convulsion. Six weeks repeat dose studies were conducted using daily, twice weekly or weekly i.p. doses in mice and i.v. doses in dogs. Higher daily doses were not tolerated in dogs for more than 3 weeks. Generally the daily dose schedule was more toxic than the weekly administration of much larger dose. Mice tolerated weekly i.p dose of 944 mg/m2 (close to twice the clinical dose) without death or clinical signs of toxicity; weekly i.v. dose of 2099 mg/m2 (about four times the clinical dose) killed two of six dogs. Across species, chronic dosing at higher doses causes decreased food consumption, mucositis, decreased red cell parameters, leukopenia, neutropenia, increased hepatic enzymes, and decreased electrolytes. Microscopic changes occur in the thymus, lymph nodes, GI tract and intestine (enteropathy, mucositis-dog), testis, bone marrow, and skin. Clinically, rash, nausea, diarrhea, asthenia, and leukopenia and neutropenia are dose-limiting.

Non-clinical studies were done to evaluate the effects of rescue agents (leucovorin and thymidine) for the treatment of severe toxicity because of LY231514. Coadministration of leucovorin, a reduced form of folate, reversed the toxicity and hematological alterations induced

by LY231514 treatment in dogs. Dogs given LY231514 combination with thymidine had no clinicopathologic alterations associated with administration of LY231514.

LY231514 i.v. doses of 0.3 mg/m2 caused testicular atrophy and reduced fertility. LY123514 is embryotoxic and teratogenic in mice at 0.6 mg/m2. Carcinogenicity studies have not been conducted. LY231514 caused no genetic damage in the standard battery of tests; however, LY231514 was clastogenic in the vivo micronucleus assay. Non-clinical irritation studies also show that LY231514 has potential to cause ocular and dermal irritation.

The pharmacokinetics of LY231514 was found to be similar in mice following both i.v. or i.p. administration. After single i.v. administration to mice, dogs and man, plasma concentration of LY231514 declined rapidly. The AUC values increased in a dose-dependent manner in all species. The elimination half-life was shorter in dogs and man compared to mice. Compound-related radiocarbon was rapidly distributed following an i.v. dose, however, tissue levels did not persist beyond 3 hrs. In both mice and dogs, the major route of elimination was via the kidney and the majority of the parent compound was excreted unchanged.

B. Nonclinnical Safety Issues Relevant to Clinical Use:

The sponsor does not need to do further non-clinical studies to support the proposed indication.

III. Administrative

/S/

A. Reviewer signature:

Doo Y. Lee Ham, Ph. D.
Pharmacology-Toxicology Reviewer

/S/

B. Supervisor signature:

Concurrence-_____
David Morse, Ph. D.
Supervisory Pharmacologist
Non-Concurrence-_____

C. cc:list

OVERALL SUMMARY AND EVALUATION:

Pemetrexed disodium (LY231514) is a structurally novel pyrrolopyrimidine antifolate antimetabolite, which is a specific inhibitor of thymidylate synthase (TS). Antimetabolites are cytotoxic drugs that are structurally similar to naturally occurring molecules by competing the sites that are necessary for the synthesis of purines, pyrimidines, and nucleic acids.

The mechanism of action of pemetrexed is not fully understood. However, many studies have shown that LY231514 is taken into the cell via the reduced folate carrier (RFC) and membrane folate-binding protein (FBP) carriers. Once in the cell, LY231514 is rapidly polyglutamated by folypolyglutamate synthetase and trapped inside the cell where exerts its antiproliferative or cytotoxic activity. Both parent and polyglutamated LY231514 behave as competitive inhibitors of several folate-dependent enzymes, including thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide transferase (GARFT), which are the key enzymes for de novo nucleotide biosynthesis.

Pharmacology:

Pemetrexed has shown its antitumor activity against a variety of tumor types in preclinical models with human tumor cells in vitro. In vivo, pemetrexed exhibited efficacy in mouse xenografts models.

In in vitro antiproliferative studies, pemetrexed was found to have antitumor activity against a broad range of tumor cell lines, including leukemia (CCRF-CEM, L1210), lung (A549), mesothelioma (NCI-H2052 and MSTO-211H), breast (MCF7), colon (GC3 and HCT8), and ovarian cancer (SKOV1) cell lines. In the colony-forming assays, clinically relevant concentrations of LY231514 gave dose-dependent responses against a wide panel of specimens, including tumor types that were chemo-resistant to methotrexate, 5-FU, and raltitrexed.
In in vivo efficacy studies, pemetrexed was active against human xenografts of colon VRC5, breast MX-1, pancreatic BxPC-3, and lung LX-1 carcinomas in nude mice. Also, pemetrexed was active against human colon xenografts (GC3 and VRC5), these tumors were sensitive to Lometrexol but resistant to Methotrexate.

LY231514 has demonstrated its activity not only as a single agent but also in combination with a variety of other chemotherapeutic agents, including platinum (cisplatin, carboplatin), 5-FU, doxorubicin, CPT-11, oxaliplatin, paclitaxel, and gemcitabine. In particular, studies with mesothelioma cell lines (MSTO-211H, NCI-H2052) showed synergistic effects when LY231514 was added simultaneously with cisplatin. In both NCI-H23 and NCI-H460 lung carcinoma cells, combination of LY231514 with cisplatin produced additive interaction, regardless of the sequence of administration.

Pharmacokinetics:

Pharmacokinetic studies of LY231514 have been performed in mice and dogs. In mice, plasma pharmacokinetics was compared following intraperitoneal doses of 20 and 200 mg/kg to that of intravenous dose of 20 mg/kg LY2315. The pharmacokinetics was determined in dogs following single (7.5, 100 mg/kg) and multiple intravenous doses (0.11-104.96 mg/kg). The pharmacokinetics of LY231514 was found to be similar in mice following intravenous or Intraperitoneal route. The terminal half-life of LY231514 in mice was 7.8 and 10 hrs for 20 mg/kg and 200 mg/kg intraperitoneal dose levels and 7 hrs for 20 mg/kg intravenous dose. After single intravenous administration to mice and dogs, plasma concentration of LY231514 declined in a biphasic manner, a rapid distribution phase followed by a longer elimination phase.

Studies Reviewed with This Submission:

Pharmacology:

Mechanism of Action:

NCPR 13: Transport of LY231514 Disodium by both Reduced Folate Carrier (RFC) and Membrane Folate Binding Protein (mFBP). Vol. 1.4, pages 1-16. 1995

NCPR 16: Mechanism of Transport of LY231514 Disodium and cross-resistance pattern in cells with markedly Impaired Transport of MTX. Vol. 1.4, pages 1-12. 2000

NCPR 18: Effect of LY231514 Disodium on Purified Folate-Requiring Enzymes and on the Proliferation of Different Tumor cell lines in culture. Vol. 1.4, pages 1-11. 1997

NCPR 20: Dipyridamole potentates in vitro activity of LY231514 Disodium by inhibition of Thymidine transport. Volume 1.4, pages 1-9.

NCPR 28: Potential Role of Dihydrofolate Reductase (DHFR) Amplification on Cellular Resistance to LY231514 Disodium. Vol. 1.5, pages 1-11.2001

NCPR 29: Role of Folic Acid in Modulating the Toxicity and Efficacy of LY231514 Disodium. Volume 1.5, pages 1-7. 1998

NCPR 30: LY231514 Disodium and Vitamin Supplements in the Human MX-1 Breast carcinomas. Volume 1.5, pages 1-12. 2002

NCPR 31: The Impact of p53 status on Cellular Sensitivity to LY231514 and other Antifolate Drugs. Volume 1.5, pages 1-13. 2001

In Vitro Efficacy Studies:

NCPR 01: Activity of the Multitargeted antifolate LY231514 in the Human tumor Cloning assay. Volume 1.3, pages 1-8.

NCPR 02: Effect of LY231514 Disodium and Cisplatin on Human mesothelioma cell lines. Volume 1.3, pages 1-15.

NCPR 03: Effects of Cisplatin/LY231514 Disodium combination in non-small cell lung cancer cell lines. Volume 1.3, pages 1-22.

NCPR 04: Effects of Carboplatin and LY231514 combinations growth of three human tumor cell lines. Volume 1.3, pages 1-62.

NCPR 05: Effects of Cisplatin, Paclitaxel, and LY2331Disodium combinations in human carcinoma cell lines. Volume 1.3, pages 1-59.

NCPR 12: Cell cycle effects of LY231514 Disodium on Gemcitabine antitumor activity in HT-29 colon carcinoma cells. Volume 1.4, pages 1-9.

NCPR 11: Phase I and pharmacologic study of sequence of Gemcitabine and the Multitargeted Antifolate agent in patients with advanced solid tumors. Vol. 1.4, pages 1-13.2000

NCPR 29: Role of Folic acid in modulation the toxicity and efficacy of LY231514 Disodium. Volume 1.5, pages 1-7.

NCPR 32: Interaction of Pemetrexed Disodium (Alimta, Multi Targeted Antifolate) and Irradiation in Vitro. Vol. 1.4, pages 1-35.

NCPR 40: Sequence dependence of Alimta (LY231514, MTA) combined with Doxorubicin in ZR-75-1 Human breast carcinoma cells. Volume 1.4, pages 1-11.

In Vivo Efficacy Studies:

NCPR 06: Effects of Oxaliplatin and 5-FU/LY231514 Disodium combinations on growth inhibition of wild type and 5-FU-resistant HT-29 cells. Volume 1.3, pages 1-8.

NCPR 08: In Vivo antitumor activity of LY231514 Disodium against various human tumor xenografts. Volume 1.4, pages 1-10.

NCPR 19: Role of Thymidylate Synthase in the Antitumor Activity of LT231514 Disodium. Volume 1.4, pages 1-9. 1999.

Safety Pharmacology:

Gen Pharm 1:

In Vitro Studies of LY231514-Na2 in the smooth and cardiac muscles of SD rats and Hartley albino guinea pigs. Volume 1.5, pages 1-36.

Gen Pharm 3:

The Acute Behavioral Effects of LY231514-Na2 following IV administration in Male CD-1 mice. Volume 1.5, pages 1-44.

Gen Pharm 4:

The Acute Effects of LY231514-Na2 on gastrointestinal motility following IV administration in male CD-1 mice. Volume 1.5, pages 1-28.

Gen Pharm 5:

A Renal Pharmacology Study in female Fischer-344 rats given a single IV injection of LY23151-Na2. Volume 1.6, pages 1-54.

Pharmacokinetics:

ADME Report 1: Relative Bioavailability of IP administration and Plasma Pharmacokinetics of LY231514 in Male CD-1 Mice after IV administration of 20 mg/kg or IP administration of 20 or 200 mg/kg (BE) LY231514 Na_2. Volume 1.6, pages 1-54.

ADME Report 2: Plasma Pharmacokinetics of LY231514 in beagle dogs after IV administration of 7.5 or 100 mg/kg (BE) LY231514 Na_2 (Toxicology Study D05091). Volume 1.6, pages 1-54.

ADME Report 3: Summary of the Whole-Body Autoradiographic distribution of [^{14}C] LY231514 Na_2 in CD-1 mice. Volume 1.6, pages 1-18. 1993

ADME Report 5: Excretion and Metabolism of [^{14}C] LY231514 Na_2 in male CD-1 Mice after a Single IV dose of 20 mg/kg comparison with a Single Oral 20 mg/kg dose. Volume 1.7, pages 1-16. 1993

ADME Report 6: Excretion and Metabolism of [^{14}C] LY231514 Na_2 in Female Beagle dogs after a single IV dose of 7.5 mg/kg and 100 mg/kg. Volume 1.7, pages 1-18. 1993

ADME Report 7: Protein Binding of 14C-LY231514 in mouse, dog and Human plasma. Volume 1.7, pages 1-8.

ADME Report 8: Urinary Metabolites of [^{14}C] LY231514 Na_2 in mice and dogs. Volume. 1.7, pages 1-16.

ADME Report 9: Quantitative Whole-Body Autoradiographic Disposition of ^{14}C-LY231514 Disodium in Male CD-1 mice after a Single IV Administration of 20 mg/kg dose (Free acid). Volume 1.7, pages 1-27.

ADME Report 10: Identification of a Urinary Metabolite of [^{14}C] LY231514 Na_2 in mice and dogs. Volume 1.7, pages 1-10.

ADME Report 11: In Vitro interaction of LY231514 with Human Cytochromes P450 CYP3A, CYP2D6, CYPP2C9, and CYP1A2. Volume 1.6, pages 1-17.

ADME Report 12: Pharmacokinetic Interaction Study of LY231514 and Aspirin in beagle dogs following a single IV bolus of 25 mg/kg. Volume 1.6, pages 1-24.

ADME Report 13: Pharmacokinetic Interaction Study of LY231514 and Ibuprofen in beagle dogs following a single IV bolus dose of 25 mg/kg. Volume 1.6, pages 1-24.

Toxicology:

TOX Report 10: A Subchronic Toxicity Study in Beagle Dog given LY231514 Na2 Daily, Twice Weekly and Weekly by Intravenous Injection for 6 Weeks. Volume 1.8, pages 1-167.

TOX Report 26: A Subchronic Toxicity Study in Beagle Dogs given 4 Weekly Intravenous Injection of LY231514 Na_2 followed by a 3-Week Recovery Phase. Volume 1.8, pages 1-103 and 1.9, pages 104-232.

TOX Report 28: A 6-Month Repeat-Dose Toxicity Study in Beagle dogs given Weekly Intravenous Doses of LY231514 Na2. Volumes 1.9, pages 1-219, and 1.10, pages 220-280.

Special Toxicology:

TOX Report 18: A Special Study in Beagle Dogs administered LY231514 Na2 Intravenously on Days 0 and 3 followed by Continuous Intravenous Infusion of Thymidine for 72 hr. Volume 1.10, pages 1-60.

Reproductive Toxicology:

TOX Report 27: A Segment I Reproductive Toxicity Study of LY231514 Na2 administered by Intraperitoneal Injection to Male CD-1 Mice. Volume 1.11, pages 1-100.

TOX Report 25: A Segment II Study of LY231514 Na2 administered Intravenously to Pregnant CD-1 Mice. Volume 1.11, pages 1-101.

Studies Reviewed Previous Submission:

Pharmacology:

Cytotoxic Effects of LY231514 on CCRF-CEM leukemia cells
Efficacy Studies of LY231514 in thymidine kinase deficient murine lymphoma model

Safety Pharmacology:

Gen Pharm 2:

Cardiovascular and Respiratory Effects of LY231514-Na2 administered IV to anesthetized dogs. Volume 1.5, pages 1-65. 1992

Pharmacokinetics:

ADME Report 1: Plasma Pharmacokinetics of LY231514 in Male CD-1 Mice after IV administration of 20 mg/kg or IP administration of 20 or 200 mg/kg (BE) LY231514 Na_2, Volume 1.6, pages 1-54.

ADME Report 2: Plasma Pharmacokinetics of LY231514 in beagle dogs after IV administration of 7.5 or 100 mg/kg (BE) LY231514 Na_2 (from Toxicology Study D05091). Volume 1.6, pages 1-54.

Toxicology:

TOX Report 5: The Acute Toxicity of LY231514 Na2 administered Intravenously to CD-1 Mice (MO2692). Volume 1.7, pages 1-25.

TOX Report 6: The Acute Toxicity of LY231514 Na2 administered Intravenously to Fisher 344 Rats (R03492). Volume 1.7, pages 1-19.

TOX Report 7: The Pilot Toxicity Study of LY231514 given Intraperitoneally to CD-1 for 2 weeks (M11090). Volume 1.7, pages 1-37.

TOX Report 9: A Subchronic Toxicity Study in CD-1 Mice given LY231514 Na2 Daily, Twice Weekly, and Weekly by Intraperitoneal Injection for 6 Weeks(M15391). Volume 1.7, pages 1-67.

TOX Report 8: Intravenous Dose-ranging toxicity studies in beagle dogs given single or multiple doses of LY231514 for up to 2 Weeks(DO2391 and DO3491) Volume 1.8, pages 1-80.

TOX Report 10: A Subchronic Toxicity Study in beagle dogs given LY231514 Na_2 Daily, Twice weekly, and Weekly by Intravenous Injection for 6 Weeks (DO5091). Volume 1.8, pages 1-167.

Special Toxicology

TOX Report 12: Leucovorin Rescue of Beagle Dogs given Lethal Doses of LY231514 Na2. Volume 1.10, pages 1-46.

Genotoxicity:

TOX Report 1: The Effect of LY231514 Na2 on the Induction of Reverse Mutations in Salmonella Typhimurium and E. Coli Using the Ames Test. Volume 1.10, pages 1-38.

TOX Report 2: The Effect of LY231514 Na2 on the Induction of Forward Mutation at the HGPRT+ Locus of Chinese Hamster Ovary Cells. Volume 1.10, pages 1-42.

TOX Report 3: The Effect of LY231514 Na2 on the In Vito Induction of Chromosomal Abberations in Chinese Hamster Ovary Cells. Volume 1.10, pages 1-36.

TOX Report 4: The Effect of LY231514 Na2 for 2 Consecutive Days on the Induction of Micronuclei in Bone Marrow of ICR Mice. Volume 1.10, pages 1-45.

Studied <u>not</u> Reviewed with This Submission:

<u>Pharmacology:</u>

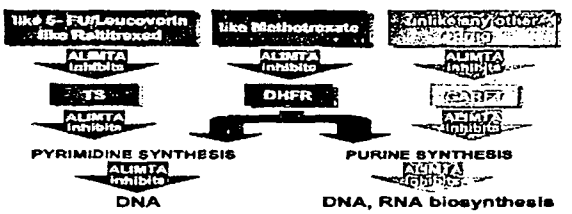
I. PHARMACOLOGY

Pemetrexed disodium (ALIMTA. MTA, LY231514) is a novel pyrrolopyrimidine antifolate antimetabolite and a specific inhibitor of thymidylate synthase (TS). It is a structural analog of Lometrexol, which is a potent inhibitor of glycinamide ribonucleotide formyltransferase (GARFT).

The mechanism of action of pemetrexed is not fully understood. However, many studies indicate that TS is the locus of action of LY231514, and that TdR is able to reverse LY231514-induced cytotoxicity in vitro and in vivo. The enzyme thymidylate synthase (TS) is a folate-requiring enzyme that catalyzes the transformation of dUMP to dTMP. This reaction requires, 5, 10-methylenetetrahydrofolate, as a co-factor. Like MTX, LY231514 is folate-based TS inhibitor and competes with the co-factor for TS.

Pemetrexed is a mild inhibitor of thymidylate synthase (TS) and dihydrofolate reductase (DHFR), while the polyglutamated forms of LY231514 are more potent inhibitors of TS, and mild inhibitors of DHFR and glycinamide ribonucleotide formyltransferase (GARFT).

Enzyme Inhibition by ALIMTA in Comparison to Other Antimetabolites



ALIMTA directly inhibits both pyrimidine and purine synthesis

LY231514 is taken into the cell by both reduced folate carrier (RFC) and membrane folate-binding protein (FPB) transport systems. Once inside the cell, LY231514 is rapidly polyglutamated by folypolyglutamate synthetase (FPGS). Studies with partially purified proteins showed that LY231514 was an efficient substrate for FPGS. The rate constant (Vm/Km) for LY231514 in the FPGS catalyzed reaction was about 100 to 400 times greater than that for MTX (Habeck et al). The more rapid and efficient polyglutamation of LY231514 allowed it to accumulate in cells faster than MTX, that exerts its antiproliferative or cytotoxic activity. Both parent and polyglutamated LY231514 behave as competitive inhibitors of several folate-dependent enzymes, which are part of the thymine and purine nucleotide biosynthesis pathways.

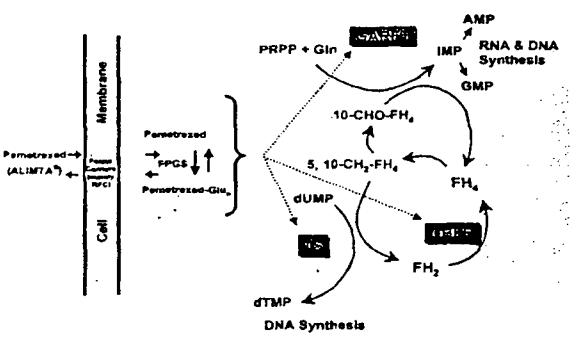


Table 1 Mechanism of Action Studies

Study No.	Parameters	Test System(s)	Results
NCPR 13	Transport of LY231514 by reduced folate carrier (RFC) and membrane folate-binding protein (FBP)	Cell lines expressing different levels of RFC & FBP: Human leukemia lines: CEM (RFC+), CEM/MTX (RFC-), & CEM-7A cells (RFC+++) Murine leukemia cell lines: L1210 cells (RFC+) L1210-B73 (RFC+/FBP+++), & L1210-FBP (RFC-/FBP+++)	LY231514 & 5-methyltetrahydrofolate had similar affinity for RFC (IC50=4 uM). LY231514 was ~1.5 fold more effective than folic acid in binding to FBP. In CEM (RFC+) cells cultured in 2 uM folic acid, CEM/MTX (RFC-), cultured in 2 uM folic acid, & CEM-7A cells (RFC+++) cultured in 0.2nM leucovorin, IC_{50} of LY231514 were 23, 470, & 5.2 nM, respectively. In L1210 cells (RFC+) grown in 2 uM folic acid, IC50 of LY231514 was 14 nM. In L1210-B73(RFC+/FBP+++) grown in 1 nM leucovorin, 20 nM leucovorin or 1 nM leucovorin + 20 nM folic acid, IC50 values of LY231514 were 4, 15, and 16 nM, respectively. In L1210-FBP (RFC-/FBP+++) grown in 1 nM leucovorin, 20 nM leucovorin or 1 nM leucovorin + 20 nM folic acid, IC50 values were 1.6, 5.6, and 162 nM, respectively.
NCPR 16	Mechanism of transport of LY231514 & cross-resistant pattern in cells with markedly impaired transport of MTX	Murine leukemia cell lines: L1210 $MTX^{r}A$ & L1210-G1a –MTX-resistant L1210 variants with point mutation (single amino acid substitution)	1. Influx rate constant (Vmax/Kt) of LY231514 was 1.3 fold higher than MTX. Higher levels of LY231514 were due to more rapid polyglutamation of LY231514 & lower rate of LY231514 efflux via RFC-independent mechanism compared to MTX. 2. Two L1210 variants with point mutation in RFC showed cross-resistance to LY231514 dependent on media folate. In folic acid, IC50 increased 5.8 – and & 22-fold for LY231514 & 12- & 71-fold for MTX. In 5-FTHF media, the IC50 increased only 3.2- & 2.4-fold for LY231514, but 10.7- & 11.4-fold for MTX. Sensitivity to both drugs showed an inverse relationship with folate pool.
NCPR 18	Effect of LY231514 on purified folate enzymes and on proliferation of different tumor cells in culture	Cell lines: Wild type leukemia,CCRF-CEM (CEM) & its polyglutamation deficient CR15 subline, colon carcinoma GC3/C1 (GC3), ileocecal carcinoma HCT8, wild type breast carcinoma ZR-75-1 & its 3 sublines AAR-FR+, MTXR, and MTXR-BB3-FR+	Growth inhibition assays: 1. LY231514 activity against CEM, GC3, & HCT8cells was prevented by 5 uM thymidine completely when LY231514 was at IC50 in all cell lines. At higher concentration, drug effect was completely blocked by the combination of 5 uM thymidine and 100 uM hypoxanthine. 2. In the polyglutamation defective CR15 cells, IC50 of LY231514 increased >7800-fold compared to that for wild type CEM cells.

Table 1. (Continued) Mechanism of Action Studies

NCPR 18	Kinetic studies of LY231514 and its synthetic γ-poly-glutamates (glu_3 and glu_5)	TS, DHFR, and GARFT were assayed [redacted] and AICARFT inhibition was assayed by monitoring the formation of [6S]-5,6,7,8-tetrahydrofolate	Table 1: Inhibition of Purified Folate-Requiring Enzymes by Alimta Ki Values (nM, n≥3) TS DHFR GARFT Alimta 109 7.0 9300 Alimta-(glu_3) 1.6 7.1 380 Alimta-(glu_5) 1.3 7.2 65 5,10-MTHF dehydrogenase 10-FTHF synthetase AICARFT Alimta 9.0 364 3.58 Alimta-(glu_3) 3.7 25 0.48 Alimta-(glu_5) 5.0 1.6 0.26 LY231514 & its polyglutamated forms behaved as a competitive inhibitor with respect to folate cofactor in folate enzymes studies. Ki values of LY231514 against TS, DHFR, GARFT & AICARFT were 109, 7, 9300, and 3580 nM, respectively.
NCPR 20	Dipyridamole potentiates in vitro activity of LY231514 by inhibition of thymidine transport	COR L23 lung cancer cell line; A549 non small cell lung ca. cell line, MCF7 & T47D breast ca. cell line	1) LY231514 inhibited tumor growth & IC50 were ~28, -46, -52,-640 nM for COR L23, T47D, MCF7, & A549, respectively. 2) Thymidine (1 uM) completely prevented growth inhibition by L231514 at IC50 in all cell lines. At 10x IC50, growth inhibition was only partially reversed by thymidine (≤1 uM), while both thymidine and hypoxanthine are required for complete reversal. 3) Dipyridamole (1 uM) inhibited thymidine transport 89% or more in all tested cell lines, whereas hypoxanthine transport was inhibited only in A549 & MCf7 cells. Dipyridamole (1 uM) prevented thymidine/hypoxanthine rescue of LY231514.
NCPR 28	Potential role of DHFR amplification on cellular resistance to LY231514	CEM-parental human leukemia T-lymphoblast cell line and two MTX-resistant sublines, CEM/R (resistance due to increase in DHFR) & CEM/T (resistance due to drug transport)	In MTX-resistant CEM/R line, cells were 8-fold less resistant to LY231514 than the selective DHFR inhibitor, MTX. LY231514 cytotoxicity was overcome by thymidine addition in contrast to combination of thymidine & hypoxanthine required for protection in parental cells. However, this line was 54-fold resistant to LY231514 compared to parental cells, suggesting LY231514 targets DHFR as well as TS. In the MTX-deficient CEM/T line, cells were 91-fold resistant to LY231514, 315 ~fold resistant to raltitrexed, and 694-fold resistant to MTX
NCPR 29	Role of folic acid in modulating efficacy of the multitargeted antifolate, LY231514 on different cell	CCRF-CEM (leukemia) GC3 (colon) IGROV1 (ovarian) KB (epidermoid)	Growth inhibitory IC50 values obtained in 2 nM folinic acid for IGROV1, KB, GC3, LX-1, & CEM were 44, 34, 12, 4 & 4 nM, respectively. Folic acid (<10 uM) in the media had little effect on growth inhibitory activity of LY231514 in cell lines tested.

	lines	LX-1 (human lung) carcinoma cell lines	However, folic acid (100 uM) in the media raised LY231514 IC50 values against IGROV1, KB, GC3, LX-1, & CEM were 25-, 17-, 9, 6-, & 22-fold, respectively. However, presence of folinic acid (10 uM) in the media raised LY231514 IC50 values against these cells >970-, 78-, 47-, 82-, & 130-fold, respectively.
NCPR 30	Effects of vitamin supplementation on activity of LY231514 in human MX-1 breast carcinoma	Nude mice bearing MX-1 breast xenograft received IP 100 or 150 mg/kg doses of LY231514 daily on days 7-11 and 14-18 alone or co-administered with nontoxic doses of either folic acid (6 or 60 mg/kg, orally), vitamin B6 (100 mg/kg, orally) or vitamin B12 (165 mg/kg, IP)	Tumor growth delay was measured (days) for the treated and for the controls in the following table. Test Compound, mg/kg — Tumor Growth Delay (Days) LY231514 100 alone — 17 + folate 6 — 17 + folate 60 — 22 + Vitamin B6 100 — 17 + Vitamin B12 165 — 22 LY231514 150 alone — 21 + folate 6 — 21 + folate 60 — 23 + Vitamin B6 100 — 21 + Vitamin B12 165 — 24 Folate alone 6 — 7 60 — 12 Vitamin alone B6 100 — 5.7 B12 165 — 12 LY231514 alone delayed tumor growth by 17 to 21 days. The combination of LY231514 with folate 60 mg or vitamin B12 165 increased TGD by 22-24 days. Vitamins or folic acid did not increase toxicity (as determined by body weight) or alter the antitumor activity of LY231514 disodium in the human MX-1 breast carcinoma.

TGD=tumor growth delay

Table 2. In Vitro efficacy studies:

Study No.	Parameter(s)	Cell Type(s)	Concentrations	Results
NCPR 01	Activity of LY231514 against patient's tumor cells in colony-forming assay	Cells from multiple tumor specimens (including colon, lung, breast, & mesothelioma) collected from different patients undergoing routine diagnostic or therapeutic procedures	LY231514 concentrations at 0.1, 1, and 10 ug/mL (0.2, 2.1, & 21 uM)	Of 358 specimens plated in the short 1 hr exposure studies, 148 (41%) were evaluable. Overall, in clinically achievable LY231514 concentrations, responses were dose-dependent: response rates were 3% of specimens (4/144) at 0.1 ug/mL, 11% (17/148) at 1.0 ug/mL, and 23% (33/141) at 10 ug/mL. At 10 ug/mL, responses included colorectal cancer at 32% (9/28), NSCLC at 25% (6/24), & and mesothelioma 66% (2/3). Scatter plots analysis demonstrated LY231514 activity (multitargeted antifolate) was not completely cross-resistance with those of cisplatin, 5-FU, irinotecan, and paclitaxel.
NCPR 02	Effects of LY231514 & cisplatin on human mesothelioma cell lines in MTT assay and Flow cytometry assay	Mesothelioma lines: MSTO-211H, NCI-H28, NCI-H2052	LY231514: 15 nM-2 uM; Cisplatin: 30 nM-10 uM	In growth inhibition study, cells were exposed for 72 hr. The growth inhibitory IC50 for LY231514 in MSTO-211H, NCI-H28, and NCI-H2052 human mesothelioma cells were 31, 180, & 209 nM, respectively. MSOT-211H was chosen for the combination studies of LY231514 and cisplatin. The IC50 for cisplatin was 1.33 uM for MSTO-211H. Using simultaneous 72-hr exposure, LY231514 and cisplatin produced synergistic growth inhibitory activity using both constant and non-constant ratio techniques in MTT assays. DNA flow cytometry studies indicated that LY231514 causes a buildup of cells near G1/S interface after 24 h incubation.
NCPR 03	Activity of LY231514 in combination with other antitumor agents against various tumor cell lines	NCI-H23 adenocarcinoma & NCI-H460 large cell carcinoma of the lung	LY231514: 11.7 nM-117 uM; Cisplatin: 16.7 nM-167 uM	Growth inhibition: LY231514 when used in combination with cisplatin produced an additive activity regardless of sequence of administration. The IC50 values of LY231514 and cisplatin in NCI-H23 cells were 235.8 and 441.2 ng/mL, respectively.
NCPR 04	Activity of LY231514 and carboplatin against three human tumor cell lines	NCI-460 (NSCLC) SKOV-3 (Ovarian cancer HT29 (Colorectal cancer)	LY231514: 0.3 nM-30 uM; Carboplatin: 0.3-3000 uM	Growth inhibitory effects of LY231514 and carboplatin and were additive when cells were exposed to both agents regardless of sequence (MTA followed by carboplatin or carboplatin followed by MTA or simultaneous exposure) in all three cell lines.
NCPR 05	Schedule-dependent cytotoxic effects of LY231514 and cisplatin of paclitaxel against various cell lines	A549, human lung carcinoma (ca.) MCF7, breast ca. PA1, ovarian ca. WiDr, colon ca.	LY231514: 20-200 nM; Cisplatin: 0.2-2 uM; paclitaxel: 5 pM-10 nM	In LY231514 and cisplatin combination, simultaneous exposure to LY231514 & cisplatin produced antagonistic effects in A549 and MCF7 cells and additive effects in PA1 and WiDr cells. Similar effects were observed in LY231514 and paclitaxel combination. Sequential exposures to MTA followed by cisplatin or paclitaxel produce synergistic effects whereas simultaneous exposure had antagonistic effects. The present findings show that the interaction of MTA and cisplatin or paclitaxel is schedule-dependent.

NCPR 06	Effects of combining MTA with either 5-FU, SN38, or oxaliplatin on growth inhibition of wild type & 5-FU-resistant HT29 cells	Parental & 5-FU-resistant HT29 colorectal carcinoma cells	a) Single agents tested: LY231514: 5-1000 nM b) Combinations tested: Concentrations were not given for LY231514 + 5FU LY231514 + SN38, & LY231514 + oxaliplatin	a) LY231514 IC50 in parental & 5-FU-resistant HT29 were 92.1 & 197 nM, respectively. b) Similar interactions were observed in both parental & 5-FU-resistant HT29 cells. Combinations of LY231514 + 5-FU & LY231514 + SN38 both gave results that were sequence independent & showed trend of antagonism progressing to synergism as fractional inhibition increased. c) Combination of LY231514 + oxaliplatin was sequence dependent with best results obtained from concurrent addition of both drugs.
NCPR 12	Cell cycle effect of LY231514 disodium on Gemcitabine antitumor activity in HT29 colon carcinoma cells	HT29 colon carcinoma	LY231514: 0.03-3 uM Gemcitabine: 3-300 nM Cells exposed for 24 h.	LY231514 at 0.2-1 uM caused HT29 to arrest at G1/S interface. Cytotoxicity was <30%. IC50 value for gemcitabine was 71 nM. Prior exposure to LY231514 at 0.3 uM decreased gemcitabine IC50 to 32 nM. Gemcitabine at 30 uM induced 70% inhibition in colony formation that was not increased by further addition of LY231514.
NCPR 32	Interaction of Pemetrexed disodium and irradiation in vitro	WiDr, human colorectal MCF7, human breast HeLa, human cervical, & LX-1, human lung carcinomas	a) Single agent tested: Cells were exposed 2 h to LY231514 (2.12 nM-10.6 uM) b) Combination tested: Cells were exposed to no drug or LY231514(106-636 nM) for 2h with radiation (0,2, 4, 6, & 8 Gy) c) Sequential combinations tested: WiDr cells were exposed to LY231514 (636 nM) for 2 h with radiation (0, 2, 4, 6 Gy) being delivered at times of drug addition.	a) LY231514 produce one log kill for LX-1, WiDr, HeLa & MCF7 at 0.45 , 1.6, 2.3, & 5.9 uM, respectively. b) For LX-1, mean inactivation dose (MID) of radiation alone and when combined with 212 nM drugs were 3.6 & 2.3. For WiDr, the MID of radiation alone and when combined with 636 nM drug,were 3.8 and 2.1. For Hela, the MID of radiation alone and when combined with 106 nM drug were 3.1 & 2.6 For MCF7, the MID of radiation alone and when combined with 106 nM drug were 3.4 and 2.9 c) Timing of LY231514 addition did not alter drug-related radiation enhancement in WiDr cells. Cell cycle progression of serum-stimulated WiDr cells was not significantly affected.
NCPR 40	Sequence dependence of LY231514 combined with Doxorubicin in ZR-75-1 human breast carcinoma cells	ZR-75-1 human breast carcinoma cells	a) Single agents tested: LY231514 and DOX at 5 nM-10 uM either alone 0-24, or 24-96 h. b) Combination tested: LY231514 (0.041-0.37 uM) & DOX (0.00051-10 uM). Cells were exposed to both drugs concurrently from 24-96 h or to one drug from 0-24 h	a) Growth inhibitory IC50 values for LY231514 and DOX were 271 and 302 nM, respectively, for 24 h exposure and 85 & 326 nM, respectively, for 72 h exposure. b) When LY231514 concentrations were below 270 nM, interaction was additive with a wide range of DOX doses. Sequence with LY231514 added prior to DOX was highly synergistic especially when both drugs were added at low drug concentrations. Reversed sequence gave mixed results ranging from strong antagonism at high LY231514 concentration to additive & weak synergistic interaction at mid & low LY231514 concentrations.

Table 3. In Vivo efficacy studies:

Study No.	Parameter(s)	Tumor Type(s)	Dose, Routes & Schedules	Results
NCPR 06	Effects of Oxaliplatin and 5-FU/LY231514 disodium combinations on growth inhibition of wild type and 5-FU-resistant HT29 cells	Parental & 5-FU-resistant HT29, human colon cancer cells grew as xenografts in nude mice	Two weekly IP doses of either LY231514 (500 mg/kg) alone or oxaliplatin (10 mg/kg) alone, or the two drugs given concurrently in combination	Tumor growth inhibition determined 28 days after treatment initiation for LY231514 alone, oxaliplatin alone, and by combinations were 23.4%, 29.5% & 52%, respectively.
NCPR 08	Activity of LY231514 against various human & murine xenografts MX-1 mammary ca. BxPC-3 pancreatic LX-1 lung VRC5 colon carcinoma xenografts grown in CD-1 nu/nu mice	The tumor xenografts were grown and removed from serial passage animals and minced into 1- to 2mm fragments. Tumor pieces were implanted subcutaneously in the axillary region of CD-1 nu/nu mice by trocar	For MX-1, BxPC-3, & LX-1,LY231514 (10-300 mg/ kg, IP) in saline. For VRC5, LY231514 (10-50 mg/kg, IP) was dissolved in 2.5% Emulpor (EL620) & dosed once daily for 10 days starting 7 days after tumor implantation	Tumors were measured 14 days after initiation of the treatment. When IP dose 300 mg/kg (qd x 10) in saline produced 79% inhibition for MX-1, 65% for BxPC-3, and 75% for LX-1. When LY231514 was formulated in emulphor against VRC5, 80% inhibition was observed at 50 mg/kg without mortality. However, LY231514 given at 100 mg/kg in this vehicle caused 30% mortality.
NCPR 19	Role of Thymidylate Synthase in the antitumor activity of LY231514	GC3 (wild type) & GC3TK-(deficient in thymidine kinase)colon xenografts grown in CD-1nu/nu mice	LY231514 (50-300 mg/kg (qd x 10), starting 7 days after tumor implantation	GC3 treated at 300 mg/kg showed tumor growth delay at ~17 days. On the other hand, GC3TK-tumor model at 300 mg/kg showed heightened sensitivity to LY231514 as shown by same course of therapy producing complete tumor regression with 4 of 10 mice being tumor free for 100 days.
NCPR 36	In Vivo Evaluation of MTA alone & in combination with gemcitabine against MX-1 human breast xenograft	MX-1 human breast ca. xenografts grown in female nu/nu mice. 5 females/group Started treatment when tumor size 62-144 mg	Group 1: control Group 2: MTA (100 mg/kg, IP, BID x 5 days Group 3: Gemzar (60 mg/kg, IP, Q3d for 4 times Group 4: Concurrent MTA and Gemzar in schedules & doses of groups 2 & 3 Group 5: Gemzar followed by MTA Group 6: MTA followed by Gemzar Group7 & 8: MTA (200 mg/kg, IP) and Gemzar (120 mg/kg IP) on days 1, 8, 15, & 22	Tumor in 1 mouse did not grow; MDS)= 18.1 (SEM=2.5, n=4) Tumor in mice did not grow or cured; MDS=17.8 (SEM=3.6, n=3) Tumor in 1 mouse did not grow or cured; MDS=21.1 (SEM= 0.8, n=4) Three mice died ; MDS=33.1 (SEM= 0.2, n=2) MDS= 37.6 (SEM= 4.4, n=5) Tumor in 1 mouse did not grow or cured; MDS= 27.9 (SEM=1.3, n=4) MDS= 16.9 (SEM= 1.9, n=5) MDS= 25.0 (SEM= 2.6, n=5)

MDS= Median Days Survival; ca.: carcinoma; MID=Mean inactivation dose (calculation of the sensitivity parameter).

Pharmacology summary:

Pemetrexed disodium (LY231514) is a novel pyrrolopyrimidine antifolate and a specific inhibitor of thymidylate synthase (TS). It is a structural analog of Lometrexol, which is a potent inhibitor of glycinamide ribonucleotide formyltransferase (GARFT).

The mechanism of action of pemetrexed is not fully understood. However, many studies indicate that TS is the locus of action of LY231514, and that TdR is able to reverse LY231514-induced cytotoxicity in vitro and in vivo. The enzyme thymidylate synthase (TS) is a folate-requiring enzyme that catalyzes the transformation of dUMP to dTMP. This reaction requires, 5, 10-methylenetetrahydrofolate, as a co-factor. Like MTX, LY231514 is folate-based TS inhibitor and competes with the co-factor for TS.

Many studies have shown that LY231514 gains entry to the cell via the reduced folate carrier and once localized is an excellent substrate. LY231514 is rapidly polyglutamated by folypolyglutamate synthase (FPGS). The rate constant (Vm/Km) for LY231514 by FPGS was about 100 to 400 times greater than that for MTX (Habeck et al, 1995). The efficient polyglutamation of LY231514 allowed it to accumulate in cells that exert its antiproliferative or cytotoxic activity by inhibiting several key folate-dependent enzymes that are essential for cell replication.

Like methotrexate, pemetrexed is a mild inhibitor of thymidylate synthase (TS) and dihydrofolate reductase (DHFR), while the polyglutamated forms of pemetrexed are more potent inhibitors of TS, DHFR and glycinamide ribonucleotide formyltransferase (GRAFT). Pemetrexed disodium has both antithymine (by inhibition of TS and DHFR) as well as antipurine effects (by inhibition of GARFT) in tumor cells. The growth inhibition activity of LY231514 was prevented concurrent presence of 5 uM thymidine and 100 uM hypoxanthine.

In in vitro studies, LY231514 was found to have growth inhibitory activity against a broad range of tumor cell lines. In growth inhibiton assays, cells were continuously exposed to the drug for 72 hrs, LY231514 was found to be active against leukemia (CCRF-CEM and L1210), lung (A549, LX-1), mesothelioma (NCI-H2052 and MSTO-211H), breast (MCF7, ZR-75-1), colorectal (GC3, HT8, WiDr), and ovarian cancer (SKOV1) cell lines. The IC50 values of LY231514 ranged from 10 nM to 300 nM. In the colony-forming assays, clinically relevant concentrations of LY231514 gave dose-dependent responses against a wide panel of specimens, including tumor types that were chemo-resistant to methotrexate, 5-FU, and raltitrexed.

LY231514 has demonstrated its activity not only as a single agent but also in combination with a variety of other chemotherapeutic agents, including platinum (cisplatin, carboplatin), 5-FU, doxorubicin, CPT-11, oxaliplatin, paclitaxel, and gemcitabine. In particular, studies with mesothelioma cell lines (MSTO-211H, NCI-H2052) showed synergistic effects when LY231514 (30 nM) was added simultaneously with cisplatin (30 nM to 500 nM). In both NCI-H23 and NCI-H460 lung carcinoma cells, combination of LY231514 (0.014 uM to 11.7 uM) with cisplatin (0.08 uM to 1.3 uM) produced an additive interaction, regardless of the sequence of administration.

In in vivo efficacy studies, LY231514 was tested against human xenografts of VRC5, MX-1, BxPC-3, and LX-1 grown in CD-1 nude mice. After ten daily IP injections (10 mg/kg to 300 mg/kg LY231514 in saline) to tumor bearing mice, LY231514 (300 mg/kg) resulted in tumor inhibition of 79% in MX-1, 65% in BxPC-3C, and 75% in LX-1. When LY231514 was formulated in Emulphor (EL620) and tested against VRC5 xenograft, 80% inhibition was noted at 50 mg/kg without mortality. However, LY231514 given at 100 mg/kg in this vehicle resulted 30% mortality without efficacy.

LY231514 was found to be highly active against a thymidine kinase deficient murine lymphoma model (L5178Y/TK-/HX-). A complete inhibition of tumor growth was observed when the compound was given IP daily x 8 from 12.5 to 200 mg/kg in DBA/2 mice bearing L5178Y/TK-/Hx- tumor. LY231514 was tested by the IV daily x 8 from 1.25 to 10 mg/kg in the L5178Y/TK-/HK- tumor system. LY231514 resulted in ~89% tumor inhibition when compared

to ≤50% by other analogs. LY231514 was highly active against human colon xenografts (GC3 and VRC5), these tumor models were sensitive to Lometrexol but resistant to MTX.

In a folic acid modulation study, mice fed a low folate diet were much more sensitive to the toxic effects of LY231514 than those on a normal (high folate) diet. Studies in mice bearing the LY5178Y/TK-/HK- lymphoma on a low folate diet indicated that significant tumor growth inhibition was achieved at the MTD of LY231514. In contrast, mice kept on a high folate diet had inhibition of tumor growth over a broader range of doses of LY231514 without lethality. This data suggests that folate supplementation may reduce toxicity without impact on the efficacy of LY231514.

The effects of vitamin on antitumor efficacy of LY231514 were evaluated in a human tumor xenograft model. Female nude mice bearing human MX-1 breast carcinoma were treated with LY231514 alone (100 or 150 mg/kg) or along with folic acid (6 or 60 mg/kg), vitamin B6 (pyridoxine) at 100 mg/kg, or vitamin B12 (cobalamin) at 165 mg/kg dose. LY231514 alone delayed tumor growth by 17 and 21 days. The combination of LY231514 with folate 60 mg or vitamin B12 165 increased TGD by 22-24 days. Vitamins or folic acid did not increase toxicity or alter the antitumor activity of LY231514 disodium in the human MX-1 breast carcinoma.

II. SAFETY PHARMACOLOGY:

LY231514 was assessed for its potential to elicit secondary receptor-mediated autonomic pharmacology by smooth and cardiac muscle tissue bath preparations in vitro. In vivo studies included a cardiovascular assessment in anesthetized dog, a battery of central nervous system and behavioral function tests and a gastrointestinal transit evaluation in mice, and a renal assessment in rats. The findings are summarized as in the following table.

Summary of Safety Pharmacology Studies with LY231514

Study Title Study #/	Model	Doses/Routes Parameters Evaluated	Findings
In Vitro Evaluations: In Vitro effects on Cardiac and Smooth Muscle Gen Pharm Report 1 GLP study Lot #211SB5 and CT05428	SD Rat or guinea pig Rat tissues: smooth muscle and uterus tissue Pig tissues: smooth muscle and atrial tissue	LY231514 concentrations: 1 x 10^{-9} M through 1 x 10^{-4} M in vitro Agonist and antagonist activity at α-, β-adrenergic, cholinergic, oxytocic and angiotensin I receptors and effects on autonomic-mediated responses in smooth and cardiac muscle	No agonist or antagonist activity on oxytocic, α-, β-adrenergic, cholinergic, and angiotensin I receptors; no effects on the positive inotropic response of atrium to isoproterenol At 10^{-5} M; weak suppression of the maximum chronotropic response of the atrium to isoproterenol
In Vivo Evaluations: Cardiovascular and Respiratory Effects Gen Pharm Report 2 GLP study	Beagle Dog 4 Males	0[b], 32 (640) or 105 mg/kg (2100 mg/m2) 10 minute infusion Arterial blood pressure, peripheral vascular resistance, stroke volume, cardiac output, heart rate, ECG including QT interval, respiratory rate, tidal volume	105 mg/kg (2100 mg/m2): mild decrease in peripheral vascular resistance and an increase in stroke volume
CNS and Behavioral Effects	CD-1 Mouse 10 Males/dose	0[a], 0[b], 60 (180), 200 (600) or 600 mg/kg, (1800 mg/m2) LY231514	No drug-induced effects were observed at 60 –600 mg/kg (180-1800 mg/m2).

Gen Pharm Report 3 GLP study Lot #CT05428		Intravenous bolus Clinical observations, spontaneous ambulatory and non-ambulatory activity, body temperature, grip strength, hexabarbital-induced sleep times, acetic acid-induced writhing, sensorimotor reactivity, and electroshock or pentylenetetrazol convulsive thresholds	≥200 mg/kg (600 mg/m2): decrease in acetic acid writhing
Gastrointestinal Effects Gen Pharm Report 4 Lot #CT05428	CD-1 Mouse 8-10 Males/dose	0[a], 0[b], 60 (180), 200 (600), or 600 mg/kg (1800 mg/ m2), LY231514 Intravenous Charcoal meal transit rate	No effects
Renal Effects Gen Pharm Report 5 Lot #CT05428	Fischer 344 Rat 8 Females/dose	0[c], 60 (360), 200 (1200) or 600 mg/kg (3600 mg/m2) LY231514 Intravenous Urinary volume, pH, sodium, potassium, chloride, creatinine and creatinine clearance, fractional excretion of sodium, serum creatinine, sodium, potassium, and osmolarity	≥200 mg/kg (1200 mg/m2): slight increase in potassium excretion 600 mg/kg: mild increase in sodium excretion and fractional excretion of sodium

[a] Purified water; [b] 5.5% mannitol in purified water; [c] 46 mg mannitol/mL in 0.9% sodium chloride.

Safety pharmacology summary:

In safety pharmacology, LY231514 concentrations (1×10^{-9}M to 1×10^{-5}M) did not produce any significant contractile activity in the isolated rat uterus, smooth muscle, or atria and smooth muscle of guinea pigs. Intravenous administration of LY231514 to beagle dogs at a dose of 105 mg/kg (2100 mg/m2) produced a mild decrease in peripheral vascular resistance and an increase in stroke volume. A single IV dose of ≤600 mg/kg (≤1800 mg/m2) to mice did not adversely alter gastrointestinal function or any CNS function. However, acetic acid induced writhing, a measure of pain perception, was reduced at intravenous doses ≥200 mg/kg (600 mg/m2). A mild increase in urinary excretion of sodium and potassium was seen at doses ≥200 mg/kg (1200 mg/m2) in rats. The pharmacological effects observed from these studies included mild renal effect, cardiovascular function and alterations in pain sensitivity.

All of these effects were observed at doses well above the proposed clinical dose (500 mg/m2), and none of these effects would be expected to influence the clinical use of LY231514.

II. PHARMACOKINETICS

Study Title: Relative bioavailability of IP administration and plasma pharmacokinetics of LY231514 in Male CD-1 mice after IV administration of 20 mg/kg or IP administration of 20 or 200 mg/kg LY231514 disodium (ADME Report 01). Volume 1.6, pages 1-54.

Key study findings:

- PK profiles of LY231514 were similar after i.v. or i.p. doses.
- Relative bioavailability was >100% in mice and < 71% in dogs.

Single-dose PK in Mice:

Method:

Intraperitoneal dosing of LY231514 was used to assess its subchronic toxicity in CD-1 mice (Study M15391). In a separate study, the pharmacokinetics of LY231514 in male CD-1 mice receiving IP doses of 20 mg/kg and 200 mg/kg were compared to that from an IV dose 20 mg/kg. The plasma level time courses are compared following two routes.

Results:

After 20 mg/kg IV or IP dosing, plasma levels declined in a biphasic manner from 41 ug/mL at 5 min to 0.028 ug/mL over 48 hrs as in Figure 1.

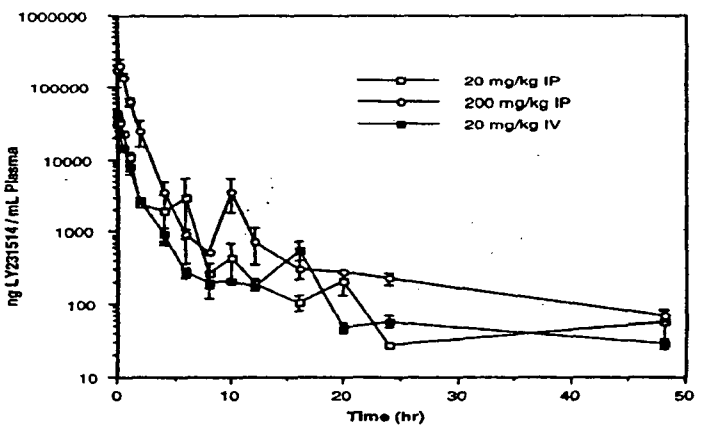


Figure 1: **Comparison of mean plasma levels of LY231514 (ng/mL) in CD-1 mice given IV or IP doses of LY231514 Na_2.**

The mean PK parameters following a single IV or IP dose to male CD-1 mice are summarized as in Table 1

Table 1. PK parameters of LY231514 in male CD-1 mice following a single IV or IP dose of LY231514

	Route of Administration		
	IV	IP	IP
Dose, mg/kg	20	20	200
Cmax, ug/mL	41	33	19
Tmax, hr	0.083	0.25	0.25
AUC, ug*hr/mL	31	44	220
T1/2α, hr	0.083-0.5		
T1/2β hr	7.0	7.8	10.0

*indicates ug

The terminal half-life of LY231514 in mice was 7.8 and 10 hrs for 20 mg/kg and 200 mg/kg IP dose levels and 7 hrs following a 20 mg/mg IV dose.

The AUC calculated for the 20 mg/kg IV data was 31 ug·hr/mL, compared to 44 ug·hr/mL for the 20 mg/kg IP data. The relative bioavailability of the 20 mg/kg IP dose (AUC_{0-t} of 44 ug·hr/mL) was found to be 140% of the 20 mg/kg IV dose (AUC_{0-t} of 31 ug·hr/mL). The calculated bioavailability greater than 100% was may due to variability of the animal data. In comparison, the AUC_{0-t} of 220 ug·hr/mL for the 200 mg/kg IP was about 7 times the AUC_{0-t} for the 20 mg/kg IV dose, resulting in a relative bioavailability of 71%. These data indicate a significant exposure of LY231514 after IP dosing to CD-1 mice, however, the range of values (71% to 140%) illustrate the variability observed in the measurement of plasma concentrations.

Study Title: Plasma Pharmacokinetics of LY231514 in beagle dogs after single intravenous administration of 7.5 or 100 mg/kg LY231514 Na_2 (from Toxicology Study D05091)(ADME Report 02). Vol. 1.6, pages 1-54. 2001.

Key study findings: The PK profile of LY231514 was biphasic and the AUC values were approximately dose proportional.

Single-dose PK in Dog:

Method:

Plasma pharmacokinetics of LY231514 was evaluated following an IV dose of 7.5 or 100 mg/kg in dogs. Blood samples were taken at 5 min., 1, 2, 4, 8, 12, 24, 30 hr up to 120 hrs post-dose. Plasma samples for LY231514 level were analyzed by HPLC/MS/MS method. For all measurements the detection limit was 0.02 ug/mL LY231514.

The PK parameters are determined following a single dose of 7.5 and 100 mg/kg LY231514 intravenously to dogs.

Results:

	LY231514			
Dose mg/kg	100 mg/kg		7.5 mg/kg	
n=3/Sex	M	F	M	F
Parameters				
Plasma levels (ug/mL)				
Cmax at 5 min				
at 12 hr				
at 24 hr				
at 30 hr				
Tmax, min	5	5	5	5
$AUC_{(0\text{-}24hr)}$, ug·hr/mL	500	499	33	30
T1/2α, min	5-20		5-20	
T1/2β, hr	2.3	2.2	2.8	1.8

M=Male; F=Female; -=No data

Plasma LY231514 declined rapidly in a biphasic manner with a short distribution half-life (T1/2α= 5-20 min) and terminal half-life (T1/2β= 2.3 hrs). The combined AUC values for males and females from the 100 mg/kg data averaged 16 times those of 7.5 mg/kg data, which shows that the exposure is approximately proportional to the dose (100 mg/kg/7.5 mg/kg=13). There were no significant differences in the PK profile of LY231514 between male and female dogs at either dose.

Multiple-dose PK/TK in Dogs:

The pharmacokinetic/toxicokinetic effect of LY231514 in beagle dogs was determined in conjunction with the 6-week toxicology study (Study D05091:Toxicokinetic study on pages 32-33 of this review).

Method:

Six dogs/dose were given slow-bolus intravenous doses of 0, 0.11, or 0.53 mg/kg daily, 3.15 or 7.87 mg/kg twice weekly, or 104.96 mg/kg weekly for 6 weeks. Dogs could not tolerate the higher daily dose (0.53 mg/kg) or the weekly dose (104.96 mg/kg). The doses lowered to 0.37 and 26.24 mg/kg, respectively. Plasma concentrations of LY231514 over time were determined on Day 1, Day 11/15, and Day32/36 from the animals of these dose groups.

Summary of PK parameters of LY231514 Following Multiple Doses of LY231514 for 6 weeks in Dogs

PK Parameters	Dose. mg/kg	Day 1 M	Day 1 F	Day 11 M	Day 11 F	Day 15 M	Day 15 F	Day 36 M	Day 36 F
C_{5min}(ug/ml)	0.11[a]	0.70	0.51			0.42	0.53	0.43	0.43
T½ (hr)		1.6	1.6			0.8	0.9	ND	ND
AUC_{0-t}		1.06	0.52			0.36	0.38	2.70	2.86
$AUC_{(0-\infty)}$ (ug·hr/ml)		1.36	0.55			0.28	0.38	ND	ND
C_{5min}(ug/ml)	0.53/ 0.37[b]	0.76	0.50			1.61	1.72		
T½ (hr)		2.0	1.9			2.3	1.5		
AUC_{0-t}		1.80	1.84			1.98	1.81		
$AUC_{(0-\infty)}$ (ug·hr/ml)		1.81	1.84			2.23	1.81		
C_{5min}(ug/ml)	3.15[c]	7.28	7.14	7.88	8.91				
T½ (hr)		2.1	1.9	2.2	1.8				
AUC_{0-t}		14.8	11.3	12.2	20.2				
$AUC_{(0-\infty)}$ (ug·hr/ml)		14.8	11.3	12.2	20.3				
C_{5min}(ug/ml)	7.87[c]	38.0	49.2						
T½ (hr)		4.0	2.46						
AUC_{0-t}		34.2	33.1						
$AUC_{(0-\infty)}$ (ug·hr/ml)		34.3	33.1						
C_{5min}(ug/ml)	104.96/ 26.24[d]	530.3	459.0			86.3	93.2	13.0	17.5
T½ (hr)		3.8	3.2			4.3	3.9	5.1	4.2
AUC_{0-t}		523.7	517.1			77.6	72.2	28.4	26.5
$AUC_{(0-\infty)}$ (ug·hr/ml)		523.7	517.1			77.6	72.2	28.4	26.5

[a] Daily IV dosing; [b] Animals were dosed daily with 0.53 mg/kg for days 1-8 and lowered to 0.37 mg/kg thereafter; [c] Twice weekly IV dosing ; [d] Animals were dosed weekly with 104.96 on day 1 and lowered to 26.24 mg/kg thereafter.

Plasma concentrations decreased rapidly from an initial high level after the intravenous dose. Plasma half-lives calculated ranged from 0.8 to 5.1 hrs. Longer half-lives were found at higher doses. Exposure to LY231514 after a single intravenous dose (as measured by $AUC_{0-\infty}$) increased proportionally with dose (1.36 ug·hr/ml in males and 0.55 ug·hr/ml in females after a 0.11 mg/kg dose up to 523 ug·hr/ml and 517 ug·hr/ml, respectively, after 104.96 mg/kg dose) in dogs. No sex differences were noted in exposure to LY231514.

Study Title: Quantitative whole-body autoradiography (QWBA) disposition of [^{14}C]-LY231514 disodium in male CD-1 mice after a single IV dose of 20 mg/kg (ADME Report 09). Volume 1.7, pages 1-27.

Key study findings: Highest concentration of radioactivity was in the liver at 5 minutes after administration of [^{14}C]-LY231514.

Method:

The AUC increased proportionally with dose. The elimination half-life was short ($T\frac{1}{2}\beta$=1.8-2.3 hrs) in dogs compared to mice ($T\frac{1}{2}\beta$= 7-10 hrs).

Toxicokinetics of LY231514 were evaluated following intravenous administration to beagle dogs in conjunction with a 6-week toxicology study. The terminal half-life in dogs was short and ranged from 2 to 4 hrs. The AUC appeared to increase proportionally with increasing doses over the dose range of 0.11 mg/kg to 104.96 mg/kg.

Unchanged [^{14}C]-LY231514 and its metabolites were rapidly distributed to various tissues and excreted via renal and biliary elimination. Highest concentrations of radioactivity were present in liver and kidney. Approximately 99% of the administered ^{14}C recovered in urine (and feces) in dogs within 24 hrs and ~93% of ^{14}C recovered in urine in mice 96 hrs after dosing. Urinary excretion of the unchanged drug is also the predominant route of elimination observed in humans. Overall, the data indicate that the disposition of LY231514 in the animal species is similar to that found in humans.

Toxicology:

Toxicity studies were conducted following single intravenous doses in mice, rats and dogs, and repeat dose studies of 2- and 6 week intraperitoneal doses in mice, and 2-, and 6 week intravenous doses using daily, twice weekly or weekly schedules in dogs. Additionally, repeat dose studies of 1-month- and 6-month intravenous toxicity studies were performed using once weekly doses in dogs.

In a single dose studies, LY231514 demonstrated low acute toxicity in both mice (MLD=>1574 mg/kg or >4722 mg/m2) and rats (MLD=>1453 mg/kg or >8718 mg/m2) but higher toxicity in dogs (MLD=>100 mg/kg or >2000 mg/m2). LY231514 was acutely more toxic in dogs than in mice or rats.

Six-weeks repeat dose studies were conducted in the mouse and dog. Mice received daily i.p. doses of LY231514 at 10.6 (31.8) and 26.2 mg/kg (78.6 mg/m2), twice weekly dose of 105 mg/kg (315 mg/m2), and weekly dose of 314.8 mg/kg (944.4 mg/m2) for 6 weeks; dogs received daily i.v. doses of 0.11 (2.2) and 0.53/0.37 mg/kg (10.6 mg/m2/7.4 mg/m2), twice weekly doses of 3.15 (63) and 7.87 mg/kg (157.4 mg/m2), and weekly dose of 104.9/26.24 mg/kg (2099 mg/m2/524.8 mg/m2) for 6 weeks. Mice tolerated daily doses of 26.2 mg/kg (78.6 mg/m2) for 6 weeks without any deaths or clinical signs of toxicity. No hematologic effects were observed in weekly or twice weekly dose but slight leukopenia was noted at daily doses. Drug-induced lesions in mice included intestinal necrosis, decreased testes weight, intestinal necrosis and hypospermatogenesis at ≥10.6 mg/kg. The minimally toxic doses (MTD) for mouse given LY231514 for 6 weeks were 10.6 mg/kg daily, 105 mg/kg twice weekly, and 314.8 mg/kg weekly in mice. In dog study, deaths occurred at all schedules (1/3& died at 0.11 mg/kg d27 and 2/3& at 0.53 mg/kg d9 & 10, daily dosing; 1/3%+ 3/3& died at 3.15 d19-22, and 6/6 at 7.87 mg/kg d15-19, twice weekly; and 1/3%,1/3& died at 104.9 mg/kg d5 & 8, weekly dose). Dogs died/killed had lymphopenia and neutropenia, and mild anemia. Most dogs (5/6) completed 6 weeks of daily doses of 0.11 mg/kg (2.2 mg/m2) with minimal clinicopathologic effects. Clinical signs included decreased food consumption, emesis, diarrhea, hypoactivity, dehydration, mucositis, abnormal stool and increased salivation. Most of the dogs given multiple doses of 7.87 mg/kg had slight increases in BUN, creatinine, ALT, AST, bilirubin, total protein and albumin values. Drug-induced lesions included moderate intestinal alterations at 0.11 mg/kg, moderate to severe enteropathy, hypocellularity of lymphoid tissues and bone marrow, mucositis, dermal skin ulcerations and hypospermatogenesis at ≥0.53 mg/kg. Higher daily doses were not tolerated in dogs for more than 3 weeks. Generally the daily dose schedule was more toxic than the weekly administration of much larger dose. The minimally toxic doses (MTD) for mouse given LY231514 once weekly for 6 weeks was 314.8 mg/kg (944.4 mg/m2). The MTD for dogs given

LY231514 once weekly for 6 weeks was not determined due to mortality in all schedules tested. Across species, dogs were more sensitive to the toxic effects of LY231514 than were mice.

In a 1-month study, dogs received weekly i.v. doses of 10 (200) or 25 mg/kg (500 mg/m2). After 4 weeks dosing, no mortality but clinical signs of toxicity included GI toxicity (watery/mucoid/soft feces), red, flaky skin and weight loss. Once weekly doses of 25 mg/kg (500 mg/m2) resulted in slight to moderate decreases in neutrophils, lymphocytes, platelets, and reticulocytes. All hematologic changes except for the decreased platelets were fully or partially reversed within a 3-week recovery period. Minimal to slight enteropathy was noted throughout the gastrointestinal tract.

A 6 month repeat dose study in dogs was planned to evaluate the chronic toxicity of LY231514 at doses of 0, 10 and 25 mg/kg (0, 200 and 500 mg/m2) given intravenously once weekly, which bridges directly to the 1-month study. However, after 3 months, dosing of 25 mg/kg group was discontinued due to the dose-limiting hematotoxicity. A dosing schedule was changed from weekly to every 3 weeks for the remainder of the study. Hematotoxicity was reversible during a 3-week non-dosing period. On day 108, three dogs (one each sex at 25 mg/kg and one female dog at 10 mg/kg) euthanized were thrombocytopenic, neutropenic, and had generalized hemorrhage and marked bone marrow hypocellularity.

Based on no mortality, slight to moderate decreases in hematology parameters and minimal to slight enteropathy, 25 mg/kg/week (500 mg/m2) once/week for one month study was considered to be a minimally toxic dose in dogs. However, hematotoxicity was the dose-limiting toxicity in a 6-month study and neither 10 nor 25 mg/kg was tolerated when given once weekly for more than 3 weeks.

Chronic toxicity studies conducted using different schedules (4-, 6-weeks, and 6-month) of LY231514 in dogs could not determine the safe clinical dose. In current clinical studies, LY231514 is administered intravenously once every 3 weeks at a dose of 500 mg/m2.

Two special toxicity studies were conducted to evaluate potential rescue agents (leucovorin and thymidine) for treatment of severe toxicity following LY231514 administration. In leucovorin study, coadministration of leucovorin (20 mg/kg, i.m. days 5-10; 25 mg/kg, i.m. days 4, & 5, and 50 mg/kg, i.v. day 4) and LY231514 (50 mg/kg, i.v. days 1 & 4) reversed both clinical signs of toxicity and hematological alterations in dogs. In the thymidine rescue study, subsequent thymidine (8 mg/kg, days 4-7) administration as a continuous infusion for 3 days was successful in rescuing dogs from life-threatening toxicity of LY231514 (50 mg/kg, i.v. days 0 & 3).

In safety pharmacology, LY231514 was assessed for its potential to elicit secondary receptor-mediated autonomic responses by smooth and cardiac muscle (tissue bath preparations), a battery of CNS and behavioral function tests in vivo assessments and, gastrointestinal transit in mice, renal assessment in rats, and cardiovascular effects in anesthetized dogs.

In Segment I male fertility, LY231514 at doses of 0.1 to 10 mg/kg caused reduced fertility rates, testicular atrophy and epididymal hypospermia. LY231514 had no effects on precoital interval or mating performance. In Segment II reproductive studies, pregnant mice were given i.v. doses ranging from 0.2 to 5 mg/kg/day (0.6 to 15 mg/m2/day) on gestation days 6 through 15. High dose LY231514 administration resulted in maternal toxicities and fetal malformations such as cleft palate, fused vertebrae and skeletal anomalies. About half of the mouse fetuses in the HD group were classified as runts. Decreased fetal weights were observed at doses ≥0.2 mg/kg (≥ 0.6 mg/m2), incomplete ossification of some skeletal structures at doses ≥ 1 mg/kg (3 mg/m2) and cleft palate at 5 mg/kg (15 mg/m2). A NOEL dose for developmental toxicity was not established because of fetal growth retardation was observed at all dose level.

LY231514 at concentrations up to 5248 ug/plate was not mutagenic in Ames assay including S. typhimurium and E. Coli. LY231514 at concentrations up to 2104 ug/mL (+S9) or 2098 ug/mL (-S9) did not induce chromosomal aberrations in CHO cells. In vitro mutation assay, LY231514 at concentrations ranging from 262 to 2099 ug/mL with or without metabolic activation, LY231514 did not induce gene mutations in HGPRT+ Chinese hamster ovary cells. In an in vivo mouse micronucleus assay, daily i.v. doses of LY231514 ranging from 393.5 to 1574.1 mg/kg for 2 days induced micronuclei in bone marrow cells of ICR mice.

In other toxicity studies, LY231514 has been evaluated in ocular and dermal irritation studies in rabbits to support workplace safety. Following a single ocular dose (0.0300 g in 0.1 mL) of LY231514 dissolved in physiological saline in the conjunctival sac of the rabbit eye, LY231514 resulted in iritis and conjunctivitis. A single dermal dose of 1000 mg/kg on the shaved dorsal trunk area produced dermal irritation. LY231514 was determined to be a moderate ocular and dermal irritant in rabbits.

Labeling Comments:

Labeling conforms to the format specified under CFR21. Part 201. Subpart B dated April 1, 1994. The proposed labeling accurately describes the preclinical observations for the most part. However, the following revisions are requested:

1. Under Pregnancy category D Section on p. 7 and 8, on lines 185 to 194 the paragraph should be changed to:

[

]

2. Under Precautions, Carcinogenesis, Mutagenesis, Impairment of Fertility Section on p. 8 and 9, Lines 236 to 245, a revised paragraph should read:

[

]

TABLE OF CONTENTS-PHARMACOLOGY/TOXICOLOGY REVIEW

Six male CD-1 mice received a single IV 20 mg/kg (60 mg/m2) dose of [^{14}C]-LY231514 (Specific activity = 7.84 uCi/mg) in saline. One animal each was euthanized at 0.083, 0.25, 0.5, 1, 3, 6, 12, 24 and 48 hrs post dose. Animals were then rapidly frozen in dry ice and hexane, and processed for whole-body Autoradiographic evaluation as described by Ullberg (1977). Sagittal whole-body sections and radiocarbon standards were simultaneously exposed to —— X-ray film for 13 days. Autoradiograms were visually and quantitatively evaluated.

Results:

Mean tissue concentrations (ug equivalent/kg) in organs and tissues and PK parameters from selected tissues after a single IV 20 mg/kg dose of [^{14}C]-LY231514 to mice and are shown below Table 1.

Table 1 Mean Tissue Concentrations of Radiocarbon (µg-eq/g) in Male CD-1 Mice Following a Single Intravenous 20 mg/kg Dose of 14C-LY231514 Disodium (free acid). Study M04096

Tissue	1001 0.083 hr	1002 0.25 hr	1003 0.5 hr	1004 1 hr	1005 3 hr	1006 6 hr	1007 12 hr	1008 24 hr	1009 48 hr
Adrenal Gland	14.39	7.90	4.26	ND	ND	ND	ND	ND	ND
Blood	59.80	24.19	10.07	ND	ND	ND	ND	ND	ND
Bone Marrow	13.50	5.97	ND	ND	ND	ND	ND	ND	ND
Brown Fat	12.89	4.79	2.85	ND	ND	ND	ND	ND	ND
Epididymis	22.16	11.50	5.53	5.69	ND	ND	ND	ND	ND
Harderian Gland	5.79	3.74	ND	ND	ND	ND	ND	ND	ND
Kidney-High	135.41	90.13	64.18	69.59	9.10	7.55	ND	ND	ND
Kidney-Low	38.29	31.81	12.19	6.90	ND	ND	ND	ND	ND
Liver-High	137.72	93.86	78.86	45.33	15.14	8.78	3.22	3.08	2.51
Liver-Low	79.81	70.67	ND	ND	ND	ND	ND	ND	ND
Lung	31.31	10.97	4.03	4.81	ND	ND	ND	ND	ND
Muscle	6.10	3.54	ND	ND	ND	ND	ND	ND	ND
Myocardium	15.42	6.39	3.28	ND	ND	ND	ND	ND	ND
Pancreas	10.29	4.38	ND	ND	ND	ND	ND	ND	ND
Preputial Gland	13.47	#	#	ND	ND	ND	ND	ND	ND
Salivary Gland	13.06	5.49	2.34	ND	ND	ND	ND	ND	ND
Skin	29.36	21.95	9.02	6.31	ND	ND	ND	ND	ND
Spleen	11.20	7.62	3.64	ND	ND	ND	ND	ND	ND
Testes	2.25	3.14	ND	ND	ND	ND	ND	ND	ND
Thymus	6.57	3.24	ND	ND	ND	ND	ND	ND	ND

ND = Not Detectable (Below mean lower limit of detection of 1.78±0.61 µg-eq/g, ranging from 0.87-2.97 µg-eq/g))
# = Tissue not sampled due to quality or size.

Autoradiographic results showed that radioactivity [^{14}C]-LY231514 and/or metabolites were readily distributed throughout the mouse at 0.5 hrs following a single IV 20 mg/kg dose. Within 1 hr, high concentrations of radioactivity were detectable in kidney, urine within the urinary bladder, liver, and gall bladder indicated that rapid urinary and biliary elimination. Detectable radioactivity did not persist in tissues and was detected only in kidney and liver at 24 hrs and 48 hrs post dose.

Study Title: Excretion and Metabolism of [^{14}C] LY231514 Na_2 in male CD-1 Mice (after a Single IV dose of 20 mg/kg comparison with a Single Oral 20 mg/kg dose (ADME 05) & in Female Dogs after a single IV 7.5 mg/kg dose (ADME 06).

Vol. 1.7, pages 1-16. 1993

Key study findings:

- Biliary excretion was the primary route of elimination of [^{14}C] LY231514 in mice via both routes.
- Rapid renal excretion was the primary route of elimination in dogs after IV dose of [^{14}C]-LY231514.

Method:

A comparative excretion study was performed following an 20 mg/kg (60 mg/m2) dose and an oral (20 mg/kg) dose of [^{14}C] LY231514 Na_2 to male mice. In a separate excretion study, 4 dogs received a single IV 7.5 mg/kg (150 mg/m2) of [^{14}C] LY231514 Na_2. Urine samples were

collected at 0-96 hr for mice and 0-168 hrs for dogs. ___ system was used to separate the samples and quantitate the radioactivity by scintillation counter.

Results:

Table 5: Cumulative Elimination of Radioactivity (Mean± SD) from CD-1 Male Mice Following single IV or Oral doses of [^{14}C] LY231514

	CD-1 Male Mice IV	CD-1 Male Mice Oral	Female Beagle Dog IV
Dose, mg/kg	20	20	7.5
N	2 groups (4 mice/group)	2 groups (4 mice/group)	4 dogs
	Percentage (%) of Dose		
Urine	34.9 ± 1.8	4.6 ± 1.9	68.8 ± 18.4
Feces	57.4 ± 13.8	88.4 ± 2.7	30.6 ± 6.9
Total[a]	92.5 ± 14.4	93.5 ± 2.6	99.2 ± 12.9
Timeframe	0-96 hr	0-96 hr	0-168 hrs

[a]= For mouse, the total also includes carcass.

After IV dose, urine and feces accounted for all recovered radioactivity (~92% total excreted). The excretion of radioactivity was rapid, as almost 90% was excreted within 24 hrs (ADME Report 08). After oral dose, only ~5% of the radioactivity was excreted into the urine, with ~88% in the feces. These data suggest that oral absorption of LY231514 was limited, with only 12% of the [^{14}C] LY231514 oral dose absorbed in mice.

The excretion of radioactivity after 7.5 mg/kg IV dose to dogs (ADME Report 08) was rapid (almost 90% excreted within 24 hrs) and excreted mostly in the urine (~69% of the dose) and to be complete (~99% recovered in the urine and feces).

Study Title: Urinary Metabolites of [^{14}C] LY231514 Na_2 in male CD-1 mice and female beagle dogs (ADME Report 08 & 10). Vol. 1.7, pages 1-16.1996

Key study findings: The majority of urinary radioactivity was unchanged parent drug, and two minor metabolites (M1 and M3).

Method:

A metabolic study was performed to assess the nature and extent of biotransformation of LY231514 following a single 7.5 mg/kg (150 mg/m2) IV dose to dogs and a 20 mg/kg (60 mg/m2) IV dose of [^{14}C] LY231514 Na_2 to male mice. Urine samples were collected from both species at 0-7 hr for the mice and 0-24 hr for the dog for radioactivity and metabolites. ___ system was used to separate and to quantitate radioactivity by scintillation counting.

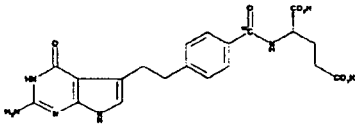
Table 1: Urinary Metabolites of [^{14}C]LY231514 Na_2 in Mice and Dogs[a]

	Percentage of [^{14}C]LY231514 Dose in Urine							
	Mouse			Dog				
Compound	Cage 1	Cage 2	Average[b]	A	B	C	D	Avg ± SD[c]
231514	29.21	15.07	22.14	37.21	64.34	58.22	17.56	44.33 ± 21.3
M-1	0.48	0.25	0.36	0.83	1.47	2.49	2.98	1.94 ± 0.97
M-3	0.35	0.11	0.23	8.35	1.77	3.14	1.58	3.71 ± 3.17
Unknowns	2.35	1.55	1.95	21.61	10.62	11.65	16.47	15.09 ± 5.04
Total %Dose	32.39	16.98	24.69	68.0	78.2	75.50	38.6	65.07 ± 18.17

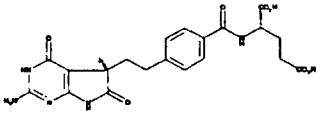
[a]Urine samples collected 0-7 hr following a single 20 mg/kg IV dose to 2 groups of 4-CD-1 male mice and dog urine samples collected from 0-24 hr from 4 dogs dosed 7.5 mg/kg IV were profiled by reverse phase HPLC. Quantitation of parent, metabolite, and unknowns was determined by radioscintillation counting.
[b]Average value from 2 cages.
[c]Mean percentage ± standard deviation.

Results:

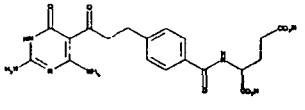
In the 0-24 hr dog urine, 65.07% of the actual dose of total radioactivity was recovered and 24.69 % was recovered in the 0-7 hr mouse urine. The parent drug was the majority of urinary radioactivity found in both species as 44.33% of the total dose in dogs (~68% of the ^{14}C found in that 0-24 hr fraction) and 22.14% in the mouse (~90% of the ^{14}C found in that 0-7 hr fraction). Recovery of radioactivity in urine and feces was essentially complete (99% = ~68 in urine and ~31% in feces) in dogs. Two minor metabolites, M-1 and M-3, were observed which accounted for less than 1% (mice) and less than 6% (dog) of the total dose in these urine samples. The structures for LY231514 and its identified metabolites are shown below.



[^{14}C]LY231514



Metabolite LY338979



Metabolite LY368962

Unchanged LY231514 (70% to 90% of the administered dose) and metabolite LY338979 were also detected in human urine from subjects from the clinical trial, H3E-MC-JMAW using LC/MS/MS method. However, metabolite LY368962 was not detected in human urine samples.

Study Title: Protein Binding of ^{14}C-LY231514 in mouse, dog and Human plasma (ADME Report 07). Vol.1.7, pages 1-8. 1995

Key study findings: The binding of [^{14}C] LY231514 to plasma proteins was 82% in human, 57% in mouse and 46% in dog.

Method:

The in vitro protein binding of [^{14}C] LY231514 was determined from plasma samples of mouse, dog and human (Study JMAW). The samples were incubated for 30 minutes at 37°C and the extent of binding was determined by ultrafiltration.
Results:

Table 4 : Mean Percentage Protein Binding of LY231514 in Human, Mouse, and Dog

	Mouse	Dog	Human
Concentration ^{14}C-LY231514 (ng/mL)	Mean percent Bound	Mean Percent Bound	Mean Percent Bound
500	57.7	46.0	81.3
5000	53.5	46.8	80.8

The percentage binding of ^{14}C-LY231514 to plasma proteins was independent of LY231514 concentrations from 500 to 5000 ng/mL.

Study Title: In Vitro interaction of LY231514 with Human Cytochromes P450 CYP3A, CYP2D6, CYPP2C9, and CYP1A2 (ADME Report 11). Vol. 1.7, pages 1-17. 1997

Key study findings: LY231514 up to 1000 uM did not induce CYP2D6 but slight inhibition was noted of CYP2C9 and CYP1A2 activities in human liver samples.

LY231514 is largely excreted unchanged in animal and human urine (ADME Study 08 and 10). Pre-clinical data suggest that unchanged LY231514 accounts for most of the urinary radioactivity, and metabolism plays a minor role in clearance of LY231514. Therefore, studies to determine whether LY231514 is a substrate for CYP450 enzymes have not been carried out. Only the ability of LY231514 to inhibit the metabolism of marker catalytic activities was assessed.

Method:

The ability of LY231514 to inhibit the metabolism of marker catalytic activities of cytochrome P450 CYP3A, CYP2D6, CYP2C9, and CYP1A2 was examined. CYP3A, CYP2D6, CYP2C9, and CYP1A2 activities were measured 30 minutes after incubation of human liver samples exposed to LY231514.

Results:

The results at the highest concentrations of LY231514 used in these inhibition studies are summarized in Table 9.

Table 9: In Vitro Inhibition of CYP 450 Enzymes by LY231514

CYP Isozyme	Marker Catalytic Activity	Substrate (Concentration)	LY231514 Concentration	Inhibition
CYP3A	Midazolam 1'-hydroxy-lase	Midazolam (5 uM)	885 uM	21%
CYP2D6	Bufuralol 1-hydroxylase	Bufuralol (5 uM)	1000 uM	Little or no inhibition
CYP2C9	Diclofenac-4'-hydroxylase	Diclofenac (2.5 uM)	1000 uM	Slight inhibition (6%)
CYP1A2	Phenacetin to acetaminophen	Phenacetin (12.5 uM)	1000 uM	Slight inhibition (8%)

Enzyme induction studies with LY231514 have not been carried out. However, LY231514 is not expected to cause any significant enzyme induction in humans with the current dosing schedule (once every 21 days).

Since peak circulating levels of LY231514 approach 200 ug/mL (468 uM) in humans, these in vitro studies suggest that LY231514 would not be expected to cause clinically significant inhibition of the metabolic clearance of drugs metabolized by CYP3A, CYP2D6, CYP2C9, and CYP1A2.

Study Title: Pharmacokinetic interaction study of LY231514 and Aspirin in beagle dogs following a single IV bolus of 25 mg/kg LY231514 as the disodium salt (ADME Report 12). Vol. 1.6, pages 1-24. 1998

Key study findings: Single and multiple oral doses of Aspirin did not alter the pharmacokinetics of LY231514.

Method:

The pharmacokinetics of LY231514 was determined after a single IV bolus dose of 25 mg/kg (500 mg/m2)(group A). Aspirin was then given as a single 10 mg/kg oral dose (group B), and daily doses of 10 mg/kg for 2 weeks and the last dose of 10 mg/kg aspirin was given 30 minutes prior to LY231514 (group C) in dogs. Blood samples were taken at 0, 5, 20 minutes, 1, 2, 4, 6, 8, 10, 12, 24, and 30 hrs post-dose for the determination of LY231514 alone (Day 0), LY231514 after a single dose of aspirin (Day 21) and LY231514 after 2 weeks of daily doses of aspirin (Day 42). Samples for the determination of total salicylates were collected at 0, 6, and 12 hrs post LY231514 dose from groups B and C only on days 21 and 42. Plasma concentrations of LY231514 were determined by LC/MS/MS method. Samples for total Salicylates were analyzed by — method. Plasma concentrations of salicylic acid in plasma less than 1 ug/mL were reported as BQL.

Results:

Table 5: Mean (±SEM) Plasma Concentrations and Pharmacokinetic Parameters for LY231514 in Four Beagle Dogs Administered a Single IV Bolus Dose of 25 mg/kg LY231514 as the Disodium Salt

	LY231514 alone			LY231514 + Single Dose Aspirin			LY231514 + Chronic Aspirin		
Time (hr)	Mean (ng/mL)	SEM	N	Mean (ng/mL)	SEM	N	Mean (ng/mL)	SEM	N
Predose	NC	NC	NC	NC	NC	NC	NC	NC	NC
0[a]	141156.5	11323.7	4	126935.4	21419.1	4	169256.3	14152.0	4
0.083	117648.7	8146.1	3	99211.6	14803.1	4	128327.5	8984.4	4
0.10	92048.9	NC	1	NV	NV	NV	NV	NV	NV
0.33	56923.9	2471.3	4	48838.5	5730.8	4	57758.8	6445.9	4
1	29736.6	3026.6	4	27956.2	2218.4	4	34409.0	6992.6	4
2	16055.6	1934.9	4	13273.6	1852.5	4	15077.0	1239.7	4
4	5000.7	575.0	4	4007.0	760.9	4	4302.8	748.2	4
6	2007.0	260.9	4	1496.4	121.1	4	2125.9	398.7	4
8	1006.9	156.8	4	947.0	139.6	4	1043.9	138.7	4
10	592.0	132.5	4	565.7	98.4	4	594.7	92.0	4
12	357.9	51.8	4	411.0	68.5	4	380.2	39.9	4
24	48.7	20.9	4	54.3	11.1	4	91.2	43.0	4
30	49.5	32.7	4	36.3	18.8	4	38.7	19.5	4
AUC_{0-30hr} (ng•hr/mL)	119678	9103	4	104802	10088	4	125778	11236	4
$AUC_{0-\infty}$ (ng•hr/mL)	119993	9164	4	104986	10124	4	126127	11187	4
Half-life (hr)	4.4	0.6	4	4.2	0.3	4	4.4	0.6	4
C_0 (ng/mL)	141156.5	11323.7	4	126935.4	21419.1	4	169256.3	14152.0	4
Clearance (mL/min/kg)	3.53	0.27	4	4.10	0.46	4	3.37	0.27	4
Volume of Distribution (L/kg)	1.43	0.21	4	1.50	0.14	4	1.40	0.27	4

NC = Not calculated
NV = No value or sample analyzed
a = extrapolated to 0 hr

Table 6: **Plasma Concentrations of Total Salicylates in Beagle Dogs after Administration of a Single Oral Dose of 10 mg/kg Aspirin and 10 mg/kg/day for 2 Weeks**

	Concentration (µg/mL)							
	Dog 275632		Dog 275662		Dog 275712		Dog 275722	
Time (hr)	Single Dose	Chronic Dose	Single Dose	Chronic Dose	Single Dose	Chronic Dose	Single Dose	Chronic Dose
0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
6								
12								

BQL = Below the limit of quantitation (<1 µg/mL)

After a single IV bolus dose of 25 mg/kg LY231514, the mean LY231514 concentration (C_0) extrapolated at time zero was 141156 ng/mL while the C_0 values after single and multiple doses of aspirin were 126935 ng/mL and 169256 ng/mL, respectively. The mean $AUC_{0-\infty}$ of LY231514 dosed alone was 119993 ng·hr/mL. The mean $AUC_{0-\infty}$ values for LY231514 after single or multiple doses of aspirin were 104986 and 126127 ng·hr/mL, respectively.
The mean half-life of LY231514 from all treatment groups ranged from 4.2 to 4.4 hrs. The mean clearance of LY231514 ranged from 3.37 mL/min/kg to 4.10 mL/min/kg in all treatment groups. The mean volume of distribution ranged from 1.40 L/kg to 1.50 L/kg for LY231514 in all treatment groups. Single and multiple oral doses of aspirin given ~30 minutes before a single bolus dose of LY231514 did not appear to significantly alter the pharmacokinetics of LY231514.

Study Title: Pharmacokinetic interaction study of LY231514 and Ibuprofen in beagle dogs following a single IV bolus dose of 25 mg/kg LY231514 as the disodium salt (ADME report 13). Vol. 1.6, pages 1-24. 1998.

Key study findings: Single and multiple oral doses of Aspirin given ~30 minutes prior to LY231514 dose did not alter the pharmacokinetics of LY231514.

Method:

The pharmacokinetics of LY231514 was determined after a single IV bolus dose of 25 mg/kg (500 mg/m2)(group A) in 4 female beagle dogs. Ibuprofen was then given as a single 5 mg/kg oral dose (group B), and daily doses of 5 mg/kg for 2 weeks and the last dose of 5 mg/kg ibuprofen was given 30 minutes prior to LY231514 in dogs (group C). Blood samples were taken at 0, 5, 20 minutes, 1, 2, 4, 6, 8, 10, 12, 24, and 30 hrs post-dose for the determination of LY231514 alone (Day 0), LY231514 after a single dose of ibuprofen (Day 21) and LY231514 after 2 weeks of daily doses of aspirin (Day 42). Samples for the determination of ibuprofen were collected at 0, 6, and 12 hrs post LY231514 dose from groups B and C only on days 21 and 42. Plasma concentrations of LY231514 were determined by LC/MS/MS method while the concentrations of ibuprofen were analyzed by HPLC/UV. Plasma concentrations of ibuprofen less than 1 ug/mL were reported as BQL.

Results:

Table 5: **Mean (±SEM) Plasma Concentrations and Pharmacokinetic Parameters for LY231514 in Four Beagle Dogs Administered a Single IV Bolus Dose of 25 mg/kg LY231514 as the Disodium Salt**

	LY231514 alone			LY231514 + Single Dose Ibuprofen			LY231514 + Chronic Ibuprofen		
Time (hr)	Mean (ng/mL)	SEM	N	Mean (ng/mL)	SEM	N	Mean (ng/mL)	SEM	N
Predose	NC	NC	NC	NC	NC	NC	NC	NC	NC
0a	149576.6	13618.5	4	155645.1	33109.6	4	111884.8	17053.2	4
0.067	100322.0	NC	1	NV	NV	NV	NV	NV	NV
0.083	114720.8	NC	2	121677.2	22640.1	4	91304.7	10866.8	4
0.10	130246.4	NC	1	NV	NV	NV	NV	NV	NV
0.33	54751.2	1547.3	4	60041.7	8697.5	4	53482.5	10431.4	4
1	29662.2	731.3	4	33195.2	2039.9	4	28044.7	2921.8	4
2	18438.3	1040.2	4	18694.5	768.7	4	14218.8	2083.4	4
4	6055.9	632.1	4	6687.2	505.3	4	6306.8	530.7	4
6	2420.4	222.7	4	3010.0	136.7	4	2536.0	323.6	4
8	1039.7	73.0	4	1328.9	79.7	4	1337.2	150.2	4
10	612.9	84.7	4	769.2	77.5	4	866.6	86.6	4
12	364.2	53.1	4	455.8	22.7	4	621.7	72.1	4
24	31.1	8.0	4	55.0	10.7	4	102.4	38.2	4
30	21.0	NC	2	33.1	6.6	4	45.5	15.3	4
AUC_{0-t} (ng•hr/mL)	125902	5289	4	137214	6705	4	114944	8945	4
$AUC_{0-\infty}$ (ng•hr/mL)	126046	5331	4	137387	6728	4	115254	9012	4
Half-life (hr)	3.7	0.2	4	4.2	0.3	4	4.4	0.3	4
C_0 (ng/mL)	149576.6	13618.5	4	155645.1	33109.6	4	111884.8	17053.2	4
Clearance (mL/min/kg)	3.32	0.14	4	3.06	0.15	4	3.68	0.29	4
Volume of Distribution (L/kg)	1.08	0.10	4	1.12	0.08	4	1.43	0.13	4

NC = Not calculated
NV= No value or sample analyzed
AUC_{0-t} = 0 hr to the last time point that was above BQL
a = extrapolated to 0 hour

Table 6: **Plasma Concentrations of Ibuprofen in Beagle Dogs after Administration of a Single Oral Dose of 5 mg/kg Ibuprofen and 5 mg/kg/day for 2 Weeks**

	Concentration (µg/mL)							
	Dog 275542		Dog 275552		Dog 275582		Dog 275622	
Time (hr)	Single Dose	Chronic Dose	Single Dose	Chronic Dose	Single Dose	Chronic Dose	Single Dose	Chronic Dose
0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
6								
12	BQL		BQL	BQL	BQL			

BQL = Below the limit of quantitation (<1 µg/mL)

After a single IV bolus dose of 25 mg/kg LY231514, the mean LY231514 concentration (C_0) extrapolated at time zero was 149576 ng/mL whereas the C_0 values after single and multiple doses of ibuprofen were 155645 ng/mL and 111884 ng/mL, respectively. The mean $AUC_{0-\infty}$ value of LY231514 dosed alone was 126046 ng·hr/mL. The mean $AUC_{0-\infty}$ values for LY231514 after single and multiple doses of ibuprofen were 137387 and 115254 ng·hr/mL, respectively. The mean half-life of LY231514 from all treatment groups ranged from 3.7 to 4.4 hrs. The mean clearance of LY231514 ranged from 3.06 mL/min/kg to 3.68 mL/min/kg. The mean volume of distribution ranged from 1.08 L/kg to 1.43 L/kg for LY231514. The mean values were similar after a single dose of ibuprofen, but increased ~32% after multiple ibuprofen. Single and multiple oral doses of ibuprofen given ~30 minutes before a single bolus dose of LY231514 did not appear to significantly alter the pharmacokinetics of LY231514.

PK summary:

Absorption, distribution, metabolism and excretion studies for LY231514 have been conducted in CD-1 mice and beagle dogs.

After single IV administration to mice and dogs, plasma concentration of LY231514 declined in a biphasic manner, a rapid distribution phase followed by a longer terminal

elimination phase. The area under the plasma concentration-time curve (AUC) increased in a dose-dependent manner over the tested dosages (20-100 mg/kg). The elimination half-life was short in dogs compared to mice. Pharmacokinetic parameters are summarized in table below.

Parameters	LY231514 Na_2						
Species	Male Mice			Dogs			
Dose, mg/kg	20	20	200	7.5		100	
Route	IV	IP	IP	IV		IV	
				Male	Female	Male	Female
Cmax, ug/mL							
at 5 min							
at 12 hr	-	-	-	-			
at 24 hr	-	-	-				
at 30 hr	-	-	-	-	-		
at 48 hr				-	-	-	-
Tmax, min	5	15	15	5		5	
$AUC_{0\text{-}24\ hr}$ ug·hr/mL	31	44	220	32		500	
T1/2α, min	5	15	15	5-20		5-20	
T1/2β, hr	7.0	7.8	10.0	2.3	1.8	2.3	2.2
F, %		100	71				

-indicates no data

In toxicokinetic (6 week) study, dogs received daily (0.53 mg/kg), twice weekly (3.15 mg/kg) and weekly doses of 104.96 mg/kg. During the study, a number of dose alterations were made due to dose-limiting hematotoxicity. Animals were dosed daily with 0.53 mg/kg through day 8 but had to lowered to 0.37 mg/kg thereafter. Additional animals were dosed weekly with 104.96 mg/kg on day 1 of this study and 26.24 mg/kg thereafter. Plasma concentrations of LY231514 over time were determined on day 1, day 11/15, and day 32/36. The systemic exposure of LY231514 as measured by $AUC_{0\text{-}\infty}$ increased proportionally with dose. The AUC was from 1.36 ug·hr/ml in males and 0.55 ug·hr/ml in females after a 0.11 mg/kg dose up to 523 ug·hr/ml and 517 ug·hr/ml, respectively, after 104.96 mg/kg dose in dogs. There seems to be no gender difference in exposure over time, however, because of experimental complications as above, there were insufficient data to make any firm conclusion on the effect of multiple doses.

In distribution study, following a single IV dose of 20 mg/kg to mice, [^{14}C]-LY231514 and its metabolites was rapidly distributed into various tissues and eliminated via both renal and biliary routes. Within 1 hr, the highest concentrations of radioactivity were found in the organs of metabolism and excretion (i.e., liver and kidneys). The data indicated that radioactivity did not persist in tissues and was detected up to 3 hrs after the dose. Only kidney and liver were detected at 6 hrs and 48 hrs, respectively, post dose.

The majority of urinary radioactivity found in both species were parent drug, constituting ~44% of the total dose in dog (~68% of the [^{14}C] found in 0-24 hr fraction) and ~22% in mouse (~90% of the [^{14}C] in 0-7 hr fraction). Two minor metabolites, M1 and M3, were observed in mice and dog samples after IV administration of LY231514. Unchanged LY231514 and metabolite M1 (LY338979) were also detected in human urine from the clinical study H3E-MC-JMAW, however, metabolite M3 (LY368962) was not detected in human urine samples.

Elimination studies were conducted in male CD-1 mice and female beagle dogs. After a 20 mg/kg IV dose of [^{14}C]-LY231514 Na2 to mice, mice excreted ~57% of the administered radioactivity in the feces and ~35% in urine. Urine and feces accounted for all of recovered radioactivity (~93% total recovery), and less than 0.2% radioactivity remained in mice 96 hrs after dosing. The excretion of radioactivity after a 7.5 mg/kg IV dose of [^{14}C]-LY231514 Na2 to dogs was rapid with approximately 90% of the administered radioactivity excreted within 24 hrs.

The majority of the administered radioactive dose (~69%) was recovered in urine and overall elimination was essentially complete (~99% recovered in the urine and feces) in dogs. Urinary excretion of the unchanged drug is also the predominant route of elimination observed in humans.

The in vitro protein binding of [^{14}C]-LY231514 study using plasma concentrations of— and — ng/mL, the percent of [^{14}C]-LY231514 bound to plasma proteins was found to be approximately 53 to 58% in mouse plasma, 46 to 47% in dog plasma, and approximately 81% in human plasma.

Studies to determine whether LY231514 is a substrate for CYP450 enzymes have not been carried out because of preclinical data suggest that unchanged LY231514 accounts for the most of the urinary radioactivity, and metabolism plays a minor role in clearance of LY231514.

In drug interaction studies, single and repeated oral doses of either 10 mg/kg/day aspirin or 5 mg/kg/day ibuprofen administered approximately 30 minutes prior to a single IV bolus dose of LY231514 did not appear to cause alteration in the pharmacokinetics of LY231514 in dogs.

PK conclusion:

The PK profile of LY231514 was a biphasic and the AUC values were dose proportional following single IP or IV administration to mice, IV administration to dogs and human. The elimination half-life was short in dogs and man compared to mice. The pharmacokinetics of LY231514 has been adequately characterized in mice and dogs.

After single IV administration to mice, dogs and human, plasma concentration of LY231514 declined in a biphasic manner, a rapid distribution phase followed by a longer terminal elimination phase. The AUC values increased in a dose-dependent manner in all species. The pharmacokinetics of LY231514 in mice, dogs and man after single doses were similar.

Species	Mice	Dog	Patients	
	CD-1	Beagle	JMAB	JMBZ
Dose, mg/kg	20	7.5		
Dose, mg/m2	60	150	40	500
n	4	3	7	4
Cmax , ng/mL	41	44	11	96
Tmax, hr	0.083	0.083	0.083	0.083
$AUC_{0\text{-}24}$, ug·hr/mL	31	32	-	-
$AUC_{0\text{-}\infty}$ ug·hr/mL	-	-	14	154
T1/2, hr	7.0	2.3	2.0	2.6
CL, mL/min	-	-	52	54
Vss, L/m2	-	-	6.6	7.1

APPEARS THIS WAY
ON ORIGINAL

III. TOXICOLOGY

Study Title: A Toxicokinetic study of a subchronic toxicity study in beagle dogs given LY231514 Na_2 daily, twice weekly and weekly by Intravenous Injection for 6 weeks in beagle dogs (Study D05091-reviwed within IND 40,061).

Key study findings:

- Systemic exposure of LY231514 increased with dose.
- Plasma half-lives ranged from 0.8 to 5.1 hrs, and longer half-lives were found at higher doses.
- No sex differences in exposure over time

Study no:	Study D05091
Volume #, and page #:	Volume 1.8, page 1-167
Conducting laboratory and location:	Lilly Research Lab., Greenfield, IN 46140
Date of study initiation:	February 1996
GLP compliance:	Yes
QA report:	Yes
Drug, lot#, and % purity:	LY231514 disodium, lot# 281NK1
Formulation/vehicle:	Sterile water for Injection, USP

Dosing:

Species/strain:	Beagle dog
# sex/group:	3♂ + 3♀/group
Age:	8 to 10 months
Weight:	10.8 kg ♂, 9.7 kg ♀
Doses in administered units:	IV doses of 0.11-0.53 mg/kg (2.2-10.6 mg/m2) daily, 3.15-7.87 mg/kg (63-157.4 mg/m2) twice week, or 26.24-104.96 mg/kg (524.8/2099 mg/m2) weekly
Route, form, and volume:	Intravenous injection, 1.0 ml/kg
Blood samples:	Taken at 0, 5, and 20 min., and 1, 2, 4, 6, 8,12 and 24 hrs postdose (once daily and twice weekly), and up to 120 hrs postdose (weekly)

Observations and times:

Clinical signs:	Once daily
Body weights:	Weekly
Food consumption:	Weekly
Physical examination:	10-11,1991 & 12-1,1992
Ophthalmoscopy:	10-11,1991 & 12-1,1992
EKG:	2nd and 5th weeks
Hematology:	None
Clinical chemistry:	None
Urinalysis:	None
Gross pathology:	Day 67
Histopathology:	Day 67

Results:

During the study, a number of dose alterations were made. One group of animals were dosed daily with 0.53 mg/kg through day 8 but lowered to 0.37 mg/kg thereafter and another group was dosed weekly with 104.96 mg/kg through day 8 but lowered to 26.24 mg/kg thereafter. Lastly a group dosed twice weekly with 3.15 mg/kg was replaced with 7.87 mg/kg twice weekly.

Plasma concentrations of LY231514 over time were determined on day 1, day 11/15, and day 32/36, and additional trough level measurements were made at various times. Plasma levels decreased over time from initial high level after the intravenous dose. Plasma half-lives calculated were ranged from 0.8 up to 5.1 hr. In general, longer half-lives were found at higher doses. The PK/TK parameters for the 6-week dog study are summarized as in following table.

Summary of PK parameters of LY231514 Following Multiple Doses of LY231514 for 6 weeks in Dogs

PK Parameters	Dose. mg/kg	Day 1 M	Day 1 F	Day 11 M	Day 11 F	Day 15 M	Day 15 F	Day 36 M	Day 36 F
C_{5min}(ug/ml)	0.11[a]	0.70	0.51			0.44	0.53	0.45	0.43
T½ (hr)		1.7	1.6			0.8	0.9	ND	ND
AUC_{0-t}		1.06	0.52			0.36	0.38	2.70	2.86
$AUC_{(0-\infty)}$ (ug·hr/ml)		1.36	0.55			0.28	0.38	ND	ND
C_{5min}(ug/ml)	0.53/	0.77	0.50			1.62	1.72		
T½ (hr)	0.37[b]	2.3	1.9			2.4	1.5		
AUC_{0-t}		1.80	1.84			1.98	1.81		
$AUC_{(0-\infty)}$ (ug·hr/ml)		1.81	1.84			2.23	1.81		
C_{5min}(ug/ml)	3.15[c]	7.29	7.14	7.89	8.91				
T½ (hr)		2.4	1.9	2.5	1.8				
AUC_{0-t}		14.8	11.3	12.2	20.2				
$AUC_{(0-\infty)}$ (ug·hr/ml)		14.8	11.3	12.2	20.3				
C_{5min}(ug/ml)	7.87[c]	39.0	49.2						
T½ (hr)		5.0	2.46						
AUC_{0-t}		34.2	33.1						
$AUC_{(0-\infty)}$ (ug·hr/ml)		34.3	33.1						
C_{5min}(ug/ml)	104.96/	530.4	459.0			86.4	93.2	14.0	17.5
T½ (hr)	26.24[d]	3.9	3.2			4.4	3.8	5.1	4.1
AUC_{0-t}		523.7	517.1			77.6	72.2	28.4	26.5
$AUC_{(0-\infty)}$ (ug·hr/ml)		523.7	517.1			77.6	72.2	28.4	26.5

[a] Daily IV dosing; [b] Animals were dosed daily with 0.53 mg/kg for days 1-8 and lowered to 0.37 mg/kg thereafter; [c] Twice weekly IV dosing ; [d] Animals were dosed weekly with 104.96 on day 1 and lowered to 26.24 mg/kg thereafter.

After a single IV dose, the systemic exposure of LY231514 (as measured by $AUC_{0-\infty}$) increased proportionally with dose. The AUC was from 1.36 ug·hr/ml in males and 0.55 ug·hr/ml in females after a 0.11 mg/kg dose up to 523 ug·hr/ml and 517 ug·hr/ml, respectively, after 104.96 mg/kg dose in dogs. Plasma half-lives ranged from 0.8 to 5.1 hrs, and longer half-lives were found at higher doses. There were no gender difference in exposure over time.

Study Title: A subchronic toxicity study in beagle dogs given 4 weekly Intravenous Injection of LY231514 Na_2 followed by a 3-week recovery phase (D03699).

Key study findings:

- Expected toxicologic profiles for folate antimetabolite were observed in clinical signs, hematology parameters, and gastrointestinal histopathology.

Study no:	Study D03699
Volume #, and page #:	Volume 1.8-9, pages 1-103, and 104-232.
Conducting laboratory and location:	Lilly Research Lab., Greenfield, IN 46140
Date of study initiation:	January 2000

GLP compliance:	Yes
QA report:	Yes
Drug, lot#, and % purity:	LY231514 disodium, lot# HK5-VGL-026
Formulation/vehicle:	Sterile water for Injection, USP

Dosing:

Species/strain:	Beagle dog
# sex/group:	6♂ + 6♀/group
Satellite groups used:	None
Age:	5 to 7 months
Weight:	7.5 to 9.7 kg ♂, 7.0 to 8.8 kg ♀
Doses in administered units:	Once weekly IV doses of 0(0), 10 (200) or 25 mg/kg (500 mg/m2)
Route, form, and volume:	Intravenous injection, 0.75 mL/kg
Dosing interval:	4 weekly doses, followed by 3 week recovery

Observations and times:

Clinical signs:	Once daily
Body weights:	Days 14, 24, and 38
Food consumption:	Days 14, 24, and 38
Physical examination:	Days -14 and 22
Hematology:	Weekly
Coagulation:	Weekly
Clinical chemistry:	Weekly
Gross pathology:	Days 24 and 43
Histopathology:	Day 43

Results:

Mortality: None died.

Clinical signs:

On days 1-3, emesis and foamy vomitus were noted infrequently for males and females at HD. Redness and/or flaking of the skin on the ventral abdomen were seen in both treated groups and redness was noted 16 days following the last dose and the flaking persisted until live-phase termination. On days 1-4, changes in fecal matters (consistency, color, soft, mucoid, watery) were observed in all treated groups, including control group. The severity and incidence was treatment-related and affected in both sexes with 50% of the dogs (LD and HD.

Body weight and food consumption:

Dose-related slight decreases (~6-15%) in mean body weight were observed in the LD and HD groups compared to the controls. On day 38, the mean body weight for the LD males remained slightly decreased.

Mean body weight (% change)	Administered Dose (mg/kg/week)			
	10		25	
Sex	M	F	M	F
Treatment phase				
Day 18	↓13*	↓12*	↓13*	↓15*
Day 24	↓18	↓12	↓14	↓6
Reversibility phase				
Day 38	↓14	↓5	↓6	↑1

*=p<.05

Physical evaluation:

Treatment-related erythema and/or flakiness of the abdominal skin were noted in one female at LD and in 3 males and 3 females at HD groups. At the end of recovery phase, this condition was not present.

Hematology:

Slight to moderate decreases in neutrophils, lymphocytes, reticulocytes, and platelet counts were observed on days 7, 14, 21, 28, 35, and 42 in both males and females given LD or HD doses of LY231514. After recovery period, these changes were reversed except for decreases in platelets in dogs given HD.

Hematological mean values following 4-week administration of LY231514 in dogs

Parameter (% change)	Administered /dose, mg/kg/week			
	10		25	
Sex	Male	Female	Male	Female
Neutrophil count				
Day 7	-	-	↓34*	-
Day 14	-	-	↓29*	-
Day 21	-	-	↓37*	↓34
Day 28	↓35*	↓5	↓54*	↓47*
Day 35	↑23	-	↓23	-
Day 42	↑30*	↑27	↓15*	↑15
Lymphocyte count				
Day 7	↓10	↓26	↓15	↓28
Day14	↓13	↓16	↓17	↓25
Day 21	↓12	↓17	↓32	↓41*
Day 28	↓5	↓25	↓11	↓17
Day 35	-	↓5	↑15	↑9
Day 42	-	↑69	-	↑19
Platelet count				
Day14	-	-	↓14	↓16
Day21	↓44*	↓42*	↓45*	↓56*
Day 28	↓33*	↓26	↓59*	↓57*
Day 35	↓17	↓19	↓45*	↓38*
Day42	-	-	↓42*	↓21
Reticulocyte count				
Day 21	↓55*	-	↓58*	↓57*
Day 28	↓32	-	↓39	↓62*
Day 35	-	-	↑41	↓20
Day 42	-	-	-	↓36

*= $p<0.05$; -=No Data

Clinical chemistry: Treatment-effects were not remarkable.

Gross pathology:

Only decreases in relative liver weights without morphologic changes were observed in dogs given LD (↓20%) and HD (↓19%) compared to control.

Histopathology:

Treatment-related lesions included minimal to slight enteropathy (mucosal hemorrhage, inflammation, edema, cryptal necrosis) of the GI tract (stomach, small intestine, large intestine), slightly higher enteropathic lesions in the jejunum and colon. After 3-week recovery period, minimal enteropathic lesions were still present in some dogs in the LD and HD groups.

Study Title: A 6-Month repeat-dose toxicity study in beagle dogs given weekly Intravenous doses of LY231514 Na_2 (Study D01301)

Key study findings:

- Once weekly or once every 3 weeks for 6 months resulted in toxic changes in clinical observations, hematology parameters, and histopathology.
- Hematotoxicity was the dose-limiting effect and neither 10 nor 25 mg/kg weekly was tolerated for more than 7 weeks.
- Drug-related skin lesions were observed.

Study no:	Study D01301
Volume #, and page #:	Volume vol. 1.9-10, pages 1-219, and 220-280
Conducting laboratory and location:	Lilly Research Lab., Greenfield, IN 46140
Date of study initiation:	January 2000
GLP compliance:	Yes
QA report:	Yes
Drug, lot#, and % purity:	LY231514 disodium, lot# RW02041
Formulation/vehicle:	Sterile water for Injection, USP

Dosing:

Species/strain:	Beagle dog
# sex/group:	4♂ + 4♀/group
Satellite groups used:	None
Age:	7 to 9 months
Weight:	9.2 to 10.8 kg ♂, 8 to 10.2 kg ♀
Doses in administered units:	Once weekly IV dose of 0 (0), 10(200) or 25 mg/kg (500 mg/m2) through day 77, then once every 3 weeks
Route, form, and volume:	Intravenous injection, 0.75 mL/kg

Observations and times:

Clinical signs:	Once daily
Body weights:	Days 14, 24, and 38
Food consumption:	Days 14, 24, and 38
Physical examination:	Days -14 and 22
Ophthalmoscopy:	Days –6 and 182
EKG:	None
Hematology:	Days –6, 27, 83 and 184
Coagulation:	Weekly
Clinical chemistry:	Days 4, 6 and 8
Urinalysis:	Weekly
Gross pathology:	Day 185
Histopathology:	Day 185

Results:

Mortality:

One male (#316273) and one female (#293673) given HD were euthanized on days 108 and 115, respectively. Prior to euthanasia, these dogs were thrombocytopenic and neutropenic, and had generalized hemorrhage and marked bone marrow hypocellularity. One female (#293602) given LD was euthanized on day 84. Each dog exhibited hypoactivity, inappetence, and persistent adverse skin lesions. All other dogs survived to the scheduled termination.

Clinical signs:

Clinical signs included skin lesions around abdominal and urogenital areas and abnormal stools (mucoid, soft, watery) in the treated dogs. Soft stools were noted in all dogs, including the controls, however, the increased incidence of watery and mucoid feces occurred in the treated groups during the treatment phase.

Body weight and food consumption:

Slight to moderate decreases in body weights were observed due to the decreased food consumption in dogs given weekly doses of 10 or 25 mg/kg. The decreases were 17-20% generally 2-4 days after each dose.

		% Change			
Daily Dose (mg/kg):		10		25	
Parameter	Sex:	M	F	M	F
Body weight[a]					
Day 41		↓9*	↓9	↓12*	↓17*
Day 83		↓12*	↓15*	↓17*	↓18*
Day 97		↓10*	↓11*	↓14*	↓16*
Day 181		↓10	↓10	↓17*	↓20*
Body weight change[b]					
Day 41		↓6*	↓5*	↓8*	↓13*
Day 83		↓3*	↓8*	↓8*	↓12*
Day 97		↑1*	↑0	↓3*	↓9*
Day 181		↑10	↑9	↑4*	↓2

Abbreviations: M = male, F = female, ↑ = increase, ↓ = decrease.

[a] Percent change relative to control group.

[b] Percent change relative to initial (Day -1) weight.

*p≤.05 = statistical significance based on actual data, not on percent change from control.

LY231514 caused decreased food consumption in dogs given 10 or 25 mg/kg. The decreases were transient and limited to 2-4 days after each dose. However, the 3 dogs that euthanized had persistent, severe deceases in food consumption (generally ≥75% of food remaining for several days) before euthanasia.

Physical evaluation:

Drug-related skin lesions were observed on days 38 to 41 in both treatment groups and again on day 83 in the HD group. The lesions included erythema, desquamation, excoriation, exudates or crusts in the ear pinnae, axillary and inguinal region, scrotum and vulva. Treatment-related hyperpigmentation was present in one or more animals in both LD and HD groups in ear pinnae, anus, vulva, scrotum, prepuce, axillary, and inguinal regions.

On day 81, one female (#293602) at LD developed signs of recumbency, hypothermia, and circulatory shock. This animal responded initially to treatment (antibiotics, etc.) however, signs of dehydration and decreased activity persisted despite continued treatment and animal was euthanized on day 84.

On day 90, one female (#293673) at HD had developed altered hemostasis: excessive red vaginal discharge during estrous, pyrexia, diarrhea and melena. This animal was treated with antibiotics and food supplementation. On day 104, ecchymotic hemorrhages were seen on the abdomen and petechial hemorrhages in the oral cavity, decreased activity and salivation. This animal was euthanized on day 115. On day 100, one male at HD (#316273) had similar signs of toxicity as the female dog (#293673). This animal did not respond to treatment and was euthanized on day 108.

Ophthalmoscopic exam: No treatment-related ocular changes were noted.

Hematology:

Moderate to severe decreases in WBC, neutrophil, lymphocyte, reticulocyte and platelet counts were observed with weekly dosing. The most severe hematotoxicity was observed in 4

dogs: one dog (#263602) at 10 mg/kg and three dogs (#293673, 316273, and 316293) at 25 mg/kg. These dogs had lowest neutrophil counts with marked decreases in platelet counts and decreases in lymphocytes and reticulocytes on day 83. Due to the dose-limiting hematologic toxicity in these animals, the dosing frequency of once weekly was changed to once every 3 weeks for the remainder of the study.

With once every 3 weeks dosing, decreases in neutrophil counts were comparable to the earlier weekly dosing period for both treated groups. However, dogs given the HD had more pronounced decreases in neutrophil counts on day 83. Decreases in platelet counts were noted in most HD and LD dogs, and the lowest platelet count (<10,000/uL) occurred in male dog at LD on day 184 (Table 3).

Table 3: Hematological mean values for once weekly and once Q 3 weeks study of LY231514 in dogs:

Group/Dose mg/kg (mg/m2)	Days	WBC 10^3/uL		Neutrophils 10^3/uL		Lymphocytes 10^3/uL		Reticulocytes 10^5/mm^3		Platelets 10^3/uL	
		M	F	M	F	M	F	M	F	M	F
Group 1	-6	10.9	11.5	6.72	7.17	3.10	3.26	55.0	79.3	293	347
Control	27	10.0	10.9	6.28	6.54	2.81	3.59	79.3	74.0	281	340
	83	10.2	11.2	6.51	6.83	2.79	2.35	52.5	51.3	291	333
	184	12.1	9.49	7.82	5.68	3.03	3.08	62.8	71.0	302	387
Group 2	-6	9.27	8.36	**5.30**	**4.55**	3.08	2.88	62.3	54.0	361	310
10 (200)	27	**6.31**	**6.04**	**3.24**	**3.28**	2.08	2.21	**50.3**	**38.5**	**219**	**188**
	83	**6.00**	**4.87**	**3.23**	**2.53**	**2.04**	**1.69**	**29.0**	**24.8**	**177**	**182**
	184	**5.78**	**6.16**	**3.15**	**3.59**	2.57	**2.04**	53.3	45.3	**73**	**139**
Group 3	-6	8.64	8.61	**4.59**	**4.88**	3.08	3.31	43.5	71.3	345	297
25 (500)	27	**5.93**	**3.98**	**3.06**	**1.56**	**2.04**	**2.08**	**29.3**	**38.5**	**174**	**124**
	83	**5.53**	**3.48**	**2.89**	**1.49**	**2.04**	**1.69**	**22.3**	**26.0**	**147**	**81**
	184	7.27	5.66	**4.33**	**3.38**	2.56	2.04	**39.7**	47.3	234	148

Clinical chemistry:

Minimal decreases in serum protein, albumin, and potassium values were observed in the treated males and transient but moderate increase in ALT value was seen on days 83 in the treated male at HD and a female given LD on days 27 and 55.

Clinical chemistry values (means) following repeat dose study of LY231514 in dogs

Group/Dose mg/kg (mg/m2)	Day	BUN mg/dL		TP G/dL		ALT U/L		AST U/L		Alb g/dL		K	
		M	F	M	F	M	F	M	F	M	F	M	F
Group 1	-6	10.4	11.5	5.6	5.6	35	24	30	24	3.3	3.5	4.3	4.3
Control	27	10.5	12.5	6.0	5.8	32	25	38	26	3.5	3.5	4.3	4.4
	55	10.9	12.8	5.9	5.8	40	32	38	32	3.3	3.4	4.1	4.0
	83	10.6	12.3	5.9	5.8	36	26	30	26	3.4	3.4	4.1	3.9
	118	12.2	13.3	6.3	6.1	32	24	29	24	3.5	3.5	4.4	4.6
Group 2	-6	10.9	11.9	5.6	5.7	32	30	31	30	3.4	3.6	4.5	4.4
10 (200)	27	13.2	9.82	5.8	**5.5**	**52**	**53**	40	53	3.3	3.5	4.5	3.9
	55	11.1	12.7		**5.5**	**47**	**102**	42	33	**3.1**	3.3	3.8	3.9
	83	11.5	**22.9**	5.9	5.8	43	45	30	33	3.2	3.3	3.9	3.7
	118	13.0	13.5	5.8	6.1	38	46	35	30	3.2	3.4	4.2	4.2
Group 3	-6	12.2	9.65	5.6	5.5	35	29	29	29	3.4	3.6	4.4	4.4
25 (500)	27	11.8	12.3	5.7	5.6	**57**	30	32	28	3.2	3.4	4.1	3.9
	55	12.3	11.8	5.8	5.6	35	33	32	35	3.1	3.3	3.9	**3.6**
	83	12.5	13.4	5.5	5.6	30	28	23	29	3.2	3.4	3.7	3.7
	118	**14.4**	11.7	5.8	6.0	30	35	31	33	**3.0**	3.4	3.9	3.7

Urinalysis: No important compound-related changes were noted.

Gross pathology:

Treatment-related organ weights were not remarkable. Gross lesions included thymus (small), lymph node (enlarged submandibular), esophagus (whole tissue alteration), stomach

(erosion), small and large intestine (focal reddening involving mucosa, inflammation), kidney (hydronephrosis), urinary bladder (hemorrhage, lesion), liver (small, lesion), prostate (small), bone marrow (tissue alteration), and skin and injection site (hemorrhage, lesion).

Histopathology:

Three dogs sacrificed during the study showed generalized hemorrhage, and slight to marked bone marrow hypocellularity was noted in two dogs at HD. Testicular degeneration in HD male occurred in developing testes, testicular morphology, missing sperm in the epididymis, and an immature prostate. The LD dog sacrificed on day 84 had enterpathy associated with jejunal intussusception and slight bone marrow hypocellularity. All three dogs had lymphoid hypocellularity affecting the lymph node, thymus and spleen.

Decreased liver glycogen depletion and depletion of zymogen granules from pancreas acini were likely secondary to decreased nutrient intake and occurred in several dogs in HD group. Skin lesions occurring early in the study were not present in dogs that survived until scheduled necropsy.

Histopathology Inventory

Study	D03699	D01301
Species	Dogs	Dogs
Study duration	Multidose study	Multidose study
Adrenals	X	X
Aorta	X	X
Bone marrow (Sternum/femur)	X	X
Brain	X	X
Cecum	X	X
Cervical spinal cord	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder	X	X
Gross lesions	X	X
Harderian gland		
Heart	X	X
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X	X
Lacrimal gland	X	X
Larynx		
Liver	X	X
Lungs	X	X
Lymph nodes, cervical	X	X
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Brachial nerve		
Ovaries	X	X
Pancreas	X	X
Parathyroid	X	X

Study	D03699	D01301
Species	Dogs	Dogs
Study duration	Multidose study	Multidose study
Peripheral nerve		
Pharynx		
Pituitary (or hypophysis)	X	X
Prostate	X	X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles		
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X	X
Sternum with bone marrow	X	X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X

Toxicology summary:

A toxicokinetic study was conducted following daily, twice weekly and once weekly doses for 6 weeks in dogs, and toxicity studies were performed following repeated intravenous doses of LY231514 disodium salt for 4-weeks and 6-months in dogs.

In study D05091subchronic toxicokinetic study, 3 dogs/sex/dose were given daily, twice weekly and weekly doses. Following IV doses of 0.11-0.53 mg/kg daily, 3.15-7.87 mg/kg twice week, or 26.24-104.96 mg/kg weekly to dogs, blood samples were taken and analyzed using HPLC/UV method. The systemic exposure of LY231514 as measured by $AUC_{0-\infty}$ increased proportionally with dose. The AUC was from 1.36 ug·hr/ml in males and 0.55 ug·hr/ml in females after a 0.11 mg/kg dose up to 523 ug·hr/ml and 517 ug·hr/ml, respectively, after 104.96 mg/kg dose in dogs. Plasma half-lives ranged from 0.8 to 5.1 hrs, and longer half-lives were found at higher doses. No gender differences in exposure over time were noted, however, because of a number of dose alterations during the study, there were insufficient data to make any firm conclusion on the PK effect of multiple doses.

In study D03699 subchronic toxicity study, beagle dogs (6/sex/group) were given weekly doses of 0, 10 (200) or 25 mg/kg (500 mg/m2) LY231514 for one month, followed by 3-week recovery period. No mortality but clinical signs included watery, mucoid, or soft feces, emesis, red, flaky abdominal skin, and weight loss. Slight to moderate decreases in neutrophils, lymphocytes, reticulocytes, and platelets counts occurred in the treated groups. There were no treatment-related changes in clinical chemistry parameters. Decreased liver weights without morphologic changes were observed in treated groups. Drug-induced lesions included minimal to slight enteropathy throughout the GI tract in both treated groups. The incidence and severity of the enteropathy was reduced at the end of 3-week recovery period.

In D01301 chronic toxicity study, beagle dogs (4/sex/group) received weekly doses of 0, 10 (200) or 25 mg/kg (500 mg/m2) for 6 months. After 3 months due to hematotoxicity, dosing schedule was changed to once 3 weeks and the dosing was discontinued for 25 mg/kg group and

resumed on day 161 on a once a every 3 weeks for the remainder of the study. Three dogs (one pair at HD and one female at LD) euthanized. These dogs were thrombocytopenic and neutropenic at the time of euthanasia and had generalized hemorrhage and slight to marked bone marrow hypocellularity. One female at LD was euthanized had jejunal intussusception associated with enteropathy. Clinical signs included GI toxicity (watery, mucoid, or soft feces), skin lesion, hypoactivity, inappetence and weight loss. Severe to marked decreases in neutrophil, lymphocyte, reticulocyte, and platelet counts were observed in all treated groups. Because of dose-limiting hematotoxicity, weekly dosing was replaced with once every 3 weeks. LY231514 appeared to be tolerated at a dose of 10 mg/kg given once every 3 weeks for approximately 3 months. Drug-induced lesions included bone marrow hypocellularity and testicular degeneration. Enteropathy was minimal in dogs that survived.

IV. SPECIAL TOXICOLOGY

Study Title: A Special Study in Beagle Dogs administered LY231514 Na2 Intravenously on Days 0 and 3 followed by Continuous Intravenous Infusion of Thymidine for 72 hr (Study #D00198):

Key study Findings:

Study no:	Study D03699
Volume #, and page #:	Volume 1.10, pages 1-60.
Conducting laboratory and location:	Lilly Research Lab., Greenfield, IN 46140
Date of study initiation:	January 2000
GLP compliance:	Yes
QA report:	Yes
Drug, lot#, and % purity:	LY231514 disodium, lot# HK5-VGL-026
Formulation/vehicle:	Sterile water for Injection, USP

Method:

Dosing:

Doses of LY231514 and Thymidine in Dogs

Group	Treatment	Animal #	Dosage	Route	Test Day
1	LY231514	All dogs	50 mg/kg	IV bolus	0 and 3
2	"	All dogs	50 mg/kg	IV bolus	0 and 3
1	Saline	294491♂ 296341♂* 277741♀* 277771♀*	0.9%	CIV infusion	4-7
2	Thymidine	297292♂ 297302♂ 277782♀ 277792♀	8mg/kg	CIV infusion	4-7

* Animals were sacrificed moribund on day 6.

Observations:

Mortality and clinical signs:	Once daily
Body weight & food consumption:	Pre-test, on days 7 & 13

Hematology:	Days 7, and 13
Clinical pathology:	Days 7, and 13
Gross pathology:	Days 7, and 13

Results:

Mortality:

Three dogs from the LY231514/saline-infusion groups were killed on day 6. All LY231514/thymidine-infusion group animals survived until the termination of the live phase.

Clinical signs:

Prior to the administration of the saline or thymidine infusion dose (days 4 to 7), all dogs received an intravenous LY231514 disodium (50 mg/kg) on days 0 and 3.

LY231514-IV bolus:

On days 0-4, the majority of dogs displayed decreased activity, yellow vomitus, and abnormal feces (soft, mucoid, and watery), and a few dogs displayed red feces, intermittent tremors, straining while defecation, and dry mouth.

On days 2-5, vomitus and emesis were noted in several animals. Many of these effects persisted during the subsequent infusion procedures, however, starting on days 5 to 6, the incidences and severity progressively worsened in the saline-infusion group but appeared decrease with intravenous thymidine.

Saline-infusion group:

On day 5, both females (#277741, #277771) and one male (#296341) had GI effects.

On day 6, the female (#277741) exhibited labored breathing, intermittent tremors, recumbency, hypoactivity, red rectal discharge, swollen perianal area, cool pinnae, rise in body temp (105.3°F). The animal was euthanized on day 6.

Another female (#277771) exhibited salivation and straining while defecation and was euthanized on day 6.

One male (#296341) developed emesis, cool pinnae, red discharge and was euthanized in moribund condition on day 6.

On days 5 to 8, the remaining dog (#294491) survived but exhibited abnormal feces, cool pinnae, straining while defecation, and salivation.

Thymidine-infusion group:

On days 5 to 11, all dogs had absent feces (not observed in the saline-infusion group), decreased activity, cool pinnae, and persisted abnormal feces.

Red and ulcerated gums were noted in all dogs after the second dose of LY231514. These lesions were not ameliorated by thymidine treatment.

Body weight and food consumption:

Marked decrease in body weight (2.8 kg loss) was noted for male dog (#294491) in saline-infusion group. All surviving dogs had decreased body weight (0.7 to 1.8 kg loss from initial body weight).

Male dog (#294491) in saline-infusion group exhibited significant decreases in food consumption (100% food remaining) through day 13.

Female dog (#277782) in thymidine-infusion group had significant decreases in food consumption (75% food remaining) through day 13. Two other dogs exhibited similar effects.

Hematology, clinical chemistry, and gross pathology:

Moderate to marked decreases in circulating neutrophils and lymphocytes were observed. Increases in red cell parameters (RBC, Hb, and Hct) and increases in BUN, creatinine, and

inorganic phosphorus were consistent with dehydration and correlated with the gross pathology findings of bloody diarrhea, intestinal hemorrhage, and oral mucositis. At necropsy, the surviving male had mild intestinal lesions and oral lesions in saline-infusion group.

None of the dogs given LY231514 in combination with thymidine had any clinico-pathologic alterations or gross findings at scheduled necropsy.

Special toxicology summary:

Following i.v. doses of 50 mg/kg LY231514 on 3-days apart, both groups of dogs developed clinical signs of toxicity: emesis, decreased activity, abnormal stools, intermittent tremors, decreased food consumption and oral mucositis. Hematologic and clinico-pathologic alterations were observed in both groups. Many of these effects persisted and progressively worsened in the saline-infusion group. However, IV thymidine rescued dogs from severe toxicity associated with administration of LY231514.

V. REPRODUCTIVE TOXICOLOGY:

Study Title: A Segment I reproductive toxicity study of LY231514 Disodium administered by IP injection to Male CD-1 mice (Study M00400, TOX Report 27):

Key Findings: In a Segment I fertility study, administration of LY231514 at doses of 0.1 to 10 mg/kg resulted in slightly reduced fertility rates and testicular atrophy and hypospermia.

Study no:	Study M00400
Volume #, and page #:	Volume 1.11, pages 1-100.
Conducted laboratory and location:	**Lilly Research Laboratories, Greenfield, Indiana**
Date of study initiation:	April 10, 2000
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, potency:	LY231514 disodium, Lot #221SB7, 83.8%
Formulation/vehicle:	Sterile water for Injection, USP

Method:

Species and strain:	Male CD-1 Mice
#/sex/group:	30 male/dose
Weight:	33.3 to 46.5 g
Dose/Route/Volume:	Daily IP doses of 0 (0), 0.1(0.3), 1 (3) or 10 mg/kg (30 mg/m2) for 6 weeks at injection volume, 1 mL/kg
Study design:	Segment I Study Males-Dosed daily for 6 weeks before mating and throughout mating

Male mice (n=30) received daily IP doses of 0. 0.1, 1, or 10 mg/kg for 6 weeks prior to cohabitation with untreated females (n=30). Evidence of mating was by inspection of expelled and retained copulatory plugs and by vaginal lavage to detect the presence of sperm. Non-pregnant females were separated from their males, is considered to be gestation/postmating day 0. Gestation/postmating day 13 pregnant females were euthanized and the uterine contents were removed, weighed and examined for the number of implantation, live fetuses, and resorption.

Observations:

Mortality: Daily
Clinical signs: Daily
Body weight: Weekly
Food consumption: Weekly
Reproductive parameters:

Morphologic pathology: The necropsy included examination of all external body surfaces and orifices; the thoracic, abdominal, and pelvic cavities and their viscera; cervical tissues and organs.

Maternal parameters: The ovaries and uterus were removed and the uterine contents were examined: the number and distribution of implantations, live conceptuses/fetuses, and resorption.

Organ weights: Day 44 or 45
Histopathology: Day 44 or 45

Results:

Mortality and clinical signs:

During mating or premating, one male animal from each group died with no clinical signs. All untreated females survived until gestation/postmating day 13.

Body weight and food consumption:

There were no important differences in absolute body weights. In general, the control and treated groups lost weight for the premating, mating, and the total treatment period. The 10 mg/kg group lost less weight overall compared to the controls as in table below.

	Administered Dose (mg/kg/day)			
Parameters	0*	0.1	1	10
Premating body weight gain(g)	-1.41	-1.81	-2.48	-1.72
Mating body weight gain (g)	-1.46	-1.04	0.04*	-0.32*
Total body weight gain (g)	-2.88	-3.04	-2.43	-2.04*

*= Vehicle; *=p≤ 0.05

The gestational body weight data of the untreated females were similar among groups. There were no adverse effects on food consumption at dose tested. Only sporadically increases in food consumption over control were noted in the 1- and 10-mg/kg groups in male mice.

Reproductive performance:

No differences in the time of mating or fertility indices were observed. The fertility indices were decreased in a dose-dependent manner.

	Administered Dose (mg/kg/day)			
Parameters	0*	0.1	1	10
Time to mating (days)	3.8	3.3	2.5	2.8
Mating index (%)	93.3	96.7	93.3	93.1
Fertility index (%)	75.0	69.0	64.3	55.6

* Vehicle

Maternal reproductive performance:

No significant differences in maternal reproductive parameters were observed as in the table below.

	Administered Dose (mg/kg/day)			
Parameters[a]	0[b]	0.1	1	10
Pregnant females	21	20	18	15
Implantations/litter	12.8	12.3	12.4	11.4
Live conceptuses/litter	11.8	11.8	11.7	10.7
Live conceptuses (%)	85.24	89.89	95.47	94.43
Postimplantation loss (%)	14.76	10.12	4.53	5.57

[a] =All data are mean values for the group except for the number of pregnant females; [b] = Vehicle.

Gross and Histopathology:

Organ weights:

Marked decreases in testis weights and slight decreases in epididymides weights were observed in all treated groups.

	Administered Dose (mg/kg/day)		
Absolute Organ Weight (% Change*)	0.1	1	10
Testes	↓59	↓66	↓68
Epididymides	↓15	↓25	↓21

*= % change values are relative to the control group

Histopathology:

Drug-induced lesions included marked or severe testicular atrophy (correlated with decreased testis weight), seminiferous tubules (collapsed), minimal to slight vacuolation of the epithelium lining the epididymides and marked hypospermia (correlated with decreased epididymides) in all treated animals.

Study Title: A Segment II reproductive toxicity study of LY231514 Disodium administered by IV injection to Female CD-1 mice (Study M01999, TOX REPORT 25). Volume 1.11, pages 1-100.

Key Study Findings: The segment II teratogenic effects of LY231514 were determined in mice given i.v. doses of 0.2 (0.6), 1(3), and 5 mg/kg (15 mg/m2) on gestation days 6 through 15. Reduced fetal weights at ≥0.2 mg/kg, incomplete ossification of several skeletal structures at ≥0.2 mg/kg, and cleft palate and other malformations were observed at 5 mg/kg LY231514.

Conducted Laboratory and Location: Lilly Research Laboratories, Greenfield, Indiana
Date of Study initiation: September 1999
GLP Compliance: Yes
Species and strain: CD-1 Mice
#/sex/group: 30/female/dose
Weight: 23.8 to 31.6 g
Drug, lot#, potency: The test material, LY231514 disodium, Lot #CT11812, 83.8% LY231514 "as is" potency
Formulation/vehicle: Sterile water for Injection, USP
Dose/Route/Volume/Duration: Daily IV doses of 0 (0), 0.2 (0.6), 1 (3) or 5 mg/kg (15 mg/m2) on days 6 through 15 (gestation) at injection volume, 2.0 mL/kg

Virgin females were cohabited with ——— males (1:1) until the required number of mated females was obtained. Evidence of mating was obtained by inspection for retained copulatory plugs. Mated females were assigned to 4 treatment groups. One mouse (#2070) in the 0.2 mg/kg group was removed due to having a firm, swollen vulva on gestation day 5. The mated females received daily LY231514 doses of 0.2(0.6), 1(3), or 5.0 mg/kg (15 mg/m2) on days 6 through 15 by injection into caudal veins.

Observations:

Live-Phase parameters:

Mortality and clinical signs: Daily
Body weight: Days 0, 6, 9, 12, and 16
Food consumption: Days 0, 6, 9, 12, and 16

Maternal and Fetal Evaluations:

Disposition of animals:

All females were euthanized and necropsied on postmating day 18. The necropsy included examination of all external body surfaces and orifices; the thoracic, abdominal, and pelvic cavities and their viscera; cervical tissues and organs.

Maternal reproductive parameters: The ovaries and uterus were removed and the uterine contents were examined: The number and distribution of implantations, live conceptuses/fetuses, and resorption.

Fetal parameters:

Live fetuses were weighed individually and fetal runts identified. Live and dead fetuses were examined externally for anatomical and skeletal anomalies and internally for visceral anomalies.

Results:

Live-Phase parameters:

All treated mice survived until the study termination. No treatment-related clinical signs were noted at any dose level. One mouse F0 (#2063) had kinked tail (present prior to treatment) and the other (#4077) had decreased skin elasticity but these were considered to be incidental. No compound related necropsy findings. A small left kidney was noted in 1 control female (#1075).

Body weight and food consumption:

The body weight on gestation day 18 and body weight gain for gestation days 16 through 18 were decreased for the 5-mg/kg group (see the table below). These decreases appeared to be due to a slight decrease in gravid uterine weight. The total body weight gain during treatment for the 5 mg/kg group was 16% less than controls.

	Administered Dose (mg/kg)		
Parameters (% Change[a])	0.2	1	5
Body weight –GD 18	↓1	↑1	↓4*
Body weight gain-GD 16 through 18	↓4	↑2	↓16*
Gravid uterine weight	↓2	↑2	↓8

Abbreviations: ↓=decrease; ↑=increase; *=$p<0.05$.

[a] Approximate % change values are relative to the control group.

No treatment-related differences in food consumption occurred at any dose level.

Maternal Reproductive and Fetal Evaluations:

Maternal reproductive parameters:

There are no LY231514-related effects on maternal parameters or fetal viability.

	Administered Dose (mg/kg)			
Parameters (n[a])	0[b]	0.2	1	5
Pregnant females	29	27	27	29
Implantations	13.2	12.9	13.4	13.5
Resorptions	1.5	0.9	0.8	1.4
Live fetuses	11.8	11.9	12.6	12.0
Dead fetuses	0.03	0.07	0.04	0.03
Postimplantation loss (%)	11.7	8.1	6.0	10.8

[a] Inidence; [b] Vehicle.

Fetal parameters:

Dose-related decreases in male and female fetal weights were seen at 5 mg/kg. The incidence of runts was greater in the HD group. There were increased incidences of fetus or litter with malformations at HD. Marked increased incidences of incomplete ossification of skull bones (frontal, parietal) were observed in the HD whereas the increase was minor in other groups. Cleft palate occurred in approximately 85% of the HD fetuses and less than 2% of the fetuses of the control and other treatment groups.

	Administered Dose (mg/kg)			
Parameters (n[a])	0[b]	0.2	1	5
Fetal weight (sexes combined, g)	1.38	1.34	1.32	1.19*
Male fetal weight (g)	1.40	1.38	1.34	1.22*
Female fetal weight(g)	1.36	1.30	1.29	1.17*
% fetal runts	0	0	0	5.0*
Male fetuses (%)	53.9	50.2	49.0	50.4
% fetuses with malformations/litter	3.1	1.9	0.6	82.7*
% Litters with malformations	32.1	18.5	7.4	96.6*
Types of malformations-fetal (litter) Incidences				
Cleft palate	4(4)	5(4)	2(2)	303(28)
Tongue protruding	1(1)	0(0)	0(0)	4(1)
Displaced kidney	2(2)	0(0)	0(0)	1(1)
Enlarged kidney	0(0)	0(0)	0(0)	3(2)
Misshapen kidney	0(0)	0(0)	0(0)	2(1)
Fused lumbar vertebra	0(0)	0(0)	0(0)	2(1)
Selected types of skeletal deviations-Fetal(litter) incidences				
Incomplete ossification of talus	9(4)	25(9)	21(10)	101(19)
Incomplete ossification of frontal bone	4(4)	6(4)	18(8)	97(24)
Incomplete ossification of parietal "	1(1)	0(0)	4(4)	18(8)
% affected fetuses/litter	14.3	9.9	6.6	84.0*

[a] Incidence; [b] Vehicle; * Significantly higher than control.

Reproductive summary:

In a Segment I study, male mice were treated daily i.p. doses of 0 (0), 0.1(3), or 10 mg/kg (30 mg/m2) LY231514 for 4 weeks prior to mating and throughout mating (approximately 6 weeks). There were no LY231514-related effects on clinical signs, body weight or food consumption. Time-to-mating and mating indices were not affected by the treatment. Dose-related decreases in the fertility indices were observed, however, there were no adverse effects on maternal reproductive performance (number of implantations, live conceptuses/litter) in the untreated females. Drug-related effects were in the male reproductive organs at all dose levels of

LY231514. Reduced testicular and epididymal weights were accompanied by testicular atrophy and hypospermia. IP doses of LY231514 at 0.1 to 10 mg/kg resulted in male reproductive toxicity by slightly reduced fertility rates and testicular atrophy and epididymal hypospermia. The NOEL for these effects was identified.

In a Segment II study, pregnant females were treated with intravenous doses of 0(0), 0.2(4), 1 (3) or 5 mg/kg (15 mg/m2) LY231514 on gestation days 6 through 15. All treated mice survived until live-phase termination. No treatment-related clinical signs, however, decrease (~16%) in body weight was observed in the HD-group. Dames were euthanized and examined the uterine contents. Dose-related decreases in fetal weights were observed doses at ≥0.2 mg/kg. Fetal gender and viability was not affected. The incidences of fetuses with incomplete ossification of several skeletal structures (incomplete ossification of frontal bone, incomplete ossification of parietal bone) were higher in the 1- and 5-mg/kg litters compared to controls. The malformations, cleft palate, fused vertebra, enlarged kidney, misshaped kidney, and tongue protruding, were observed mostly in the HD group. A NOEL for developmental toxicity was not established because of fetal growth retardation was observed at all dose levels.

VI. SUPPLEMENTAL TOXICITY STUDIES

Study Title: A Primary Eye Irritation study in Rabbits with LY289739 (LY231514 Disodium) Study 3130.163 (TOX Report 13).

Key study findings: An ocular dose (0.0300 g in 0.1 mL) of LY289739 in the conjunctival sac of the rabbit eye resulted in iritis and conjunctivitis. LY231514 was a mild irritant to the ocular tissue of the rabbit.

Study no:	Study 3130.163
Volume # , and page #:	Volume 1.11, pages 1-47.
Conducting lab. and location:	———
Date of study initiation:	November 3, 1993
GLP compliance:	Yes
QA report:	Yes
Drug, lot#, and % purity:	Test article, LY289739 is the disodium salt of LY231514 and all references should be directed to the free base LY23151; lot #281NK2
Formulation/vehicle:	The test article was ——— — The weight of processed test article occupied a volume of 0.1 mL (0.0300 g) was then determined and utilized for dose administration
Dosing:	
Species/strain:	Adult NZW rabbits
# sex/group:	3♂ + 3♀/group
Age/weight:	~8-20 weeks old, ~2.0 to 3.5 kg
Administered dose:	Liquid, gel and pastes will be administered at a volume of 0.1 mL
Route/volume:	0.1 mL of test article will be instilled into the conjunctival sac of right of each animal. The left eye will serve as control and instilled sterile saline
Observations:	
Mortality/Clinical signs:	Twice daily
Body weight:	Prior to dosing on day 0 and prior to euthanasia

Ocular observations: The eyes were examined macroscopically at 1, 24, 48 and 72 hrs and on day 7after dosing according to the Draize Ocular Irritation Grading System

Results:

Each of six rabbits received a 0.0300 g dose (0.1 mL equivalent) of the test article in the conjunctival sac of the right eye. The left eye of each animal was not treated but given 0.9% saline and served as a control. Exposure to the test article produced iritis in 1/6 test eyes at the 1 hr scoring interval which resolved by the 24 hr scoring interval. Conjunctivitis (redness, swelling and/or discharge) was noted in 6/6 test eyes at the 1 hr scoring interval. The conjunctival irritation diminished during the remainder of the test period and resolved completely in all test eyes by study day 7. No corneal opacity, iritis or conjunctivitis was observed in the control eyes.

Based on this test, LY289739 is considered to be a mild irritant to the ocular tissue of the rabbit.

Study Title: An Acute Dermal Toxicity/Irritation study in Rabbits with LY289739 (LY231514 Disodium) TOX Report 14.

Key study findings: A single dermal dose of 1000 mg/kg LY289739 on the shaved dorsal trunk area produced dermal irritation.

Study no:	Study 3130.164
Volume # , and page #:	Volume 1.11, pages 1-42.
Conducting lab. and location:	[redacted]
Date of study initiation:	November 3, 1993
GLP compliance:	Yes
QA report:	Yes
Drug, lot#, and % purity:	Test article, LY289739 is the disodium salt of LY231514 and all references should be directed to the free base LY23151; lot #281NK2
Formulation/vehicle:	The test article was administered as received or diluted with an appropriate vehicle.
Dosing:	
Species/strain:	Adult NZW rabbits
# sex/group:	5♂ + 5♀/group
Age/weight:	~8-20 weeks old, ~2.0 to 3.0 kg
Administered dose:	On day 0, the test article will be given dermally at a level of 1000 mg/kg to approximately 10% of the body surface area (BSA)
Route/volume:	The fur was removed from the dorsal trunk area of the animals. On the following day, the test article will be administered on the clipped area (≥10% of the animals BSA).
Observations:	
Mortality/Clinical signs:	Twice daily on study day 0 and daily thereafter (days 1-14)
Body weight:	Prior to dosing on day 0, and days 7 & 14
Dermal observations:	Animals were examined for signs of erythema and edema and the responses scored on study days 1-14 according to the Dermal Irritation Grading System.
Scheduled euthanasia:	On day 14

Results:

The single dose dermal toxicity of LY289739 was evaluated in NZW rabbits following a single dermal dose of 1000 mg/kg into the shaven dorsal trunk of the animals. No mortality occurred during the test. Treatment-related clinical signs included soft stools, fecal staining, dark material around the facial area, and dermal irritation at the site of drug application. Animals were examined for signs of erythema and edema and the responses scored on study days 1-14 according to the Dermal irritation grading system.

A summary of Acute Toxicity/Irritation study following a single dermal dose of 1000 mg/kg in Rabbits

Clinical Observations	Day of Study	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Animal # 8881♂	Dose:															
Erythema Grade 1	1000 mg/kg				P	P										
Erythema Grade 2			P	P												
Edema Grade 1				P												
Edema Grade 2			P													
Animal #8883♂	1000 mg/kg															
Soft stools													P		P	
Irritation taped area			P	P	P	P	P	P								
Erythema Grade 1						P	P	P								
Erythema Grade 2					P											
Erythema Grade 3			P	P												
Edema Grade 1				P	P											
Edema Grade 2			P													
Animal #8890♂	1000 mg/kg															
Irritation taped area			P	P	P	P	P	P	P	P						
Erythema Grade 1					P	P	P	P	P							
Erythema Grade 2			P	P												
Edema Grade 1			P	P	P											
Animal #8626♂	1000 mg/kg															
Erythema Grade 1						P	P	P	P							
Erythema Grade 2			P	P	P											
Edema Grade 1			P	P	P	P	P	P								
Animal #8884♂	1000 mg/kg															
Fecal stain				P	P	P	P	P	P	P	P					
Dark material around mouth and nose				P	P	P										
Swelling mouth area						P										
Edema Grade 1				P	P	P	P									
Animal #8908♀	1000 mg/kg															
Dark material around mouth		P														
Erythema Grade 1			P	P	P											
Erythema Grade 2					P											
Edema Grade 1			P	P												
Animal #8914♀	1000 mg/kg															
Soft stool			P													
Dark around mouth		P		P	P											
Dark around nose				P	P											
Erythema Grade 1				P	P	P										
Erythema Grade 2			P													
Edema Grade 1			P	P												
Animal #8915♀	1000 mg/kg															
Dark around mouth				P	P	P										
Erythema Grade 1					P	P	P	P	P	P	P					
Erythema Grade 2			P	P												
Edema Grade 1			P	P												
Animal #8919♀	1000 mg/kg															
Erythema Grade 1					P	P	P	P	P							
Erythema Grade 2			P	P												
Edema Grade 1			P	P												
Animal #8920♀	1000 mg/kg															
Fecal stain			P	P	P											
Swelling mouth area				P												
Erythema Grade 1							P	P	P							
Erythema Grade 2			P	P	P	P										
Edema Grade 1			P	P	P	P	P	P								

Grade code: p-present

Dermal irritations of erythema (grade 1, 2 and 3) and edema (grade 1 and 2) were observed at the site of drug application. The acute dermal Median lethal dose (MLD) of LY231514 was estimated to be >1000 mg/kg in the rabbits. Based on this test, LY231514 was determined to be a moderate irritant to the dermal tissue of the rabbit.

Summary of Supplemental toxicity studies:

LY231514 has been evaluated in ocular and dermal irritation studies. Following a single ocular dose (0.0300 g in 0.1 mL) of LY231514 in the conjunctival sac of the rabbit eye, LY231514 resulted in iritis and conjunctivitis. LY231514 was considered to be a mild irritant to the ocular tissue of the rabbit.

The single dose dermal toxicity of LY289739 was evaluated in NZW rabbits. A single dermal dose of 1000 mg/kg on the shaved dorsal trunk area produced dermal irritation. Different grades of erythema and edema were observed on the site of drug application. LY231514 was determined to be a moderate dermal irritant.

LY231514 has been evaluated in ocular and dermal irritation models to support workplace safety.

APPEARS THIS WAY
ON ORIGINAL

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

/s/

David Morse
12/19/03 02:19:13 PM
PHARMACOLOGIST
Entering Primary Review

Pharmacology/Toxicology Review of NDA 21-462

Date: 15 Dec. 2003

From: David E. Morse, Ph.D.
Supervisory Pharmacologist
Div. of Oncology Drug Products, HFD-150

To: Robert Temple, M.D.
Director, Office of Drug Evaluation I

Through: Richard Pazdur, M.D.
Director, Div. of Oncology Drug Products, HFD-150

Cc: Grant Williams, M.D., Dep. Dir., DODP (HFD-150)
DooYoung LeeHam, Ph.D., Pharm./Tox., DODP (HFD-150)

Subject: NDA 21-462
ALIMTA® for Injection (pemetrexed disodium)
Secondary Review of Pharm./Tox. Information and Product Label

I. Materials Included in Review

1. Pharm./Tox. Review of NDA 21-462, written by DooYoung LeeHam, Ph.D.
2. Product Labeling, draft of 15 Dec. 2003

II. Introduction

The sponsor (Eli Lilly and Company) is seeking approval of ALIMTA® (intravenous injection) for use in the treatment of malignant pleural mesothelioma in combination with cisplatin infusion. Pemetrexed disodium (ALIMTA®) is a novel pyrrolopyrimidine antifolate antimetabolite, which is an inhibitor of thymidylate synthase (TS), dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GARFT), crucial enzymes involved in the de novo synthesis of thymidine and purine nucleotides. Through inhibition of purine synthesis, pemetrexed exerts a cytotoxic effect in replicating cells.

III. Background

ALIMTA® (pemetrexed disodium) is a pyrrolopyrimidine antifolate. Although its' mechanism of action is not fully understood, multiple non-clinical studies suggest pemetrexed exerts antineoplastic activity by interfering with folate-dependent metabolic processes essential for cell replication. After entrance into the cell (via reduced folate carrier [RFC] and membrane folate-binding protein [FBP]), pemetrexed is rapidly polyglutamated by folypolyglutamate synthetase. Both parent and polyglutamated pemetrexed act as competitive inhibitors of several folate-dependent enzymes, including thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide transferase (GARFT), which are key enzymes for de novo nucleotide biosynthesis. These actions are similar to methotrexate, which has inhibitory effects on thymidylate synthase (TS) and dihydrofolate reductase (DHFR).

When tested in a series of in vitro and in vivo (xenograft) models of cancer, pemetrexed demonstrated activity against a variety of tumor types, including leukemia (CCRF-CEM, L1210), lung (A549), mesothelioma (NCI-H2052 and MSTO-211H), breast (MCF7), colon (GC3 and HCT8), and ovarian cancer (SKOV1).

Non-clinical toxicity studies were conducted to determine the acute and repeat-dose effects of pemetrexed when administered to mice, rats, and dogs. Toxicity studies of pemetrexed included: single and repeat dose studies of 2 and 6-weeks (intraperitoneal) dosing in mice, and, 4 and 6-weeks and 6-months (intravenous) dosing in dogs. In single dose studies, pemetrexed demonstrated limited acute toxicity in mice and rats, but more extensive toxicity in dogs. Six weeks repeat dose studies were conducted using daily, twice weekly or weekly intraperitoneal doses in mice and intravenous doses in dogs. Mice tolerated weekly intraperitoneal doses of up to 944 mg/m2 (approximately twice the clinical dose) without death or clinical signs of toxicity, whereas weekly intravenous dosing at 2099 mg/m2 (approximately four times the clinical dose) resulted in the early termination of several dogs. Repeat-dose adverse effects at higher doses caused decreased food consumption, emesis, diarrhea, mucositis, decreased red cell parameters, leukopenia, neutropenia, and increased hepatic enzymes in dogs. In mice, weight loss and leukopenia were the predominant indices of drug induced toxicity. Histopathologic indices of toxicity generally occurred in the thymus, lymph nodes, GI tract and intestine (enteropathy and mucositis), testis (atrophy and/or degenerative changes), bone marrow, and skin. Clinically, rash, nausea, diarrhea, asthenia, leukopenia and neutropenia were/are dose-limiting; thus, supporting the adequacy and predictive utility of the non-clinical toxicology studies used in the development of pemetrexed.

Pemetrexed (intravenous) doses of $\geq$0.3 mg/m2 caused testicular atrophy and reduced fertility. Further, pemetrexed was embryotoxic and teratogenic in mice when administered at 0.6 mg/m2. Pemetrexed caused no genetic damage in a standard battery of in vitro tests mutation and clastogenicity assays, although, pemetrexed was clastogenic in the vivo micronucleus assay. Carcinogenicity studies of pemetrexed disodium have not been conducted.

The intravenous administration of pemetrexed to beagle dogs at a dose of 105 mg/kg (2100 mg/m2) resulted in a slight decrease in total peripheral vascular resistance and a concomitant increase in cardiac stroke volume. A slight increase in the urinary excretion of sodium and potassium was seen at doses $\geq$200 mg/kg (1200 mg/m2, IV) in rats. In contrast, in a series of safety pharmacology studies, pemetrexed (1×10^{-9}M-10^{-5}M) did not induce any significant changes in the contractile activity of the isolated uterus, smooth muscle, or atria of rats, or smooth muscle of guinea pigs. Single IV doses of $\leq$600 mg/kg ($\leq$1800 mg/m2) to mice did not alter gastrointestinal function or CNS/motor function. Nociception (as measured by acetic acid induced responses) was slightly reduced at intravenous doses $\geq$200 mg/kg (600 mg/m2) in mice. These, or related effects, were not observed in clinical trials at the intended clinical dose (500 mg/m2), and are therefore not expected to influence the clinical use of pemetrexed.

Limited non-clinical investigations of "rescuing agents" (leucovorin and thymidine) were conducted in conjunction with the administration of pemetrexed. Study results suggest that the coadministration of leucovorin (20 mg/kg, i.m. days 5-10; 25 mg/kg, i.m. days 4, & 5, and 50 mg/kg, i.v. day 4) reduced/reversed the toxicity and hematological alterations

induced by pemetrexed treatment (50 mg/kg, i.v. days 1 & 4) in dogs. Dogs given pemetrexed (50 mg/kg, i.v. days 0 & 3) with thymidine (8 mg/kg, days 4-7, administration as a continuous infusion) had no toxic alterations associated with administration of pemetrexed when compared to the saline-treated controls.

AUC values for pemetrexed were approximately dose proportional following single intraperitoneal or intravenous administration to mice, and intravenous administration to dogs and humans. Elimination half-life was significantly shorter in dogs and man when compared to mice. The PK profile of pemetrexed was biphasic following radiocarbon tracer administration, with rapid tissue distribution following an intravenous dose and subsequent elimination (tissue levels generally did not persist beyond 3 hrs post-dose). In both mice and dogs, the major route of elimination of drug was via the kidney as unchanged 'parent' compound.

IV. Comments and Conclusions

1. Review of NDA 21-462, ALIMTA® for Injection (pemetrexed disodium), indicates the product has been adequately evaluated in multiple repeat-dose non-clinical safety studies (including: acute and repeat-dose/repeat-cycle (IV or IP) toxicology studies (mice and dogs) up to 6 months duration, reproductive toxicity tests in mice (Segments I [male only]-II; ICH endpoints A and C), and genotoxicity tests (in vitro and in vivo), for approval in the treatment of patients with malignant pleural mesothelioma.

2. Specific comments pertaining to the product review follow.

   a) It should be noted that for the requested indication (
   — the general CFR specified requirement for carcinogenicity testing and a full spectrum of reproductive toxicity studies were not deemed necessary by the Review Division.
   b) The application included multiple non-clinical studies of the proposed 'mechanism of action' of pemetrexed disodium. Specifically, these non-clinical studies suggest that pemetrexed exerts its' antineoplastic activity by interfering with folate-dependent enzymatic activity (thymidylate synthase [TS], dihydrofolate reductase [DHFR], and glycinamide ribonucleotide transferase [GARFT]), necessary for the de novo synthesis of thymidine and purine nucleosides essential for cell replication. Although supportive of the sponsor's supposition regarding the anti-cancer activity of pemetrexed, these studies do not provide an adequate explanation of the activity of pemetrexed such that it is possible to account for differences in individual tumor responses between patients, between tumor sites, or in an individual tumor over a series of assessment intervals. In vivo data correlating tumor response with drug disposition, drug conversion/activation, cellular enzymatic activity, and intracellular nucleoside 'pool' concentrations, would potentially enhance the early identification of likely pemetrexed sensitive (responder) and insensitive (non-responder) tumors.

3. Specific comments pertaining to the product label follow.

   Review of the draft product label suggests that it adequately reflects the non-clinical safety profile of pemetrexed (ALIMTA®) for injection.

IV. Summary

A review of the action package for NDA 21-462, ALIMTA® for Injection (pemetrexed disodium), suggests that the product has been adequately evaluated in multiple non-clinical safety studies for potential approval in the treatment of malignant pleural mesothelioma in combination with intravenous cisplatin (ALIMTA® 500 mg/m2; cisplatin 75 mg/m2).

APPEARS THIS WAY
ON ORIGINAL

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

/s/

David Morse
12/22/03 04:08:10 PM
PHARMACOLOGIST