CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-886

PHARMACOLOGY REVIEW(S)

Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original, Review No. 1

NDA No. 20886

Serial No(s).: 000 **Type:** NDA **Date(s) of Submission:** NDA dated: 5/27/98

CDR stamp date: 5/27/98

Information to be Conveyed to Sponsor: Yes (x), No ( )

Reviewer: Chang H. Ahn, Ph.D.

Date Review Completed: November 12, 1998

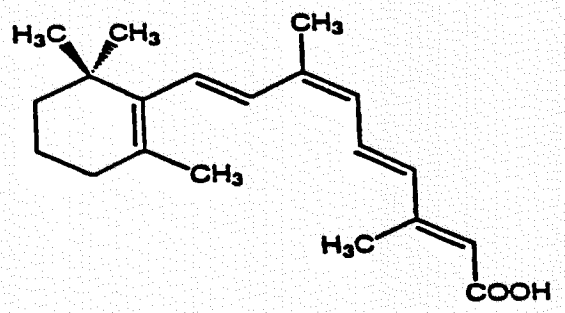
Sponsor: Ligand Pharmaceuticals, Inc. **Manufacturer (if different):**

Drug Name: Primary: Alitretinoin Other Names: Panretin gel 0.1%, 9-cis-retinoic acid, LGD 1057, ALRT 1057, LGD 100057, AGN 192013

Chemical Name: (2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-2, 4, 6, 8 - nonatetraenoic acid

CAS Number: 5300-03-8

Structure:



Molecular Weight (and Formula optional): 300.44, $C_{20}H_{28}O_2$

Related INDs/NDAs/DMFs: IND

DMF

Class: Antineoplastic agent (retinoid analogue)

Indication: First line topical treatment of cutaneous lesions in patients with AIDS-related Kaposi's sarcoma

Clinical Formulation: 0.1% topical gel contains LG1057 %w/w), dehydrated alcohol USP %w/w), polyethylene glycol 400 USP %w/w), hydryoxypropyl cellulose NF %w/w), and butylated hydroxytoluene NF %w/w).

Route of Administration: topical

Drug Administration: *Dose*: 0.1% gel *Schedule*: 2-4 times a day *Duration of treatment*: Therapeutic effects may be seen in 2-14 weeks. Panretin gel was applied for up to 96 weeks in clinical trials. Estimated average topical dose to patients treating cutaneous Kaposi's sarcoma lesions with 9cRA 0.1% gel is estimated to be 0.04 mg/kg/day (i.e., 1.48 mg/m2/day) based on assumption that a 60 g tube of gel will be sufficient for 30 days of treatment (vol. 1, p185).

Studies Reviewed in this NDA:

Pharmacology

1. Antikeratinizing activities of LG1057 and ATRA in the rhino mouse (RR-740-93-001; vol. 14:2-15)

Pharmacokinetics and Toxicokinetics

1. Tissue distribution of [^{3}H]ALRT 1057 in rats (RR-845-97-003; vol. 35:365-442)
2. Metabolism by rat liver microsomes and the effects of repeated treatment on rat liver microsome drug metabolizing enzymes (RR-845-97-006; vol. 36:75-94)

3. Oxidative and reductive metabolism in rats (RR-845-96-001; vol. 36: 46-74)
4. Oxidized metabolites and the biliary metabolite profile in dogs (RR-845-97-008; vol. 36:95-108)
5. Identification of cytochrome P450 isozymes involved in human ALRT 1057 metabolism (RR-845-97-005; vol. 36:109-132)

Reproductive Toxicology

1. Preliminary study of embryo-fetal toxicity in rabbit by oral gavage administration (RR-815-97-015; vol. 30:2-394)
2. Preliminary study of embryo-fetal toxicity in rats by oral gavage administration (RR-815-97-011; vol. 31:1-359)
3. The teratogenic activity of 9-cis-retinoic acid (Kochhar DM et al. Teratology 47:439-440, 1993)
4. 9-cis-retinoic acid: a direct acting dysmorphogen (Kraft JC and Jucha MR. Biochem. Pharmacol. 46:709-716, 1993)

Genetic Toxicology

1. Mouse micronucleus test (RR-815-97-013; vol. 32:77-110)
2. In vitro mammalian chromosome aberration test in human lymphocytes (RR-815-97-014; vol. 32:111-147)
3. Mammalian cell mutation assay (RR-815-97-012; vol. 32:148-180)

Phototoxicity

1. In vitro phototoxicity : Summary of non-GLP and GLP studies (RR-815-98-001; vol.32:182-291)

Studies Previously Reviewed: reviewed by Chang H. Ahn, Ph.D., IND

Toxicology

1. 28-Day repeated dose dermal toxicity study in rats (RR-815-94-003)
2. Guinea pig primary skin irritation test (RR-REG-815-94-005)
3. Dermal sensitization study in guinea pig (modified Buehler's technique) (RR-815-94-004)
4. Acute oral toxicity in rats (RR-815-93-001, RR-815-93-008)
5. Acute oral toxicity in dogs (RR-815-93-002, RR-815-93-009)
6. 28-Day oral toxicity in rats (RR-815-93-003)
7. 28-Day oral toxicity in dogs (RR-815-93-005)
8. 91-Day repeated dose oral toxicity study in rats (RR-815-95-001)
9. 91-Day repeated dose oral toxicity study in dogs (RR-815-95-002)
10.

Pharmacokinetics and Toxicokinetics

1. In vitro human skin penetration study (RR-845-94-004)
2. Pharmacokinetics of topical LGD1957 in rats (RR-845-94-0003)
3. Absolute bioavailability in rats (RR-845-93-004)
4. Bioavailability and metabolism in dogs (RR-845-93-008)
5. Toxicokinetics in rats (RR-845-93-009)
6. Toxicokinetics in dogs (RR-845-93-010)
7. Relative bioavailability in rats and dogs (RR-845-93-001, RR-845-93-002)
8. Metabolism in rats (RR-845-93-007)
9. Plasma protein binding (RR-845-91-011)

Pharmacology

1. A high affinity ligand for retinoid X receptors in mammalian cells and yeast (Cell 68:397-406, 1992; J. Biol. Chem. In press, 1993)

2. Transactivation profiles of retinoid receptors (RR-750-93-001)
3. Retinoic acid receptor binding profile (RR-750-93-002)
4. Potency and efficacy in differentiation of HL60 (RR-750-93-003)
5. Inhibition of Kaposi's sarcoma (RR-750-93-006)
6. Effects on the growth of primary human head and neck squamous cell carcinoma xenografts in athymic mice (RR-740-93-010, RR-740-93-011)
7. Effects on the proliferation and clonogenicity of human head and neck squamous cell carcinoma lines (RR-740-93-003)

Genetic Toxicology

1. E.coli mutation assay (RR-815-93-006)
2. Salmonella mutation assay (RR-815-93-007)

Studies Not Reviewed in this NDA: The following studies were not reviewed due to duplicative nature or similar findings observed in the studies reviewed.

Pharmacology

1. Regulation of cellular proliferation and apoptosis in cervical carcinoma and multiple myeloma cell lines (RR-750-93-009)
2. Regulation of T-cell apoptosis (RR-750-97-001)
3. Induction of CD38 mRNA expression in APL cells (NB4) and human peripheral blood (RR-750-97-005)
4. ALRT1047 induces growth inhibition of human breast cancer cells in culture (RR-750-97-008)
5. Chemoprevention study of LG1057, 13-cis-retinoic acid and ATRA in the Sencar mouse two-stage model of carcinogenesis (RR-740-93-008)
6. Effects on growth of human head and neck squamous cancer cell line (1483) in an athymic nude mouse xenograft model (RR-740-93-010; RR-740-93-011)
7. Enhanced antitumor efficacy of cisplatin in combination with LG1057 in human oral squamous carcinoma xenografts in nude mice (RR-750-97-007)
8. Induction of RAR-β expression in a human squamous cell carcinoma xenograft tumor model (RR-750-97-004)
9. Induction of kidney 24-hydroxylase and RAR-β RNA in mice (RR-700-97-001)
10. Inhibition of human breast cancer xenografts in nude mice (RR-750-97-003)

Pharmacokinetics and Toxicokinetics

1. Pharmacokinetics in athymic mouse plasma and HN6N xenografts (RR-845-95-008)
2. Dose proportionality in rats (RR-845-93-003)
3. Induction potential in rats (RR-845-93-006)
4. Pharmacokinetic and metabolic cross-induction effects of ALRT1057 and ATRA in male rats (RR-845-96-023)
5. A relative oral bioavailability study of three formulations in dogs (RR-845-93-012)
6. Effect of drug substance specific surface area on plasma AUC values following oral administration to dogs (RR-845-97-004)
7. Plasma retina-vitreous and choroid concentrations after iv administration to rats (RR-845-97-007)

Toxicology

1. 10-Day repeated dose oral range-finding toxicity study in rats (RR-815-93-011) (similar findings in longer-term studies)

2. 10-Day repeated dose oral range-finding toxicity study in dogs (RR-815-93-004)) (similar findings in longer-term studies)
3. 28-Day repeated dose dermal toxicity study in rats (RR-815-98-002) (the study was prematurely terminated due to deaths in control and treatment groups, in part, due to vehicle (DEET) used.

Note: Portions of this review were excerpted directly from the sponsor's submission.

INTRODUCTION/ DRUG HISTORY

9-cis-Retinoic acid (9cRA), an active ingredient of Panretin® 0.1% gel, is a pan-agonist for both retinoic acid receptor (RAR) and retinoid X receptor (RXR) and possesses antiproliferative and differentiating properties. The sponsor conducted clinical trials with Panretin gel in patients with AIDS-related Kaposi's sarcoma. The sponsor believes that those clinical studies demonstrated clinical benefits (e.g., reduction of size of cutaneous lesions, reduction of the rate of progression, and improved quality of life, etc) to the patients and thus intends to obtain approval for marketing of the product.

Pharmacology

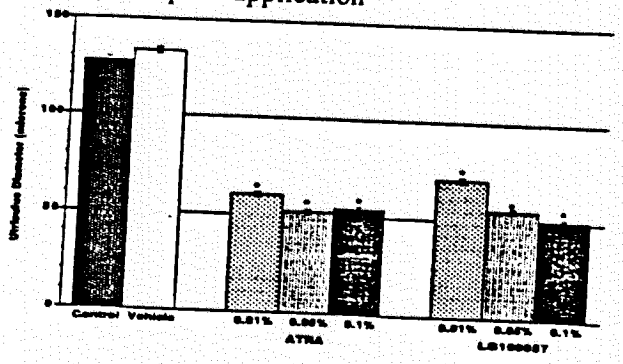
1. Antikeratinizing activities of LG1057 and ATRA in the rhino mouse (RR-740-93-001; vol. 14:2-15)

method:

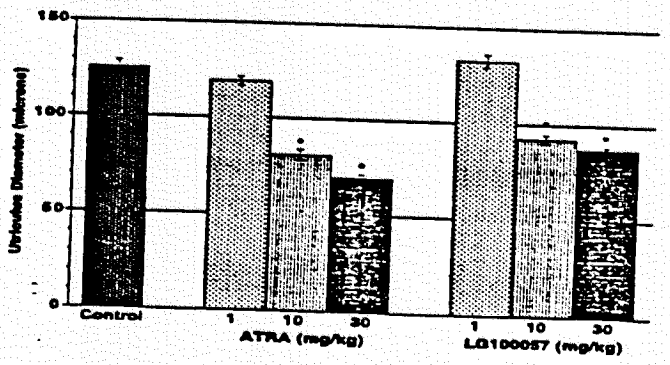
GLP statement: no

Results: All doses were tolerated in this study. Both oral and topical ATRA and LGD1057 induced antikeratinizing effects in mice with similar potency as evidenced by their ability to decrease utriculus diameter, i.e., induced smoother skin compared to the very wrinkled skins in control mice. Topical doses of 0.01, 0.05 and 0.1% LGD 1057 resulted in 48%, 60% and 63% decreases in utriculus diameter, respectively (control 128 microns), whereas oral doses of 10 and 30 mg/kg/day LGD 1057 resulted in 28% and 32%, respectively. Oral dose of 1 mg/kg/day was not effective. * (asterisk) indicates statistical significant vs control ($P<0.05$)

i) Topical application



ii) Oral administration



Pharmacokinetics and Toxicokinetics

1. Tissue distribution of [^{3}H]LGD 1057 in rats (RR-845-97-003; vol. 36:365-442)

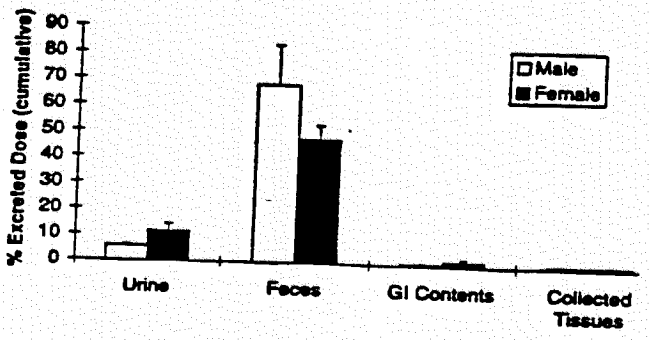
method:

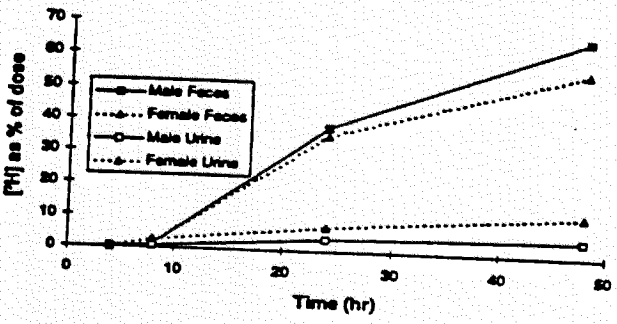
GLP Statement: no

Results:

i. Excretion of radioactivity cumulative to 48h post-dose

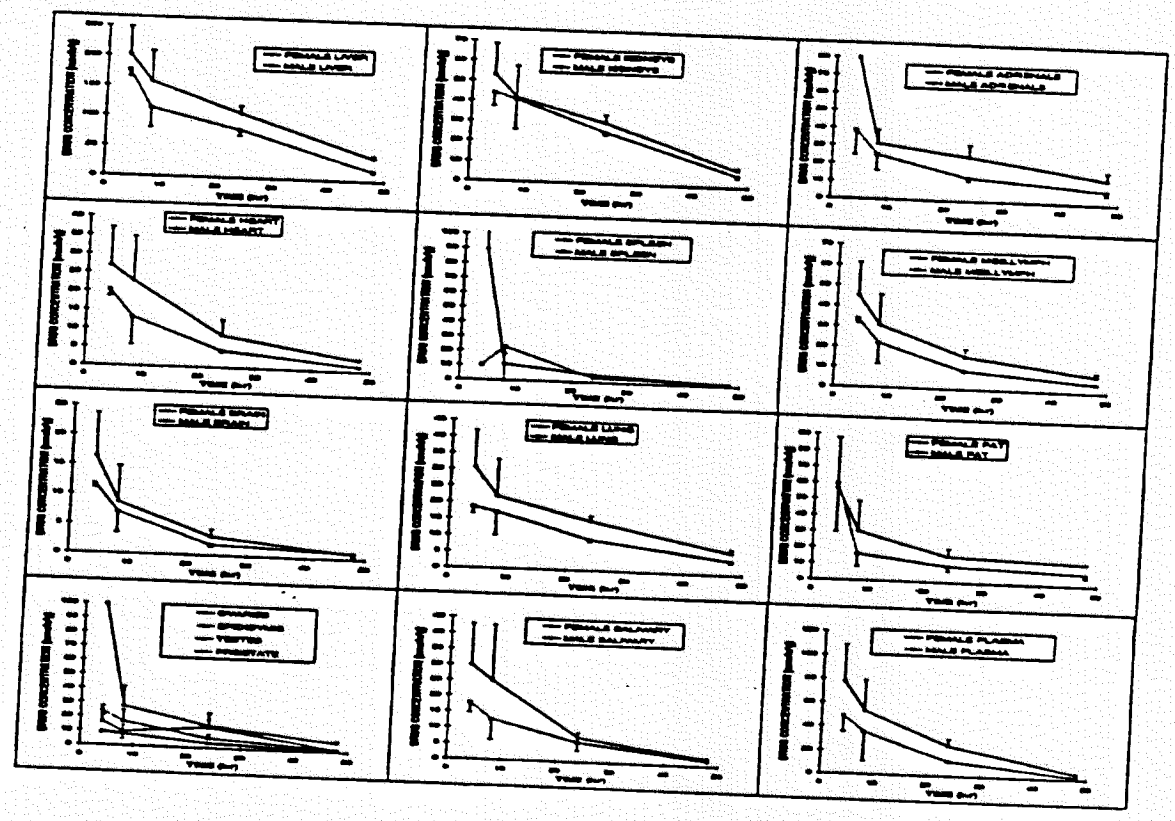
48 h after single oral doses of [^{3}H]LGD 1057, 68.1 % (male) and 47.7% (female) of total administered radioactivity were excreted in the feces and 5.6%(male) and 11.7% (female) in the urine, respectively.





ii. Tissue distribution : liver > adrenals=fat > kidneys > ovaries > mesenteric lymph node

There was no apparent accumulation of radioactivity in blood or any tissue by 48h.



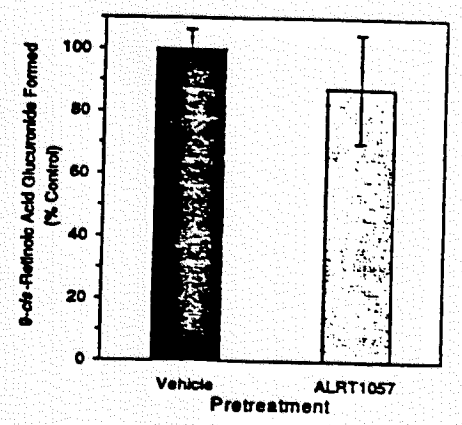
2. Metabolism by rat liver microsomes and the effects of repeated treatment on rat liver microsome drug metabolizing enzymes (RR-845-97-006; vol. 36:75-94)
method:

GLP Statement: no

Results: Levels of hepatic microsomal P450 in the LGD treated rats (0.484 nmol/mg protein) were 18% lower ($P<0.005$) compared to control group (0.589 nmol/mg protein). The levels of P450 isozymes CYP1A1, CYP2B1/2 and CYP4A were induced, whereas levels of CYP1A2, CYP2C11 and CYP2E were decreased. The rate of 4-hydroxy-9cRA formation was similar between the treated and control groups (20.8 pmol/min/mg protein vs 19.9 pmol/min/mg protein, respectively). The amount of 9-cRA glucuronide formed was also similar between the treated and control groups (the treated group was 87% of that of the control group).

	Optical Density•mm/mg protein[a]			
P450 isozyme	Vehicle	ALRT1057	Change (%)	p value
CYP1A1	6.6 ± 0.87	11 ± 2.1	+67	0.03
CYP1A2	16 ± 4.9	12 ± 5.4	-28	0.36
CYP2B1/2	3.5 ± 0.2	7.9 ± 2.3	+122	0.08
CYP2C11	6.9 ± 1.0	5.6 ± 1.7	-18	0.33
CYP2E	34 ± 3.1	23 ± 2.9	-33	0.01
CYP3A	32 ± 3.8	31 ± 3.4	-4	0.69
CYP4A	33 ± 10	75 ± 4.8	+129	0.002

[a] Values (mean±SD, n=3) were obtained using Western blot analysis.

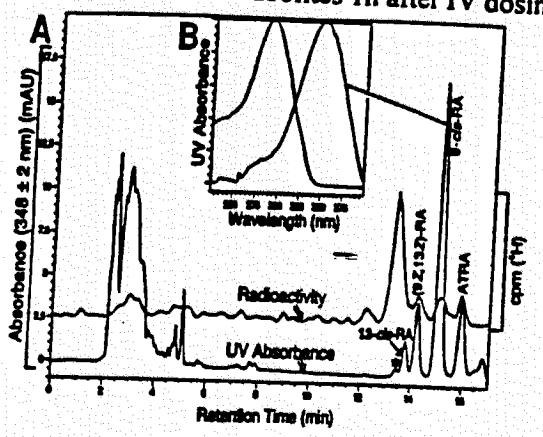


3. Oxidative and reductive metabolism in rats (RR-845-96-001; vol. 36:46-74)
method:

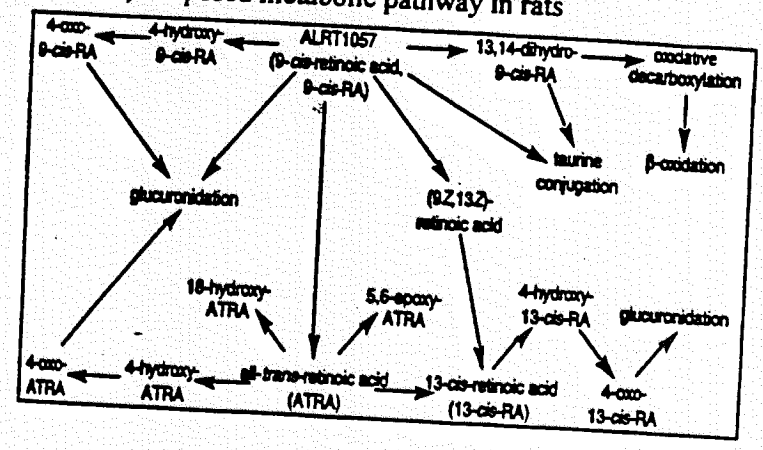
GLP statement: no

Results: LGD 1057 metabolites: plasma metabolites - 4-hydroxy-ATRA, 4-oxo-ATRA, 4-hydroxy-9cRA, and 4-oxo-9cRA, 4-hydroxy-9cRA; liver metabolites after oral dosing- 13, 14-dihydro-9cRA, taurine conjugate (N-(13,14-dihydro-9-cis-retinoyl)taurine.

i) Plasma metabolites 1h after IV dosing



ii) Proposed metabolic pathway in rats



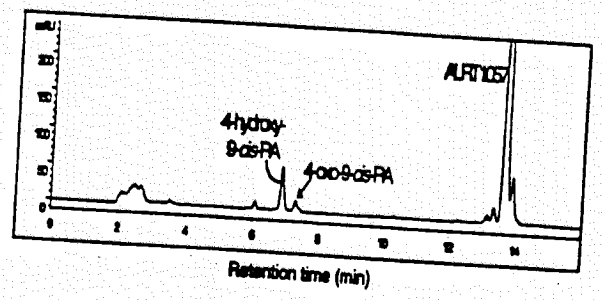
4. Oxidized metabolites and the biliary metabolite profile in dogs (RR-845-97-008; vol. 36:95-108)

method:

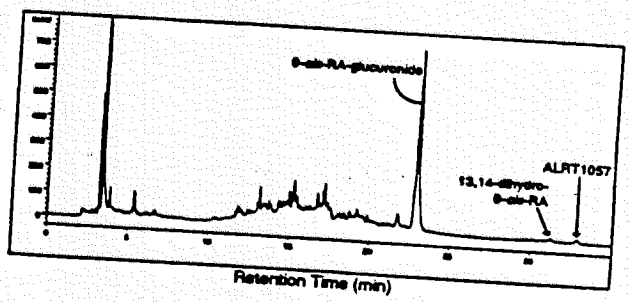
GLP statement: no

Results: In liver microsome study, 4-hydroxy-9cRA and 4-oxo-9cRA were produced (4-hydroxy >> 4-oxo metabolites) presumably by P450 system. In bile samples, 9cRA-glucuronide and 13,14-dihydro-9cRA were detected.

i) Hepatic microsome metabolites



ii) Bile metabolites



5. Identification of cytochrome P450 isozymes involved in human ALRT 1057 metabolism (RR- 845-97-005; vol. 36:109-132)

method:

GLP statement: no

Results: Based on the three types of experiments conducted, at least 4 P450 isozymes appear to be involved in oxidative metabolism of LGD 1057- CYP 1A1, CYP1A2, CYP2C9 and CYP 3A4.

a. Kinetics and chemical inhibition study

i) Kinetics

Transformation	Km, uM	Vmax, nmol/min/mg protein
Production of 4-hydroxy- and 4-oxo-9cRA from LGD 1057	7	0.2
Production of 4-oxo-9cRA from 4-hydroxy-9cRA	hi affinity 4	0.09
	low affinity 67	0.4

ii) Chemical inhibition study

Inhibitor	CYP isozyme	ALRT1057 remaining (% control)	4-hydroxy-9-*cis*-RA formation (% control)	4-oxo-9-*cis*-RA formation (% control)
α-naphthoflavone	1A1			
acetanilide	1A2(n=4)			
coumarin	2A6			
orphenadrine	2B			
sulfaphenazole	2C9			
S-mephenytoin	2C19			
quinidine	2D6			
4-methylpyrazole	2E1(n=4)			
troleandomycin	3A4			

* $p<0.05$ relative to control (no inhibitor)

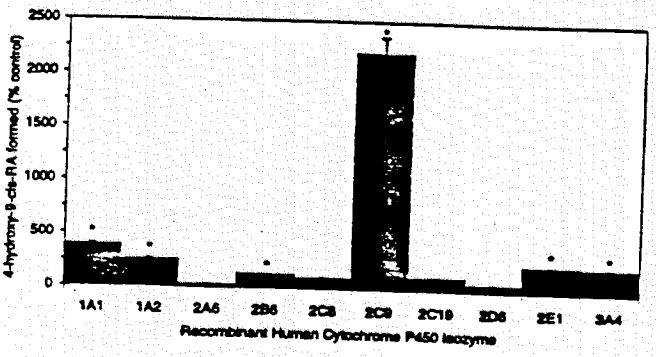
b. Correlation study

Isozyme	ALRT1057 Disappearance	4-hydroxy-9-*cis*-RA Formation	4-oxo-9-*cis*-RA Formation
1A2	0.317		
2A6	0.570*		
2C9	0.808**		
2C19	0.276		
2D6	0.429		
2E1	0.065		
3A4	0.924**		
3A4/5	0.845**		
4A9/11	0.111		

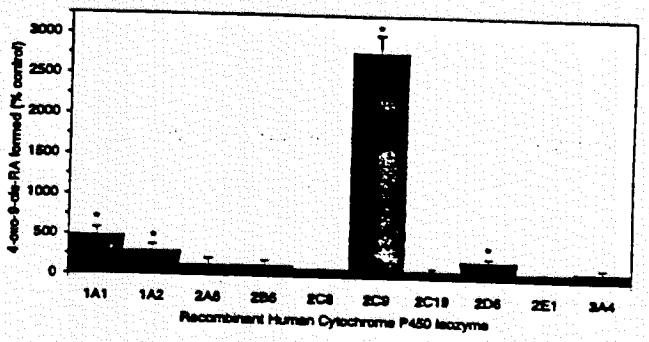
[a] See Section 4.5.3., Table 4.
* Statistically-significant but not biologically-relevant positive correlation (R>0.4575).
** Statistically-significant and biologically-relevant positive correlation (R>0.78).

c. Recombinant isozyme study

i) Production of 4-hydroxy-9cRA



ii) Production of 4-oxo-9cRA



Reproductive Toxicology

1. Preliminary study of embryo-fetal toxicity in rabbit by oral gavage administration (RR-815-97-015; vol. 30:2-394;

animal: New Zealand white rabbits (6 mated females/group)

GLP statement: yes

Results: **Teratogenic effects** (increased incidence of fused sternebrae) were observed at or greater than 0.5 mg/kg dose, maternal toxicity at or greater than 1.5 mg/kg dose, and embryolethality (early resorption) at or greater than 1.5 mg/kg dose.

Dose, mg/kg/day	0	0.5	1.5	5.0	15
Mortality	none	none	none	none	6/6 (d15-23)
Clinical signs lethargy	UR	UR	UR	UR	6/6
Pregnancy rate	6/6	6/6	5/6	2/6	4/6
Body weight gain, g. d6-d18 d18-d30	 248 143	 180 136	 54 128	 -41 212	 -1054 -
Food consumption, d6-d18, g/animal/day d18-d30, g/animal/day	 733 747	 671 687	 488 691-	 673 760	 84 -
Litter dams with viable fetuses corpora lutea, #/animal #implantation, #/animal live fetuses, #/animal dead fetuses, #/animal sex ratio, % males postimplantation loss #/animals resorption, early #/animal % of implant./animal resorption, late #/animal % of implant./animal mean fetal body wt., g Wt. gravid uterus, g fetal observation: external malformation # fetuses with abnormality # litters with abnormality visceral malformation # fetuses with abnormality # litters with abnormality skeletal malformation # fetuses with abnormality # litters with abnormality total # with abnormality # fetuses with abnormality # litters with abnormality sternebrae 5 or 6 unossified litter incidence fetal incidence interparietal ↓ ossification litter incidence fetal incidence	 6 9.8 8.2 7.7 0 54.5 0.5 0.2 1.4 0.3 3.3 52.4 579 0 0 0 0 0 0 0 0 0 0 0 0	 6 9.2 8.5 7.5 0.3 42.8 1.0 0.3 3.9 0.3 4.2 50.5 598 0 0 1 1 6 4 7 5 0 0 0 0	 5 12.2 9.8 8.0 0 54.6 1.8 1.6 16.3 0.2 2.2 41.3 552 8 2 11 4 9 3 13 5 1 1 3 9	 0 9.5 9.5 0 0 - 9.5 9.5 100 0 0 - 23	 0 0 0 0 - - - - - - - - 35
Toxicokinetics *First dose (gestation d6)* Cmax, ng/ml AUC, ng.h/ml (24h) Tmax, h *Last dose (gestation d18)* Cmax, ng/ml AUC, ng.h/ml (24h) Tmax, h	 	 130 497 2 39.7 179 1	 207 922 1 96.6 487 1	 693 4760 2 156 1750 1	 1780 17900 2 *not calculable due to death of all animals

2. Preliminary study of embryo-fetal toxicity in rats by oral gavage administration (RR-815-97-011; vol. 31:1-359)

animal: CD® (SD-derived (8 mated females/group and 6 mated females/group for toxicokinetic evaluation)

GLP statement: yes

Results: Fetal data included only external examination (i.e., no visceral and skeletal examinations were conducted). Based on the submitted data, 9cRA appears not to cause external malformations in rats at doses up to 15 mg/kg/day, but produces embryotoxicity (early resorptions at 5 mg/kg and post-implantation loss at 15 mg/kg) at or greater than 5 mg/kg/day dose and growth alterations at 15 mg/kg/day dose.

Dose, mg/kg/day	0	0.5	1.5	5.0	15
Mortality	none	none	none	none	none
Clinical signs	UR	UR			
red staining, extremities			3/14	7/14	10/14
red vaginal discharge			UR	UR	3/14
Pregnancy rate	7/8	8/8	7/8	8/8	8/8
Body weight gain, g. 6-d20	110	105	107	101	79
Food consumption, 6-d20, g/animal/day	154	158	157	151	142
Litter					
Dams with viable fetuses	7	8	7	8	6
Corpora lutea, #/animal	15.1	14.4	14.3	13.3	13.0
Implantation, #/animal	14.1	13.0	13.6	12.6	12.1
Live fetuses, #/animal	14.0	12.8	13.3	12.0	6.5
Dead fetuses, #/animal	0	0	0	0	0
Sex ratio, % males	52.2	49.6	43.8	49.3	57.4
Postimplantation loss					
#/animals	0.1	0.3	0.3	0.6	5.6
Resorption, early					
#/animal	0.1	0.3	0.3	0.6	3.5
% of implant./animal	1.0	1.9	1.9	5.3	32.5
Resorption, late					
#/animal	0	0	0	0	2.1
% of implant./animal	0	0	0	0	18.0
Mean fetal body wt., g	3.9	3.9	4.1	4.0	3.3
Wt. gravid uterus, g	86	79	85	81	50
Fetal observation:					
External malformation					
# fetuses with abnormality	0	0	0	0	0
# litters with abnormality	0	0	0	0	0
Visceral malformation	not examined	not examined	not examined	not examined	not examined
Skeletal malformation	not examined	not examined	not examined	not examined	not examined

Toxicokinetics					
First dose (gestation d6)					
Cmax, ng/ml		18.8	44.4	199	260
AUC, ng.h/ml (24h)		90.6	101	362	1690
Tmax, h		1	1	1	2
Last dose (gestation d18)					
Cmax, ng/ml		13.5	55.7	101	125
AUC, ng.h/ml (24h)		69.0	123	272	718
Tmax, h		1	1	1	1

3. The teratogenic activity of 9-cis-retinoic acid (Kochhar DM et al. Teratology 47:439-440, 1993 (abstract))

method:

Results: Single oral doses of 9cRA (50 mg/kg) administered on day 11 of gestation produced teratogenic effects (e.g., limb and craniofacial defects) in term fetuses which were similar in pattern to those of ATRA. In the limb bud micromass assay, 9cRA was one-half potent as ATRA in suppression of chondrogenesis. After an oral dose of 9cRA (50, 100 or 200 mg/kg) to mice on day 11 of gestation, 9cRA was detectable in the embryo within 30 min after administration. Among metabolites (ATRA, 13cRA, and trans and cis isomers of 4-oxo-RA), ATRA was the major metabolite of which Cmax in the embryo at 1h after the dose was about 6-fod greater than that of 9cRA, suggesting that a substantial degree of isomerization of 9cRA to ATRA accompanies teratogenesis.

4. 9-cis-retinoic acid: a direct acting dysmorphogen (Kraft JC and Jucha MR. Biochem. Pharmacol. 46:709-716, 1993)

method: