Results: 9cRA produced tranchial arch, cephalic and somite defects similar to those elicited by equal concentrations of ATRA. After intraamniotic microinjection of 600 ng/ml, 9cRA produced cardiac defect (involving gross enlargement of the heart) in 53% of cultured embryos. These results suggest that 9cRA is a direct-acting dysmorphogen.

Parameter	Control	Dose* (ng/mL)		
		150	300	600
Embryos cultured	40	38	48	31
Survival (%)	40 (100)	38 (100)	48 (100)	30 (97)
Any defects (%)	3 (8)	19† (50)	29† (60)	27† (90)
Multiple defects (%)	0 (0)	12† (32)	11† (23)	12† (40)
Anterior schisis (%)	0 (0)	2 (5)	7† (15)	2 (7)
Cephalic defects‡ (%)	0 (0)	3 (8)	16† (33)	6† (20)
Rhombencephalic schisis (%)	0 (0)	2 (5)	3 (6)	4 (13)
Branchial arch defects (%)	0 (0)	11† (29)	20† (42)	10† (33)
Irregular somites (%)	3 (8)	12† (32)	10 (21)	13† (43)
Abnormal rotation (%)	0 (0)	6† (16)	8† (17)	8† (27)
Open optic vesicles (%)	0 (0)	8† (21)	13† (27)	2 (7)
Cardiac defects (%)	0 (0)	0 (0)	0 (0)	16† (53)
Mean yolk sac diameter (mm)	3.5 ± 0.3	3.4 ± 0.3	3.3 ± 0.5	3.1 ± 0.3†
Mean embryonic length (mm)	3.0 ± 0.3	2.9 ± 0.3	2.8 ± 0.3†	2.9 ± 0.4
Mean somite number	28 ± 3	28 ± 3	29 ± 1	23 ± 1†
Mean yolk sac protein (μg)	395 ± 82	535 ± 138†	478 ± 150†	241 ± 85†
Mean embryonic protein (μg)	381 ± 83	537 ± 55†	467 ± 100†	299 ± 50†

Genetic Toxicology

1. Mouse micronucleus test (RR-815-97-013; vol. 32:77-110)
method:

GLP statement: yes

Results: negative

Sampling Time	Treatment (mg/kg)		% Immature Erythrocytes	Incidence *(mean)
24 h	Control		45	1.7
	LGD 1057	375	47	1.5
		750	47	1.7
		1500	48	1.4
	Mitomycin C	12	42	35.1 (P<0.001)
48 h	Control		44	0.8
	LGD 1057	1500	46	1.1

*Incidence- # micronucleated cells per 2000 immature erythrocytes examined

2. In vitro mammalian chromosome aberration test in human lymphocytes (RR-815-97-014; vol. 32:111-147)

method:

GLP Statement: yes

Results: negative

Treatment (ug/ml)		MI, %	Aberrations Without S9			With S9			# Aberrant Cells Without S9		With S9	
		-S9/+S9	ctb/cte	csb	ctg	ctb/cte	csb	ctg	Exc. gaps	Inc. gaps	Exc. gaps	Inc. gaps
Control		100/100	2/0	0		0	0	0	2	3	0	0
LGD 1057	150	99/77	0/0	1	1	1	0	0	1	2	1	1
	300	96/83	0/0	1	2	1/0	1	0	1	3	2	2
	600	85/60	1/0	1	1	0/1	0	0	2	3	1	1
Mitomycin C	0.4	-	5/4	11	4				19	22		
Cytoxan	25	-	26/3	3	1						25	25

* MI- relative mitotic index ; ctb- chromatid break, cte- chromatid exchange, csb- chromosome break, cse- chromosome exchange, ctg- chromatid gap, csg- chromosome gap

3. Mammalian cell mutation assay (RR-815-97-012; vol. 32:148-180)

method:

GLP Statement: yes

Results: negative. It was observed that mean mutant frequency at 450 ug/ml LGD 1057 with S9 fraction was somewhat greater than that of control, however, dose-dependent effects were not established. Repeat test also showed 'negative'.

Treatment, ug/ml		Mean Cell Survival (%) Without S9	With S9	Mean Mutant Frequency/10^6 survivors Without S9	With S9
Control		100	100	8	3
LGD 1057	0.1	107	102		
	5	123	82		
	50	155	68		
	150	142	52	8	3
	300	108	15	6	4
	450	130	75	4	9
	600	109	44	1	4
EMS	250	174		214	
MC	5		75		195

Phototoxicity

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

method:

GLP Statement- yes

Results: LGD 1057 has shown its weak phototoxic potential in Epiderm® skin model, hemoglobin assay and histidine assay.

Treatment, ug/ml		EPIDERM® SKIN MODEL % Viable Cells (mean)		HEMOGLOBIN ASSAY % Hemolysis (mean)		HISTIDINE ASSAY % Histidine (mean)	
		Irradiated	non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated
LGD 1057	30	100	103	105	101	67	100
	300	108	114	122	122	60	93
	3000	84	107	n/a	n/a	61	102
Piroxicam	4	93	113	106	100	100	113
	400	95	110	129	111	95	123
8-MOP	4	105	101	106	87	93	97
	400	42	104	100	87	74	123
Rose Bengal	4	70	114	131	95	34	110
	400	41	112	317	235	81	152

Overall Summary

I. PHARMACOLOGY

A. Interaction with retinoid receptors

9-cis-retinoic acid (9cRA), a retinoid, interacts with all retinoid receptor subtypes (RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ). Unlike 9cRA, all-trans retinoic acid (ATRA) only interacts with RARs, but not with RXRs. 9cRA is capable of inhibiting cell growth, inducing differentiation and inducing apoptosis through interaction with one or more of these retinoid receptors that function as ligand-dependent transcription factors and thus modulate expression of target genes. The following table shows binding affinity and transcriptional activator function of 9cRA:

	Transactivation EC_{50} (nM)		Saturation Binding Kd (nM)	
Retinoid	9cRA	ATRA	9cRA	ATRA
RARα	137	352	0.31	0.37
RARβ	22	82	0.2	0.37
RARγ	37	10	0.78	0.22
RXRα	193	916	1.62	>100
RXRβ	114	1492	2.36	>100
RXRγ	121	1130	2.29	>100

B. Effects on cancer cells

9cRA inhibited proliferation of AIDS Kaposi's sarcoma-derived cells, multiple myeloma cells (RPMI 8226), human promyelocyte cells (HL-60), some human breast cancer cells, and a human head and neck squamous carcinoma cells HNSCC), as summarized below:

Cancer Cell Line		Inhibition of Proliferation, IC_{50}
AIDS Kaposi's sarcoma-derived cells		1-2.5 uM
multiple myeloma cells,	RPMI 8226	5-10 nM
human promyelocyte cells,	HL-60	30 nM
Human breast cancer cells,	T-47D, SK-BR-3, Hs578T	3 nM-100 nM
	MDA-MB lines, BT-20	no inhibition
HNSCC,	1483, SCC25, SqCC/Y1	0.1-10 uM

9cRA also induced differentiation and apoptosis of HL-60 cells with EC_{50} values of 100 nM and 10 uM, respectively.

Nude mouse xenograft models (mouse athymic NCr-NU) with HNSCC cell lines (1483) were studied with 9cRA.

HNSCC Xenografted Cell Line	Treatment	Results
HN9N	oral 9cRA 60 mg/kg/day x 13 weeks	about 50% inhibition of tumor growth when compared to vehicle control
HN9N	oral 9cRA 60 mg/kg/day x 9 weeks	Tumor regressed completely after 6 weeks of treatment. At the end of treatment, tumor volume of 4 mm3 with 9cRA vs 96 mm3 with vehicle control ($P<0.05$)
HN21P	oral 9cRA 60 mg/kg/day x 9 weeks	Tumor regressed completely after 6 weeks of treatment and no residual tumor was observed 15.5 weeks later at necropsy.

In other xenograft model studies with these HNSCC tumor cell lines, duration of up to 28 days and 30 mg/kg/day 9cRA did not produce significant inhibition of tumor growth. No study of xenograft model with Kaposi's sarcoma cells was conducted.

C. Antikeratinizing effects

Retinoids are known to inhibit the conversion of squamous cells to keratinocytes, suggesting retinoids as a potentially important therapeutic agent for treatment of skin disorders of keratinization (e.g., psoriasis, acne vulgaris). In an in vivo model study to examine antikeratinizing activity of 9cRA, hairless rhino mice were applied daily topical doses of 0.01, 0.05 and 0.1% 9cRA for 2 weeks and skin utriculus diameter that decreases in size in response to

antikeratinzing agents was quantified. A topical application of 0.01, 0.05 and 0.1% 9cRA doses resulted in 48%, 60% and 63% decreases in utriculus diameter, respectively, suggesting potential therapeutic benefits with 9cRA treatment of keratinization disorders.

D. PPAR activation and hepatocyte mitogen

Peroxisome proliferators induce proliferation of peroxisomes and hepatocyte proliferation (Lock et al. Ann. Rev. Pharmacol. Toxicol. 29:145-163, 1989). Many of these compounds have been shown to induce liver tumors in rats and mice and rodent chemical carcinogenesis also is closely correlated with the proliferation of liver peroxisomes (Reddy et al. Nature 283:397-398, 1980; Rao and Reddy. Carcinogenesis 8:631-636, 1987). The majority of these compounds show no mutagenic activity and thus are classified as non-genotoxic carcinogens. For peroxisome proliferator-induced cancer, one major underlying mechanism proposed is peroxisome proliferator activated receptor (PPAR) and its association with RXR, which 9cRA activates (Kliewer et al. Nature 358:771-774, 1992; O'Brien et al. Carcinogenesis 17:185-190, 1996). Both PPAR and RXR are nuclear receptors/transcription factors. PPAR binds to peroxisome proliferator-response elements (PPRE) as heterodimers with RXR and activate gene transcription in response to peroxisome activators. Both 9cRA and RXR ligand LGD 1069 activate the PPAR/RXR signal transduction pathway (Kliewer et al. Nature 358:771-774, 1992; Mukherjee et al. Arterioscl. Thromb. Vasc. Biol. 18:272-276, 1998). Furthermore, 9cRA also acts as a direct hepatocyte mitogen in rats through receptor-mediated mechanisms (i.e., PPAR-RXR pathway) similar to peroxisome proliferators, that is, both activate genes that regulate hepatocyte proliferation (Ohmura et al. Life Sci. 58:PL 211-PL 216, 1996). These evidences suggest that 9cRA is a potential non-genotoxic carcinogen that activates PPAR-RXR pathways similar to other non-genotoxic peroxisome proliferators.

II. PHARMACOKINETICS

A. Preclinical and clinical pharmacokinetics

Topical Pharmacokinetics

	Rat	Human
Dose	6 mg/m2/day (0.5% solution)	1.48mg/m2/day (0.1% gel- recommended human dose)
Cmax, nM	M: 7.2-94 (d1), 31-957 (d5) F: BLQ (d1), BLQ-11.3 (D5)	BLQ (*no relationship found between quantitatable 9cRA concentrations and gel strength, time since application, frequency of application, or extent or duration of application)
Tmax, h	21 (d1), 2.3 (d5)	
AUC, uMxh	0.23 (d1), 1.75 (d5)	

BLQ- below limit of quantitation (<2.5 ng/ml)

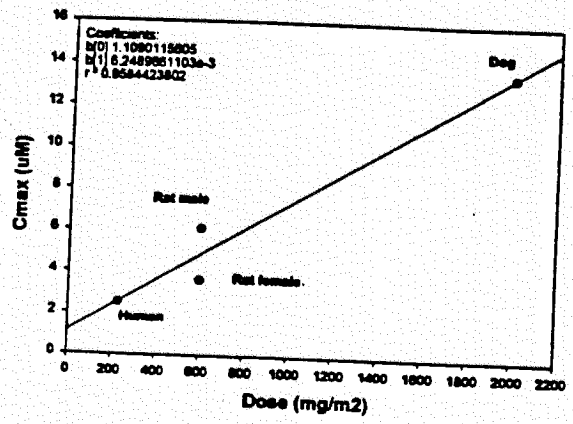
Oral Pharmacokinetics

	Rat (Male/Female)	Dog (Male)	Human (Male/Female)
Single Dose PK			
Dose, mg/m2	600	2000	230
Cmax, uM	6.12 (M), 3.63 (F)	13.6	2.52
Tmax, h	5.25(M), 3.63 (F)	1.4	2.0
AUC, uM.h	49 (M), 29 (F)	46.5	8.15
T1/2, h	0.57 (M), 0.67 (F)	0.87	1.2
Cl, ml/min/kg	37.2 (M), 32.3 (F)	6.96	-
Vd, l/kg	9.72 (M), 10.5 (F)	0.27	-
Bioavailability, %	27 (M), 16 (F)	5.5	-

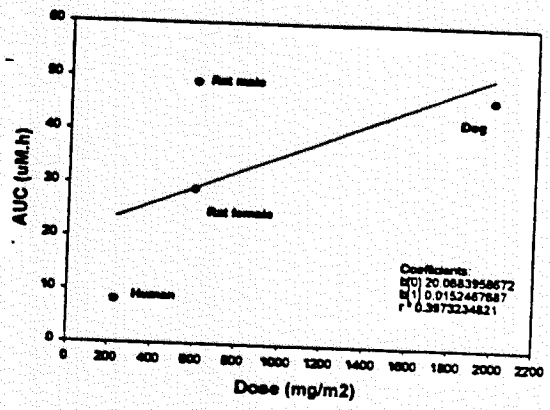
Repeat Dose PK			
Dose, mg/m2	180	500	230
Cmax, uM	0.68 (M), 0.59 (F)	26.4	0.67
Tmax, h	-	0.94	1.5
AUC, uM.h	-	57.4	2.06
T1/2, h	-	1.54	1.3

Interspecies oral dose proportionality of Cmax and AUC:

Cmax



AUC



B. Tissue distribution, metabolism and excretion following oral administration

	Rat	Dog	Human
Plasma protein binding, %	>92	>95	>97
Tissue distribution	liver>adrenal=fat>kidneys>ovaries> mesenteric lymph node	-	
Metabolism In Vivo	ATRA, 13-cis-RA, 4-hydroxy-9cRA, 4-oxo-9cRA, 13, 14-dihydro-9cRA, and 13,14-dihydro-9cRA and N-(13,14-dihydro-9-cis-retinoyl)taurine	ATRA, 13-cis-RA, 4-hydroxy-9cRA, 4-oxo-9cRA, 13,14-dihydro-9cRA, 9cRA glucuronide	
Liver Slice	ATRA, 13-cis-RA, 4-hydroxy-9cRA, 4-oxo-9cRA, 13,14-dihydro-9cRA		
Isolated Hepatocytes	ATRA, 13-cis-RA, 4-oxo-9cRA, 13,14-dihydro-9cRA, 9-cRA taurine conjugates, putative glucuronides		
Liver Microsome	4-hydroxy-9cRA, 4-oxo-9cRA, 9cRA glucuronide	4-hydroxy-9cRA, 4-oxo-9cRA	4-oxo-9cRA, 4-hydroxy-9cRA
P450 isozymes involved			CYP 1A1, CYP1A2, CYP 2C9, CYP 3A4
Excretion	Primarily hepatobiliary-feces (68% M, 47% F), some in urine (5% M, 11% F)	Primarily hepatobiliary, feces, some in urine	

III. TOXICOLOGY

A. Dermal studies

A 28-day dermal toxicity in rats and a dermal sensitization study in guinea pigs were conducted. In the 28-Day dermal toxicity study, rats received the daily topical application of 0.01%, 0.05% or 0.5% 9cRA (0.5 ml/animal) to the dorsal skin (approximately 10% of body surface area) for 28 days. Systemic toxicities (e.g., hunched posture, emaciation, weight loss, reduced food consumption, and increases in PMNL counts, ALT and BUN, etc) and dermal toxicities (e.g., erythema with fissure) were observed in 0.5% group. Erythema and epidermal scaling/thickening were observed in 0.01% and 0.05% groups. NOAEL appeared to be < 0.01%.

In dermal sensitization study, guinea pigs received the induction and challenge doses of 0.05% or 0.5% 9cRA. There was suggestive changes of dermal irritation, but no indication of hypersensitive reactions.

B. Phototoxicity study

To examine phototoxic potential of 9cRA, Epiderm skin model assay, hemoglobin oxidation and photohemolysis and histidine assay were conducted. 9cRA has shown a weak phototoxic potential in these assays.

C. Oral toxicity studies

Species	Duration	Dose (mg/m2/day)	Toxic Target	Toxic Level (mg/m2)
Rat	single dose	30, 300, 3000, 6000, 9000	skin (peeling)	STD_{10} = 9000 NOAEL= 3000
Dog	single dose	200, 1800, 3600, 7200, 14400	skin (erythema), blood (↑PMNL, ↑triglyceride), kidney (↑BUN), liver (↑AST)	STD_{10}=14400
Rat	28-Day	6, 30, 180	long bones (fracture, osteoporosis), spleen/liver (extramedullary hematopoiesis, ↑AST, ↑cholesterol, gastric mucosa (hyperplasia), kidney (↑BUN), heart (cardiomyopathy)	STD_{10}= 180 TDL= 6
Dog	28-Day	6, 30, 200, 1000	kidney (↑BUN, nephropathy), liver (cytoplasmic rarefaction)	STD_{10}= 1000 TDL= 30 NOAEL= 6
Rat	91-Day	0.18, 1.8, 6	↑triglyceride, ↑alkaline phosphatase	TDL= 6 NOAEL= 1.8
Dog	91-Day	2, 6, 30	not determined since the highest dose tested was NOAEL	NOAEL= 30

IV. Reproductive Toxicity

Developmental studies following oral administration of 9cRA were conducted in rats and rabbits. Teratogenic effects were observed at or greater than 0.5 mg/kg/day in rabbits, but not in rats at doses up to 15 mg/kg/day. However, rat fetuses were only examined exteranlly and not for visceral or skeletal defects. Maternal toxicity was observed in rabbits at or greater than 1.5 mg/kg/day. Embryotoxicity was also observed in rabbits at 1.5 mg/kg/day and in rats at 5 mg/kg/day.

Kochhar et al (Teratology 47:439-440, 1993) studied the teratogenic activities of 9cRA in pregnant ICR mice and in the limb bud mesenchymal cell micromass cultures using chondrogenesis as an end-point. 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.

Kraft and Juchau (Biochem. Pharmacol. 46:709-716, 1993) conducted experiments in vitro with cultured rat conceptuses (i.e., whole embryo culture system) and demonstrated that 9cRA (300 ng/ml amniotic fluid) produced tranchial arch and somite defects similar to those elicited by equal concentrations of ATRA. After intraamniotic microinjection of 600 ng/ml on day 10 of gestation, 9cRA produced an unusual heart defect. These results suggest that 9cRA is a direct-acting dysmorphogen.

V. Genetic Toxicity

Bacterial mutation assays, mammalian cell mutation assay, chromosome aberration test in human lymphocytes and mouse micronucleus test were conducted. Under the experimental conditions, 9cRA was not mutagenic or clastogenic in these genotoxic assays.

Labeling Issues:

Published studies- A teratogenic effect following a single oral administration of 50 mg/kg 9cRA (approximately 100 times the recommended human topical dose on a body surface area basis) to mice on day 11 of gestation was reported. In addition, teratogenic effects of 9cRA from in vitro teratogenicity assays of the limb bud mesenchymal cell micromass and of whole rat embryo culture were also reported.

RECOMMENDATION

1. Panretin can be approved for Kaposi's sarcoma from the pharm/tox perspective.
2. Revise labeling as recommended (a separate labeling review will follow).

/S/

Chang H. Ahn, Ph.D.
Expert Pharmacologist

11/17/98
Date

Original NDA 20886
c.c. /Division File
/PAndrews
/RWhite
/CAhn

11/17/98

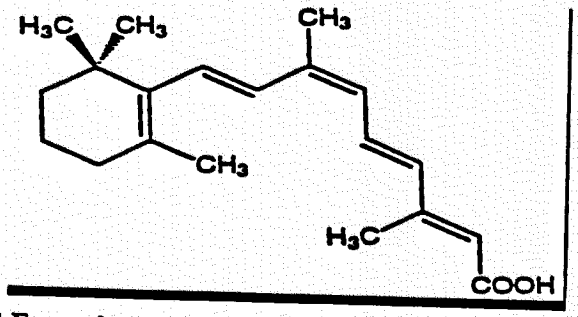
Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Review No. 2 (Labeling Review)

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 24, 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), hydroxypropyl 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 2 mg/day (i.e., 1.18 mg/m2/day) based on assumption that a 60 g tube of gel will be sufficient for 30 days of treatment (vol. 1, p185).

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

Labeling Review:
The labeling for Panretin® 0.1% gel conforms to 21 CFR 201.57. The following changes to the text are recommended:

Redacted 1

pages of trade

secret and/or

confidential

commercial

information

/S/

Chang H. Ahn, Ph.D.
Expert Pharmacologist

11/24/98
Date

Original NDA 20886
c.c. /Division File
/PAndrews
/RWhite
/CAhn
/AChapman

/S/

11/24/98