

SPECIAL TOXICOLOGY STUDIES

Phototoxicity

1. In vitro phototoxicity studies (summary; vol 1.41, pp165-243, RR-815-98-006. [

method: MatTEK phototoxicity- Using MatTEK's EPI-2000 in vitro tissue skin model assay, phototoxic potential of LG1069 was tested. Phototoxicity is evaluated by applying 300 and 3000 ug/ml LG1069 (Lot # LG100069-000Z035; diluent- DMSO/PBS) to two sets of tissue- UVA/UVB irradiated and non-UVA/UVB irradiated tissues after the drug treatment. Phototoxicity is determined by measuring differences in cytotoxicity between irradiated and non-irradiated tissue (i.e., phototoxic when significant increase in toxicity ($P \leq 0.05$) occurs). Cytotoxicity is measured by MTT assay. Hemoglobin Oxidation and Photohemolysis- Freshly drawn, heparinized blood from volunteers is centrifuged at 3000 rpm (1500x g) for 10 min and the plasma is removed. The red blood cells obtained is suspended in 5 ml PBS and is centrifuged again at 3000 rpm for 10 min. After washes of the RBC suspension, aliquots of the RBC suspension is diluted (1:1000) and 10 ml aliquots of the diluted suspension is transferred to Petri plates. The triplicate set of plates is treated with 300 ug/ml LG1069 (Lot # LG100069-000Z035; diluent- DMSO/PBS) to two groups- UVA/UVB irradiated (1.6 mW/cm² x 150 min to give a dose of about 15 Joules/cm²) and non-UVA/UVB irradiated groups. Phototoxic material will oxidize hemoglobin. The absorbance of hemoglobin is read at 420 nm vs. a reagent blank (2ml PBS + 2 ml Drabkin's reagent). Phototoxicity is indicated by a significant ($P \leq 0.05$) increase in hemoglobin oxidation with the irradiated set compared to the non-irradiated set.

Histidine Assay- L-histidine (1 mM) is treated with 300 and 3000 ug/ml LG1069 (Lot # LG100069-000Z035; diluent- DMSO/PBS) in 6-well plates to two sets- UVA/UVB irradiated (3 mW/cm² for 2-3 h to give a dose of about 21-31 Jules/cm²) and non-UVA/UVB irradiated sets. Phototoxic material will oxidize histidine. The amount of histidine remaining in the reaction samples is determined by Pauly reaction and is calculated by reading the optical densities of each sample at 540 nm vs a blank solution (2 ml PBS +200 ul 1% sulfanilic acid in 0.87 N HCl + 200 ul 5% sodium nitrite in water + 0.6 ml 20% sodium carbonate + 2 ml 75% ethyl alcohol). Phototoxicity is indicated by a significant ($P \leq 0.05$) increase in oxidation of histidine by singlet oxygen in the presence of test material compared to the non-irradiated set. In these assays, positive controls were 8-methoxypsoralen (400 ug/ml) and rose bengal (4 ug/ml) and negative control was DMSO in PBS (7813 ug/ml).

Protein Photobinding Assay- To determine photoallergenic potential of LG1069, human serum albumin (2 mg/ml in PBS) was incubated with 10 uM [³H]LG1069 (specific activity 0.27 Ci/mmol; lot # LG100069-000Z037) at 37°C in the dark for 1 h. The mixture was transferred to 3-ml cuvettes: half of the cuvettes were incubated for an additional 1 h, while the other half were irradiated in a Rayonet photochemical chamber, equipped with sixteen 35-watt UV lamps. The contents of the cuvettes were acidified to pH 3, transferred to glass tubes containing 0.5g dextran-coated charcoal, inverted 10 times and stirred magnetically for 1 h. The tubes were centrifuged and aliquots (three 25 ul replicates/sample) were measured for their radioactivity in scintillation counter. Additional 50 ul aliquots were subjected to protein measurements using a protein assay kit. By normalizing to protein concentration, an estimate of the stoichiometry of photobinding was determined.

GLP Statement- yes

Results- LG1069 was not phototoxic from assays of MatTEK phototoxicity, but showed a phototoxic potential from assays of photohemolysis/hemoglobin oxidation and histidine. LG1069 also showed a photoallergenic potential from assays of protein photobinding assay.

a. MatTEK EPI-2000 phototoxicity assay- not phototoxic

Treatment	Concentration ug/ml	Exposure Time h	Average % Viable Cells	
			Irradiated with UVA	Non-irradiated with UVA
LG1069	300	48	87.64	88.08
	3000	48	56.09	56.40
8-MethoxyPsoralen	400	48	87.41	116.20
DMSO:PBS	7813 in PBS	48	100	100

b. hemoglobin oxidation assay- phototoxic

Treatment	Concentration ug/ml	Exposure Time h	Average % Hemolysis	
			Irradiated with UVA	Non-irradiated with UVA
LG1069	300	2.5	1964.71	1578.95
Rose Bengal	4	2.5	2341.18	1536.84
DMSO:PBS	7813 in PBS	2.5	100	100

c. histidine assay- phototoxic

Treatment	Concentration ug/ml	Exposure Time h	Average % Histidine	
			Irradiated with UVA	Non-irradiated with UVA
LG1069	300	2.5	82.69	95.15
	3000	2.5	79.97	101.86
Rose Bengal	4	2.5	31.40	86.00
DMSO/PBS	7813 in PBS	2.5	100	100

d. photobinding assay- The radioactivity content of the samples which had been exposed to UV light was 100-fold greater than that of the control samples. The stoichiometry of photobinding was 1 molecule of LG1069 to 9.05 molecules of albumin (i.e., (albumin/LG1069) = $16.2 \mu\text{M}/1.79 \mu\text{M} = 9.05$). These results suggest that LG1069 is a potential photo-allergen.

Measurement	No Light Exposure	Post-UV Light Exposure	Fold Increase due to UV-irradiation
Albumin, mg/ml	1.21	1.12 (16.2 uM)	-
dpm/mg protein, after charcoal extraction	8949	898664	100
Mean LG1069 Conc. (uM) after extraction	0.0194	1.79 (0.622 ug/ml)	
stoichiometry of photobinding (Albumin/LG1069)	-	9.05 (= 16.2/1.79)	

Overall Special Toxicology Summary- - LG1069 was phototoxic in in vitro phototoxicity assays and showed a photosensitizing potential in the protein photobinding assay.

OVERALL SUMMARY AND EVALUATION

I. Pharmacology

A. Interaction with retinoid receptors

LG1069 interacts with retinoid X receptor subtypes (RXR α , RXR β , and RXR γ), but not with retinoic acid receptor (RAR). LG1069 is capable of inhibiting AP-1 activity, inducing apoptosis, cell cycle arrest and differentiation, and inhibiting growth of tumor cells, through interaction with RXRs (and potentially with RAR at concentrations $\geq 1 \mu\text{M}$) that function as ligand-dependent transcription co-factors and thus modulate expression of target genes. The following table shows binding affinity and transcriptional activator function of LG1069:

Receptor	Transactivation EC ₅₀ (nM)	Saturation Binding Kd (nM)
RAR α	>10,000	6298
RAR β	>10,000	>10,000
RAR γ	>10,000	4804
RXR α	25	30
RXR β	27	14
RXR γ	19	15

B. Effects on cancer cells

Cancer Cell Line	Inhibition of Proliferation
Breast T47D, SK-BR-3, MCR-7 Primary tumor samples	IC ₅₀ = 0.1-1 uM No inhibition with 0.1 uM LG1069
Cervix ME-180 Primary tumor samples	IC ₅₀ = 0.5 uM No inhibition with 0.1 uM LG1069
Colon Primary tumor samples	No inhibition at 0.1 uM LG1069
Head and Neck 1483, SCC25, SqCC/Y1	10-30% inhibition with 1 uM LG1069
Kaposi's sarcoma-derived cells	40% inhibition by 1 uM and 73% by 10 uM LG1069
Leukemia Primary AML cells	0-50% inhibition with 0.1 uM LG1069
Melanoma Primary tumor samples	No inhibition with 0.1 uM LG1069
Ovary Primary tumor samples	10-50% inhibition with 0.1 uM LG1069

C. In vivo tumor models

Head and Neck cancer xenograft in nude mice- LG1069 (60 mg/kg/day, po) induced complete regression of HN9N and HN21P-xenografted tumors by day 49 and day 37, respectively. The complete regressions were maintained up to 68 days post-implantation of tumor cells. No growth inhibitory effects on 1483-xenografted tumors were observed.

NMU-induced rat mammary tumor- Dose-dependent effects of LG1069 were observed on complete response, tumor burden and number tumors per animal. LG1069 dose at 100 mg/kg/day resulted in complete regression in 72% of primary tumors, 81% decrease in tumor burden, and significant decrease in number tumors/animal (0.69 vs 3.63 in control).

D. Epidermal 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., acne vulgaris, psoriasis). In an in vivo model study to examine antikeratinization activity of LG1069, hairless rhino mice received daily oral doses of LG1069. Antikeratinizing effect was not observed at doses up to 100 mg/kg/day. However, when administered topically (0.05 – 0.5%), antikeratinizing effect was observed.

II. Pharmacokinetics

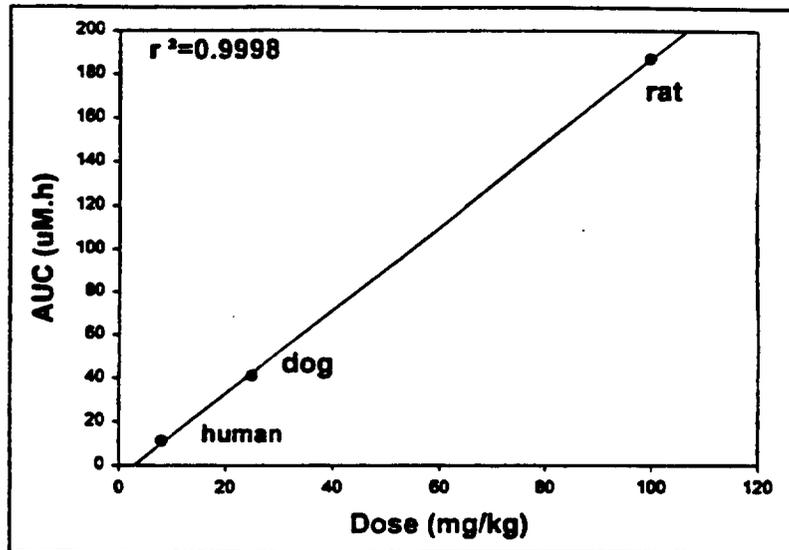
A. Preclinical and clinical pharmacokinetics

Pharmacokinetics	Rat (PEG/water vehicle)		Dog (capsule)	Human* (capsule)	
	600, single dose	600, multiple dose	500, single dose	300, single dose	300, multiple dose
	100	100	25	8.1	8.1
Route	PO (Male/Female)	PO (Male/Female)	PO	PO	PO
C _{max} , uM	15.6/16.9	4.8/10.0	70.4	2.6	3.2
T _{max} , h	3.7/2.6	3.2/2.1	1.5		
T _{1/2} , h	7.6/5.0	4.4/5.0	2.5	1-3	1-3
AUC _{0-∞} , uM.h	205/169	44.6/82.1	41.1	11.1	10.9
Bioavailability, %			83.1		
Protein binding	-	-	-	>99.8 (5-5000 ng/ml)	
Tissue Distribution in Rats	8h - GI (85.4%M/85.0%F) > tissues (4.8%M/5.3%F) > plasma (0.27%M/0.29%F) 24h- GI (20.7%M/65.8%F) > tissues (1.4%M/3.7%F) > plasma (0.01%M/0.7%F)				
Metabolism	CYP2B, CYP3A and/or CYP4A-mediated oxidation: 6-hydroxy LG1069, 7-hydroxy LG1069, 6-oxo-LG1069, 7-oxo-LG1069 Glucuronidation: acyl glucuronide, ether glucuronide Conjugation: taurine conjugate, 7-sulfate conjugate				
Excretion, Feces	91.1(M)/100(F)			0.04	
Urine	0.14 (M)/0.59(F)			-	

*human pharmacokinetic parameters from vol. 1.57 (pp104-107)

B. Interspecies oral dose proportionality of C_{max} and AUC

C_{max} of rats and dogs cannot be directly extrapolated to that of humans ($r^2=0.13$ on mg/m² basis, $r^2=0.1570$ on mg/kg basis), while AUC of these animals may be directly extrapolated to humans ($r^2=0.75$ on mg/m² basis, $r^2=0.9998$ on mg/kg basis). Dosing on mg/kg basis may provide much greater interspecies correlation of AUC than dosing on mg/m² basis.



III. Toxicology

Toxicity Study	Treatment	Toxicity Targets and Toxic Effects	Toxic Levels
Rat 28-Day	3, 10, 30 or 100 mkd, po, for 28 days	Blood (hemorrhage in many tissues, prolongation of PT and aPTT, increased levels of fibrinogen), liver (hepatocellular necrosis), pancreas (acinar cell necrosis), heart (cardiomyopathy), bone (tibial osteopathy), skin (dermatopathy), lymphoid organs (lymphoid depletion in spleen, lymph nodes and thymus)	LD ₁₀ = 10 mkd HNSTD= 3 mkd
Dog 91-Day	0.1, 0.3 or 1.5 mkd, po, for 91 days	Testes (tubular degeneration)	HNSTD= 1.5 mkd
Rat 6-Month	3, 30, 100 or 300 mkd po, for 6 months	Blood (prolonged clotting time, ↓hemoglobin and RBC), Eye (lens opacity- cataractogenic), liver (hypertrophy, ↑AST/ALT), skin (acanthosis), stomach (acanthosis, hyperkeratosis)	HNSTD= 300mkd*
Dog 6-Month	1, 3 or 10 mkd, po, for 6 months	Adrenal cortex (vacuolation), liver (hepatocellular hypertrophy, ↑ALT/AST), eye (cataract), platelets (↓ in counts).	HNSTD= 10 mkd

* mkd- mg/kg/day; * increased incidence of gavage-related deaths at 300 mkd

IV. Genetic Toxicology

LG1069 is not mutagenic in bacterial (*Salmonella* and *E.coli*) or mammalian cell (mouse lymphoma L5178Y tk^{-/+}) mutation assays, or clastogenic in vitro (CHO cells) or in vivo (micronucleus test in mice).

Note that the CHO cell assay in the absence of S9 was not an adequate test due to the absence of cytotoxicity at the highest concentration tested. This result should therefore not be included in the label.

V. Reproductive Toxicology

Retinoids, as a class, have demonstrated their capability of inducing teratogenic effects in animals and humans. LG1069 is maternally toxic in rats when given orally at 4 mg/kg/day (1/12th the recommended clinical oral dose on a mg/m² basis). LG1069 caused developmental mortality and altered growth (≥ 10 mg/kg/day, 1/5th the recommended clinical dose on a mg/m² basis) and was dysmorphogenic (≥ 4 mg/kg/day, 1/12th the recommended clinical dose on a mg/m² basis). LG1069 induces testicular degeneration in dogs (1.5 mg/kg/day, 1/10th the recommended clinical dose on a mg/m² basis).

VI. Safety Pharmacology

Study	Model	Effects
Cardiovascular Assay	Normotensive male SD rats received daily oral doses of 0, 10, 30 and 60 mg/kg/day LG 1069 by gavage. Mean arterial blood pressure and heart rate (plus clinical pathology) were measured.	<ol style="list-style-type: none"> 1. No effects on mean arterial blood pressure and heart rate. 2. 23% and 20% Increases in aPTT in 30 (p<0.01) and 60 mg/kg (p<0.01) groups, respectively.
Blood Coagulation	Male SD rats received daily oral doses of 0, 10 or 100 mg/kg/day LG1069, admixed with powdered feed, for 29 days. When PT and aPTT increased 2-fold, animals received 10 mg/kg vitamin K ₁ by ip (d17/18) or sc (d19/20).	<ol style="list-style-type: none"> 1. aPTT increased 3-fold in 10 mg/kg group and 4-fold in 100 mg/kg group over control at week 3. PT also increased 2-fold in 100 mg/kg group. Vitamin K restored PTs and aPTTs to control levels after 4 days of treatment. 2. Due to bleeding disorders, 6/8 animals in 100 mg/kg group died. The bleeding disorders were fully reversed when vitamin K was co-administered.
Hepatocyte Proliferation	Female SD rats received daily oral doses of 100 mg/kg/day by gavage for 1, 2, 4, or 12 days. Two h prior to sacrifice, rats were injected with 50 uCi [³ H]thymidine by ip. At necropsy, livers were excised, homogenized and analyzed for [³ H]thymidine incorporation and DNA contents.	<ol style="list-style-type: none"> 1. absolute liver weight increased significantly following 4 or 12 days dosing (8.4 g vs. 5.1 g control). 2. Increased rate of [³H]thymidine incorporation into liver occurred during LG1069 administration and was reversible over a 5-day recovery period. 3. liver concentrations of protein, DNA and glycogen from LG1069-treated groups were not significantly different from control group.
Plasma Lipids	Male rabbits received oral daily doses of 0, 1, 10 or 100 mg/kg/day LG1069 by gavage for 4 days. Blood for evaluation of lipids was collected at termination.	<ol style="list-style-type: none"> 1. increases in plasma cholesterol (3-fold) and triglycerides (10-fold) in 100 mg/kg group. LDL and HDL were not affected.
Neuro-pharmacology	Male SD rats received daily oral doses of 0, 10, 30 or 100 mg/kg/day LG1069 by gavage for 4 days. Ataxia, convulsions, alertness and spontaneous motor activity were measured.	<ol style="list-style-type: none"> 1. No treatment-related effects on ataxia, convulsions, alertness, spontaneous motor activity, or body temperature were observed.

VII. Phototoxicity

LG1069 was phototoxic in in vitro phototoxicity assays and showed a photosensitizing potential in the protein photobinding assay.

Phototoxic Assay	Results
MatTEK phototoxicity test	Not phototoxic
Hemoglobin oxidation and photohemolysis	Phototoxic
Histidine Assay	Phototoxic
Protein photobinding assay	The stoichiometry of photobinding was 1 molecule of LG1069 to 9.05 molecules of albumin (i.e., (albumin/LG1069) = 16.2 uM/1.79 uM = 9.05). These results suggest that LG1069 is a potential photo-allergen.

VIII. Labeling Issues

A. Pharmacological and Toxicological Effects

Retinoids are an integral part of regulating mechanisms of cell proliferation and differentiation via control of the expression of the genes for many cytokines (e.g., TGFβ, TGFα, FGF, IGF, IL-1, IL-2, IL-8, IFNγ, NGF and PDGF), their receptors (e.g., TGFβ-R, EGFR, PDGF-R, IL-6R, and NGF-R) and oncogenes (e.g., jun, fos, fgr (src TK family)). Retinoids interact with retinoid receptors that are classified as retinoic acid receptor (RAR) and retinoid X receptor (RXR) subtypes. Retinoid receptors act as transcription factors by forming homodimers (RAR-RAR and RXR-RXR) or heterodimers (RAR--RXR). RXR also forms complexes with many transcriptional factors (e.g., vitamin D receptor, thyroid hormone receptor, PPARs, etc) for subsequent regulation of gene transcription. LG1069 is a RXR-

specific agonist that interacts with RXR subtypes with K_d (affinity) ranging from 14 nM to 30 nM and with EC₅₀ (transactivation) ranging from 19 nM to 27 nM.

LG1069 inhibits proliferation of cancer cells of breast, head and neck, ovary and Kaposi's sarcoma, and of AML cells with IC₅₀ of ≥ 0.1 μ M. Oral LG1069 did not inhibit the conversion of squamous cells to keratinocytes.

Following oral administration in animals, LG1069 produced toxicities in blood (hemorrhage in many tissues, \downarrow hemoglobin and RBC prolongation of PT and aPTT, increased levels of fibrinogen), bone (tibial osteopathy), eye (cataract), heart (cardiomyopathy), liver (hepatocellular necrosis, hypertrophy, \uparrow AST/ALT), pancreas (acinar cell necrosis), skin (dermatopathy, acanthosis), lymphoid organs (lymphoid depletion in spleen, lymph nodes and thymus), stomach (acanthosis, hyperkeratosis), and testes (tubular degeneration). Series of assays (MatTEKskin model assay, hemoglobin oxidation/photohemolysis, histidine assay and protein photobinding assay) conducted to assess phototoxic potential of LG1069 suggest that LG1069 is a phototoxin and photoallergen.

B. Carcinogenicity, Mutagenicity, and Impairment of Fertility

Carcinogenicity- Long-term studies in animals to assess the carcinogenic potential of LG1069 have not been conducted.

Mutagenicity- LG1069 was not genotoxic in a bacterial (Salmonella and E.coli) mutation assay, mammalian cell (mouse lymphoma L5178Y tk^{-/+}) mutation assay, chromosome aberration assay (CHO cell) or an *in vivo* micronucleus test in mouse bone marrow. The CHO cell assay in the absence of S9 activation was not an adequate test for genetic toxicity.

Impairment of Fertility- LG1069 induces testicular degeneration in dogs (1.5 mg/kg/day, 1/10th the recommended clinical dose on a mg/m² basis).

C. Pregnancy Category

Pregnancy Category- D

Retinoids, as a class, have demonstrated their capability of inducing teratogenic effects in animals and humans. LG1069 is maternally toxic in rats when given orally at 4 mg/kg/day (1/12th the recommended clinical oral dose on a mg/m² basis). LG1069 is fetotoxic (≥ 10 mg/kg/day, 1/5th the recommended clinical dose on a mg/m² basis) and teratogenic (≥ 4 mg/kg/day, 1/12th the recommended clinical dose on a mg/m² basis). At 4 mg/kg the AUC in rats is approximately 0.3 times the human AUC at 300 mg/m².

D. Cataract Formation

Cataract development has been reported in association with the oral administration of LG1069 at 3 mg/kg/day in rats (1/17th the recommended clinical dose on a mg/m² basis) and 3 mg/kg/day in dogs (1/5th the recommended clinical dose on a mg/m² basis). The mechanism of LG1069-induced cataracts was not known.

E. Drug Interaction

LG1069 induced bleeding in rats (10 mg/kg/day, 1/5th the recommended clinical dose on a mg/m² basis), probably due to the prolonged PT and aPTT (3 mg/kg/day, 1/17th the recommended clinical dose on a mg/m² basis). The concurrent use of LG1069 with drugs that affect coagulation such as NSAIDs should be discouraged.

E. Phototoxicity

Retinoids as a class have been associated with photosensitivity. LG1069 was phototoxic in *in vitro* phototoxicity assays and showed a photosensitizing potential in the protein photobinding assay.

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Draft Labeling

Study Reviewed

I. Pharmacology

- A. Transcriptional activation (vol. 1.4, RR-750-93-004)
- B. Retinoic acid receptor binding profile (vol 1.4, RR-750-93-013)
- C. Effects on human leukemia cells, Kaposi's sarcoma, cervical carcinoma cells, head and neck squamous carcinoma and breast carcinoma cells (vol. 1.4, RR-750-93-008/-012; vol. 1.5, RR-750-93-007/-013)
- D. Effects on human head and neck squamous cell carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-014)
- E. Effects on human cervical carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-015)
- F. Effects on cardiovascular and central nervous systems (vol. 1.5, RR-740-93-006/-007)

II. Pharmacokinetics

- A. Toxicokinetics in rats (vol. 1.7, RR-845-93-021)
- B. Toxicokinetics in dogs (vol. 1.6, RR-845-93-023)
- C. Bioavailability in rats (vol. 1.6, RR-845-93-018)
- D. Bioavailability and metabolism in dogs (vol. 1.6/1.7, RR-845-93-022/-024)
- E. Tissue distribution and metabolism in mice (vol. 1.7, RR-845-93-017)
- F. Metabolism in rats (vol. 1.7, RR-945-93-020)
- G. Plasma protein binding (vol. 1.7, RR-845-93-025)

III. Toxicology

- A. Acute oral toxicity study in rats (vol. 1.8, RR-815-93-020/-021)
- B. Acute oral toxicity study in dogs (vol. 1.8, RR-815-93-015)
- C. 28-Day repeat dose oral toxicity study in rats (vol. 1.9, RR-815-93-022)
- D. 28-Day repeat dose oral toxicity study in rats (interim report, IT-001 supplement)
- E. 28-Day repeat dose oral toxicity study in dogs (vol. 1.12, RR-815-93-016)

IV. Genetic Toxicology

N/A

V. Reproduction Toxicology

N/A

VI. Human Experience

N/A

Protocol

This is an open-label, uncontrolled phase I study to evaluate the tolerability, safety, potential toxicity, pharmacokinetics and metabolic fate of LGD1069. Patients with advanced

cancer will receive oral dose of 5 mg/m²/d LGD1069 (escalated to 10, 20, 30, 45, 60, 80 and 105 mg/m²/d; qd) for 4 weeks.

Safety Review

I. Pharmacology

A. Transcriptional activation (vol. 1.4, RR-750-93-004)

Luciferase gene expression resulted from activation of the each human retinoid receptor subtype was measured utilizing the cis-trans assay. In the cis-trans assay, a cDNA encoding an intracellular receptor (IR) and a reporter gene (luciferase) under the control of a hormonally responsive (HRE-containing) promoter are introduced into a cell lacking the IR. The ability of the hormone to regulate gene expression can be directly monitored by measuring luciferase activity. Following exposure of the transfected cells to an appropriate agonist, an increase in reporter activity can be measured in cell extracts, reflecting ligand-dependent IR-mediated increases in reporter mRNA transcription.

Receptor Subtype	Mean EC ₅₀ (nM)		
	LGD 1069	9-cis-RA	ATRA
RAR α	>10,000	191	352
RAR β	>10,000	51	82
RAR γ	>10,000	45	10
RXR α	25	253	916
RXR β	27	221	1492
RXR γ	19	147	1130

B. Retinoic acid receptor binding profile (vol 1.4, RR-750-93-013)

The ability of retinoid to bind to the human retinoid receptor subtypes was determined by measuring competitive displacement of radiolabeled 9-cis-RA.

Receptor Subtype	Mean K _d (nM)		
	LGD 1069	9-cis-RA	ATRA
RAR α	>10,000	15.2	15.4
RAR β	>10,000	13.4	13.2
RAR γ	>10,000	14.7	18.0
RXR α	30	7.0	>10,000
RXR β	14	7.0	>10,000
RXR γ	15	17	>10,000

C. Effects on human leukemia cells, Kaposi's sarcoma, cervical carcinoma cells, head and neck squamous carcinoma and breast carcinoma cells (vol. 1.4, RR-750-93-008/-012; vol. 1.5, RR-750-93-007/-013)

i) Human acute promyelocytic leukemia HL 60 cell line

- a. LGD 1069 does not inhibit thymidine incorporation into DNA at concentrations up to 10 μ M, suggesting it does not inhibit DNA synthesis of HL60 cells.
- b. Up to 1 μ M, LGD 1069 does not induce cellular differentiation measured by NBT reduction assay in both HL60 and NB4 cells (cf, EC_{50} of ATRA = 0.2 μ M).
- c. LGD 1069 induces a concentration-dependent increase in transglutaminase activity with an EC_{50} value of 6 nM (cf, RAR selective TTNPB does not induce the enzyme activity over a broad concentration range), DNA laddering, and morphological changes such as condensed nuclei and cytoplasm, multiple blebbing of the cell membrane and formation of apoptotic bodies, indicating that it induces apoptosis, probably through RXR receptors. Transglutaminase is involved in protein cross-linking and its induction is correlated with induction of apoptosis. (cf. cell proliferation and differentiation are related to RAR and apoptosis is to RXR).

ii) Cervical carcinoma ME-180 cells

For 96 hours LGD 1069 treatment up to 1 μ M, there were 20% decrease in cell viability and 24% decrease in DNA synthesis (thymidine uptake), respectively. However, by day 6 of exposure to LGD 1069 1 μ M, the number of viable cells decreased by 98%. LGD 1069-treated cells DNA laddering and morphological changes indicating apoptosis.

iii) Kaposi's sarcoma, Human head and neck squamous carcinoma 1483 cells and breast carcinoma MCF-7 cells

Cell Line	%Growth Inhibition at 1 μ M
Kaposi's	40
Head and Neck	30
MCF-7	79

D. Effects on human head and neck squamous cell carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-014)

Moderately differentiated squamous cell carcinoma derived from a metastatic mandibular lymph node of a 68-year old male (HN9N) or from a primary lesion of the tongue of a 69-year old male (HN21-P) were inoculated to nude mice. Effects of LGD 1069 or ATRA on rates of tumor - growth were determined following daily doses of 60 or 30 mg/kg, respectively, which was given

d2 following tumor implantation. The results are shown below:

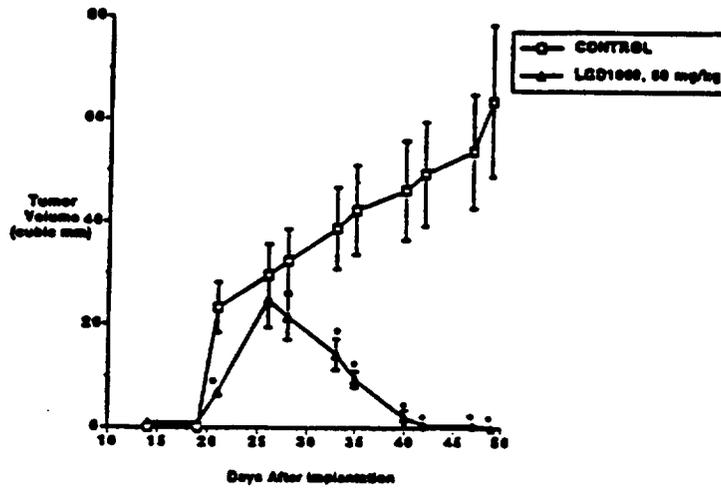


FIGURE 11. Effect of LGD1069 on Growth of Human Primary Squamous Cell Tumor Xenografts in Nude Mice (Tumor HN9N)

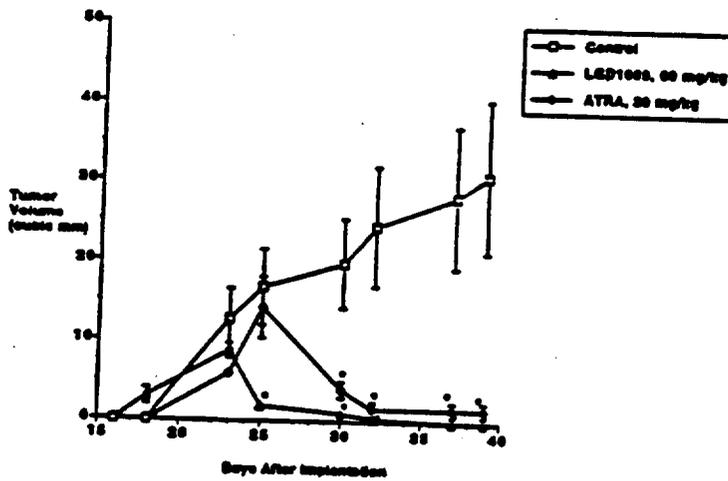


FIGURE 12. Effect of Retinoids on the Growth of Primary Human Squamous Cell Xenografts in Nude Mice (Tumor HN21-P)

E. Effects on human cervical carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-015)

Human cervical carcinoma ME-10 was xenografted to nude mice. LGD 1069 (10, 30 and 100 mg/kg po) or ATRA (10 and 30 mg/kg po) was administered 5d/wk, beginning on d2 following implantation of ME 10 cells. The results were shown below:

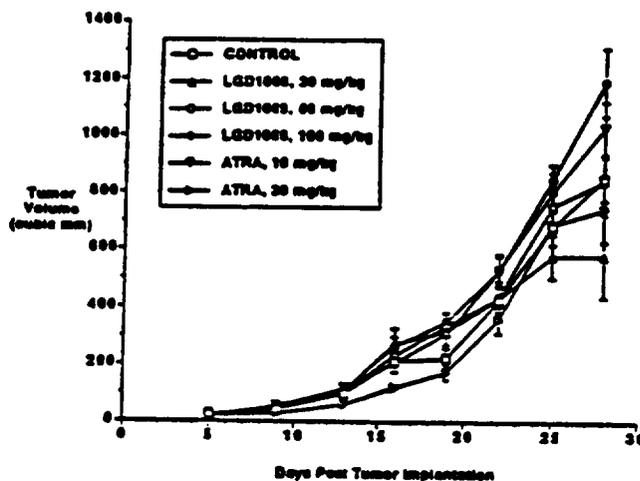


FIGURE 13. Effects of Either 30, 60, or 100 mg/kg LGD1069 PO, 10 or 30 mg/kg ATRA PO, or Sesame Oil Vehicle on Growth of ME-180 Xenografts

Mice were inoculated with ME-180 cells and treatment with drugs was begun two days thereafter. Tumors were measured at the indicated times. Each point represents the mean \pm SEM of 8 - 10 tumors.

F. Effects on cardiovascular and central nervous systems (vol. 1.5, RR-740-93-006/-007)

i) Cardiovascular effects in normotensive rats (10 males/group)

LGD 1069 (10, 30 or 60 mg/kg/d x 4d, po) were given to animals. No clinical signs observed. APTT was increased by 23% in animals with 60 mg/kg, but it was within the normal range. Clinical pathology showed increases in ALK, ALT, AST, Na, globulin and total bilirubin.

ii) CNS effects in rats

Rats received 10, 30 or 100 mg/kg/d LGD 1069 (x 4d, po) and social behavior, ataxia, convulsions, alertness and spontaneous motor activity were measured daily. All animals showed normal social behavior and body weight gain. LGD 1069 did not protect animals from challenge with 150 mA for a 0.2 sec duration with transcorneal electroshock to measure anticonvulsant activity.

II. Pharmacokinetics

A. Bioavailability and metabolism in rats and dogs (vol. 1.6, RR-845-93-018, vol. 1.7, RR-945-93-020, vol. 1.6/1.7, RR-845-93-022/-024) with [11-¹⁴C]LGD1069

i) Pharmacokinetics in rats

Parameter	IV(10 mg/kg)		PO(100 mg/kg)	
	M	F	M	F
C _{max} , uM	22.9	38.4	8.09	10.6
T _{max} , hr	-	-	2.67	2.5
T _{1/2} , hr	2.16	2.60	-	-
AUC, uM.hr	28.6	52.9	65.9	249
V _d , L/kg	6.2	3.44	-	-
%Bioavail.	-	-	22.7	47.0

The dose proportionality study showed that AUCs from LGD 1069 dose in range of 3 to 100 mg/kg in sesame oil solution or solution/suspension in male rats appeared roughly proportional to dose.

Plasma: a less abundant metabolite eluted at 3.6 min, while 25-35% of the peak area of the parent drug was detected at 4.15 min.

Isolated rat hepatocytes: two major peaks and three minor metabolites were detected. Two of the metabolites were mon-oxidized products. Glucuronide metabolites were not detected, although those were expected since LGD 1069 was excreted primarily via the hepatobiliary route. Those metabolites did not bind to retinoid receptor subtypes of RAR and RXR up to 300 nM.

Liver slices: Only a small percentage of the parent drug was metabolized during 18-hr course. One of the three peaks were corresponded to a similar peak of a mono-oxidized metabolite formed in isolated hepatocytes.

ii) Pharmacokinetics in 4 male dogs

Parameter	IV(6 mg/kg)	PO(100 mg/kg)
C _{max} , uM	44.75	23.6
T _{max} , hr	-	2
AUC, uM.hr	63.7-133	58.6-243
V _d , L/kg	0.388	-
%Bioavail.	-	7.55 (5.13-11.0%)

Plasma: Putative glucuronides of LGD 1069 and its metabolites were detected.

Liver: A mono-oxidized metabolite(s) was presented.

Feces: Three metabolites were observed. Approximately 20-60% of the IV LGD 1069 was estimated to be excreted as intact drug in 48-hr period after dosing in feces.
 Urine: After IV, the concentrations of LGD 1069 were below the limit of quantitation. After po, approximately 0.04% of the administered dose appeared in urine. No metabolites after IV or PO dose were detected.

iii) Bioavailability in Babassu oil and clinical formulation in female dogs

25 mg/kg LGD 1069 in two prototype clinical formulas and babassu oil in female dogs

Parameter	Suspension	Solution	Babassu Oil	
			Fed	Fasted
C _{max} , uM	7.39	60.0	12.4	12.0
T _{max} , hr	2.0	1.3	1.17	2.17
AUC, uM.hr	42.8	236	51.7	55.8

Comments: The solution formulation produced much greater AUCs and C_{max} than the suspension and babassu oil formulations, whereas there were no significant differences between suspension and babassu oil formulations (BA_{rel} of the suspension = 83.4% of babassu oil formulation). Feeding did not affect the extent of absorption from babassu oil formulation.

B. Tissue distribution and metabolism in mice (vol. 1.7, RR-845-93-017)

Animals received 30 mg/kg oral dose of [11-¹⁴C] LGD 1069 and organ kinetics were measured.

TABLE 6. Mean Organ-to-Plasma Ratios of Nanomole Equivalents

Time (hr)	Mean organ-to-plasma ratios of nmol equivalents								
	Stomach	Duodenum	Jejunum	Small Intestine	Large Intestine	Liver	Skin	Dermis	Epidermis
0.25	468	96.1	138	83.6	25.7	40.1	11.8	1.18	8.92
0.5	183	30.0	32.8	40.4	7.58	36.5	4.74	0.683	1.34
1	134	38.4	32.3	17.8	3.95	29.0	1.42	0.848	2.78
2	180	36.2	33.0	25.4	14.3	32.7	3.84	1.33	12.0
4	100	63.0	75.8	107	32.6	28.7	1.33	0.481	0.287
8	71.8	68.8	87.0	178	238	48.5	0.573	0.570	3.22
24	537	124	211	242	737	162	8.70	3.83	20.0

TABLE 7. Kinetics of Percentages of Dose in Organs

Time (hr)	Percent of Dose								Total
	Stomach	Duodenum	Jejunum	Small Intestine	Large Intestine	Liver	Skin	Plasma	
0.25	36.3	13.8	20.6	8.84	8.98	7.34	3.18	0.224	96.2
0.5	43.7	8.12	4.90	8.07	2.74	14.6	2.80	0.636	90.3
1	38.9	18.0	10.0	4.61	1.82	13.8	1.21	0.568	87.1
2	67.3	18.4	17.1	8.12	11.2	20.7	4.88	0.848	148*
4	23.8	16.7	18.2	21.8	13.0	14.1	0.847	0.801	108*
8	8.11	8.39	8.38	11.8	34.8	8.01	0.167	0.216	78.7
24	0.546	0.244	0.451	0.432	2.10	0.778	0.044	0.010	4.60

*Total percentages of dose at 2 and 4 hr may have exceeded 100% due to some deacidization of organs and/or underestimation of cpm in the administered dose.

Accumulation of the parent drug and its metabolites occurred at later timepoints in stomach, liver and skin. In skin, epidermal concentration was approximately of 5-fold that in dermis. Homogenized liver showed time-dependent metabolism to 4 metabolites, which were also present in plasma at much lower levels.

E. Plasma protein binding (vol. 1.7, RR-845-93-025)

<u>Concentration</u> (uM)	<u>% Protein Binding</u>	
	Rat	Dog
1	99.9	100
10	99.0	100
100	99.0	100

ie, LGD 1069 almost completely binds to protein in rat and dog plasma at concentrations which occur in toxicology studies.

III. Toxicology

A. Acute oral toxicity study in rats (vol. 1.8, RR-815-93-020/-021)

animal: SD rats (5/sex/group)

dose/route/duration: a single dose of 30, 100, 250, 500, 1000 and 1500 mg/kg LGD1069 in sesame oil suspension by gavage

observation: observation period- 14-d; clinical signs- daily; body weight- d1, 3,5,7,14; necropsy- d15

GLP statement: Yes

Results: No mortality was observed. Toxicity was unremarkable except lower body weight gain in animals at 1000 and 1500 mg/kg groups (approximately 10 g less than controls, started on d1-d3, persisted till d14, and was not statistically significant).

B. Acute oral toxicity study in dogs (vol. 1.8, RR-815-93-015)

animal: beagle dogs (2/sex/group)

dose/route/duration: a single dose of 720 mg/kg LGD1069 in babassu oil suspension, given as capsules, po

observation: observation period- 14d; clinical signs- daily; body weight- d-1, 3, 5,7,14; food consumption- daily; clinical pathology- d-1, d2, d15; necropsy- d15

GLP statement: yes

Results: No mortality was observed. Decreased body weight (0.05 kg males and 0.1 kg females) up to d7, but recovered at d14. One treated female dog showed very low food intake during wk

1. No changes in hematology was observed. Clinical chemistry showed increases in phosphorus (M: 14.4-31.3%, F: 9-49.2%) and triglycerides (M: 18-163.6%, F: 24.1-129.2%), and decreases in HDL (M: 2-17%, F: 2.8-14%), LDL (M: 32.8-69.1%, F: 0.8-41.6%) and cholesterol (M: 0.7-17.9%, F: 1.5-17.9%). Necropsy showed small testes (2/2) and a single white focus on the left atrium of the heart (1/2F).

Toxicokinetics:

<u>Parameter</u>	<u>Male</u>	<u>Female</u>
Dose, mg/kg	720	720
C _{max} , ng/ml	35527-41384	51808-69740
AUC _{0-∞} , ug.hr/ml	388.7- 459.3	528.3-600.9
T _{max} , hr	3	2
T _{1/2γ} , hr	3.39-9	4.38-6.03

C. 28-Day repeat dose oral toxicity study in rats (vol. 1.9, RR-815-93-022)

animal: SD rats (15/sex/group)

dose/route/duration: 1, 5 and 30 mg/kg/d LGD1069 in sesame oil by gavage for 28 days

observation: observation period- 28d recovery; clinical signs- daily; body weight/food

consumption- weekly; clinical pathology- d27/29 or recovery; necropsy/

histopathology- at the end of treatment or recovery

GLP statement: yes

Results:

Mortality: 11 at 30 mg/kg group (M: 10/19, F: 1/19, d27), 10 at 100 mg/kg group (M: 8/19, F: 2/19, d23) and 15 at 150 mg/kg group (M: 14/19, F: 1/19, d21), indicating highly gender-dependent risks.

30 mg/kg group: ruffled coat, pale mucous membrane, rales, labored breathing, lethargy and red/black discharge from the nose; significant decrease in body weight gains during wk 5 (20 vs 7.1 g); biologically relevant increases in cholesterol, triglycerides, ALT, AST, ALK, HDL and albumin, and decrease in globulin; increased platelet count (M: 1154 vs 1409, F: 1143 vs 1737); increases in absolute liver and adrenal weights (M/F) with increase in hepatocellular glycogen and adrenocortical cellular hypertrophy in adrenal gland, increase in absolute ovarian weights (F) with no microscopic lesions, hemorrhagic lesions (ie, dark, red or purple discoloration of tissue) and edema/foci in esophagus (hemorrhage and perforation, M/F), thymus, mesenteric lymph node, salivary gland, skeletal muscle and skin (subcutaneous edema of skin of neck, 5/10M), pale discoloration (as a result of decreased RBC due to hemorrhage) in the spleen, liver, heart and kidney, increased extramedullary hematopoiesis in the spleen, centrilobular necrosis in the liver, acanthosis and hyperkeratosis of the skin, osteopathy, and gastropathy of the forestomach.

100 mg/kg group: ruffled coat, chest/neck hair loss, emaciation, pale mucous membrane, rales, labored breathing, lethargy and red/black discharge from the nose; significant decrease in body weight gains during wk 4 (19.7 vs 4.0 g); biologically relevant or statistically significant increases in cholesterol, triglycerides, albumin, HDL, ALT, AST and ALK, and decreases in K, phosphorus and globulin; increased platelet count (M: 1154 vs 1712, F: 1143 vs 1542), and decreases in RBC (M: 8.9 vs 6.9, F: 8.2 vs 7.4), hemoglobin (M: 16.4 vs 12.8, F: 15.0 vs 13.2) and hematocrit (M: 51.1 vs 41.5, F: 48.2 vs 43.1); increases in absolute liver and adrenal weights (M/F) with increase in hepatocellular glycogen and adrenocortical cellular hypertrophy in adrenal gland, increase in absolute ovarian weights (F) with no microscopic lesions, hemorrhagic lesions (ie, dark, red or purple discoloration of tissue) and edema/foci in esophagus (hemorrhage and perforation, M/F), thymus, mesenteric lymph node, salivary gland, skeletal muscle and skin (subcutaneous edema of skin of neck, 5/10M), pale discoloration (as a result of decreased RBC due to hemorrhage) in the spleen, liver, heart and kidney, increased extramedullary hematopoiesis in the spleen, centrilobular necrosis in the liver, acanthosis and hyperkeratosis of the skin, osteopathy, mild cardiomyopathy (focal myofiber degeneration and necrosis of the right ventricle of the heart, 2/8M) and gastropathy of the forestomach.

150 mg/kg group: ruffled coat, chest/neck/head hair loss, emaciation, pale mucous membrane, rales, labored breathing, lethargy and red/black discharge from the nose; significant decrease in body weight gains during wk 3 (27.6 vs 9.8 g); biologically relevant or statistically significant increases in cholesterol, triglycerides, albumin, total bilirubin, HDL, ALT, AST and ALK, and decreases in K, phosphorus and globulin; increased platelet count (M: 1154 vs 1853, F: 1143 vs 1758), and decreases in RBC (M: 8.9 vs 6.7, F: 8.2 vs 7.7), hemoglobin (M: 16.4 vs 12.6, F: 15.0 vs 13.8) and hematocrit (M: 51.1 vs 40.6, F: 48.2 vs 44.8); increases in absolute liver and adrenal weights (M/F) with increase in hepatocellular glycogen and adrenocortical cellular hypertrophy in adrenal gland, increase in absolute ovarian weights (F) with no microscopic lesions, hemorrhagic lesions (ie, dark, red or purple discoloration of tissue) and edema/foci in esophagus (hemorrhage and perforation, M/F), thymus, mesenteric lymph node, salivary gland, skeletal muscle and skin (subcutaneous edema of skin of neck, 5/10M), pale discoloration (as a result of decreased RBC due to hemorrhage) in the spleen, liver, heart and kidney, increased extramedullary hematopoiesis in the spleen, centrilobular necrosis in the liver, acanthosis and hyperkeratosis of the skin, osteopathy, mild cardiomyopathy (focal myofiber degeneration and necrosis of the right ventricle of the heart), and gastropathy of the forestomach.

Coagulation Profiles (seconds):

Group	Prothrombin Time		Activated Partial Thromboplastin Time	
	Male	Female	Male	Female
control	16.1	14.1	23.2	19.3
30 mg/kg	26.0	16.0	54.3	25.4
100 mg/kg	29.5	27.1	53.6	33.5
150 mg/kg	28.4	20.4	76.9	27.9

Hemorrhagic incidence in various tissues based on gross and histopathology:

Tissue	0 mg/kg		30 mg/kg		100 mg/kg		150 mg/kg	
	M(10)	F(10)	M(10)	F(10)	M(8)	F(12)	M(14)	F(10)
Lung	10	0	7	1	5	1	8	2
thyroid gl.	0	0	9	0	6	0	12	0
trachea	0	0	3	0	5	0	8	0
esophagus	0	0	9	2	8	8	14	3
spleen	0	0	2	0	3	0	6	0
thymus	0	0	10	0	7	6	10	2
liver	0	0	4	0	3	1	5	0
testes	0	0	2	0	3	0	2	0
epididymis	0	0	5	0	8	0	11	0
mesentericLN	0	0	10	0	6	8	14	8
mandibularLN	0	1	7	0	7	1	11	1
skin, s.c.	0	0	4	0	2	1	8	0
bone	0	0	1	0	1	0	3	0
skeletal muscl	0	0	5	0	4	0	6	0
forestomach	0	0	0	0	1	0	3	0

Toxicokinetics:

Parameter	30 mg/kg			100 mg/kg			150 mg/kg		
	d1	d14	d28	d1	d14	d28	d1	d14	d28
Cp, uM M	6.72	4.45	na	16.41	5.52	6.11*	18.26	5.95	na
F	5.26	2.21	2.17	13.79	8.92	5.18	18.43	9.23	4.27

(AUC_{0-∞}, uM.hr following 100 mg/kg po (5d/wk x 5 wk) 132 on d1 and 25.9 on d33)

* measured on d22.

Comments: Target toxic organs of LGD1069 were liver (rarefaction, necrosis, elevated liver enzymes), adrenals (hypertrophy), spleen (extramedullary hematopoiesis), heart (mild cardiomyopathy), skin (dermatopathy: acanthosis and hyperkeratosis), esophagus/forestomach (inflammation, gastropathy), ovary (hypertrophy) and bones (osteopathy). Widespread hemorrhage was observed in numerous tissues in terminal sacrifice males and females in all treated groups, however, hemorrhagic incidence at various tissues in males was much higher than that in females as summarized in the above table. Prothrombin time and APTT increased significantly.

LD₁₀ of LGD1069 in female rats was 100 mg/kg/d and LD₅₀ in male rats was 30 mg/kg/d (the low dose), indicating that male rats were much more sensitive to the drug. However, the plasma concentrations from each dose level were not much different between male and female rats. Based on this study, no safe starting dose can be determined, unless only female patients are

selected for the proposed study.

D. 28-Day repeat dose oral toxicity study in rats (interim report, IT-001 supplement)

animal: SD rats (15 male/group + 4 males/group for pharmacokinetics)
dose/route/duration: 3, 10, 30 and 100 mg/kg/d LGD1069 in sesame oil by gavage for 28d
observation: observation period- 28d recovery; clinical signs- daily; body weight/food
consumption- weekly; clinical pathology- d27/29 or recovery; necropsy/
histopathology- at the end of treatment or recovery
GLP statement: yes

Results: Histopathology results not submitted.

Mortality: 2 at 10 mg/kg (2/19), 7 at 30 mg/kg (7/19) and 13 at 100 mg/kg (13/19).

3 mg/kg group: discharge in the eye; unremarkable in body weight and food consumption; decrease in WBC (15.5 vs 13.0) and increase in platelets (1147 vs 1481); increases in cholesterol (76 vs 92), ALT (29 vs 40) and AST (65 vs 77); gross pathology unremarkable except increases in weights of adrenal glands and liver.

10 mg/kg group: discharge in the eye, dried blood in nose/mouth (7/15)/foot/paw; unremarkable in body weight and food consumption; decrease in WBC (15.5 vs 12.7) and increase in platelets (1147 vs 1469); increases in cholesterol (76 vs 102), triglyceride (69 vs 103), ALT (29 vs 56), AST (65 vs 77), ALK (158 vs 184) and CK (236 vs 454); gross pathology unremarkable except red foci in esophagus (1/10) and increased adrenal gland and liver weights.

30 mg/kg group: discharge in the eye (5/15), dried blood in nose/mouth (9/15)/foot/paw, ruffled coat (5/15), pale mucous membranes (5/15), laceration (7/15), and alopecia (3/15); unremarkable in body weight and food consumption; decreases in RBC (8.86 vs 7.72), hemoglobin (16.4 vs 13.8) and WBC (15.5 vs 12.2), and increases in PMN (11 vs 20) and platelets (1147 vs 1766); increases in cholesterol (76 vs 98), triglyceride (69 vs 179), ALT (29 vs 53), AST (65 vs 83), and ALK (158 vs 234), and decrease in LDL (4.4 vs -15.5); increased adrenal gland and liver weights, red foci in epididymis (2/12), esophagus (3/12), eyes (2/12) and thymus, and pale kidneys (3/12), liver (4/12) and spleen (3/12).

100 mg/kg group: discharge in the eye (6/15), dried blood in nose/mouth (12/15)/foot/paw (5/15), urine stain (7/15), ruffled coat (3/15), pale mucous membranes (9/15), emaciation (3/15), lethargy (1/15), and laceration (6/15); unremarkable in body weight and food consumption; decreases in RBC (8.86 vs 6.07), hemoglobin (16.4 vs 11.0) and WBC (15.5 vs 9.3), and increases in PMN (11 vs 39) and platelets (1147 vs 1583); increases in glucose (119 vs 319), BUN (12 vs 20), globulin (1.9 vs 2.4), cholesterol (76 vs 153), triglyceride (69 vs 788), total bilirubin (0.1 vs 0.3), ALT (29 vs 197), AST (65 vs 183), ALK (158 vs 384) and CK (236 vs 734), and decreases in LDL (4.4 vs -84.2) and phosphate (9.5 vs 8.5); increased adrenal gland and liver weights, red

foci in epididymis (7/15), esophagus (6/15), eyes (3/15), pancreas (4/15) and thymus (5/15), pale kidneys (3/15), liver (6/15) and spleen (4/15), red/purple testes (10/15), red foci/fluid in thoracic cavity, and red mandibular and mesenteric lymph nodes (2/15 each).

Coagulation:

<u>Dose</u> mg/kg	<u>PT</u> sec	<u>APTT</u> sec	<u>Fibrinogen</u> mg/dl
0	15.6	40.6	331
3	29.1	66.2	441
10	53.4	102.2	450
30	77.2	-	578
100	111	-	911

Comments: LD₁₀ in male rats from this study appears to be 10 mg/kg/d (60 mg/m²/d x 28d, qd, po). Therefore, based on this study, the safe suggest starting dose (ie, 1/10th of LD₁₀) will be 6 mg/m²/d for 28 d study.

One of the most significant, dose-dependent toxicities is hemorrhage, indicated by higher PT and APTT values, in various tissues such as esophagus, testes, abdominal cavity, pancreas, and thymus (red foci). Major toxic target organs are the liver and kidneys as demonstrated in clinical pathology and gross pathology.

E. 28-Day repeat dose oral toxicity study in dogs (vol. 1.12, RR-815-93-016)

animal: beagle dogs (6/sex/group)

dose/route/duration: 10, 30, 100 and 200 mg/kg/d LGD1069 in babassu oil suspension in capsules by po

observation: observation period- 28 d recovery; clinical signs- 2/d; body weight/food consumption- weekly/daily; clinical pathology- d 1, d22 and at sacrifice; necropsy/histopathology- d 29 and at the end of recovery;

GLP statement: yes

Results:

Mortality: 1 at 30 mg/kg group (M: 1/6 on d29), 3 at 100 mg/kg group (M: 2/6, F: 1/6; all animals sacrificed on d22 due to deteriorating conditions) and 3 at 200 mg/kg group (F: 3/6; all animals sacrificed on d22 due to deteriorating conditions).

10 mg/kg group: diarrhea, dry flaky skin, erythema, emaciation, ruffled coat; increase in globulin and decrease in albumin; increase in absolute liver weight.

30 mg/kg group: diarrhea, dry flaky skin, erythema, emaciation, ruffled coat, oily fur, conjunctivitis; lost weight (F: 0.3 kg); decreases in RBC, hemoglobin and MCH in females; increases in globulin (M), AST (F) and ALK(F), decreases in albumin (M/F), cholesterol (M/F), HDL (F) and LDL (M); increase in absolute liver weight, red or purple foci in the stomach and intestines, purple/red foci of the heart; diffuse hepatocellular degeneration diffuse hepatocyte vacuolation and deposition of brown (bile) pigment in the hepatocytes (M), minimal to mild tubular changes, tubular regeneration and tubular mineralization in the kidneys (M/F), mucosal hyperplasia, and degenerative changes and lymphoid depletion or vacuolation of lymphoid follicles in the GIT, and aspermatogenesis in testes, epididymides and prostate (1M).

100 mg/kg group: diarrhea, dry flaky skin, erythema, emaciation, ruffled coat, oily fur, lethargy, conjunctivitis; lost weight (M: 0.3 kg) and significant decrease in food consumption during week 1 and 2 (M/F); decrease MCH in F; increases in globulin (M/F) and ALK(F), decreases in albumin (M/F), cholesterol (M/F), HDL (M/F), LDL (M) and phosphorus (F); increase in absolute liver weight, increase in absolute liver weight, red or purple foci in the stomach and intestines, purple/red foci of the heart; diffuse hepatocellular degeneration diffuse hepatocyte vacuolation and deposition of brown (bile) pigment in the hepatocytes (M/F), minimal to mild tubular changes, tubular regeneration and tubular mineralization in the kidneys (M/F), mucosal hyperplasia, and degenerative changes and lymphoid depletion or vacuolation of lymphoid follicles in the GIT, aspermatogenesis in testes, epididymides and prostate (2M), thymic lymphoid depletion (M/F), and high level keratohyalin granules in the surface epithelium of the skin.

200 mg/kg group: diarrhea, dry flaky skin, erythema, emaciation, ruffled coat, oily fur, lethargy, conjunctivitis; lost weight (F: 0.8 kg) and significant decrease in food consumption during week 1 (M/F); decreases in RBC, hemoglobin and MCHC in females; increases in BUN (M), globulin (M), ALT (M), GGT (M) and ALK(M), decreases in albumin (M/F), cholesterol (M/F), HDL (F), LDL (M) and phosphorus (F); significant decrease in ovary weight, red or purple foci in the stomach and intestines, purple/red foci of the heart; diffuse hepatocellular degeneration diffuse hepatocyte vacuolation and deposition of brown (bile) pigment in the hepatocytes, minimal to mild tubular changes, tubular regeneration and tubular mineralization in the kidneys (M/F), mucosal hyperplasia, and degenerative changes and lymphoid depletion or vacuolation of lymphoid follicles in the GIT, aspermatogenesis in testes, epididymides and prostate (1M), thymic lymphoid depletion (M/F), minimal to mild hemorrhage, degeneration and mineralization in the heart, and high level keratohyalin granules in the surface epithelium of the skin.

Coagulation Profiles (seconds):

Group	Prothrombin Time		Activated Partial Thromboplastin Time	
	Male	Female	Male	Female
control	8.7	8.4	11.9	11.4
10 mg/kg	9.6	9.4	10.8	10.9
30 mg/kg	10.7	9.4	11.4	11.4
100 mg/kg	11.1	9.9	10.8	11.6

200 mg/kg 12.7 8.3 13.2 10.8

Toxicokinetics of 720 mg/kg/d dose from 2-week toxicity study in dogs:

<u>Parameter</u>	<u>Male</u>	<u>Female</u>
C _{max} , uM	110.50	174.64
T _{max} , hr	3	2
T _{1/2} , hr	6.12	5.2
AUC, uM.hr	1218.57	1622.51

<u>Dose</u> kg/mg/d	<u>AUC (0-12h) (uM.hr)</u>		<u>C_{max} (uM)</u>		<u>T_{max} (hr)</u>	
	d1	d28	d1	28	d1	28
10 M	8.2	4.5	1.19	0.68	2	2
	F	5.0				
30 M	19	2.4	4.03	0.57	2	2
	F	19				
100 M	95	7.4	8.45	0.27	3	12
	F	66				

Comments: Target toxic organs in dogs are liver (hepatocellular degeneration, vacuolation and pigment deposition, AST, ALT, ALK), kidneys (tubular dilation, mineralization, BUN), GIT (congestion, hemorrhage, mucosal hyperplasia and degeneration), heart (degeneration and mineralization), and skin.

Pharmacokinetic results showed that there were dose-proportional increases in AUC and treatment-duration-dependent decrease in AUC, as observed in other retinoids such as ATRA and 9-cis-RA.

Non-lethal dose as well as TDL in dogs is 10 mg/kg/d (200 mg/m²/d) and LD₁₀ is approximately 30 mg/kg (600 mg/m²/d). TDH can not be determined from this study. Based on this study, the safe starting dose will be 66 mg/m²/d for 28d study.

Summary and Evaluation

A. Pharmacology

Unlike ATRA and 9-cis-RA, LGD 1069 specifically interacts with retinoid receptors RXR and increases RXR-mediated transcriptional activation as summarized below:

Receptor Transactivation Mean EC ₅₀ (nM)			Receptor Receptor Mean Kd (nM)			
Subtype	LGD 1069	9-cis-RA	Subtype	LGD 1069	9-cis-RA	ATRA
RAR α	>10,000	191	RAR α	>10,000	15.2	15.4
RAR β	>10,000	51	RAR β	>10,000	13.4	13.2
RAR γ	>10,000	45	RAR γ	>10,000	14.7	18.0
RXR α	25	253	RXR α	30	7.0	>10,000
RXR β	27	221	RXR β	14	7.0	>10,000
RXR γ	19	147	RXR γ	15	17	>10,000

LGD 1069 induces apoptotic changes in cell lines such as HL 60 and ME-180 at submicromolar concentrations and causes tumor regression in human head and neck squamous cell carcinoma and cervical carcinoma xenografts in nude mice at daily dose of 10-100 mg/kg.

B. Pharmacokinetics

The pharmacokinetics of LGD 1069 in rats and dogs were determined as follows:

Rats (10 mg/kg iv and 100 mg/kg po):

Parameter	IV		PO	
	M	F	M	F
Cmax, uM	22.9	38.4	8.09	10.6
Tmax, hr	-	-	2.67	2.5
T _{1/2} , hr	2.16	2.60	-	-
AUC, uM.hr	28.6	52.9	65.9	249
Vd, L/kg	6.2	3.44	-	-
%Bioavail	-	-	22.7	47.0

Male Dogs

Parameter	IV(6 mg/kg)	PO(100 mg/kg)
Cmax, uM	44.75	23.6
Tmax, hr	-	2
AUC, uM.hr	63.7-133	58.6-243
Vd, L/kg	0.388	-
%Bioavail	-	7.55 (5.13-11.0%)

Dogs:

Parameter	Male	Female
Dose, mg/kg	720	720
Cmax, ug/ml	35.5-41.3	51.8-69.7
AUC _{0-∞} , ug.hr/ml	388.7-459.3	528.3-600.9
Tmax, hr	3	2
T _{1/2} , hr	3.39-9	4.38-6.03

There are significant differences in AUC of male and female rats (ie, AUC of female rats is approximately 2-fold for iv and 4-fold for po higher than that of male rats, respectively), although Cmax and T_{1/2} are significantly different between male and female rats. The AUC difference could explain, in part, the significantly increased mortality observed in male rats at the same dose levels in addition to vitamin K deficiency syndrome only developed in male rats indicated by higher PT and APTT.

Bioavailabilities in male and female rats are 22.7% and 47%, respectively, whereas bioavailability in male dogs is 7.55%. The extent of bioavailability of LGD 1069 is similar to that of 9-cis-RA (ie, <20% in rats and 5% in dogs). Plasma protein binding of LGD 1069 is 99.9% in both rat and dog plasma. Tissue distribution and metabolism study shows that LGD 1069 accumulates in stomach, liver and skin and that there are 4 metabolites including 2 mon-oxidized metabolites. LGD 1069 is excreted primarily via the hepatobiliary route.

C. Toxicology

Acute oral toxicity studies in rats and dogs suggested that LGD 1069 did not cause death of animals up to 1500 mg/kg in rats and 720 mg/kg in dogs. In 28-day toxicity studies in rats and dogs, LD10 was determined to be 10 mg/kg/d in male rats and 30 mg/kg/d in female rats, whereas TDL (TDH could not be determined) and LD10 in dogs were 10 mg/kg/d and 30 mg/kg/d, respectively.

Target toxic organs of LGD1069 in rats were liver (rarefaction, necrosis, elevated liver enzymes), adrenals (hypertrophy), spleen (extramedullary hematopoiesis), heart (mild cardiomyopathy), skin (dermatopathy: acanthosis and hyperkeratosis), esophagus/forestomach (inflammation, gastropathy), ovary (hypertrophy) and bones (osteopathy). Widespread hemorrhage was observed in numerous tissues in terminal sacrifice males and females in all treated groups, however, hemorrhagic incidence at various tissues in males was much higher than that in females. Prothrombin time and APTT increased significantly as summarized below:

Group	Prothrombin Time		Activated Partial Thromboplastin Time	
	Male	Female	Male	Female
control	16.1	14.1	23.2	19.3
30 mg/kg	26.0	16.0	54.3	25.4
100 mg/kg	29.5	22.1	53.6	33.5
150 mg/kg	28.4	20.4	76.9	27.9

Target toxic organs in dogs are liver (hepatocellular degeneration, vacuolation and pigment deposition, AST, ALT, ALK), kidneys (tubular dilation, mineralization, BUN), GIT (congestion, hemorrhage, mucosal hyperplasia and degeneration), heart (degeneration and mineralization), and skin. PT and APTT were not significantly affected by LGD 1069 and thus no widespread hemorrhagic incidence was observed, except foci in GIT.

Sex-dependent sensitivity to LGD 1069 was observed only in rats (not in dogs). Dose-dependent, significant increases in PT and APTT were observed in male rats and much widespread hemorrhagic incidence was observed only in male rats as summarized below:

Tissue	0 mg/kg		30 mg/kg		100 mg/kg		150 mg/kg	
	M(10)	F(10)	M(10)	F(10)	M(8)	F(12)	M(14)	F(10)
Lung	10	0	7	1	5	1	8	2

thyroid gl.	0	0	9	0	6	0	12	0
trachea	0	0	3	0	5	0	8	0
esophagus	0	0	9	2	8	8	14	3
spleen	0	0	2	0	3	0	6	0
thymus	0	0	10	0	7	6	10	2
liver	0	0	4	0	3	1	5	0
testes	0	0	2	0	3	0	2	0
epididymis	0	0	5	0	8	0	11	0
mesentericLN	0	0	10	0	6	8	14	8
mandibularLN	0	1	7	0	7	1	11	1
skin, s.c.	0	0	4	0	2	1	8	0
bone	0	0	1	0	1	0	3	0
skeletal muscl	0	0	5	0	4	0	6	0
forestomach	0	0	0	0	1	0	3	0

Similar gender-dependent toxicity in rats was observed with butylated hydroxytoluene (BHT), ie, BHT-induced hemorrhagic death only in male rats (hemorrhagic death at 693 mg/kg/d x 3wk in male, but not significant physiological changes at 1000 mg/kg/d x 3 wk in female rats) (Ed. Cosmet. Toxicol. 18:229-235, 1080). This phenomenon (bleeding and death in male sex) has been also observed with hypervitaminosis A: vitamin K deficiency syndrome in male rats due to interference of vitamin K absorption by vitamin A. In male rats treated with BHT, vitamin K1 or K2, but not K3 supplement completely corrected the clinical hemorrhage (Toxicol. Applied Pharmacol. 50:261-266, 1079). In beagle dogs, BHT had only a minor effect on PT but not APTT at 760 mg/kg/d x 2 wk and no hemorrhagic death in male dogs, as observed in the 28-day toxicity study in dogs conducted by the sponsor. The submitted toxicity studies and published articles indicate that rats are more sensitive than other species to vitamin K deficiency (since rats have an absolute requirement for dietary vitamin K) and that male rats are more susceptible than female rats to the deficiency, probably due to a protective effect of estrogen which may enhance vitamin K absorption (Am. J. Clin. Nutr. 9:109-116, 1961; Am. J. Physiol. 232: H12-H17, 1977). In humans, total elimination of dietary vitamin K (in adults only) does not produce vitamin K deficiency (J. Amer. Dietetic. Asso. 92:585-590, 1992). Interestingly, drug-induced vitamin K deficiency in vivo is correctable by vitamin K1 or K2 intake, but not by K3, whereas nutritional vitamin K deficiency is corrected by administration of either vitamin K1, K2 or K3.

D. Overall Summary

Based on the submitted 28-day toxicity studies, LD10 is 10 mg/kg/d (60 mg/m²/d) in male rats and 30 mg/kg/d (180 mg/m²/d) in female rats, whereas TDL (TDH is not available) in dogs is 10 mg/kg/d (200 mg/m²/d). 1/10th of LD10 in rats and 1/3 of TDH in dogs are 6 mg/m²/d (male rats), 18 mg/m²/d (female rats), and 66 mg/m²/d (dogs), respectively. Therefore, the safe suggested starting dose for human trial will be 6 mg/m²/d. The sponsor proposes the study starting with 5 mg/m²/d.

Since LGD 1069 may alter the coagulation time as observed in animal toxicity study, the

sponsor should carefully monitor PT and APTT in patients. Furthermore, LGD 1069 (and also 9-cis-RA) induces cardiomyopathy/degeneration of heart in animals (both rodents and dogs), careful monitoring of cardiac function is required.

Recommendation

The proposed study appears to be safe to initiate with starting dose as proposed or at 6 mg/m²/d level.

Memo to MO: Since LGD 1069 may alter the coagulation time as observed in animal toxicity study, the sponsor should carefully monitor PT and APTT in patients. Furthermore, LGD 1069 (and also 9-cis-RA) induces cardiomyopathy/degeneration of heart in animals (both rodents and dogs), careful monitoring of cardiac function is required.

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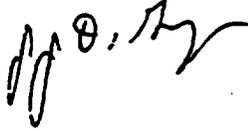
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