CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 21055

PHARMACOLOGY REVIEW(S)
Division of Oncology Drug Products, HFD-150
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review No. 1
Keywords: Targretin, bexarotene, RXR agonist, NDA

NDA #: 21-055
Serial #: 000 Type: NDA Letter Dated: 6/22/99 Received by CDR: 6/23/99

Information to be conveyed to the sponsor: Yes
Reviewer: Chang H. Ahn, Ph.D.
Review Completion Date: December 10, 1999
Sponsor: Ligand Pharmaceuticals Inc. San Diego, CA
Manufacturer:

Drug:
Code Name: LG100069, LGD1069
Generic Name: bexarotene
Trade Name: Targretin® capsules
Secondary therapies: none
Chemical Name: 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthaleneyl)vinyl]benzene carboxylic acid
CAS Registry Number: 153559-49-0
Molecular formula/weight: C_{24}H_{28}O_{2} (348.48)

Structure:

Related INDs, NDAs, DMFs: IND

Drug Class: Antineoplastic agent (retinoid analogue with RXR selectivity)
Indication: The treatment of patients with cutaneous manifestations of refractory or persistent early and refractory advanced stage CTCL.

Clinical Formulation: Ingredient Amount (mg/capsule)
Bexarotene 75.00
PEG 400, NF
Polysorbate 20, NF
Povidone (K-90), USP
Butylated hydroxyanisole, NF
Gelatin, NF
Sorbitol Special-glycerin blend
Titanium oxide, USP trace

Dosage and administration: 300 mg/m²/day taken as a single oral daily dose with a meal for up to 97 weeks for CTCL patients.
Studies Reviewed within this submission:

**Toxicology**
1. 28-Day oral toxicity study in rats (vol. 1.16, pp 2-409, RR-815-94-013)
2. 91-Day oral toxicity study in dogs (vol. 1.30, pp 105, to vol. 1.32, RR-815-95-003)
3. 6-Month oral toxicity study in rats (vol. 1.21, pp 2, to vol. 1.27, RR-815-98-004)
4. 6-Month oral toxicity study in dogs (vol. 1.33, pp 2- to vol. 1.36, RR-815-98-003a)

**Pharmacology**

**Mechanism of Action**
1. Transcriptional activation and receptor binding profile (vol. 1.10, pp276-341, RR-750-93-013-004/011)
2. Repression of AP-1 activity (vol. 1.10, pp 390-397, RR-750-93-011)
3. Regulation of Fas-ligand expression and activation-induced T-cell apoptosis (vol. 1.11, pp 2-21, RR-750-98-002)

**In vitro efficacy studies**
1. Growth inhibitory effects on carcinoma cells of breast, head/neck and on primary tumor samples (vol. 1.10, pp398, to 1.11, pp38-80, RR-750-98-009/014, 93-013)
2. Induction of cell cycle arrest and differentiation in NB4 APL cells (vol. 1.11, pp 22-37, RR-750-98-003)

**In vivo/Ex vivo studies**
2. Effects on mammary carcinoma in the NMU-induced rat mammary tumor model (vol. 1.11, pp 160-223, RR-750-98-001/004/010)
3. Antikeratinizing activities in the rhino mouse (vol. 1.11, pp 146-159, RR-740-93-002)

**Safety Pharmacology**

**Pharmacokinetics**
1. Relative bioavailability of micronized LGD 1069 in sesame oil and in 10% PEG/Aqueous formulation in male rats (vol. 1.43, pp 207- , RR-845-96-003a)
3. Single and repeat dose oral pharmacokinetics in rats (vol. 1.45, pp2-33, RR-845-98-007)
4. Three-way crossover oral bioequivalence study of two clinical formulations in dogs (vol. 1.46, pp83-163, RR-845-94-011)
5. Five-way crossover oral study in female beagle dogs (vol. 1.46, pp 205-315, RR-845-98-014)
7. Effects on hepatic microsomal cytochrome P450 and in vitro metabolism (vol. 1.48, pp 2-20, RR-845-98-009)

**Reproductive Toxicology**
1. Oral developmental toxicity study in rats (vol.1.38, pp 134- , RR-815-97-003b)
2. Oral dose-range development toxicity study in rats (vol. 1.39, pp 2-260, RR-915-97-004a)

**Genetic Toxicology**
2. CHO chromosome aberration assay (vol. 1.40, pp 66-141, RR-815-97-008)
4. In vivo bone marrow micronucleus assay in mice (vol. 1.41, pp 2-163, RR-815-97-010)

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

Studies Not Reviewed within this submission:
Toxicology
1. 5-Week repeated dose toxicity study in female rats (vol. 1.17, pp 2-97, RR-815-93-019a)
2. Effects in male rats via feed for 37 days (vol. 1.17, pp 98-125, RR-815-93-018a)
3. 6-Month oral toxicity study in rats: 3-Month interim report (vol. 1.18-1.20, RR-815-97-016)
4. 30-Day repeated dose oral range-finding study in dogs (vol. 1.30, pp 2-104, RR-815-93-017)
5. 28-Day dermal toxicity study in rats (vol. 1.37, pp 2-2, RR-94-008)
6. 28-Day dermal toxicity study in rats (0.1% and 1% gel containing DEET) (vol. 1.38, pp 2-36, RR-815-98-005a)
7. Dermal sensitization study in guinea pigs (Buehler's technique modified) (vol. 1.38, pp 37-108, RR-815-94-009)
8. Guinea pig primary skin irritation for LGD 1069 and Retin-A 0.1% cream (vol. 1.38, pp 109-132, RR-815-94-010)

Pharmacology
1. Development of leiomyoma in the Eker rat uterine fibroid model (vol. 1.11, pp 224-234, RR-750-98-007)

Pharmacokinetics
1. Relative bioavailability study in dogs (vol. 1.46, pp 166-204, RR-845-98-013)
2. Pharmacokinetics of topical LGD1069 in male and female rats (vol. 1.48, pp 167-207, RR-845-94-008a)

Studies Previously Reviewed: The following studies were reviewed under IND[ ]
Pharmacology
1. Transcriptional activation (vol. 1.4, RR-750-93-004)
2. Retinoic acid receptor binding profile (vol. 1.4, RR-750-93-013)
3. 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)
4. Effects on human head and neck squamous cell carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-014)
5. Effects on human cervical carcinoma xenografts in nude mice (vol. 1.5, RR-740-93-015)
6. Effects on cardiovascular and central nervous systems (vol. 1.5, RR-740-93-006/-007)

Pharmacokinetics
1. Toxicokinetics in rats (vol. 1.7, RR-845-93-021)
2. Toxicokinetics in dogs (vol. 1.6, RR-845-93-023)
3. Bioavailability in rats (vol. 1.6, RR-845-93-018)
4. Bioavailability and metabolism in dogs (vol. 1.6/1.7, RR-845-93-022/-024)
5. Tissue distribution and metabolism in mice (vol. 1.7, RR-845-93-017)
6. Metabolism in rats (vol. 1.7, RR-945-93-020)
7. Plasma protein binding (vol. 1.7, RR-845-93-025)

Toxicology
1. Acute oral toxicity study in rats (vol. 1.13, RR-815-93-020/-021)
2. Acute oral toxicity study in dogs (vol. 1.13, RR-815-93-015)
3. 28-Day repeat dose oral toxicity study in rats (vol. 1.14, RR-815-93-022)
4. 28-Day repeat dose oral toxicity study in rats (interim report, vol. 1.16, RR-815-94-013)
5. 28-Day repeat dose oral toxicity study in dogs (vol. 1.12, RR-815-93-016)

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

**INTRODUCTION AND DRUG HISTORY**

Targetretin is a synthetic retinoid analogue that is claimed to selectively activate retinoid X receptors. Targetretin is the first among RXR-specific retinoids for which an NDA has been submitted. RAR-specific retinoids (e.g., all-trans retinoic acid, 13-cis-retinoic acid) and pan-RAR/RXR agonist (e.g., 9-cis-retinoic acid) are either NDA-approved or in clinical trials for oncologic indications. Targetretin has demonstrated its antitumor activity against squamous cell carcinoma xenografts in nude mice and carcinogen-induced mammary tumors in rats. It has also shown to possess antikeratinizing activity in rhino mice. The sponsor initiated clinical trials and conducted two open-label, multicenter, multinational, historically-controlled, phase II/III studies in patients with CTCL. Based on the results of these phase II/III studies, the sponsor seeks an NDA approval for patients with refractory or persistent early and refractory advanced stage CTCL.

**PHARMACOLOGY**

**Mechanism of Action**

1. Transcriptional activation and receptor binding profile (vol. 1.10, pp276-341, RR-750-93-013/-004/011)

- **Transactivation-** The recombinant DNA constructs were transiently transfected into sub-confluent (about 70%) CV-1 cells by calcium-phosphate coprecipitation. Following transfection, all subsequent steps were performed on a Biomek Automated Workstation (Beckman). Medium containing the DNA was removed from transfected cells after six hours; cells were washed and LG1069 (10^-12 - 10^-5 M) was added. After 38 hours the cells were washed and then lysed with 0.5% Triton-X 100 and assayed for luciferase and B-galactosidase activities using a luminometer and ELISA plate reader. The EC50 was determined graphically. Receptor expression plasmids used in the cotransfection assay included pRS-hRARa, pRS-hRARb, pRS-hRARb, pRS-hRARa, pRS-hRARb, pRS-hRARb, and pRS-hRARb.

- **Receptor binding-** Si21 cells (1.2 x 10^6 cells/ml) transfected with vectors containing receptor DNA encoding hRARa, hRARb, hRARb, mRARa, mRARb, or mRARb were grown in suspension culture, harvested and washed by centrifugation (1000 x g, 10 min, 4°C). Cell pellets were suspended in lysis buffer (10 mM Tris, pH 7.5, 5 mM DTT, 2 mM EDTA, 1 mM PMSF, 1 uM/ml aprotinin, 1 uM/ml leupeptin) and homogenized. Lysates were obtained by centrifugation at 100,000 x g for 1 hour at 4°C. The final volume for binding assays was 250 ul (10-40 ug extract protein, 5 nM [3H]-ATRA or 10 nM [3H]-9-cis-RA plus varying concentrations of competing LG1069 (0 - 10^-5M) in 96-well minitube system. Incubations were carried out at 4°C until equilibrium was achieved. At the end of the incubation period, 50 ul of 6.25% hydroxyapatite was added in the wash buffer (100 mM KCl, 10 mM Tris HCl, either 5 mM CHAPS (for RXRs) or 0.5% Triton X-100 (for RARs)) which binds the receptor-ligand complex. The mixture was vortexed and incubated for 30 min at room temperature, centrifuged and the supernatant removed. The hydroxyapatite pellet was washed and the amount of receptor-ligand complex determined by liquid scintillation counting. After correcting for non-specific binding, IC50 values (concentrations of competing ligand required to decrease specific binding by 50%) were determined graphically and Kd values were determined by application of the Cheng-Prussof equation.

- **GLP statement-** No

**Results-** LG1069 is a RXR-specific ligand with Kd values of 14-30 nM for RXRs (vs. 480-10,000 nM for RARs). Transactivation assays also confirmed RXR-mediated activity of LG1069 with EC50 values of 19-27 nM for RXR (vs. >10,000 nM RARs).

<table>
<thead>
<tr>
<th>Receptor</th>
<th>EC50 (nM)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RARa</td>
<td>&gt;10,000</td>
<td>6298</td>
</tr>
<tr>
<td>RARb</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>RARy</td>
<td>&gt;10,000</td>
<td>42c</td>
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<tr>
<td>RXRa</td>
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<td>30</td>
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<td>RXRb</td>
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</tr>
<tr>
<td>RXRy</td>
<td>19</td>
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</tbody>
</table>
2. Repression of AP-1 activity (vol. 1.10, pp 390-397, RR-750-93-011)

Method- Plasmid DNA constructs (a plasmid expressing RXRα under the control of a constitutive promoter) and a plasmid which expresses the reporter enzyme luciferase under the control of a conditional promoter (collagenase) containing an AP-1 responsive element) were transiently transfected into HeLa cells by calcium-phosphate coprecipitation. After removing medium containing the DNA from transfected cells after 16 h, cells were washed and LG1069 (10^{-9} to 10^{-7} M) was added. After another 16 h, AP-1 activity was induced by PMA, a phorbol ester, and cells were lysed 4-6 h later and luciferase activity was measured.

GLP Statement- No

Results- LG1069 down-regulated transcriptional regulation by AP-1 in a dose-dependent manner in cells transfected with RXRα. The results suggest that LG1069 binding to the RXRα receptor can interfere with AP-1 activated gene expression.

3. Regulation of Fas-ligand expression and activation-induced T-cell apoptosis (vol. 1.11, pp 2-21, RR-750-98-002)

Method- Apoptosis assay— (a) A T-cell hybridoma line 2B4, which rapidly expresses the Fas receptor and Fas-ligand (FasL), their interaction transduces a death signal, was stimulated to undergo activation-induced apoptosis by culturing the cells in the presence of anti-T cell receptor monoclonal antibody in the presence or absence of 1 uM LG1069. Apoptosis was determined by flow cytometry of cells exposed to propidium iodide as a vital dye. (b) The expression of functional FasL on 2B4 cells was assayed using Fas-expression L1210.fas target cells, which undergo apoptosis when exposed to FasL. 2B4 cells (1 x 10^5/well) were exposed to FasL to give the ratio of effector:target cells were 2:1, 1:1 and 1:2 and 4:1 were activated by culturing the cells on anti-CD3 antibody-coated plates in the presence or absence of 1 uM LG1069. After 4 h, 2.5x10^4 [3H]Tdr-labeled L1210.fas target cells were added per well. Controls for non-specific apoptosis included L1210 wild-type cells (Fas negative) and target cells cultured in the absence of 2B4 effector cells. The data is expressed as the percent DNA fragmentation per culture relative to controls.

GLP Statement- No

Results- LG1069 (1 uM) inhibited activation-induced apoptosis in 2B4 T-cell hybridoma cells. At 1 uM, this apoptotic activity could be produced through interaction with RARs as well as RXR, suggesting its pan-retinoid activity rather than RXR-specific activity.

a. Inhibition of activation-induced apoptosis in 2B4 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Apoptotic Cells (% total cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Un-activated 2B4 cells</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.77 (0.58)</td>
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<tr>
<td>LG1069 1 uM</td>
<td>3.6 (2.94)</td>
</tr>
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</table>
b. Inhibition of functional FasL expression on anti-CD3 activated 2B4 cells detected as lysis of L1210.fas target cells

<table>
<thead>
<tr>
<th>Treatment Effector:Target Ratio</th>
<th>L1210 wild-type and L1210.fas Target Cell Apoptosis (% Control)</th>
<th>L1210 Wild-type</th>
<th>L1210.fas</th>
<th>L1210 Wild-type</th>
<th>L1210.fas</th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
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<td>-0.99</td>
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<tr>
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<td>0.74</td>
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In vitro efficacy studies

1. Growth inhibitory effects on Kaposi’s sarcoma derived cells, and carcinoma cells of breast, head/neck and on primary tumor samples (vol. 1.10, pp343, to 1.11, pp38-80, RR-750-98-009/014, 93-007/013) method– Subconfluent monolayer cells (500 - 4 x 10^4 cells/well) were cultured in 96-well plates and incubated for 24 h in the presence of various concentrations of LG1069. The effects of LG1069 on the growth of these cells were monitored in thymidine incorporation and/or cell number. For thymidine incorporation, 1 uCi[^3]H]Thymidine (43 Ci/mmol specific activity) per well was added for 18 h. The cells were released with trypsin/EDTA and precipitated with 10% trichloroacetic acid onto glass fiber filter mass using a multi-well cell harvester. Radioactivity incorporated into DNA as a direct measurement of cell growth, was measured by liquid scintillation counting. For cell counting, the viable cell number was determined with trypan blue and a hemocytometer or a Coulter cell counter after 1-3 days culture.

GLP statement- No

Results-

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Study No.</th>
<th>Growth Inhibition (IC&lt;sub&gt;50&lt;/sub&gt; or % Inhibition at various LG1069 concentration)</th>
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<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>LG1069 (µM)</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Primary tumor samples</td>
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<tr>
<td>Primary tumor samples</td>
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</table>
2. Induction of cell cycle arrest and differentiation in NB4 APL cells (vol. 1.11, pp 22-37, RR-750-98-003) method- Cell cycle arrest- APL NB-4 cells (1-2 x 10^6 cells) were treated in the presence or absence of LG1069 (10^-10 - 10^-5 M) for 4 days and then fixed by adding 3 ml 100% ethanol for 30 min and the fixed cells were washed in 1 ml PBS containing 5 µl RNase A/T1 cocktail and incubated 30 min on ice. 1 ml of the propidium iodide solution (25 ug/ml) was added. the cells incubated overnight at 4°C and then analyzed for DNA contents by flow cytometry. Differentiation- Expression of the CD11b was used as a measure of differentiation. NB4 cells were treated with 1 uM LG1069 for 4 days and then were stained with a 10 µl phycoerythrin-conjugated mouse anti-human CD11b mAb for 45 min on ice. Cells were fixed in 1 ml paraformaldehyde (1% in PBS) and analyzed for CD11b by flow cytometry.

GLP statement- No

Results- Cell cycle G1 arrest- EC50 of LG1069 was approximately 1.5 uM (vs. RAR-specific TTNPB 650 pM and pan-agonist LG1057 30 nM). There was a modest increase in the number of cells (18.7%) expressing CD11b at 1 uM LG1069 compared to a vehicle control (vs. pan-agonist LG1057 10 nM with 33.1% CD11b positive cells and vehicle control with 1.1% CD11b positive cells). These results suggest that LG1069 is a weak inducer of G1 arrest and differentiation of NB4 cells and that the LG1069 may induce these effects through RAR-specific mechanism.

In vivo/Ex vivo studies
1. Effects on growth of human head and neck carcinoma xenografts in nude mice (vol. 1.11, pp 95-108, 132-145, 255-271, RR-740-94-007a, 93-014a, 750-98-005) method- 1483 cell line- Xenografts were established by sc injection of 1 x 10^6 1483 cells at two sites dorsally on nude mice. LG1069 (3, 30 and 60 mg/kg in sesame oil; lot # LG100069-000Z2004) was administered orally by gavage 48h later on a regimen of 5 days dosing/week. Xenograft growth was assessed by measurement of tumor volume. HN9N and HN21P cell lines- nude mice were implanted sc with 1 mm^3 tumor pieces. Two days after transplantation, animals were treated with 60 mg/kg LG1069 (in sesame oil), po, daily. Tumor volumes were measured 2/week using Vernier calipers.

GLP statement- No

Results- LG1069 did not alter proliferation of rapidly-growing 1483 tumors, whereas tumors treated with LG1069 regressed to a non-measurable size by day 49 for HN9N and day 37 for HN21P, respectively. The regression was maintained up to 68 days after implantation.

1484 Tumors

HN9N Tumors

HN21P Tumors

3. Effects on mammary carcinoma in the NMU-induced rat mammary tumor model (vol. 1.11, pp 160-223, RR-750-98-001/004010) method- Female SD rats (6-13/group, 55 days old) received a single iv injection (via tail vein) of 50 mg/kg N-nitroso-N-methylurea (NMU) to induce mammary tumors. After tumors were established (75 mm^2), the tumor bearing rats were treated with daily oral doses of 0, 10, 30 or 100 mg/kg/day LG1069 (in 10% v/v PEG/Tween 80 and 90% of 1% w/v carboxymethylcellulose) by gavage (5 ml/kg) or 150 ug/kg or 800 ug/kg/day tamoxifen (s.c) for 6 weeks. At the end of the treatment period tumor response was measured.
Results- Tumor response- complete regression, partial regression and progression of primary tumors were 72.2%, 16.7% and 11.1% in 100 mg/kg/day LG1069 group and 33.3%, 33.3% and 33.3% in 800 ug/kg/day tamoxifen group, respectively. Complete regression of tumors in LG1069 groups was dose-dependent with 10.5%, 27.8% and 72.2% at 10, 30 and 100 mg/kg/day, respectively.

Tumor burden- Animals of LG1069-treated groups showed a dose-dependent decrease of up to 81% in tumor burden with 376, 121 and 95 mm² at 10, 30 and 100 mg/kg/day, respectively (vs. 498 mm² control animals). Number of tumors per animal- Animals of LG1069-treated groups showed a dose-dependent decrease in the mean numbers of tumor per animal (0.69 in 100 mg/kg/day LG1069 vs 3.63 in control animals). Tamoxifen decreased the number to 1.33 at 800 ug/kg/day. These results suggest potential chemopreventive effects of LG1069.

4. Antikeratinizing activities in the rhino mouse (vol. 1.11, pp 146-159, RR-740-93-002)

Method- Hairless female rhino mice (18 g, 6-8 weeks old) were topically applied by daily dose of 0, 0.05, 0.1 or 0.5% LG1069 (in ethanol:propylene glycol =70:30 v/v, 0.1 ml) (or 0.01, 0.05 and 0.1% ATRA) or received daily oral doses of 0, 10, 30 or 100 mg/kg/day LG1069 (in super refined sesame oil) by gavage (5 ml/kg) for 3 weeks. Mice were sacrificed 72h after the last dose and dorsal trunk skin was removed and placed into 0.5% acetic acid for 24-36h at 4°C. An area of skin (2x 5 cm) was partitioned and the epidermis was carefully peeled off. The epidermis was placed on a glass slide with the dermal side facing up, cleared in alcohol/xylene, and coverslipped with Permount for microscopic evaluation. Each mount the diameters of 10 orifices in 5 random fields were measured with an Optimax Image Analysis System and mean orifice diameter was calculated for each treatment group.

GLP statement-

Results- Topical administration- LG1069 produced dose-related decreases in orifice diameter from 40% at 0.05% LG1069 to 61% at 0.5% LG1069 (vs. 0.01-0.1% ATRA were almost equally efficacious with approximately 60% decrease).

Oral administration- 100 mg/kg/day LG1069 produced only 13% decrease in orifice diameter and 10 and 30 mg/kg doses were inactive. ATRA produced dose-dependent decreases with 6, 36 and 44% at 1, 10 and 30 mg/kg/day.

These results suggest that LG1069 may be an effective antikeratinizing agent when administered topically, but not an active agent following oral administration.
Overall Pharmacology Summary:

<table>
<thead>
<tr>
<th>Study</th>
<th>Results/Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytotoxic Mechanisms</strong></td>
<td>Receptor interaction- Kd of 14-30 nM for RXR and of &gt;4800 nM for RAR</td>
</tr>
<tr>
<td></td>
<td>Transactivation- EC50 of 19-27 nM for RXR and of &gt;10,000 nM for RAR</td>
</tr>
<tr>
<td></td>
<td>Apoptosis in 50% of activated 2B4 T-cell hybridoma cells by 1 uM LG1069</td>
</tr>
<tr>
<td></td>
<td>AP-1 inhibition- IC50 of approximately 0.3 nM in RXRa-transfected HeLa cells</td>
</tr>
<tr>
<td></td>
<td>G1 cell cycle arrest- EC50 of 1.5 uM in NB4 APL cells</td>
</tr>
<tr>
<td><strong>Growth Inhibition in vitro</strong></td>
<td>LG1069 1 uM- inhibited growth of cancer cells from breast (SKBr-3 50%, MCF-7 80%), head and neck (10-30%) and Kaposi's sarcoma (40%).</td>
</tr>
<tr>
<td></td>
<td>LG1069 0.1 uM- inhibited AML cells (up to 50%), breast cancer T47D (50%) and ovarian cancer cells (10-50%), but did not inhibit primary tumor-derived cells from breast, cervix, colon and melanoma.</td>
</tr>
<tr>
<td><strong>In vivo Tumor Models</strong></td>
<td>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, but had no effect on 1483-xenografted tumors. The complete regressions were maintained up to 68 days post-implantation of tumor cells.</td>
</tr>
<tr>
<td></td>
<td>NMU-induced rat mammary tumor- There observed dose-dependent effects of LG1069 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)</td>
</tr>
<tr>
<td><strong>Epidermal Effect</strong></td>
<td>Antikeratinizing effect in rhino mice was observed by LG1069 when administered topically (0.05 - 0.5%), but not when administered orally at dose up to 100 mg/kg/day.</td>
</tr>
</tbody>
</table>

SAFETY PHARMACOLOGY


<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Results/Effects</th>
<th>Study No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular Assay</strong></td>
<td>Normotensive male SD rats (10/group, b.w. 200-300 g) received daily oral doses of 0, 10, 30 and 60 mg/kg/day LG 1069- (lot # LG100069-000200; in sesame oil) by gavage (10 ml/kg) for 4 days. Mean arterial blood pressure and heart rate (plus clinical pathology) were measured directly from a chronic indwelling cannula in the abdominal aorta.</td>
<td>1. No effects on mean arterial blood pressure and heart rate. 2. 23% and 20% increases in aPTT in 30 (p&lt;0.01) and 60 mg/kg (p&lt;0.01) groups, respectively. 3. No necropsy performed.</td>
<td>RR-740-93-006</td>
</tr>
</tbody>
</table>
### Blood Coagulation

Male SD rats (8/group, b.w. 250 g, 8-9 wks of age) received daily oral doses of 0, 10 or 100 mg/kg/day LG1069 (lot # LG100069AD15) in sesame oil suspension admixed with powdered feed for 29 days. When PT and aPTT increased 2-fold, animals received 10 mg/kg vitamin K<sub>1</sub> by ip (d17/18) or sc (d19/20).

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. 6/8 animals in 100 mg/kg group died due to bleeding disorders, while none died in 100 mg/kg LG1069 + vitamin K group.</td>
</tr>
<tr>
<td></td>
<td>2. 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.</td>
</tr>
</tbody>
</table>

### Hepatocyte Proliferation

Female SD rats received daily oral doses of 100 mg/kg/day LG1069 (lot # LG100069-0002007, bw 180-200, 2 months of age) by gavage for 1, 2, 4, or 12 days. Two h prior to sacrifice, rats were injected with 50 uCi [³H]thymidine by ip. At necropsy, livers were excised, homogenized and analyzed for [³H]thymidine incorporation and DNA contents.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. absolute liver weight increased significantly following 4 or 12 days dosing (8.4 g vs. 5.1 g control).</td>
</tr>
<tr>
<td></td>
<td>2. Increased rate of [³H]thymidine incorporation into liver occurred during LG1069 administration and was reversible over a 5-day recovery period.</td>
</tr>
<tr>
<td></td>
<td>3. Liver concentrations of protein, DNA and glycogen from LG1069-treated groups were not significantly different from control groups.</td>
</tr>
</tbody>
</table>

### Plasma Lipids

Male rabbits (5/group, bw 1.25-1.55 kg) received oral daily doses of 0, 1, 10 or 100 mg/kg/day LG1069 (in sesame oil; lot # LG100069-0002004) by gavage (5 ml/kg) for 4 days. Blood for evaluation of lipids was collected at termination.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Increases in plasma cholesterol (3-fold) and triglycerides (10-fold) in 100 mg/kg group. 1 and 10 mg/kg/day LG1069 dose levels were unremarkable. LDL and HDL were not affected.</td>
</tr>
<tr>
<td></td>
<td>2. Dose-related increases in liver weight (control 40.1g, 1 mg/kg group 48.8g, 10 mg/kg group 50.2g, 100 mg/kg group 58.7g).</td>
</tr>
<tr>
<td></td>
<td>3. Approximately 10% body weight loss by day 5 in 100 mg/kg group compared to controls.</td>
</tr>
</tbody>
</table>

### Neuropharmacology

Male SD rats (10/group, bw 150-250g) received daily oral doses of 0, 10, 30 or 100 mg/kg/day LG1069 (in sesame oil; lot # LG100069-0002004) by gavage (10 ml/kg) for 4 days. Ataxia, convulsions, alertness and spontaneous motor activity were measured.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. No treatment-related effects on ataxia, convulsions, alertness or spontaneous motor activity.</td>
</tr>
<tr>
<td></td>
<td>2. No change in body temperature observed.</td>
</tr>
</tbody>
</table>

Overall Safety Pharmacology Summary: Increases in aPTT (3-fold with 10 mg/kg/day, 4-fold with 100 mg/kg/day) in rats, increases in plasma cholesterol (3-fold) and triglycerides (10-fold) with 100 mg/kg/day LG1069 in rabbits and increases in absolute liver weights and rate of thymidine incorporation into liver in rats were observed. Neuropharmacological effects by LG1069 with doses up to 100 mg/kg/day was unremarkable.
PHARMACOKINETICS AND TOXICOKINETICS

1. Relative bioavailability of micronized LGD 1069 in sesame oil and in 10% PEG/Aqueous formulation in male rats (vol. 1.43, pp 207- , RR-845-96-003a)

  方法- 雄性SD大鼠（4/组，体重237-276g）接受了单次口服剂量为35 mg/kg LGD1069（批号：LG100069-0002011）在
   植物油（微粉化）或在PEG400/水（微粉化或微粉化）LGD1069在10% PEG400
   （含0.5% Tween 80/1%羧甲基纤维素钠）的溶液中。溶液（5 ml/kg）胃灌胃。血样（0.4 ml）被收集以确定药代动力学参数。

   GLP声明- 没有

   本- 系统性暴露LG1069最高。微粉化形式的PEG400/水溶液，由植物油和微粉化形式的PEG400/水溶液提供，暗示了降低
   颗粒尺寸可能增强吸收（并提高生物利用度）的趋势。

| 参数 | 植物油 | PEG400/水，微粉化 | PEG/水，微粉化doors 
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, μM</td>
<td>6.37 ± 1.10</td>
<td>3.16 ± 2.35</td>
<td>7.12 ± 0.25</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>3</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>AUCo-6, μM·h</td>
<td>25.4 ± 6.06</td>
<td>20.9 ± 8.28</td>
<td>31.5 ± 3.01</td>
</tr>
</tbody>
</table>

2. 单一口服药代动力学研究在大鼠（vol. 1.44, pp 2- , RR-815-98-013）

   方法- SD大鼠（10/性/组，M- 197.5-258g，F- 167.1-205.9g，年龄约为5周）接受了单次口服剂量为
   30, 100或300 mg/kg LGD1069（批号LG100069-0002035）的溶液。溶液含PEG400, polysorbate 20, butylated
   hydroxynitrosol和povidone，胃灌胃。血样在9, 0.5, 1, 2, 3, 4, 6, 9和12小时后采集。

   GLP声明- 是

   本- 系统性暴露LG1069呈剂量-反应关系。剂量范围为3-300 mg/kg。Cmax和AUC在男性中高于女性。

<table>
<thead>
<tr>
<th>剂量, mg/kg</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, ng/ml</td>
<td>488</td>
<td>411</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AUCo-12, ng·h/ml</td>
<td>2470</td>
<td>1830</td>
</tr>
</tbody>
</table>

3. 单一和重复给药的药代动力学研究在大鼠（vol. 1.45, pp2-33, RR-845-98-007）

   方法- 3/性/组大鼠接受了每天0, 10, 30或100 mg/kg的微粉化LG1069（批号LG100069-
   0002011）PEG400/水溶液。溶液，由胃灌胃14天。在第15天，对照组大鼠接受了单个
   剂量为10, 30或100 mg/kg的微粉化LG1069的PEG400/水溶液。血样（0.7-1 ml）被收集
   通过股静脉在0, 1, 2, 3, 6, 9和12小时后。

   GLP声明- 没有

   本- Cmax和AUC在重复给药组（包括雄性）中都显著低于单剂量组。Cmax和AUC在两性中的水平相似。

<table>
<thead>
<tr>
<th>剂量, mg/kg</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, μM</td>
<td>3.47/3.14</td>
<td>1.55/2.01</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>2.4/1.6</td>
<td>1.8/1.7</td>
</tr>
<tr>
<td>Tik, h</td>
<td>2.86/6.20</td>
<td>5.22/5.94</td>
</tr>
<tr>
<td>AUC0-72, μM·h</td>
<td>25.0/22.5</td>
<td>14.1/15.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>剂量, mg/kg</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, μM</td>
<td>2.42/3.53</td>
<td>15.6/16.9</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>2.4/1.4</td>
<td>3.7/2.6</td>
</tr>
<tr>
<td>Tik, h</td>
<td>3.4/0.65</td>
<td>7.60/5.09</td>
</tr>
<tr>
<td>AUC0-72, μM·h</td>
<td>22.6/31.3</td>
<td>205/169</td>
</tr>
</tbody>
</table>

4. 单一和重复给药的药代动力学研究在大鼠（vol. 1.45, pp2-33, RR-845-98-007）

   方法- 3/性/组大鼠接受了每天0, 10, 30或100 mg/kg的微粉化LG1069（批号LG100069-
   0002011）PEG400/水溶液。溶液，由胃灌胃14天。在第15天，对照组大鼠接受了单个
   剂量为10, 30或100 mg/kg的微粉化LG1069的PEG400/水溶液。血样（0.7-1 ml）被收集
   通过股静脉在0, 1, 2, 3, 6, 9和12小时后。

   GLP声明- 没有

   本- Cmax和AUC在重复给药组（包括雄性）中都显著低于单剂量组。Cmax和AUC在两性中的水平相似。

<table>
<thead>
<tr>
<th>剂量, mg/kg</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, μM</td>
<td>3.47/3.14</td>
<td>1.55/2.01</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>2.4/1.6</td>
<td>1.8/1.7</td>
</tr>
<tr>
<td>Tik, h</td>
<td>2.86/6.20</td>
<td>5.22/5.94</td>
</tr>
<tr>
<td>AUC0-72, μM·h</td>
<td>25.0/22.5</td>
<td>14.1/15.8</td>
</tr>
</tbody>
</table>
4. Two and three-way crossover oral bioequivalence studies of two clinical formulations in dogs (vol. 1.46, pp4-163, RR-845-94-010/011)

method- Eight female beagle dogs received a single iv dose of 5 mg/kg LG1069 (lot#: LG100069-000Z010- crystalline) or received a single oral dose of 25 mg/kg LG1069 (lot#: LG100069-000Z015; formulation lot #: 9309-012 (non-micronized, capsules formulation SG2) and 9408-003 (micronized, capsules formulation SG3)) on two occasions separated by 1 week. On week 3, a single iv dose of 5 mg/kg LG1069 (lot#: 9408-003 (micronized)) was administered to all dogs. Serial blood samples (3 ml) were collected via a cephalic vein cannula or by jugular venipuncture prior to and at 0.5, 1, 2, 4, 6, 8, 12 and 24 h post-dose.

GLP statement- No

Results- Cmax and AUC values of a clinical formulation (SG3) containing micronized LG1069 was 7.6-fold and 6-fold greater than those values of a clinical formulation (SG2) containing non-micronized LG1069, respectively, in female dogs.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Non-micronized</th>
<th>Micronized</th>
<th>Micronized</th>
<th>Micronized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose. mg/kg</td>
<td>25</td>
<td>25</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Route of administration</td>
<td>PO, capsule</td>
<td>PO, capsule</td>
<td>IV</td>
<td>PO, capsule</td>
</tr>
<tr>
<td>Cmax, uM</td>
<td>9.24</td>
<td>70.4</td>
<td>32.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>2.38</td>
<td>1.5</td>
<td>0.5</td>
<td>1.13</td>
</tr>
<tr>
<td>T1/2, h</td>
<td>4.03</td>
<td>2.53</td>
<td>3.82</td>
<td>3.44</td>
</tr>
<tr>
<td>AUC0-∞, uM.h</td>
<td>41.1</td>
<td>243</td>
<td>73.5</td>
<td>54.6</td>
</tr>
<tr>
<td>Cl. ml/min/kg</td>
<td></td>
<td></td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td>Vd, l/kg</td>
<td></td>
<td></td>
<td>0.563</td>
<td></td>
</tr>
<tr>
<td>Bioavailability (%)*</td>
<td>10.4</td>
<td>83.1</td>
<td></td>
<td>74.3</td>
</tr>
</tbody>
</table>

*Bioavailability (%)= absolute oral bioavailability relative to a 5 mg/kg iv LG1069.

5. Five-way crossover oral study in female beagle dogs (vol. 1.46, pp 205-315, RR-845-98-014)

method- 8 female beagle dogs received a single oral dose of 35 mg/kg LG1069 (lot#: LG100069-000Z2018/020/026/032) of each formulation (soft gelatin capsules (SG), soft gelatin capsules which formed pellets during in vitro dissolution test (SG2), hard gelatin capsules (HG), tablet A, or tablet B) with one week washout period between dosings. Serial blood samples (2.5 ml) were collected from the cephalic vein prior to dosing, at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 9 and 12 h post-dose. SG is a clinical formulation.

GLP statement- No

Results- Cmax and AUC values of formulations of a hard gelatin capsules and two tablets were significantly higher than those of soft gelatin capsules.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Soft gelatin capsules, SG</th>
<th>Soft gelatin capsules, SG2</th>
<th>Hard gelatin capsules, HG</th>
<th>Tablet A</th>
<th>Tablet B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose. mg/kg</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Cmax, uM</td>
<td>97.1</td>
<td>95.6</td>
<td>114.0</td>
<td>117.0</td>
<td>127.4</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>1.5</td>
<td>1.5</td>
<td>1.8</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>T1/2, h</td>
<td>2.9</td>
<td>3.6</td>
<td>2.1</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>AUC0-∞, uM.h</td>
<td>344</td>
<td>374</td>
<td>436</td>
<td>422</td>
<td>483</td>
</tr>
</tbody>
</table>

*HG and tablet A and B contain LG1069 50%, povidone K30 USP, sodium lauryl sulfate NF, corn starch NF, microcrystalline cellulose NF, lactose NF (none in tablet B), croscarmellose sodium NF (none in HG and tablet B), sodium starch glycolate NF (none in HG and tablet A), colloidal silicon dioxide NF and magnesium stearate NF (none in HG).


method- SD rats (3/sex/time point, bw 200-250g, 6-8 weeks of age) received a single oral dose of 100 mg/kg [14C]LG1069 (in sesame oil suspension; lot#: LG100069-914Z001 and LG100069-000Z004, 67.5 uCi/kg) by gavage (5 ml/kg). Animals were sacrificed 4, 8, 24 or 48 h post-dose. Blood was drawn from the abdominal aorta and tissues and excreta were collected. Radioactivity from urine, feces, blood and tissue homogenates was measured using a scintillation counter.

GLP statement- No
Results- Orally administered LG1069 was eliminated almost exclusively in the feces in rats. Radioactivity in tissues decreased with time (in which the highest level was observed at 4 h) and no apparent accumulation of radioactivity in tissues after 48 h was observed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>%Dose Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4h (M/F)</td>
</tr>
<tr>
<td>Blood</td>
<td>0.64/0.67</td>
</tr>
<tr>
<td>Tissues*</td>
<td>13.2/7.6</td>
</tr>
<tr>
<td>GIT contents</td>
<td>80.1/75.6</td>
</tr>
<tr>
<td>Feces</td>
<td>0.001/0.006</td>
</tr>
<tr>
<td>Urine</td>
<td>0.004/0.005</td>
</tr>
<tr>
<td><strong>Total activity recovered, 0-48h</strong></td>
<td>94.0/83.7</td>
</tr>
</tbody>
</table>

*The liver, kidneys, adrenal glands, fat, mesenteric lymph node, pancreas, heart, salivary gland, gastrointestinal tract and ovaries

7. Effects on hepatic microsomal cytochrome P450 and in vitro metabolism (vol. 1.48, pp 2-20, RR-845-98-009)

Method- In vivo effects- Male SD rats (3/group, bw approximately 250g) received oral doses of 0 (PEG400/water vehicle only) or 100 mg/kg/day LG1069 (in PEG400/water microparticulate suspension; lot#: LG100069-000Z011) or, ip doses of 80 mg/kg/day phenobarbital, 50 mg/kg/day dexamethasone, 25 mg/kg/day 3-methylcholanthrene or oil vehicle by gavage (5 ml/kg) for 4 days. After the final dose, hepatic microsomes were prepared by differential centrifugation. Microsomes were examined spectrophotometrically for total cytochrome P450 and for P450 isozyme levels using Western blot analysis. In vitro metabolism- the microsomes obtained from the in vivo study were incubated with 100 uM LG1069 under conditions supporting either P450-mediated metabolism or glucurononitransferase (GT)-mediated metabolism. Rates of LGD1069 metabolism were determined by monitoring the formation of metabolites using HPLC-UV.

GLP statement- No

Results- LG1069 (100 mg/kg/day) induced increase in total P450 concentration. Among P450 isozymes, concentrations of CYP2B1/2B2, CYP3A and CYP4A increased significantly and concentrations of CYP1A2 and CYP2C11 decreased. Both oxidative metabolism (hydroxy- and o xo-LG1069) and glucuronidation of LG1069 by liver microsomes were induced in microsomes from rats treated with 100 mg/kg/day LG1069.

a. Effects on P450 in hepatic microsomes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total hepatic P450 (pmol/mg protein)</th>
<th>Change from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG400/water control</td>
<td>577</td>
<td>-</td>
</tr>
<tr>
<td>LG1069, 100 mg/kg/day</td>
<td>1102</td>
<td>91%</td>
</tr>
<tr>
<td>Oil vehicle control</td>
<td>596</td>
<td>-</td>
</tr>
<tr>
<td>Phenobarbital, 80 mg/kg/day</td>
<td>1283</td>
<td>112%</td>
</tr>
<tr>
<td>Dexamethasone, 50 mg/kg/day</td>
<td>1251</td>
<td>110%</td>
</tr>
<tr>
<td>3-methylcholanthrene, 25 mg/kg/day</td>
<td>1132</td>
<td>90%</td>
</tr>
</tbody>
</table>

b. Relative concentration of P450 isozymes in hepatic microsomes

<table>
<thead>
<tr>
<th>P450 Isozyme</th>
<th>Optical Density x mm/mg protein (and Change from Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEG400/Water</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>3.1</td>
</tr>
<tr>
<td>CYP2B1/2B2</td>
<td>0.9</td>
</tr>
<tr>
<td>CYP2C11</td>
<td>5.7</td>
</tr>
<tr>
<td>CYP2E</td>
<td>67.0</td>
</tr>
<tr>
<td>CYP3A</td>
<td>11.4</td>
</tr>
<tr>
<td>CYP4A</td>
<td>2.1</td>
</tr>
</tbody>
</table>
c. rates of formation of LG1069 metabolites

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate of Metabolite Formation (mAU x s/min/mg protein)</th>
<th>P450-Mediated</th>
<th>Change from Control</th>
<th>GT-Mediated</th>
<th>Change from Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>PEG400/water vehicle</td>
<td>12.0</td>
<td></td>
<td></td>
<td>13.0</td>
<td>77%</td>
</tr>
<tr>
<td>LG1069. 100 mg/kg/day</td>
<td>43.0</td>
<td>252%</td>
<td></td>
<td>23.0</td>
<td></td>
</tr>
<tr>
<td>Oil vehicle</td>
<td>15.0</td>
<td></td>
<td></td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>Phenobarbital, 80 mg/kg/day</td>
<td>27.0</td>
<td>80%</td>
<td></td>
<td>15.2</td>
<td>39%</td>
</tr>
<tr>
<td>Dexamethasone, 50 mg/kg/day</td>
<td>68.5</td>
<td>357%</td>
<td></td>
<td>9.0</td>
<td>-17%</td>
</tr>
<tr>
<td>3-MC, 25 mg/kg/day</td>
<td>7.7</td>
<td>-49%</td>
<td></td>
<td>11.1</td>
<td>2%</td>
</tr>
</tbody>
</table>

*Rates are expressed in terms of increase in metabolite (sum of hydroxy-LG1069 and oxo-LG1069) HPLC peak areas over time.


Method- Metabolites of LG1069 (lot#: LG100069-00020072011, 100 uM/incubation mixture for 4 h) were generated using rat liver slices (from a male SD rat), rat liver microsomes (from male SD rats) and by collecting bile from rats dosed with 100 mg/kg LG1069. Metabolite profiles were analyzed using mass spectrometry. Metabolites were identified using electron ionization and negative ion electron capture ionization mass spectrometry and gas chromatography/mass spectrometry.

GLP statement- No

Results- A number of metabolites were formed by rat liver slices incubated with LG1069 (putative metabolites were assigned numbers as peaks 2, 3, 4, 6, 7, 8, and 9 in analysis. Peaks (metabolites presumed to be formed by P450) in liver microsomes form rats were peaks 2, 3, 4, 7/7' and 9/9'. Peaks from analysis of bile collected from a rat dosed with 100 mg/kg LG1069 were peaks 2, 3, 4, 6 and 8. Tentative structural assignments for major metabolites observed during analysis were made as below:

<table>
<thead>
<tr>
<th>Peak</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Ether glucuronide</td>
</tr>
<tr>
<td>3</td>
<td>Ether glucuronide</td>
</tr>
<tr>
<td>4</td>
<td>6- and/or 7-hydroxylated acyl glucuronide</td>
</tr>
<tr>
<td>6</td>
<td>taurine conjugate</td>
</tr>
<tr>
<td>7</td>
<td>6-hydroxy-LG1069</td>
</tr>
<tr>
<td>7'</td>
<td>7-hydroxy-LG1069</td>
</tr>
<tr>
<td>8</td>
<td>acyl glucuronide of LG1069</td>
</tr>
<tr>
<td>9</td>
<td>6-oxo-LG1069</td>
</tr>
<tr>
<td>9'</td>
<td>7-oxo-LG1069</td>
</tr>
</tbody>
</table>

Suggested Metabolic Pathway for LG1069 in rats:

*may be two isomers
*a,b,d Differentiation between C-6 and C-7 positions for the hydroxy, oxo and Phase II metabolites was not achieved; absolute stereochemistries, where applicable, were not determined.
*b Metabolites were predicted as intermediary metabolites but were not observed.
*Up to four isomers
method- Drug-free fasting plasma was obtained from 3 healthy male and female volunteers and pooled. The pooled human plasma was re-warmed to 37°C and pH was adjusted to 7.4. Plasma samples were spiked with [3H]LG1069 to achieve concentrations of 5, 10, 100, 1000 and 5000 ng/ml. After a 15 min incubation, plasma and PBS (dialysate) were added to respective sides of the dialysis cells of Spectra/Por Equilibrium Dialyzer with teflon cells and Spectra/Por 2 membranes and were incubated at 37°C for 4 h. At 4h, aliquots of plasma and dialysate were removed and analyzed for radioactivity by liquid scintillation counting and calculation of protein binding.

GLP statement- No

Results: Binding of LG1069 to human plasma proteins was very high (>99.8%), which was independent of LG1069 concentration. Free fractions ranged from 0.12% to 0.18%.

<table>
<thead>
<tr>
<th>Plasma [3H]LG1069 Concentration</th>
<th>Plasma Protein Binding (%)</th>
<th>Free Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ng/ml</td>
<td>99.87</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>99.88</td>
<td>0.12</td>
</tr>
<tr>
<td>100</td>
<td>99.82</td>
<td>0.18</td>
</tr>
<tr>
<td>1000</td>
<td>99.87</td>
<td>0.17</td>
</tr>
<tr>
<td>5000</td>
<td>99.85</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Overall Pharmacokinetic Summary:

<table>
<thead>
<tr>
<th>Pharmacokinetics</th>
<th>Rat (PEG/water vehicle)</th>
<th>Dog (capsule)</th>
<th>Human* (capsule)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose, mg/m2</td>
<td>600, single dose 100</td>
<td>600, multiple dose 100</td>
<td>500, single dose 25</td>
</tr>
<tr>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Route</td>
<td>PO (Male/Female)</td>
<td>PO (Male/Female)</td>
<td>PO</td>
</tr>
<tr>
<td>Cmax, uM</td>
<td>15.6/16.9</td>
<td>4.8/10.0</td>
<td>70.4</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>3.7/2.6</td>
<td>3.2/2.1</td>
<td>1.5</td>
</tr>
<tr>
<td>T1/2, h</td>
<td>7.6/5.0</td>
<td>4.4/5.0</td>
<td>2.5</td>
</tr>
<tr>
<td>AUC0-∞, uM·h</td>
<td>205/169</td>
<td>44.6/82.1</td>
<td>41.1</td>
</tr>
<tr>
<td>Bioavailability, %</td>
<td></td>
<td></td>
<td>83.1</td>
</tr>
<tr>
<td>Protein binding</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tissue Distribution in Rats</td>
<td>8h - GI (85.4%/M/85.0%/F) &gt; tissues (4.8%/M/5.3%/F) &gt; plasma (0.27%/M/0.29%/F)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metabolism</td>
<td>CYP2B, CYP3A and/or CYP4A-mediated oxidation: 6-hydroxy LG1069, 7-hydroxy LG1069, 6-oxo-LG1069, 7-oxo-LG1069</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucuronidation</td>
<td>acyl glucuronide, ether glucuronide</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Conjugation</td>
<td>taurine conjugate, 7-sulfate conjugate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Excretion, Feces</td>
<td>91.1(M)/100(F)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>0.14(M)/0.59(F)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

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

Cmax of rats and dogs cannot be directly extrapolated to that of humans ($r^2=0.13$ on mg/m$^2$ 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/m$^2$ 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/m$^2$ basis.
In hepatic microsomes, cytochrome P450 CYP2B, CYP3A and CYP4A were markedly induced (821% - 7138%) by LG1069 (100 uM). LG1069 was almost exclusively eliminated via feces.

**TOXICOLOGY**

**Multiple Doses**

1. **28-Day oral toxicity study in rats** (vol. 1.16, pp 2-409, RR-815-94-013)

   Animal- male SD rats (15/group except 10/group in low dose, plus 4/group in mid and high dose groups for pharmacokinetics; B.W. 83.6 - 116.2 g, 3-5 weeks of age).

   Treatment- animals received oral daily doses of 0, 3, 10, 30 or 100 mg/kg/day LG1069 (lot #. LG100069-000Z007, formulated in sesame oil) by gavage (10 ml/kg) for 28 days.

   Observation- recovery period- 28 days, clinical signs/mortality- daily, body weight/food consumption- weekly, clinical pathology- d29, necropsy- d29 and d57 (animals in recovery), pharmacokinetics- 1 ml blood collected 2h post dose on days 1, 14 and 28 from 4/rats/group.

   GLP statement- yes

   Results: HNSTD and LD10 were 3 mg/kg/day and 10 mg/kg/day, respectively. Major toxic targets included blood clotting system (hemorrhage in numerous tissues, prolongation of PT and aPTT, increased levels of fibrinogen), liver (hemorrhage, hepatocellular necrosis), pancreas (acinar cell necrosis), heart (cardiomyopathy), bone (tibial osteopathy), skin (dermatopathy), and lymphoid organs (lymphoid depletion in spleen, lymph nodes, thymus).

<table>
<thead>
<tr>
<th>Dose. mg/kg/day</th>
<th>0</th>
<th>3</th>
<th>10</th>
<th>30</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>0/15</td>
<td>0/10</td>
<td>2/19</td>
<td>7/19</td>
<td>14/19</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>urine stain</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>discharge</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>bleeding</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>pale mucous memb</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>B.W. gain. d-1/28. g</td>
<td>121</td>
<td>166</td>
<td>176</td>
<td>173</td>
<td>161</td>
</tr>
<tr>
<td>Food consumption week 4 (g)</td>
<td>140</td>
<td>171</td>
<td>177</td>
<td>173</td>
<td>168</td>
</tr>
<tr>
<td>Hematology</td>
<td>RBC, x10^6</td>
<td>8.86</td>
<td>8.96</td>
<td>8.92</td>
<td>7.72</td>
</tr>
<tr>
<td></td>
<td>hemoglobin, g/dl</td>
<td>16.4</td>
<td>16.4</td>
<td>16.0</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>WBC, x 10^3</td>
<td>15.5</td>
<td>13.0</td>
<td>12.7</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes, x 10^3</td>
<td>13.6</td>
<td>11.57</td>
<td>11.14</td>
<td>9.70</td>
</tr>
<tr>
<td></td>
<td>PT, sec</td>
<td>15.6</td>
<td>29.1</td>
<td>53.4</td>
<td>77.2</td>
</tr>
<tr>
<td></td>
<td>APPT, sec</td>
<td>40.6</td>
<td>66.2</td>
<td>102.2</td>
<td>&gt;120</td>
</tr>
<tr>
<td></td>
<td>fibrinogen, mg/dl</td>
<td>331</td>
<td>441</td>
<td>450</td>
<td>578</td>
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<tr>
<td>Clinical chemistry</td>
<td>Glucose, mg/dl</td>
<td>119</td>
<td>119</td>
<td>128</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>BUN, mg/dl</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Cholesterol, mg/dl</td>
<td>76</td>
<td>92</td>
<td>102</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Triglyceride, mg/dl</td>
<td>69</td>
<td>73</td>
<td>103</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>Phosphate, mg/dl</td>
<td>9.5</td>
<td>9.1</td>
<td>8.6</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Potassium, mEq/l</td>
<td>6.3</td>
<td>5.7</td>
<td>5.2</td>
<td>5.49</td>
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<tr>
<td></td>
<td>ALT, U/l</td>
<td>29</td>
<td>40</td>
<td>56</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>AST, U/l</td>
<td>65</td>
<td>77</td>
<td>97</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>ALK, U/l</td>
<td>158</td>
<td>152</td>
<td>184</td>
<td>234</td>
</tr>
<tr>
<td>Gross pathology</td>
<td>adrenal wt. g</td>
<td>N=10</td>
<td>0.053</td>
<td>0.075</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>liver wt. g</td>
<td>UR</td>
<td>12.93</td>
<td>17.52</td>
<td>17.86</td>
</tr>
<tr>
<td></td>
<td>red focu/color*</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>2/5</td>
</tr>
<tr>
<td>Histiopathology</td>
<td>Hemorrhage**</td>
<td>UR</td>
<td>UR</td>
<td>5/8</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>Lymphoid depletion</td>
<td>UR</td>
<td>UR</td>
<td>4/5</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>UR</td>
<td>UR</td>
<td>2/5</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>UR</td>
<td>UR</td>
<td>2/5</td>
<td>3/3</td>
</tr>
<tr>
<td>Mesenteric LN</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>1/5</td>
<td>2/3</td>
</tr>
<tr>
<td>Mandibular LN</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>0/5</td>
<td>2/3</td>
</tr>
<tr>
<td>Adrenal cortex</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>1/8</td>
<td>2/5</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>UR</td>
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<tr>
<td>Liver</td>
<td>UR</td>
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<td>UR</td>
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<tr>
<td>Cellular necrosis</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
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</tr>
<tr>
<td>Acinar pancreas</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
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<td>Acinar necrosis</td>
<td>UR</td>
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<tr>
<td>Skin, dermatopathy</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
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<tr>
<td>Tibia, osteopathy</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
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</tr>
<tr>
<td>Heart, cardiomyopathy</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
<td>d1/d14/d28</td>
<td>d1/d14/d28</td>
<td>d1/d14/d28</td>
<td>d1/d14/d28</td>
<td>d1/d14/d28</td>
</tr>
<tr>
<td>Plasma conc. ng/ml</td>
<td>879/643/578</td>
<td>879/643/578</td>
<td>879/643/578</td>
<td>879/643/578</td>
<td>879/643/578</td>
</tr>
</tbody>
</table>

UR: unremarkable; *: red foci in esophagus, epididymides, pancreas, seminal vesicles, skeletal muscle, skin, thymus, and testes; **: hemorrhage- esophagus, forestomach, heart, kidney, liver, lung, mandibular lymph node, mesenteric lymph node, acinar pancreas, prostate, salivary gland, skeletal muscle, skin, seminal vesicles, thymus, testes;

2. 91-Day oral toxicity study in dogs (vol. 1.30, pp 105, to vol.1.32, RR-815-95-003)

Animal- beagle dogs (6/six/group, M 8.3-10.2 kg, F 6.4-8.8 kg)
treatment- animals received oral daily doses of 0, 0.1, 0.3 or 1.5 mg/kg/day LG1069 (suspensions in babassu oil and filled in capsules; lot #. LG100069-002008) for 91 days.

Observation- recovery period- 28 days, clinical signs/mortality/food consumption- daily, body weight- weekly,
ophthalmic/cardiovascular (ECG, blood pressure, heart rate) examinations - prior to treatment, during wk 6 and prior to necropsy, clinical pathology- d-1, wk 4 and 8, and at necropsy, urinalysis- prior to treatment and within 48h of necropsy, pharmacokinetics- 2.8 ml blood/sample collected, time 0, 0.5, 1.2,3,4,6,8 and 12 h postdose on days 1, 45 and 91.

GLP statement- yes

Results: HNSTD was 1.5 mg/kg/day. Toxic target organ was testes (tubular degeneration- nuclear pyknosis, vacuolization and loss of germinal epithelium, recovered at the end of recovery period- Labeling: infertility). After repeated dosing, AUC of LG1069 at day 91 after subtracting trough levels was reduced by 45% in males and 39% in females when compared to day 1.

<table>
<thead>
<tr>
<th>Dose, mg/kg/day</th>
<th>0</th>
<th>0.1</th>
<th>0.3</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>B.W.</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Food consumption</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Cardiovascular exam.</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Hematology</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Clinical chemistry</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Gross pathology</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Testes wt, g, male</td>
<td>15.11</td>
<td>11.77</td>
<td>15.50</td>
<td>11.00</td>
</tr>
<tr>
<td>Adrenal wt, g, females</td>
<td>0.943</td>
<td>0.985</td>
<td>1.172</td>
<td>1.219</td>
</tr>
<tr>
<td>Histopathology</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
<td>UR</td>
</tr>
<tr>
<td>Testes, male tubular degeneration</td>
<td>UR</td>
<td>1/4</td>
<td>1/4</td>
<td>3/4</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
<td>d1/d91</td>
<td>M</td>
<td>F</td>
<td>133/107</td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>ND/22.1</td>
<td>ND/28.4</td>
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<td>5.5/2.6</td>
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<tr>
<td>Cmin, ng/ml</td>
<td>1099/832</td>
<td>1174/1049</td>
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<td>1174/1049</td>
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<td>Tmax, h</td>
<td>N/A/572</td>
<td>N/A/715</td>
<td>N/A/572</td>
<td>N/A/715</td>
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</table>

UR: unremarkable; *AUC- AUC after subtracting of predose trough levels
### Results

Significant increases in mortality were observed in 300 mg/kg/group. Majority of mortalities appeared as a result of gavage damage (e.g., perforation of esophagus, etc). LG 1069 was cataractogenic (lens opacity - Labeling) (30 mg/kg or higher). Toxicity targets included, blood (prolonged clotting time, decreased hemoglobin and RBC), eye (cataract), liver (liver enzymes, hypertrophy), skin (acanthosis), and stomach (acanthosis, hyperkeratosis).

### Dose mg/kg/day

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>3</th>
<th>30</th>
<th>100</th>
<th>300 (8 weeks only)</th>
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<tr>
<td>Alopecia</td>
<td>5/42</td>
<td>3/42</td>
<td>20/40</td>
<td>36/39</td>
<td>22/22 (wk9)</td>
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<td>Erythema</td>
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<td>UR</td>
<td>3/40</td>
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<td>30/37 (wk 7)</td>
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<tr>
<td>Ocular opacity</td>
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<td>B W. gain. g. M/F</td>
<td>434.1/189.4</td>
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<td>454.2/188.5</td>
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</tr>
<tr>
<td>g/kg/day wk 8</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
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<tr>
<td>wk 26</td>
<td>60.2/74.3</td>
<td>64.2/81.0</td>
<td>66.9/77.3</td>
<td>69.3/75.8</td>
<td>74.0/98.6</td>
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<td>M/F</td>
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<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M(F 8)</td>
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<td>130/101</td>
<td>136/123</td>
<td>160/132</td>
<td>152/166</td>
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<td>59/42</td>
<td>91/74</td>
<td>115/94</td>
<td>143/108</td>
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<td>129/55</td>
<td>273/152</td>
<td>364/295</td>
<td>446/681</td>
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<td>Cholesterol, mg/dl</td>
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<td>152/117</td>
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<td>Triglyceride, mg/dl</td>
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<td>Cataract incidence %</td>
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<td>wk26/wk30</td>
<td>wk26/wk30</td>
<td>wk26/wk30</td>
<td>wk26/wk12</td>
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<td>Gross pathology</td>
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<td>M/F, wk 27</td>
<td>M/F, wk 27</td>
<td>M/F, wk 27</td>
<td>M/F, wk 8</td>
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<td>0.093/0.109</td>
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<td>Histopathology</td>
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<tr>
<td>Eye, cataract</td>
<td>wk 27</td>
<td>wk27</td>
<td>wk27</td>
<td>wk27</td>
<td>wk8</td>
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<td>Skin, acanthosis</td>
<td>UR</td>
<td>M UR, F 2/19</td>
<td>M 6/13, F 3/17</td>
<td>M 8/12, F 10/17</td>
<td>M 2/9, M 3/10</td>
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<td>Stomach, acanthosis</td>
<td>UR</td>
<td>UR</td>
<td>M 1/13, F UR</td>
<td>M 1/12, F 8/17</td>
<td>M 4/9, F 4/10</td>
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<td>Hyperkeratosis</td>
<td>UR</td>
<td>UR</td>
<td>M 5/12, F 12/17</td>
<td>M 5/12, F 9/17</td>
<td>M 9/9, 10/10</td>
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</table>
4. 6-Month oral toxicity study in dogs (vol. 1.33, pp 2- to vol. 1.36, RR-815-98-003a)

Animal - beagle dogs (6/sex/group, M 8.5-11.0 kg, F 7.2-9.8 kg, 7-9 months old)

Treatment - animals received oral daily doses of 0, 1, 3 or 10 mg/kg/day LG1069 (suspensions containing PEG 400 NF, polysorbate 20 NF, butylated hydroxyanisole NF and povidone USP, which was filled in capsules; lot # LG100069-002015) for 26 weeks.

Observation - recovery period: 28 days, clinical signs/mortality/food consumption - daily, body weight - weekly, ophthalmic cardiovascular (ECG, blood pressure, heart rate) examinations - prior to treatment, during wks 1, 12, 26 (6 month) and 30 (recovery), clinical pathology - days 1, 12, 29, 57, 89, 119, 148, 182 (6 month) and 210 (recovery), urinalysis - days 14, 86, 181 (6 month) and 209 (recovery), pharmacokinetics - 2 ml blood/sample collected, time 0, 0.5, 1, 2, 3, 4, 6, 9 and 12 h postdose on days 1, 180 and 210 and 2 h post-dose on days 30, 58, 90, 120 and 149.

GLP statement - yes

Results: HNSTD was 10 mg/kg/day. Toxic targets include adrenal cortex (vacuolation), liver (enzymes, hepatocellular hypertrophy), eyes (cataract), ears (excessive ceruminous material), and platelets (increased). In female animals, body weight gain in 10 mg/kg group was lower than that in control animals, while food consumption in 10 mg/kg animals was greater than that in control group (no apparent reason was provided by the sponsor).

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<th>Dose, mg/kg/day</th>
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<th>1</th>
<th>3</th>
<th>10</th>
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<td>Mortality</td>
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<td>None</td>
<td>None</td>
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<td>Clinical signs</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
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<tr>
<td>Skin, reddening</td>
<td>2/5</td>
<td>4/6 (longer duration)</td>
<td>3/5 (longer duration)</td>
<td>3/6 (longer duration)</td>
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<tr>
<td>B. W. gain, kg, d1-d179</td>
<td>M 1.2</td>
<td>F 1.5</td>
<td>M 1.7</td>
<td>F 1.4</td>
</tr>
<tr>
<td>Food consumption</td>
<td>M 24.9</td>
<td>F 27.7</td>
<td>M 27.5</td>
<td>F 28.4</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>UR</td>
<td>UR</td>
<td>2/12</td>
<td>5/12 (M 4, F 1)</td>
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<tr>
<td>Cardiovascular exam.</td>
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<td>UR</td>
<td>UR</td>
<td>UR</td>
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<tr>
<td>Hematology, month 6</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
</tr>
<tr>
<td>Platelet, 10^3/μl</td>
<td>265/490</td>
<td>242/251</td>
<td>386/343</td>
<td>479/490</td>
</tr>
<tr>
<td>Clinical chemistry, mo 6</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
</tr>
<tr>
<td>AST, u/l</td>
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<td>30/28</td>
<td>29/31</td>
<td>50/59</td>
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<td>ALT, u/l</td>
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<td>35/28</td>
<td>34/37</td>
<td>178/92</td>
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<td>Alk. P, u/l</td>
<td>72/68</td>
<td>78/76</td>
<td>131/139</td>
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<td>HDL, mg/dl</td>
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<td>Gross pathology, 4/sex/gr</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
</tr>
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<td>Ear, abnormal content</td>
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<td>UR</td>
<td>UR/1</td>
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<td>Liver, enlarged</td>
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<tr>
<td>Histopathology, 4/sex/gr</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
<td>M/F</td>
</tr>
<tr>
<td>Liver, bile stasis</td>
<td>UR</td>
<td>UR</td>
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</table>
Overall Toxicology Summary:

<table>
<thead>
<tr>
<th>Toxicity Study</th>
<th>Study No.</th>
<th>Treatment</th>
<th>Toxicity Targets and Toxic Effects</th>
<th>Toxic Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 28-Day</td>
<td>RR-815-94-013</td>
<td>3, 10, 30 or 100 mkd in sesame oil, po, for 28 days</td>
<td>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)</td>
<td>LD$_{10}$ = 10 mkd HNSTD = 3 mkd</td>
</tr>
<tr>
<td>Dog 91-Day</td>
<td>RR-815-95-003</td>
<td>0.1, 0.3 or 1.5 mkd, po, for 91 days, in babassu oil in capsules</td>
<td>Testes (tubular degeneration)</td>
<td>HNSTD = 1.5 mkd</td>
</tr>
<tr>
<td>Rat 6-Month</td>
<td>RR-815-98-004</td>
<td>3, 30, 100 or 300 mkd (in aqueous suspension in PEG 400), po, for 6 months</td>
<td>Blood (prolonged clotting time, ↑hemoglobin and RBC), Eye (lens opacity- cataractogenic), liver (hypertrophy, ↑AST/ALT), skin (acanthosis), stomach (acanthosis, hyperkeratosis)</td>
<td>HNSTD = 300 mkd*</td>
</tr>
<tr>
<td>Dog 6-Month</td>
<td>RR-815-98-003a</td>
<td>1, 3 or 10 mkd (in aqueous suspension in PEG 400), po, for 6 months</td>
<td>Adrenal cortex (vacuolation), liver (hepatocellular hypertrophy, ↑ALT/AST), eye (cataract), platelets (↑ in counts)</td>
<td>HNSTD = 10 mkd</td>
</tr>
</tbody>
</table>

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

Labeling Issues:

Infertility- LG1069 induced testes degeneration in dogs (1.5 mg/kg/day, 1/10th the recommended clinical dose on a mg/m2 basis)

Cataract- 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.

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 anticoagulant or drugs that prolong clotting time such as NSAIDs should be discouraged.
## Histopathology Inventory

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Species</th>
<th>Rat</th>
<th>Dog</th>
<th>28-day oral tox</th>
<th>91-Day oral tox</th>
<th>6-month oral tox</th>
<th>6-month oral tox</th>
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<tbody>
<tr>
<td>Schedule/Duration of dosing</td>
<td>Daily x 28 days</td>
<td>Daily x 91 days</td>
<td>Daily x 26 weeks</td>
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REPRODUCTIVE TOXICITY

1. Oral developmental toxicity study in rats (vol.138, pp 134-, RR-815-97-003b)

Animal- female SD rats (25/group, BW- 237-268 g, Age- approximately 65 days)
Treatment- animals received daily oral doses of 0, 1, 4 or 16 mg/kg/day LG1069 (lot # LG 100069-0002020, suspension of PEG 400: Tween:carboxymethyl cellulose: water = 9.95:0.05:0.99:91) by gavage (dosage volume- 1.5 ml/kg) on gestation days 7 through 17.
Observation- mortality/clinical signs/2/day, body weight- daily, food consumption- d0,7,10,12,15,18 and 20, necropsy- d20
GLP statement- yes

Results- 4 mg/kg/day- increased fetal incidences of skeletal variations, 16 mg/kg/day- reduced embryo/fetal viability (increased resorptions), reduced fetal body weights, increased incidences of variations and malformations (cleft palate, depressed eye bulges, microphthalmia, and incomplete or no ossifications). Body weight gains- reduced gains at doses of 4 and 16 mg/kg/day during the drug treatment.

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<td>6/25 (p&lt;0.01)</td>
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<td>100</td>
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<td>d7-d17</td>
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<td>9 (36.0)</td>
<td>14 (56.0)</td>
<td>14 (56.0)</td>
<td>15 (62.5)</td>
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<td>%resorbed conceptuses/litter</td>
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<td>5.0</td>
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<td>mean fetal body wt., g/litter</td>
<td>3.32</td>
<td>3.26</td>
<td>3.22</td>
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<td>litters with fetuses with any alteration, # (%)</td>
<td>14 (56)</td>
<td>15 (60)</td>
<td>24 (96) (p&lt;0.01)</td>
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<td>24 (6)</td>
<td>39 (10.3)</td>
<td>88 (23.3)</td>
<td>229 (67.4) (p&lt;0.01)</td>
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<td>%fetuses with any alteration/litter</td>
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* IO- incompletely ossified, NO- not ossified, IS- irregularly shaped

2. Oral dose-range development toxicity study in rats (vol. 1.39, pp 2-260, RR-915-97-004a)
Animal- female SD rats (8/group, 4/group for pharmacokinetics, B.W.- 258-292 g, Age- about 65 days)
Treatment- animals received daily oral doses of 0, 3, 10, 30 or 65 mg/kg/day LG 1069 (lot # LG10069-
000120) in suspension of PEG 400: twice:caroxymethyl cellulose: water =
9.95:0.05:0.9:89.1) by gavage (dosage volume- 5 ml/kg) on gestation days 7 through 17.
Observation- mortality/clinical signs-2/day, body weight- daily, food consumption- d0,7,10,12,15,18 and
20, necropsy- d20, blood samples- d17, prior to dosing, 1, 2, 3 and 4 h post-intubation
GLP statement- yes

Results- 30 and 65 mg/kg/day- significantly reduced body weight gain of dam during gestation period (in
particular the treatment period), reduced embryo/fetal viability, reduced fetal body weights, increased fetal
incidence of external alterations, and reduced male sex ratio (65 mg/kg/day group only).
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gross pathology</td>
<td>17.26</td>
<td>16.64</td>
<td>17.34</td>
<td>17.45</td>
<td>15.95</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>17.26</td>
<td>16.64</td>
<td>17.34</td>
<td>17.45</td>
<td>15.95</td>
</tr>
<tr>
<td>Litter</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87.5</td>
<td>42.8</td>
</tr>
<tr>
<td>dams with viable fetuses (%)</td>
<td>17.0</td>
<td>18.1</td>
<td>19.4</td>
<td>17.1</td>
<td>18.0</td>
</tr>
<tr>
<td>corpora lutea, #/animal</td>
<td>16.4</td>
<td>16.1</td>
<td>17.2</td>
<td>14.9</td>
<td>16.6</td>
</tr>
<tr>
<td>#implantation, #/animal</td>
<td>15.6</td>
<td>15.5</td>
<td>16.1</td>
<td>4.2</td>
<td>1.6</td>
</tr>
<tr>
<td>live fetuses, #/animal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dead fetuses, #/animal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sex ratio, % males/litter</td>
<td>52.5</td>
<td>52.9</td>
<td>59.6</td>
<td>49.3</td>
<td>36.1</td>
</tr>
<tr>
<td>resorption, early, #/animal</td>
<td>0.8</td>
<td>0.6</td>
<td>1.0</td>
<td>8.9</td>
<td>13.7</td>
</tr>
<tr>
<td>resorption, late, #/animal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>dams with any resorptions, # (%)</td>
<td>4 (50)</td>
<td>4 (50)</td>
<td>4 (50)</td>
<td>8 (100)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>*absorbed concepuses/litter</td>
<td>4.7</td>
<td>3.9</td>
<td>6.4</td>
<td>68.9</td>
<td>77.3</td>
</tr>
<tr>
<td>mean fetal body wt., g/litter</td>
<td>3.38</td>
<td>3.29</td>
<td>3.01</td>
<td>2.15</td>
<td>1.96</td>
</tr>
<tr>
<td>fetal observation:</td>
<td>3 (42.8)</td>
<td>2 (66.7)</td>
<td>9 (26.5)</td>
<td>10 (90.0)</td>
<td></td>
</tr>
<tr>
<td>external alterations</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>eyes depressed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>litter incidence, # (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>fetal incidence, # (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Toxicokinetics</td>
<td>387</td>
<td>1181</td>
<td>1613</td>
<td>1527</td>
<td></td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>-</td>
<td>-</td>
<td>2650</td>
<td>4867</td>
<td></td>
</tr>
<tr>
<td>AUC0-4h, ng h/ml*</td>
<td>-</td>
<td>-</td>
<td>1.7</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Tmax, h</td>
<td>-</td>
<td>-</td>
<td>2.3</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

* AUC = 134.8 x Dose (mg/kg/day) + 440.4 (r² = 0.9428)

Overall Reproductive Toxicity Summary- development study was conducted in rats only (i.e., not in rabbits). Embryo/fetotoxic≥ 10 mg/kg/day, Teratogenic≥ 4 mg/kg/day, NOAEL≤ 1 mg/kg/day, and Maternally toxic≥ 4 mg/kg/day.

<table>
<thead>
<tr>
<th>Toxic Effect</th>
<th>1 mg/kg/day</th>
<th>4 mg/kg/day</th>
<th>10 mg/kg/day</th>
<th>16 mg/kg/day</th>
<th>30/65 mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dams</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight gains, decreased, d7-17</td>
<td>-</td>
<td>+ (3%)</td>
<td>+ (5%)</td>
<td>++ (-25%)</td>
<td>+++ (&gt; 68%)</td>
</tr>
<tr>
<td>Food consumption, reduced, d7-d17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (15%)</td>
<td>+ (+35%)</td>
</tr>
<tr>
<td>Litter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implantation, reduced</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Embryo/fetal viability, reduced</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Fetal body weight, decreased</td>
<td>-</td>
<td>-</td>
<td>+ (10%)</td>
<td>+ (15%)</td>
<td>++ (&gt;36%)</td>
</tr>
<tr>
<td>Variations/ Malformations</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Sex ratio, Male, reduced</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (65 mg/kg only)</td>
</tr>
</tbody>
</table>

* Changes: + slight, ++ mild, +++ moderate

Reproduction Risk Integration:
Signal: dysmorphogenesis (skeletal and soft tissue malformations and alterations) and altered growth

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Effects/Relevance</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal Strength part A</td>
<td>cross species concordance: ++ LG1069 was tested only in the rat.</td>
<td>+1</td>
</tr>
<tr>
<td>- multiplicity of effects: ↑ (effects were seen on skeletal, visceral, external structures)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Signal Strength part B</td>
<td>maternal toxicity: ++ (dysmorphogenesis occurred in the presence of)</td>
<td>+1</td>
</tr>
</tbody>
</table>
Draft Labeling Recommendations- 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 caused developmental mortality and altered growth (≥ 10 mg/kg/day, 1/5th the recommended clinical dose on a mg/m² basis) and was dysmorphic (≥ 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).

GENETIC TOXICOLOGY

1. Salmonella/E. coli mutation assays (vol. 1.39, pp262-28.1, pp 2-65, RR-815-95-005/RR-815-95-004, method: test strains of TA 98, TA 100, TA 1353, TA 1357 (for histidine reversion at GC sites) and WP2uvrA (for tryptophan reversion at AT sites) were used in the presence or absence of S9 fraction. A mixture (2 ml of molten top agar, 0.1 ml indicator organism (about 10⁹ bacteria), and 0.5 ml S9 or buffer, and LG1069 (lot # LG100069-002015)) was poured into a plate containing about 25 ml of minimal glucose agar supplemented with a trace of Oxoid nutrient broth and then incubated at 37°C for 48 h. The histidine or tryptophan independent revertant colonies on each plate were then counted.

GLP statement: yes

Results: Negative

<table>
<thead>
<tr>
<th>Strains</th>
<th>TA 98</th>
<th>TA 100</th>
<th>TA 1353</th>
<th>TA 1357</th>
<th>TA 1538</th>
<th>WP2uvrA</th>
</tr>
</thead>
<tbody>
<tr>
<td>S9 Fraction</td>
<td>-S9</td>
<td>+S9</td>
<td>-S9</td>
<td>+S9</td>
<td>-S9</td>
<td>+S9</td>
</tr>
<tr>
<td>Control, 100 µl DMSO</td>
<td>16</td>
<td>29</td>
<td>121</td>
<td>110</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>LG1069, ug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>27</td>
<td>121</td>
<td>118</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>28</td>
<td>129</td>
<td>137</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>23</td>
<td>25</td>
<td>121</td>
<td>126</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>100</td>
<td>24</td>
<td>24</td>
<td>121</td>
<td>128</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>500</td>
<td>20</td>
<td>35</td>
<td>110</td>
<td>121</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>1000</td>
<td>21</td>
<td>25</td>
<td>114</td>
<td>125</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Positive control</td>
<td>2-NF</td>
<td>2-A</td>
<td>NaN³</td>
<td>NaN³</td>
<td>2-A</td>
<td>2-A</td>
</tr>
</tbody>
</table>

*2-anthramine (2-4 ug); 2-A, N-ethyl-N'-nitro-N-nitrosoguanidine (5 ug); ENNG, 2-nitrofluorene (5 ug); 2-NF; 9-aminoacridine (50 ug); 9-AA; sodium azide (5 ug); NaN³
2. CHO chromosome aberration assay (vol. 1.40, pp 66-141, RR-815-97-008)

Method: CHO-K1 Chinese hamster cells were exposed to 4.9, 19, 78, 312, and 1250 µg/ml (for cytotoxic assay), or 1.25, 2.5, 5, 10, 20, 40 and 60 µg/ml (for chromosome aberration assay) LG1069 (lot # LGD 100069-0002035) in a vehicle (1% DMSO) in the presence or absence of S9 fraction (positive controls: methyl methane sulfonate (MMS 20 µg/ml) without S9 and cyclophosphamide (CP 12.5 µg/ml) with S9) for 3 hours and further allowed to grow for 21 hours and 45 hours. Colcemid (0.2 µg/ml) was added 2.5 hours before harvest to induce mitotic arrest of cells. End points: cytotoxic assay—mitotic index (% cells in mitosis) in at least 500 cells per culture, chromosome aberration assay—chromosome aberrations in 100 cells per culture and polyploidy (45 hours harvest only) from a minimum of 100 mitosis.

GLP statement: yes

Results: Negative. The highest concentration (5 µg/ml) selected in the absence of S9 activation appears to be not adequate since the concentration did not cause at least 50% reduction in cell number or confluency, as recommended by ICH S2A.

Cytotoxicity Assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, h</th>
<th>Mitotic Index (%)</th>
<th>Relative Confluence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-S9</td>
<td>+S9</td>
</tr>
<tr>
<td>LG 1069, µg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21</td>
<td>8.4</td>
<td>10.8</td>
</tr>
<tr>
<td>4.9</td>
<td>21</td>
<td>7.6</td>
<td>12.3</td>
</tr>
<tr>
<td>19</td>
<td>21</td>
<td>4.5</td>
<td>11.3</td>
</tr>
<tr>
<td>78</td>
<td>21</td>
<td>suppressed mitosis/toxicity</td>
<td>suppressed mitosis/toxicity</td>
</tr>
<tr>
<td>312</td>
<td>21</td>
<td>precipitation toxicity</td>
<td>precipitation toxicity</td>
</tr>
<tr>
<td>1250</td>
<td>21</td>
<td>precipitation toxicity</td>
<td>precipitation toxicity</td>
</tr>
</tbody>
</table>

Chromosome Aberration Assay (21 h exposure; 200-cell analyzed)

<table>
<thead>
<tr>
<th>S9</th>
<th>-S9</th>
<th>+S9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>DMSO</td>
<td>LG1069</td>
</tr>
<tr>
<td>Conc. µg/ml</td>
<td>1%</td>
<td>1.25</td>
</tr>
<tr>
<td>Mitotic index (%)</td>
<td>8.4</td>
<td>6.0</td>
</tr>
<tr>
<td>% cells, structurally abnormal chromosome</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>% cells, chromosome exchanges</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Aberrations (frequency/cell)</td>
<td>Structural aberrations</td>
<td>0.03</td>
</tr>
<tr>
<td>chromosome exchange</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>chromatid deletion</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>% cells, chromatid gaps</td>
<td>2.0</td>
<td>3.5</td>
</tr>
</tbody>
</table>


Method: L5178Y mouse lymphoma cells (6 x 10^6 cells/culture of 10 ml RPMI 1640 medium supplemented with 0.1% Pluronic F68, 0.22 mg/ml sodium pyruvate and 5% heat-inactivated donor horse serum; clone 3.7.2.C heterozygous at the tk locus) were exposed to LG1069 (lot 13, 16, 21, 26, 32 and 40 µg/ml LG1069, lot # LG100069-0002035), or vehicle (1% DMSO) only, or a positive control (methyl methanesulfonate (MMS 5 µg/ml), ethyl methanesulfonate (EMA 200 µg/ml), or 20-methyl cholanthrene (MCA 5 µg/ml)) for 4 hours in the presence or absence of S9 fraction (0.1 ml per 10 ml culture medium). After removing treatment solutions by centrifugation, the suspended cell were rotated in a roller drum for 2 days for expression of any mutations. Approximately 3 x 10^6 cells from each culture were seeded in 100 ml of cloning medium supplemented with trifluorothymidine (TFT 5 µg/ml) for selection of TFT-resistant cells and a serial dilution containing approximately 600 cells was seeded in 100 ml of nonselective cloning medium to determine the percentage of viable cells. Cloning medium with cells was poured into 100-mm Petri dishes (3 dishes/100 ml of culture medium). Cells were incubated for 12 days. At the end of the 12-day culture, the colonies of cells in each Petri dish were counted using an Artel Model 880 automatic colony counter with a standard 50-mm lens.
Criteria for positivity:
a. a significant (p<0.05) dose-related increase in the mutant frequency (frequency of TFT-resistant colonies) occurred,
b. the mean mutant frequency of a set of duplicate cultures treated with one or more of the three highest acceptable concentrations of LG1069 was statistically significant (p<0.05),
c. at least one concentration induced an average absolute increase in mutant frequency greater than 70 x 10^{-6}, and
d. the results were reproducible in a second experiment.

GLP statement- yes

Results- Negative

<table>
<thead>
<tr>
<th>S9 Activation</th>
<th>-S9 Fraction</th>
<th>+S9 Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Cloning Efficiency (%)</td>
<td>Average Mutant Frequency (%)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Control 1% DMSO</td>
<td>LG1069, ug/ml</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>LG1069, ug/ml</td>
<td>13</td>
<td>138</td>
</tr>
<tr>
<td>16</td>
<td>96</td>
<td>63</td>
</tr>
<tr>
<td>21</td>
<td>74</td>
<td>67</td>
</tr>
<tr>
<td>26</td>
<td>4.5</td>
<td>88</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>40</td>
<td>not cloned</td>
<td>not cloned</td>
</tr>
<tr>
<td>MMS, 5 ug/ml</td>
<td>97</td>
<td>318</td>
</tr>
<tr>
<td>EMS, 200 ug/ml</td>
<td>105</td>
<td>488</td>
</tr>
<tr>
<td>MCA, 5 ug/ml</td>
<td></td>
<td>76</td>
</tr>
</tbody>
</table>

4. In vivo bone marrow micronucleus assay in mice (vol. 1.41, pp 2-163, RR-815-97-010, 1993.6 g, 6-8 weeks of age) received a single oral dose of 0, 250, 500 and 1000 mg/kg LG1069 (in DMSO, lot # LG100069-0002036) or positive control (urethane 30 mg/kg) by gavage (5 ml/kg). At 24, 48 or 72 h postdose, mice (5/sex/group per each sampling time) were euthanized, both femurs were removed, and 3 bone marrow slides were prepared. For cytological analysis, the number of polychromatic erythrocytes (PCE) among 200 erythrocytes per animal and the number of micronucleated PCE among a total of 2000 PCE per animal were determined.

Criteria for positivity:
a. there was a statistically significant (p<0.05) increase in micronucleated PCE,
b. the increase was dose-related, and
c. the micronucleated PCE frequency was greater than the mean historical micronucleus vehicle frequency + 2SD (e.g. 0.19 + 0.46).

GLP statement- yes

Results- negative

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of PCE with micronucleus</th>
<th>Clinical Signs</th>
<th>Body weight gain (d1-4), g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h (M/F)</td>
<td>48 h (M/F)</td>
<td>72 h (M/F)</td>
</tr>
<tr>
<td>LG1069, mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25/15</td>
<td>28/15</td>
<td>37/14</td>
</tr>
<tr>
<td>250</td>
<td>28/19</td>
<td>28/21</td>
<td>26/23</td>
</tr>
<tr>
<td>500</td>
<td>16/22</td>
<td>26/20</td>
<td>31/23</td>
</tr>
<tr>
<td>1000</td>
<td>24/22</td>
<td>32/19</td>
<td>36/21</td>
</tr>
<tr>
<td>Urethane, 30 mg/kg</td>
<td>57</td>
<td>29</td>
<td>27</td>
</tr>
</tbody>
</table>

Overall Genetic Toxicity Summary- LG1069 is not mutagenic in bacterial (Salmonella and E.coli) or mammalian cell (mouse lymphoma L5178Y tk-/+ mutant) 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.