TABLE 2. Mean Endogenous Plasma Concentrations and Pharmacokinetic Parameters of Retinoids and Retinoid Metabolites Following Ingestion of Fried Turkey Liver (2 g raw weight/kg body weight) by Healthy Male Volunteers (N=10)[a]

Retinoid[b]	Endogenous Concentration (ng/mL)	Post-Liver Consumption C_{max} (ng/mL)	Post-Liver Consumption $AUC_{(0-24)}$ (ng·hr/mL)
9-*cis*-RA	<0.30	2.7	10.7
Retinol	641	800	16822
retinyl palmitate	32.2	3540	21114
14-HRR	1.3 (N=3)	3.7	61.7
ATRA	0.8	2.0	19.7
4-oxo-ATRA	0.6 (N=3)	0.8	14.7
13-*cis*-RA	1.1	21.5	204
4-oxo-13-*cis*-RA	2.4	32.1	435
9,13-di-*cis*-RA	< 0.5	17.1	68.2

[a] N = 10 for all mean retinoid concentration except 14-HRR and 4-oxo-ATRA where N=3 as indicated within the table.

[b] 9-*cis*-RA = 9-*cis*-retinoic acid; 14-HRR = 14-hydroxy-4,14-retro-retinol; ATRA = all-*trans*-retinoic acid; 4-oxo-ATRA = 4-oxo-all-*trans*-retinoic acid; 13-*cis*-RA = 13-*cis*-retinoic acid; 4-oxo-13-*cis*-RA = 4-oxo-13-*cis*-retinoic acid; 9,13-di-*cis*-RA = 9,13-di-*cis*-retinoic acid.

2. Absorption and Pharmacokinetics of 9-*cis*-Retinoic Acid Following Topical Application

a. 9-cis-Retinoic Acid Penetration of Human Cadaver Skin and 9-cis-Retinoic Acid Epidermal Concentrations Following Topical Application to Healthy Volunteers

Two studies evaluated the ability of 9-*cis*-retinoic acid to penetrate into the skin following application of alcohol-based drug solutions.

In a non-GLP pilot study, the *in vitro* penetration of nine 9-*cis*-retinoic acid test topical formulations (seven % gels, two % ointments) was assessed using human cadaver skin. The study demonstrated drug delivery into the dermis and epidermis for all nine test formulations. A gel formulation containing 9-*cis*-retinoic acid in a vehicle consisting of dehydrated alcohol, polyethylene glycol 400 (PEG 400), and hydroxypropyl cellulose provided flux through the skin (approximately % penetration) with the largest recovery in the stratum corneum (%) and epidermis (%).

In the second study, skin concentrations of 9-*cis*-retinoic acid and other retinoids were determined to assess the effect of topically-applied retinoids on epidermal 4-hydroxylase activity. Solutions (0.1%) of either ATRA, 13-*cis*-retinoic acid or 9-*cis*-retinoic acid were prepared in a vehicle consisting of %

with mg/mL butylated hydroxytoluene. The solutions were applied to 3x2 inch areas on the buttocks of volunteers (N=7) and occluded for a period of two or four days. Retinoid concentrations and 4-hydroxylase activity were determined in the biopsy samples. In vehicle-treated skin, the total retinoid concentration in the biopsy samples was nM (about ng/g), consisting of 30% ATRA and 70% 13-*cis*-retinoic acid. Two days following application of 9-*cis*-retinoic acid, the total retinoid concentration in the skin biopsy was nM (about ng/g), consisting of 52% 9-*cis*-retinoic acid, 36% ATRA, and 12% 13-*cis*-retinoic acid. Total- skin retinoid concentrations following application of ATRA or 13-*cis*-retinoic acid were similar to those observed following 9-*cis*-retinoic acid application, but the proportion of each retinoid isomer observed was markedly different. Application of all isomers resulted in an increase in skin 4-hydroxylase activity. However, only ATRA appeared to be metabolized by the enzyme. Thus, topical application of an alcoholic solution of 9-*cis*-retinoic acid resulted in quantifiable epidermal concentrations of 9-*cis*-retinoic acid. 9-*cis*-Retinoic acid induced 4-hydroxylase activity in the skin but did not appear to be a substrate for the enzyme.

b. Plasma 9-cis-Retinoic Acid Concentrations in Patients with Cutaneous T-Cell Lymphoma Following Topical Application of PANRETIN™ Gel

Phase 1-2 trials of PANRETIN™ Gel (0.01%, 0.05% and 0.1% [w/w]) for the treatment of cutaneous T-cell lymphoma (CTCL, mycosis fungoides) were conducted in adult patients diagnosed with histologically-confirmed CTCL to evaluate systemic exposure following repeat topical application of PANRETIN™ Gel.

Single-time point plasma samples were obtained from patients at Weeks 2 and 4 and every two to four weeks thereafter while patients remained on study. Plasma concentrations of 9-*cis*-retinoic acid and ATRA were determined using a method with LLQ values of ng/mL for 9-*cis*-retinoic acid and ng/mL for ATRA.

A total of 107 plasma samples from seven patients with CTCL were analyzed. A majority (51%) of the samples was obtained following repeat application of the lowest strength gel (0.01%). In all samples, plasma concentrations of 9-*cis*-retinoic acid and ATRA were below their respective LLQ values. Individual patient treatment areas ranged from % of their body surface area (median: 2%). Thus, even following extensive topical application of PANRETIN™ Gel, there was no quantifiable systemic exposure to 9-*cis*-retinoic acid.

c. Plasma 9-cis-Retinoic Acid Concentrations in Patients with Kaposi's Sarcoma Following Topical Application of PANRETIN™ Gel

Phase 1-2 trials of PANRETIN™ Gel (0.01%, 0.05% and 0.1% [w/w]) for the treatment of the cutaneous lesions of KS were conducted in patients with acquired

immunodeficiency syndrome (AIDS) to evaluate systemic exposure following repeat topical application of PANRETIN™ Gel.

Single-time point plasma samples were obtained from 94 patients at Weeks 2 and 4 and every two to four weeks thereafter while patients remained on study. Plasma concentrations of 9-*cis*-retinoic acid and its metabolite 4-oxo-9-*cis*-retinoic acid (LG100182), or its isomeric metabolite ATRA, were determined using methods. A total of 483 plasma samples from 72 patients were analyzed with methods with a 9-*cis*-retinoic acid LLQ value of ng/mL. Samples represented treatment periods of up to weeks and a majority (60%) of the samples was obtained following repeat application of the 0.1% gel strength. Approximately half of the samples (N=239) were obtained within four hours of the most recent gel application. The samples were obtained without dietary restrictions.

A total of 153 plasma samples from 22 patients were analyzed using method with a 9-*cis*-retinoic acid LLQ value of ng/mL. Samples represented treatment periods of up to 44 weeks and over one-half of these samples (N=81; 53%) were obtained within four hours of the most recent gel application. At least 70% of the samples were obtained following repeat application of the highest dose strength gel (0.1%). The samples were obtained without dietary restrictions. Baseline samples were not available for any of the patients.

9-*cis*-Retinoic acid concentrations were not quantifiable in any of the 483 samples analyzed using the analytical methods with LLQ values of ng/mL. In addition, plasma concentrations of ATRA and 4-oxo-9-*cis*-retinoic acid were less than their respective LLQ values: ng/mL and ng/mL. Using these assay methods, there was no quantifiable systemic exposure to 9-*cis*-retinoic acid, ATRA or 4-oxo-9-*cis*-retinoic acid following repeat topical application of PANRETIN™ Gel.

9-*cis*-retinoic acid was quantifiable in 26 of the 153 samples (17%) analyzed using analytical method (LLQ of ng/mL). The highest observed 9-*cis*-retinoic acid concentration was ng/mL. Ten of 22 (45%) evaluated patients had quantifiable 9-*cis*-retinoic acid concentrations in at least one plasma sample. 4-oxo-9-*cis*-retinoic acid was not quantifiable in any of the 153 samples (LLQ: ng/mL). Quantifiable 9-*cis*-retinoic acid concentrations were observed following application of either the % gel strengths at time points ranging from hours following the most recent gel application to 339 hours following the most recent gel application. Quantifiable concentrations were seen at the earliest sampling period (Week 2) and following up to 40 weeks of PANRETIN™ Gel application. Of the 26 samples with quantifiable 9-*cis*-retinoic acid concentrations, 19 samples were obtained from 4 patients who had primarily applied the 0.1% gel strength. In these 4 patients, 19 of 39 samples (49%) contained quantifiable drug concentrations, in contrast to 4 of 69 samples (6%) for samples obtained from the other 15 patients who applied the 0.1%

gel. Within the 4 patients with the majority of quantifiable concentrations, quantifiable concentrations were sporadically observed as early as 0.5 hours following the most recent gel application out to time points as late as 339 hours following the most recent gel application. Both the range of observed 9-*cis*-retinoic acid concentrations and the frequency of quantifiable concentrations are similar to those observed in patients with psoriasis prior to receiving oral 9-*cis*-retinoic acid therapy. The observed 9-*cis*-retinoic acid concentrations were less than those which had been observed in healthy volunteers following ingestion of a vitamin- A-rich meal. Thus, the quantifiable 9-*cis*-retinoic acid concentrations observed in the Phase 1-2 topical studies of PANRETIN™ Gel probably represented endogenous systemic exposure to 9-*cis*-retinoic acid, with no discernible contribution from topical application of the product. There did not appear to be any relationship between quantifiable 9-*cis*-retinoic acid concentrations and gel strength, time since application, frequency of application, or extent or duration of application. The lack of correlation between 9-*cis*-retinoic acid systemic exposure and treatment emphasizes that quantifiable concentrations may have been due, in whole or in part, to dietary or nutritional supplement sources of this endogenous substance rather than exogenous application of 9-*cis*-retinoic acid.

3. Pharmacokinetics of 9-*cis*-Retinoic Acid Following Oral Application

The pharmacokinetics of 9-*cis*-retinoic acid and its isomeric metabolite, ATRA, were evaluated following repeat daily or twice daily oral administration of PANRETIN™ Capsules to patients with advanced cancer and following repeat daily administration to patients with severe plaque psoriasis. In addition, three literature publications provide 9-*cis*-retinoic acid pharmacokinetic parameters following oral administration of soft gelatin capsules containing 9-*cis*-retinoic acid (from a source independent of Ligand Pharmaceuticals Inc.) to healthy volunteers or patients with solid tumors. Two of these publications also provided an evaluation of the metabolites of 9-*cis*-retinoic acid.

The single dose pharmacokinetics are approximately dose-proportional over a mg/m^2 dose range. C_{max} values occur within hours following dosing. Across studies, terminal elimination phase half-life values are approximately 1-2 hours.

Following repeat once-daily oral dose administration of 9-*cis*-retinoic acid at doses mg/m^2, C_{max} and AUC values were similar to Day 1 values. A dose-related apparent induction of oral clearance of 9-*cis*-retinoic acid was observed in most studies at higher total daily dose levels (mg/m^2). Following repeat twice-daily oral dose administration, the extent of induction appeared to be related to the total daily dose administered. In all studies, repeat dose terminal elimination half-life values were similar to values observed following single dose administration.

Repeat dose predose concentrations were generally less than 5% of C_{max} values and were routinely detected only at dose levels mg/m², indicating that there is minimal accumulation of drug on a repeat once-daily or twice-daily dose regimen.

4-oxo-9-*cis*-Retinoic acid was quantitated in many of the studies. Following both single-dose and multiple-dose administration, plasma concentrations of this metabolite were approximately one-half to equivalent to concentrations of parent compound. Little isomerization of 9-*cis*-retinoic acid to ATRA, 13-*cis*-retinoic acid or 9,13-di-*cis*-retinoic acid was observed.

Administration of low oral doses mg) of 9-*cis*-retinoic acid did not appear to affect total body retinoid pool as assessed by plasma retinol concentrations. Administration of higher oral doses mg) of 9-*cis*-retinoic acid resulted in dose-related decreases in plasma retinol concentrations of up to 30%.

4. Pharmacokinetics of 9-*cis*-Retinoic Acid in Special Populations

No formal studies were conducted to evaluate the pharmacokinetics of 9-*cis*-retinoic acid in special populations. In order to assess the impact of patient demographic characteristics on 9-*cis*-retinoic acid pharmacokinetics, compiled data from the two Ligand-sponsored Phase 1-2 studies evaluating PANRETIN™ Capsules in patients with advanced cancer were partitioned and analyzed according to age, gender and race. Overall mean age of the combined patient population was 57 yr (median: 59.0 yr; range: yr). Of the 79 patients, 61 (77%) were Caucasian, 6 (7.5%) were Black, 3 (3.8%) were Filipino, 3 (3.8%) were Indian, 2 (2.5%) were Asian, 2 (2.5%) were Lebanese, 1 (1.3%) was Hispanic and 1 (1.3%) was Mediterranean. Baseline age for 57 of the patients was less than 65 yr, while 22 patients had a baseline age greater than or equal to 65. Forty-three of the patients were male, 36 were female. While there were some differences in parameters between the different age, gender and race groups at individual dose levels, no consistent differences in the single or multiple dose pharmacokinetics of 9-*cis*-retinoic acid between the groups were noted.

5. Metabolic Fate of 9-*cis*-Retinoic Acid

a. in vitro metabolism

In vitro studies were conducted to identify the human cytochrome P450 (CYP) isozymes involved in human 9-*cis*-retinoic acid metabolism. Three experimental designs were employed to assess the capability of different CYP isozymes to metabolize 9-*cis*-retinoic acid.

In first study, CYP isozymes capable of metabolizing 9-*cis*-retinoic acid were identified through the use of competitive inhibitors and mechanism-based inhibitors of specific

CYP isozymes in pooled human liver microsome preparations. It was concluded that CYP 1A1, 1A2, 2B, 2C9, 2C19 and 3A4 were likely to be involved in the metabolism of 9-*cis*-retinoic acid. CYP 2D6 was thought not to be involved in 9-*cis*-retinoic acid metabolism and the results for CYP 2A6 and 2E1 were inconclusive.
The second study use the variability in ability of individual donor human liver microsome preparations to metabolize isozyme-specific substrates to determine if the metabolism of a test compound is correlated with activity of a specific isozyme across a panel of individual donor preparations and therefore possibly metabolized by those isozymes. Correlations were considered biologically relevant if correlation coefficients were greater than A strong correlation between the extent of metabolite formation and the amount of isozyme-specific metabolic activity across the panel of preparations suggested CYP 2C9 and 3A4 were likely to be involved in 9-*cis*-retinoic acid metabolism. In contrast, there did not appear to be a correlation between metabolite formation or 9-*cis*-retinoic acid disappearance and the relative activity for CYP 2C19, 2E1, 4A9 and 4A11.

The third study used human B-lymphoblastoma cell lines expressing recombinant individual CYP isozymes to assess the potential for individual isozymes to metabolize test compounds. Microsomes from cell lines expressing CYP 2C9 produced the greatest quantity of 4-hydroxy-9-*cis*-retinoic acid and 4-oxo-9-*cis*-retinoic acid. In addition, microsome preparations from cells expressing CYP isozymes 1A1, 1A2, 2B6, 2E1, and 3A4 were capable of metabolizing 9-*cis*-retinoic acid to 4-hydroxy-9-*cis*-retinoic acid; microsome preparations from cell lines expressing CYP 1A1, 1A2, and 2D6 were capable of metabolizing 9-*cis*-retinoic acid to 4-oxo-9-*cis*-retinoic acid.

The collective data from the three individual metabolic studies were evaluated to identify CYP isozymes which, based on all experimental data, were likely to be involved in metabolism of 9-*cis*-retinoic acid. Based on data from all three studies, CYP isozymes 1A1, 1A2, 2C9, and 3A4 are likely to be involved in human metabolism of 9-*cis*-retinoic acid. Based on lack of activity, or conflicting information, CYP isozymes 2A6, 2B, 2C8, 2C19, 2D6, 2E1, and 4A9/11 were unlikely to be involved in the human metabolism of 9-*cis*-retinoic acid.

b. In Vivo Metabolic Observations

The *in vivo* metabolism of 9-*cis*-retinoic acid following oral administration has been evaluated in a number of studies. The major identified circulating 9-*cis*-retinoic acid metabolite is 4-oxo-9-*cis*-retinoic acid. While plasma concentrations of this metabolite are below the LLQ value following topical PANRETIN™ Gel application, plasma concentrations of this metabolite are approximately one-half that of parent compound to equivalent to that of parent compound following oral 9-*cis*-retinoic acid administration. Isomerization of 9-*cis*-retinoic acid to ATRA has been observed, but ATRA concentrations are generally less than 10% those of parent compound. Only minimal

conversion to other isomers (9,13-di-*cis*-retinoic acid and 13-*cis*-retinoic acid) has been observed. All retinoic acid isomers were present in fecal samples following oral administration of 9-*cis*-retinoic acid. Other minor metabolites observed in human plasma include 4-oxo-ATRA and possibly other 4-oxo retinoid isomers. At least four urine glucuronide conjugates are associated with oral 9-*cis*-retinoic acid administration. Two of the glucuronide conjugates have been identified as conjugates of 9-*cis*-retinoic acid and 4-oxo-9-*cis*-retinoic acid, with the latter being a major urinary metabolite. However, it should be noted that urinary excretion appears to be a minor elimination pathway overall.

Based on *in vivo* and *in vitro* studies, metabolism of 9-*cis*-retinoic acid occurs through oxidation to 4-hydroxy and 4-oxo metabolites; isomerization to ATRA, 9,13-di-*cis*-retinoic acid and 13-*cis*-retinoic acid; and glucuronidation of parent compound, 4-oxo-9-*cis*-retinoic acid and possibly other aglycones. 4-oxo-9-*cis*-Retinoic acid and 4-hydroxy-9-*cis*-retinoic acid are capable of binding to and activating retinoid receptors, however, their activity and potency are less than that for 9-*cis*-retinoic acid.

6. Binding of 9-*cis*-Retinoic Acid to Human Plasma Proteins

The *in vitro* free fraction of 9-*cis*-retinoic acid in human plasma was determined using methods.

V. GENERAL COMMENTS

1. The NDA provided an understanding of the systemic exposure of 9-*cis*-retinoic acid after topical application of PANRETIN™ Gel in patients with AIDS-related Kaposi's sarcoma. This was supported by the studies in which PANRETIN™ Gel was used in patients with CTCL and the studies in which oral PANRETIN™ capsule was used.

2. The applicant's request for a waiver of *in vitro* release test is acceptable based on that this product consists of % Dehydrated Alcohol, USP, However, in order to control the quality of the product, data should show that the concentration of the drug is invariant from batch to batch.

3. The samples for determining systemic exposure in the KS studies were obtained from the patients without dietary restrictions. Baseline samples were not available for any of the patients. These deficiencies weakened the conclusion that quantifiable concentrations may be due, in whole or in part, to dietary or nutritional supplement sources of this endogenous substance rather than exogenous application of 9-*cis*-retinoic acid.

APPEARS THIS WAY
ON ORIGINAL

VI. LABELING COMMENTS

1. In the Pharmacokinetics subsection of the CLINICAL PHARMACOLOGY Section, the following statements should be added:

2. The following statements in Drug Interaction subsection of PRECAUTIONS Section:

should be changed to:

3. The following statements in Drug Interaction subsection of PRECAUTIONS Section:

was based on the preclinical animal studies. Is there any human data to show the drug interaction between DEET and Panretin® gel? If there is, the information should be provided to support this statement.

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