were 25.38, 26.90, and 26.83 g/day, respectively (table 5). The ratios of LSMs for A/C and B/C were 0.95 and 1.00, respectively. Plasma concentrations of unchanged orlistat and its primary metabolite M1 (Ro 42-3988) for groups A, B, and C were similar among the three groups. On the basis of the results of the test parameters of fat absorption, the 120 mg formulations (the phase III formulation and that intended for market use in either the U.S. or Europe) were considered to be pharmacologically equivalent.

Two aspects of this bioequivalence study need comment; the pharmacodynamic endpoint and the need for the study. According to the Emax dose-response model established for orlistat (see page 19), the percent of ingested fecal fat that is excreted is an insensitive pharmacodynamic marker at high doses (i.e. doses at which Emax is approached). At 360 mg daily, approximately 98% of Emax has been attained; a 25% decrease in 'luminal exposure' of orlistat (e.g. a dose of 270 mg daily or decreased dissolution of a capsule) would still be expected to attain 93% of Emax. Therefore, its utility in testing the bioequivalence of generic, or other, formulations of orlistat remains dubious.

Secondly, there is a need to comment on the necessity for this bioequivalence study. The sponsor claims that the only differences between the clinical and TBM formulations are the color of and imprint upon the capsule, the manufacturing equipment size, and manufacturing site. According to SUPAC Guidance (November, 1995), any one of these changes would not require *in vivo* documentation of bioequivalence in the postapproval period; here, multiple changes have been made by the sponsor and it remains uncertain whether these multiple changes have a significant impact on formulation quality and performance, and require *in vivo* bioequivalence documentation.

Table 5. Summary stats of results for pivotal 120 mg BE from NP15400

Parameter	A (U.S. Market)	[redacted]	C (Phase III)
24-h Fecal Fat (g/day)[a]			
LSMs	25.38	[redacted]	26.83
Ratio of LSMs		A/C = 0.95	
90% CI for Ratio of LSMs		A/C = 0.87, 1.02	
Plasma orlistat (Ro 18-0647)			
# of measurable/N	11/18	[redacted]	11/18
Conc. Range (ng/mL)	0.26 - 3.33		0.21 - 1.75
Plasma M1 metabolite (Ro 42-3988)			
Mean ± SD (ng/mL)	45.46 ± 21.57	[redacted]	40.08 ± 17.81

[a] The change from baseline

II. Pharmacokinetics

[redacted] BEST POSSIBLE

Phase II / III Plasma Monitoring

General Methods and Design

One blood sample was taken from each patient after ~ 24, 52, and / or 104 weeks of treatment with orlistat or placebo tid with meals. In BM14119B (1 year efficacy) and BM14119C (2 year efficacy), trough samples were drawn prior to the morning dose (~16 hours after the last dose taken in the previous day) and only unchanged orlistat was determined. In BM14150 (6 month dose-response), NM14161 (2 year efficacy), and NM14336 (1 year in NIDDM), samples were drawn at 2-4 hours after the lunch or dinner dose; both orlistat

and metabolites M1 (Ro 42-3988) and M3 (Ro 42-2556) were measured. Limits of quantification for orlistat, M1, and M3 in plasma were 0.2, 0.32, and 10 ng/mL, respectively, and assays for orlistat and M1 were validated. (See appendix for study synopses)

Protocol BM14150 (6 Month, Phase II)

Plasma samples collected after 24 weeks of treatment were analyzed for the presence of unchanged orlistat and its metabolites M1 and M3. Quantities of orlistat in the range of 0.20 to 8.77 ng/mL were detected in the plasma of a few of the orlistat-treated patients. The overall data suggest that there is a dose-related increase in 1) plasma concentrations of M1 metabolite after 24 weeks of treatment with orlistat, and 2) percent of measurable plasma concentrations of intact orlistat. This dose-proportionality is lacking for M3 metabolite.

Protocol BM14119B (1 Year, Phase III)

Plasma samples collected after 24 and 52 weeks of treatment were analyzed for the presence of unchanged orlistat. Minute quantities of orlistat were detected in the plasma of 5 out of 89 (5.6%) orlistat-treated patients after 24 weeks, but none after 52 weeks, of treatment. This suggests that systemic exposure of orlistat at trough levels was extremely low and that there was no accumulation from 24 to 52 weeks of treatment.

Protocol BM14119C (2 Year, Phase III)

Plasma samples collected after 24, 52, and 104 weeks of treatment were analyzed for the presence of unchanged orlistat. Quantities of orlistat in the range of 0.21 to 2.08 ng/mL were detected in the plasma of a few samples from orlistat-treated patients (32/668 or 4.8%) after 24, 52, and 104 weeks of treatment. The overall data tend to indicate that there was lack of accumulation of plasma concentrations of intact orlistat from 24 to 52 to 104 weeks of treatment with orlistat.

Protocol NM14161 (2 Year, Phase III)

Plasma samples collected after 20, 48, and 100 weeks of treatment were analyzed for the presence of unchanged orlistat and metabolites M1 and M3. There was low systemic exposure to orlistat, but the number of samples that contained measurable orlistat appeared to be proportional to the dose of orlistat. Plasma levels of metabolite M1 and M3 were all measurable. M1, but not M3, appeared to be proportional to the dose of orlistat. No further accumulation was found from 6 mos to 1 year and to 2 years for plasma concentrations of both orlistat and metabolites M1 and M3.

Protocol NM14336 (1 Year, Phase III)

To examine any potential changes in orlistat exposure and metabolism in a subgroup of obese patients, plasma monitoring was conducted in obese subjects with non-insulin dependent diabetes mellitus (NIDDM) maintained on oral hypoglycemic agents. Samples for measurement of drug levels were taken after 20 weeks of treatment. Quantities of intact orlistat in the range of 0.21 to 8.3 ng/mL were detected in 76/118 (64%) of the plasma samples analyzed, each subject contributing one sample.

Kinetics of M1 and M3 in Plasma (Protocol BD14419)

Eleven blood samples were taken from each of 24 volunteers on day 1 (prior to the start of treatment), day 5 (pre-dose, i.e., C_{trough}, and 2, 4, 6, 8, 10, and 12 h after the morning dose), and day 10 (pre-dose, and 7.75 and 8 hours postdose) of treatment with 120 mg orlistat or placebo tid (except that no lunch dose was given on days 5 and 10). The day-5 samples were analyzed to evaluate the kinetics of orlistat, M1 and M3.

Detectable orlistat plasma levels (0.2 - 3.37 ng/mL) were most frequent at 2 to 6 h after the morning dose on

day 5. M1 and M3 were present in all, except for a few subjects' samples; mean pharmacokinetic parameters derived from day-5 data are summarized in Table 6.

Table 6. PK analysis of plasma from BD14419

	Ro 42-3988 (M1)			Ro 42-2556 (M3)		
Parameter	Mean	Range	C.V. (%)	Mean	Range	C.V. (%)
C_{trough} (ng/mL)	26.30	2.41-45.20	60	151	11-437	88
C_{max} (ng/mL)	44.66	9.75-59.35	31	204	38-544	80
t_{max} (h)	5.09[a]	4-8	27	7.83	4-12	30
AUC_{0-12} (ng·h/mL)	328.7	59.2-446.7	33	1953	235-4915	78
λ_z (1/h)	0.24	0.17-0.32	21	0.07[b]	0.03-0.16	70
$t_{½}$ (h)	3.04	2.16-4.20	22	13.52[b]	4.32-20.07	46

[a] N = 11.
[b] N = 7.

Summary of Monitoring Plasma Concentrations

Systemic exposure to orlistat was minimal with a lack of accumulation of orlistat in plasma after a long-term treatment with a therapeutic dose of orlistat (i.e., 120 mg tid) in phase II and III trials.

Bile concentrations of orlistat and M1 (Protocol ND14278) were measured via NG tube aspiration. Orlistat concentrations in bile measured 16 hours post-dose (mean: 19.6 ng/mL, range: <2 to 43.5 ng/mL, n=9) tended to be greater than those seen in plasma, while M1 concentrations in bile (mean 21.5 ng/mL: range <2 to 46.2 ng/mL, n=8) were similar to observed plasma concentrations from other studies, indicating both orlistat and M1 undergo biliary excretion.

A. Single vs. Multiple Dose Administration

Although Phase I single and multiple dose studies did collect plasma samples for orlistat analysis, most/all assayed samples contained orlistat at concentrations below the LOQ (5 ng/mL) using [redacted] assay. Most phase II studies investigated efficacy and tolerability and did not include pharmacokinetic sampling.

B. Healthy volunteers vs. patients

Plasma concentrations of orlistat and metabolites tended to be comparable in obese and nonobese healthy subjects.

C. Food Effects

The proposed labeling states that orlistat may be taken "during or up to 1 hour after the meal" As the mechanism of action of orlistat is to prevent the absorption of fatty acids from meals, it is reasonable that it be taken with meals. In the 7 efficacy and safety trials orlistat was administered "with breakfast, lunch, and dinner". Three phase I studies examined the effects of taking orlistat mid-meal, after meals, 0.5, 1, or 2 hours after a meal; results indicated that fecal fat excretion was not influenced by the time of administration. However, none of these studies was submitted to OCPB for review - only study synopses were provided. These phase I studies were done with low-dose, not-to-be-marketed formulations and measured fecal fat, a pharmacodynamic endpoint that was used in bioequivalence studies but is not validated as a surrogate marker for long-term efficacy. This was discussed with the Medical Officer (Dr. Colman); he is comfortable with the proposed labeling.

III. Metabolism

One mass balance study (NK14883) was used to determine the metabolic profile of [redacted]C-labeled orlistat in the plasma and urine of obese volunteers. The overall proposed metabolic pathway is presented in Figure 3.

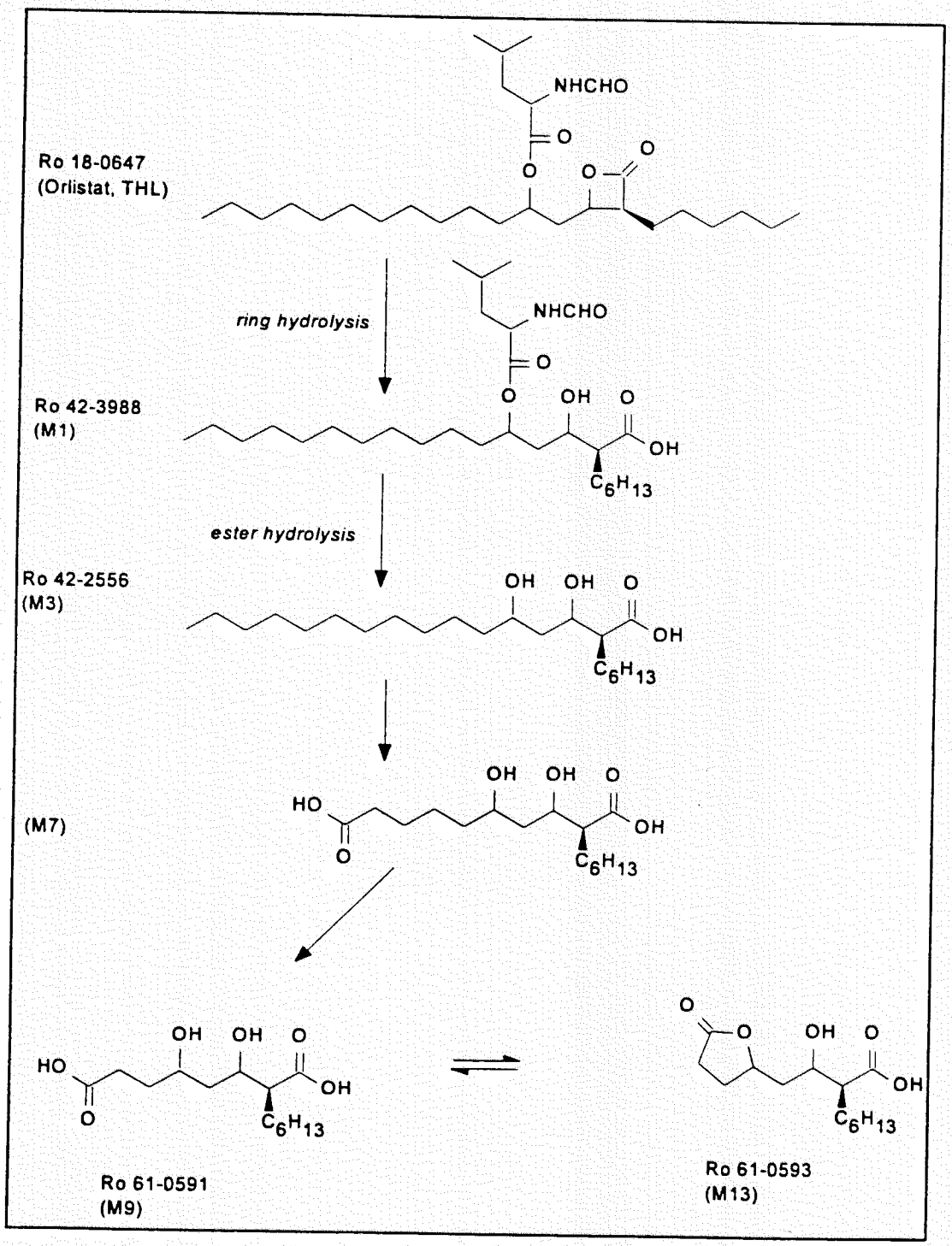

Figure 3. The Proposed Metabolic Pathway of Orlistat.

In the plasma of both male and female volunteers, two major metabolites were identified: the β-lactone ring hydrolysis product of orlistat, M1, and the subsequent ester hydrolysis metabolite after the cleavage of the N-formyl leucine side-chain (M3). Orlistat, M1, and M3 were also found in human bile (study ND14278). Metabolites M7, M9, M13, and the glucuronic acid conjugate of M13 (M13-GA) were the major metabolites found in human urine. All these metabolites contain a hydrolyzed β-lactone ring and are, therefore, inactive.

Three single-dose scintillation studies using radio-labeled orlistat (Ro 18-0647/004, lactone ring ^{14}C-labeled) were conducted in male and female subjects. Orlistat (NB: not the TBM formulation) was administered orally half-way through a breakfast. Samples of blood, urine, and feces were collected before and at intervals for 5 - 9 days after drug administration until no residual radioactivity was present in any of the three biological specimens. Overall results from the three mass balance studies (table 7) indicate high recovery of the labeled material from the feces with only low recovery from urine ; this is consistent with minimal absorption of orlistat.

Table 7. Summary of pharmacokinetic parameters for [redacted]C-labeled compounds (total radioactivity) in three studies

Variable	P-7166	NK14178	NK14883
No. of Subjects (Gender)	6 (M)	8 (M)	8 (5M/3F)
Volunteer Status	normal	normal	obese
Formulation	[redacted]	[redacted]	[redacted]
Dose (exact)			
μCi	47.6	176	366
mg	48.4	350	357
Urinary excretion recovery (%)[a]	4.12 ± 0.76	1.53 ± 0.21	1.13 ± 0.50[d]
Fecal excretion recovery (%)[a]	100.4 ± 6.8	97.4 ± 21.0[b,*]	96.4 ± 18.1[e]
		104.4 ± 10.7[c]	101.9 ± 11.7[f]
Plasma concentration			
C_{max} (ng eq/mL)	51 ± 15	151 ± 27	150 ± 51[g]
t_{max} (hrs)	7.5 ± 1.2	8.2 ± 4.6	6.8 ± 1.5[g]

Pharmacokinetic parameters are reported as mean ± S.D.
[a] Percent of dose administered.
[b] n = 7, excluding subject 6, who had several missing samples.
[c] n = 6, excluding subjects 5 and 6 who had several missing samples.
[d] 0-24 hours.
[e] n = 7, excluding subject 1 whose fecal recovery was unusually low.
[f] n= 6, excluding subjects 1 and 2 who had low fecal recovery.
[g] Based on a limited number of plasma sample 4-10 hours post-dose.
* 83 ± 8.1% as intact orlistat.

IV. Dose and Dosage Form Proportionality

Orlistat and M1, but not M3, plasma concentrations tended to be dose proportional, upto 240 mg, although orlistat remained below LOQ in many samples.

[redacted]

V. Special Populations

Renal / Hepatic insufficiency

No studies conducted in these population.

Age

The effect of age was not studied.

Race

The effect of race was not studied.

Gender

Pharmacokinetic differences due to gender were examined in a few small biostudies, with no differences being found. No specific studies were mounted to explore the effects of gender on the pharmacokinetics of orlistat.

Pediatric

No subjects under the age of 18 were enrolled in clinical trials.

VI. Drug Interactions / Binding Studies

A. In vitro

Protein Binding

Protein binding of orlistat in the human plasma was determined *in vitro* after addition of ^{14}C-labeled drug to plasma, human serum albumin solution, and isolated human proteins. Because of the limit of quantification (5 ng eq/mL), binding data could only be obtained at high drug concentrations (many μg/mL) which were several thousands times higher than plasma concentrations observed at therapeutic doses. Analysis of drug concentrations was carried out by liquid scintillation counting. The binding of orlistat (intact and small amount of degradation products) to plasma proteins was > 99%, suggesting that free concentrations of the drug in plasma were lower than 1%. Lipoproteins and serum albumin appeared to be major binding proteins in human plasma. Although orlistat appears to be highly bound in plasma, minimal systemic exposure at therapeutic doses precludes any clinical implications through displacement.

Partitioning into Red Blood Cells

The red blood cell/plasma partitioning was measured using freshly drawn human, dog, and rat blood. Equilibration of orlistat between red blood cells and plasma at 37°C was rapid ($\leq$ 5 min) and fully reversible. The blood/plasma partitioning at 37°C was 0.63 - 0.78 in all three species.

No *in vitro* drug interaction studies were performed.

B. In vivo

A total of fourteen drug interactions studies were performed by the firm. Four studies (using atenolol, captopril, furosemide and nifedipine) were small (n=6) sequential studies and used only 50 mg of orlistat. Although these studies are presented for completeness in the Appendix, due to the small sample size and sequential design, they are considered to be of limited usefulness. The remaining studies were generally

performed in 12-30 normal subjects and utilized a crossover design (the alcohol and pravastatin studies were parallel). The firm's approach to selecting drugs to perform interaction studies was to choose drugs which obese patients might be expected to be using, drugs with a narrow therapeutic index, and fat-soluble vitamins. The results of all studies are listed in Table 8.

Drugs likely to be used in the target population

Pravastatin

In a parallel study in 24 volunteers given 40 mg pravastatin for 10 days with either orlistat 120 mg TID or placebo, an increase in the bioavailability of pravastatin of about 31% was seen in the treatment group as compared with placebo. A 30% drop in lipid parameters (total cholesterol, LDL, triglycerides) was also noted in the treatment group as compared with placebo; however, it is unknown whether this effect is due to the increased bioavailability of pravastatin or a consequence of decreased fat absorption. This increase in pravastatin absorption was not associated with any adverse events and is probably (at worst) of no clinical consequence, and may be associated with a beneficial effect; therefore, no dose adjustment is needed.

Nifedipine GITS

A study was performed to examine the effect of chronic (4 days) orlistat administration on the single-dose pharmacokinetics of nifedipine GITS[1] in 18 normal volunteers in a randomized crossover fashion. The results, as depicted in Table 8, are somewhat inconclusive due to the lack of statistical power. One subject (subject 2) was excluded by the firm as an outlier on the basis of what was felt to be unusually high concentrations when dosed with orlistat, and low levels when dosed with placebo. Excluding this subject allows the conclusion of no interaction, as the 90% confidence interval is between 80-125%. However, examining all subjects, the levels seen in Subject 2 do not appear unusual. Furthermore, one might expect such "unusual" levels in a highly variable drug like nifedipine and so this subject was left in for the analysis by the reviewer. Examining the individual and mean plasma concentration vs. time plots, it appears that there is no consistent effect of orlistat on nifedipine pharmacokinetics.

Glyburide

A study was performed to examine the effect of chronic (4 days) orlistat administration on the single-dose pharmacokinetics of glyburide in 12 normal volunteers in a randomized crossover fashion. The results of this study were also somewhat inconclusive, again due to high intra-subject variability and a lack of statistical power. Examining the individual plasma concentration vs. time plots, it appears the treatments are equivalent in all subjects except for Subject 1. This subject showed a 6-fold drop in glyburide absorption in the treatment arm as compared with placebo. Again, the firm declared this subject an "outlier" and excluded this subject from the analysis. Again, justification for this seems unclear for a compound with high intra-subject variability (52% intrasubject CV for AUC). Overall, the results suggest that the effects of orlistat on glyburide absorption, if real, are not large.

Ethanol

In 30 subjects given either a) orlistat and placebo ethanol (n=10), b) orlistat and 30 g ethanol (n=10), or c) placebo orlistat and 30 g ethanol (n=10), no effect of orlistat on ethanol absorption was observed. In addition, fecal fat measurements taken during the study suggest that ethanol does not affect the efficacy of orlistat in preventing the absorption of fat.

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[1] Procardia XL

Narrow Therapeutic Index Drugs

Oral Contraceptives

In 20 female volunteers on a stable regimen of oral contraceptives, no change in estradiol or progestins were noted when given with orlistat. Levels of progesterone and LH were also unchanged.

Digoxin

In 12 healthy men given a single 0.4 mg dose of digoxin after 4 days of orlistat or placebo, no effect of orlistat administration on the pharmacokinetics of digoxin was noted.

Phenytoin

Orlistat had no effect on the pharmacokinetics of a single 300 mg dose of phenytoin as compared to placebo when given to 12 normal male volunteers.

Warfarin

Orlistat or matching placebo was given with meals to 12 normal volunteers for 10 days. On the morning of the eleventh day a single 30 mg dose of warfarin was given along with the orlistat or placebo. After a 3 week washout, the subjects were crossed over to the other treatment.

Both R and S-warfarin were measured. In addition, serial measurements of the prothrombin time were made. Serum concentrations of vitamin K1 and its epoxide were determined on days 1, 4, 6 and 10 of each treatment arm.

As seen in Table 8, orlistat had no effect on the pharmacokinetics of either stereoisomer of warfarin. The prothrombin times after each treatment were also nearly identical. The vitamin K and K-epoxide measurements were associated with appreciable variability, but the ratio of the K1 treatment means and 90% confidence intervals (0.69 (0.39, 1.25)) suggest that there may be some adverse effect on K1 absorption. Based on the short duration of the study and the suggested effect on vitamin K absorption, an interaction with warfarin (based on affecting the absorption of vitamin K) can not be ruled out. Overall, the data suggest that patients on chronic stable warfarin therapy might experience an increase in PT after starting orlistat.

Fat-soluble vitamins and minerals

Vitamins A and E

In 12 subjects given a single 400 IU dose of Vitamin E (as the acetate) given on the fourth day of orlistat or placebo given TID for 9 days, orlistat decreased vitamin E absorption by about 60% (table 8). Subjects were also dosed with vitamin A in this study, but the results were inconclusive as the dose used (25000 IU) did not raise retinol levels significantly above baseline. However, based on the results of the β-carotene study (below), it is likely that orlistat will affect vitamin A absorption.

β-Carotene

In 48 subjects given 0, 30, 60 or 120 mg (12 subjects per dose) β-carotene on the fourth day of orlistat or placebo given TID for 6 days, orlistat resulted in a 30-40% decrease in extent of absorption as compared with placebo.

Calcium

No studies were performed. As steatorrhea is known to increase the fecal loss of calcium, it appears likely

that orlistat will promote calcium loss. The effect of orlistat on calcium supplementation (e.g., what percentage of the daily supplement dose is lost) is unknown.

Vitamin K

No studies were performed, but the results of the warfarin study suggest that a decrease in vitamin K absorption may occur with chronic orlistat administration.

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water, bile acids, epithelial cell proliferation and SCFA. Of these, SCFA were not evaluated in this PD study. But the results from the other three PD parameters do not seem to give reasons for concern.

Recently, the validity of cell proliferation as a major risk factor for colon and other cancers has been questioned [E. Farber, Cancer Res. 55:3759-3762 (1995)]. It is axiomatic that cell proliferation plays an important and even critical role in many steps in cancer development. It can also be stated that cell proliferation with altered control is the prime property that is the first characteristic phenotype feature of a malignant neoplastic cell population. Indeed, much information accumulated during the last 15 years reveals that cell proliferation is often associated with carcinogenesis in rodents and humans.[32] When added to the colonic lavage, sennosides, which chronic use has been associated with increased colon cancer risk, induce acute massive cell loss by apoptosis, causing a decrease in crypt length, followed by increased cell proliferation and inhibition of apoptosis to restore cellularity [B.A.P. van Gorkom et al., Gastroenterology 110:A608 (1996)].[33] But the overall accuracy of proliferative parameters in discriminating between "normal" and "high risk" for colon cancer is rather low: in a recent study, abnormal proliferative patterns were found in only half of patients with adenomatous polyps, and the most predictive feature, an upward extension of the proliferative zone, was also present in 23% of normal controls [G. Marra et al., Gastroenterology 106:A412 (1994)]. Furthermore, as pointed out by Ward et al. [Envir. Health Persp. 101(Suppl. 5):125-136 (1993)], an increasing number of examples can be found that suggest cell proliferation is often not associated with carcinogenesis. The group of Earnest et al. have reported a lack of correlation between colonic epithelial cell proliferation and drug-induced inhibition of colon cancer in rats treated with azoxymethane. Treatment with CA significantly increased colon tumors, cancer and the WCMC but not BrdU labeling index. Both piroxicam and UDCA decreased tumor and cancer development. However, the labeling in piroxicam and UDCA-treated rats did not correlate with neoplasm tumor development. These results do not support that labeling index in normal appearing rectal mucosa is a surrogate endpoint biomarker for predicting effects of cancer chemoprevention drugs. They also suggest that suppression of cancer development by piroxicam and UDCA does not occur through a pathway primarily affecting epithelial cell proliferation [Gastroenterology 108:A463 (1995)]. Also, sulindac (an NSAID) exhibits tumor incidence and causes tumor regression but does not act on proliferation in dimethylhydrazine treated rat colon [W. Fischbag and J. Rheinländer, Gastroenterology 108:A468 (1995)]. In summary then, chronic cell proliferation does occur [in colon cancer] but, in itself, it does not seem to be associated with an increased risk for carcinogenesis. In conclusion, clinically, cell proliferation seems to be out of vogue.

3) **Is There a Risk of Colon Cancer?**

No one can, of course, answer this question with certainty. This is because the available information to assess carcinogenesis in humans is very incomplete. But the 1997 most accepted view is that cancer of the colon is a **multistage carcinogenicity process**. The cause of colorectal cancer is now

widely accepted to be the accumulation of mutations in specific genes controlling cell division, apoptosis, and DNA repair [K.W. Kinzler and B. Vogelstein, Cell 87:159-170 (1996)]. In the case of orlistat's long-term administration it appears that the colon cancer concern might be at least partially counteracted by several pieces of information, including:

1. Orlistat does not seem to have mutagenicity or genotoxicity potential. This statement is based on negative results of a battery of short-term assays.[1] [This information is important because of the observation that most mutagens are also carcinogenic.]

2. There are also nonmutagenic carcinogens for which there are no predictive assays and for which conventional extrapolations to potential effects in human being may not apply. The most obvious biological activity for many of these nongenotoxic agents is the induction of cell proliferation. But, according to the results in study W-144999, orlistat does not seem to induce cell proliferation in obese subjects given a hypocaloric diet and the compound at the recommended doses for six weeks.

3. Because the unhydrolyzed TG being offered to the colon is structurally normal, this situation is like in other malabsorption syndrome, especially pancreatic insufficiency. No effects of the fat on colonic architecture are expected.

4. Although higher than before treatment, the amount/concentration of FFAs being offered to the colon does not increase much with orlistat. Not much cytotoxic effect due to this low amount/concentration of these FFAs is expected.

   [On the other hand, the increase in unhydrolyzed TG and some FFAs would be accompanied by an increase in fecal water. It is axiomatic that steatorrhea will eventually induce diarrhea.]

5. Orlistat's PD effects result in a significant decrease in total BAs, particularly DCA not only in the solid but - most importantly - the liquid phase of the stool. Orlistat shares this PD effect with compounds now being tested in the prevention of colorectal cancer, such as UDCA.

6. Orlistat inhibits the secretory phospholipase A_2, an enzyme involved in the production of arachidonic acid, which is a substrate for the production of PGs and LTs [A.J. Watson and R.N. DuBois, Lancet 349:444-445 (1997)].[2]

---

[1]
- Ames tests (± metabolic activation) in tester strains TA97, TA98, TA100, TA102, TA1535, TA1537 and TA1538.
- Mammalian cell (V79/HPRT) gene mutation assay (± metabolic activation)
- Unscheduled DNA synthesis in primary cultures of rat hepatocytes (UDS assay)
- Clastogenesis *in vitro* in human peripheral lymphocytes (± metabolic activation)
- Chromosome aberration assay *in vivo*, in mice (mouse micronucleus test)

[2] How this enzyme modifies tumor susceptibility is unknown, but it is interesting that it is secreted in Paneth cells into the intestinal lumen, where it could digest dietary fats or where its bactericidal effects could alter bacterial flora [S.S. Harwig et al., J. Clin. Invest. 95:603-610 (1995)].

> Recent findings indicate that COX-2 may play an important part in the development or maintenance of adenomas and that increased COX-2 expression could result directly from an inability of adenomatous polyposis coli to carry out its normal function. In man, use of NSAIDs has been linked to a 40 to 50% reduction in relative risk for colorectal cancer [R.N. DuBois et al., Gastroenterol. Clin. North Amer. 25:773-791 (1996)].[34]

7. Finally, due to a decrease in the intestinal absorption of liposoluble vitamins with orlistat treatment, higher than normal amounts of vitamin A, D, E and K are being offered to the colon. Possible associations between vitamins and cancer incidence have been discussed in numerous reviews. None of the vitamin-cancer relationships are proved and only a few associations can be said to be supported by a reasonably sound body of evidence. But lower consumption of vitamin A (dietary retinol and carotenoids plus supplements) appears associated with increased risk of colonic adenomas [J.W. Kikendall, Gastroenterology 106:A401 (1994)]. Vitamin D and retinoid x receptor gene expression in human colonic mucosa and tumors provides a rational basis for therapy with 1, 25 $(OH)_2$ vitamin D_3 analogs. The latter have been shown to inhibit azoxymethane-induced colonic tumorigenesis [R.K. Wali, Gastroenterology 108:A550 (1995)] and to prevent adenomas from progressing to carcinomas, probably through protein kinase C isoforms [R.K. Wali et al., Gastroenterology 111:118-126 (1996)]. To exert its salutory proposed cytoprotective effects, the liposoluble vitamins (or, more likely, their derivatives) would need to be in the liquid phase of the stool, in contact with the colonic epithelial cell. This hypothesis is worth testing. Again, more liposoluble vitamins offered to the colon does not seem to represent a situation of concern.

IV. RECOMMENDATIONS FOR REGULATORY ACTION

This consult review assessed the results of a pharmacodynamic study (Protocol W-144999) in obese subjects given a hypocaloric diet (1900 Kcal/day; ca. 30% fat) and orlistat, at 20 mg t.i.d., the proposed recommended dose for six weeks. Analysis of total weight, total fat, free fatty acids total and individual bile acid content and calcium and pH in the fecal material as well as free fatty acids, total and individual bile acid concentration and pH in the fecal water did not reveal findings of concern under orlistat treatment, in comparison to those induced by a placebo control. A detailed analysis of the evidence, from rectal biopsies, based on crypt compartment analysis of three biomarkers (BrdU, PCNA and WCMC) together with numerous pertinent publications allows the following conclusions a) the biopsy site (rectum) is appropriate; b) the endpoints to assess cell proliferation (quantification of proliferation with a numerical parameter, the labeling index and compartmentalization of crypts), are also appropriate; c) under the experimental conditions used in this trial, orlistat did not induce colonic epithelial cell proliferation. Although cell proliferation as a biomarker for colon cancer seems out of vogue, the consultant believes that the lack of changes in cell proliferation and the significant decrease in deoxycholic acid in both the aqueous and solid phase of the stool, give the general feeling that we are not talking about a dangerous situation here. But in reality, the

available PD information is incomplete and the long-term effects of orlistat on colonic architecture are not known. Since obesity is a lifetime disease, treatment with orlistat is expected to last years during which time there is ample opportunity for the occurrence of presently unforeseeable mucosal colonic changes. The following is recommended:

1. Post marketing surveillance for people to whom the compound may be most dangerous in the long term. These include those with risk factors (i.e. low fiber diet), those with predisposing conditions (i.e. ulcerative colitis greater than 10 years) and those with premalignant lesions (i.e. dysplasia, adenomatous polyps, bilious adenomas, familial polyposis, previous colon cancer and schistosomiasis).

2. The orlistat induced decrease in DCA in the liquid and solid phase of the stool may be potentially beneficial. The compound is neither genotoxic nor mutagenic. The sponsor should consider doing animal experiments to answer the following questions, in a progressive fashion:

   a) Does orlistat produce growth inhibition of malignant colonic epithelial cells *in vitro and/or in vivo*?

   b) Does orlistat prevent cancer in an animal (rat) model?

   c) Is orlistat a chemopreventive agent in colorectal cancer in man?

/S/ [redacted] March 6, 1997

Hugo E. Gallo-Torres, M.D., Ph.D.

cc:
HFD-180
HFD-180/SFredd
HFD-180/HGallo-Torres
HFD-510/EColman
HFD-510/MHess
HFD-180/CSO Consult File
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APPEARS THIS WAY ON ORIGINAL