Attachment 2 of 3

Orlistat Preclinical and Clinical Summary Report on Colon Cancer
ORLISTAT (XENICAL®)
PRECLINICAL AND CLINICAL SUMMARY REPORT ON COLON CANCER

August 3, 2006

Hoffmann-La Roche, Inc.
GlaxoSmithKline Consumer Healthcare

Confidentiality Statement

The information contained in this document, especially unpublished data, is the property of Hoffmann-La Roche Inc. (or under its control), and is therefore provided to you in confidence as a regulatory authority for review by you and your staff. It is understood that this information will not be disclosed to others without written authorization from Hoffmann-La Roche Inc.
# Table of Contents

1. Executive Summary ............................................ 7  
   1.1 Results .................................................. 7  
   1.2 Conclusions ............................................. 8  

2. Background, Purpose And Scope Of Report ................. 8  

3. Overview of Orlistat ............................................ 9  
   3.1 Physiochemical and Pharmacokinetic Profile of Orlistat ............. 10  
   3.2 Summary of Orlistat Safety ................................ 11  
   3.3 Summary of Orlistat Efficacy ................................ 11  
   3.3.1 Weight Loss ............................................ 11  
   3.3.2 Improvements in Obesity-Related Risk Factors ................. 13  
   3.4 Overall Risk-Benefit Profile ................................ 14  

4. Regulatory History Of Orlistat Capsules ..................... 14  

5. Epidemiology ................................................. 16  
   5.1 Incidence of Colorectal Cancer ................................ 16  
   5.2 Risk Factors for Colorectal Cancer ............................ 17  

6. Preclinical Data................................................ 19  
   6.1 Animal Data on Orlistat and Colonic Mucosal Cell Proliferation .... 19  
   6.1.1 Carcinogenicity Studies ................................... 20  
   6.1.1.1 Two Year Oral (Dietary Admix) Carcinogenicity Study with Orlistat in NMRI Mice .......... 21  
   6.1.1.2 Two Year Oral (Dietary Admixture) Carcinogenicity Study with Orlistat in Wistar Rats .......... 22  
   6.1.1.3 Conclusions ........................................... 23  
   6.1.2 Genotoxicity Studies...................................... 25  
   6.1.3 Exploratory Studies ...................................... 25  
   6.1.3.1 Short-Term Study in Rats ................................ 25  
   6.1.3.2 Short-Term Study in Rats with Recovery Period .......... 26  
   6.1.3.3 Three-Week Study in Rats ............................... 27  
   6.1.3.4 Four-Week Study in Rats ............................... 28
6.1.3.5 Parallel Nine-Month Studies in Rats Maintained on Either a Low or Normal Calcium Diet ................................................................. 30
6.1.4 Summary .................................................................................. 32
6.2 Aberrant Crypt Foci: Background and Characteristics ..................... 32
6.3 Recently Published Animal Data on Orlistat and Colonic Mucosal Proliferation (Garcia et al 2005) .................................................. 36

7. Human Clinical Data ...................................................................... 38
7.1 Evaluation of Colonic Cell Proliferation (Human) ............................ 38

8. Pre- and Postmarketing Adverse Events ........................................... 39
8.1 Reports from Phase II, III, IIIb and IV Clinical Trials ...................... 39
8.1.1 Phase II and III Clinical Trials .................................................. 39
8.1.2 Phase IIIb-IV Clinical Trials ..................................................... 40
8.2 Spontaneous Reports from Post-marketing Surveillance ................. 42

9. Discussion of Preclinical and Clinical Data ..................................... 44
9.1 Animal Data ................................................................................ 44
9.2 Human Data .............................................................................. 45

10. Conclusions .................................................................................. 45
11. References .................................................................................... 46
List of Tables

Table 1 Percentage of Patients Achieving = 5% Weight Loss .............. 12
Table 2 Mean Change in Risk Factors From Randomization Following 4-Years Treatment ................................... 13
Table 3 Incidence Rates of Colorectal Cancer, Age-Adjusted to the 2000 US Standard, 1997-2001 .................................. 17
Table 4 Incidence Rates of Neoplasms of Colon (ICD-10: C18), the Rectosigmoid Junction (ICD-10: C19), and Rectum (ICD-10: C20). All Ages, Age-Standardized. Newly Diagnosed Cases in 2003 .............................................. 17
Table 5 Multivariate Adjusted* Rate Ratios of Colon and Rectal Cancer According to BMI Groups by Menopausal Status ........... 18
Table 6 Fecal Parameters and Cell Proliferation in Short Term Rat Study (Mean Values) .............................................. 26
Table 7 Fecal Parameters and Cell Proliferation in Short Term Rat Study with Recovery Period (Mean Values) .................................. 27
Table 8 Fecal Parameters and Cell Proliferation in 3-Week Rat Study (Mean Values) ..................................... 28
Table 9 Fecal Parameters and Cell Proliferation in 4-Week Rat Study (Mean Values) .............................................. 29
Table 10 Results Summary of 9 Month Study in Rats ACF Results - Study Weeks 39 and 48..................................... 31
Table 11 Examples of Conditions Shown to Increase Colonic ACF in Animal Models .............................................. 36
Table 12 Reports of Colon/Rectal Cancers in Reported in Controlled Clinical Trials of =6Month Duration .................................. 41
Table 13 Spontaneous Reports of Colon/Rectal Cancers or Neoplasms in the Roche Adverse Event Safety Database.................. 43
List of Figures

Figure 1 Incidence Rates of Colorectal Cancer by Age Groups per 100,000 Population in England in 2003. Black bars: Men, grey bars: women. ............................................. 16

Figure 2 PCNA Labeling Index of Rat Colonic Mucosa ............................................. 37

Figure 3 Number ACF per cm2 of Rat Colonic Mucosa ............................................. 37
GLOSSARY OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACF</td>
<td>Aberrant crypt foci</td>
</tr>
<tr>
<td>BCAC</td>
<td>β-catenin accumulated crypts</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BrdU</td>
<td>5-bromo-2-deoxyuridine</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>DMH</td>
<td>Dimethyl hydrazine</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>HACAs</td>
<td>Histologically altered crypts with ACF appearance</td>
</tr>
<tr>
<td>HACNs</td>
<td>Histologically altered crypts with macroscopically normal-like appearance</td>
</tr>
<tr>
<td>HCA</td>
<td>Peripheral human lymphocyte assay</td>
</tr>
<tr>
<td>IND</td>
<td>Investigational New Drug</td>
</tr>
<tr>
<td>NDA</td>
<td>New drug application</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>PCNA-LI</td>
<td>Proliferating cell nuclear antigen labeling index</td>
</tr>
</tbody>
</table>
1. EXECUTIVE SUMMARY

In two preclinical studies conducted by Hoffman La Roche (Roche) in support of the original development program for Xenical®, an increase in total aberrant crypt foci (ACF) has been observed. These observations were evaluated by international regulatory agencies including FDA during the review and approval of the Xenical® 120 mg dossier. Currently, there is no consensus in the scientific community that total ACF is an accurate predictor of colonic tumor development. However, some scientists are of the opinion that ACF may be a potential biomarker for the development of colon cancer [1].

Therefore, Roche and GSK (collectively referred to as the “Sponsors”) have reviewed all the relevant preclinical, clinical and post-marketing data for orlistat, including the ACF data, to assess the potential risk of developing colon cancer as a result of orlistat therapy. In addition, a brief epidemiology review of risk factors for colorectal cancer is provided, in which obesity is identified as a major risk factor.

This report provides a summary of the data reviewed as part of this comprehensive assessment and firmly supports the conclusion that there is no evidence to support a causal link between the use of orlistat and colon cancer.

The results of this review are summarized below (Section 1.1).

1.1 Results

- Although some preclinical studies with orlistat showed an increase in total ACF, there was no increase in ACF multiplicity. This is of importance because there are a number of publications in the scientific literature that have concluded that ACF crypt multiplicity predicts tumor development more reliably than total number of ACF [2, 3].
- There was no evidence of hyperplasia, neoplasia or tumors from the histological examination of the colonic and rectal mucosa of orlistat treated animals in both short and long term studies.
- The 2005 publication by Garcia et al [1] reported an increase in total ACF in rats treated with the carcinogenic substance dimethyl hydrazine (DMH) in addition to orlistat, and/or a high fat diet supplemented with 10% cotton oil. In this study, ACF were observed only in the colons of animals treated with the carcinogen DMH.
- In the 2 two-year orlistat carcinogenicity studies, there is no histological evidence of neoplasia or tumors to support the hypothesis that total ACF detected in some shorter term studies would likely progress to neoplasia, adenoma or carcinoma.
- There is no consensus in the scientific community that total ACF alone is an accurate predictor of colonic tumor development.
- Relevant clinical data following treatment with orlistat were reviewed for evidence of colonic proliferation and/or the development of colorectal cancer. These clinical data do not support an association between treatment with orlistat and an increase in colonic proliferation, or the development of colorectal cancer.
These data included:

- short-term fecal marker and rectal biopsy study assessing colonic mucosal cell turnover
- data from 16 long-term Phase II and III controlled clinical trials of at least 6 months duration, (6,146 orlistat patients and 4,516 patients on placebo treated for up to 4 yrs) a total of 3 reports of colon or rectal carcinoma were identified
- data from Phase IIIb and IV studies of at least 6 months duration (3,571 orlistat patients, 3,396 on placebo), a total of 4 reports of colon or rectal carcinoma were identified

- Since its launch in 1997 through March 31, 2006 more than 25.8 million patients have been treated with Xenical®. From that 25.8 million patient treatments, a total of 7 spontaneous reports of colon or rectal carcinoma were identified in Roche’s cumulative post-marketing surveillance database

The three cut-off dates for data included in this report are:

a. March 31, 2006 for 25.8 million patients treated with orlistat
b. April 18, 2006 for the sponsor’s international safety database
c. June 2, 2006 for published literature

1.2 Conclusions

Based on a comprehensive review of orlistat preclinical, clinical and market surveillance data, the Sponsors conclude that there is no evidence of a causal link between the use of orlistat and colorectal cancer. The increase in total ACF noted in preclinical studies is not an accurate predictor of colonic tumor development based on the absence of neoplasia in appropriately conducted rodent carcinogenicity studies. Therefore no change in labeling of Rx orlistat is warranted. Additionally, the overall risk-benefit profile of orlistat remains favorable and appropriate for over-the-counter use.

2. Background, Purpose and Scope of Report

In two preclinical studies conducted by Hoffman La Roche (Roche) in support of the original development program for Xenical®, an increase in total aberrant crypt foci (ACF) has been observed. These observations were evaluated by international regulatory agencies including FDA during the review and approval of the Xenical® 120 mg dossier. Currently, there is no consensus in the scientific community that total ACF is an accurate predictor of colonic tumor development. However, some scientists are of the opinion that ACF may be a potential biomarker for the development of colon cancer [1].

Therefore, Roche and GSK (collectively referred to as the “Sponsors”) have reviewed all the relevant preclinical, clinical and post-marketing data for orlistat for the purpose of independently evaluating the safety profile of orlistat with respect to ACF, colonic cell proliferation and the potential for development of colorectal cancer. Background
epidemiology data regarding risk factors for colorectal cancer, which includes obesity, is also provided.

The preclinical data reviewed in this report includes:

- genotoxicity, carcinogenicity and exploratory studies on colonic cell proliferation conducted by the sponsor
- the data from the 2005 publication by Garcia et al [1]
- published data on ACF

The clinical trial data reviewed in this report includes:

- short term colonic biopsy/biomarker study in obese subjects
- safety data from all long-term double-blind, placebo controlled Phase II and III clinical trials of ≥6 months duration supporting the labeling for obesity management, treatment of type 2 diabetes and delay in onset of diabetes; these studies ranged from 6 months to 4 yrs in duration
- safety data from double-blind, placebo controlled Phase IIIB-IV studies of at least 6 months duration

Post-marketing surveillance includes:

- a review of the Roche international safety database for spontaneous reporting and clinical study reporting of colon/rectal cancers or neoplasms since first launch in 1997 through April 18, 2006, representing an estimated exposure of 25.8 million patients treated with orlistat.

Epidemiology background information includes:

- incidence of colorectal cancer in various populations
- risk factors associated with colorectal cancer

3. OVERVIEW OF ORLISTAT

Orlistat, a potent, specific, long-acting and reversible inhibitor of lipases, is a member of a new class of drugs available for the treatment of obesity. It exerts its therapeutic activity in the lumen of the stomach and small intestine by forming a covalent bond with the active serine site of gastric and pancreatic lipases. The inactivated enzymes are unavailable to hydrolyze dietary fat in the form of triglycerides into absorbable free fatty acids and monoglycerides. As undigested triglycerides cannot be absorbed, the resulting caloric deficit has a positive effect on weight control. Systemic absorption of the drug is not needed for activity.
3.1 Physiochemical and Pharmacokinetic Profile of Orlistat

Solubility: Orlistat is highly lipophilic. The partition coefficient between octanol and buffer (pH 7.45) is larger than 1000 and the solubility in water at 23°C is extremely low at <1 mg/100 mL. Similar or even higher preferential partitioning of orlistat was observed with tributyrin/buffer pH 7.4: log P >4. Orlistat disperses in micellar bile salt solutions to a certain extent; however, the solubility in bile salt solutions is low. In triglyceride emulsions containing either triolein or tributyrin emulsified with bile salts at concentrations of 3 to 20 mM, orlistat was almost exclusively present in the triglyceride phase. In lipid emulsion assays simulating the duodenal conditions as closely as possible, the rapid and complete partitioning of orlistat into the triglyceride phase was also evident. Thus, in the presence of emulsified triglycerides and bile salt micelles, orlistat dissolves predominantly in the emulsion lipid and does not incorporate into mixed micelles.

Absorption: Systemic exposure to orlistat is minimal. Following oral dosing with 360 mg 14C-orlistat, plasma radioactivity peaked at approximately 8 hours: plasma concentrations of intact orlistat were near the limits of detection (<5 ng/mL). In therapeutic studies involving monitoring of plasma samples, detection of intact orlistat in plasma was sporadic and concentrations were low (<10 ng/mL or 0.02 μM), without evidence of accumulation, and consistent with minimal absorption. The average absolute bioavailability of intact orlistat was assessed in studies with male rats at oral doses of 150 and 1000 mg/kg/day and in male dogs at oral doses of 100 and 1000 mg/kg/day and found to be 0.12%, 0.59% in rats and 0.7%, 1.9% in dogs, respectively.

Distribution: In vitro orlistat was >99% bound to plasma proteins (lipoproteins and albumin were major binding proteins). Orlistat minimally partitioned into erythrocytes.

Metabolism: Based on animal data, it is likely that the metabolism of orlistat occurs mainly within the gastrointestinal wall. Based on a 14C-orlistat mass balance study in obese patients, of the minute fraction of the radio-labeled dose that was absorbed systemically, the presence of two metabolites, M1 and M3, accounted for approximately 42% of total radioactivity in plasma. In view of the extremely weak systemic lipase inhibitory activity (1000- and 2500-fold less than orlistat respectively) and the low plasma levels at the therapeutic dose, these metabolites are considered pharmacologically inconsequential. The primary metabolite M1 had a short half-life (approximately 3 hours) whereas the secondary metabolite M3 disappeared at a slower rate (half-life approximately 13.5 hours).

In obese patients, steady-state plasma levels of M1, but not M3, increased in proportion to orlistat doses.

Elimination: Following a single oral dose of 360 mg 14C-orlistat in both normal weight and obese subjects, fecal excretion of the unabsorbed drug was found to be the major route of elimination. Approximately 97% of the administered radioactivity was excreted in feces and 83% of that was found to be unchanged orlistat. The cumulative renal excretion of total radioactivity was <2% of the given dose of 360 mg 14C-orlistat. The time to reach complete excretion (fecal plus urinary) was 3 to 5 days. The disposition of orlistat appeared to be similar between normal weight and obese subjects. Orlistat, M1
3.2 Summary of Orlistat Safety

The safety of orlistat is supported by a comprehensive body of data including preclinical (animal) testing, controlled clinical trials (human) and long-term post-marketing surveillance.

In controlled clinical trials, 4.8% and 8.8% of patients treated with orlistat 60mg and 120 mg, respectively, discontinued treatment due to adverse events, compared to 5.0% of placebo-treated patients. For orlistat-treated subjects, the most common adverse events resulting in discontinuation of treatment were gastrointestinal. The commonly observed treatment-emergent adverse events associated with the use of orlistat in the Phase III controlled clinical trials which are primarily a manifestation of the mechanism of action include oily spotting, flatus with discharge, fecal urgency, fatty/oily stool, oily evacuation, increased defecation and fecal incontinence. Only a very small percentage of patients discontinued due to a serious adverse event. Orlistat has been studied extensively and its safety and efficacy are supported by more than 100 clinical studies conducted in 30 countries. When used as prescribed, treatment with orlistat is considered to be generally well tolerated.

Since launch, orlistat has been used safely by more than 25 million patients. There is no evidence of increased risk of colon cancer from post-marketing surveillance data involving these patients, or from clinical trials of orlistat with participants using the drug continuously for as long as four years.

Compelling evidence from these multiple sources support the safety of orlistat 60mg (OTC) and 120mg (Rx) for their intended uses.

3.3 Summary of Orlistat Efficacy

The efficacy of orlistat is robust and consistent as demonstrated in multiple, long-term, placebo-controlled clinical trials. Orlistat plus diet has repeatedly demonstrated significantly greater weight loss, when compared to placebo plus diet. Moreover, the weight loss effects of orlistat (60mg and 120mg capsules) are clinically meaningful and meet the FDA standards of efficacy for prescription weight control drugs. It is important to note that the use of orlistat in overweight and obese subjects is associated with a number of benefits in addition to weight loss itself.

The efficacy of orlistat (120mg and 60mg), in terms of weight loss and improvement of obesity-related risk factors, is discussed in more detail below.

3.3.1 Weight Loss

Orlistat, 120mg administered tid with meals, has been approved as an Rx product in the US since April, 1999 and is indicated for:

- obesity management including weight loss and weight maintenance
- reduce the risk of weight regain after prior weight loss
Treatment and weight loss with orlistat has been also been shown to result in:

- significant weight loss in obese patients with type 2 diabetes when used in conjunction with sulfonylureas, insulin and metformin
- improve OGTT status in obese patients
- delay the onset of type 2 diabetes in obese, non-diabetic patients (XENDOS Study)
- improve obesity related risk factors
- decrease BMI in obese adolescents (12 to 16 years of age)

In the Phase III studies of 1, 2 and 4 years duration, the percentage of adult patients achieving a ≥ 5% weight loss are summarized in Table 1.

Table 1 Percentage of Patients Achieving ≥ 5% Weight Loss

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Orlistat (n=)</td>
<td>Placebo (n=)</td>
<td>Orlistat</td>
<td>Placebo</td>
</tr>
<tr>
<td>Xendos (14150)</td>
<td>1649</td>
<td>1655</td>
<td>72.8</td>
<td>45.1</td>
</tr>
<tr>
<td>14119B</td>
<td>110</td>
<td>108</td>
<td>35.5</td>
<td>21.3</td>
</tr>
<tr>
<td>14119C</td>
<td>343</td>
<td>340</td>
<td>54.8</td>
<td>27.4</td>
</tr>
<tr>
<td>14149</td>
<td>241</td>
<td>236</td>
<td>50.6</td>
<td>26.3</td>
</tr>
<tr>
<td>14161</td>
<td>210</td>
<td>212</td>
<td>37.1</td>
<td>16.0</td>
</tr>
<tr>
<td>14185</td>
<td>657</td>
<td>223</td>
<td>42.6</td>
<td>22.4</td>
</tr>
<tr>
<td>Diabetes Studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14336</td>
<td>162</td>
<td>159</td>
<td>31</td>
<td>13</td>
</tr>
<tr>
<td>(sulfonylureas)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37002</td>
<td>189</td>
<td>180</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>(sulfonylureas)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37047 (metformin)</td>
<td>250</td>
<td>254</td>
<td>39</td>
<td>16</td>
</tr>
<tr>
<td>37047 insulin</td>
<td>266</td>
<td>269</td>
<td>33</td>
<td>13</td>
</tr>
</tbody>
</table>

- denotes no data for the time frame

Orlistat 60 mg capsules are proposed for over-the-counter use as a weight loss aid in overweight adults, 18 years and older, when used along with a reduced calorie and low fat diet. The application for OTC Orlistat is currently under FDA review (see Section 4).

The results from the controlled clinical studies on orlistat 60 mg show that when used as an adjunct to diet, subjects taking orlistat 60 mg consistently lost more weight and had greater reductions in BMI scores and waist and hip circumferences compared to the placebo group. These differences between treatments were statistically significant and clinically meaningful. A clinically and statistically significant (p<0.001) number of overweight and obese subjects lost >5% of baseline body weight on orlistat 60 mg compared to placebo and therefore, fulfill the approval criteria used by the FDA for the approval of weight loss drugs.
3.3.2 Improvements in Obesity-Related Risk Factors

In addition to weight loss, changes in obesity related risk factors were assessed in the Phase III studies in the population as a whole, that is, all patients regardless of their baseline risk factor status and in the population with abnormal risk factors at randomization.

The mean change from baseline for obesity related risk factors after 4 years of treatment in the XENDOS study for the population as a whole (Table 2) and the population with abnormal baseline values are reported below.

### Table 2 Mean Change in Risk Factors From Randomization Following 4-Years Treatment

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>XENICAL 120 mg+</th>
<th>Placebo†</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>-7.02%</td>
<td>-2.03%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>-11.66%</td>
<td>-3.85%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>+5.92%</td>
<td>+7.01%</td>
<td>&lt;0.055</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>-0.53</td>
<td>-0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>+3.64%</td>
<td>+1.30</td>
<td>&lt;0.075</td>
</tr>
<tr>
<td>Fasting Glucose, mmol/L</td>
<td>+0.12</td>
<td>+0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting Insulin, pmol/L</td>
<td>-24.93</td>
<td>-15.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic Blood Pressure, mm Hg</td>
<td>-4.12</td>
<td>-2.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mm Hg</td>
<td>-1.93</td>
<td>-0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anthropometric:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist Circumference, cm</td>
<td>-5.78</td>
<td>-3.99</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Treatment designates XENICAL 120 mg three times a day plus diet or placebo plus diet
† Intent-to-treat population

The changes from randomization following 4-years treatment in the population with abnormal lipid levels (LDL ≥130 mg/dl, HDL <35 mg/dl, LDL/HDL ≥3.5, triglycerides ≥2.54 mmol/L) were greater for XENICAL compared to placebo with respect to LDL-cholesterol (-14.86 vs -7.34, p<0.00), LDL/HDL (-0.87 vs -0.59, p<0.00) and triglycerides (-15.63 vs -13.92, p=0.562). HDL increased in the placebo group by 12.9% and in the XENICAL group by 11.6%, p=0.339. In the population with abnormal blood pressure at baseline (systolic >140 mm Hg, the change in SBP from baseline was greater for XENICAL than placebo (-11.44 vs -8.70, p=0.002). For patients with a diastolic blood pressure ≥90 mm Hg at baseline, the decrease in DBP in the XENICAL patients was -8.00 mm Hg vs -6.26 in the placebo-treated patients, p=0.006. Fasting insulin decreased more for XENICAL than placebo (-47 vs -37, p=0.002) from randomization to year 4 in the population with abnormal baseline values (≥120 pmol/L).
Also in the XENDOS trial, at the end of 4 years of treatment, weight loss with orlistat resulted in a delay in the onset of type 2 diabetes with a relative risk reduction of 42% in patients with impaired glucose tolerance at baseline.

Obesity and/or overweight is the most important modifiable risk factor for type 2 diabetes and weight loss has been associated with a reduction in total mortality in such patients. Weight loss in patients with type 2 diabetes is extremely difficult as many anti-diabetic medications and the natural progression of the disease results in undesirable effects on body weight, lipid levels and blood pressure.

Several studies in obese patients with type 2 diabetes treated with anti-diabetic medications such as sulfonylureas, insulin and metformin were conducted. In these studies, patients were randomized to orlistat plus diet or placebo plus diet in addition to their anti-diabetic medications. Compared to placebo, treatment with orlistat resulted in significant weight loss as shown in Table 1, and improvement in glycemic control (reductions in HbA1c, FPF, and antidiabetic medications) and improvement in cardiovascular risk factors (LDL, LDL/HDL, triglycerides, systolic and diastolic blood pressure).

Although the treatment of co-morbid conditions is outside the scope of the OTC indication to promote weight loss, in Phase III trials evaluating orlistat 60 mg tid, it's use has been associated with a favorable profile in terms of comorbidities. In controlled clinical studies at 6 months, orlistat 60 mg provided a reduction of total cholesterol by at least 3.1% and a reduction of LDL cholesterol by at least 5.2% in overweight and obese individuals. Positive effects were also observed in terms of blood pressure; in a short-term study (4 months) in overweight subjects (BMI 25-28 kg/m²) orlistat 60 mg provided a reduction in systolic blood pressure of -4.51 mmHg and a reduction of -2.77 mmHg for diastolic blood pressure.

3.4 Overall Risk-Benefit Profile

The prevalence of obesity and overweight in the United States has reached epidemic proportions. Greater than 120 million adults (nearly two-thirds of adults) in the United States are affected and these numbers are increasing.

Overweight and obesity (clinically defined as BMI ≥25 kg/m² and ≥30 kg/m², respectively) substantially raise the risk of morbidity from hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and endometrial, breast, prostate, and colon cancers.

Even a modest 5% weight loss, can have considerable medical benefits in terms of ameliorating these weight-related medical complications.

In conclusion, orlistat is an effective and generally well tolerated treatment for obese and overweight patients and the benefits of weight loss with orlistat outweigh the risks.

4. REGULATORY HISTORY OF ORLISTAT CAPSULES

Developed by Hoffmann-La Roche (Roche), orlistat (tetrahydrolipstatin) was first synthesized in 1983 and received its first approval as a prescription weight loss aid in Argentina in 1997. Since that time, orlistat 120 mg capsules have been approved in over
145 countries worldwide, including the United States. This foreign marketing experience includes 6 countries where Xenical® (120 mg) has recently been reclassified from prescription to OTC status as a ‘pharmacy-only’ medicine. These 6 countries are Thailand, Philippines, Malaysia, Australia, New Zealand and Singapore. Xenical® has not been withdrawn from marketing in any country for any reason related to safety or effectiveness.

The US IND for orlistat was opened in 1988 and all preclinical and clinical research and development was conducted and reviewed under that IND. During the development of orlistat, Roche had numerous discussions with FDA on an ongoing basis and the input received was incorporated in the development program. In addition Roche consulted with numerous preclinical and clinical experts regarding various aspects of the orlistat development program who also provided input on study design, data analysis and the overall development program. In 1996, Roche submitted its new drug application (NDA 20-766) for Xenical® (orlistat) 120 mg capsules to the United States Food and Drug Administration. Xenical was the first weight loss drug for which controlled clinical of 2 years duration were included in the original NDA. NDA 20-766 included four Phase III trials of 2 years duration.

The drug approval process for the original Xenical 120mg capsules NDA was rigorous. During the initial review cycle, the primary FDA reviewing division (Division of Metabolism and Endocrinology Drug Products) solicited internal (FDA) expert consultation from the Division of Gastrointestinal and Coagulation Drug Products and from the Oncology Division. Additionally, FDA convened two independent meetings of the Endocrinologic and Metabolic Drugs Advisory Committees in 1997 and 1998 to consider issues related to the product’s safety and efficacy.

Roche received approval for Xenical in the US in April 1999 for the indications of obesity management, including weight loss and weight maintenance, when used in conjunction with a reduced calorie diet as well as the reduction of weight regain after prior weight loss. Other major labeling supplements to NDA 20-766 included the submission of data in pediatric patients aged 12 to 16 years (June 2003) and data from the 4 year XENDOS study (December 2003). Both of these supplements were approved and data from these applications is included in the approved Xenical label.

In 2001, Roche initiated a program to investigate the potential over-the-counter use of orlistat 60 mg as a weight loss aid. In 2004, GlaxoSmithKline (GSK) licensed the rights to OTC orlistat and assumed ownership for the OTC development program. In June 2005, GSK submitted a separate NDA (NDA 21-887) for the use of orlistat 60 mg capsules as an over-the-counter weight loss aid.

In January 2006, a joint meeting of the Nonprescription Drugs and the Endocrinologic & Metabolic Drugs Advisory Committee was convened by FDA to consider the appropriateness of orlistat 60 mg capsules as an OTC weight loss aid. At the conclusion of the proceedings, the Joint Advisory Committee voted 11 to 3 that orlistat was safe and effective for OTC use to promote weight loss in overweight adults when used along with a reduced calorie and low fat diet.
In April 2006, GSK received an approvable letter for its OTC application, a reflection of the acceptability of orlistat's safety and efficacy profile.

In summary, the safety and efficacy of orlistat 60mg (OTC) and 120mg (Rx) capsules have been comprehensively assessed as part of a scientifically rigorous and transparent process.

5. EPIDEMIOLOGY

5.1 Incidence of Colorectal Cancer

Cancers of the colon and rectum combined are the third most common cause of cancer and the second most common cause of cancer death in the US. The incidence of colorectal cancer is approximately 50 per 100,000 in the USA: 63.4 per 100,000 in men and 46.4 per 100,000 in women [4]. The figures for England are similar: 62.3 per 100,000 for men and 49.5 per 100,000 for women [5]. The incidence of colorectal cancer is increasing with increasing age: Men aged 40-44 years have an incidence of 10 per 100,000. This increases fourteen-fold to 143 per 100,000 in the age group 60-64 years (Figure 1).

Figure 1 Incidence Rates of Colorectal Cancer by Age Groups per 100,000 Population in England in 2003. Black bars: Men, grey bars: women


African Americans have the highest colorectal cancer incidence rates with 72.9 cases per 100,000 for males and 56.5 per 100,000 for females (Table 3). White males have a 13% and white females a 19% lower incidence rate than African Americans.
Table 3  Incidence Rates of Colorectal Cancer, Age-Adjusted to the 

<table>
<thead>
<tr>
<th>Race/ethnicity</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>African Americans</td>
<td>72.9 per 100,000</td>
<td>56.5 per 100,000</td>
</tr>
<tr>
<td>Whites</td>
<td>63.1 per 100,000</td>
<td>45.9 per 100,000</td>
</tr>
<tr>
<td>Asian Americans/Pacific Islanders</td>
<td>56.3 per 100,000</td>
<td>38.6 per 100,000</td>
</tr>
<tr>
<td>Hispanics/Latinos</td>
<td>49.6 per 100,000</td>
<td>32.5 per 100,000</td>
</tr>
<tr>
<td>American Indians/Alaska Natives</td>
<td>38.3 per 100,000</td>
<td>32.7 per 100,000</td>
</tr>
<tr>
<td>All races/ethnicities</td>
<td>63.4 per 100,000</td>
<td>46.4 per 100,000</td>
</tr>
</tbody>
</table>

Source: American Cancer Society, 2005 [4]

Neoplasms of the colon are approximately two to three times more frequently diagnosed than neoplasms of the rectum (Table 4). The gender differences are less pronounced in colon cancers (males to females = 1.1:1) than in cancers of the rectum (1.7:1) [5]. Neoplasms of the rectosigmoid junction are less common.

Table 4  Incidence Rates of Neoplasms of Colon (ICD-10: C18), the 
Rectosigmoid Junction (ICD-10: C19), and Rectum (ICD-10: 
C20). All Ages, Age-Standardized. Newly Diagnosed Cases 
in 2003

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>Gender</th>
<th>Incidence rate per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplasm of colon</td>
<td>Males</td>
<td>30.1</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>21.6</td>
</tr>
<tr>
<td>Neoplasm of rectosigmoid junction</td>
<td>Males</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>2.5</td>
</tr>
<tr>
<td>Neoplasm of rectum</td>
<td>Males</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>8.2</td>
</tr>
</tbody>
</table>


Nonpolypoid lesions seem to be more frequent than previously thought. Kudo et al found percentages of polypoid and nonpolypoid lesions of 55.5% and 44.5%, respectively [6]. The authors reported their 14-years of experience treating 14,014 colorectal adenomas and early carcinomas in northern Japan. Rembacken et al studied prospectively 1,000 consecutive UK patients who underwent colonoscopy for a variety of indications and used chromoscopy to study suspicious areas for colorectal neoplasia [7]. They identified 290 adenomas. Sixty-three percent were polypoid, 36 percent were flat, and one percent was depressed.

5.2  Risk Factors for Colorectal Cancer

The major risk factors for colorectal cancer are a family history of colorectal cancer and a previous personal history of colorectal cancer, colorectal polyps, or chronic inflammatory bowel disease [4]. Five to ten percent of patients with colorectal cancer have an inherited genetic abnormality such as familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer that causes the cancer [4].

Current medical opinion suggests that colorectal cancers need several years to develop. Matsui et al, who investigated the natural history of 49 cancers using a retrospective radiological method, estimated a doubling-time of 31.2 months when the cancer was
limited to the mucosa [8]. The cancer doubling-time was 25.8 months when the tumor grew down to the submucosa. The pathological growth pattern (nonpolypoid or polypoid growth) did not affect the tumor growth speed.

Calle *et al.* found an association between body mass index (BMI) and mortality from colorectal cancer [9]. Overweight people (BMI between 25-29.9) had a 1.20-fold higher risk (95% CI: 1.12-1.30) of dying from colorectal cancer compared with people of normal weight (BMI between 18.5-24.9). This risk increased with increasing BMI: People with a BMI between 30-34.9 had a 1.47-fold higher risk (95% CI: 1.3-1.66), and people with a BMI between 35.0-39.0 had a 1.84-fold higher risk (95% CI: 1.39-2.41). The authors studied prospectively a population of more than 900,000 US adults who were free of cancer at enrollment in 1982, with 16 years of follow-up. The authors used a Cox proportional-hazards model and adjusted for age, education, smoking status and number of cigarettes smoked, physical activity, alcohol use, marital status, race, aspirin use, fat consumption and vegetable consumption but not for gender. Terry *et al.* studied approximately 90,000 women, aged 40-59 with an average follow-up time of 10.6 years [10]. They found an association between obesity (BMI ≥30) and colorectal cancer in pre-menopausal but not in post-menopausal women (Table 5). The association was slightly higher for colon cancer than for rectal cancer. Generally, obese pre-menopausal women had an approximately two-fold higher risk compared with non-obese pre-menopausal women. The authors adjusted for age, smoking, educational level, vigorous physical activity, oral contraceptive use, hormone replacement therapy and parity.

### Table 5 Multivariate Adjusted* Rate Ratios of Colon and Rectal Cancer According to BMI Groups by Menopausal Status

<table>
<thead>
<tr>
<th></th>
<th><strong>Pre-menopausal</strong></th>
<th><strong>Post-menopausal</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colorectal Colon Rectum</td>
<td>Colorectal Colon Rectum</td>
<td></td>
</tr>
<tr>
<td>Number of cases</td>
<td>176 118 58</td>
<td>275 184 91</td>
<td></td>
</tr>
<tr>
<td>BMI &lt;25</td>
<td>1.0 (referent)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI 25-30</td>
<td>1.06</td>
<td>1.19</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>(0.74-1.53)</td>
<td>(0.77-1.85)</td>
<td>(0.43-1.61)</td>
</tr>
<tr>
<td>BMI &gt;30</td>
<td>1.88</td>
<td>1.95</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>(1.24-2.86)</td>
<td>(1.15-3.29)</td>
<td>(0.88-3.49)</td>
</tr>
</tbody>
</table>

* Adjusted for age, smoking, educational level, vigorous physical activity, oral contraceptive use, hormone replacement therapy and parity. Source: Terry *et al.* (2002) [10]

Will *et al.* found a 1.3-fold higher risk (95% CI: 1.03-1.65) for colorectal cancer in male diabetics and a 1.16-fold higher risk (95% CI: 0.87-1.53) in female diabetics compared with non-diabetics [11]. The authors analyzed data from the 1959-1972 Cancer Prevention Study which included more than 860,000 subjects with a 13-year follow-up period. The authors adjusted the results for colorectal cancer risk factors such as race, educational level, body mass index, smoking, alcohol use, dietary intake, aspirin use, physical activity and family history of colorectal cancer.

US men have an incidence of colorectal cancer more than 35% higher than in women (American Cancer Society, 2005) [4].

Age is a risk factor for colorectal cancer (Figure 1). For example, men in the age group 60-64 years have a fourteen-fold higher incidence than men in the age group 40-44 years.
African Americans have the highest risk of developing colorectal cancer; almost two-fold higher than American Indians/Alaska Natives who have the lowest incidence (Table 3). The risks of American whites, Asian Americans/Pacific Islanders, Hispanics/Latinos, lie somewhere in between.

Patients with ulcerative colitis have an approximately five-fold higher risk of colorectal cancer. The risk increases with the duration of the disease. Eaden et al estimated an overall incidence rate of colorectal cancer for any patient with colitis of 3 per 1000 patient years (95% CI: 2/1000-4/1000) [12]. The authors estimated a cumulative risk of experiencing colorectal cancer of 3% (95% CI: 2.2-3.8) at 10 years’ disease duration, 5.9% (95% CI: 4.3-7.4) at 20 years’ disease duration, and 8.7% (95% CI: 6.4-10.9) at 30 years’ disease duration.

Colditz et al reviewed the literature regarding a possible association between physical activity and colon cancer [13]. People with the highest level of activity across numerous studies that used different measures of activity (occupational or leisure-time activity) had an approximately 50% lower incidence of colon cancer. This association persisted in studies using multivariate analyses to control for diet and other known or suspected risk factors for colon cancer.

Chao et al reported a multivariate-adjusted risk ratio of 1.32 (95% CI: 1.16-1.49) among men who were current smokers compared with those who have never smoked and 1.41 (95% CI: 1.26-1.58) for women [14]. The risk among current and former smokers increased with the duration of smoking and average number of cigarettes smoked per day. The authors adjusted for age, race, BMI, education, family history of colorectal cancer, exercise, aspirin and multivitamin use, alcohol consumption, and intake of vegetables, high-fiber grain foods, and fatty meats.

According to Eunyoung et al, alcohol consumption is a risk factor for colorectal cancer [15]. The authors found an increased risk of 1.4 (95% CI: 1.16-1.72) for people who consumed 45 g or more alcohol per day (≥3 drinks per day) compared with nondrinkers. The authors performed a pooled analysis of eight cohort studies.

Evaluating 134,365 participants from the Nurses’ Health Study and the Health Professionals Follow-up Study, Wei et al identified differences in risks of colon and rectal cancer [16]. They found age, sex, family history, height, BMI, physical activity, folate, beef, pork or lamb as a main dish, processed meat, and alcohol to be related to colon cancer, whereas only age and gender showed statistically significant associations with rectal cancer.

6. PRECLINICAL DATA

6.1 Animal Data on Orlistat and Colonic Mucosal Cell Proliferation

Inhibition of gastrointestinal lipases, which are responsible for the hydrolysis of dietary triglycerides, is expected to increase the quantity of fat presented to the colonic mucosa. It has been suggested that orlistat, as an inhibitor of gastric and pancreatic lipases, might potentially increase the risk of colon cancer through a mechanism of increased colonic
mucosal cell proliferation. This is based on animal data indicating that excess dietary lipids may affect colonic mucosal structure as well as colonic lymphocytes and the production of inflammatory mediators in the colon [17,18,19]. For these reasons, a number of preclinical studies were submitted under NDA 20-766 to determine the carcinogenic and genotoxic potential of orlistat as well as the effects of orlistat on colonic mucosal proliferation. It should be noted that multiples of the recommended human clinical dose that are cited in the following sections refer to the recommended human prescription dose of 360 mg/day and not to the proposed human over-the-counter (OTC) dose of 180 mg/day.

6.1.1 Carcinogenicity Studies

Among the preclinical studies that were submitted under NDA 20-766, two 2-year oral carcinogenicity studies were conducted with orlistat in mice and rats. These 2-year carcinogenicity studies are the scientifically accepted method for the evaluation of potential cancer risk of pharmaceutical agents [20,21]. Dose selection for these carcinogenicity studies was based on animal to human area-under-the-curve (AUC\(_{0-24}\)) exposure ratios, not the maximum tolerated dose (MTD), which is in accordance with the ICH guidelines for high dose selection for carcinogenicity studies. According to the ICH guidelines, compounds that are non-genotoxic can use an animal to human AUC\(_{0-24}\) exposure ratio of 25 times or more the human exposure to select the high dose level for a carcinogenicity study [22]. Dose selection and study design for both of these carcinogenicity studies with orlistat were reviewed and approved by FDA prior to initiation of the studies. In these carcinogenicity studies, orlistat was administered in the diet at concentrations resulting in doses of 0, 25, 375, 750 and 1500 mg/kg/day in mice and 0, 150, 500 and 1000 mg/kg/day in rats. The diet used in these studies was standard certified chow containing 22% fat and 0.98% calcium. In addition to evaluating the carcinogenic potential of orlistat, these studies also determined colonic mucosal cell proliferation using proliferating cell nuclear antigen (PCNA) immunohistochemical staining [23,24].

The following are very brief summaries of the carcinogenicity studies conducted in NMRI mice and Wistar rats. The information presented in the study summaries relate to cell proliferation, toxicity and carcinogenicity. In addition, the resulting animal doses as multiples of the human AUC\(_{0-24}\) exposure and body surface area (mg/m\(^2\)) are presented.
6.1.1.1 Two Year Oral (Dietary Admix) Carcinogenicity Study with Orlistat in NMRI Mice

A two-year oral (dietary admixture) carcinogenicity study was conducted in NMRI mice (50/sex/group; oncogenicity animals) at doses of 0, 0, 25, 75, 375, 750 and 1500 mg/kg/day. Male mice were treated for 104 weeks and female mice were treated for 95 instead of 104 weeks because of intercurrent mortality in all female dose groups, including the controls. Additional mice (6/sex/group) were designated for Interim Sacrifice after 4, 26, 52, and 78 weeks [23].

Oncogenicity Animals

There was no effect of orlistat on survival or adverse clinical signs. The female groups were however, sacrificed after 96 weeks of dosing because all female groups, including controls, were approaching 25% survival. Dose-related increased food consumption was observed in all male treated groups and in females at doses of 375 mg/kg/day and greater. At the high dose of 1500 mg/kg/day, body weight of male, but not female, mice was slightly decreased during the first 41 weeks of the study but not thereafter. At necropsy, there were no treatment-related macroscopic or organ weight changes in male or female mice considered related to orlistat. Histologically, there were no non-neoplastic findings that were considered to be related to treatment with orlistat. The non-neoplastic changes that were observed, were characteristic of mice of this age and strain and their nature and incidence did not distinguish treated from control mice. With respect to neoplastic findings, there were no neoplastic changes considered to be related to treatment with orlistat. The type, incidence, and organ distribution of the neoplastic lesions were considered to be similar in treated and control mice. The same was true for the number of primary neoplasms, the number of mice with primary neoplasms, the number of mice with more than one primary neoplasm, the number of mice with metastases, and the number of benign and malignant neoplasms/sex/group.

Interim Sacrifice Animals

Findings in mice designated for interim sacrifice after 4, 26, 52, or 78 weeks of dosing were similar to the mice in the oncogenicity groups. There was no treatment-associated effect on survival or adverse clinical signs. There was an increase in food consumption without a concomitant increase in body-weight. Body-weight of male mice in the 1500 mg/kg/day dose group was slightly lower than that of the control groups. Plasma levels of vitamins E and D3 and hepatic levels of vitamin E, were decreased at all dose levels despite supplementation of the three highest dose groups with both vitamins. Irrespective of treatment, vitamin A in the liver tended to increase during the course of the study.

A minimal increase in colonic mucosal cell proliferation was observed in male mice receiving 1500 mg/kg/day orlistat after 26, 52 and 78 weeks of treatment. A similar increase was observed in female mice receiving 1500 mg/kg/day orlistat after 52 and 78 weeks of treatment. A minimal increase in colonic mucosal cell proliferation was also observed in male mice receiving 25, 375 and 750 mg/kg/day orlistat after 78 weeks of treatment and in female mice receiving 750 mg/kg/day orlistat after 52 weeks of treatment.
At necropsy, there were no macroscopic findings or organ weight changes considered to be treatment related. There were no non-neoplastic or neoplastic findings considered related to treatment with orlistat.

Toxicokinetics

Systemic exposure to unchanged orlistat was assessed after 26, 52, and 78 weeks of dosing and in the oncogenicity groups, at termination of the study. No unchanged drug was detected in the low-dose groups (25 mg/kg/day). Dose-related increases in plasma levels of orlistat were observed in the other dose-groups with the levels higher (1.5 to 2-fold) in males than in females. A 1.3- to 2.8-fold increase in systemic exposure was observed over the course of the study. Despite the very high dose levels administered, rather low concentrations of unchanged drug were observed. However, systemic exposure of the mice at the high dose of 1500 mg/kg/day as compared to humans is relatively high. The exposures that were achieved in the carcinogenicity study were AUC\(_{(0-24)}\) values of 1030 and 803 ng.h/mL for male and female mice, respectively. With a human AUC\(_{(0-24)}\) of 13 ng.h/mL at a dose of 120 mg t.i.d, this [25] provides an AUC\(_{(0-24)}\) multiple of 79-fold and 62-fold for male and female mice, respectively. The dose multiple based on mg/m\(^2\) at the high dose of 1500 mg/kg/day (4500 mg/m\(^2\)) and the human dose of 360 mg/day (200 mg/m\(^2\)) provides a dose multiple of 22.5-fold [23].

6.1.1.2 Two Year Oral (Dietary Admixture) Carcinogenicity Study with Orlistat in Wistar Rats

A two-year, oral (dietary admixture) oncogenicity study of orlistat was conducted in Wistar rats (50 rats/sex/group; oncogenicity animals) at doses of 0, 0, 150, 500, and 1000 mg/kg/day. Male and female rats were treated for 104 weeks. Additional rats were designated for interim sacrifice at 26 and 52 weeks (5/sex/group) and at 78 weeks (15/sex/group). Rats treated with orlistat received a weekly oral supplementation of fat-soluble vitamins by gavage [24].

Oncogenicity Animals

Survival and adverse clinical signs were not affected by treatment with orlistat. Food consumption was increased in all treated rats within the first weeks with effects on body weight towards the end of the study. At necropsy, adrenal weight was increased in female rats at doses of 500 and 1000 mg/kg/day and mean kidney weight was increased in male rats. Diffuse adrenal cortical hypertrophy was diagnosed in both male and female rats at 500 and 1000 mg/kg/day. Other histopathological diagnoses, considered to be associated with orlistat, included alveolar histiocytosis and renal nephropathy. With respect to neoplastic findings, there was a statistically significant decrease in the incidence of mammary fibroadenomas in female rats in the high-dose group. There was no increase in the incidence of tumors at any site considered to be related to treatment with orlistat. Thus, there was no evidence of an oncogenic effect of orlistat administered orally at doses up to 1000 mg/kg/day (8599 mg/kg/day) to Wistar rats for up to two years.
Interim Sacrifice Animals

Findings in rats designated for interim sacrifices were similar to those in mice in the oncogenicity groups. There was no effect on survival or adverse clinical signs. Food intake was increased in all treated groups within the first week of dosing and body weight decreases were seen in the later stages of the study. Increased adrenal weight was observed in males and females at the 500 and 1000 mg/kg/day dose levels and, in rats sacrificed at 26, 52, and 78 weeks, histopathological findings in the adrenals consisted of diffuse adrenal cortical hypertrophy. Alterations in a variety of clinical pathology parameters were observed primarily in the 500 and 1000 mg/kg/day dose groups. These included: slight increases in glucose and urea levels, moderate to marked increases in total cholesterol, marked increases in total bilirubin levels, markedly increased triglyceride levels, and slight changes in some electrolytes and the protein electrophoretic pattern. Hepatic levels of vitamin A were slightly decreased in treated male but not in treated female rats. However, in spite of vitamin supplementation, vitamin E levels in the liver were moderately to markedly reduced in all treated groups with males affected more than females.

Orlistat treatment did not result in any increases in colonic mucosal cell proliferation following 26, 52, 78 and 104 weeks of treatment. However, mucosal cell proliferation of the rectum was increased in male and female rats receiving 500 or 1000 mg/kg/day orlistat after 52, 78 and 104 weeks of treatment.

Toxicokinetics

The toxicokinetic analyses showed that throughout the study and at all dose levels, the rats were systemically exposed to orlistat. When measured at 104 weeks, very high systemic exposures were achieved in the carcinogenicity study at the high dose of 1000 mg/kg/day. AUC\(_{(0-24)}\) values were 24500 and 29800 ng.h/mL for male and female rats, respectively. As compared to the AUC\(_{(0-24)}\) of 13 ng.h/mL in humans at a 360 mg dose provides an AUC\(_{(0-24)}\) exposure multiple of 1885 fold and 2292 fold for male and female rats, respectively. The dose multiple based on mg/m\(^2\) at the high dose of 1000 mg/kg/day (6000 mg/m\(^2\)) and the human dose of 360 mg/day (200 mg/m\(^2\)) provides a dose multiple of 30 fold. No significant gender differences were observed but, in some instances, plasma levels of orlistat were 20 to 30% higher in female than in male rats. Systemic exposure increased 2.4- to 5.2-fold over the course of the study. Similar accumulation was noted in a 1-year study in rats and is consistent with the slow rate of elimination of orlistat.

6.1.1.3 Conclusions

Treatment with orlistat for up to two years at doses up to 1500 mg/kg/day in NMRI mice and 1000 mg/kg/day in Wistar rats did not result in any treatment related tumor responses and specifically did not result in treatment related tumors of the gastrointestinal tract despite very large multiples of the human exposure based either on AUC\(_{(0-24)}\), ratios or body surface area ratios. The high dose levels administered in these carcinogenicity studies achieved AUC\(_{(0-24)}\) multiples of 79-fold and 62-fold for male and female mice, respectively, and 1185-fold and 2292-fold for male and female rats, respectively, greater
than the human $\text{AUC}_{(0-24)}$. These $\text{AUC}_{(0-24)}$ multiples are significantly greater than the 25-fold animal to human $\text{AUC}_{(0-24)}$ ratio recommended in the ICH guidelines for carcinogenicity study high dose selection for non-genotoxic pharmaceuticles. As such, the doses selected provide a valid basis for the assessment of the carcinogenic potential of orlistat. Using the conversion factors of 3 for mice, 6 for rats and 37 for humans that are used by FDA to extrapolate doses expressed on a body weight basis (mg/kg) to doses expressed on a body surface area basis (mg/m$^2$) [25], the highest doses administered to mice and rats in these studies were 4500 mg/m$^2$ and 6000 mg/m$^2$, respectively. These doses are approximately 22.5- and 30-fold greater than the maximum clinical dose of 200 mg/m$^2$. 
6.1.2 Genotoxicity Studies

The genotoxic potential of orlistat was also evaluated under NDA 20-766 and the results of these evaluations demonstrated that orlistat was not genotoxic in the Ames test, a mammalian forward mutation assay (V79/HPRT), an in vitro clastogenesis assay in human peripheral lymphocytes (HCA), an in vitro unscheduled DNA synthesis assay in rat hepatocytes and an in vivo mouse micronucleus test [26]. This test battery met the requirements of the ICH guidance for evaluation of genotoxic potential [27]. Based on the results of these studies, orlistat is considered to be nongenotoxic.

6.1.3 Exploratory Studies

To assess the potential effects of orlistat on colonic mucosal cell proliferation, a series of exploratory animal studies was conducted under NDA 20-766 at the request of the FDA before the results of the carcinogenicity studies were available for review. The overall development program (including the 9 month exploratory study) to address this issue was discussed with and agreed to by FDA. The objective of these exploratory studies was to evaluate the colonic luminal effects of orlistat using doses that would produce inhibition of fat absorption similar to what is observed in humans consuming a western-style diet. Inhibition of fat absorption in these exploratory studies ranged from approximately 5 to 15% in animals receiving the lower doses of orlistat to more than 75% in animals receiving the highest doses of orlistat. These studies were not intended to evaluate the systemic effects of orlistat nor the effects of orlistat following administration at doses representing large multiples of the human dose used in the repeated dose toxicity studies and carcinogenicity studies.

6.1.3.1 Short-Term Study in Rats

In the first of these exploratory studies, male Wistar rats were administered 0, 8.5, or 117 mg/kg/day orlistat for 9 or 10 days in a diet that provided approximately 40% of calories as animal fat, in the form of butter fat, approximately 0.1% calcium and 10% cellulose. A separate group of animals received 8.5 mg/kg/day orlistat in the same diet but with only 5% cellulose. Cellulose was added to the diet to evaluate the effect of fiber on the colonic mucosa. Fecal output of total fat, free fatty acids, bile acids and calcium were determined in freeze dried feces. Lytic activity of fecal water was determined in vitro by evaluation of lysis of human erythrocytes by fecal water from treated animals. Alkaline phosphatase activity in fecal water was also measured in the absence and presence of l-phenylalanine, an uncompetitive inhibitor of alkaline phosphatase. The difference in activity represented the activity of alkaline phosphatase in fecal water which reflects epitheliolysis. Colonic mucosal proliferation was evaluated by measuring the incorporation of tritiated thymidine into DNA obtained from scrapings of the colonic mucosa. The following table summarizes the mean value results of the key parameters evaluated in this study:
Xenical® (orlistat)
(Ro 18-0647)

Preclinical and Clinical Summary
Report on Colon Cancer

### Table 6  Fecal Parameters and Cell Proliferation in Short Term Rat Study (Mean Values)

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium (mM)</th>
<th>Bile Acids (mM)</th>
<th>Free Fatty Acids (mM)</th>
<th>Lytic Activity (%)</th>
<th>Alkaline Phosphatase (U/ml)</th>
<th>DNA (µg/scraping)</th>
<th>Proliferation (dpm/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 10% Cellulose</td>
<td>1.87</td>
<td>6.46</td>
<td>1.79</td>
<td>11.37</td>
<td>41.08</td>
<td>296.67</td>
<td>20.14</td>
</tr>
<tr>
<td>8.5 mg/kg/day orlistat 10% Cellulose</td>
<td>0.88</td>
<td>1.75</td>
<td>41.51</td>
<td>88.77</td>
<td>134.33</td>
<td>269.33</td>
<td>52.64</td>
</tr>
<tr>
<td>8.5 mg/kg/day orlistat 5% Cellulose</td>
<td>1.46</td>
<td>2.11</td>
<td>97.94</td>
<td>87.80</td>
<td>100.57</td>
<td>268.50</td>
<td>58.44</td>
</tr>
<tr>
<td>117 mg/kg/day orlistat 10% Cellulose</td>
<td>0.93</td>
<td>1.34</td>
<td>7.27</td>
<td>12.92</td>
<td>9.82</td>
<td>324.50</td>
<td>198.96</td>
</tr>
<tr>
<td>Control 10% Cellulose 0.15% Sodium Cholate</td>
<td>2.29</td>
<td>18.37</td>
<td>0.88</td>
<td>87.30</td>
<td>204.53</td>
<td>292.50</td>
<td>75.52</td>
</tr>
</tbody>
</table>

Statistical significance not reported

The results of this study revealed that colonic proliferation was increased approximately 2.7- and 10-fold in animals of the low and high dose orlistat groups, respectively, when compared with the controls. Absorption of dietary fat was inhibited by 24% in animals of the low dose orlistat groups and 80% in animals of the high dose orlistat group. The concentration of bile acids in the feces was decreased by 3.1- to 3.7-fold in animals of the low dose orlistat groups and by 4.8-fold in animals of the high dose orlistat group when compared to the controls [28]. The doses administered to animals of the low and high dose orlistat groups in this study were approximately 30% and more than 4-fold greater than the human prescription dose, respectively, based on body surface area.

### 6.1.3.2 Short-Term Study in Rats with Recovery Period

Similar effects were observed in a subsequent 10-day study of the same design in which male Wistar rats were administered 0, 8.5, 25, or 127 mg/kg/day orlistat in a diet that provided 40% of calories as fat (90% milk fat, 10% corn oil), approximately 0.1% calcium and 5 or 10% cellulose. In this study additional groups of animals, undergoing the same treatment, were maintained for a 10-day recovery period. Fecal output of total fat, free fatty acids, bile acids, triglycerides, diglycerides, monoglycerides and calcium were determined in freeze dried feces. Bile acids, fatty acids, triglycerides, diglycerides and monoglycerides were measured in fecal water. Lytic activity of fecal water was determined in vitro by evaluation of lysis of human erythrocytes by fecal water from treated animals. Alkaline phosphatase activity was measured in feces and fecal water. Total colonic weight, mucosal scraping weight, mucosal scraping protein and colonic proliferation was determined on days 9, 10, 19 and 20 of the study by measuring the...
incorporation of tritiated thymidine into DNA of scrapings of the colonic mucosa. The following table summarizes the mean value results of the key parameters evaluated in this study:

### Table 7  
**Fecal Parameters and Cell Proliferation in Short Term Rat Study with Recovery Period (Mean Values)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium (mM)</th>
<th>Bile Acids (mM)</th>
<th>Free Fatty Acids (mM)</th>
<th>Lytic Activity (%)</th>
<th>Alkaline Phosphatase (U/ml)</th>
<th>DNA (µg/scraping)</th>
<th>Proliferation (dpm/µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.18</td>
<td>4.70</td>
<td>3.89</td>
<td>1.0</td>
<td>24.3</td>
<td>572</td>
<td>18.7</td>
</tr>
<tr>
<td>8.5 mg/kg/day orlistat</td>
<td>0.89</td>
<td>1.52</td>
<td>89.40</td>
<td>98.6</td>
<td>43.8</td>
<td>692</td>
<td>35.4</td>
</tr>
<tr>
<td>25 mg/kg/day orlistat</td>
<td>0.54</td>
<td>0.71</td>
<td>48.40</td>
<td>99.0</td>
<td>26.2</td>
<td>628</td>
<td>45.4**</td>
</tr>
<tr>
<td>5% Cellulose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>127 mg/kg/day orlistat</td>
<td>1.18</td>
<td>0.95</td>
<td>9.90</td>
<td>48.1</td>
<td>11.4</td>
<td>590</td>
<td>93.8***</td>
</tr>
<tr>
<td>Control (Recovery)</td>
<td>0.93</td>
<td>6.03</td>
<td>1.60</td>
<td>4.1</td>
<td>23.1</td>
<td>636</td>
<td>24.2</td>
</tr>
<tr>
<td>8.7 mg/kg/day orlistat</td>
<td>0.79</td>
<td>5.67</td>
<td>1.80</td>
<td>2.6</td>
<td>40.0</td>
<td>720</td>
<td>22.7</td>
</tr>
<tr>
<td>(Recovery)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121 mg/kg/day orlistat</td>
<td>1.50</td>
<td>5.30</td>
<td>3.43</td>
<td>23.2</td>
<td>33.2</td>
<td>722</td>
<td>32.3**</td>
</tr>
<tr>
<td>(Recovery)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** *, ***, p < 0.01, <0.001

Under the conditions of this study, treatment with orlistat was shown to inhibit absorption of dietary fat by 29%, 58% and more than 75% in animals of the low, mid and high orlistat dose groups, respectively. This inhibition of the absorption of dietary fat was accompanied by a dose-dependent increase in colonic mucosal proliferation. In high dose animals, this increase was approximately 5-fold greater than that of the controls. After termination of treatment, the effects of orlistat on the intestinal parameters and colonic epithelial proliferation were largely reduced [29]. The doses of orlistat administered in this study were approximately 30%, 88% and more than 4-fold greater than the recommended human dose of orlistat, in animals of the low, mid and high dose orlistat groups, respectively, based on body surface area.

#### 6.1.3.3 Three-Week Study in Rats

In contrast, colonic mucosal proliferation was not observed in another study in which animals were administered orlistat in the diet at lower doses for approximately 3 weeks [30]. In this study, male Wistar rats were administered 0, 8.2, or 24.4 mg/kg/day orlistat in a diet containing 40% of calories as fat (90% milk fat, 10% corn oil) and a low level (0.1%) of calcium or 0, 8.5, or 24.7 mg/kg/day orlistat in a diet containing 40% of calories as fat (90% milk fat, 10% corn oil) and a normal level (1.0%) of calcium for 20 to 22 days. Fecal output of fat, free fatty acids, bile acids, triglycerides, diglycerides,
monoglycerides, calcium and alkaline phosphatase were determined in freeze dried feces. Bile acids, free fatty acids, phospholipids and alkaline phosphatase were measured in fecal water. Cytotoxicity of fecal water was determined in vitro as release of hemoglobin from human erythrocytes. Colonic proliferation was determined by measuring the incorporation of tritiated thymidine into DNA of scrapings of the colonic mucosa. The following table summarizes the mean value results of the key parameters evaluated in this study:

Table 8 Fecal Parameters and Cell Proliferation in 3-Week Rat Study (Mean Values)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Orlistat Dose (mg/kg/day)</th>
<th>Calcium (mM)</th>
<th>Bile Acids (mM)</th>
<th>Free Fatty Acids (mM)</th>
<th>Lytic Activity (%)</th>
<th>Alkaline Phosphatase (U/mM)</th>
<th>DNA (µg/scraping)</th>
<th>Proliferation (dpm/µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% Control</td>
<td>0.97</td>
<td>4.37</td>
<td>2.29</td>
<td>45</td>
<td>25.6</td>
<td>952</td>
<td>41.2</td>
<td></td>
</tr>
<tr>
<td>Milk Fat, 8.2</td>
<td>0.83</td>
<td>1.54</td>
<td>120.00</td>
<td>96</td>
<td>22.9</td>
<td>1083</td>
<td>29.8</td>
<td></td>
</tr>
<tr>
<td>0.1% Calcium</td>
<td>24.4</td>
<td>1.24</td>
<td>0.80</td>
<td>74.00</td>
<td>15.1</td>
<td>992</td>
<td>33.7</td>
<td></td>
</tr>
<tr>
<td>40% Control</td>
<td>1.06</td>
<td>1.38</td>
<td>4.29</td>
<td>0</td>
<td>10.9</td>
<td>836</td>
<td>30.2</td>
<td></td>
</tr>
<tr>
<td>Milk Fat, 8.5</td>
<td>1.91</td>
<td>1.45</td>
<td>5.10</td>
<td>2</td>
<td>8.2</td>
<td>830</td>
<td>40.7</td>
<td></td>
</tr>
<tr>
<td>1.0% Calcium</td>
<td>24.7</td>
<td>2.71</td>
<td>0.48</td>
<td>8.62</td>
<td>7</td>
<td>999</td>
<td>39.2</td>
<td></td>
</tr>
<tr>
<td>Standard Control</td>
<td>Control</td>
<td>8.30</td>
<td>0.65</td>
<td>0.1</td>
<td>0.1</td>
<td>1042</td>
<td>37.2</td>
<td></td>
</tr>
</tbody>
</table>

No statistically significant differences

Under the conditions of this study, administration of orlistat with either diet had no significant effect on colonic mucosal proliferation as measured by tritiated thymidine incorporation. Absorption of dietary fat in this study was inhibited by 30% and 50% in animals of the low and high dose orlistat groups, respectively [30]. The doses of orlistat administered in this study were approximately 30% and 87% the recommended human dose of orlistat, in animals of the low and high dose orlistat groups, respectively, based on body surface area.

6.1.3.4 Four-Week Study in Rats

To further investigate the effects of prolonged orlistat treatment, another study was conducted in which animals were administered orlistat in the diet for 4 weeks. In this study, male and female Wistar rats were administered orlistat in a high fat and low calcium diet which resulted in doses of approximately 0, 3.3, 8.9, 21.0, or 43.7 mg/kg/day for males and approximately 0, 4.2, 11.2, 24.9, or 57.7 mg/kg/day orlistat for females. Fecal output of total lipids, unesterified fatty acids (ionized and salt forms), triglycerides, diglycerides and bile acids were determined in freeze dried feces. Free fatty acid and bile acid content of fecal water was also determined. Colonic cell proliferation was evaluated...
using PCNA and 5-bromo-2-deoxyuridine (BrdU) immunohistochemistry to measure labeling index and crypt grade. The following table summarizes the mean value results of the key parameters evaluated in this study:

### Table 9
**Fecal Parameters and Cell Proliferation in 4-Week Rat Study (Mean Values)**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Orlistat Dose (mg/kg/day)</th>
<th>Fecal Water</th>
<th>PCNA</th>
<th>BrdU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fatty Acids (mM)</td>
<td>Bile Acids (mM)</td>
<td>Labeling Index (%)</td>
</tr>
<tr>
<td>Males</td>
<td>0</td>
<td>0.29</td>
<td>2.39</td>
<td>31.77</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>1.92</td>
<td>1.93</td>
<td>33.76</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>3.12</td>
<td>1.35</td>
<td>37.65*</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>0.82</td>
<td>1.16</td>
<td>40.37*</td>
</tr>
<tr>
<td></td>
<td>43.7</td>
<td>0.55</td>
<td>1.00</td>
<td>53.33*</td>
</tr>
<tr>
<td>Females</td>
<td>0</td>
<td>0.46</td>
<td>1.77</td>
<td>34.79</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>2.26</td>
<td>1.64</td>
<td>36.27</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>2.82</td>
<td>1.14</td>
<td>36.28</td>
</tr>
<tr>
<td></td>
<td>24.9</td>
<td>1.08</td>
<td>0.89</td>
<td>36.46</td>
</tr>
<tr>
<td></td>
<td>57.7</td>
<td>0.62</td>
<td>1.11</td>
<td>39.93</td>
</tr>
</tbody>
</table>

* Significant increase p < 0.05 compared to control    ** Significant decrease p < 0.05 compared to control

The results of PCNA immunohistochemical staining revealed a statistically significant increase in cell proliferation, as measured by labeling index and crypt grade, in male rats receiving 8.9, 21.0 or 43.7 mg/kg/day orlistat. A statistically significant increase in cell proliferation, as measured by crypt grade, was also observed in female rats receiving the 24.9 or 57.7 mg/kg/day orlistat, while mean labeling index was significantly increased in female rats receiving 57.7 mg/kg/day orlistat. The results of BrdU immunohistochemical staining revealed a statistically significant increase in cell proliferation, as measured by crypt grade, in male rats receiving 21.0 and 43.7 mg/kg/day orlistat, while mean labeling index was significantly increased only in male rats receiving 43.7 mg/kg/day orlistat. In contrast there was no detectable increase in cell proliferation observed in treated female rats, as determined by BrdU labeling index. In fact, the BrdU labeling index was significantly less in animals receiving the 4.2, 11.2 or 24.9 mg/kg/day orlistat when compared with the controls. In this study, absorption of dietary lipids was inhibited by 5% to 12% in animals of the low dose orlistat group and by 42% to 59% in animals of the high dose orlistat group. A biphasic response in the excretion of unesterified fatty acids was observed, with animals receiving the low intermediate orlistat dose excreting the greatest amount of fatty acids. Daily excretion of bile acids was dose-dependently reduced, but only in males [31]. The doses administered in this study were approximately 12% and 15% of the human dose for males and female of the low dose group, respectively, and 1.5- to 2-fold greater than the human dose for males and females of high dose group, respectively, based on body surface area.
6.1.3.5 Parallel Nine-Month Studies in Rats Maintained on Either a Low or Normal Calcium Diet

The potential colonic effects of orlistat were also investigated following chronic treatment in two parallel studies that were conducted to evaluate the long-term effects of orlistat on the histopathology of the colon of rats maintained on a diet consisting of 40% animal fat and either low (0.1%) or normal (1.0%) calcium. The doses of orlistat administered in these studies were intended to inhibit fat absorption similar to what is observed in humans consuming a western-style diet. In these studies, male and female Wistar rats were fed either of these diets containing 0, 70, 140, or 280 ppm orlistat for 9 months. These dietary concentrations resulted in orlistat doses approximately equal to 0, 3, 7, and 15 mg/kg/day for males and 0, 4, 10 and 22 mg/kg/day for females. The experimental design incorporated two interim sacrifices at 13 and 26 weeks, and a terminal sacrifice after 39 weeks of treatment. At each sacrifice, an equal number of rats was randomly selected from each group and maintained untreated for a recovery period of at least 8 weeks after which they were sacrificed and evaluated at study weeks 21, 34 and 48. At each sacrifice, sections of colon were examined for the effects of colonic cell proliferation using PCNA immunohistochemical staining and, beginning with the second interim sacrifice (week 26), for aberrant crypt foci (ACF) using whole mount-preparations stained with methylene blue. Five colons per sex per group were evaluated at weeks 26 and 34 while up to ten colons per sex per group were evaluated at weeks 39 and 48 [32,33].

The results of these studies showed that after 26 weeks of treatment, orlistat had no effect on colonic mucosal proliferation. At each interim evaluation up to week 34, there were no differences in PCNA labeling index (PCNA-LI), crypt height, crypt grade, number of ACF/group, number of ACF/rat, percentage of rats with at least one ACF, or number of aberrant crypts/ACF (multiplicity) that were considered related to treatment with orlistat. However, at the week 39 sacrifice, mean crypt height of females receiving approximately 22 mg/kg/day orlistat in either the normal or low calcium diet was significantly greater than the controls. This effect was not apparent in the results of PCNA-LI which demonstrated no treatment-related differences in the labeling index at this study interim. At the final recovery sacrifice at 48 weeks, there were no differences in PCNA labeling index, crypt height, or crypt grade that were considered related treatment. Several differences in ACF parameters were observed at study weeks 39 and 48 which are summarized in Table 10:
A dose proportional increase in the number of ACF/group and the number of ACF/rat was also observed at week 39 in females receiving 10 or 22.1 mg/kg/day orlistat in the high fat and normal calcium diet. However, females receiving 22.1 mg/kg/day orlistat had the lowest number of aberrant crypts/ACF (multiplicity) and number of ACF composed of 4 or more aberrant crypts at this interim. At the final recovery sacrifice at 48 weeks, there was an increase in the number of ACF/group, the number of ACF/rat, the percentage of rats with at least one ACF and the number of ACF composed of 4 or more aberrant crypts in females receiving 4.4 or 10 mg/kg/day orlistat in the high fat and normal calcium diet. However, similar effects were not observed in females receiving the high fat and low calcium diet containing orlistat at any dose level or in females receiving 22.1 mg/kg/day orlistat in the high fat and normal calcium diet. There was also a decrease in number of aberrant crypts/ACF (multiplicity) in orlistat-treated females receiving the high fat and normal calcium diet when compared with the controls [32,33]. The highest doses of orlistat administered in the high fat and low calcium diet study were 50.7% and 77.6% of the maximum clinical dose for males and females, respectively, on the basis of body surface area. The highest doses administered in the high fat and normal

### Table 10 Results Summary of 9 Month Study in Rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Sex</th>
<th>Orlistat Dose (mg/kg/day)</th>
<th>Total Number of ACF/Group</th>
<th>Incidence of ACF/Rat</th>
<th>Incidence (%) of Rats with ACF</th>
<th>Multiplicity (AC/ACF)</th>
<th>% ACF &gt; 4 AC</th>
<th>Total Number of ACF/Group</th>
<th>Incidence of ACF/Rat</th>
<th>Incidence (%) of Rats with ACF</th>
<th>Multiplicity (AC/ACF)</th>
<th>% ACF &gt; 24 AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% Pork Fat 0.1% Calcium</td>
<td>Male</td>
<td>0</td>
<td>10</td>
<td>1.0</td>
<td>50</td>
<td>3.8</td>
<td>50.0</td>
<td>7</td>
<td>0.7</td>
<td>50</td>
<td>10.1</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8</td>
<td>5</td>
<td>0.5</td>
<td>30</td>
<td>8.6</td>
<td>80.0</td>
<td>4</td>
<td>0.4</td>
<td>22</td>
<td>7.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.3</td>
<td>2</td>
<td>0.2</td>
<td>30</td>
<td>19.5</td>
<td>100.0</td>
<td>9</td>
<td>0.9</td>
<td>50</td>
<td>2.7</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.3</td>
<td>1</td>
<td>0.1</td>
<td>11</td>
<td>3.0</td>
<td>0</td>
<td>2</td>
<td>0.3</td>
<td>29</td>
<td>3.0</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
<td>16</td>
<td>1.6</td>
<td>80</td>
<td>6.0</td>
<td>43.8</td>
<td>15</td>
<td>1.5</td>
<td>80</td>
<td>4.9</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>13</td>
<td>1.3</td>
<td>70</td>
<td>6.1</td>
<td>53.8</td>
<td>14</td>
<td>1.4</td>
<td>80</td>
<td>6.9</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.2</td>
<td>14</td>
<td>1.4</td>
<td>80</td>
<td>3.8</td>
<td>28.6</td>
<td>6</td>
<td>0.6</td>
<td>50</td>
<td>6.8</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.9</td>
<td>1</td>
<td>0.1</td>
<td>10</td>
<td>2.0</td>
<td>0</td>
<td>7</td>
<td>0.7</td>
<td>60</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>40% Pork Fat 1.0% Calcium</td>
<td>Male</td>
<td>0</td>
<td>9</td>
<td>1.1</td>
<td>50</td>
<td>2.6</td>
<td>33.3</td>
<td>4</td>
<td>0.4</td>
<td>22</td>
<td>3.3</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8</td>
<td>12</td>
<td>1.2</td>
<td>60</td>
<td>2.2</td>
<td>16.7</td>
<td>8</td>
<td>0.8</td>
<td>60</td>
<td>4.9</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.7</td>
<td>7</td>
<td>0.7</td>
<td>50</td>
<td>4.0</td>
<td>42.9</td>
<td>8</td>
<td>0.8</td>
<td>70</td>
<td>6.0</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.3</td>
<td>7</td>
<td>0.7</td>
<td>50</td>
<td>2.6</td>
<td>42.9</td>
<td>7</td>
<td>0.8</td>
<td>67</td>
<td>5.3</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
<td>8</td>
<td>0.8</td>
<td>70</td>
<td>3.4</td>
<td>25.0</td>
<td>10</td>
<td>1.0</td>
<td>50</td>
<td>6.3</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4</td>
<td>9</td>
<td>1.0</td>
<td>78</td>
<td>3.9</td>
<td>44.4</td>
<td>32</td>
<td>3.2</td>
<td>80</td>
<td>4.8</td>
<td>71.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>18</td>
<td>2.0</td>
<td>78</td>
<td>4.7</td>
<td>44.4</td>
<td>38</td>
<td>4.2</td>
<td>78</td>
<td>5.5</td>
<td>60.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.1</td>
<td>50</td>
<td>5.0</td>
<td>80</td>
<td>2.9</td>
<td>24.0</td>
<td>16</td>
<td>1.8</td>
<td>56</td>
<td>3.3</td>
<td>31.3</td>
</tr>
</tbody>
</table>

Statistical significance not reported.
calcium diet study were 54.2% and 78.3% of the maximum clinical dose for males and females, respectively, on the basis of body surface area.

6.1.4 Summary
Collectively, the results of the available preclinical studies do not suggest a potential human cancer risk. Orlistat was shown to be nongenotoxic in a standard battery of genotoxicity studies and the results of two oral carcinogenicity studies employing maximum doses that were 62- to 79-fold greater in mice and 1885- to 2292-fold greater in rats than the human AUC(o-24) demonstrated that orlistat was not carcinogenic. These doses are also approximately 22.5- and 30-fold greater than the maximum clinical dose of 200 mg/m². The observed increases in colonic mucosal cell proliferation observed in the mouse carcinogenicity study were not associated with hyperplasia or neoplasia of the colon. The results of the exploratory studies show that orlistat produced an increase in cell proliferation in the short-term exploratory studies. While mean crypt height of females receiving 22 mg/kg/day orlistat in either diet in the 9 month chronic studies was significantly greater than the controls at week 39, there were no differences in colonic cell proliferation as evidenced by no increase in the PCNA-LI in these animals. While an increase in the incidence of total ACF was observed in females receiving the mid and high doses of orlistat with a high fat and normal calcium diet at week 39 and in females receiving the low and mid doses of orlistat with a high fat and normal calcium diet at week 48, this effect was absent in females receiving the same doses of orlistat with a high fat and low calcium diet at these and all other study intervals. In fact, among animals receiving the high fat and low calcium diet, it was females of the vehicle control group that exhibited the greatest incidence of total ACF in weeks 39 and 48.

The observed increases in the experimental markers of colonic cell proliferation in the 9-month studies is considered to be of limited significance compared with the large body of clinical data and experience with orlistat, because histological examination of the colonic and rectal mucosa of orlistat-treated animals in these studies revealed no evidence of hyperplasia or neoplasia. Furthermore, even though total ACF were increased in low, mid and high dose females in the latter interims of the high fat and normal calcium 9-month dietary study, ACF multiplicity was not. This is of importance because there are a number of publications in the scientific literature that have concluded that ACF crypt multiplicity predicts tumor development more reliably than total number of ACF [2,3]. The absence of any evidence for an increased incidence of tumors in the gastrointestinal tracts in the two carcinogenicity studies in mice and rats supports the conclusion that the ACF that were observed in the 9-month exploratory studies do not lead to tumor formation in the gastrointestinal tract. Thus total ACF were not predictive for tumor formation in orlistat treated rats.

6.2 Aberrant Crypt Foci: Background and Characteristics
Aberrant crypt foci (ACF) were initially described in 1987 by Bird et al in mice treated with a colon-specific carcinogen and subsequently they were identified in the colonic mucosa of human subjects [34]. Structurally, ACF appear as isolated, grouped, or raised colonic crypts that are increased in size and have altered morphological features [34]. Since their discovery, there has been a great deal of debate regarding the significance of
ACF as possible precursor lesions of colorectal adenomas and cancers. In humans, the relationship between ACF and the development of adenomas and cancers of the colon is not well defined. In addition to their appearance in the colonic mucosa of patients with neoplastic disease, ACF are frequently observed in patients with non-neoplastic diseases such as sigmoid diverticulosis, diverticulitis, chronic constipation and rectal prolapse [35]. It has also been reported that 10% of normal subjects under the age of 40 had ACF, and that the prevalence rose significantly to 56.3% in normal subjects that were 40 to 49 years of age, and to 65.7% in the 60 to 69 age cohort [2]. Furthermore, it has been reported that some compounds such as 2-((carboxyphenyl) retinamide and genistein, which have been shown to prevent the occurrence of ACF, actually enhance the development of colon cancers and that cholic acid, a known promoter of colon carcinogenesis, inhibits the formation of ACF [36]. The following discussion summarizes the current body of data regarding ACF, their histological, morphological and biological differences, as well as their heterogeneity and multiple classifications.

In animals, ACF have been observed in sections of colonic mucosa that have been stained with methylene blue using high or low magnification microscopy. More recently, ACF have been observed in humans by in vivo examination using high magnification chromoscopic colonoscopy. Histologically, ACF are heterogeneous, displaying variable features, and can therefore be grouped into different categories. One histological classification of ACF that has been reported by DiGregorio et al divides ACF into 3 categories consisting of those that are almost histologically normal (typical ACF), those resembling hyperplastic polyps (hyperplastic ACF) and those resembling microadenomas (dysplastic ACF) [37]. Another classification system that was developed by Otori et al is essentially the same with the exception of an additional stage I abnormality category [38].

Typical ACF consist of foci that are almost normal in appearance but have enlarged crypt diameters. Epithelial cells of typical ACF have regular nuclei with only mild crowding and with no stratification, mucin depletion or dysplasia. Goblet cells in this type of ACF are slender, uniform and normal in appearance with small, basally oriented nuclei and apical collections of mucus. The main histological feature that differentiates typical ACF from normal crypts is a subtle crypt widening [3]. ACF that resemble hyperplastic polyps have a serrated luminal configuration, distended goblet cells in the upper crypt and proliferating epithelium in the low area of the crypt. Epithelial cells in this type of ACF are slender, uniform and normal in appearance with small, basally oriented nuclei and apical collections of mucus. The main histological feature that differentiates typical ACF from normal crypts is a subtle crypt widening [3]. ACF that resemble hyperplastic polyps have a serrated luminal configuration, distended goblet cells in the upper crypt and proliferating epithelium in the low area of the crypt. Epithelial cells in this type of ACF contain enlarged and crowded nuclei with no stratification. Mitotic activity is limited to the lower two-thirds of the crypts and no dysplasia or mucin depletion is present [3]. ACF resembling adenomas have a slit-like appearance and contain absorptive cells (enterocytes) with crowded, enlarged, vesicular and stratified nuclei, which may lose their polarity depending on the degree of dysplasia present. Epithelial cells in this type of ACF exhibit mucin depletion, a scarcity of goblet cells and frequent mitoses [3]. Additionally, the classification by Otori et al adds the stage I abnormality category of ACF, which is a precursor of the dysplastic or adenomatous ACF, exhibiting high proliferation indices and zonal expression [38].

From the available published data, the majority of the investigators have concluded that dysplasia is considered a feature of neoplasia, although it is not sufficient to define cancer. Moreover, there is agreement on dysplasia (including dysplastic ACF) being necessary for the definition of preneoplastic lesion [37]. The majority of ACF found in
subjects with familial adenomatosis polyposis, a hereditary predisposition for colorectal cancer, are dysplastic, while sporadic ACF are mostly typical and hyperplastic [2]. Of the categories of ACF that have been defined, it has been postulated that only dysplastic ACF or microadenomas progress to an adenoma or carcinoma, while sporadic ACF are usually hyperplastic and relatively infrequently become neoplastic [2]. This postulation is clearly supported by the results of a recently published study in which the sequential development from early lesion to tumor was evaluated in F344 rats for up to 28 weeks following subcutaneous administration of the colon-specific carcinogen azoxymethane. Histological examination of methylene blue-stained colon preparations in this study revealed two types of early lesions consisting of the classic elevated ACF and small flat lesions, which were defined as flat ACF and which were characterized by bright blue staining, compressed crypt openings, and crypts not elevated above the surrounding mucosa. As time progressed, large flat ACF and tumors developed and showed a uniform picture of severe dysplasia, while classic elevated ACF did not display these changes and were mainly hyperplastic. Additionally, during the time course of the study, the number of flat ACF and tumors was virtually constant with approximately 2.5 lesions per rat. In contrast, the number of classic elevated ACF was initially approximately 180 lesions per rat and at termination was approximately 80 lesions per rat. The flat ACF also grew significantly faster than the classic elevated ACF. The investigators concluded that the results of the study indicated a continuous developmental growth from small flat dysplastic ACF to the stage of a tumor, while in contrast classic elevated ACF do not seem to be as closely related to tumorigenesis [39].

In addition to histological differences, recent advances in ACF research have led to the discovery of various genetic mutations and morphologic changes that can occur within different subsets of ACF which are indicative of possible prospective pathological changes. Over expression of the transcriptional activator β-catenin, due to mutations that cause a loss of function in the adenomatosis polyposis coli (APC) tumor suppressor gene, has been shown to be critical in the initiation and progression of human colorectal carcinogenesis [40]. In several recent publications, early-appearing β-catenin accumulated crypts (BCAC) have been described in the colonic mucosa of rodents, which appear different from the classic ACF. In one recently published study, the formation of BCAC, ACF and tumors of the colon in two strains of mice with differing sensitivities to azoxymethane was evaluated. SWR/J mice are known to be relatively susceptible to the colonic carcinogenic effects of azoxymethane, whereas AKR/J mice are reported to be virtually resistant. In this study, mice were subcutaneously administered azoxymethane once weekly for 3 weeks after which the colons of treated mice were evaluated at 16 and 41 weeks of age for the presence of these lesions. Although AKR/J mice had a lower incidence of colonic tumors than SWR/J mice, AKR/J mice showed a similar frequency of ACF to that of SWR/J mice. More importantly, the incidence of BCAC in SWR/J mice was significantly higher than that in AKR/J mice and the highest frequency was observed in the distal segments of the colon where tumors mainly developed [36]. The results of this study indicate that a marker, other than total ACF, was more predictive of colonic tumor development.

An analysis of the morphological and biological properties of BCAC was performed in another recently published study in which male F344 rats were administered
Toxicology Report on Colon Cancer

Xenical® (orlistat) (Ro 18-0647)

azoxymethane once weekly for 3 weeks and colonic crypts were evaluated at 5, 10 and 20 weeks following carcinogen treatment. Immunohistochemical analysis using the labeled streptavidin biotin method was performed using primary antibodies against β-catenin. Histological examination was performed to evaluate distinctive features of colonic dysplasia and neoplasia. Additionally, at the week 10 evaluation, AgNOR staining, which is considered to be a useful biomarker of cell proliferation, and hexosaminidase activity, which is reported to be decreased in the early stages of colon carcinogenesis in rats, were evaluated. The results of these analyses showed that both the number of crypts per lesion and the diameter of BCAC were significantly increased with time course after carcinogen exposure. Similarly, the histological abnormality in those crypts was also increased with time. In contrast, ACF did not show any increase in histological abnormality during the time course and maintained a consistent histology throughout the study. Histological abnormality of BCAC was significantly greater than that for ACF at every time point and the number of AgNOR/nucleus in BCAC was significantly higher than in ACF. BCAC were accompanied frequently by Paneth cells and had decreased hexosaminidase activity. Collectively, these results strongly suggest that BCAC, which are independent of ACF, are more reliable early markers of colon cancer [41].

In another published study, mutational analysis of the β-catenin gene and immunohistochemistry for β-catenin protein were performed in early appearing lesions, including ACF, of colonic mucosa in rats following azoxymethane administration. In this study, male F344 rats were subcutaneously administered azoxymethane once weekly for 3 weeks and sacrificed 10 weeks thereafter. The colonic mucosa of treated animals was examined in en face preparations and in serial sections following observation in whole mount preparations. Histological examination of the colonic mucosa revealed two types of histologically altered crypts. The first type of altered crypts, which macroscopically resembled ACF but histologically appeared altered, were termed histologically altered crypts with ACF appearance (HACAs). The second type of altered crypts, which did not macroscopically resemble ACF and could not be clearly distinguished from adjacent normal crypts in whole mount preparations, were termed histologically altered crypts with macroscopically normal-like appearance (HACNs). β-Catenin gene mutations were found in 10 of 15 HACNs (67%) and 3 of 15 HACAs (20%). Frequent immunoreactivity of β-catenin protein was also observed in the cytoplasm of HACNs, whereas apparent accumulation was not confirmed in any HACAs analyzed. The results of this study indicate that there were two types of altered crypts in the colonic mucosa of azoxymethane-treated rats and that β-catenin signaling may contribute to the initial stage of colon carcinogenesis. The investigators of this study concluded that HACNs are more likely to be direct precursors of colon tumors than HACAs in rat colon carcinogenesis [42].

In addition to the published data on the heterogeneity of ACF, a number of published studies have evaluated the effects of certain substances or conditions that can affect the incidence of ACF. The following table presents some of these substances or experimental conditions that were also shown to increase the incidence of colonic ACF in animal models:
Table 11 Examples of Conditions Shown to Increase Colonic ACF in Animal Models

<table>
<thead>
<tr>
<th>Strain/Species</th>
<th>Substance / Condition</th>
<th>Treatment Duration</th>
<th>Carcinogen</th>
<th>Effect on ACF</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar Rats</td>
<td>Exercise (Swim to Exhaustion)</td>
<td>Single Episode</td>
<td>DMH&lt;sup&gt;1&lt;/sup&gt;</td>
<td>192% increase</td>
<td>Demarzo et al 2004</td>
</tr>
<tr>
<td>IL-10 KO Mice</td>
<td>7% Fish Oil&lt;sup&gt;2&lt;/sup&gt; Diet</td>
<td>12 week</td>
<td>None</td>
<td>129% increase</td>
<td>Hegazi et al 2006</td>
</tr>
<tr>
<td>F344 Rats</td>
<td>Corn Oil&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9 weeks</td>
<td>AOM&lt;sup&gt;4&lt;/sup&gt;</td>
<td>116% increase</td>
<td>Kohno et al 2000</td>
</tr>
<tr>
<td>F344 Rats</td>
<td>Fasted Refed&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Up to 1 year</td>
<td>AOM&lt;sup&gt;6&lt;/sup&gt;</td>
<td>167% increase</td>
<td>Caderni et al 1999</td>
</tr>
<tr>
<td>F344 Rats</td>
<td>Refined Carbohydrate&lt;sup&gt;7&lt;/sup&gt;</td>
<td>10 weeks</td>
<td>IQ&lt;sup&gt;8&lt;/sup&gt;</td>
<td>&gt; 10-fold increase</td>
<td>Kristiansen et al 1996</td>
</tr>
</tbody>
</table>

1. Single administration of 50 mg/kg dimethylhydrazine
2. Contained an abundance of ω-3 polyunsaturated fatty acids
3. Diet containing 23% corn oil
4. 15 mg/kg azoxymethane administered once weekly for 3 weeks
5. Rats fasted for 4 days and then refed for 1 day
6. Single administration of 5, 7.5, 10 or 20 mg/kg AOM
7. 45% Corn starch, 45% potato starch, 5% sucrose, 5% dextrin
8. 2-Amino-3-methyl-imidazo[4,5-f]quinoline (diet-related colon cancer initiator formed during meat cooking) administered at 0.03% of the diet.

Collectively, the available data support the position that total ACF incidence is not a universally recognized early marker of neoplasia. This is supported by the fact that ACF are frequently observed in patients with nonneoplastic diseases and that there are certain compounds that enhance the development of colon cancer that actually inhibit the formation of ACF. Although recent literature suggests that other markers (i.e., BCAC, HACN) may be more relevant early predictors of neoplasia, these markers also have not been validated.

6.3 Recently Published Animal Data on Orlistat and Colonic Mucosal Proliferation (Garcia et al 2005)

A number of investigations have been published in recent years that have evaluated the potential association of ACF as possible precursor lesions of colorectal adenomas and cancers. Of these, one recently published study evaluated the potential association between orlistat and ACF induction [1]. Specifically, this study evaluated the effects of a high fat diet when administered alone, in combination with orlistat, in combination with the carcinogen dimethyl hydrazine (DMH), or in combination with both orlistat and DMH on the formation of colonic ACF in rats following 30 days of treatment. This treatment was also compared with the effects of standard rat diet with the same combinations of orlistat and DMH. In this study, male Wistar rats were randomized into 8 groups. Animals in groups 1 through 4 received standard rat diet and animals of groups 5 through 8 received a high fat diet enriched with 10% cotton oil. Animals in groups 3, 4, 7 and 8 were also administered 25 mg/kg DMH once weekly for 2 weeks. Additionally, the diets of animals in groups 2, 4, 6 and 8 were supplemented with 200 ppm orlistat. Animals of all groups were maintained on their assigned diets for 30 days. At termination of the treatment period, all animals were sacrificed and necropsied. Sections of the distal colon were prepared to provide a view of transversally cut colonic...
crypts, which were then histologically examined to identify and quantify ACF. Colonic cell proliferation was evaluated by examining cell nuclei in 100 colonic crypts using PCNA-LI expressed as a ratio of cell nuclei positively immunostained with anticyclin/PCNA [1].

The following figures summarize the key findings of this study.

**Figure 2**  
PCNA Labeling Index of Rat Colonic Mucosa

![Graph showing PCNA Labeling Index of Rat Colonic Mucosa](image)

**Figure 3**  
Number ACF per cm$^2$ of Rat Colonic Mucosa

![Graph showing Number ACF per cm$^2$ of Rat Colonic Mucosa](image)

The results of the histological examination revealed that ACF were observed only in the colons of animals treated with the carcinogen DMH. Animals treated with DMH in combination with a high fat diet exhibited an increase in ACF that was comparable to...
animals treated with DMH in combination with orlistat. Animals treated with DMH in combination with both a high fat diet and orlistat exhibited the highest incidence of ACF. Similarly, the results of PCNA-LI showed that animals treated with a high fat diet alone and animals treated with orlistat alone exhibited an increase in the PCNA-LI compared with the controls. Both the high fat diet and orlistat caused a further increase in the PCNA-LI in animals treated with DMH. Orlistat in combination with a high fat diet produced a cumulative effect on the increase of the PCNA-LI when compared with orlistat alone. The investigators of this study concluded that orlistat increases these colonic markers at the same level as a high fat diet [1].

The results of this study are considered to be of limited relevance to the clinical use of orlistat, because ACF were observed only in the colons of animals treated with the carcinogen DMH. Additionally, the investigators of this study only evaluated total ACF and not ACF crypt multiplicity. This important because there are a number of publications in the scientific literature that have concluded that ACF crypt multiplicity predicts tumor development more reliably than total number of ACF [2,3]. Furthermore, the investigators of this study also published another study in which they reported a statistically significant increase in colonic ACF in rats that were subjected to a single session of exhaustive swimming and then administered DMH [43]. As previously stated, ACF are frequently observed in patients with nonneoplastic diseases and there are certain compounds that enhance the development of colon cancer that actually inhibit the formation of ACF.

7. **HUMAN CLINICAL DATA**

7.1 **Evaluation of Colonic Cell Proliferation (Human)**

The ability of orlistat to inhibit the action of gastrointestinal lipases and thereby limit the absorption of dietary triglycerides has led to interest in the possible effect of increased fecal fat on the risk of colon and rectal cancer. The most widely mentioned hypothesis is that an increase in dietary fat leads to an increase in cytotoxic fecal bile acids that can promote damage to the colonic mucosal epithelial cells and produce a compensatory hyperproliferative response [44]. This hypothesis was evaluated in the clinical development program.

A 6-week randomized, double blind, placebo-controlled, parallel group study was performed in 24 obese (BMI 30-40 kg/m²) subjects who received orlistat 120 mg or placebo by mouth three times daily [45]. In collected fecal material, total fat, free fatty acids and total as well as constituent bile acids were measured. In addition, these same parameters were measured in fecal water, perhaps an even more meaningful compartment to measure since it is in direct contact with the luminal epithelium. Rectal biopsy samples were taken in order to assess colonic mucosal cell turnover by the identification of crypt cells in S-phase and by whole crypt mitotic count before and after six weeks of treatment.

As expected, total fecal fat and fecal free fatty acids were increased significantly in the subjects receiving orlistat versus those receiving placebo Importantly, excretion of bile acids, which are recognized as toxic to the colonic mucosa, was significantly lower with orlistat treatment (decrease of 64.4 mg per 24 h) than with placebo treatment (increase of 52.3 mg per 24 h) [p<0.05], with the largest decrease being observed with deoxycholic.
acid and lithocholic acid; this is considered a potentially beneficial effect of orlistat on colon proliferation risk. In the free water component, there was no significant increase in free fatty acids in the orlistat compared with placebo group but there was again a very significant decrease in total bile acids, most noticeably, in deoxycholic acid. No significant changes in any indices of colonic cell proliferation were detected during the study period in subjects receiving orlistat compared with those receiving placebo, and there was no correlation between changes in fecal lipids and indices of colonic cell turnover. In addition, results from each of the 3 different proliferative assays performed were similar, providing additional assurance that there was no evidence of an undesirable effect of orlistat treatment on colonic cellular proliferation. Importantly, orlistat treatment also did not increase colonic crypt size, mucosal proliferation or expand the colonic proliferative compartment within the crypt as compared with placebo treatment. The results of this multiple dose evaluation indicate that inhibition of dietary fat absorption by orlistat did not adversely alter or change the proliferative status of the colonic epithelium in these obese subjects.

8. **PRE- AND POSTMARKETING ADVERSE EVENTS**

8.1 **Reports from Phase II, III, IIIb and IV Clinical Trials**

Safety data from all double-blind, placebo controlled clinical trials of ≥6 months duration were reviewed for incidence of colorectal cancer and the findings are summarized in the following sections.

8.1.1 **Phase II and III Clinical Trials**

The clinical development program of orlistat included three large regulatory submissions in overweight or obese adult patients. These regulatory submissions included the original submission evaluating orlistat as a treatment for obese patients and overweight patients with obesity-related risk factors, a subsequent submission evaluating orlistat as a treatment for type 2 diabetes in overweight and obese patients and a third submission evaluating orlistat as a treatment to prevent the development of type 2 diabetes.

These included a total of 16 double blind placebo-controlled studies of between 6 months to four years of treatment. The studies included were NM14185, BM14119B, BM14119C, BM14150, NM14302, BM14149, NM14161, NM14336, M37002, M37005, M37022, M37047, M37048, M37059 and BM15421. In addition, a one year phase III study conducted in Japan (JM16255) is also included. In total, there were 4516 patients who received placebo and 6146 patients who received orlistat (1052 at 60mg tid and 5094 at 120mg tid).

The safety data for these studies was reviewed for incidences of colonic or rectal carcinoma. A review of these studies revealed that a total of 3 patients had documented colonic or rectal carcinoma during those studies. Two patients receiving orlistat 60 mg three times a day (0.19%), one patient in study NM14302 and one patient in study JM16255 and one patient receiving orlistat 120mg three times a day (0.02%) in study BM15421. All of these patients had confounders and two of the patients had a very short latency between the time of diagnosis and the start of the study drug.
These cases and the relevant medical history are summarized in Table 12.

### 8.1.2 Phase IIIb-IV Clinical Trials

The post-marketing randomized double-blind, placebo-controlled studies of orlistat (≥6mo duration) in obese or overweight patients with co-morbid risk factors from the Phase IIIb/IV clinical trials program were screened to determine the incidence of colon cancer/rectal cancer in placebo versus orlistat patients. The studies examined included M37001, M37004, M37006, M37008, M37009, M37011, M37012, M37013, M37017, M37018, M37020, M37029, M37030, M37031, M37033, M37034, M37044, M37049, M37058, M37059, M37068, M37074 and M37086. Terms used for the searches included colon cancer, colon neoplasm, rectal cancer, bowel cancer as well as a screening of the final study report (FSR) tables containing adverse events and serious adverse events. The search revealed a very low incidence of colon cancer.

There was one case of colonic carcinoma (0.03%) out of 3,396 placebo treated patients and three (0.08%) out of 3,571 orlistat patients. All of these patients had confounders and most had a short latency between the diagnosis and the start of study drug and there was evidence of potential pre-existing lesions. Details of the individual cases are as reported in Table 12.
### Table 12: Reports of Colon/Rectal Cancers in Reported in Controlled Clinical Trials of ≥6 month Duration

<table>
<thead>
<tr>
<th>MCN</th>
<th>Study #</th>
<th>Country</th>
<th>Treatment /Dosage</th>
<th>Age/ Gender /BMI</th>
<th>Preferred Term</th>
<th>Latency in days after start of 1st dose</th>
<th>Latency in days after therapy stopped</th>
<th>Outcome</th>
<th>Reporter Relatedness</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>51961</td>
<td>NM14302</td>
<td>US</td>
<td>Orlistat /60 mg</td>
<td>50/F/ 34.5</td>
<td>Colon cancer</td>
<td>197</td>
<td>160</td>
<td>Improved</td>
<td>No</td>
<td>History of uterine fibroids and hysterectomy are confounders. Rectal bleeding started on day 89. Short latency of 6 months. The early age onset of colon cancer in the patient's father suggests a genetic disposition.</td>
</tr>
<tr>
<td>217117</td>
<td>BM15421</td>
<td>Sweden</td>
<td>Orlistat /120 mg</td>
<td>57/M/ 33.8</td>
<td>Rectal cancer</td>
<td>694</td>
<td>0</td>
<td>Unknown</td>
<td>Yes</td>
<td>Morbidly obese (134kg)</td>
</tr>
<tr>
<td>311226</td>
<td>JM16255</td>
<td>Japan</td>
<td>Orlistat /60 mg</td>
<td>47/M/ 28</td>
<td>Colon cancer</td>
<td>110</td>
<td>26</td>
<td>Outcome death</td>
<td>Yes</td>
<td>At time of diagnosis patient had lung and liver metastases</td>
</tr>
<tr>
<td>113097</td>
<td>M37029</td>
<td>Germany</td>
<td>Orlistat /120 mg</td>
<td>57/F/ 29.7</td>
<td>Rectal cancer</td>
<td>112</td>
<td>30</td>
<td>Improved</td>
<td>No</td>
<td>History of rectal bleeding for several months is a confounder. Short latency of 4 months.</td>
</tr>
<tr>
<td>228833</td>
<td>M37011</td>
<td>France</td>
<td>Orlistat /120 mg</td>
<td>65/F/ 37.6</td>
<td>Colorectal cancer</td>
<td>26</td>
<td>0</td>
<td>Unknown</td>
<td>No</td>
<td>Liver metastases diagnosed 19 days after orlistat start. Causality due to orlistat is less likely.</td>
</tr>
<tr>
<td>308864</td>
<td>M37044</td>
<td>Sweden</td>
<td>Placebo</td>
<td>58/M/ NR</td>
<td>Colon cancer</td>
<td>271</td>
<td>NR</td>
<td>Persisting</td>
<td>No</td>
<td>Patient had low hemoglobin at start of therapy, which is suggestive of possible undetected carcinoma before start...</td>
</tr>
<tr>
<td>237344</td>
<td>M37034</td>
<td>Australia</td>
<td>Orlistat /120 mg</td>
<td>70/M/ 34.6</td>
<td>Bowel cancer</td>
<td>169</td>
<td>NR</td>
<td>Improved</td>
<td>No</td>
<td>Examination revealed a mass in left iliac fossa and confirmed on CT scan.</td>
</tr>
</tbody>
</table>

NR: Not recorded  
MCN: Manufacturer Control Number
8.2 Spontaneous Reports from Post-marketing Surveillance

The Roche adverse event safety database was searched for spontaneous reports of colon cancer, colon neoplasm, rectal neoplasm, colorectal cancer, and rectal cancer cumulatively with the cut-off date of April 18, 2006 and is based on approximately 25.8 million patient treatments as of March 31, 2006. The search retrieved 7 cases as depicted in Table 13.

The cases were assessed with regards to alternative explanations or confounders such as family history of cancer or risk factors for cancer such as smoking, alcohol use, previous history of other cancers, etc. in addition to obesity. Two cases had a family history of cancer. In one case, smoking was identified as a risk factor. Five cases were 50 years of age or above, which is in itself a risk factor for development of cancer. Age was not specified in two cases. In two cases, there was insufficient information regarding age, medical history, orlistat administration dates, onset and diagnosis dates of colon neoplasm. In several of the cases, metastatic disease was identified at the time of diagnosis. Obesity is also a risk factor for cancer.
Table 13  Spontaneous Reports of Colon/Rectal Cancers or Neoplasms in the Roche Adverse Event Safety Database

<table>
<thead>
<tr>
<th>MCN / Country</th>
<th>Age (years) / Gender</th>
<th>BMI (kg/m²)</th>
<th>Treatment /Dosage</th>
<th>Preferred Term</th>
<th>Latency in days after start of 1st dose</th>
<th>Latency in days after therapy stopped</th>
<th>Outcome</th>
<th>Reporter Relatedness</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>250287 / Germany</td>
<td>69/F</td>
<td>26.4</td>
<td>Orlistat /120 mg</td>
<td>Colon cancer</td>
<td>~244</td>
<td>~0</td>
<td>Persisting</td>
<td>Unknown</td>
<td>Age and obesity are confounders. Cancer of the appendix.</td>
</tr>
<tr>
<td>253627 / UK</td>
<td>56/F</td>
<td>36.5</td>
<td>Orlistat /120 mg</td>
<td>Colon cancer</td>
<td>312</td>
<td>0</td>
<td>Persisting</td>
<td>Unknown</td>
<td>Family history of bowel cancer, history of smoking and alcohol use are confounders</td>
</tr>
<tr>
<td>300465 / UK</td>
<td>68/F</td>
<td>32.4</td>
<td>Orlistat /Unknown</td>
<td>Colon cancer</td>
<td>~110</td>
<td>~6</td>
<td>Improved</td>
<td>No</td>
<td>Family history of bowel cancer and age are confounders. Metastases to the liver at time of diagnosis.</td>
</tr>
<tr>
<td>313831 / USA</td>
<td>Unknown /M</td>
<td>Unknown</td>
<td>Orlistat /120 mg</td>
<td>Colon cancer</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Yes</td>
<td>Insufficient information regarding age, medical history, orlistat administration dates, onset and diagnosis dates of colon cancer</td>
</tr>
<tr>
<td>328812 / USA</td>
<td>54/F</td>
<td>82.6</td>
<td>Orlistat /Unknown</td>
<td>Colon cancer</td>
<td>~441</td>
<td>~38</td>
<td>Death</td>
<td>No</td>
<td>Most likely metastases from ovarian carcinoma.</td>
</tr>
<tr>
<td>337630 / Ireland</td>
<td>67/M</td>
<td>Unknown</td>
<td>Orlistat /Unknown</td>
<td>Colon cancer</td>
<td>~9</td>
<td>Unknown</td>
<td>Persisting</td>
<td>No</td>
<td>Short latency is suggestive of pre-existing cancer, which was diagnosed 9 days after start of orlistat therapy</td>
</tr>
<tr>
<td>358244 / USA</td>
<td>Unknown /F</td>
<td>Unknown</td>
<td>Orlistat /120 mg</td>
<td>Colon neoplasm</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Yes</td>
<td>Not able to confirm if actually a carcinoma.</td>
</tr>
</tbody>
</table>

~ designates estimated value
9. DISCUSSION OF PRECLINICAL AND CLINICAL DATA

9.1 Animal Data

The results of the available animal studies do not demonstrate an association between the clinical use of orlistat and colon cancer. Moreover, when tested in subchronic and chronic toxicity studies and in mutagenicity and carcinogenicity studies, orlistat did not demonstrate a genotoxic, or carcinogenic potential.

The highest doses administered in the carcinogenicity studies that demonstrated no evidence of colon cancer, were 62– to 79–fold greater in mice and 1885– to 2292–fold greater in rats than the human AUC(0-24), which are significantly greater than the 25-fold animal to human AUC(0-24) ratio recommended in the ICH guidelines for carcinogenicity study high dose selection [22]. The high doses in these studies were also 22.5- and 30-fold greater than the recommended human dose of orlistat, based on body surface area. The duration of dosing in these lifetime studies represents the average lifespan for mice and approximately two-thirds of the average lifespan for rats.

The results of the exploratory studies that were conducted with orlistat in Wistar rats demonstrated that orlistat produced an increase in cell proliferation in the short-term exploratory studies. In the 9-month exploratory studies, an increase in the incidence of total ACF was also observed in females receiving the mid and high doses of orlistat with a high fat and normal calcium diet at week 39 and in females receiving the low and mid doses of orlistat with a high fat and normal calcium diet at week 48. However, this effect was absent in females receiving similar doses of orlistat with a high fat and low calcium diet. Furthermore, even though total ACF were increased in low, mid and high dose females in this study, ACF multiplicity was not. This is of importance because there are a number of publications in the scientific literature that have concluded that ACF crypt multiplicity predicts tumor development more reliably than total number of ACF [2,3].

The absence of any evidence for an increased incidence of tumors in the gastrointestinal tracts in the two carcinogenicity studies in mice and rats supports the conclusion that the ACF that were observed in the 9-month exploratory studies do not lead to tumor formation in orlistat-treated rats.

The recent publication of a study in which male rats were treated with the carcinogen DMH in combination with a high fat diet supplemented with 10% cotton oil and/or orlistat represents a unique unsubstantiated finding [1]. The increases in total ACF reported in this study are considered to be of limited significance compared with the extensive clinical experience with orlistat, because they were observed only in animals that were administered the carcinogen. Additionally, the investigators of this study only evaluated total ACF and not ACF crypt multiplicity, which, as noted above, may be a more reliable predictor of early tumor development [2,3]. Furthermore, the investigators of this study previously published another study in which they reported a statistically significant increase in ACF in the colons of rats that were subjected to a single session of exhaustive swimming and then administered DMH [43].
9.2 Human Data

Moreover, in humans, ACF occur independent of colon cancer. ACF are frequently observed in patients with non-neoplastic diseases such as sigmoid diverticulosis, diverticulitis, chronic constipation and rectal prolapse [35]. It has also been reported that 10% of normal subjects under the age of 40 had ACF, and that the prevalence rose significantly to 56.3% in normal subjects that were 40 to 49 years of age, and to 65.7% in the 60 to 69 age cohort [2]. Furthermore, it has been reported that some compounds such as 2-(carboxyphenyl) retinamide and genistein, which have been shown to prevent the occurrence of ACF, actually enhance the development of colon cancers and that cholic acid, a known promoter of colon carcinogenesis, inhibits the formation of ACF, but enhances the development of BCAC [36]. Thus there is agreement among a number of investigators that total ACF do not correlate with colon cancer risk.

Detailed and specific studies conducted in man evaluating the potential for orlistat to produce negative changes in the colonic environment provide no meaningful evidence that such effects occur. There is a significant decrease in putative bile acids in patients treated with orlistat as compared to those patients receiving placebo. Direct evidence from rectal biopsies provided no evidence of colonic cellular proliferation and orlistat treatment did not increase crypt size, did not increase mucosal proliferation and did not expand the colonic proliferative compartment. These data are consistent with the benign nature of even very high doses of orlistat as seen in the carcinogenicity studies discussed above. In addition, very few cases of documented colonic or rectal cancer were identified during the clinical development of orlistat and the vast majority was identified early during treatment and had evidence of being pre-existing at the time of study entry. In addition, there is no evidence of a signal based on post-marketing surveillance data.

The concept of a biomarker is that it can be used as a surrogate for an outcome or an event when it is not possible or feasible to conduct the appropriate long-term studies. In the case of orlistat, ACFs cannot be considered well accepted biomarkers for the development of colonic carcinoma based on the fact that very long-term studies in two species of rodents studied at a wide range of doses in a large number of animals showed no evidence of colonic carcinoma. In fact, the vast majority of evidence shows that treatment with orlistat under many different conditions, even those designed to provoke negative findings, do not provide meaningful evidence of an undesirable effect. Since the time of the original regulatory approval for orlistat, there are no new or compelling data that would change or alter the original safety evaluation regarding colonic findings.

10. CONCLUSIONS

Based on a comprehensive review of orlistat preclinical, clinical and market surveillance data, the Sponsors conclude that there is no evidence of a causal link between the use of orlistat and colorectal cancer. The increase in total ACF noted in preclinical studies is not an accurate predictor of colonic tumor development based on the absence of neoplasia in appropriately conducted rodent carcinogenicity studies. Therefore no change in labeling of Rx orlistat is warranted. Additionally, the overall risk-benefit profile of orlistat remains favorable and appropriate for over-the-counter use.
11. REFERENCES


30. Three week oral (dietary fat admix) study of Ro 18-0647/008 (Orlistat) in rats maintained on purified high fat diets containing either low or high concentrations of calcium. Effects on fecal parameters and colonic mucosa proliferation. Hoffman-La Roche Report No. 162,809


38. Otori K et al. Emergence of adenomatous aberrant crypt foci (ACF) from hyperplastic ACF with concomitant increase in cell proliferation. Cancer Research. 1995;55:4743-4746.


