

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

***APPLICATION NUMBER:***

**201699Orig1s000**

**PHARMACOLOGY REVIEW(S)**

Comments on N201699 Fidoxomicin

From Abigail Jacobs, AD 4/8/11

1. I agree that there are no remaining pharm/tox issues.
2. I concur that the animal pharmacology section of labeling should be left blank and agree with other labeling suggestions given in the pharm/tox review.
3. I have discussed some other comments with the reviewer and they will be addressed as appropriate.

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/s/

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ABIGAIL ABBY C C JACOBS

04/08/2011

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH**

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 201699  
Supporting document/s: Supporting document #1  
Applicant's letter date: September 20, 2010 (rolling submission)  
Product: Fidaxomicin  
Indication: Treatment of *Clostridium difficile* infection and prevention of recurrences.  
Applicant: Optimer Pharmaceuticals, Inc.  
Review Division: Division of Anti-infective and Ophthalmologic Products  
Reviewer: Wendelyn Schmidt, Ph.D.  
Secondary Reviewer: Amy Nostrandt, DVM, Ph.D.  
Acting Division Director: Wiley Chambers, M.D.  
Project Manager: Carmen DeBellas

*Template Version: December 7, 2009*

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## 1 Executive Summary

### 1.1 Recommendations

**1.1.1 Approvability:** From the pharmacology/toxicology perspective, Difidid can be approved.

**1.1.2 Additional Non Clinical Recommendations:** None.

### 1.1.3 Labeling

The Pregnancy section reads as shown below. In consultation with the Clinical Pharmacology reviewer, Dr. Aryn Kim, The AUC(0-t) value in healthy humans was 48.3 ± 18.4 ng.h/mL.

(b) (4)

(b) (4)

Thus, the pregnancy section should read:

Pregnancy Category B. Reproduction studies have been performed in rats and rabbits by the intravenous route at 12.6 and 7.0 mg/kg respectively. The plasma exposures ( $AUC_{0-t}$ ) at these doses were approximately 200 and 66 fold that in humans respectively, and have revealed no evidence of harm to the fetus due to fidaxomicin. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Section 13 reads as follows for Carcinogenesis, mutagenesis and impairment of fertility.

### 13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

(b) (4)

The label should read as shown below. The AUC ratios used were based on the AUC 0-t on the final day of dosing

(b) (4)

Long-term carcinogenicity studies in animals have not been conducted to evaluate the carcinogenic potential of fidaxomicin.

Neither fidaxomicin nor OP-1118 was mutagenic in the Ames assay. Fidaxomicin was also negative in the rat micronucleus assay. However, fidaxomicin and its metabolite, OP-1118, were clastogenic in Chinese hamster ovary cells.

Fidaxomicin did not affect the fertility of male and female rats at intravenous doses of 6.3 mg/kg. The exposure (AUC<sub>0-t</sub>) was approximately 100 times that in humans.

The section under Animal pharmacology (13.2) should be completely deleted.

## 1.2 Brief Discussion of Nonclinical Findings

The pharmacodynamics and mechanism of activity of fidaxomicin are discussed in the clinical microbiology section of the NDA. Cardiologic effects were minimal as tested in the hERG assay, telemeterized dogs (single 1 mg/kg intravenous dose), and in oral dog and monkey toxicology studies. No other respiratory, CNS or renal toxicities were identified in the safety pharmacology or general toxicology studies.

Absorption was variable and low by the oral route in most species tested. Metabolism by gut and intestinal enzymes in rats and dogs included the species formed by humans. Excretion was primarily via the fecal route. In dogs, less than 1% of the dose was excreted via the urine.

Toxicity studies of up to 3 months duration have been conducted by the oral and intravenous routes in rats, dogs and cynomolgus monkeys. All studies were conducted at the maximum feasible dose, but due to variable absorption, low solubility and presumed low bioavailability, studies by routes other than the clinical oral route were requested to better define the toxic potential of fidaxomicin. The initial one month oral gavage studies in rats and monkeys with labrasol as vehicle showed minimal toxicities at the maximum feasible dose of 90 mg/kg. Intravenous studies in rats for 14 days with 3 different vehicles were conducted. No fidaxomicin-related toxicities were noted at the maximum feasible doses (<4 mg/kg as an i.v. bolus). A 3 month oral capsule study in the dog showed no toxicity at the maximum feasible dose of approximately 1 g/kg/day.

Segment I and II reproductive toxicity studies were conducted in rats and rabbits. OPT-80 had no effects on fertility or development through implantation in the rat at intravenous doses in 1% solutol HS15 of up to 6.3 mg/kg. In the rat by the intravenous route in 1% solutol HS15, when administered during the period of organogenesis, OPT-80 had no effect on maternal or fetal parameters at the highest dose tested, 12.6 mg/kg. In the rabbit, the highest dose tested, 7.0 mg/kg, was a NOAEL for both dams and offspring.

Fidaxomicin and its main metabolite, OP-1118 were negative for genotoxicity in the Ames bacterial assay. In the chromosomal aberration assay, fidaxomicin was positive, while OP-1118 was negative. Fidaxomicin was negative in the rat micronucleus assay.

## 2 Drug Information

### 2.1 Drug: Dificid

**2.1.1 CAS Registry Number (Optional): 56646-60-4****2.1.2 Generic Name: Fidaxomicin, tiacumicin B****2.1.3 Code Name: OPT-80, PAR-101**

Chemical Name: : 3-[[[6-deoxy-4-O-(3,5-dichloro-2-ethyl-4,6-dihydroxybenzoyl)-2-O-methyl- $\beta$ -L-mannopyranosyl]oxy]-methyl]-12-[[6-deoxy-5-c-methyl-4-O-(2-methyl-1-oxopropyl- $\beta$ -D-lyxo-hexopyranosyl]oxy]-11-ethyl-8-hydroxy-18-(1-hydroxyethyl)-9,13,15-trimethyloxacyclooctadeca-3,5,9,13,15-penataen-2-one

Molecular Formula/Molecular Weight: C<sub>52</sub>H<sub>74</sub>Cl<sub>2</sub>O<sub>18</sub>; mw = 1058

Structure or Biochemical Description: macrolide

Pharmacologic Class: anti-infective

**2.2 Relevant IND/s, NDA/s, and DMF/s: IND 64435****2.3 Clinical Formulation: Tablet**

**2.3.1 Drug Formulation:** The current clinical formulation is a film coated tablet containing 200 mg Dificid. Excipients include microcrystalline cellulose, pre-gelatinized starch, hydroxypropyl cellulose, butylated hydroxytoluene, sodium starch glycolate, and magnesium stearate. (b) (4) polyvinyl alcohol, talc, polyethylene glycol, lecithin (soy) and titanium dioxide.

**2.3.2 Comments on Novel Excipients:** There are no novel excipients.

**2.3.3 Comments on Impurities/Degradants of Concern:** There are no excipients or degradants of concern based on the multiples of the human dose tested in the dog studies.

**2.4 Proposed Clinical Population and Dosing Regimen:** The current dosing regimen for C. difficile treatment is 200 mg given orally twice daily for 10 consecutive days.

**2.5 Regulatory Background:**

The original IND was submitted in 2003. While the two initial oral supporting studies in rats and monkeys showed no significant toxicities, the insensitive plasma assay and highly variable (and low) levels of fidaxomicin in the rat feces were worrisome in the event of systemic absorption. Other toxicity studies were requested at higher exposures to further investigate the toxicologic profile of the drug. Although there were issues with formulation and toxicity in the control animals, a 3 month study in the dog using the tablet formulation contained in gelatin capsules demonstrated no significant toxicity with a maximum feasible dose.

### 3 Studies Submitted

#### 3.1 Studies Reviewed in the NDA

##### Safety Pharmacology:

1. Cardiovascular assessment of orally administered PAR-101 in conscious radiotelemetry-implanted naïve male and female beagle dogs. Study # (b) (4) 609004.
2. OPT-80: Respiratory assessment following intravenous administration to plethysmograph-restrained male Sprague Dawley rats. Study # (b) (4) 609008.
3. An acute central nervous system pharmacological and toxicokinetics study of OPT-80 using a functional observational battery. Study # (b) (4) 609009.

##### Toxicology:

1. A pilot 14-day repeated dose oral (capsule) toxicity study of fidaxomicin in dogs. Study # (b) (4) -609005.
2. A 14-day repeated dose oral (capsule) toxicity study of fidaxomicin in dogs. Study # (b) (4) -609007.
3. A 14-day repeated dose oral (gavage) toxicity study of fidaxomicin in dogs. Study # (b) (4) -609002.
4. A 3 month repeated dose oral (capsule) toxicity study of fidaxomicin in dogs. Study # (b) (4) -609006.
5. A 3-month repeated dose oral (gavage) toxicity study of fidaxomicin in dogs. Study # (b) (4) 609003.
6. OPT-80: A 2-week intravenous toxicity study in rats. Study # 1176-003.

##### Genetic Toxicology

1. Bacterial reverse mutation assay of aged fidaxomicin. Study # AC29mw.503btl.
2. Bacterial reverse mutation assay of OP-1118. Study # AC29me.503btl
3. In vitro mammalian chromosome aberration test on OP-1118. Study # AC29me.331btl.
- 4.

**Studies previously reviewed in the IND:****Pharmacology:**

These studies are reviewed by the Clinical Microbiologist.

**Safety Pharmacology:**

1. Evaluation of the effects of PAR-101 and OP-1118 on cloned hERG channels expressed in human embryonic kidney (HEK293) cells. Study # 1176-014.
2. Cardiovascular effects of PPAR-101 (OPT-80) administered intravenously in the beagle dog. Study # 1176-020, supporting document #120, 4/11/08.

**Pharmacokinetics:**

1. Gastrointestinal pharmacokinetics of OPT-80 in rats following a single oral dose in labrasol.
2. Determination of the potential metabolites of OPT-80 using intestinal and liver microsomes from rat, dog, monkey and human.
3. In vitro drug metabolism report: Reaction phenotyping of OPT-80 using human intestinal and liver microsomes and CYP-450 specific chemical inhibitors.
4. Excretion, pharmacokinetics, and tissue distribution in dogs administered a single oral dose of <sup>3</sup>H-OPT-80.
5. Gastrointestinal pharmacokinetics of OPT-80 in hamsters following a single oral dose in labrasol

**General Toxicology:**

1. OPT-80: an acute intravenous toxicity study in rats. Study # 015386.
2. OPT-80: an acute oral toxicity study in rats. Study # 015353.
3. OPT-80: A 28-day oral toxicity study in rats. Study # 015352.
4. OPT-80: a 28-day oral toxicity and toxicokinetics study in cynomolgus monkeys. Study # 2002-4923.
5. OPT-80: a 14 day intravenous toxicity study in Sprague Dawley rats. Study # 1003-2571.
6. OPT-80: a 14-day intravenous infusion toxicity study in Sprague-Dawley rats. Lab study # 1004-0901.
7. OPT-80: A 2 week intravenous toxicity study in rats. Study # 1176-003

**Genetic Toxicology:**

1. Bacterial reverse mutation assay. OPT-80. Study # AA64WW.503.BTL
2. In vitro mammalian chromosome aberration test. OPT-80. Study # AA64WW.331.BTL.
3. Mammalian erythrocyte micronucleus test. Study # AA64WW.125M.BTL.

**Reproductive Toxicology:**

1. PAR-101 (formerly OPT-80): A study of fertility and early embryonic development to implantation in rats. Study # 1069-013, supporting document # 63.
2. PAR 101 (formerly OPT-80): study for effects on embryo-fetal development in rats. Study # 1069-005. Supporting document #063
3. PAR 101 (formerly OPT-80): study for effects on embryo-fetal development in rats. Study # 1069-007. Supporting Document #063
4. PAR 101 (formerly OPT-80): A study for effects on embryo-fetal development in New Zealand White rabbits. Study # 1069-008, supporting document # 063.
5. PAR-101 (OPT-80): A range-finding study for effects on embryo-fetal development in New Zealand white rabbits. Study # 1069-016. Supporting document #092.
6. PAR 101 (formerly OPT-80): a study for effects on embryo-fetal development in New Zealand white rabbits. Study # 1069-018, supporting document #092.

**3.2 Studies Not Reviewed:** Assorted pharmacokinetic studies, as the toxicokinetics encompass most of this data.

## 4 Pharmacology

**4.1 Primary Pharmacology:** Please see the Clinical Microbiology Review for this information.

### 4.3 Safety Pharmacology

**1. Cardiovascular assessment of orally administered PAR-101 in conscious radiotelemetry-implanted naïve male and female beagle dogs. Study # [REDACTED] (b) (4)**

Key study findings: Doses up to 9600 mg/day did not affect cardiac parameters.

The study was conducted at [REDACTED] (b) (4) in 2008 according to US GLP. A modified Latin square design was used to investigate the cardiac effects of 0, 5, 16 or 48 tablets (200 mg fidaxomicin/tablet packaged into gelatin capsules, Lot # 184732, 95.9% pure) in 4/sex telemeterized Beagle dogs with a 1 week washout period between doses. Dogs were 9-20 months old and weighed from 5.6-10.5 kg (males) or 6.6-11.0 kg (females). Vehicle was the empty gelatin capsules. Observations included weekly body weights, hematology, serum chemistry and urinalysis prior to each week's dose, and heart rate/ECG/arterial pressure and body temperature for 30 seconds every 10 minutes from 2 hours prior to dosing through 24 hours post-dose. TK values were collected at 0 and 2 hours post-dose.

Results: All dogs survived to the end of the study. One HD male and female showed dilated pupils at 2 hours post-dose. There were no significant differences in heart rate, blood pressure, body temperature, PR interval, or ECG waveform.

Toxicokinetics showed no detectable levels of drug in the control dogs or at any dose prior to dosing. The 2 hour values are shown in the table below.

Plasma levels in dogs with oral capsule fidaxomicin at 2 hours (ng/mL)				
Dose	Males		Females	
	PAR-101	OP 1118	PAR-101	OP 1118
5 tablets (1000 mg)	301 ± 199	3.2 ± 3.2	51 ± 20.	BLQ
16 tablets (3200 mg)	1062 ± 547	53 ± 33	1168 ± 320	48 ± 17
48 tablets (9600 mg)	2942 ± 698	145 ± 97	6055 ± 1855	336 ± 104

**2. OPT-80: Respiratory assessment following intravenous administration to plethysmograph-restrained male Sprague Dawley rats. Study # <sup>(b) (4)</sup> 609008.**

Key study findings: Fidaxomicin had no effect on respiratory parameters at intravenous doses up to 7.5 mg/kg.

The study was conducted at <sup>(b) (4)</sup> in January, 2009 according to US GLP. OPT-80 (batch # 509789, 91.5% pure, doses adjusted for purity) was administered intravenously in 1% solutol HS-15 phosphate buffered saline at 0, 1, 4, and 7.5 mg/kg as a slow bolus (over 3 minutes) at a dose volume of 10 mL/kg to 8 male Sprague Dawley rats/dose with an additional 9 males/dose for toxicokinetics. Respiratory function data (respiratory frequency, tidal volume, minute volume) was collected for 1 hour pre-dose and 5 hours post-dose. Toxicokinetic samples were taken at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8, and 24 hours post-dose.

Results: The dosing solutions were within 10% of the targeted dose. All animals survived to the scheduled sacrifice and there were no dose related clinical observations. There were no remarkable differences in respiratory frequency, tidal volume, or minute volume.

**Table 2. Toxicokinetic Parameters for OPT-80 in Male Rats after an Intravenous Dose**

Dose (mg/kg)	AUC <sub>0-∞</sub> (hr·ng/mL)	AUC <sub>0-∞</sub> /Dose ((hr·ng/mL)/mg/kg)	AUC <sub>0-tlast</sub> (hr·ng/mL)	AUC <sub>0-tlast</sub> /Dose ((hr·ng/mL)/mg/kg)	CL (mL/min/kg)	t <sub>1/2</sub> (hr)	V <sub>ee</sub> (mL/kg)
1	230	230	222	222	72.4	0.0941	616
4	1630	407	1620	406	41.0	0.268	512
7.5	4080	544	4040	538	30.6	0.452	408

**Table 3. Toxicokinetic Parameters for OP-1118 in Male Rats after an Intravenous Dose of OPT-80**

Dose (mg/kg)	AUC <sub>0-∞</sub> (hr·ng/mL)	AUC <sub>0-∞</sub> /Dose ((hr·ng/mL)/mg/kg)	AUC <sub>0-tlast</sub> (hr·ng/mL)	AUC <sub>0-tlast</sub> /Dose ((hr·ng/mL)/mg/kg)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose ((ng/mL)/mg/kg)
1	72.0	72.0	63.9	63.9	335	335
4	595	149	581	145	2450	613
7.5	1400	186	1380	184	4350	580

**Table 3 (continued). Toxicokinetic Parameters for OP-1118 in Male Rats after an Intravenous Dose of OPT-80**

Dose (mg/kg)	t <sub>1/2</sub> (hr)	T <sub>max</sub> (hr)	OP1118 AUC <sub>0-tlast</sub> /OPT-80 AUC <sub>0-tlast</sub> (%)
1	0.141	0.0830	28.8
4	0.191	0.0830	35.8
7.5	0.374	0.0830	34.2

**3. An acute central nervous system pharmacological and toxicokinetics study of OPT-80 using a functional observational battery. Study # <sup>(b) (4)</sup> 609009.**

Key study findings: Intravenous fidaxomicin at doses up to 7.5 mg/kg had no effect on observations in the Irwin test.

The study was conducted at <sup>(b) (4)</sup> in January, 2009 according to US GLP. OPT-80 (batch # 509789, 91.5 % pure, ) in 1% solutol HS-15 with phosphate buffered saline was administered as a single i.v. dose at 0, 1, 4, or 7.5 mg/kg (dose volume 10 mL/kg) to Sprague Dawley rats (6 males/dose for main study, 3 males/dose for TK, weight range 215-282 g for main study) and a FOB was conducted at 0.25, 1, 2, and 8 hours post-dose. Plasma samples for comparison were taken at 15 minutes post-dose. Oral chlorpromazine hydrochloride at 20 mg/kg was used as a positive control.

Results: The dosing solutions were within 5% of the targeted dose and homogenous. Fidaxomicin had no effect on home cage observations, handling, open field behavior, startle stimulus, or mean body temperature while the positive control showed the expected depression of behavior. TK values are shown below.

**Table 8. Study Sample Concentrations**

Subject Group	Treatment Description	Subject	Gender	Day	Minute	Custom ID	OPT-80 Concentration (ng/mL)	OP-1118 Concentration (ng/mL)	Comments
1	0mg/kg	39227	Male	0	15	2008350002	<LLOQ (20.0)	<LLOQ (20.0)	
1	0mg/kg	39231	Male	0	15	2008350004	<LLOQ (20.0)	<LLOQ (20.0)	
1	0mg/kg	39245	Male	0	15	2008350008	<LLOQ (20.0)	<LLOQ (20.0)	
2	1mg/kg	39223	Male	0	15	2008350001	212	128	
2	1mg/kg	39229	Male	0	15	2008350003	183	126	
2	1mg/kg	39236	Male	0	15	2008350006	226	171	
3	4mg/kg	39233	Male	0	15	2008350005	975	669	Hemolyzed
3	4mg/kg	39253	Male	0	15	2008350010	1140	1090	
3	4mg/kg	39261	Male	0	15	2008350011	1140	946	
4	7.5mg/kg	39243	Male	0	15	2008350007	2470	2160	
4	7.5mg/kg	39250	Male	0	15	2008350009	2410	2030	
4	7.5mg/kg	39267	Male	0	15	2008350012	1770	2020	

## 6 General Toxicology

### 6.1 Single-Dose Toxicity: previously reviewed.

### 6.2 Repeat-Dose Toxicity

#### 1. A pilot 14-day repeated dose oral (capsule) toxicity study of fidaxomicin in dogs. Study # <sup>(b) (4)</sup>-609005.

This study was not reviewed thoroughly as it was not conducted according to GLP and another study with higher doses and longer duration by the same route was done according to GLP. Two Beagle dogs/sex/dose were administered 0, 2, 5, or 16 tablets/day in gelatin capsules for 14 consecutive days. A standard toxicology battery of observations (except histopathology, but including fecal and plasma levels of drug) was observed. All dogs survived to scheduled sacrifice. There were no noteworthy observations in any group.

#### 2. A 14-day repeated dose oral (capsule) toxicity study of fidaxomicin in dogs. Study # <sup>(b) (4)</sup>-609007.

This study was not reviewed thoroughly as it was not conducted according to GLP and another study with similar doses and longer duration by the same route was done according to GLP. Two Beagle dogs/sex/dose were administered 0, 32, or 48 tablets/day in gelatin capsules for 14 consecutive days. A standard toxicology battery of observations (except histopathology, but including fecal and plasma levels of drug) was observed. All dogs survived to scheduled sacrifice. There were no noteworthy observations in any group.

**3. A 14-day repeated dose oral (gavage) toxicity study of fidaxomicin in dogs.**  
**Study # <sup>(b) (4)</sup> -609002.**

This study was conducted according to GLP. Three dogs/sex/dose were treated daily by oral gavage with 0, 30, 60 or 120 mg/kg fidaxomicin in LT-2 (9.16% labrafac WL1349, 24.27% Labrasol, 13.65% Labrafil M1944CS, 32.92% Tween 80, 10.0% Plurol Oleique CC497 and 10.0% purified water). The only observation was sporadic vomiting and changes in stools (soft/mucoid/diarrhea, white material), which did not consistently affect dosing. There were no other noteworthy changes with treatment.

**4: A 3- month repeated dose oral (capsule) toxicity study of PAR-101 in Beagle dogs with a 28 day recovery period.**

Study no.: <sup>(b) (4)</sup> 609006

Study report location: Electronic NDA

Conducting laboratory and location:

<sup>(b) (4)</sup>

Date of study initiation: March, 2008

GLP compliance: Yes, USA

QA statement: Yes

Drug, lot #, and % purity: PAR-101, lot # R0242001, 98.3% pure

**Key Study Findings:** The NOAEL was the highest dose tested, 48 tablets of 200 mg each or 9.6 g/dog/day (estimate based on weight of dogs of around 1 g/kg/day).

#### Methods

Doses: 0, 5, 16 or 48 tablets/day (0, 1.0, 3.2, 9.6 g/animal/day)

Frequency of dosing: Once daily

Route of administration: Oral

Formulation/Vehicle: Tablets (200 mg each) were placed in size 12 gelatin capsules

Species/Strain: Beagle dogs

Number/Sex/Group: 4/sex/dose

Age: 8.5-11 months

Weight: M: 8.2-11.5 kg; F: 5.9-9.4 kg

Satellite groups: 3/sex at control HD for recovery

Deviation from study protocol: None significant.

#### Observations and Results

**Mortality (twice daily):** All dogs survived to scheduled sacrifice.

**Clinical Signs (4X/day during dosing, once daily during recovery; detailed exam weekly):** Clinical signs were primarily excretion (defecation decreased, diarrhea,

soft/pale/yellow feces) and emesis (some contained tablets). Incidences of emesis were not frequent enough so that drug delivery was impaired. Signs decreased or were absent during the recovery period.

**Body Weights (weekly):** There were no remarkable differences in body weights between dosing groups during treatment or recovery.

**Feed Consumption (daily):** There were no remarkable differences in food consumption between dosing groups.

**Ophthalmoscopy (pretest, week 12):** There were no ocular lesions.

**ECG (pretest at week -2 and -1, week 4, 12, 17 at 1 hour post-dose for at least 30 seconds):** There were no remarkable differences in heart rate or Q-T intervals with treatment.

**Hematology (pretest, week 4, week 12):** There were no remarkable differences with dose.

**Clinical Chemistry (pretest, week 4, week 12):** There were no noteworthy changes with dosing.

**Urinalysis (pretest, week 4, week 12):** There were no remarkable differences between dosing groups.

**Gross Pathology:** At the end of the treatment period, one HD male had reddened cecal mucosa while another male had a dark red area of the stomach. At the end of the recovery period, 1 HD male had a dark red area of the colon, while another had a dark red area of the rectum. There were no remarkable observations in the females. There were no microscopic correlates.

**Organ Weights:** There were no remarkable dose dependent changes.

## **Histopathology**

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: Most findings were minimal in severity, affecting single animals/dose and not increased with increasing dose, thus were not of toxicologic significance.

**Toxicokinetics (study day 0, 86 at 0, 0.08, 0.25, 0.5, 1, 2, 4, 6, 24 hours post-dose):**

The toxicokinetics parameters are shown in the tables below. As shown by the wide error values, interanimal variability was significant and may be partly due to emesis of the capsules. There were no remarkable differences with gender. Cmax was closer to linear than AUC with dose. Values for Tiacumycin C and F were analyzed in 2009. The analysis was highly variable, but values are shown below.

Fecal samples were also analyzed at Day 3 and 86. At Day 3, the majority of the control females showed some OPT-80 in their feces, suggesting either cross contamination or assay interference. The lab report from the analysis group suggested that the contamination came from handling prior to their obtaining the samples. There was some overlap in fecal OPT-80 values across doses. Data was presented without means or standard deviations and did not include the re-analysis values for out of range samples.

Text Table 1. Mean Toxicokinetic Results for PAR-101 and OP-1118 in Male Dogs\*

PAR-101					
Dosage (tablets/day)	AUC <sub>last</sub> (ng·h/mL)	Metabolite/ Parent Ratio**	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	T <sub>1/2</sub> (h)
PAR-101					
<u>Day 0</u>					
5	122 (40.6-236)	NA	126 (40.6-281)	2.0 (2-2)	NC
16	2935 (1448-4549)	NA	1725 (1250-2010)	3.0 (2-6)	0.6 <sup>†1</sup>
48	23262 (6992-38884)	NA	4263 (2870-6900)	3.7 (2-6)	3.6 <sup>†3</sup>
<u>Day 86</u>					
5	136 (5.0-343)	NA	170 (10-297)	1.4 (0.5-2)	NC
16	2631 (1627-3553)	NA	1827 (779-2480)	1.6 (0.5-2)	0.7
48 <sup>†6</sup>	8161 (3941-15320)	NA	3012 (1820-4120)	3.0 (2-4)	0.7 <sup>†1</sup>
OP-1118					
<u>Day 0</u>					
5	1.79 (0.00-7.15)	0.01 (0.00-0.03)	3.58 (0.0-14.3)	2.0 <sup>†1</sup>	NC
16	234 (48.6-509)	0.07 (0.02-0.11)	156 (76.1-241)	3.0 (2-6)	NC
48	1887 (633-4145)	0.09 (0.04-0.17)	407 (216-624)	4.3 (2-6)	NC
<u>Day 86</u>					
5	3.12 (0.00-8.40)	0.01 (0.00-0.03)	7.13 (0.00-16.8)	1.5 (1-2) <sup>†2</sup>	NC
16	161 (79.4-285)	0.06 (0.02-0.10)	115 (47.3-216)	1.8 (1-2)	NC
48 <sup>†6</sup>	945 (290-1935)	0.12 (0.06-0.20)	361 (179-686)	3.0 (2-4)	0.9 <sup>†1</sup>

C<sub>max</sub> = The maximum measured concentration of the analyte in plasma.

T<sub>max</sub> = The sampling time at which C<sub>max</sub> was reached.

T<sub>1/2</sub> = The half-life for the analyte in plasma.

AUC<sub>last</sub> = The area under the plasma analyte concentration vs. time curve from the time of dosing to the time of the last concentration >LLOQ.

N=4 at 5 and 16 tablets/day, N=7 at 48 tablets/day, except where reported as '†(n)'.

NA = Not applicable; NC = Not calculable.

\* Due to high inter-animal variability, mean and range are reported for AUC<sub>last</sub>, metabolite/parent ratio, C<sub>max</sub> and T<sub>max</sub>.

\*\* Ratio of OP-1118 AUC<sub>last</sub> /PAR-101 AUC<sub>last</sub>.

Text Table 2. Mean Toxicokinetic Results for PAR-101 and OP-1118 in Female Dogs\*

PAR-101 Dosage (tablets/day)	AUC <sub>last</sub> (ng·h/mL)	Metabolite/ Parent Ratio**			
		C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	T <sub>1/2</sub> (h)	
PAR-101					
<u>Day 0</u>					
5	203 (97.3-454)	NA	114 (77.1-145)	2.0 (1-4)	NC
16	3733 (1527-8809)	NA	1930 (910-4700)	2.0 (2-2)	0.6 <sup>†2</sup>
48	16960 (4789-46210)	NA	4116 (1360-7610)	3.3 (1-4)	2.7 <sup>†3</sup>
<u>Day 86</u>					
5	1242 (131-3469)	NA	1305 (185-3390)	1.0 (1-1)	1.4 <sup>†1</sup>
16	8161 (2201-13898)	NA	4070 (960-7110)	2.8 (1-6)	0.6 <sup>†1</sup>
48	14307 (5929-29749)	NA	4669 (1670-8120)	2.6 (2-4)	0.7 <sup>†3</sup>
OP-1118					
<u>Day 0</u>					
5	0.00	0.00	0.00	NA	NA
16	271 (35.7-773)	0.06 (0.02-0.09)	171 (51.6-483)	2.5 (2-4)	NC
48	1443 (335-3409)	0.09 (0.06-0.13)	475 (136-1050)	4.3 (2-6)	NC
<u>Day 86</u>					
5	85.3 (0.00-292)	0.04 (0.00-0.08)	44.1 (0.00-122)	1.7 (1-2) <sup>†3</sup>	NC
16	438 (106-1070)	0.05 (0.03-0.08)	235 (45.0-558)	2.0 (2-2)	NC
48	1590 (734-2305)	0.13 (0.05-0.18)	566 (357-861)	3.7 (2-6)	NC

C<sub>max</sub> = The maximum measured concentration of the analyte in plasma.

T<sub>max</sub> = The sampling time at which C<sub>max</sub> was reached.

T<sub>1/2</sub> = The half-life for the analyte in plasma.

AUC<sub>last</sub> = The area under the plasma analyte concentration vs. time curve from the time of dosing to the time of the last concentration >LLOQ.

N=4 at 5 and 16 tablets/day, N=7 at 48 tablets/day, except where reported as '†(n)'.

NA = Not applicable; NC = Not calculable.

\* Due to high inter-animal variability, mean and range are reported for AUC<sub>last</sub>, metabolite/parent ratio, C<sub>max</sub> and T<sub>max</sub>.

\*\* Ratio of OP-1118 AUC<sub>last</sub> /PAR-101 AUC<sub>last</sub>.

**Table 2. Mean ( $\pm$ SD) Toxicokinetic Parameters for Tiacumycin C and Tiacumycin F in Male and Female Dogs Given 86 Daily Oral Doses (9600 mg/dog) of OPT-80**

Analyte	Sex	AUC <sub>0-tlast</sub> (hr·ng/mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)
Tiacumycin C	Male	76.8 $\pm$ 45.7	27.1 $\pm$ 20.5	2.57 $\pm$ 1.90
	Female	271 $\pm$ 260	84.9 $\pm$ 74.0	3.86 $\pm$ 1.46
	Combined	174 $\pm$ 26	56.0 $\pm$ 60.2	3.21 $\pm$ 1.76
Tiacumycin F	Male	384 $\pm$ 297	107 $\pm$ 62.3	2.43 $\pm$ 1.99
	Female	764 $\pm$ 615	202 $\pm$ 156	3.29 $\pm$ 1.25
	Combined	574 $\pm$ 504	154 $\pm$ 124	2.86 $\pm$ 1.66

**5: A 3 month repeated dose oral (gavage) toxicity study of PAR-101 in beagle dogs with a 28 day recovery period.**

Study no.: (b) (4)-609003

Study report location: Electronic submission w/ NDA

Conducting laboratory and location:

Date of study initiation: June, 2007

GLP compliance: Yes, USA

QA statement: Yes

Drug, lot #, and % purity: PAR-101, batch # 509789, 93.0% pure

(b) (4)

### Key Study Findings

The vehicle was chosen as it gave the highest bioavailability at approximately 80% of maximum solubility. Previous single dose data also showed emesis with a 3.0 ml/kg dose within 1 hour.

That said, the vehicle appeared to be toxic in that there were anaphylactoid responses in all dosing groups even with premedication. The study was stopped early and another oral method of dosing was chosen. There were no other significant toxicities that could be attributed to drug rather than vehicle.

### Methods

Doses: 0, 10, 30 , 120 mg/kg/day

Frequency of dosing: Once daily for 90 consecutive days

Route of administration: Oral gavage

Dose volume: 1 mL/kg

Formulation/Vehicle: LT-2 which consists of 9.16% Labrafac WL1349; 24.27% Labrasol, 13.65% Labrafil M1944CS; 32.92% Tween 80, 10.0% Plurol Oleique CC497 and 10.0% water

Species/Strain: Beagle dogs

Number/Sex/Group: 4/sex/dose

Age: 11 months

Weight: M: 7.9-12.2 kg; F: 6.5-9.4 kg

Satellite groups: 3/sex in control and HD groups

- Unique study design:** Dogs showing allergic reactions at dosing were also administered 1.1 mg/kg diphenhydramine
- Deviation from study protocol:** Due to severe reactions in the dogs, dosing ceased after 14 or 15 consecutive days. Animals were allowed to recover for 5-6 days, then were sacrificed (3/sex/group). Remaining animals were returned to stock colony. No other deviations were noted that would change the outcome of the study.

## Observations and Results

**Mortality (twice daily):** The data on the dogs either found dead or euthanized in extremis are shown in the following table from the sponsor.

**Text Table 3: Selected Macroscopic Findings for Animals Found Dead or Euthanized in Extremis**

Animal Number	Dosage Level (mg/kg/day)	Sex	Date of Disposition	Disposition	Findings
3600	120	Male	9 June 2007 (study day 2)	Euthanized in extremis (animal was replaced)	<ul style="list-style-type: none"> <li>• Cecum: reddened mucosa</li> <li>• Colon: reddened mucosa</li> <li>• Duodenum: dark red contents and reddened mucosa</li> <li>• Ileum: reddened mucosa</li> <li>• Jejunum: reddened mucosa</li> <li>• Lungs: dark red discoloration</li> <li>• Rectum: reddened mucosa</li> <li>• Skin: edematous</li> <li>• Stomach: dark red contents</li> </ul>
3621	10	Female	10 June 2007 (study day 2)	Found dead (animal was replaced)	<ul style="list-style-type: none"> <li>• Cecum: reddened mucosa</li> <li>• Ileum: reddened mucosa</li> <li>• Rectum: reddened mucosa</li> <li>• Spleen: white areas</li> </ul>
3617	120	Female	11 June 2007 (study day 3)	Euthanized in extremis (animal was replaced)	<ul style="list-style-type: none"> <li>• Cecum: reddened mucosa</li> <li>• Colon: reddened mucosa</li> <li>• Jejunum: reddened mucosa</li> <li>• Mesenteric Lymph Node: dark red discoloration</li> <li>• Lungs: dark red discoloration</li> <li>• Rectum: reddened mucosa</li> </ul>
3589	10	Male	11 June 2007 (study day 4)	Found dead	<ul style="list-style-type: none"> <li>• Cecum: reddened mucosa</li> <li>• Lungs: dark red discoloration</li> <li>• Mediastinal Lymph Node: dark red discoloration</li> <li>• Bronchial Lymph Node: dark red discoloration</li> <li>• Rectum: reddened mucosa</li> <li>• Skin: red matting</li> </ul>
3624	control	Female	17 June 2007 (study day 9)	Euthanized in extremis	<ul style="list-style-type: none"> <li>• Colon: foreign material</li> <li>• Lungs: dark red discoloration and not fully collapsed</li> </ul>
3620	10	Female	21 June 2007 (study day 13)	Euthanized in extremis	<ul style="list-style-type: none"> <li>• Cecum: dark red areas</li> <li>• Lungs: dark red discoloration, firm and not fully collapsed</li> <li>• Skin: clear matting</li> <li>• Trachea: foamy contents</li> </ul>

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**Clinical Signs (4X daily during dosing, detailed exams weekly):** The primary clinical signs, which increased in frequency/severity with dose, were soft/mucoid feces or diarrhea. Emesis was also noted sporadically at most doses. In the females that were euthanized, labored/shallow breathing, reddened ears and swollen face and ears were also observed. Histamine levels were not examined.

**Body Weights (weekly):** There were no remarkable differences in weight between groups.

**Feed Consumption (daily):** There were no dose dependent changes in food consumption with treatment.

**Ophthalmoscopy:** There were no findings in any group.

**ECG (week -2, -1):** The drug effects were not measured due to early termination of the study.

**Hematology (week -1, week 2):** In the males, the reticulocyte number increased dose dependently with increases of 16 and 42% in the MD and HD respectively. No dose dependent changes (especially increases) were noted in the females, suggesting that the male results may have been an artifact.

**Clinical Chemistry (week -1, week 2):** There were no remarkable dose dependent changes with treatment.

**Urinalysis (week -1, week 2):** There were no noteworthy findings with treatment.

**Gross Pathology:** The changes in the early death animals are summarized in the sponsor's table above and consisted mainly of reddening of the lower gi tract mucosa and dark red discoloration of the lymph nodes. The latter is usually stress related. In the animals that survived to rescheduled necropsy, there were no noteworthy observations.

**Organ Weights:** In the HD males, the kidney weights (absolute and relative) were increased by roughly 25%. Liver weights were increased by approximately 15%. Spleen weights were also decreased by approximately 25% in the MD and HD males. In the females, there were no major differences in organ weights with dose.

**Histopathology:** Samples were collected but not analyzed.

**Toxicokinetics (Day 0 at 0.08, 0.25, 0.5, 1, 2, 4, 6, and 24 hours post-dose, LLOQ = 9.90 ng/mL; fecal samples):** The data was highly variable, but there did not appear to be any gender specific differences. The values are shown in the table below. No OPT-80 was found in the control samples. Data from fecal samples was not found.

**Table 2. Toxicokinetic Parameters for OPT-80 and OP-1118 in Male and Female Dogs after Oral Administration of OPT-80 (N = 4-7/sex/group)**

Compound	Dose (mg/kg)	Sex	AUC <sub>0-<i>last</i></sub> (hr·ng/mL)	AUC <sub>0-<i>last</i></sub> /Dose ((hr·ng/mL)/mg/kg)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose ((ng/mL)/mg/kg)	T <sub>max</sub> (hr)
OPT-80	10	M	864±760	86.4±76.0	602±510	60.2±51.0	1.31±0.851
		F	210±189	21.0±18.9	195±63.8	19.5±6.38	1.06±0.718
		Combined	537±621	53.7±62.1	398±400	39.8±40.0	1.19±0.741
	30	M	4400±7160	147±239	3280±2970	109±99.1	0.875±0.829
		F	13900±17000	463±567	6690±7130	223±238	0.813±0.375
		Combined	9150±13100	305±437	4990±5380	166±179	0.844±0.597
	120	M	5890±6140	49.1±51.1	7360±5540	61.4±46.2	0.500±0.661
		F	8340±7480	69.5±62.3	5690±3360	47.5±28.0	1.11±0.840
		Combined	7110±6690	59.3±55.8	6530±4490	54.4±37.4	0.804±0.792
OP-1118	10	M	36.3±50.6	3.63±5.06	76.9±86.4	7.69±8.64	1.50±0.577
		F	9.98±12.4	0.998±1.24	15.2±11.7	1.52±1.17	1.67±0.577
		Combined	23.1±36.9	2.31±3.69	46.0±65.9	4.60±6.59	1.57±0.535
	30	M	660±1180	22.0±39.4	292±352	9.73±11.7	1.00±0.866
		F	1240±1540	41.3±51.4	556±588	18.5±19.6	1.63±0.750
		Combined	949±1310	31.6±43.6	424±470	14.1±15.7	1.36±0.802
	120	M	591±572	4.93±4.77	404±414	3.37±3.45	1.25±1.35
		F	768±598	6.40±4.98	469±347	3.91±2.90	1.36±0.627
	Combined		680±570	5.66±4.75	436±369	3.64±3.07	1.30±1.01

**Stability and Homogeneity:** Samples were within 10% of the targeted doses.

## 6: OPT-80: A 2-week intravenous toxicity study in rats.

Study no.: **Study # 1176-003.**

Study report location: Supporting document #060, submitted  
11/17/06

Conducting laboratory and location:

(b) (4)

Date of study initiation: June, 2005

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: OPT-80, lot # 94180005, 94.4% pure

**Key Study Findings:** This study was actually the dose-ranging for the reproductive toxicity studies. The actual dose was less than 1 mg/kg/day as drug was retained by the (b) (4). There were no toxicologic findings.

**Methods**

Doses: 0, 4 mg/kg/day  
Frequency of dosing: Once daily  
Route of administration: Intravenous over 3-5 minutes  
Dose volume: 20 mL/kg  
Formulation/Vehicle: 0.2% Tween 80/5% dextrose  
Species/Strain: Sprague Dawley rats  
Number/Sex/Group: 3/sex/dose  
Age: 6 weeks old at acquisition  
Weight: M: 180-196 g, F: 164-178 g  
Unique study design: Dose was chosen based on maximal volume and maximal solubility  
Deviation from study protocol: There were no deviations that affected the conclusions.

## Observations and Results

**Mortality (twice daily):** One male control rat died on Day 12. There were no remarkable clinical signs or gross abnormalities.

**Clinical Signs (twice daily):** There were no remarkable clinical signs in either group.

**Body Weights (weekly):** Body weights did not differ significantly between the control and treated groups.

**Feed Consumption (weekly):** There were no noteworthy differences between treated and control food consumption.

**Hematology (Day 1, 2, 14, 15):** While the males had slightly elevated WBC values at day 15, there were no remarkable differences in the hematology values in females with treatment.

**Clinical Chemistry (Day 1, 2, 14, 15):** Data was not collected.

**Urinalysis:** Differences between treated and controls were negligible.

**Gross Pathology:** There were no noteworthy observations in either the control or treated rats.

**Organ Weights:** There were no remarkable differences in organ weights with treatment.

**Histopathology:** Samples were collected, but not examined.

**Toxicokinetics (24 hours after last dose on Day 14):** All samples were below the level of quantitation.

**Stability and Homogeneity:** From the day 1 solution, only 11.2% of the nominal dose was recovered at homogeneity analysis. However, the solution was homogeneous. At Day 14, 26.5% of the nominal solution was recovered and it was homogenous. It

appeared with further testing that filtering was responsible for the loss of drug. Thus, the administered dose was roughly 0.4 to 1 mg/kg/day.

## 7 Genetic Toxicology

### 7.1 In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

#### 1: Bacterial reverse mutation assay of OP-1118.

Study no.: AC29me.503btl  
Study report location: EDR, NDA submission dated Sept. 2010  
Conducting laboratory and location: (b) (4)  
Date of study initiation: September, 2009  
GLP compliance: Yes, USA  
QA statement: Yes  
Drug, lot #, and % purity: OP-1118, lot # Sy121 52A, 96.86% pure by HPLC

**Key Study Findings:** The study was valid and negative for mutagenicity.

#### Methods

Strains: S. typhimurium TA 98, TA100, TA1535, TA 1537; E. coli WP2 uvrA.  
Concentrations in definitive study: 50, 150, 500, 1500, 5000 ug/plate  
Basis of concentration selection: Toxicity, precipitation  
Negative control: DMSO  
Positive control: 2-aminoanthracene, 2-nitrofluorene, sodium azide, 9-aminoacridine, methylmethanesulfonate  
Formulation/Vehicle: DMSO

**Study Validity:** The study was valid in that sufficiently high doses (without background lawn toxicity or precipitation) were used, negative controls were within historical values and the positive controls significantly increased the number of revertants.

**Results:** There were no significant increases in the number of revertants with OP1118.

**2: Bacterial mutation with aged fidaxomicin**

Study no.: AC29MW.503.BTL  
Study report location: EDR, NDA submission Sept. 2010  
Conducting laboratory and location: [REDACTED] (b) (4)  
Date of study initiation: July, 2010  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: OPT-80, lot # 509789, 86.6% pure (lot was 3.5 years aged)

**Key Study Findings:** Aged fidaxomicin was not mutagenic in a valid assay.

**Methods**

Strains: *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA  
Concentrations in definitive study: 0, 15, 50, 150, 500, 1500, 5000 ug/plate  
Basis of concentration selection: Bacterial lawn toxicity, precipitate  
Negative control: DMSO  
Positive control: 2-aminoanthracene, 2-nitrofluorene, sodium azide, 9-aminoacridine, methylmethanesulfonate  
Formulation/Vehicle: DMSO, water

**Study Validity:** Based on lack of bacterial lawn toxicity, precipitant, and increased # of revertants with the positive controls, the study was valid. In the initial test with strain TA1535, the positive control was not sufficiently increased, thus the study was repeated and obtained acceptable levels of revertants.

**Results:** There were no significant increases in the number of revertants in the presence or absence of S9 fraction with aged fidaxomicin.

## 7.2 *In Vitro Chromosomal Aberration Assays in Mammalian Cells*

### 3: In vitro mammalian chromosome aberration test for OP-1118

Study no.: AC29ME.331.BTL  
Study report location: EDR, NDA submission dated Sept. 2010  
Conducting laboratory and location: [REDACTED] (b) (4)  
Date of study initiation: July, 2009  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: OP-1118, lot # SY121\_52A, 96.86% pure

**Key Study Findings:** The study was positive for clastogenicity.

#### Methods

Cell line: CHO (Chinese hamster ovary cells)  
Concentrations in definitive study: Non-S9 at 500-4000 ug/mL; 100-1200 ug/mL for S9 4 hr and 20 hr groups  
Basis of concentration selection: Cytotoxicity, precipitate  
Negative control: DMSO  
Positive control: Mitomycin C, cyclophosphamide  
Formulation/Vehicle: DMSO  
Incubation & sampling time: 4, 20 hours

**Study Validity:** The study was valid as precipitate in growth media was noted at concentrations of 1500 ug/mL or greater and growth inhibition was noted at the 5000 ug/mL concentration without metabolic activity, 1200 ug/mL in the presence of S9. Cells with aberrations in the control groups were within the historical control range.

**Results:** With a 4 hour treatment in the presence of S9 fraction, the 900 ug/mL group showed a slight increase in the percentage of cells with numerical and structural aberrations. Similar results were seen with a 20 hour treatment without metabolic activation. However, only the 20 hour treatment was considered statistically significant. The sponsor deemed the study to be negative.

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TABLE 7  
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH OP-1118  
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

## 4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment ( $\mu\text{g/mL}$ )	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell	
			Numerical	Structural	Numerical	Structural	Gaps	Br	Ex	Br	Dic	Ring			
DMSO	A	13.8	100	100	0	0	0	0	0	0	0	0	0	0	0.000
	B	14.2	100	100	1	0	0	0	0	0	0	0	0	0	0.000
OP-1118 250	A	13.2	100	100	0	0	0	0	0	0	0	0	0	0	0.000
	B	12.0	100	100	0	0	0	0	0	0	0	0	0	0	0.000
700	A	13.2	100	100	2	0	0	0	0	0	0	0	0	0	0.000
	B	11.6	100	100	0	0	0	0	0	0	0	0	0	0	0.000
900	A	11.4	100	100	3	1	0	1	0	0	0	0	0	0	0.010
	B	10.6	100	100	3	2	0	6	0	0	0	0	0	0	0.060
CP, 10	A	3.0	100	50	1	22	0	11	4	0	0	0	0	0	0.300
	B	3.2	100	50	1	22	0	10	3	0	0	0	0	0	0.260

**Treatment:** CHO cells were treated for 4 hours at  $37 \pm 1^\circ\text{C}$  in the presence of an exogenous source of metabolic activation. Additional dose levels of 100, 500, 600 and 800  $\mu\text{g/mL}$  were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination. Dose levels 1000 and 1200  $\mu\text{g/mL}$  were not analyzed due to excessive toxicity.

**Mitotic index** = number mitotic figures  $\times 100/500$  cells counted.

**%Aberrant Cells:** numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

**Chromatid breaks (Br)** include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

**Chromosome breaks (Br)** include breaks andacentric fragments; Dic, dicentric chromosome.

**Severely damaged cells** includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

**Average aberrations per cell:** severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 9  
CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH OP-1118  
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

## 20-HOUR CONTINUOUS TREATMENT

Treatment ( $\mu\text{g/mL}$ )	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell	
			Numerical	Structural	Numerical	Structural	Gaps	Br	Ex	Br	Dic	Ring			
DMSO	A	15.8	100	100	0	2	0	1	0	0	1	0	0	0	0.020
	B	14.0	100	100	0	1	0	1	0	0	0	0	0	0	0.010
OP-1118 250	A	10.8	100	100	0	2	0	2	0	0	0	0	0	0	0.020
	B	12.6	100	100	0	3	0	2	0	0	1	0	0	0	0.030
700	A	8.8	100	100	0	5	0	10	0	0	0	0	0	0	0.100
	B	9.2	100	100	4	1	0	1	0	0	0	0	0	0	0.010
900	A	6.8	100	100	4	3	0	4	0	0	0	0	0	0	0.040
	B	7.0	100	100	1	4	0	8	1	0	0	0	0	0	0.090
MMC, 0.1	A	6.0	100	50	0	22	0	6	5	0	0	0	0	0	0.220
	B	4.8	100	50	0	22	0	13	4	0	0	0	0	0	0.340

**Treatment:** CHO cells were treated for 20 hours at  $37 \pm 1^\circ\text{C}$  in the absence of an exogenous source of metabolic activation. Additional dose levels of 100, 500, 600 and 800  $\mu\text{g/mL}$  were tested as a safeguard against excessive toxicity at higher dose levels but were not required for microscopic examination. Dose level 1000  $\mu\text{g/mL}$  was not required for analysis. Dose level 1200  $\mu\text{g/mL}$  was not analyzed due to excessive toxicity.

**Mitotic index** = number mitotic figures  $\times 100/500$  cells counted.

**%Aberrant Cells:** numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.

**Chromatid breaks (Br)** include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.

**Chromosome breaks (Br)** include breaks andacentric fragments; Dic, dicentric chromosome.

**Severely damaged cells** includes cells with one or more pulverized chromosome and cells with 10 or more aberrations.

**Average aberrations per cell:** severely damaged cells and pulverizations were counted as 10 aberrations.

#### 7.4 Other Genetic Toxicity Studies

##### **Derek for Windows Consultancy report. OPT-80. Study No. Derek Analysis 2010.**

An in silico analysis of 14 impurities of OPT-80 was conducted. All but one compound had a polyhalogenated aromatic group which gave a non-genotoxic carcinogenicity alert, while all compounds had an alpha,beta-unsaturated ester or thioester (class II or II) which gave a chromosome damage alert. The carcinogenicity risk was deemed plausible in mice and rats based on the similarity to para-dichlorobenzene. However, with further analysis, the test company deemed the impurities to have a low probability of being human carcinogens due to the positioning of the halogens on the benzene ring. The chromosomal damage was considered plausible to rodents.

**8 Carcinogenicity:** No carcinogenicity studies were needed in support of this NDA as the duration of use of the drug is less than 2 weeks

**9 Reproductive and Developmental Toxicology:** These studies were reviewed previously and will be discussed in the final summary.

#### 11 Integrated Summary and Safety Evaluation

The original IND for fidaxomicin was submitted in August of 2003. Fidaxomicin is a macrolide antibiotic with activity against *Clostridium difficile* through inhibition of RNA polymerase. Fidaxomicin has low systemic bioavailability, so use in treating *C. difficile* associated diarrhea is logical. Further discussion of the mechanism of action and spectrum of activity of fidaxomicin can be found in the clinical microbiology review.

Due to the low solubility of fidaxomicin, a series of issues regarding formulation, metabolism and route of administration have arisen. It was initially thought that there might be significant differences across species in bioavailability, especially since the early plasma assays were relatively insensitive. An additional concern was the potential for increased intestinal permeability in humans with severe *C. difficile* overgrowth. To address the full potential spectrum of toxicities for fidaxomicin, additional studies by alternate routes (e.g. intravenous) were requested. Unfortunately, toxicity issues with the various formulations for solubilization made interpretation of these studies difficult. Ultimately, a tablet was used for the clinical formulation, and an adequate study was conducted in the dog using tablets enclosed in gelatin capsules for the course of 3 months. This is considered the definitive study on the toxicity of fidaxomicin, and renders the other issues with formulation moot.

Safety pharmacology studies were conducted with intravenous fidaxomicin in the rat and oral tablets in gelatin capsules in the dog. Both the hERG assay and telemeterized

dogs showed no effects for cardiac changes. Respiratory, and CNS assays in the rat by the intravenous route (1% Solutol HS-15 formulation) were also negative. No cardiac, respiratory, or central nervous system effects of fidaxomicin were noted in the toxicologic studies; however, vehicle effects on the CNS were observed in control animals.

In the initial submission, the assay for drug in plasma had a lower limit of quantitation in the ug/mL range. In one rat study submitted in the original IND, there was also <10% recovery of drug from fecal samples within the first 48 hours post-dose, suggesting either metabolism, absorption and sequestration, or an inaccurate assay. A mass balance study with radioactive drug was recommended. The mass balance study in the dog showed that >85% of the dose was found in the feces (range 80-98%) after oral gavage administration of 6.5 mg/kg. Plasma levels at this dose were only detectable in 2/6 dogs at single timepoints. Other tissue levels were not measured until day 7 and no drug was found.

When metabolism by liver and intestinal microsomes was investigated across species, up to 5 metabolites were seen with liver microsomes, while only 3 were present after incubation with intestinal microsomes. The metabolite profile, when liver and intestinal profiles were considered, did not differ significantly between Sprague Dawley rat, Beagle dog, Cynomolgus monkey and human; however, the half-life with liver microsomes in dog was much longer than that in the rat or human. Microsomal activity was not inhibited by fidaxomicin, and other inhibitors had no effect on the metabolism of fidaxomicin. Parent drug and the main metabolites, OP-1118, were measured in all the toxicology studies.

The toxicology studies and formulations are summarized in the following table. The initial oral studies were conducted in rats and monkeys by oral gavage with a labrasol formulation. The plasma concentrations were analyzed with an insensitive assay (lower limit of quantitation around 2 ug/mL). However, little drug was noted in the feces of rats, suggesting that metabolism or absorption in the rat might differ from other species. No toxicity was noted in the oral gavage studies in rats or monkeys at doses up to 90 mg/kg. While solubility issues prevented higher doses from being tested with a single daily dose, multiple dose/day or intravenous routes were recommended to elucidate toxicity with a "leaky gut" or to cover inter-species differences. In rats and rabbits using a 1% solutol/PBS formulation, intravenous administration at maximum feasible doses resulted in minimal toxicity and no obvious target organs of toxicity. In the dog, oral gavage administration was not feasible due to anaphylactic reactions which were more evident in the 3 month study than in the dose ranging study.

In the rabbit intravenous study, more than half of the control dams lost their litters, while in a second study, no toxicities were identified at the same dose. It should be noted that in the second study, solutions were made fresh just prior to injection. When the final formulation (capsules) was tested in the dog by the oral route over 3 months, no toxicity was noted. Sporadic (with respect to time and dose) vomiting was noted in this study, but was not severe or frequent enough to confound the data interpretation.

Toxicology Studies Conducted with Fidaxomicin				
Species	Duration	Route	Doses (mg/kg)	Issues
Rat	1X	IV <sub>a</sub>	0, 20, 62.5, 200	Deaths at 200 only
	1X	PO <sub>b</sub>	0, 167, 500, 1000	No toxicity. NOAEL = 1000 mg/kg
	DX5 (14)	IV <sub>a</sub>	0, 4, 20, 75	Initially designed as DX14 study stopped at D6; deaths on study 8/36 C, 3/20 L, 4/20 M, 12/35 H; deaths were associated with inactivity, labored breathing, hunchback, ptosis.
	DX14	IV <sub>c</sub>	0, 19.2 mg/kg/day (civ)	3/10 C, 3/10 H died D8-10; deaths attributed to inappropriate catheterization; No other significant differences between treated, control.
	DX14	IV <sub>c</sub>	0, 4	No significant toxicity. NOAEL = 4 mg/kg
	DX28	PO <sub>b</sub>	0, 10, 30, 90	2/10 control F died on D4; body weight gain decreased in HD males. 1 HD male with enlarged cecum. NOAEL = 30 mg/kg in males, 90 mg/kg in females
	Seg 1 repro	IV <sub>d</sub>	0, 1, 4, 7.5	No tox, no change in fertility parameters; NOAEL = 7.5. Actual concentrations closer to 6 mg/kg at HD.
	Seg 2 repro	IV <sub>d</sub>	4, 8, 15	1/27 dead at 4 mg/kg; no differences in uterine parameters, fetal body weights, gender ratios, or malformations/variations between treated and control. NOAEL maternal and fetal = 15 mg/kg
Rabbit	Seg 2 repro #1	IV <sub>d</sub>	2, 4, 7.5	1/23 death @ M; plasma levels @ H = 9-14 ug/mL; OPT-1118 = 38-45 ug/mL. 13/20 C, 2/22 L, 2/23 M 0/22 H dams had all feti resorbed. There were no significant incidences of malformations/variation in treated animals
	Seg 2 reprod #2	IV <sub>d</sub>	2, 4, 7.5	1/23 MD animal sacrificed due to inability to dose via ear vein. Plasma levels of parent 7-11 ug/mL. No remarkable differences between treated, control offspring. NOAEL maternal and fetal = actual dose of 7.0 mg/kg
Dog	1X cardio	IV <sub>d</sub>	1	Rash, itching hives, hypotension. Retching, emesis, convulsive movements.
	DX14	PO <sub>f</sub>	0, 32, 48 tablets/day	There were no significant toxicities at any dose. Non-GLP.
	DX14	PO <sub>e</sub>	0, 30, 60 120	No significant toxicity (sporadic vomiting/soft

				stool)
	3 month	PO <sub>e</sub>	0, 10, 30 120	Control thru HD: death after swelling, hypotension, prostration. D 2-4 of study. (no problems in pilot). Study stopped at 2 weeks dosing, 1 week recovery
	3 month	PO <sub>f</sub>	0, 5, 16, 48 tablets/day	No significant toxicity. Sporadic vomiting, but not consistent enough to affect interpretation/dosing. NOAEL = 48 tablets/day
Monkey	DX28	PO <sub>b</sub>	0, 10, 30, 90	2/6 HD monkeys dead with gavage errors; no other signs of toxicity. NOAEL= 90 mg/kg.

a vehicle = 10% dimethyl acetamid/20% ethanol/70% PEG400

b vehicle = labrasol

c vehicle = 0.2% Tween polysorbate 80/5% dextrose

d vehicle = 1% solutol HS-15/PBS

e vehicle = 9% labrafac WL1349, 24.3% labrasol, 13.6% labrafil M1944-CS, 33% Tween 80, 10% plurol oleique CC497, and 10% water.

f tablet in gelatin capsules

At maximum feasible doses, there were no effects of fidaxomicin on fertility in the rat, or embryo-fetal development in the rat or rabbit by the intravenous route. All of the reproductive toxicity studies used a formulation of 1% solutol HS-15 in PBS.

Reproductive Toxicity with Fidaxomicin by the intravenous route			
Study	Species	Max. Dose/NOAEL	AUC <sub>0-t</sub> of fidaxomicin at NOAEL (ng.hr/mL)
Fertility	Rat	6.3 mg/kg	4750/5080
Embryo-fetal development	Rat	12.6 mg/kg	9330
Embryo-fetal development	Rabbit Study # 1069-008	7.0 mg/kg	3233
Embryo-fetal development	Rabbit Study # 1069-018	7.0 mg/kg	3170

(/) = (AUC in males/AUC in females)

The AUC values for the rat study (not included in the original write-up) are shown in the following table.

NDA # 201,699

Reviewer: Wendelyn Schmidt

**Table 2. Toxicokinetic Parameters for OPT-80 in Pregnant Rats after Intravenous Administration from Gestation Day 6 through Gestation Day 17 (N = 3/group/time point)**

Gestation Day	Dose (mg/kg/day)	AUC <sub>0-∞</sub> (hr·ng/mL)	AUC <sub>0-∞</sub> /Dose ((hr·ng/mL)/mg/kg)	AUC <sub>0-tlast</sub> (hr·ng/mL)	AUC <sub>0-tlast</sub> /Dose ((hr·ng/mL)/mg/kg)	CL (mL/min/kg)	t <sub>1/2</sub> (hr)	V <sub>ss</sub> (mL/kg)
6	4	2700	675	2700	675	24.7	0.197	175
	8	10400	1300	10400	1300	12.8	0.209	92.3
	15	16700	1110	16700	1110	15.0	0.456	154
17	4	1160	290	1140	288	57.5	0.318	1070
	8	5150	644	5110	638	25.9	0.302	267
	15	9350	623	9330	622	26.7	0.596	450

**Table 3. Toxicokinetic Parameters for OP-1118 in Pregnant Rats after Intravenous Administration of OPT-80 from Gestation Day 6 through Gestation Day 17 (N = 3/group/time point)**

Gestation Day	Dose (mg/kg/day)	AUC <sub>0-tlast</sub> (hr·ng/mL)	AUC <sub>0-tlast</sub> /Dose ((hr·ng/mL)/mg/kg)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose ((ng/mL)/mg/kg)	t <sub>1/2</sub> (hr)	T <sub>max</sub> (hr)
6	4	910	228	2270	567	0.236	0.0800
	8	2700	337	6440	805	0.289	0.0800
	15	6320	421	16300	1090	0.447	0.0800
17	4	1280	321	2070	517	0.362	0.0800
	8	2760	345	6930	866	0.371	0.0800
	15	6680	445	13200	878	0.710	0.0800

Parent fidaxomicin, the major metabolite, OP-1118, and aged fidaxomicin were all negative in the Ames test. In chromosomal aberration assay in Chinese hamster ovary cells, both parent fidaxomicin and OP-1118 were positive, although the signal was stronger with the parent compound. The rat micronucleus assay with up to 75 mg/kg fidaxomicin intravenously was negative.

In conclusion, fidaxomicin, whether delivered by an oral or intravenous route, causes almost no damage in the rat, dog or cynomolgus monkey. There were no reproductive effects, and while there may be some clastogenic effects, both with the parent and, to a lesser extent the OP-1118 metabolite, there were no significant changes to marrow in the rat micronucleus assay. There are no pharmacology/toxicology objections to the approval of fidaxomicin.

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/s/

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WENDELYN J SCHMIDT

04/06/2011

AMY C NOSTRANDT

04/07/2011

I concur with the reviewer's assessment of the adequacy of the data and agree with the conclusions drawn in this review.