Summary of histopathology findings

Organ/Tissue	Principle Drug Related Finding	TPV/RTV	TPV	RTV
Multiple tissues	Excessive hemorrhage	X	X	
	Lymphoid depletion	X	X	X
Liver	Hypertrophy, hepatocellular, centrilobular	X	X	X
	Karyomegaly	X		X
	Cholangiohepatitis, subacute			X
	Increased mitotic index			X
	Hyperplasia, Kupffer cell			X
Lung	Microgranuloma, histiocytic			X
Lymph node, mesenteric	Hyperplasia, reticuloendothelial cell	X		X
Skin	Fat depletion, subcutis	X	X	X
Stomach	Necrosis, mucosal, glandular	X	X	X
Testis	Degeneration, seminiferous tubule, bilateral	X		
Thymus	Lymphocytolysis	X	X	X
Thyroid gland	Hypertrophy, follicular epithelial	X	X	X

Toxicokinetics:

Plasma Concentrations of TPV and RTV on Drug Week 26 at 8 hours after TPV dose and 9 hours after RTV dose:

Dose Level TPV/RTV (mg/kg/day)	Gender	TPV (μM)	RTV (ng/ml)
120/32	F	133 (Week 14)	1882 (higher than expected - protocol deviation)
	M	198	585
600/160	F	406	418
	M	303	141
1200/320	F	488	462
	M	344	0 (below 10)
1200/0	F	361	0
	M	290	0
0/160	F	0	1689
	F	0	1555

Other:

Histopathology inventory

Note: Tissues were examined histopathologically for animals that died or were sacrificed moribund and for all terminal sacrifice Control, 1200/320 mg/kg/day TPV/RTV, TPV only and

RTV only group animals. A "+" denotes tissues from the 120/32 and 600/160 mg/kg/day TPV/RTV groups that were also examined.

Study U04-3111	Dose (mg/kg/day)			
Species Rat	Control	TPV/RTV 1200/320	TPV 1200	RTV 160
Adrenals	X	X	X	X
Aorta	X	X	X	X
Bone Marrow, femur +	X	X	X	X
Bone Marrow, sternum				
Bone (femur with stifle joint) +	X	X	X	X
Bone, sternum	X	X	X	X
Brain +	X*	X*	X*	X*
Cecum +	X	X	X	X
Cervix	X	X	X	X
Colon +	X	X	X	X
Duodenum +	X	X	X	X
Epididymis +	X	X	X	X
Esophagus	X	X	X	X
Eye +	X	X	X	X
Fallopian tube				
Gall bladder				
Gross lesions	X	X	X	X
Harderian gland +	X	X	X	X
Heart	X	X	X	X
Ileum +	X	X	X	X
Injection site				
Jejunum +	X	X	X	X
Kidneys +	X*	X*	X*	X*
Lachrymal gland				
Larynx				
Liver +	X*	X*	X*	X*
Lungs +	X	X	X	X
Lymph nodes, bronchial	X	X	X	X
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric +	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves +	X	X	X	X
Ovaries +	X*	X*	X*	X*
Pancreas +	X	X	X	X
Parathyroid +	X	X	X	X
Peripheral nerve				

Pharynx				
Pituitary	X	X	X	X
Prostate	X	X	X	X
Rectum +	X	X	X	X
Salivary gland +	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X	X	X	X
Skeletal muscle	X	X	X	X
Skin +	X	X	X	X
Spinal cord +	X	X	X	X
Spleen +	X	X	X	X
Sternum	X	X	X	X
Stomach +	X	X	X	X
Testes +	X*	X*	X*	X*
Thymus +	X	X	X	X
Thyroid +	X*	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X	X	X	X
Vagina	X	X	X	X
Zymbal gland				

X, histopathology performed
*, organ weight obtained

Study title: 26-Week oral (gavage) interaction/toxicity study in the Beagle dog on tipranavir and ritonavir.
(See IND 51,979 (474) Summary of Immunotoxicity Findings for Tipranavir in Appendix for discussion of findings related to immunotoxicity in this study.).

Key study findings: Target organs when TPV and RTV were co-administered were the liver and urinary bladder. There was no significant increase in toxicity when the two compounds were co-administered. Additional target organs noted when TPV and RTV were administered alone included the gallbladder, bone marrow (sternum, rib) and spleen. There was no NOAEL for the co-administration of TPV and RTV due to the presence of hepatocellular hypertrophy in the liver of one male dog in the low dose TPV/RTV group.

Study no.: U04-3011
Volume # and page #: Module 4, M002, vol. 1.24, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877
Date of study initiation: November 27, 2001

GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV, Lot no. 113010, [redacted] purity; RTV, Lot no. 66729 TL and TSA-02-001, [redacted] purity.

Methods

Doses: Initial doses were 0, 15/4, 37.5/10 and 75/20 mg/kg/day TPV/RTV, 75 mg/kg/day TPV or 20 mg/kg/day RTV. These doses continued to the end of Drug Week 12 due to a high incidence of emesis. Doses were escalated in Drug Week 13 from 75/20 mg/kg/day TPV/RTV, 75 mg/kg/day TPV or 20 mg/kg/day RTV to 150/40, 150 and 40, respectively.
Species/strain: Beagle dogs [redacted]
Number/sex/group or time point (main study): 3
Route, formulation, volume, and infusion rate: Tipranavir in aqueous solution, pH 10.5; ritonavir in propylene glycol. 2 ml/kg for tipranavir and 2ml/kg for ritonavir; animals received RTV first followed by TPV. Due to the bitter taste of TPV a cherry syrup wash was added in Drug Weeks 7 and 8.
Satellite groups used for toxicokinetics or recovery: None.
Age: 7 – 9 months
Weight: 6 – 11 kg
Sampling times:
Unique study design or methodology (if any):

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.
Mortality: Room checks for morbidity and mortality were performed once daily during the Pretest Phase and twice daily during the Drug Phase.
Clinical signs: Clinical observations were recorded at least once daily during the Pretest Phase. Clinical observations were made 1 and 4 hours after dosing. Physical examinations, including measurement of reflexes (patella, pupillary and pain reflex), heart rate, rectal temperature and respiratory rate were performed in Pretest Weeks -4 and -2 and Drug Weeks 3, 7, 11, 19 and 24 at 2 – 4 hours after dosing.
Body weights: Body weights were measured once weekly during Pretest and Drug Phases.
Food consumption: Food consumption was measured daily in the Pretest and Drug Phases.
Ophthalmoscopy: Both eyes of all dogs were examined in Pretest Week -4 and Drug Weeks 7, 13 and 26.
EKG: Blood pressure and electrocardiograms were measured in Pretest Weeks -4 and -2 and in Drug Weeks 3, 7, 11, 19 and 25, 2 – 4 hours after dosing.
Hematology: In Pretest Weeks -4 and -1 and in Drug Weeks 4, 8, 12, 18 and 26, blood samples were collected from the jugular vein for performance of a standard hematology battery of tests.
Clinical chemistry: In Pretest Weeks -4 and -1 and in Drug Weeks 4, 8, 12, 18 and 26, blood samples were collected from the jugular vein for performance of a standard clinical chemistry battery.

Urinalysis: In Pretest Weeks -4 and -1 and in Drug Weeks 4, 8, 12, 18 and 26, animals were placed in metabolism units overnight with access to water but without food, for collection of urine.
Gross pathology: A complete necropsy was performed on early death and terminal sacrifice animals.
Organ weights (specify organs weighed if not in histopath table):
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no ( )

Results

Mortality: No drug-related deaths occurred during the study. Two dogs died from dosing accidents.

Clinical signs: Clinical signs considered related to TPV and RTV administration were increased salivation, emesis and soft stool/diarrhea. The incidence of these signs was dose-related. No drug-related effects were observed on physical examinations during the course of the study.

Body weights: No drug-related effects were observed on body weights.

Food consumption: No drug-related effects were observed on food consumption.

Ophthalmoscopy: No drug-related changes in ophthalmology were observed in the study.

EKG: No drug-related changes in electrocardiograms or blood pressure were observed in the study.

Hematology: There were no drug-related changes in hematology parameters.

Clinical chemistry: Significant drug-related changes in clinical chemistry when data from both sexes were combined included elevations of 93% to 134% in mean alkaline phosphatase in the 37.5/10 mg/kg/day TPV/RTV, 75-150/20-40 mg/kg/day TPV/RTV and 20-4 mg/kg/day RTV. One 20-40 mg/kg/day RTV male was noted to have elevations of 84% and 200% AST and ALT, respectively, as compared to its Pretest values. A slight decrease (13%) in mean albumin level was noted in 75-150/20-40 mg/kg/day TPV/RTV animals in Drug Week 26 only.

Urinalysis: There were no drug-related changes in urinalysis.

Gross pathology: Drug-related macroscopic enlargement and/or prominent lobular architecture in the liver were observed in males and females from the high dose TPV/RTV group and in

males from the TPV group and the RTV group. These changes correlated with diffuse centrilobular hypertrophy microscopically.

Organ weights (specify organs weighed if not in histopath table): There were no changes in organ weights except for liver weights. The percentage differences for the group mean absolute liver weights compared to control group means were as follows: 1) +20% (M) and +14% (F) in the low dose TPV/RTV group; 2) +6% (M) and +3% (F) in the middle dose group; 3) +19% (M) and +10% (F) in the high TV/RTV dose group; 4) +14% (M) and +7% (F) in the TPV group and 5) +31% (M) and +25% (F) in the RTV group.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no ( )

Drug-related microscopic changes were observed in the liver, bone marrow (sternum, rib), lymph nodes, gallbladder, thymus, spleen and urinary bladder.

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Histopathology Findings

Group: Dose (mg/kg/day): Test Article: Formulation: **TPV Aqueous pH 10.5/RTV Propylene glycol**	**Control** **0**		**Low-Dose TPV/RTV** **15/4 TPV/RTV**		**Mid-Dose TPV/RTV** **37.5/10 TPV/RTV**		**High-Dose TPV/RTV** **75/20 150/40 TPV/RTV**		**High TPV** **75/0 150/0 TPV**		**High RTV** **0/20 0/40 RTV**	
Gender:	M	F	M	F	M	F	M	F	M	F	M	F
No. in Group	3	3	3	3	3	3	3	3	3	3	3	3
Bone Marrow, Rib Granulocytic hyperplasia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++ +	1/3 ++	0/3	0/3

Bone Marrow, Sternum **Granulocytic hyperplasia**	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	1/3 ++	0/3	0/3
Lymph Node **Lymphoid hyperplasia**	0/3	0/3	0/3	1/3 + 1/3 ++	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++ +
Mesenteric Lymph Node **Lymphocytic depletion, cortical**	0/3	0/3	1/3 ++	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	0/3	0/3
Spleen **Lymphocytic depletion**	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	1/3 ++ 1/3 ++ +	1/3 +
Thymus **Lymphocytic depletion, cortical**	1/3 ++ 1/3 ++ +	1/3 ++ 1/3 ++ + 1/3 ++ ++	1/3 + 1/3 ++ + 1/3 ++ ++	3/3 ++	1/3 ++ 1/3 ++ + 1/3 ++ ++	1/3 ++ 1/3 ++ ++	1/3 + 1/3 ++ 1/3 ++ +	1/3 ++ 1/3 ++ ++	1/3 ++ 2/3 ++ ++	1/3 + 1/3 ++ ++	1/3 ++ 2/3 ++ +	1/3 ++ +
Liver **Centrilobular hypertrophy**	0/3	0/3	1/3 +	0/3	1/3 ++	1/3 ++	1/3 ++ 1/3 ++ +	1/3 ++ +	2/3 ++ +	2/3 +	1/3 + 2/3 ++ +	1/3 +
Urinary Bladder **Hypertrophy of transitional epithelium**	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	0/3
Gall Bladder **Cystic glandular hyperplasia**	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	2/3 ++	1/3 ++	1/3 ++

Number affected/Number examined.
+ = Minimal; ++ = Mild; +++ = Moderate; ++++ = Marked

Toxicokinetics: Blood was collected for the measurement of plasma levels of TPV and RTV in Drug Weeks 3, 7, 13, 18 and 26. Blood samples were collected prior to dosing and approximately 1, 2, 4, 8, and 24 hours after dosing.

TPV and RTV co-administration caused increases in plasma levels of TPV and decreases in RTV, as compared to the individual drugs administered alone. In most instances, TPV and RTV

plasma levels increased with increasing dose level and there were no gender differences for either compound given alone or co-administered.

C_{max} and AUC of TPV and RTV on Drug Week 26:

Dose Level TPV/RTV (mg/kg/day)	Gender	TPV		RTV	
		C_{max} (M)	AUC_{0-24h} (M.h)	C_{max} (ng/ml)	AUC_{0-24h} (ng.h/ml)
15/4	F	34	157	554	1,603
	M	19	72	61	274
37.5/10	F	41	217	438	2,031
	M	37	236	372	2,014
75-150/20-40	F	64	598	416	5,121
	M	66	722	852	7,780
75-150/0	F	16	74	6	18
	M	40	346	0	0
0/20-40	F	0	0	10,970	97,340
	F	0	0	10,574	79,014

Histopathology inventory

Study U04-3011	**Dose mg/kg/day**					
Species Dog	**0**	**15/4**	**37.5/10**	**75/20–150/40**	**75-150**	**20-40**
	Control	**TPV/RTV**	**TPV/RTV**	**TPV/RTV**	**TPV**	**RTV**
Adrenals	X	X	X	X	X	X
Aorta	X	X	X	X	X	X
Bone Marrow, rib	X	X	X	X	X	X
Bone Marrow, sternum	X	X	X	X	X	X
Bone, femur	X	X	X	X	X	X
Bone, sternum	X	X	X	X	X	X
Brain	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix	X	X	X	X	X	X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X

Epididymis	X	X	X	X	X	X
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
Fallopian tube						
Gall bladder	X	X	X	X	X	X
Gross lesions	X	X	X	X	X	X
Harderian gland						
Heart	X	X	X	X	X	X
Ileum	X	X	X	X	X	X
Injection site						
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*
Lachrymal gland						
Larynx						
Liver	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X	X
Lymph nodes, bronchial	X	X	X	X	X	X
Lymph nodes mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves	X	X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X
Peripheral nerve						
Pharynx						
Pituitary	X	X	X	X	X	X
Prostate	X	X	X	X	X	X
Rectum	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles						
Skeletal muscle	X	X	X	X	X	X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X	X	X	X	X	X
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X*
Thymus	X	X	X	X	X	X
Thyroid	X	X	X	X	X	X
Tongue	X	X	X	X	X	X
Trachea	X	X	X	X	X	X

Urinary bladder	X	X	X	X	X	X
Uterus	X	X	X	X	X	X
Vagina	X	X	X	X	X	X
Zymbal gland						
Tonsils	X	X	X	X	X	X

X, histopathology performed
*, organ weight obtained

Study title: Toxicokinetics of tipranavir in a 26-week oral (gavage) interaction/toxicity study in beagle dogs with ritonavir co-administration.
(Reviewed under U04-3011 26-Week oral (gavage) interaction/toxicity study in the beagle dog on tipranavir and ritonavir.)

Study no.: U03-3193

Study title: 26-Week Oral (Capsule) Safety Study in the Beagle Dog on Tipranavir and Ritonavir in SEDDS.

Key study findings: The purpose of this study was to determine the safety of the self-emulsifying drug delivery system (SEDDS) formulation, with TPV/RTV co-administration. Exposure to CrEL in the SEDDS formulation was of particular interest because IV administered CrEL is know to cause anaphylactoid reactions and it seemed possible that orally administered CrEL might pass from the GI tract into systemic circulation with long treatment duration. Consequently, exposures to the SEDDS formulation were chosen to achieve 1 (91 mg/kg/day SEDDS containing — mg/g CrEL), 10 (910 mg/kg/day SEDDS) and 30-fold (2720 mg/kg/day SEDDS) exposure to CrEL in humans.

Hematology and clinical chemistry changes were observed in three animals exposed to the highest dose level of SEDDS vehicle, including one Control female and two high dose animals. These changes included elevated white blood cell counts, accompanied by neutrophilia and monocytosis and increased liver alkaline phosphatase. In the two high dose animals, these changes were reversible and without microscopic changes. The Control female was sacrificed moribund in Week 13. In view of the similar changes noted in this female and two other animals receiving the high dose of SEDDS, this death was judged to be related to SEDDS vehicle administration. Target organs of SEDDS vehicle toxicity noted in the early death female were the stomach, intestine and mesenteric lymph nodes. These organs displayed microscopic changes consistent with an infectious etiology but in the absence of a causative agent.

CrEL plasma levels were detectable 2 hours after the first or second dose in several animal administered 2720 mg/kg/day SEDDS and one animal receiving 910 mg/kg/day SEDDS. Plasma CrEL levels ranged from [ ] mg/ml. Given that a 10 kg Beagle has a blood volume of 850 ml, the total dose in the blood at the point of measurement would range from 87 to 192 mg. This is considerably lower than the IV doses of CrEL, e.g. approximately 27,000 mg

in Paclitaxil, known to cause anaphylactoid reactions in humans. It should also be noted that there were no signs of a hypersensitivity reaction (e.g. edema, hives, rash) in the animals in the study and no SEDDS-related fecal occult blood was detected.

In conclusion, the NOEL for SEDDS is considered to be 91 mg/kg/day SEDDS due to the appearance of detectable levels in CrEL in the plasma of one animal dosed at 910 mg/kg/day. However, due to the lack of observed toxicities in animals dosed at 910 mg/kg/day, the NOAEL is considered to be 910 mg/kg/day SEDDS. This dose gives a 10-fold safety factor for the amount of CrEL — in the proposed human dose of 500/200 mg/kg/day TPV/RTV in the SEDDS vehicle. However, in light of the large amount of CrEL in this oral drug, including the amount of CrEL in the recommended human dosage of TPV/RTV 500/200 mg BID in the label should be considered.

Study no.: U04-3184
Volume #, and page #: Module 4, M002, Vol., 1.40; page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., Toxicology & Safety Assessment, Ridgefield, CT 06877-0368.
Date of study initiation: 2-10-03
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV stock SEDDS formulation, lot no. 6257-15 (purity [ ]
SEDDS vehicle formulation, lot no. 5804-91; RTV lot no. TSA-02-001 (purity [ ]

Methods

Doses: 0 TPV/RTV in 2720 SEDDS; 15/6 TPV/RTV in 91 SEDDS; 15/6 TPV/RTV in 910 SEDDS; 15/6 TPV/RTV in 2720 SEDDS mg/kg/day (twice daily dosing with half the dose administered followed by half the dose 4 hours later).

Species/strain: Beagle dog
Number/sex/group or time point (main study): 3/sex/group.
Route, formulation, volume, and infusion rate: Oral via gelatin capsules.
Satellite groups used for toxicokinetics or recovery:
Age: 7 – 9 months.
Weight: 6 to 10 kg
Sampling times:
Unique study design or methodology (if any):

The purpose of the study was to investigate the safety of the TPV SEDDS formulation, with the dose levels selected based on exposure to Cremophor EL (CrEL) at the proposed human dose level of 500/200 mg TPV/RTV. The clinical TPV SEDDS capsule of 250 mg TPV contains — mg CrEL in one gram. The RTV formulation contains 100 mg RTV and — ng CrEL per capsule. Therefore, at 500/200 mg TPV/RTV, humans receive per day four tipranavir capsules and four RTV capsules. Consequently, humans are being exposed to a total of — mg CrEL/day or — mg/kg/day based on a 60 kg human. Exposures to the SEDDS formulation

were chosen to achieve 1, 10 and 30-fold the exposure to CrEL in humans. A low dose of 91 mg/kg/day SEDDS (containing — mg/g CrEL) approximates (— mg/kg/day) the equivalent human exposure (— mg/kg/day) to CrEL at the 500/200 TPV/RTV BID dose level on a body weight basis (mg/kg/day). The middle dose of 910 mg/kg/day SEDDS approximates 10-fold the human exposure; the high dose of 2720 mg/kg/day SEDDS approximates 30-fold the human exposure.

The dose level of TPV/RTV was selected based on results of a 26-week oral toxicity study in dogs such that the lowest dose would result in minimal toxicity and a low incidence of emesis. The dose level was adjusted to — mg/kg/day to reflect the ratio of 500/200 mg TPV/RTV BID or 2.5:1.

Observations and times:

Mortality: Morbidity and mortality checks were performed once daily during the Pretest Phase and twice daily during the Drug Phase.

Clinical signs: Clinical observations performed once daily during the Pretest Phase and at least once before and approximately 30 minutes after each dose administration during the Drug Phase throughout the study. Physical examinations, including measurement of reflexes, heart rate, rectal temperature and respiratory rate were performed in Pretest Weeks -4 and -2 and Drug Weeks 3, 7 11, 19 and 25 at 1 to 2 hours after the second dose.

Body weights: Body weights were recorded once weekly during the Pretest and Drug Phases of the study.

Food consumption: Food consumption was evaluated daily in the Pretest and Drug Phases of the study.

High dose control female 03-00001 received special food due to retained deciduous loose canines. The canines were removed surgically on Drug Day 23 and the animal was maintained on wet food or a mix of wet food/kibble. On Drug Day 33 subcutaneous fluids were administered because of dehydration. This animal continued to exhibit decreased food consumption and showed high white blood cell count with neutrophilia and monocytosis. The animal was sacrificed on Drug Day 81.

High-dose female 03-00019 displayed a high white blood cell count with neutrophilia as well as neck swelling and decreased food consumption starting in Drug Weeks 3 to 4. These effects were determined to be due to a neck lesion and with appropriate treatment the lesion healed and the adverse events resolved.

Ophthalmoscopy: Ophthalmoscopy was not performed.

EKG: EKGs and blood pressure were measured in Pretest Weeks -4 and -2 and in Drug Weeks 3, 7, 11, 19 and 25 1 to 2 hours after the second dose. Heart rate, PQ, QRS and QT durations were quantitated and evaluated.

Fecal analysis: Occult blood monitoring was performed for all dogs in Pretest Weeks -4 and -2 and in Drug Weeks 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26.

Hematology: Blood samples were collected from the jugular vein in Pretest Weeks -4 and -2 and in Drug Weeks 4, 8, 12, 18 and 26. Blood was analyzed for standard hematology parameters.

Clinical chemistry: Blood samples were collected from the jugular vein in Pretest Weeks -4 and -2 and in Drug Weeks 4, 8, 12, 18 and 26. Blood was analyzed for standard clinical chemistry parameters and for alkaline phosphatase (AP) isoenzymes.
Urinalysis: Urine samples were collected in Pretest Weeks -4 and -2 and in Drug Weeks 4, 8, 12, 18 and 26. Animals were placed in metabolism units overnight with access to water, but without food, for collection of urine. Urine was analyzed for standard urinalysis parameters.
Gross pathology: A complete necropsy was performed on all animals at the end of the study and on early death Control female 03-00001.
Organ weights (specify organs weighed if not in histopath table): See table at the end of this study.
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Mortality: One Control female was sacrificed moribund in Drug Week 13. This death was considered related to administration of high dose SEDDS. This animal displayed clinical signs of decreased motor activity, thinness/emaciation, decreased food consumption, decreased body weight and clinical pathology changes including elevated white blood cell counts with neutrophilia and monocytosis, increased alkaline phosphatase and secondary effects considered related to the anorexia observed.

Clinical signs: Clinical signs considered related to test article and/or vehicle administration were soft stools and diarrhea. These signs increased in frequency with the dose of SEDDS and are considered related to the SEDDS vehicle.

Emesis was not a predominant sign as it had been in earlier studies using an aqueous solution of TPV, where the taste of the solutions was judged to be the cause of excessive emesis. Taste was not a factor in this study since TPV was administered in a capsule.

No overt evidence of hypersensitivity (e.g. edema, hives or erythema) to the SEDDS formulation was noted.

Body weights: Body weights were decreased in two animals, including an early death control female (Week 13) and a High dose female. The effects on these animals were considered related to high dose SEDDS.

For Control female 03-00001, an overall body weight loss of 2 kg between Drug Days 1 to 78 was observed.

High dose female 03-00019 lost a total of 0.9 kg between Drug Day 1 and 36 but recovered by Drug Week 13.

Food consumption: Food consumption was decreased in two animals, including an early death control female (Week 13) and a High dose female. The effects on these animals were considered related to high dose SEDDS.

Control female 03-00001 exhibited decreased food consumption starting on Drug Day 10. The adjustments to her diet are described under methods. Decreased food consumption continued until this animal was sacrificed moribund.

High dose female -3-00019 exhibited decreased food consumption transiently until recovery in Drug Week 13.

Ophthalmoscopy: Ophthalmoscopy was not performed.

EKG: No drug- or vehicle-related effects were observed.

Fecal analysis: Results of occult blood testing in fecal samples did not reveal any definitive pattern that can be related to drug or vehicle. Fecal samples from a number of animals in all groups displayed positive results for occult blood during the Pretest phase. However, the same animals did not necessarily test positive during the Drug Phase. The sponsor states that part of this may be explained by sampling effects.

Hematology: No hematology changes occurred that were considered related to TPV/RTV administration. There were no coagulation changes that were judged drug or vehicle related.

In three animals, one Control and two High dose animals, all of which received 2720 mg/kg/day, white blood cell counts were elevated at various time points, accompanied by neutrophilia and monocytosis (see Table below). These changes were judged by the sponsor to be related to the SEDDS vehicle.

Control female 03-00001 displayed increased white blood cell counts 4-fold and 7-fold above Pretest levels in Drug Weeks 8 and 12, respectively. The increase in white cell counts was due to an increase in absolute neutrophil and monocyte counts, which in Drug Week 12 were 10-fold to 11-fold higher than Pretest levels, respectively. Red blood cell parameters (RBC, hemoglobin and hematocrit) were decreased between 32 to 35% in Drug Week 12.

High dose male 03-00022 displayed an elevated white blood cell count in Drug Week 18 that was 3-fold above Pretest Week -1 value. An increase in absolute neutrophil and monocyte counts of 4.6-fold and approximately 3-fold above Pretest Week -1 levels was observed. White blood cell parameters had returned to normal in Drug Week 26.

High dose female 03-00019 displayed an elevated white blood cell count in Drug Week 4 that was 3.5-fold above its Pretest Week -1 value, due to approximately 6-fold increases in absolute

neutrophil and monocyte counts. The white blood cell count returned to normal by Drug Week 8 measurement. Slight decreases of 15 to 17% in red blood cell parameters were observed during the study but the values were within normal limits.

Clinical chemistry: Changes in clinical chemistry parameters included increased alkaline phosphatase (AP), shown to be of hepatic origin through isoenzyme analysis. Total AP levels administered TPV/RTV remained elevated over time, although not in all individual animals, but those of Control animals tended to decrease over the course of the study. This was due to TPV/RTV-related increases in hepatic AP isoenzymes concurrent with a normal expected decrease in bone AP isoenzymes as dogs aged. Hepatic origin AP isoenzymes levels ranged from 6 to 34 U/L in all animals during Pretest Phase and in Control animals during Drug Phase (except for the early death Control female, whose levels were 236 and 332 U/L in Drug Weeks 8 and 12, respectively), while hepatic AP isoenzymes levels ranged from 1 to 150 U/L in TPV/RTV-treated animals.

Many clinical chemistry changes were noted in three dogs, Control female 03-00001, High dose male 03-00022 and High dose female 03-00019 (see Table below).

Hematology and clinical chemistry findings in individual animals:

Animal/Sex	03-00001/F	03-00022/M	03-00019/F
Dose TPV/RTV (mg/kg/day)	0 (Control)	15/6	15/6
SEDDS (mg/kg/day)	2720	2720	2720
Sampling Time Drug Phase versus (Pretest Week)	Week 12[a] (Pretest Week -1)	Week 18 (Pretest Week -1)	Week 4 (Pretest Week -1)
WBC count ($10^3/\mu l$)	69.84 (9.95)	27.44 (8.77)	30.61 (8.77)
Neutrophils ($10^3/\mu l$)	58.56 (5.48)	23.31 (5.10)	24.50 (4.38)
Monocytes ($10^3/\mu l$)	5.48 (0.48)	1.55 (0.49)	1.86 (0.30)
Alkaline phosphatase (U/L)	389 (116)	140 (111)	188 (91)
Hepatic AP isoenzymes (U/L)	332 (16)	94 (12)	150 (20)
BUN (mg/dL)	5 (13)	9 (15)	10 (15)
ALT (U/L)	15 (33)	-	17 (39)
Glucose (mg/dL)	26 (98)	-	51 (95)
Total protein (g/dL)	5.5 (5.4)	-	6.4 (5.3)
Albumin (g/dL)	1.5 (3.2)	-	2.8 (3.1)
Globulin (g/dL)	4.0 (2.2)	-	3.6 (2.2)
Calcium (mg/dL)	9.3 (11.6)	-	-
Phosphorus (mg/dL)	5.4 (7.4)	-	4.6 (6.4)
Cholesterol (mg/dL)	219 (144)	-	251 (117)
Total bilirubin (mg/dL)	0.9 (0.1)	-	-

Urinalysis: No drug- or vehicle-related changes were observed.

Gross pathology: Drug-related macroscopic changes were not observed in the study. However, vehicle-related changes were evident in Control animal 03-00001 that was sacrificed moribund. Mesenteric lymph node enlargement and discoloration with local adhesion to the cecum and pancreas were noted. This correlated with the histopathological finding of subacute lymphadenitis. Bronchial lymph node enlargement was also noted and correlated with subacute lymphadenitis.

Organ weights (specify organs weighed if not in histopath table): There were no significant treatment-related weight changes in organ weight parameters.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )
Drug-related microscopic observations were not observed. Vehicle-related changes were observed.

Subacute to marked pyogranulomatous inflammation in multiple organs (brain, cecum, colon, duodenum, heart, liver, bronchial lymph nodes, mandibular lymph nodes, mesenteric lymph nodes, spleen, stomach, thyroid glands, thymus and urinary bladder) was observed in Control female 03-00001. The inflammation was centered in mesenteric lymph nodes and the stomach with secondary lesions in other organs and consisted of infiltrates of neutrophils, macrophages and multinucleate giant cells mixed with edema fluid, hemorrhage and cellular debris. Special stains did not demonstrate organisms within the lesions. Amyloidosis was present in the liver, bronchial lymph node, mandibular lymph node and spleen. The bone marrow demonstrated granulocyte hyperplasia secondary to the inflammatory processes present in other organs.

A finding of uncertain nature was present within the lung. The change occurred in animals from middle dose (0/3 males and 1/3 females) and high dose (2.3 males and 0.3 females) groups and consisted of infiltrates of macrophages and granulocytes into alveolar spaces and septa. This change is most likely iatrogenic in nature.

Another change worthy of mention was minimal lymphoid depletion in Peyer's patches of the ileum. This change was evident in animal from the low dose (1/3 males and 0/3 females), middle dose (1/3 males and 0/3 females), high dose (1/3 males and 2/3 females) groups but not in Control animals. This change was not considered drug-related as it was not evident in other lymphoid tissues.

Toxicokinetics: Blood was collected for the measurement of plasma levels of TPV, RTV and CrEL via the jugular vein prior to dosing and 1, 2, 4, 6, 8 and 24 hours after the first daily dose in Drug Weeks 3, 7, 13, 18 and 26 from all animals on study.

Cmax levels and AUC exposures of TPV and RTV were similar across all time points, all groups and both sexes, as they all received the same dose level of both compounds but in different volumes of SEDDS vehicle. Levels of both test articles were highly variable. Neither compound was detected in the plasma of Control animals.

Levels of CrEL plasma levels were below detectable limits (LOD = — mg/ml) in the low dose group receiving 91 mg/kg/day SEDDS. One male of the middle dose group receiving 910 mg/kg/day CrEL displayed a CrEL level above the limit of detection (— mg/ml) at one time point. CrEL levels were variably above the limit of detection in males of the high dose group and females of the Control group, both receiving 2720 mg/kg/day SEDDS. CrEL levels detected ranged from [ ] mg/ml and were noted at 2 hours after the first or second capsule administration.

Toxicokinetic parameters for tipranavir, ritonavir and cremophor EL.

Dose TPV/RTV mg/kg/day	0/0 (Control)		15/6		15/6		15/6	
Dose SEDDS mg/kg/day	2720		91		910		2720	
Sex: No. of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
TPV Cmax (μM)	0	0	18.7	15.6	22.9	16.4	18.9	14.5
TPV AUC_{0-24} (μM.h)	0	0	99.1	107	161	118	143	96.0
RTV Cmax (ng/ml)	0	0	160	92.5	440	141	167	84.3
RTV AUC_{0-24} (ng.h/ml)	0	0	654	710	2715	829	1080	520
Cremophor EL (mg/ml)[a]	[							]

a Expressed as number of animals displaying CrEL levels above the limit of detection (LOD = — mg/ml) – total number of time points for all animals; note that all time points > LOD occurred 2 hours after first or second dose.

Other:

Histopathology inventory

Study: U04-3184		Dose Group mg/kg/day			
Species: Dog	TPV/RTV (mg/kg/day)	0	15/6	15/6	15/6
	SEDDS (mg/kg/day)	2720	91	910	2720
Adrenals		X	X	X	X

Aorta	X	X	X	X
Bone Marrow smear Rib & Sternum	X	X	X	X
Bone Rib & Sternum	X	X	X	X
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder	X	X	X	X
Gross lesions	X	X	X	X
Harderian gland				
Heart	X	X	X	X
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland				
Larynx				
Liver	X*	X*	X*	X*
Lungs	X	X	X	X
Lymph nodes, Bronchial	X	X	X	X
Lymph nodes, Mandibular	X	X	X	X
Lymph nodes, Mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves	X`	X	X	X
Ovaries	X*	X*	X*	X*
Pancreas	X	X	X	X
Parathyroid	X	X		X*
Peripheral nerve				
Pharynx				
Pituitary	X	X	X	X
Prostate	X	X	X	X
Rectum	X	X	X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles				
Skeletal muscle	X	X	X	X

Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X	X	X	X
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X	X	X	X
Thyroid	X	X	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X*	X*
Vagina	X	X	X	X
Zymbal gland				
Additional Tissues				
Tonsils	X	X	X	X

X, histopathology performed
*, organ weight obtained

Study title: Toxicokinetics of tipranavir in a 26-week safety study in beagle dogs following oral (capsule) dosing of tipranavir SEDDS formulation with ritonavir co-administration.
(Reviewed under U04-3184 26-Week oral (capsule) safety study in the beagle dog on tipranavir and ritonavir in SEDDS.)

Study no.: U04-3295

Study title: 39-Week Oral Toxicity Study in the Dog Followed by a 9-Week Recovery Period

Key study findings: No drug-related deaths occurred. Clinical signs were related to local gastrointestinal effects of the dosing solution and increased in frequency with dose. All signs stopped when dosing stopped. Reversible decreases in body weight gain were noted in mid- and high-dose groups. Mild, reversible decreases in red blood cell numbers and hemoglobin levels were observed in the high-dose group. Reversible, dose-related increases in serum alkaline phosphatase and decreases in albumin and total protein were observed at the mid- and high-doses. A reversible, dose-related increase in liver weight was observed in the mid- and high-dose groups. Histopathology revealed reversible hepatocyte hypertrophy in the liver and increased hematopoiesis in the spleen and incompletely reversible cystic hyperplasia of the gall bladder epithelium at the mid and high doses. Reversible, minimal bile duct hyperplasia in the liver of high-dose females was also noted. The NOAEL for both sexes was 20 mg/kg/day which gives an AUC corresponding to 0.03 to 0.04 of the expected human AUC (500/200 mg TPV/RTV BID). The high dose used in this dog study produces an AUC approximately equivalent to the human AUC.

Study no.: U00-3270 (Pharmacia & Upjohn Study Report a0017797)

Volume #, and page #: Module 4, M002, Vol. 1.25, page 1.
Conducting laboratory and location: Drug Development Toxicology, Worldwide Toxicology, Pharmacia & Upjohn, Kalamazoo, MI
Date of study initiation: May 7, 1997
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TPV (PNU-140690E), (A1)5134-AS-163 [ ] purity) and (A1)5134-AS-1650 [ ] purity).

Methods

Doses: 0, 20, 75 or 320 mg/kg/day (0, 10, 37.5 or 160 mg/kg twice daily)

Species/strain: Beagle dog

Number/sex/group or time point (main study): 7/sex/group

Route, formulation, volume, and infusion rate: Oral via gastric intubation, purified water with pH adjusted to approximately 10.5 with 10% sodium hydroxide.

Satellite groups used for toxicokinetics or recovery: Blood samples were collected from the last 3 dogs/sex/group and 3 of the original 7 animals/group constituted the recovery group.

Age: 9 – 10 months.

Weight: Males – 8.2 to 11.6 kg; Females – 6.0 to 8.4 kg

Sampling times: Blood and urine samples were collected on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338. Toxicokinetics samples were collected predose (AM) and 2, 8, 10 and 14 hours after the AM dose on Days 1, 182 and 273 and predose (AM) and 10 hours after the AM dose on Days 28 and 84.

Unique study design or methodology (if any):

Observations and times:

Mortality: Clinical observations were performed 3 times prior to dose initiation and at least 2 times daily, 1 hour after each dose. Recovery dogs were observed once daily during the recovery period.

Clinical signs: Clinical observations performed 3 times prior to dose initiation and at least 2 times daily, 1 hour after each dose. Recovery dogs were observed once daily during the recovery period.

Body weights: Body weights were recorded four times prior to dose initiation and once weekly throughout treatment and recovery periods and prior to each scheduled necropsy following an overnight fast.

Food consumption: Food consumption was evaluated daily starting on Day -26 and continued throughout treatment and recovery periods except for Days when the dogs were fasted overnight for blood collection the following day. Each dog received 500 grams [ ] Diet [ ] 1 hour before the morning dosing. Uneaten food was weighed the next morning. Canned food was used as dietary supplements at the discretion of the Study Director. Data on amounts and days are retained in raw data.

Ophthalmoscopy: All dogs were given an ophthalmoscopic examination on Days -8, 269 and 332.
EKG: ECGs were collected from all dogs during the pretest period on Days -18 and -4, during treatment at 2.5 hours after dosing on Days 88, 179 and 270 and from 3/sex/group during recovery on Day 333.
Hematology: Blood samples were collected from the jugular vein on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338 from all surviving dogs. Blood was analyzed for standard hematology parameters.
Clinical chemistry: Blood samples were collected from the jugular vein on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338 from all surviving dogs. Blood was analyzed for standard clinical chemistry parameters.
Urinalysis: Urine samples were collected on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338 from all surviving dogs. Urine was analyzed for standard urinalysis parameters.
Gross pathology: A complete necropsy was performed on all animals euthanized on Days 274, 275 and 339 and in dog number 54 following her death on Day 245.
Organ weights (specify organs weighed if not in histopath table): See table at the end of this study.
Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Mortality: Dog number 54 possibly aspirated some of the dosing solution during the morning dosing on Day 245. She was found dead 1 hour after the afternoon dosing period. No other unscheduled deaths occurred in the study.

Clinical signs: Clinical signs were observed at all drug levels and included emesis, soft feces and diarrhea and salivation before and after dose administration. These clinical signs occurred with greater frequency in the treated groups than in the control group and generally frequency increased as the dose increased.

Body weights: There were no statistically significant changes in body weight data when individual dose groups were compared to controls. However, the mid- and high-dose males and high-dose females lost a small amount of weight as shown in the table below.

Food consumption: High dose males consumed more food per day (398 g) than the other groups (302 – 334 g). Thus, their weight loss and slower weight gain occurred in spite of adequate food consumption.

Ophthalmoscopy: No drug-related changes were observed.

EKG: No drug-related effects were observed.

Hematology: See the table below. All effects reversed during the recovery period.

Clinical chemistry: See the table below. All effects reversed during the recovery period.

Urinalysis: No drug-related effects were observed.

Gross pathology: No treatment-related effects were observed at interim and final necropsies. The unscheduled necropsy of dog number 54 revealed gross lesions associated with aspiration of the dosing solution and included dark red coloration of multiple lung lobes, red mottling in other areas of the lung and red foci in the thymus.

Organ weights (specify organs weighed if not in histopath table): Liver weights showed a statistically significant dose-related increase in males and females in the mid- and high-dose groups. These increases were apparently related to hepatocyte hypertrophy seen microscopically. This change reversed during the recovery period. No other organ weights showed a treatment effect.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

See the table below for histophathological findings. The sponsor states that the data on testicular degeneration and/or atrophy were re-evaluated by an expert panel. The findings of the panel are reported in study U04-3531. The panel concluded that the findings in the dog were within normal limits of variation.

Table: Percent change (+/-) compared to controls.

Parameter (Week 39)	Dose (mg/kg/day)			
	75		320	
	Male	Female	Male	Female
Body weight	-9.2%	+3.6 %	-9.5%	-8.3%
Red blood cells	-2.0 %	-11.2 %	-4.2 %	-15.7 %
Hemoglobin	-0.6 %	-6.8 %	-4.4 %	-9.0 %
Mean cell volume	+0.7	+5.9 %	+0.5	+10.8 %
Alkaline phosphatase	+285 %	+167 %	+ 875 %	+791 %
Albumin	-12.6 %	-6.7 %	- 19.2 %	-24.2 %
Total protein	-8%	-2%	-10%	-14%
Albumin/globulin ratio	-8.6 %	-11.4 %	-19.0 %	-22.8 %
Calcium	-1.8%	-3.2 %	-4.5 %	-3.8 %
Cholesterol	+9%	+15%	-13%	-40%
Liver weight (% body weight)	+38.4 %	+41.6 %	+75.2 %	+57.5 %

Hematopoiesis spleen (increased)	0/4	1/4 mild	2/4 minimal 1/4 mild	3/4 mild 1/4 moderate
Hepatocellular hypertrophy	2/4 minimal 2/4 mild	1/4 minimal 2/4 mild	1/4 minimal 3/4 moderate	1/4 mild 3/4 moderate
Bile duct hyperplasia	0	0	0	3/4 minimal
Cystic hyperplasia of gallbladder epithelium	2/4 mild 1/4 moderate NR	3/4 minimal 1/4 mild NR	2/4 mild 2/4 moderate NR	2/4 mild 2/4 moderate
Mild vacuolar degeneration of seminiferous tubules	1/4		2/4 NR	
Atrophy of seminiferous tubules	1/4 mild		1/4 moderate NR	

NR = Not completely reversible after the 9-week recovery period.

Toxicokinetics: Cmax and AUC increased with dose with values for mid-dose males being greater than predicted by linear dose relationship. No gender differences were noted and there was no evidence of increased clearance or decreased absorption over time.

Dose mg/kg/day	0 (Control)		20		75		320	
	Males	Female	Male	Female	Male	Female	Male	Female
Cmax (μM)	0	0	6.5	7.9	69	38	127	101
AUC_{0-24} (μM.h)	0	0	50	55	490	290	1130	1180
Fold versus Human AUC*			0.03	0.04	0.32	0.19	0.73	0.77

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 μM.h.

Histopathology inventory

Study: U00-3270	Dose Group mg/kg/day			
Species: Dog	0	20	75	320
Adrenals	X*	X*	X*	X*
Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)				

Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder	X	X	X	X
Gross lesions	X	X	X	X
Harderian gland				
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland	X	X	X	X
Larynx				
Liver	X*	X*	X*	X*
Lungs	X	X	X	X
Lymph nodes, cervical				
Lymph nodes, Mandibular	X	X	X	X
Lymph nodes, Mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves				
Ovaries	X*	X*	X*	X*
Pancreas	X	X	X	X
Parathyroid	X*	X*	X*	X*
Peripheral nerve				
Pharynx				
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Rectum	X	X	X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles				
Skeletal muscle				
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X*	X*
Sternum	X	X	X	X
Stomach	X	X	X	X