

Species / strain: Rat/ —:CD® (SD) **b(4)**

Age: 6 weeks

Weight: 189-229g (M) and 129-175g (F)

Group designation and dose levels:

Group	Dose ^{a)} (mg/kg/day)	Concentration (w/v%)	Dose volume (ml/kg)	No. of animals	
				Male	Female
Control	Physiological saline	—	5	24 (6 ^{b)} , 6 ^{c)})	24 (6 ^{b)} , 6 ^{c)})
Lowest	0.0032	0.000064	5	12	12
Low	0.010	0.0002	5	12	12
High	0.032	0.00064	5	24 (6 ^{b)} , 5 ^{c,d)})	24 (6 ^{b)} , 6 ^{c)})
Highest	0.10	0.002	5	24 (6 ^{b)} , 6 ^{c)})	24 (6 ^{b)} , 6 ^{c)})

a): Expressed as actual weight and not corrected for purity

b): 2-week recovery group

c): 4-week recovery group

d): Died owing to intubation error

Table excerpted from sponsor's package

Unique study design or methodology: None

Observations and times:

Clinical signs: Twice daily
Body weights: Twice weekly
Food consumption: Twice weekly
Hematology: End of Dosing and recovery week 2 and 4
Clinical chemistry: End of Dosing and recovery week 2 and 4
Urinalysis: Dosing week 4 and recovery week 2 or 4.
Ophthalmology: Daily
Organ weights: At sacrifice
Gross pathology: At sacrifice
Myelogram: At sacrifice
Histopathology: At sacrifice

Results:

Mortality: One high-middle dose (0.032 mg/kg) treated male died after dosing on day 11. Copious foam was observed in the right auricle of the heart and posterior cardinal vein. The sponsor believes the cause of death was an intubation error.

Clinical Signs: Unremarkable

Body weight: HD males and females lost ~15% and ~8%, respectively, by end of dosing period. This effect was reversible or showed a trend of reversibility.

Food consumption: HD males and females showed ~19% and ~17%, respectively, decrease in food consumption by end of dosing period. This effect was reversible or showed a trend of reversibility.

Hematology: All changes were reversible or showed a trend of reversibility.

% changes in hematology data vs. control

	Males				Females			
	LD	LMD	HMD	HD	LD	LMD	HMD	HD
White blood cells	-19	-33	-43	-60	-25	-27	-39	-57
Lymphocytes	x	x	x	-20	x	x	x	-17
Platelets	x	x	x	x	x	x	x	-31

LD, LMD, HMD, HD – low dose, low-middle dose, high-middle dose, and high dose, respectively
 x - no toxicologically significant change

Clinical Chemistry: All changes were reversible or showed a trend of reversibility.

% changes in clinical chemistry vs. control

	Males		Females	
	HMD	HD	HMD	HD
Aspartate aminotransferase	+47	+180	+46	+96
Alkaline phosphatase	-19	-27	x	x
Lactate dehydrogenase	x	+1152	+34	+162
Creatine phosphokinase	+27	+91	+22	+71
Total bilirubin	x	+132	x	+58
Triglycerides	-19	-65	-25	-62
Phospholipids	x	-19	x	x

HMD, HD – high-middle dose, and high dose, respectively
 x - no toxicologically significant change

Urinalysis: Reversible increase in urine volume in HD males (+77%) and MD (+55%) and HD (+82%) females.

Ophthalmology: Unremarkable

Organ weights: The majority of changes occurred in HD animals, as shown below. At the high-middle dose (0.032 mg/kg) an approximate 20% decrease was noted in salivary glands,

thymus, and adrenal glands. Except for an irreversible decrease in ovary weight, all other changes were reversible or showed a trend of reversibility. An additional finding, only seen in the recovery period, was an increase in female pituitary weight (+22%).

% changes in organ weights, relative to body weight

	HD Male	HD Female
Salivary glands	-26	-21
Thymus	-19	-23
Liver	-18	x
Spleen	+33	x
Adrenal glands	-39	-20
Seminal vesicle	-21	/
Prostate	-31	/
Ovary	/	-60

x - no toxicologically significant change
 / - irrelevant to sex of animals

Gross Pathology: All changes occurred in HD animals and were reversible or showed a trend of reversibility. Clouding of the thymic parenchyma was observed in 11/12 males and 7/12 females. Slight enlargement of the spleen was seen in 5/12 and 2/12 females.

Myelogram: Unremarkable

Histopathology: All changes were reversible or showed a trend of reversibility.

Adequate battery: Yes
 Peer review: Yes

End of Dosing Phase (Males): Incidence and grade of histopathology

Diagnosis	Grade	# animals affected			
		LD N=12	LMD N=12	HMD N=12	HD N=12
Thymus					
-degeneration and necrosis of lymphocyte	1-2	0	0	11	12
-increase in # tingible body macrophages	1-2	0	4	11	12
Spleen					
-increased extramedullary hematopoiesis	1-2	0	0	4	12

-degeneration and necrosis of lymphocyte -increase in # tingible body macrophages	1	0	0	1	12
Mandibular lymph node -degeneration and necrosis of lymphocyte -increase in # tingible body macrophages	1-2 1-2	0 2	0 8	8 9	10 12
Mesenteric lymph node -degeneration and necrosis of lymphocyte -increase in # tingible body macrophages	1-2 1	0 0	0 0	6 5	5 4
Bone Marrow -degeneration and necrosis of lymphocyte	1	0	0	0	12

LD, LMD, HMD, HD – low dose, low-middle dose, high-middle dose, and high dose, respectively
Grade 1, 2, refers to slight, moderate, respectively

End of Dosing Phase (Females): Incidence and grade of histopathology

Diagnosis	Grade	# animals affected			
		LD N=12	LMD N=12	HMD N=12	HD N=12
Thymus -degeneration and necrosis of lymphocyte -increase in # tingible body macrophages	1-2 1-2	0 0	0 0	12 10	12 12
Spleen -increased extramedullary hematopoiesis -degeneration and necrosis of lymphocyte -increase in # tingible body macrophages	1 1 1	0 0 0	0 0 0	0 0 2	11 11 12
Mandibular lymph node -degeneration and necrosis of lymphocyte -increase in # tingible body macrophages	1 1	0 0	0 0	10 12	8 6
Mesenteric lymph node -degeneration and necrosis of lymphocyte -increase in # tingible body macrophages	1 1	0 0	0 0	7 3	8 9

<i>macrophages</i>					
Bone Marrow <i>-degeneration and necrosis of lymphocyte</i>	1	0	3	9	9
*Ovary <i>-maturation arrest of ovarian follicle</i>	1-2	0	0	0	12

LD, LMD, HMD, HD – low dose, low-middle dose, high-middle dose, and high dose, respectively

Grade 1, 2, refers to slight, moderate, respectively

* After 2 weeks and 4 weeks of recovery the incidence was reduced to 6/12 and 5/12, respectively.

Study title: FK228: A 26-week intravenous injection toxicity study in the albino rat.

Key study findings:

- Rats were administered FK228 (romidepsin) I.V. at doses of 0.1, 0.33, and 1 mg/kg/day for 26 weeks.
- Mortality occurred in 1/20 MD females, 1/20 LD males, and 1/20 control males. Necropsy of the MD female revealed a blot clot in the ventral cervical region extending into the ventral thoracic region with the presence of hemorrhage in the surrounding skeletal muscle. No clinical signs were noted in the LD male and the cause of death is unknown. The control male was sacrificed due to limited usage of its hindlimbs. The cause of this is also unknown.
- Hematopoietic toxicities included dose-responsive decreases in white blood cells, lymphocytes, eosinophils, and platelets. These findings coincided with bone marrow hypocellularity and atrophy in the spleen and thymus. Pigment deposits were seen in the bone marrow, spleen, and thymus. A dose-responsive increase in reticulocytes was also seen.
- Liver toxicity was noted with a dose-responsive increase in AST and ALT in males with histopathological findings including cystic degeneration, mononuclear cell infiltration, vacuolation and pigment deposits (males and females).
- Reproductive organs were targeted as evidenced by atrophy of the uterus, ovary, testis, and mammary glands in ♀s at all dose levels and testicular degeneration and atrophy in ♂s at MD and HD.
- Females showed elevated cholesterol levels and a dose-responsive increase in pituitary size and weight with histopathological findings noting hyperplasia in the pars distalis.

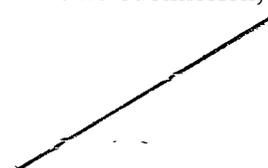
Study no.:

501650

Volume and page #:

Electronic Submission, Module 4

Conducting laboratory and location:



b(4)

Date of study initiation:

March 9, 2006

GLP compliance:

Yes

QA report: Yes
Drug, lot #, and % purity: FK228
 Lot No.: 005033L
 Purity: 99.7%

Methods:

Doses: 0, 0.1, 0.33, and 1 mg/kg/day
Dose justification: Based on previous experience in shorter term toxicity studies that determined the maximum tolerated dose in rats to be 1 mg/kg.
Route / formulation / volume: I.V. / 2% (v/v) ethanol and 98% (v/v) 0.9% NaCl / 5 mL/kg
Schedule: Weekly for 3 weeks out of every 4 weeks, for 26 weeks.
Species / strain: Rat/Sprague Dawley (CD® (SD) IGS BR) **b(4)**
Age: ~6 weeks
Weight: 192-232g (M) and 147-185g (F)
Group designation and dose levels:

Group Number Identification	Dose Level (mg/kg/dose)	Number of Animals			
		Main Study ^a		Toxicokinetic	
		Males	Females	Males	Females
1/ Vehicle Control	0	20	20	10	10
2/ FK228	0.1	20	20	10	10
3/ FK228	0.33	20	20	10	10
4/ FK228	0.67/1 ^b	20	20	10	10

a Euthanized on Day 183

b As the dose level of 0.67 mg/kg was considered well tolerated after the second dose (Day 8) the dose level was increased to 1 mg/kg for all subsequent doses

Table excerpted from sponsor's package

Unique study design or methodology: Following two weekly doses of FK228 at 0.67 mg/kg in the high dose group, the test article was considered well tolerated. Therefore, beginning with the third weekly dose, the high dose was increased to 1.0 mg/kg.

Observations and times:

Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Hematology: Week 13 and at sacrifice
Clinical chemistry: Week 13 and at sacrifice
Urinalysis: Week 13 and 26
Ophthalmology: Predose, Week 13 and 25
Organ weights: At sacrifice
Gross pathology: At sacrifice
Histopathology: At sacrifice
Toxicokinetics: Dosing Day 1 and 176

Results:

Mortality: A total of 3 unscheduled deaths occurred, as detailed below.

0.33 mg/kg (MD) animals: One female at 0.33 mg/kg was found dead on Day 89. No clinical signs of deteriorating condition were noted prior to death. Necropsy revealed a blot clot in the ventral cervical region extending into the ventral thoracic region. Microscopic examination confirmed the presence of hemorrhage (Grade 4 of 5) in the surrounding adjacent skeletal muscle. The sponsor believes the death of this rat was considered due to the blood collection procedure and not treatment-related.

0.1 mg/kg (LD) animals: One male at 0.1 mg/kg was found dead on Day 134. No clinical signs of deteriorating condition were noted prior to death. Macroscopic and microscopic evaluation failed to reveal the cause of death.

Control group animals: One male in the control group was euthanized on Day 169 due to limited usage of its hindlimbs. The cause is unknown.

Clinical Signs: The following clinical signs noted were dose-responsive and occurred in both sexes; yellow staining of fur in urogenital area, red staining of muzzle fur, thin fur on muzzle and forelimbs, scabs on tail, and tail skin appearing dry, red, or blue.

Body weight: HD males lost ~12% by week 13 and ~15% by end of study, week 26. Female body weights were unremarkable.

Food consumption: HD males consumed ~15% less food by week 13 and ~15% less food by end of study, week 26. Female food consumption was unremarkable.

Hematology:

Week 13: % changes in hematology data vs. control

	Males			Females		
	LD	MD	HD	LD	MD	HD
White blood cells	-37	-62	-80	-14	-54	-71
Neutrophils	-23	-51	-61	+19	-11	-45
Lymphocytes	-39	-65	-81	-18	-60	-76

Monocytes	-29	-35	-53	x	x	x
Eosinophils	-13	-56	-71	-25	-50	-67
Basophils	-29	-59	-71	-31	-56	-63
Platelets	x	-12	-36	x	-21	-46
Reticulocytes	x	+20	+42	+35	+63	+113

x - no toxicologically significant change

Week 26: % changes in hematology data vs. control

	Males			Females		
	LD	MD	HD	LD	MD	HD
White blood cells	-12	-35	-40	-29	-42	-50
Neutrophils	-22	x	+62	-33	-38	x
Lymphocytes	x	-43	-60	-28	-45	-59
Monocytes	-20	x	x	-33	-20	-29
Eosinophils	x	-33	-43	-41	-40	-43
Basophils	x	-29	-57	x	x	x
Platelets	x	x	x	x	x	x
Reticulocytes	+39	+75	+92	+62	+88	+160

x - no toxicologically significant change

Clinical Chemistry:

Week 13: % changes in clinical chemistry vs. control

	Males			Females		
	LD	MD	HD	LD	MD	HD
Triglycerides	x	-8	-34	-31	x	-22

x - no toxicologically significant change

Week 26: % changes in clinical chemistry vs. control

	Males			Females		
	LD	MD	HD	LD	MD	HD
Triglycerides	x	x	x	x	x	-31
Aspartate aminotransferase	x	+71	+93	x	x	x
Alanine aminotransferase	x	+228	+412	x	x	x
Total bilirubin	x	x	x	+43	+21	+29
Cholesterol	x	x	x	x	+39	+18

x - no toxicologically significant change

Urinalysis: Unremarkable

Ophthalmology: Unremarkable

Organ weights:

Week 26: % changes in organ weights, relative to body weight

	Males			Females		
	LD	MD	HD	LD	MD	HD
Thymus	-66	-72	-60	x	-42	+126
Pituitary	x	x	x	x	+25	+25
Ovary	/	/	/	-21	-36	-64
Uterus	/	/	/	x	x	-19

x - no toxicologically significant change
/ - irrelevant to sex of animals

Gross Pathology:

Week 26: Incidence of gross pathology findings

	Males			Females		
	LD	MD	HD	LD	MD	HD
Small Thymus	1/20	6/20	4/20	x	x	x
Enlarged Pituitary	x	x	x	2/20	9/20	10/20
Small Ovary	/	/	/	2/20	18/20	19/20

x - no toxicologically significant change
/ - irrelevant to sex of animals

Histopathology: Adequate battery: Yes
Peer review: Yes

End of Dosing Phase (Males): Incidence and grade of histopathology

Diagnosis	Grade	# animals affected			
		Cont N=20	LD N=20	MD N=20	HD N=20
Bone Marrow					
-hypocellularity: hematopoietic	1-2	1	7	5	6
-deposits: pigment	1	0	1	11	15
Liver					
-degeneration: cystic	2	0	0	1	5
-deposits: pigment	1	0	8	5	9
-infiltration: mononuclear cell	1	2	2	2	7
Mammary Gland					
-atrophy	1-4	0	15	12	15
Spleen					
-deposits: pigment	1-3	7	18	18	20
-atrophy/necrosis: lymphoid	1-2	1	1	8	20
Thymus					
-atrophy/necrosis: lymphoid	1-5	2	15	19	19
-hyperplasia: lymphoid	1-3	0	6	10	16
Testis					
-degeneration/atrophy: seminiferous epithelium	1	0	0	1	10

Grade 1, 2, 3, 4, 5 refers to minimal, slight, moderate, marked, severe, respectively

End of Dosing Phase (Females): Incidence and grade of histopathology

Diagnosis	Grade	# animals affected			
		Cont N=20	LD N=20	MD N=20	HD N=20
Bone Marrow					
-hypocellularity: hematopoietic	1-2	0	0	6	9
-deposits: pigment	1	0	0	8	9
Spleen					
-deposits: pigment		0	4	19	17
-atrophy/necrosis: lymphoid	1-2	0	0	7	11
-hematopoiesis/extramedullary:	1-2	0	0	9	12
increased	1-2				
Thymus					
-atrophy/necrosis: lymphoid	1-2	1	5	14	19
-hyperplasia: lymphoid	1-3	0	0	3	19
Pituitary					
-hyperplasia: pars distalis	1-2	1	2	10	14
Liver					
-deposits: pigment		0	4	5	6
-vacuolation: hepatocellular	1-2	1	2	2	6
Kidney					
-dilatation: tubular	1-2	1	5	12	7
Mammary Gland					
-atrophy	2-5	0	20	20	20
Ovary					
-atrophy	1-5	0	15	20	20
-cyst	1-4	0	11	6	1
Uterus					
-atrophy	1-4	0	14	20	19
Vagina					
-atrophy	2-3	0	3	7	3
-degeneration and/or necrosis	1-2	0	0	2	3
-mucification: epithelial	1-4	0	5	9	2

Grade 1, 2, 3, 4, 5 refers to minimal, slight, moderate, marked, severe, respectively

Toxicokinetics:

- Cmax and AUC were generally dose proportional from LD to HD in males and females.
- Significant drug accumulation was observed at Day 176 in males and females.
- No significant sex differences were seen.
- Details are listed in the sponsor's table below:

Group Mean Toxicokinetic Parameters of Depsipeptide (FK228) in Plasma Following Intravenous Injection of Depsipeptide (FK228) to the Albino Rat – Day 1

Males (Day 1)

Group No.	Dose Level (mg/kg)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-last} (ng•h/mL)	k _{el} (1/h)	t _{1/2el} (h)	AUC _{0-inf.} (ng•h/mL)	% Extrapolation AUC _{0-inf.}
2	0.1	0	9.50	0.50	1.76	a	a	a	a
3	0.33	0	53.0	2	11.7	0.960	0.722	12.4	5.10
4	0.67	0	60.7	4	17.0	0.800	0.867	17.2	1.22

Females (Day 1)

Group No.	Dose Level (mg/kg)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-last} (ng•h/mL)	k _{el} (1/h)	t _{1/2el} (h)	AUC _{0-inf.} (ng•h/mL)	% Extrapolation AUC _{0-inf.}
2	0.1	0	17.9	0.50	2.49	a	a	a	a
3	0.33	0	37.1	1	7.96	3.33	0.208	8.21	3.06
4	0.67	0	93.0	2	18.7	1.45	0.479	19.0	1.51

a It was not possible to estimate the k_{el} with an acceptable degree of confidence due to an inability to characterize the terminal phase. Consequently, all parameters derived from this, t_{1/2el}, AUC_{0-inf.} and % extrapolation AUC_{0-inf.} were not estimated.

Group Mean Toxicokinetic Parameters of Depsipeptide (FK228) in Plasma Following Intravenous Injection of Depsipeptide (FK228) to the Albino Rat – Day 176

Males (Day 176)

Group No.	Dose Level (mg/kg)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-last} (ng•h/mL)	k _{el} (1/h)	t _{1/2el} (h)	AUC _{0-inf.} (ng•h/mL)	% Extrapolation AUC _{0-inf.}
2	0.1	0	19.0	1	5.16	3.36	0.206	5.33	3.22
3	0.33	0	129	4	28.7	0.625	1.11	29.4	2.19
4	1	0	377	6	86.9	0.356	1.95	88.7	2.02

Females (Day 176)

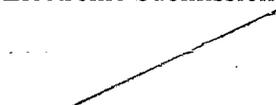
Group No.	Dose Level (mg/kg)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-last} (ng•h/mL)	k _{el} (1/h)	t _{1/2el} (h)	AUC _{0-inf.} (ng•h/mL)	% Extrapolation AUC _{0-inf.}
2	0.1	0	19.2	1	4.22	3.03	0.229	4.41	4.25
3	0.33	0	92.3	2	20.4	1.02	0.681	21.3	4.48
4	1	0	362	6	73.2	0.588	1.18	74.4	1.67

Tables excerpted from sponsor's package

Study title: Four-Week Intravenous Toxicity Study of FR901228 in Dogs with 4-Week Recovery Study (Fujisawa Study GLR910296; July 1991). Reviewed by W. David McGuinn, Ph.D. and slightly modified for this NDA review.

Key study findings:

- Slight atrophy of the thymus in MD and HD females.
- Histopathological findings included slight-moderate increase in tingible body macrophages and slight-moderate degeneration and necrosis of the lymphocytes in the thymus, spleen, and lymph nodes. A slight-moderate decrease in erythroblasts and cellular phagocytosis was also noted.

Species: GLR910296
Volume and page #: Electronic Submission, Module 4
Conducting laboratory and location: 
Date of study initiation: March 1, 1990
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: FR901228
Lot No.: 004196L
Purity: 98.1%

b(4)

Methods:

Species: Beagle dog
Drug: Depsipeptide (Lot No.004196L, purity: 98.1%)
Doses: 0, 0.0032, 0.01 and 0.032 mg/kg I.V. daily for 28 days
Schedule: daily for 28 days
Route, formulation, volume: I.V. / Ethanol / propylene glycol (1:4) in saline;
Concentrations of 0.025 or 0.05 (w/v %) / 2 ml/kg
at 10 ml/min
Group designation and dose levels: 3/sex/dose group, 3/sex in high dose recovery
group

Observations and times:

Clinical signs: Daily
Body weights: Weekly
Food consumption: Daily
Hematology: Before dosing, week 2 and before necropsy
Clinical chemistry: Before dosing, week 2 and before necropsy
Urinalysis: Before dosing, week 2 and before necropsy
Heart Rate: Weekly
Ophthalmology: Before the start of dosing and before necropsy
Necropsy: Day 28 for main group animals and day 32 for recovery
animals

Histopathology: All groups

Results:

Mortality: None

Clinical Signs: No changes

Body weight: Slight decreases in three HD dogs.

Food consumption: Occasional decreases in individual animals.

Hematology: White blood cells decreased slightly in the males and females of the 0.032 mg/kg group.

Clinical Chemistry: AST and ALT decreased slightly in the males and females of the 0.032 mg/kg group during the dosing period; potassium decreased slightly in the males; and glucose increased slightly and alkaline phosphatase decreased slightly in the females.

Urinalysis: No changes

Ophthalmology: No changes

Heart Rate: No changes

Gross Pathology: Slight atrophy of the thymus in one MD and one HD female.

Organ weights: No significant changes.

Histopathology: *Thymus:* Slight or moderate increase of tingible body macrophages in 1 MD female and in all the HD animals; slight degeneration and necrosis in 1 HD male and 2 HD females; slight decrease of cortical lymphocytes in 1 HD female.
Spleen: Slight increase of tingible body macrophages in 1 MD male and in 1 HD male and all the HD females; slight degeneration and necrosis of the lymphocytes in 2 HD males and all HD females; slight hemosiderin deposition in the red pulp of 2 HD females.
Lymph node: Slight to moderate degeneration and necrosis of the lymphocytes in the retropharyngeal lymph node of 2 LD males and 1 LD female and of all MD and HD animals. Similar changes were observed in the mesenteric lymph node and intestinal submucous lymph nodules of the MD

and HD animals. The severity of these lesions increased with increasing dose. Tingible body macrophages increased in the lymph nodes of many of the animals.

Bone marrow: Slight cellular phagocytosis in 1 MD female; slight to moderate decrease of erythroblasts and cellular phagocytosis in all HD animals.

Study title: Four-Week intermittent Intravenous Toxicity Study of FR901228 in Dogs - Additional Study (——— Study GLR910298; September 1991). Reviewed by W. David McGuinn, Ph.D. and slightly modified for this NDA review.

b(4)

Key study findings:

- Alterations in clinical chemistry parameters included decreased iron, PO_4^{+++} , Ca^{++} , K^+ , fibrinogen and increased AST were seen in both dose group. An increase in LDH and BUN was also seen in HD dogs.
- Marked decrease in erythroblastic cells.
- QT interval was increased after dosing in the LD dog (range 0 to 90 ms, average 33 ± 25 ms above baseline, 10%) and HD dog (range -20 to 70 ms, average 28 ± 24 ms, 12%). ST segment tended to increase in the HD dog after day 20.
- In the heart of the HD dog, there were dark red spots, white coloration and thickening of the epicardium and pericardium with retention of 117 ml of dark red fluid in the pericardial cavity.
- Additional histopathological findings included dark red foci on the corticomedullary zone of the kidney, node of splenic abscess, dark red to yellowish white foci in the left anterior lobe of the lung, and hepatization in part of the lung.

Methods:

Species: Beagle dog

Drug: Depsipeptide (Lot No.004196L, purity: 98.1%)

Doses: 1 or 2 mg/kg (total dose: 8 or 16 mg/kg)

Schedule: Day 1, 5, 8, 12, 15, 19, 22, and 27

Route, formulation, volume: I.V. / Ethanol/propylene glycol (1:4) in saline; Concentrations of 0.025 or 0.05 (w/v %) / 4 ml/kg at 10 ml/min

Group designation and dose levels: 1 male beagle dog/dose group

Observations and times:

Clinical signs: Daily

Body weights: Days before dosing, days 5, 8, 12, 15, 19, 22, 26, and 29

Food consumption: Daily

Hematology: Days 2, 5, 6, 8, 9, 15, 16, 22, 23, 26, 27, and 29 of dosing

Clinical chemistry: Days 2, 5, 6, 8, 9, 15, 16, 22, 23, 26, 27, and 29 of dosing

ECG: Before dosing on each dosing day and 24 hours after dosing
Necropsy: Day 29
Histopathology: Bone marrow only

Results:

Mortality: None

Clinical Signs: Gastrointestinal toxicity (emesis, abnormal feces). Injection site swelling occurred in dogs treated with 2 mg/kg/day (biwx4).

Body weight: Decreased body weight 15% at 1 mg/kg, 26% at 2 mg/kg

Food consumption: Decreased

Hematology: Lymphopenia with decreased monocyte counts, increased neutrophils.

Clinical Chemistry: Decreased iron, PO_4^{+++} , Ca^{++} , K^+ , fibrinogen and increased AST were seen in both dosed dogs. In addition, LDH and BUN increased in dogs in the 2.0 mg/kg/day dose group.

Bone Marrow: Marked decrease in erythroblastic cells

ECG: QT interval was consistently increased after dosing in the low dose dog (range 0 to 90 ms, average 33 ± 25 ms above baseline, 10%). The change in the HD dog in QT interval was similar (range -20 to 70 ms, average 28 ± 24 ms, 12%). ST segment tended to increase in the HD dog after day 20.

Gross Pathology: At necropsy, small thymus was seen in both dogs. There were no changes in the heart of the low dose dog. In the heart of the high dose dog, there were dark red spots, white coloration and thickening of the epicardium and pericardium with retention of 117 ml of dark red fluid in the pericardial cavity. There were dark red foci on the corticomedullary zone of the kidney, node of splenic abscess, dark red to yellowish white foci in the left anterior lobe of the lung, and hepatization in part of the lung.

2.6.6.4 Genetic toxicology

Study title: FK228 Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 100, TA 1537 and TA 98 and *Escherichia coli* WP2uvrA.

Key study findings:

- FK228 (romidepsin) was not mutagenic in tester strains of *Salmonella* or *E. coli* in the presence and absence of S-9 mix.

Study no.: 775325
Volume and page #: Electronic Submission, Module 4
Conducting laboratory and location: / **b(4)**
Date of study initiation: December 17, 2004
GLP compliance: Yes (OECD)
QA report: Yes
Drug, lot #, and % purity: FK228
Lot No.: 005033L
Purity: 99.7%

Methods:

Tester Strains: *Salmonella typhimurium* tester strains, TA98, TA100, TA1535, TA1537, and *Escherichia coli* tester strain WP2 *uvrA*.

Metabolic activation system: Aroclor 1254-induced rat liver S9.

Concentrations used in definitive study: 17, 50, 167, 500, 1667, and 5000 µg per plate +/- S9 mix.

Basis of concentration selection: Based on results of initial toxicity test using TA100 only.

Negative Control: Dimethyl sulfoxide

Positive Controls:

Strain	S9	Positive Control	Concentration (µg/plate)
TA1535, TA1537	+	2-Aminoanthracene	2
TA98, TA100	+	2-Aminoanthracene	0.5
WP2 <i>uvrA</i> /pKM101	+	2-Aminoanthracene	20
TA1535, TA100	-	Sodium Azide	1
TA98	-	2-Nitrofluorene	1
TA1537	-	9-Aminoacridine	80
WP2 <i>uvrA</i> /pKM101	-	ENNG	2

Using *S. typhimurium* TA 1538, the enzymatic activity of each batch of S9 was characterized with additional mutagens that require metabolic activation as shown below.

Substance	Quantity per Plate	Revertant Colonies per Plate			
					Mean
Dimethylsulphoxide	100 µL	17	29	25	24
2-Aminoanthracene	0.5 µg	623	604	617	615
2-Acetylaminofluorene	10 µg	1250	1281	1239	1257
4-Acetylaminofluorene	1 mg	155	115	192	154
Benzo(a)pyrene	5 µg	411	414	406	410
Dimethylaminoazobenzene	100 µg	89	94	95	93

Incubation and sampling time: Plate incorporation method with 48 hr incubation period @ 37°C and pre-incubation method with 20 minute incubation period @ 37°C prior to plating and incubating for an additional 48 hours @ 37°C.

Analysis:

- 3 replicates for each test compound concentration.
- revertant colonies were scored using a Cardinal Colony Counter and a validated software system (York Electronics).

Criteria for positive results: For the test article to be evaluated positive it must have caused a concentration-related increase ($\geq 2 \times$ mean vehicle control value except TA100, where it must show $\geq 1.5 \times$ mean vehicle control value) in the mean revertants per plate and be reproducible.

Study validity:

- An adequate selection of bacterial tester strains were used, strain integrity was documented, and culture titers were $\geq 3 \times 10^8$ cells/mL.
- The maximum concentration level was acceptable ($\geq 5000 \mu\text{g}/\text{plate}$) and at least 2 concentrations were tested per log in each tester strain \pm S-9 mix.
- Both negative (vehicle) and positive control data were within the laboratory historical range.
- The mean positive control value (\pm S9-mix) exhibited ≥ 3 -fold increase over the respective mean vehicle control value for each tester strain.
- There was a minimum of three nontoxic concentrations ($\leq 50\%$ reduction in mean number of revertants/plate relative to the mean vehicle control value with an accompanying abrupt dose-dependent drop in the revertant count or a reduction in the background lawn) in each tester strain \pm S-9 mix.

Results:

As detailed in the sponsor's table below, precipitate was seen at concentrations $\geq 1667 \mu\text{g}/\text{plate}$ using the pre-incubation method and at $5000 \mu\text{g}/\text{plate}$ using the plate incorporation method. Cytotoxicity was only noted using the pre-incubation method. For all *S. typhimurium* tester strains, cytotoxicity was seen at $5000 \mu\text{g}/\text{plate}$ in the presence of S9 and at concentrations $\geq 1667 \mu\text{g}/\text{plate}$ in the absence of S9. No

increase in revertant numbers was seen in any tester strain, using either activation condition or plating method.

Conclusion:

FK228 was not mutagenic in tester strains of *Salmonella* or *E. coli* in the presence or absence of S-9 mix.

Plate incorporation method in the presence of S9 Mix:

First Mutation Assay

Mean Number of Revertant Colonies Per Plate in the Presence of S9 Mix (FLI 102).

Test Item	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± SD				
DMSO	100 µL	12 ± 2	11 ± 4	27 ± 2	105 ± 13	10 ± 2
FK228	17	10 ± 4	17 ± 1	26 ± 6	103 ± 8	9 ± 2
	50	14 ± 3	16 ± 2	25 ± 9	96 ± 3	14 ± 2
	167	8 ± 4	16 ± 2	28 ± 8	108 ± 5	12 ± 3
	500	14 ± 3	14 ± 5	31 ± 6	91 ± 18	10 ± 4
	1667	9 ± 3	13 ± 1	30 ± 6	110 ± 8	10 ± 2
	5000 (P)	7 ± 3	11 ± 6	27 ± 7	81 ± 3	8 ± 3
Positive controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level µg per plate	2	2	0.5	0.5	20
	Mean ± SD	352 ± 26	303 ± 15	573 ± 8	909 ± 58	512 ± 15

SD Standard Deviation
 2AAN 2-Aminoanthracene
 P Precipitation

Table excerpted from sponsor's package

Plate incorporation method in the absence of S9 Mix:

First Mutation Assay (continued)

Mean Number of Revertant Colonies Per Plate in the Absence of S9 Mix.

Test Item	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µL	8 ± 2	8 ± 4	18 ± 2	67 ± 1	13 ± 2
FK228	17	12 ± 4	6 ± 3	15 ± 3	85 ± 14	8 ± 2
	50	6 ± 4	12 ± 3	19 ± 2	82 ± 15	7 ± 2
	167	11 ± 2	9 ± 3	14 ± 4	87 ± 5	7 ± 1
	500	5 ± 2	12 ± 6	17 ± 7	97 ± 9	12 ± 3
	1667	8 ± 2	13 ± 3	17 ± 6	93 ± 3	9 ± 1
	5000 (P)	3 ± 3	3 ± 1	19 ± 2	72 ± 9	5 ± 3
Positive controls	Compound	NaN ₃	9AA	2NF	NaN ₃	ENNG
	Dose Level µg per plate	1	80	1	1	2
	Mean ± SD	359 ± 25	2081 ± 162	561 ± 60	1149 ± 16	106 ± 9

SD Standard Deviation
 NaN₃ Sodium azide
 9AA 9-Aminoacridine
 2NF 2-Nitrofluorene
 ENNG N-Ethyl-N-nitro-N-nitrosoguanidine
 P Precipitation

Table excerpted from sponsor's package

Pre-incubation method in the presence of S9 Mix:

Second Mutation Assay

Mean Number of Revertant Colonies Per Plate in the Presence of S9 Mix (FLI 102).

Test Item	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± SD				
DMSO	100 µL	7 ± 2	8 ± 1	19 ± 5	80 ± 6	10 ± 3
FK228	17	7 ± 4	9 ± 5	21 ± 6	73 ± 6	7 ± 1
	50	7 ± 3	7 ± 2	19 ± 8	72 ± 16	7 ± 3
	167	7 ± 3	10 ± 5	17 ± 3	73 ± 18	9 ± 2
	500	9 ± 1	7 ± 4	18 ± 4	68 ± 3	7 ± 3
	1667 (P)	*	*	*	*	*
	5000 (P)	*(STL)	*(TL)	*(TL)	*(TL)	*
Positive controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level µg per plate	2	2	0.5	0.5	20
	Mean ± SD	190 ± 30	143 ± 18	572 ± 45	467 ± 27	584 ± 42

SD Standard Deviation
 2AAN 2-Aminoanthracene
 TL Thin Lawn
 STL Slightly Thin Lawn
 P Precipitation
 * Colonies could not be accurately enumerated due to precipitation

Table excerpted from sponsor's package

Pre-incubation method in the absence of S9 Mix:

Second Mutation Assay (continued)

Mean Number of Revertant Colonies Per Plate in the Absence of S9 Mix

Test Item	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µL	9 ± 1	7 ± 2	15 ± 5	73 ± 24	8 ± 6
FK228	17	8 ± 1	3 ± 2	9 ± 2	65 ± 7	10 ± 2
	50	7 ± 3	10 ± 2	19 ± 9	61 ± 4	7 ± 3
	167	9 ± 2	6 ± 4	17 ± 3	59 ± 7	7 ± 3
	500	9 ± 5	7 ± 6 (STL)	17 ± 4 (STL)	65 ± 7	10 ± 6
	1667 (P)	* (STL)	* (VTL)	* (TL)	* (STL)	*
	5000 (P)	* (STL)	* (NL)	* (VTL)	* (STL)	*
Positive controls	Compound	NaN ₃	9AA	2NF	NaN ₃	ENNG
	Dose Level µg per plate	1	80	1	1	2
	Mean ± SD	261 ± 1	2421 ± 155	624 ± 15	780 ± 26	306 ± 21

SD Standard Deviation
 NaN₃ Sodium azide
 9AA 9-Aminoacridine
 2NF 2-Nitrofluorene
 ENNG N-Ethyl-N-nitro-N-nitrosoguanidine
 TL Thin Lawn
 STL Slightly Thin Lawn
 P Precipitation
 VTL Very Thin Lawn
 NL No Lawn Present
 * Colonies could not be accurately enumerated due to precipitation

Table excerpted from sponsor's package

Study title: FK228: Mouse Lymphoma Cell Mutation Assay

Key study findings:

- FK228 (romidepsin) was not mutagenic in the mouse lymphoma L5178Y *tk* locus assay in the presence or absence of S9 mix, following a 4-hour exposure.
- FDA/CDER QSAR analysis indicated negative mutagenic results in an *in vitro* mammalian cell gene mutation assay.

Study no.: 775330
Volume and page #: Electronic Submission, Module 4
Conducting laboratory and location: /

b(4)

Date of study initiation: February 17, 2004
GLP compliance: Yes (OECD)
QA report: Yes
Drug, lot #, and % purity: FK228
Lot No.: 005033L
Purity: 99.7%

Methods:

Cell line / method: Mouse lymphoma L5178Y cells / microwell

Metabolic activation system: Aroclor 1254-induced rat liver S9

Concentrations (ng/mL) used in definitive study:

Assay 1 (in the absence of S9 mix):	6.25, 12.5, 25, 50, 100, 150, 250 and 400
Assay 2 (in the presence of S9 mix):	25, 50, 100, 200, 300, 400, 500 and 700
Assay 3 (in the absence of S9 mix):	12.5, 25, 50, 75, 100, 125, 150 and 175
Assay 4 (in the presence of S9 mix):	100, 150, 200, 250, 300, 350, 400 and 450
Assay 5 (in the absence of S9 mix):	50, 75, 100, 125, 150, 175, 200 and 225
Assay 6 (in the absence of S9 mix):	70, 100, 130, 160, 190, 220, 250 and 280

*****ALL EXPERIMENTS UTILIZED A 4 HOUR EXPOSURE PERIOD**

Basis of concentration selection: Assay 1 and 2 were based on initial toxicity tests in the presence and absence of S9. Concentrations for additional assays were based on all previous assay results.

Negative control: Dimethyl sulfoxide

Positive control: Without S9 activation – 250 µg/mL ethyl methanesulphonate (EMS) and 10 µg/mL methyl methanesulphonate (MMS).
With S9 activation – 2.5 µg/mL 3-methylcholanthrene (3-MC).

Incubation and sampling time: 4 hrs in the presence and absence of S9 activation

Analysis:

- Duplicate flasks for each test compound concentration.
- Cells were counted using an illuminated background (light-box) or microscope on day 9 and 14 post-plating.

Criteria for positive results: According to the sponsor, an experiment was considered positive if one or more concentrations were statistically significant and there was a significant linear trend.

Study validity:

- Cloning efficiency of the solvent controls was greater than 65%.
- Mutant frequency (MF) of the solvent control was within the range of the historical solvent control and IWGT acceptance criteria ($50-170 \times 10^{-6}$ using microwells or $35-140 \times 10^{-6}$ using agar).
- Induced mutant frequency (IMF) of the positive controls was at least 300×10^{-6} with at least 40% of the colonies being small colonies.
- An adequate number of concentrations were analyzable.
- Test article was examined up to concentrations that produced $\leq 20\%$ relative total growth (RTG).

Results: See results in sponsor's tables below.

Significance of MF induction based on IWGT criteria using the microwell technique (IMF > global evaluation factor (GEF) of 126).

Assay 1 - In the absence of S9 mix, a weak linear trend for an induction of MF was seen. However, no significant increases in MF were seen. Additionally, the test article was not examined up to concentrations that produced $\leq 20\%$ RTG.

Assay 2 - In the presence of S9 mix, while a weak linear trend for MF induction was noted, no significant increases in MF were seen.

Assay 3 - In the absence of S9 mix, a concentration-related increase in MF was seen. The highest dose (175 ng/mL) showed a significant increase in MF. However, this dose was bordering the acceptable limit of cytotoxicity (<10% RTG).

Assay 4 - This assay was in the presence of S9 mix. Cytotoxicity was seen at concentrations > 400 ng/mL while the heterogeneity factor for the cloning efficiency assay was outside historical acceptance limits at 350 ng/mL. No significant increases in MF were seen at any concentration.

Assay 5 - This assay was in the absence of S9 and due to an unusual pattern of toxicity (RTG >20 even though highly cytotoxic, suspension growth was low, sponsor claims possible dosing errors) this assay was considered inconclusive.

Assay 6 - Due to inconclusive results in assay 5, assay 6 was also completed in the absence of S9 mix. No significant increases in MF were seen at any concentration. Additionally, due to cytotoxicity, this assay did not contain enough analyzable concentrations to be valid. Worth noting the concentration giving 8% RTG is negative.

Conclusion:

Overall, a total of 6 assays were conducted, 4 in the absence of S9 mix, and 2 in the presence of S9 mix (all with a 4 hour exposure period).

In the presence of S9, assay 2 showed a weak linear trend for an induction in MF. If higher concentrations were analyzable, a positive result may have been seen. Thus, this assay was considered inconclusive. For the other assay in the presence of S9, assay 4, the sponsor claims the results were inconclusive due to cytotoxicity at concentrations > 400 ng/mL and the heterogeneity factor for the cloning efficiency assay being outside historical acceptance limits at 350 ng/mL. There were still enough analyzable concentrations however to conclude this assay was negative for a mutagenic response. Therefore, it was concluded that FK228 was not mutagenic in the mouse lymphoma L5178Y *tk* locus assay in the presence of S9 mix.

In the absence of S9, assay 1 showed a weak linear trend for an induction in MF. However, this assay was not valid since the test article was not examined up to concentrations that produced $\leq 20\%$ RTG. Assay 5 was considered inconclusive due to an unusual pattern of cytotoxicity and assay 6 did not contain enough analyzable concentrations to be valid. Assay 3 showed a concentration-related increase in MF and a significant increase in MF at the highest dose (175 ng/mL). This weakly positive result however occurred at a concentration on the threshold (<10% RTG) of an acceptable level of cytotoxicity. Therefore, the result of assay 3 was considered equivocal.

Of all 4 assays conducted in the absence of S9, only one concentration that bordered an acceptable limit of cytotoxicity, from one assay, showed a marginally positive increase in MF, resulting in an equivocal result of the assay. In contrast to this marginally positive response, assay 6 showed concentrations exceeding the acceptable limit of cytotoxicity that were still negative for an induction of MF. In consideration of these data and the overall inconsistency in mutagenicity and lack of reproducibility across the repeated assays, it was concluded that FK228 was not mutagenic in the mouse lymphoma L5178Y *tk* locus assay in the absence of S9 mix.

Since 24-hour exposure in the absence of S9 was not completed, as suggested in the ICH S2(B) guidance when the result of the short treatment without metabolic activation is negative, FDA/CDER quantitative structure activity relationship (QSAR) analysis was utilized to assess if FK228 had structural alerts for genotoxicity. QSAR results are shown following the mouse lymphoma assay results below.

Assay 1:

FK228

**Mouse Lymphoma Assay Mutation Test in the Absence of S9 Mix (4h Exposure)
Summary of Means of Data (Assay 1)**

Chemical	Concentration (ng/mL)	Relative Total Growth %	Mutant Fraction (x 10 ⁻⁶)	IMF (Induced Mutant Fraction x10 ⁻⁶)	Ratio of Small to Large Colonies	Statistical Comparison
DMSO	(100 µL added)	100	69	-	0.81	-
EMS	250,000	80	439	370	0.44	*
MMS	10,000	43	581	512	2.57	*
FK 228	6.25	88	66	-	0.63	-
	12.50	66	77	8	1.06	-
	25.00	56	71	3	0.88	-
	50.00	48	111	43	0.96	-
	100.00	24	113	44	0.59	-
	150.00	NPT	NPT	-	-	-
	250.00	NPT	NPT	-	-	-
	400.00	NPT	NPT	-	-	-

IMF = Mutant fraction of treatment minus mutant fraction of vehicle control group
 * = Significant difference in log mutant fraction compared with vehicle control (P <0.05)
 Test for linear trend of mutant fraction with concentration of FK 228 = significant (P = 0.008)
 NPT = Not Plated - Toxic

Table excerpted from sponsor's package

Assay 2:

FK228

**Mouse Lymphoma Assay Mutation Test in the Presence of S9 Mix (4h Exposure)
Summary of Means of Data (Assay 2)**

Chemical	Concentration (ng/mL)	Relative Total Growth %	Mutant Fraction (x 10 ⁻⁶)	IMF (Induced Mutant Fraction x10 ⁻⁶)	Ratio of Small to Large Colonies	Statistical Comparison
DMSO	(100 µL added)	100	50	-	0.72	-
3-MC	2,500	45	869	819	1.93	*
FK 228	25	89	56	6	0.88	-
	50	85	53	3	0.48	-
	100	75	66	16	2.05	-
	200	42	89	39	1.05	*
	300	18	102	52	0.42	*
	400	NPT	NPT	-	-	-
	500	NPT	NPT	-	-	-
	700	NPT	NPT	-	-	-

IMF = Mutant fraction of treatment minus mutant fraction of vehicle control group
 * = Significant difference in log mutant fraction compared with vehicle control (P <0.05)
 Test for linear trend of mutant fraction with concentration of FK 228 = significant (P = 0.003)
 NPT = Not Plated - Toxic

Table excerpted from sponsor's package

Assay 3:

FK228

**Mouse Lymphoma Assay Mutation Test in the Absence of S9 Mix (4h Exposure)
Summary of Means of Data (Assay 3)**

Chemical	Concentration (ng/mL)	Relative Total Growth %	Mutant Fraction ($\times 10^{-6}$)	IMF (Induced Mutant Fraction $\times 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
DMSO	(100 μ L added)	100	61	-	1.23	-
EMS	250,000	91	444	382	0.65	*
MMS	10,000	48	803	742	3.20	*
FK 228	12.5	NPS	NPS	-	-	-
	25.0	NPS	NPS	-	-	-
	50.0	NPS	NPS	-	-	-
	75.0	47	85	24	0.66	-
	100.0	49	130	68	1.47	*
	125.0	36	114	53	1.03	*
	150.0	21	144	83	2.34	*
	175.0	11	226	165	3.52	*

IMF = Mutant fraction of treatment minus mutant fraction of vehicle control group

* = Significant difference in log mutant fraction compared with vehicle control (P <0.05)

Test for linear trend of mutant fraction with concentration of FK 228 = significant (P <0.001)

NPS = Not Plated - Surplus

Table excerpted from sponsor's package

Assay 4:

FK228

**Mouse Lymphoma Assay Mutation Test in the Presence of S9 Mix (4h Exposure)
Summary of Means of Data (Assay 4)**

Chemical	Concentration (ng/mL)	Relative Total Growth %	Mutant Fraction ($\times 10^{-6}$)	IMF (Induced Mutant Fraction $\times 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
DMSO	(100 μ L added)	100	65	-	0.77	-
3-MC	2,500	26	1257	1192	1.55	*
FK 228	100	NPS	NPS	-	-	-
	150	74	120	55	1.12	*
	200	66	94	29	0.93	-
	250	35	109	44	1.31	*
	300	25	91	26	1.25	-
	350	17	84	19	1.42	†
	400	NPT	NPT	-	-	-
	450	NPT	NPT	-	-	-

IMF = Mutant fraction of treatment minus mutant fraction of vehicle control group

* = Significant difference in log mutant fraction compared with vehicle control (P <0.05)

Test for linear trend of mutant fraction with concentration of FK 228 = significant (P = 0.012)

NPS = Not Plated - Surplus

NPT = Not Plated - Toxic

† = Survival heterogeneity factor (Hs) outside historical acceptance limits. Dose excluded from statistical analysis

Table excerpted from sponsor's package

Assay 5:

FK228

**Mouse Lymphoma Assay Mutation Test in the Absence of S9 Mix (4h Exposure)
Summary of Means of Data (Assay 5)**

Chemical	Concentration (ng/mL)	Relative Total Growth %	Mutant Fraction ($\times 10^{-6}$)	IMF (Induced Mutant Fraction $\times 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
DMSO	(100 μ L added)	100	65	-	1.31	-
EMS	250,000	88	598	532	0.48	*
MMS	10,000	59	936	870	2.26	*
FK 228	50	NPS	NPS	-	-	-
	75	56	106	40	0.70	-
	100	56	105	40	0.93	-
	125	56	91	26	1.51	-
	150	(68)	(88)	(23)	(2.60)	†
	175	(33)	(147)	(81)	(1.50)	†
	200	(33)	(88)	(22)	(0.86)	†
	225	24	105	40	1.65	-

IMF = Mutant fraction of treatment minus mutant fraction of vehicle control group

* = Significant difference in log mutant fraction compared with vehicle control ($P < 0.05$)

Test for linear trend of mutant fraction with concentration of FK 228 = significant ($P = 0.018$)

NPS = Not Plated – Surplus

† = Dose with only one surviving replicate culture. Not included in statistical analysis

Table excerpted from sponsor's package

Assay 6:

FK228

**Mouse Lymphoma Assay Mutation Test in the Absence of S9 Mix (4h Exposure)
Summary of Means of Data (Assay 6)**

Chemical	Concentration (ng/mL)	Relative Total Growth %	Mutant Fraction (x 10 ⁶)	IMF (Induced Mutant Fraction x10 ⁻⁶)	Ratio of Small to Large Colonies	Statistical Comparison
DMSO	(100 µL added)	100	55	-	0.78	-
EMS	250,000	60	642	588	0.30	*
MMS	10,000	45	620	565	1.55	*
FK 228	70	31	117	63	0.71	*
	100	40	121	66	0.54	*
	130	(8)	(119)	(64)	(0.60)	†
	160	(2)	(167)	(112)	(1.32)	†
	190	NPT	NPT	-	-	-
	220	NPT	NPT	-	-	-
	250	NPT	NPT	-	-	-
	280	NPT	NPT	-	-	-

IMF = Mutant fraction of treatment minus mutant fraction of vehicle control group

* = Significant difference in log mutant fraction compared with vehicle control (P <0.05)

† = Test for linear trend of mutant fraction with concentration of FK 228 = significant (P <0.001)

NPT = Not Plated - Toxic

† = Dose level giving unacceptable toxicity (<10% RTG). Results presented for information, but excluded from statistical analysis

Table excerpted from sponsor's package

QSAR Analysis for Genetic Toxicity Results:

Drug name: Romidepsin

Computational Toxicology Software:

The *MC4PC* and *MDL-QSAR* software programs of FDA/CDER were utilized.

Summary:

A computational toxicology analysis was performed on romidepsin. *MC4PC* version 2.1 and *MDL-QSAR* version 2.2 were used for these evaluations. The *MC4PC* and *MDL-QSAR* models use significantly different approaches to identify molecular attributes associated with biological activity, and are intended as complementary systems. Consequently, a positive prediction by one model system is not negated by a negative prediction from the other. The molecular composition of romidepsin was checked for representation (coverage) among the molecular structural features found in the control database (training set).

Results:

Genetic toxicity was predicted using both *MC4PC* and *MDL-QSAR*. The *MC4PC* models for *Salmonella* mutagenicity and micronucleus *in vivo* predicted romidepsin to be negative. *MDL-QSAR* predicted romidepsin to be negative in the *Salmonella* mutagenicity, hprt, chromosome aberrations *in vivo*, and UDS models. *MDL-QSAR* made no definitive call for romidepsin with the micronucleus *in vivo* model.

Conclusion:

The QSAR analysis indicated that romidepsin does not contain a structural alert for genotoxicity in *Salmonella* or the hprt assay.

Study title: Micronucleus test in bone marrow cells of CD rats 0 h I.V. dosing and 24 h and 48 h sampling.

Key study findings:

- FK228 (romidepsin) did not induce micronuclei in bone marrow cells at the MTD in male and female CD rats.

Study no.:	775351
Volume and page #:	Electronic Submission, Module 4
Conducting laboratory and location:	 b(4)
Date of study initiation:	December 17, 2005
GLP compliance:	Yes (OECD)
QA report:	Yes
Drug, lot #, and % purity:	FK228 Lot No.: 005033L

Purity: 99.7%

Methods:

Doses: 0, 0.25, 0.5, and 1 mg/kg in males and 0, 0.75, 1.5, and 3 mg/kg in females

Dose justification: Dosed to MTD

Route / formulation / volume: I.V. / 80% propylene glycol and 20% ethanol in 0.9% saline / 5 mL/kg

Negative control: vehicle

Positive control: 50 mg/kg cyclophosphamide

Schedule: Single dose with sacrifice at 24 or 48 hours post-dosing

Species / strain: Rat / CD

Age: 6-7 weeks

Group designation and dose levels:

Males:

Dose Group	Daily Test Dose	Treatment and Number of Rats		
		Dose (h)	24 h Sample	48 h Sample
Vehicle Control	5 mL PG/ETOH/kg	0	5 M	5M
Low Dose	0.25 mg FK228/kg	0	5 M	
Mid Dose	0.5 mg FK228/kg	0	5 M	
High Dose ^b	1 mg FK228/kg	0	5 M	5 M
Positive Control	50 mg Cyclophosphamide/kg	0	5 M	
Untreated	-	0	3M	

Females:

Dose Group	Daily Test Dose	Treatment and Number of Rats		
		Dose (h)	24 h Sample	48 h Sample
Vehicle Control	5 mL PG/ETOH/kg	0	5 F	5 F
Low Dose	0.75 mg FK228/kg	0	5 F	
Mid Dose	1.5 mg FK228/kg	0	5 F	
High Dose ^b	3 mg FK228/kg	0	5 F	5 F
Positive Control	50 mg Cyclophosphamide/kg	0	5 F	
Untreated	-	0	3F	

^b= An additional contingency group of 5 M + 5 F were dosed and sampled at either 24 h or 48 h as required.

Table excerpted from sponsor's package

Analysis:

- Using the better of the 2 prepared slides for examination, at least 2000 polychromatic erythrocytes (PCE) were scored for micronuclei to determine the frequency of micronucleated cells (MN-PCE). The ratio of PCE to normochromatic cells (NCE) was determined by counting at least 1000 erythrocytes (PCE + NCE) per marrow preparation.

Criteria for positive results: A biologically and significantly relevant increase in the number of micronucleated polychromatic erythrocytes (MN-PCE) that was greater than 10% over the expected historical control range.

Study validity:

- Positive control group, administered via the same route of administration, demonstrated a statistically significant increase of MN-PCE, relative to the concurrent negative control.
- Vehicle control MN-PCE values fell within the historical control data range.
- Highest dose tested was the MTD.

Results:

As detailed in the sponsor's table below, no significant increases in MN-PCE above concurrent control values were seen following any dose or time point.

Conclusion:

I.V. administration of FK228 did not induce micronuclei in bone marrow cells at the MTD of 1 mg/kg in male and 3 mg/kg in female CD rats at 24 h and 48 h sampling times.

FK228
Micronucleus Test in Bone Marrow of CD Rats
Summary of Assessment Data

Treatment	Dose (h)	Sample (h)	Sex	No. of Rats Scored	Erythrocytes				PCE/NCE Mean ± S.D.
					Normochromatic Cells (NCE)		Polychromatic Cells (PCE)		
					No. of MN-NCE	PCE Analysed	No. of MN-PCE	% MN-PCE	
5 mL PG/ETOH kg/day	0	24	M	5	2	10009	3	0.03	0.69 ± 0.15
				5	2	10004	3	0.03	0.59 ± 0.06
	0	24	F	5	0	10002	3	0.03	0.81 ± 0.10
				5	1	10003	2	0.02	0.73 ± 0.09
0.25 mg FK228 /kg/day	0	24	M	5	4	10011	2	0.02	0.63 ± 0.11
				F	5	2	10001	7	0.07
0.5 mg FK228 /kg/day	0	24	M		5	1	10000	1	0.01
				F	5	4	10007	4	0.04
1.5 mg FK228 /kg/day	0	24	M		5	0	10006	2	0.02
				F	5	4	10006	5	0.05
3 mg FK228 /kg/day	0	24	F		5	4	10009	7	0.07
				5	5	10010	9	0.09	0.43 ± 0.08
50 mg Cyclophosphamide/ Kg/day	0	24	M	5	28 α	10002	201 Φ	2.01 Φ	0.54 ± 0.09
				F	5	11 α	10003	90 Φ	0.90 Φ
Untreated	-	24	M		3	0	6004	2	0.03
				F	3	2	6005	1	0.02

PCE = Polychromatic erythrocytes
 MN-PCE = Micronucleated PCE
 NCE = Normochromatic erythrocytes
 MN-NCE = Micronucleated NCE
 Φ = Positive response in PCE
 α = Evident response in NCE

Table excerpted from sponsor's package

2.6.6.5 Carcinogenicity

No carcinogenicity studies were conducted.

2.6.6.6 Reproductive and developmental toxicology

Embryofetal development

Study title: FK228 Preliminary Developmental Toxicity Study in Rats.

Key study findings:

- There were no evident maternal effects of romidepsin.
 - Changes included a decrease in corrected body weight on gestational day 20 (4%) and food consumption at the high dose.
- There were no evident romidepsin effects on embryofetal development.
 - A decrease in fetal weight (9%) at the high dose was noted.

Study no.: IRN25115

Volume #, and page #: Electronic submission, Module 4

Conducting laboratory and location:

b(4)

Date of study initiation: February 8, 2005

GLP compliance: No

QA reports: Yes

Drug, lot #, and % purity: FK228 (FR901228), Batch 005033L, 100.0 %

Methods

Doses: Vehicle control, 0.0032, 0.01, 0.032, 0.1 mg/kg once daily (0.0192, 0.06, 0.192, 0.6 mg/m²/day)

Species/strain: Rat / —:CD(SD)

Number/sex/group: 6

Route, formulation, Volume, and infusion rate: Intravenous injection, FK228 was formulated as a stock solution in 80% (v/v) propylene glycol / 20% (v/v) ethanol which was diluted with 0.9% saline (vehicle control was 80% (v/v) propylene glycol / 20% (v/v) ethanol diluted in saline at the equivalent concentration to that of the FK228 high dose), 5 mL/kg, 2 mL/min.

TK satellite groups: None

Study design: Time-mated female rats were obtained, where day 0 was the day of mating detection. Animals were dosed once daily by intravenous injection via tail vein from GD 6 through GD 16. Females and fetuses were examined after euthanization on GD 20.

Dose Justification: Dose levels were selected based on previous 4-week toxicity study in rats.

Parameters evaluated: Females: viability and clinical signs, body weight, food consumption, gross pathology, reproductive tract weight, # of corpora lutea, implantations, live or dead fetuses, resorptions (early or late)

Fetuses: External examinations, weight

Statistical Analysis: None

b(4)

Results:

Maternal effects –

Mortality (dams): None

Clinical signs (dams):

- 1 – 0.01 mg/kg/day female and 1 – 0.032 mg/kg/day female had sparse hair on GD 16-19

- 1 – 0.1 mg/kg/day female had red staining on ventral abdomen on GD 14
- 1 – 0.0032 mg/kg/day female had reddened thymus

Body weight (dams):

- ↓ body weight gain at 0.032 mg/kg/day (12%) and 0.1 mg/kg/day (24%) female from GD 6-17 compared to controls
- only ↓ of 4% in corrected body weight on GD20 in 0.1 mg/kg/day females compared to control

Group Mean Uncorrected Body Weight (g) (pregnant animals only)

Day of Gestation	Group/Dose Level (mg/kg/day)				
	1 (0)	2 (0.0032)	3 (0.01)	4 (0.032)	5 (0.1)
4	223 ± 9	231 ± 16	233 ± 11	232 ± 9	233 ± 14
6	240 ± 13	244 ± 17	247 ± 8	246 ± 7	247 ± 14
9	249 ± 13	257 ± 21	262 ± 10	255 ± 6	253 ± 17
13	278 ± 16	285 ± 22	292 ± 11	279 ± 7	274 ± 17
17	315 ± 22	321 ± 23	330 ± 13	312 ± 10	304 ± 20
20	361 ± 33	367 ± 29	379 ± 14	357 ± 12	354 ± 23
Weight Gain Days 6-17	75 ± 15	77 ± 6	82 ± 8	66 ± 6	57 ± 9
% of Control	-	103	109	88	76

Table excerpted from sponsor's package

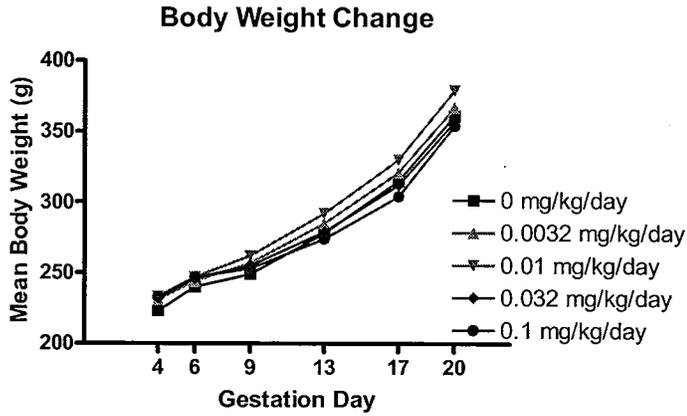
Group Mean Body and Uterine Weight, GD20 Data

	Group/Dose level (mg/kg/day)				
	0	0.0032	0.01	0.032	0.1
Uncorrected mean body weight (GD20)(g)	361	367	379	357	354
Mean total uterus weight (g)	76	76	82	71	77
Corrected mean body weight (GD20)(g)	285	291	297	286	277
(% of control)	-	100	104	100	96

Corrected Weight Gain From GD6 to GD20

	Group/Dose level (mg/kg/day)				
	0	0.0032	0.01	0.032	0.1
GD6 BW (g)	240	244	247	246	247
GD20 corrected BW (g)	285	291	297	286	277
GD6-20 BW gain (g)	45	47	50	40	30

BW: body weight.



Food consumption (dams):

- ↓ food consumption at 0.032 mg/kg/day (15%) and 0.1 mg/kg/day (26%) throughout treatment period compared to controls.

Mean Food Consumption (g) (pregnant animals only)

Day of Gestation	Group/Dose Level (mg/kg/day)				
	1 (0)	2 (0.0032)	3 (0.01)	4 (0.032)	5 (0.1)
4	24	23	25	23	21
5	27	25	26	26	25
6	27	26	26	24	25
7	24	24	24	23	21
8	25	26	26	21	19
9	24	25	24	22	20
10	26	25	25	22	19
11	26	25	24	22	18
12	28	27	27	25	19
13	28	27	25	23	20
14	28	27	26	22	19
15	29	26	27	23	19
16	34	33	32	30	28
17	32	29	30	26	23
18	34	32	32	29	26
19	31	31	30	29	27
20	26	27	28	25	24
Days 7-17	304	294	290	259	225
% of Control	-	97	95	85	74

Table excerpted from sponsor's package

Toxicokinetics: Not conducted

Uterine effects –

Terminal necropsy:

- Slight ↓ (9%) in fetal weight at 0.1 mg/kg/day
- Unremarkable uterine effects

Reproduction data

	0	0.0032	0.01	0.032	0.1
	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
Females mated	6	6	6	6	6
Pregnant females	6	6	6	6	6
Aborted	0	0	0	0	0
Premature birth	0	0	0	0	0
Pregnancy frequency (%)	100	100	100	100	100
Pregnant females at C-section	6	6	6	6	6
w/ viable fetuses	6	6	6	6	6
w/ total fetal death	0	0	0	0	0
Corpora lutea (total)	86	83	86	75	83
#/dam (mean)	14.3	13.8	14.3	12.5	13.8
Implantation sites	76	78	81	72	81
#/dam (mean)	12.7	13.0	13.5	12.0	13.5
Pre-implantation loss (%)	12	6	6	4	2
Post-implantation loss (total)	2	1	0	1	2
#/animal (mean)	0.3	0.2	0	0.2	0.3
% (mean)	3	1	0	1	2
Live fetuses (total)	74	77	81	71	79
#/dam (mean)	12.7	12.8	13.5	11.8	13.2
% impl./dam (mean)	97	99	100	99	98
Dead fetuses (total)	0	0	0	0	0
Resorptions (early) (total)	2	1	0	1	2
#/dam (mean)	0.3	0.2	0	0.2	0.3
% (mean)	3	1	0	1	2
Resorptions (late) (total)	0	0	0	0	0
#/dam (mean)	0	0	0	0	0
% (mean)	0	0	0	0	0
Mean total uterus weight (g)	76	76	82	71	77
Mean fetal weight (g)	3.91	3.82	3.80	3.81	3.57
(% of control)	-	98	97	97	91

Embryo-fetal effects –

Externally visible abnormalities:

- Unremarkable

Study title: FK228 Developmental Toxicity Study in Rats.

Key study findings:

- There were no evident maternal effects of romidepsin.
 - BW gains were comparable among all groups when corrected for uterine weight.
 - Statistically significant ↓ uncorrected (13%) body weight gain was seen in 0.06 mg/kg/day females from GD 6-17 compared to controls. This reduction was secondary to reduced uterine weight.
- There were no evident romidepsin effects on embryofetal development.
 - Decreases in fetal weight (not statistically significant – 5%) at the mid and high dose were noted.

Study no.: IRN25501

Volume #, and page #: Electronic submission, Module 4

Conducting laboratory and location:

b(4)

Date of study initiation: April 29, 2005

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: FK228 (FR901228), Batch 005033L, 98.6 %

Methods

Doses:	Vehicle control, 0.006, 0.02, 0.06 mg/kg once daily (0.036, 0.12, 0.36 mg/m ² /day)	
Species/strain:	Rat, — CD(SD)	b(4)
Number/sex/group:	20	
Route, formulation, volume, and infusion rate:	Intravenous injection, FK228 was formulated as a stock solution in 80% (v/v) propylene glycol / 20% (v/v) ethanol which was diluted with 0.9% saline (vehicle control was 80% (v/v) propylene glycol / 20% (v/v) ethanol diluted in saline at the equivalent concentration to that of the FK228 high dose), 5 mL/kg, 2 mL/min.	
TK Satellite groups:	None	
Study design:	Time-mated female rats were obtained in three batches on gestation day (GD) 3, 2 or 1, respectively, where day 0 was the day of mating detection. Animals were dosed once daily by intravenous injection via tail vein	

from GD 6 through GD 16. Females and fetuses were examined after euthanization on GD 20.

Dose Justification: Dose levels were selected based on preliminary developmental toxicity data from study IRN25115 and relevant toxicological data. The dose range finding study, IRN25115, tested the effects of daily intravenous injection of 0.0032, 0.01, 0.032 or 0.1 mg/kg FK228 in pregnant rats from gestation day 6 through 16. This study reported a reduction in maternal body weight (↓ 24% and 12%) not adjusted for uterine weight and food consumption (↓ 26% and 15%) at 0.1 and 0.032 mg/kg/day, respectively, when compared to concurrent controls. A reduction in mean fetal weight was seen at 0.1 mg/kg/day (↓ 9%) compared to concurrent controls.

Parameters evaluated:

- Females: Viability and clinical signs, body weight, food consumption, gross pathology, reproductive tract weight, # of corpora lutea, implantations, live or dead fetuses, resorptions (early or late)
- Fetuses: External and visceral examinations, weight, skeletal abnormalities and ossification, soft tissue abnormalities, (malformations and variations)

Statistical Analysis:

- Maternal body weight gains – Analysis of variance
- Mean litter and fetal weights – Kruskal-Wallis non-parametric test (2-sided against control at 5% significance)
- No other parameters were analyzed statistically

Results

Maternal effects –

Mortality (dams): None

Clinical signs (dams):

0.06 mg/kg/day:

- 1 female had sparse hair on GD 20 and hairloss on abdomen at necropsy
- 1 female had hunched body on GD 14 and left head tilt on GD 14-16

0.02 mg/kg/day:

- 1 female had bald areas on GD 6-16 and 20 and hairloss on forelimbs at necropsy

Body weight (dams):

- Statistically significant ↓ uncorrected (13%) body weight gain in 0.06 mg/kg/day females from GD 6-17 compared to controls
- BW gains were comparable among all groups when corrected for uterine weight.

Group Mean Uncorrected Body Weight (g) (pregnant animals only)

Day of Gestation	Group/Dose Level (mg/kg/day)			
	1 (0)	2 (0.006)	3 (0.02)	4 (0.06)
4	242 ± 23	236 ± 31	235 ± 25	232 ± 17
6	256 ± 25	252 ± 34	247 ± 26	244 ± 17
9	273 ± 24	269 ± 37	262 ± 27	256 ± 18
13	298 ± 28	297 ± 41	289 ± 28	278 ± 20
17	339 ± 33	336 ± 48	329 ± 32	316 ± 24
20	385 ± 37	376 ± 54	373 ± 38	361 ± 27
Gain Days 6-17†	82 ± 11	84 ± 18	82 ± 10	71 ± 12**
% of Control	-	102	100	87

† = Analysed statistically

** = P<0.01

Table excerpted from sponsor's package

Group Mean Body and Uterine Weight, GD20 Data

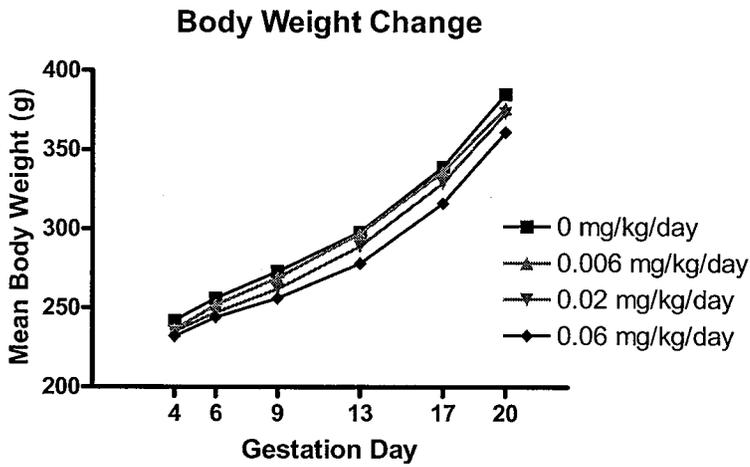
	0 mg/kg/day	0.006 mg/kg/day	0.02 mg/kg/day	0.06 mg/kg/day
Uncorrected mean body weight (GD20)(g)	385	376	373	361
Weight gain (GD6-17) (g)	82	84	82	71 (↓13%)
Mean total uterus weight (g)	85	74	78	74 (↓13%)
Corrected mean body weight (GD20)(g)	300	302	295	287
(% of control)	-	100	98	96

Corrected Weight Gain From GD6 to GD20

	Group/Dose level (mg/kg/day)			
	0	.006	.02	.06
GD6 BW (g)	256	252	247	244
GD20 corrected BW (g)	300	302	295	287
*GD6-20 BW gain (g)	*44	*50	*48	*43

BW: body weight.

*BW gains were comparable among all groups when corrected for uterine weight



Food consumption (dams):

- ↓ (16%) food consumption at 0.06 mg/kg/day throughout treatment period compared to controls (not analyzed statistically)

Mean Food Consumption (g) (pregnant animals only)

Day of Gestation	Group/Dose Level (mg/kg/day)			
	1 (0)	2 (0.006)	3 (0.02)	4 (0.06)
4	26	24	25	24
5	25	25	25	26
6	27	26	25	26
7	26	25	24	23
8	26	26	24	22
9	27	27	24	22
10	27	26	24	22
11	26	27	24	22
12	28	28	26	23
13	28	29	26	23
14	27	28	26	23
15	27	27	27	24
16	28	29	26	25
17	33	32	27	25
18	32	32	31	28
19	30	30	29	29
20	27	26	25	27
Days 7-17	303	304	278	254
% of Control	-	100	92	84

Table excerpted from sponsor's package

Toxicokinetics: Not conducted

Uterine effects –

Terminal necropsy:

- Slight ↓ in fetal weight at ≥ 0.02 mg/kg/day (not statistically significant)
- Unremarkable uterine effects

Reproduction data

	0 mg/kg/day	0.006 mg/kg/day	0.02 mg/kg/day	0.06 mg/kg/day
Females mated	20	20	20	20
Pregnant females	20	20	19	20
Aborted	0	0	0	0
Premature birth	0	0	0	0
Pregnancy frequency (%)	100	100	95%	100
Pregnant females at C-section	20	20	19	20
w/ viable fetuses	20	20	19	20
w/ total fetal death	0	0	0	0
Corpora lutea (total)	285	282	271	265
#/dam (mean)	14.25	14.10	14.26	13.25
Implantation sites	279	253	257	265
#/dam (mean)	13.95	12.65	13.52	13.25
Pre-implantation loss (%)	2	10	5	5
Post-implantation loss (total)	11	11	14	9
#/dam (mean)	0.6	0.6	0.7	0.5
% (mean)	4	4	5	4
Live fetuses (total)	268	242	243	243
#/dam (mean)	13.4	12.1	12.8	12.2
% impl./animal (mean)	96	96	95	96
Males (total)	126	120	114	126
#/dam (mean)	47	50	47	52
Females (total)	141	122	129	117
#/dam (mean)	53	50	53	48
Dead fetuses (total)	0	0	0	0
Resorptions (early) (total)	11	10	13	9
#/dam (mean)	0.6	0.5	0.7	0.5
% (mean)	4	4	5	4
Resorptions (late) (total)	0	1	1	0
#/dam (mean)	0	0.1	0.1	0
% (mean)	0	0.4	0.4	0
Mean total uterus weight (g)	85	74	78	74 (↓13%)
* Mean fetal weight (g)	3.96	3.83	3.78	3.78
(% of control)	-	96	95	95

* analyzed statistically (p > 0.05)

Embryo-fetal effects –

(Not analyzed statistically)

Visceral alterations:

- ↓ incidence (fetal and litter) of locally thinned tendinous regions of the diaphragm with protrusion of the median liver lobe at 0.06 mg/kg/day compared to control
 - no historical control data provided for this finding

Skeletal alterations:

- ↓ incidence (fetal and litter) of incomplete ossification in skull and vertebral arches at ≥ 0.006 mg/kg/day compared to control
 - Incidence of incomplete ossification was higher in the control group for this study than in the 7 previous studies and 1 study following this one (i.e. outside the historical control range)
 - Values for ≥ 0.02 mg/kg/day groups are lower than historical control range

Incidence of fetal abnormalities and variants in the current study

Abnormality/Variant	0	0.006	0.02	0.06
	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
Incidence of Fetuses (Litters)				
<u>Visceral</u>				
Tendinous region of diaphragm locally thinned with minimal protrusion of median liver lobe	8(6)	3(3)	9(7)	1(1)
Tendinous region of diaphragm locally thinned with protrusion of median liver lobe	6(5)	2(2)	3(3)	0
Total number examined viscerally	268(20)	242(20)	243(19)	243(20)
<u>Skeletal</u>				
Incomplete Ossification				
Sacral vertebral arches	29(10)	15(8)	6(5)	5(4)
Cervical vertebral arches	2(2)	2(2)	1(1)	0
≥ 4 skull bones	6(5)	3(2)	0	2(2)
≤ 3 skull bones	46(18)	16(10)	10(7)	10(8)
Total number examined	135(20)	120(20)	120(19)	120(20)

Historical control ranges compared to control group from the current study

	Incidence of incomplete ossification Foetus (litter)	
	≤ 3 skull bones	SVA's
Background Control Range prior to study	19 (9) – 42 (15)	9 (7) – 21 (13)
Control data from a study performed 1 month later	14 (10)	10 (6)
Study Values at:		
0 mg/kg/day	46 (18)	29 (10)
0.006 mg/kg/day	16 (10)	15 (8)
0.02 mg/kg/day	10 (7)	6 (5)
0.06 mg/kg/day	10 (8)	5 (4)

SVA: sacral vertebral arches
Table excerpted from sponsor's package

2.6.6.7 Local tolerance

Study title: FK228 Local Lymph Node Assay

Key study findings:

- FK228 was considered a sensitizer in mice and therefore has the potential to cause delayed contact hypersensitivity.

Study no.:

IRN25565

Volume and page #:

Electronic Submission, Module 4

Conducting laboratory and location:

/

b(4)

Date of study initiation:

April 26, 2005

GLP compliance:

Yes (OECD)

QA report:

Yes

Drug, lot #, and % purity:

FK228

Lot No.: 005033L

Purity: 98.6%

Methods:

Doses: 0, 0.025, 0.05, 0.1, and 0.25%

Dose justification: Original dose justification based on data obtained from the acute rat oral toxicity study (509484) and were anticipated not to cause systemic toxicity or excessive local irritation. Amended dose levels were chosen based on results from animal groups 2-4 which were sacrificed due to severity in clinical signs.

Route / formulation / volume: Subcutaneous / acetone:olive oil 4:1 (v/v) / 25µL

Schedule: Daily for 3-days
 Species / strain: Mice/ CBA/Ca
 Age: 6 -8 weeks

Group designation and dose levels:

Group	Treatment	Dose Level (%)	Animal
5	Acetone:Olive oil (4:1, v/v)	0	21-25
6	FK 228	0.025	26-30
7		0.05	31-35
8		0.1	36-40
9		0.25	41-45

Table excerpted from package

Lymph Node Stimulation Index: Results below are expressed as a Stimulation Index (SI), which was obtained by dividing the disintegrations per minute (DPM) of each test item group by the DPM of the vehicle control group. The SI for the vehicle control group was one. A positive response included an SI ~3, with consideration of statistical significance, when appropriate. This test method was validated in Feb 2005 using the positive control hexylcinnamaldehyde at 10, 25, and 50%.

Unique study design or methodology: Study was originally designed to test animals groups 1-4 with 0, 1, 2.5, and 5% dose levels of FK228, respectively. However, animals in groups 2-4 were sacrificed on days 4 and 5 due to severity in clinical signs. Therefore, animal groups 5-9 were added to the study to test lower concentrations of FK228, as shown above.

Observations and times:

Clinical signs: Daily
Body weights: Day 1 and Day 6
Lymph node
Stimulation index: Day 6

Results:

Mortality: At dose levels of 1, 2.5, and 5% FK228, all animals were sacrificed on days 4 and 5 due severity in clinical signs. These clinical signs included hunched appearance, subdued behavior, skin cold to touch, thin appearance, shallow and labored respiration, piloerection, and rolling gait.

Clinical signs: In animals receiving 0.025, 0.05, 0.1, and 0.25% FK228, clinical signs included a hunched appearance and subdued behavior.

Body weight: Unremarkable

Lymph Node
Stimulation Index: As shown below, stimulation indices for 0.025%, 0.05%, 0.1% and 0.25% FK 228 were calculated to be 1.5, 2.4, 2.8

and 2.2, respectively. Rounding the stimulation index up, the 0.1% FK228 dose level was considered a sensitizer. This sensitizing effect appeared to be dose responsive from 0.025-0.1 %. The decreased stimulation index in animals treated with the highest dose (0.25%) was likely due to an increased severity in clinical signs.

Conclusion:

Under the conditions of this study, FK228 was considered a sensitizer in mice and therefore has the potential to cause delayed contact hypersensitivity.

FK228

Local Lymph Node Assay

Individual Scintillation Counts (DPM)

Treatment	Dose Level (%)	Animal	Disintegrations per minute (DPM)	Group Mean (DPM)	Stimulation Index (SI)
Acetone:Olive oil (4:1, v/v)	0	21	8283	10208	1
		22	16788		
		23	9602		
		24	-		
FK 228	0.025	25	6158	14807	1.5
		26	16084		
		27	10372		
		28	26874		
		29	4226		
	0.05	30	16477	24935	2.4
		31	24872		
		32	18040		
		33	21656		
		34	26862		
	0.1	35	33243	28555	2.8
		36	22724		
		37	28805		
		38	41355		
		39	23068		
	0.25	40	26825	22681	2.2
41		31064			
42		28721			
43		17105			
44		23760			
		45	12754		

- Sample lost during preparation

Table excerpted from package

Study title: FK228 Acute Dermal Irritation Test in Rabbits

Brief Summary:

This GLP (OECD) study, conducted by _____ investigated the acute dermal irritation potential of FK228 following a single 4 hour exposure to 0.5g of FK228 in two male New Zealand White rabbits. FK228 was applied to the upper and lower region of the dorsal trunk under a water moistened semi-occlusive patch. Sites of administration were examined for irritation approximately 1, 24, 48, and 72 hours following patch removal. Since no erythema or edema was noted at any timepoint in either animal, FK228 was not considered to be irritating to rabbit skin.

b(4)

2.6.6.8 Special toxicology studies

Study title: Myelotoxicity of Cyclic Peptide (NSC-630176) to Human, Canine, and Murine CFU-GM Progenitor Cells (Hipple Study; August 4, 1994).
Reviewed by W. David McGuinn, Ph.D.

Brief Summary:

Researchers exposed mouse, dog and human bone marrow cells (CFU-GM) to 0.001, 0.01, 0, 1, 1.0, 3.0, or 10 nM concentrations of Depsipeptide for 12 to 14 days. They estimated the IC₅₀, IC₇₅ and IC₉₀ values by log-linear extrapolation between the two nearest flanking data points. The following table shows these estimated values.

Species	IC ₇₅ (nM)	IC ₉₀ (nM)
mouse	5.5	9.0
dog	1.5	2.2
human	1.5	6.0

The sponsor states that these values were not significantly different because none were greater than 10 fold different, but they did not establish this lack of significance statistically.

Study title: *In Vitro* Study of Cyclic Peptide (NSC-630176) Induced Toxicity in Cardiac Myocyte Cultures Derived from Fetal Rat, Newborn Dog, and an Immortalized Human Cell Line (SRI-Chm-93-362-8000-XLI; August 31, 1994). Reviewed by W. David McGuinn, Ph.D.

Brief Summary:

Researchers at _____ exposed cardiac myocytes derived from neonatal rats (Fisher 344 and Sprague Dawley), juvenile Beagle dog and immortalized human fetal SV₄₀ transformed cells (W1) to depsipeptide (0.1-100 pM). Doxorubicin (0.001-10 pM) and minoxidil (0.1-100 pM) were used as positive controls. Myocytes were exposed for 6 hours to the test compounds. They determined cell culture viability by the MTT (3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) dye

b(4)

exclusion assay and by determining extracellular LDH levels. Both assays were done at 24, 48, and 72 hours after the initiation of treatment (30, 50, and 70 hr. for dog cell cultures). Depsipeptide was cytotoxic and caused a concentration dependent release of LDH in all three myocyte models. Depsipeptide was more cytotoxic to myocytes derived from all three species than minoxidil or doxorubicin, the positive controls. The following table shows representative results from these experiments at 24 hours. In the MTT assay, human fetal cells seemed to be more sensitive than the other species. In the LDH assay they appeared less sensitive. The assays both showed poor reproducibility and there were probably inter-species differences in the response to the assays. Nevertheless, the assays both showed that DPT is toxic to cardiomyocytes and even more so than doxorubicin and minoxidil (not shown).

MTT Dye Exclusion assay, cells were exposed to the test drug for 6 hr.

% inhibition at 24 hours of myocytes

DPT Concentration μ M	F344 rat		Beagle (30 hr)		Human	
		sd		sd		sd
0	0	38.4	0	3	0	6.8
0.1	34.9	29	36.3	25	73.6	2.5
1	56	17.8	50.2	1.3	99.3	1
10	95.7	11.8	19.8	33.6	100	1.3
100	100	19.5	87	2.6	100	0.6
Doxirubicin						
Doxirubicin Concentration μ M						
0	0	15.5	0	3	0	11.3
10	26.9	20.7	77.2	9.9	82.2	2.7

LDH Release assay, cells were exposed to the test drug for 6 hr.

Mean LDH B-B* units at 24 hours

DPT Concentration μ M	F344 rat		Beagle		Human	
		sd		sd		sd
0	88.2	15.1	221	6.6	40	2.9
0.1	98.5	16.3	246	5.7	114	3.2
1	102.5	38.9	276	11	107	6
10	248.5	16.3	350	10	94	4.7
100	253	0	500 ^a		64	6.9
Doxirubicin Concentration μM						
0.001	71.5	16.5	214	2.1	43	0.5
10	137.5	10.6	253	3.5	40	4.5

*Berger-Broida Units/ml: the amount of LDH that reduces 4.8×10^{-4} μ mol of pyruvate per minute at 25C

^a n = one, due to limits of the assay range linearity.

Study title: Cardiotoxicity Study of NSC-630176D in Female Nude Mice (SRI Study SRI-Chm-91-1145; November 1991). Reviewed by W. David McGuinn, Ph.D. and slightly modified for this NDA review.

Key study findings:

- Histopathological findings in cardiac tissue included mild-moderate focal inflammation, minimal-mild focal mineralization, and minimal neutrophilic infiltration.

Methods:

Species / strain: 138 female NCR-nu mice

Drug: romidepsin (no lot given)

Vehicle: deionized water (q3hx8, q4dx3 IP schedule); deionized water containing 25% PEG 400 (all other schedules)

Dose & Schedule:

“Toxicology Study”

- 1) IV q4dx3 (0, 2.4, 3.6, 5.3, or 8.0 mg/kg/day); Necropsy day 12, 5 mice per group
- 2) IV qdx5 (0, 0.64, 0.96, 1.44, or 2.16 mg/kg/day); Necropsy day 8, 5 mice per group
- 3) IP q4dx3 (0, 1.6, 2.4, 3.6, or 5.3 mg/kg/day); Necropsy day 13, 5 mice per group

4) IP q3hx8, q4dx3 (0, 0.2, 0.3, 0.45, or 0.66 mg/kg/dose); Necropsy day 14, 5 mice per group.

“Efficacy Study”

5) IV q4dx3 (0, 2.4, 3.6, 5.3, or 8.0 mg/kg/day); Necropsy day 37, 9 mice per group

6) IV qdx5 (0, 1.44, or 2.16 mg/kg/day); Necropsy day 37, 1 or 2 mice per group

7) IP q4dx3 (5.3 mg/kg/day); Necropsy day 37, 1 mouse

The report briefly describes a second portion of this study in which 38 tumor bearing mice were treated (Groups 5, 6 and 7). Histopathology results (heart only) for the “Efficacy Study” were included in tables and summaries so they were included in this review.

GLP compliance: No

QA report: No

Observations and times:

Clinical signs: 3 to 5 days after the final dose (groups 1-4)

Hematology: 3 to 5 days after the final dose, sera from two animals/dose group were used to determine CK and LDH isoenzyme activity (groups 1-4)

Organ weights: At necropsy (groups 1-4)

Histopathology: Heart (groups 1-7) and spleen and femurs (groups 1-4)

Results:

Mortality: Group 2 – 1/5 HD mice, day 6
Group 4 – All 0.45 and 0.66 mg/kg/dose mice

Body weight: Group 1 – Mean weight decreases in all treated animals, 15% in 8 mg/kg/day group
Group 2 – Mean weight decreases in all treated animals, 20% in 2.16 mg/kg/day group
Group 3 – No toxicologically significant changes
Group 4 – Mean weight decreases in all treated animals, > 20% in 0.45 and 0.66 mg/kg/dose groups

Hematology: -Mild anemia (10-27% decrease compared to controls) was seen on all schedules (Days 8-14) except in mice on the IP q4dx3 schedule.
-Leukopenia was seen in mice on the IV q4dx3 schedule (43-67% decrease on Day 12) and on the IP q3hx8, q4dx3 schedule (48-62% decrease on Day 14).
-Lymphopenia was seen only in mice on the IP schedules; a 58-81% decrease on Day 14 in mice on the q4dx3 schedule

and a 74-88% decrease on Day 14 in mice on the q3hx8, q4dx3 schedule.

-Thrombocytopenia on IV dosing schedules, 17-35% decrease in platelets on Day 12 on the q4dx3 schedule, 54-92% decrease on the qdx5 schedule, Platelets decreased ~28% in mice given 2.4 mg/kg/day on the IP q3hx8, q4dx3 dosing schedule.

Clinical Chemistry: -LDH-1 and LDH-2 elevated in 2/2 mice each receiving 1.6 and 2.4 mg/kg/day. LDH-3 increased in 1/2 mice on 5.3 mg/kg/day, IP q4dx3 schedule.
-LDH isozyme changes in mice given drug on the IV qdx5 dosing schedule were limited to increases in LDH-3 in 1/2 mice in the 0.96 mg/kg/day and 2/2 mice each in the 1.44 and 2.16 mg/kg/day dose groups.

Organ weights: Group 1 – No changes
Group 2 – Decreased absolute and relative heart weight in 2.16 mg/kg/day mice
Group 3 – Decreased relative heart weight in 3.6 and 5.3 mg/kg/day mice
Group 4 – Decreased absolute heart weight in 0.3 and 0.66 mg/kg/dose mice, but an increased relative heart weight in 0.3 mg/kg/dose mice due to weight loss
There were also dose related changes in absolute and relative spleen weight

Bone Marrow: Group 1 – Dose related decrease in total femoral count (64% of control in 8 mg/kg/day); myeloid/erythroid ratio decreased with increasing dose; erythroid precursors were about 60% of control values in all treated groups; myeloid precursors were 9 to 17% higher than controls in 2.4, 3.6, 5.3 mg/kg/day animals while they were 14% lower in 8 mg/kg/day animals.
Group 2 – Dose related decrease in total femoral count (63% of control in 2.16 mg/kg/day); myeloid/erythroid ratio decreased with increasing dose; erythroid precursors were about 25%, 8%, 13% and 8% of control values in 0.64, 0.96, , 1.44 and 2.16 mg/kg/day respectively; lymphocyte counts were 69, 77, 65, and 80 percent lower than controls in the

0.64, 0.96, , 1.44 and 2.16 mg/kg/day animals respectively.

- Group 3 – No differences in total femoral counts but the myeloid/erythroid ratios were 88, 144, 480, and 452 percent higher than controls in 1.6, 2.4, 3.6 and 5.3 mg/kg/day animals respectively. These changes were due to decreases in erythroid precursors, which were 61, 46, 33 and 38 per of control values in the 1.6, 2.4, 3.6 and 5.3 mg/kg/day animals, respectively. Myeloid precursors were approximately 25, 14, 59, and 55 percent higher than those of the control mice.
- Group 4 – No differences in total femoral counts but the myeloid/erythroid ratios were 91 and 891 percent higher than controls in 0.2 and 0.3 mg/kg/dose animals respectively. These changes were due to decreases in erythroid precursors, which were 64 and 34 percent per of control values in the 0.2 and 0.3 mg/kg/dose animals, respectively. Lymphocyte counts were 50 and 17 percent lower of controls in the 0.2 and 0.3 mg/kg/dose animals respectively. The other animals died and were not evaluated.

Histopathology:

Cardiac-

- Group 1 – No findings
- Group 2 – Mild to moderate focal inflammation 2/5 2.16 mg/kg/day mice; focal necrosis 1/5 2.16 mg/kg/day mice mice; minimal focal mineralization 1/5 mice
- Group 3 – No findings
- Group 4 – No findings
- Group 5 – Neutrophilic infiltration 1/9 2.4 mg/kg/day, 3/9 5.3 mg/kg/day, 2/9 8 mg/kg/day
- Group 6 – Minimal neutrophilic infiltration 1/1 1.44 mg/kg/day, 1/2 2.16 mg/kg/day, mild focal chronic inflammation 1/2 2.16 mg/kg/day, mild focal mineralization 1/2 2.16 mg/kg/day, minimal focal necrosis 1/2 2.16 mg/kg/day.
- Group 7 – Minimal neutrophilic infiltration

Bone Marrow-

- Group 1 – Mild hyperplasia, 5/5 2.4 mg/kg/day, moderate hyperplasia 5/5 3.6 mg/kg/day, 5/5 5.3 mg/kg/day, 5/5 8 mg/kg/day

- Group 2 – Mild hyperplasia, 5/5 0.64 mg/kg/day, moderate hyperplasia 5/5 0.96 mg/kg/day, 5/5 1.44 mg/kg/day, 4/4 2.16 mg/kg/day
- Group 3 – Mild hyperplasia, 2/5 2.4 mg/kg/day, moderate hyperplasia 5/5 3.6 mg/kg/day, 5/5 5.3 mg/kg/day
- Group 4 – Mild hyperplasia, 1/4 0.45 mg/kg/dose, moderate hyperplasia 2/5 0.66 mg/kg/dose; mild cellular depletion 2/4 0.45 mg/kg/dose, 3/5 0.66 mg/kg/dose.
- Spleen-
- Group 1 – Mild hematopoietic cellular proliferation 5/5 2.4 mg/kg/day, moderate hematopoietic cellular proliferation 5/5 3.6 mg/kg/day, 5/5 5.3 mg/kg/day, 5/5 8 mg/kg/day
- Group 2 – Mild hematopoietic cellular proliferation 5/5 0.64 mg/kg/day, moderate hematopoietic cellular proliferation 5/5 0.96 mg/kg/day, 5/5 1.44 mg/kg/day, 4/5 2.16 mg/kg/day; cellular depletion 1/5 2.16 mg/kg/day
- Group 3 – Mild hematopoietic cellular proliferation 4/5 2.4 mg/kg/day, 1/5 3.6 mg/kg/day, moderate hematopoietic cellular proliferation 5/5 2.4 mg/kg/day, 5/5 3.6 mg/kg/day, 4/5 5.3 mg/kg/day; cellular depletion 1/5 5.3 mg/kg/day
- Group 4 – Mild hyperplasia, 1/4 0.45 mg/kg/dose, moderate hyperplasia 2/5 0.66 mg/kg/dose; mild cellular depletion 2/4 0.45 mg/kg dose, 3/5 0.66 mg/kg/dose.

2.6.6.9 Discussion and Conclusions

Romidepsin is an HDAC inhibitor. Recently, HDAC4, a class II HDAC, has been shown to regulate the survival of retinal neurons in the mouse (Chen and Cepko, 2009). However, no ocular toxicity was observed in animal studies.

The non-clinical program of romidepsin identified the target organ/tissues of toxicity to be the bone marrow, thymus, spleen, liver, heart, and ♂ and ♀ reproductive systems. Reduced calcium, iron, phosphate, and potassium, and hyperglycemia were noted in some studies. Female-specific toxicities of romidepsin included pituitary hyperplasia, increased cholesterol levels, and atrophy of the ovary, uterus, mammary gland and vagina. Considering an *in vitro* binding assay determined that romidepsin competes with β -estradiol for binding to estrogen receptors, these female-specific toxicities may be a result of estrogen signaling modulation. Based on the mechanism of action of romidepsin, its effects on female reproductive organs in toxicology studies, and its binding to estrogen receptors, romidepsin is potentially embryo-fetal toxic. However, no embryo-fetal toxicity was seen with this drug under the conditions tested, with the

exception of a slightly reduced (↓5%) fetal weight at high dose. There was no indication of maternal toxicity. Body weight gains (GD 6 to GD 20) were comparable among dams in all groups, when corrected for the uterine weight. Likely animals were not exposed to adequate levels of romidepsin. Presently there are no data to show dams had adequate exposure to romidepsin; e.g. lack of TK data and gross pathology findings. Moreover, an indirect assessment of systemic exposure was not possible either, mainly due to lack of TK data in relevant general toxicology studies or discordance in dose/ schedules between reproductive toxicology and general toxicology studies that contained TK data. Romidepsin was not mutagenic or clastogenic in the *in vitro* and *in vivo* assays tested.

└ in the drug product: b(4)

The final ISTODAX drug product contains a └ not currently listed in ICH Q3C. The proposed specification limit for mg/vial with a maximum allowed dose of 40 mg romidepsin (four vials). This would result in a maximum dose of └ per patient. Since the safety of I.V. administered └ has not been adequately established, the total level of └ per patient should not exceed levels previously given to patients in clinical trials, i.e. └ Therefore, based on information provided by the applicant on patient exposure of up to 33.8 mg romidepsin in the GPI-04-0001 study (approximately 3.4 vials for 10 mg romidepsin per vial) and the recommended dose of 14 mg/m², the level of: └ in the final drug product may not exceed └ 10 mg romidepsin vial. b(4)

└ may have been present in non-clinical batches of romidepsin and could have contributed to toxicities observed, particularly at high doses for which CNS effects were evident in rats and dogs. There is no information in the package regarding the levels of └ in batches of the drug used for the non-clinical studies. b(4)

2.6.6.10 Tables and Figures

See text of review for pertinent tables and figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

Acute Toxicity Studies					
Species	Route / Duration	N/sex/ dose	Dose (mg/kg)	Dose (mg/m ²)	Significant findings
Rat	IV Single dose	5	5.1	30.6	30.6 mg/m ² : 90% mortality; tonic convulsion; foci on thymus; red coloration of lung 21.6 mg/m ² : 60% mortality; tonic convulsion; foci on thymus; red coloration of lung; petechiae in glandular stomach ≤ 15.6 mg/m ² (+ vehicle control): no toxicities
			3.6	21.6	
			2.6	15.6	
			1.9	11.4	
			1.4	8.4	
			1	6.0	
Dog	IV Single dose	1	1	20	20 mg/m ² : ↓ body weight 1-2 days postdose; ↓ motility; irregular rhythm of heart and respiration; emesis; tremor; ↓ body temperature; mucosal
			0.1	2	
			0.01	0.2	

					congestion; ↓ lymphocytes, calcium, and potassium; ↑ AST, glucose; ↓ thymic weight; thymic atrophy; ↓ cortical lymphocytes of thymus <u>2 mg/m²</u> : ↓ body weight 1-2 days postdose (♀) and motility; emesis; thymic atrophy; ↓ thymic weight; ↓ cortical lymphocytes of thymus <u>0.2 mg/m²</u> : no toxicities
<i>Repeat Dose Toxicity Studies</i>					
Species	Route / Duration	N/sex/dose	Dose (mg/kg)	Dose (mg/m ²)	Significant findings
Mouse	IV Once or twice weekly x 4 weeks	10 Males	8 5.3 3.6	24 15.9 10.8	<u>24 mg/m²/day</u> : 40% mortality (dosed once per week); 20% mortality (dosed twice per week); injection site swelling and necrosis; ↑ neutrophils; splenic cellular proliferation and necrosis; bone marrow cellular hyperplasia and depletion, hepatic hematopoietic foci and fatty degeneration, testicular degeneration, and thymic depletion <u>15.9 mg/m²/day</u> : injection site swelling and necrosis; ↓ RBC; ↑ platelets <u>10.8 mg/m²/day</u> : injection site swelling and necrosis; ↓ RBC; ↑ platelets
Rat	IV Daily x 4 weeks	24 (12 for 0.0032 and 0.010 mg/kg)	0.1 0.032 0.01 0.0032	0.6 0.192 0.06 0.0192	<u>0.6 mg/m²/day</u> : ↓ WBC and triglycerides; ↑ AST, lactate dehydrogenase, creatinine phosphokinase, total bilirubin, urine volume; ↓ weight of salivary glands, thymus, adrenal glands, liver, seminal vesicle, prostate, ovary; ↑ weight and size of spleen; clouding of the thymic parenchyma; degeneration and necrosis of lymphocyte and increase in # tingible body macrophages in thymus, spleen, mandibular and mesenteric lymph nodes; increased extramedullary hematopoiesis in spleen; degeneration and necrosis of lymphocyte in bone marrow; maturation arrest of ovarian follicle <u>0.192 mg/m²/day</u> : 1 ♂ mortality; ↓ WBC and triglycerides; ↑ AST, lactate dehydrogenase, creatinine phosphokinase, total bilirubin, urine volume; ↓ weight of salivary glands, thymus, adrenal glands; degeneration and necrosis of lymphocyte and increase in # tingible body macrophages in thymus, spleen, mandibular and mesenteric lymph nodes <u>0.06 mg/m²/day</u> : ↓ WBC <u>0.0192 mg/m²/day</u> : ↓ WBC
Rat	IV Weekly for 3 of every 4 weeks x 26 weeks	240	1 0.33 0.1	6 1.98 0.6	<u>6 mg/m²/day</u> : ↓ WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, and triglycerides; ↑ AST, ALT, reticulocytes, and cholesterol (♀); ↓ weight of uterus, weight (♂) and size of thymus; ↑ pituitary weight (♀); hematopoietic hypocellularity in bone marrow; pigment deposits in bone marrow, liver, and spleen; cystic degeneration and mononuclear cell infiltration in liver; lymphoid atrophy/necrosis in spleen and thymus; lymphoid hyperplasia in thymus; degeneration/atrophy in seminiferous epithelium of

					testis; tubular dilatation in kidney; hyperplasia in pars distalis of ♀ pituitary; atrophy of mammary gland, ovary, uterus, and vagina <u>1.98 mg/m²/day</u> : 1 ♀ mortality with blot clot in ventral cervical region extending into the ventral thoracic region; ↓ WBC, neutrophils, lymphocytes, monocytes, eosinophils, and basophils; ↑ AST, ALT and reticulocytes; ↓ weight (♂) and size of thymus; ↑ pituitary weight (♀); hematopoietic hypocellularity in bone marrow; pigment deposits in bone marrow, liver, and spleen; lymphoid atrophy/necrosis in spleen and thymus; lymphoid hyperplasia in thymus; tubular dilatation in kidney; hyperplasia in pars distalis of ♀ pituitary; atrophy of mammary gland, ovary, uterus, and vagina; mucification of vagina epithelial; ovarian cyst <u>0.6 mg/m²/day</u> : 1 ♂ mortality, cause unknown; ↓ WBC, lymphocytes, monocytes, eosinophils, and basophils; ↓ thymus weight (♂); hematopoietic hypocellularity in bone marrow; pigment deposits in liver, and spleen; lymphoid atrophy/necrosis and hyperplasia in thymus; tubular dilatation in kidney; atrophy of mammary gland, ovary, uterus, and vagina; mucification of vagina epithelial; ovarian cyst
Dog	IV Daily x 4 weeks	24	0.032 0.01 0.0032	0.64 0.20 0.064	<u>0.64 mg/m²/day</u> : ↓ WBC and AST/ALT; ↓ erythroblasts and cellular phagocytosis; increase in tingible body macrophages in thymus, spleen, and lymph nodes; degeneration and necrosis in thymus; lymphocyte degeneration and necrosis in spleen and lymph nodes <u>0.20 mg/m²/day</u> : lymphocyte degeneration and necrosis in lymph nodes <u>0.064 mg/m²/day</u> : lymphocyte degeneration and necrosis in lymph nodes
Dog	IV Twice weekly x 4 weeks	2 (male)	2 1	40 20	<u>40 mg/m²/day</u> : injection site swelling; 26% ↓ in body weight; marked ↓ in erythroblastic cells; ↓ lymphocytes and monocytes; ↑ neutrophils, lactate dehydrogenase, blood urea nitrogen; ↓ iron, potassium, calcium, phosphate, fibrinogen, AST parameters, ↑ QT interval (-20 to 70 ms, average of 28 ms above baseline) and ST segment; small thymus; dark red foci on heart, kidney, and lung; white coloration and thickening of the epicardium and pericardium <u>20 mg/m²/day</u> : marked ↓ in erythroblastic cells; ↓ lymphocytes and monocytes; ↑ neutrophils; ↓ iron, potassium, calcium, phosphate, fibrinogen, AST parameters, ↑ QT interval (0 to 90 ms, average of 33 ms above baseline); small thymus
Genetic Toxicology Studies					
Type of Study		Concentration / Dose			Results
Bacterial mutagenesis assay (Ames)		17, 50, 167, 500, 1667, and 5000 µg per plate			Negative in the presence or absence of S9 mix

Mouse lymphoma cell mutation assay	6.5-700 ng/mL		Negative in the presence or absence of S9 mix		
<i>In vivo</i> micronucleus test in bone marrow cells of rats	0.25, 0.5, 1 mg/kg in males 0.75, 1.5, and 3 mg/kg in females		Negative		
<i>Reproductive and Developmental Toxicity Studies</i>					
Species	Route / Duration	N/dose group	Dose (mg/kg)	Dose (mg/m ²)	Significant findings
Rat	IV daily from gestation day 6-16	6	0.1 0.032 0.01 0.0032	0.6 0.192 0.06 0.0192	<u>0.6 mg/m²/day</u> : ↓ fetal weight (9%) <u>≤ 0.192 mg/m²/day</u> : no toxicities
Rat	IV daily from gestation day 6-16	20	0.06 0.02 0.006	0.36 0.12 0.036	<u>0.36 mg/m²/day</u> : ↓ fetal weight (5%), ↓ uterine weight of 13%. <u>0.12 mg/m²/day</u> : ↓ fetal weight (5%) <u>0.036 mg/m²/day</u> : no toxicities

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Pharmacology, safety pharmacology, pharmacokinetic/ ADME, and toxicology studies with romidepsin were conducted in *in vitro* systems and/or in animal species. The receptor binding assay in conjunction with results of toxicology study suggests the potential for romidepsin to interfere with estrogen pathway. The reproductive toxicology studies were deemed inadequate. The following comments were communicated to the applicant and will be the basis of PMR studies:

- The reproductive toxicology studies conducted in rats did not result in significant maternal or embryo-fetal toxicity, and are therefore deemed inadequate to assess potential risk to a developing embryo or fetus associated with romidepsin treatment. Adequate embryo-fetal risk assessment should be provided. Embryo-fetal toxicology studies are typically conducted in two species. If romidepsin causes embryo-fetal lethality or is teratogenic in one species, a study in the second species may not be warranted. Provide dates for protocol submission, study completion, and submission of the final study report.
- Romidepsin was shown to bind to estrogen receptors *in vitro*. Toxicology studies suggested romidepsin modulation of estrogen signaling as evidenced by female-specific findings (e.g. atrophy of mammary gland, uterus, ovary and vagina; pituitary hyperplasia; elevated cholesterol and triglycerides). Therefore, romidepsin may increase the risk of estrogen-agonist-like serious risks, such as uterine cancer, clotting, and cardiovascular disease, or the risk of estrogen-antagonist-like serious risks, such as osteoporosis and fracture. In addition, romidepsin may interfere with hormonal contraceptives, resulting in high-risk pregnancies. Please assess estrogenic and anti-estrogenic effects of romidepsin. The assessment could be based on clinical or non-clinical data. Provide dates for protocol submission, study completion, and submission of the final study report

This application had a deficiency regarding the level of: _____ present in the drug product. An acceptable level of: _____ /10 mg romidepsin vial (or less) based on the highest amount delivered to patients in clinical trials and the recommended romidepsin dose of 14 mg/m². The following deficiency was communicated to the applicant:

b(4)

“For safety reasons, the level of: _____ in the drug product may not exceed _____ mg per 10 mg romidepsin vial. This is based on patient exposure during clinical trials and a maximum proposed dose of 14 mg/m². Provide revised drug product specifications which include a limit on: _____ to not exceed _____ per 10 mg romidepsin vial.”

b(4)

This deficiency was resolved as the applicant agreed to limit the amount of _____ in the drug product to _____ per 10 mg romidepsin vial.

b(4)

Considering that the toxicity profile of I.V. administered _____ has not been adequately characterized and this _____ may have contributed to adverse reactions observed in patients, the applicant needs to assess the toxicity profile of I.V. administered _____ in nonclinical study(ies). The following was communicated to the applicant and will be the basis of the PMR:

- The final ISTODAX drug product contains the _____
_____ This _____ is not currently listed in ICH Q3C, and the safety of I.V. administered _____ has not been adequately established. The amount of _____ delivered to patients in clinical trials was _____ of the dose of your drug product, and may have contributed to toxicities seen in clinical trials. Please characterize toxicities associated with I.V. administered _____ in at least one non-clinical toxicology study, using an appropriate animal species, and propose a safe clinical dose based on your data. Provide dates for protocol submission, study completion, and submission of the final study report.

b(4)

Unresolved toxicology issues: None.

Recommendations: We recommend approval of romidepsin (ISTODAX) for the proposed indication, i.e. treatment of cutaneous T-cell lymphoma in patients who have received at least one prior systemic therapy.

Suggested labeling:

A separate review will be conducted if deemed necessary. Currently, the nonclinical information presented in the label is supported by this review document.

Signatures:

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes X No _____

APPENDIX/ATTACHMENTS

None

Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-22393

ORIG-1

GLOUCESTER
PHARMACEUTICA
LS INC

ROMIDEPSIN FOR INFUSION

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ALEXANDER H PUTMAN
10/19/2009

TODD R PALMBY
10/19/2009

HALEH SABER
10/20/2009

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

NDA/BLA Number: 22-393

Applicant: Gloucester
Pharmaceuticals, Inc

Stamp Date: 1/12/09

Drug Name: Romidepsin

NDA/BLA Type: Standard

On initial overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		Appears to be acceptable.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Based on agreements made at end of phase 2 and pre-NDA meetings.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		Appears to be acceptable.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	This is a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _____ YES _____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Alexander Putman
2/26/2009 04:54:45 PM
CSO

Haleh Saber
2/27/2009 01:32:00 PM
PHARMACOLOGIST