

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**22-393**

**PHARMACOLOGY REVIEW(S)**

## MEMORANDUM

Istodax (romidepsin)

**Date:** October 20, 2009

**To:** File for NDA 22-393

**From:** John K. Leighton, PhD, DABT

Associate Director for Pharmacology/Toxicology  
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review and labeling provided by Drs. Putman and Palmby and team leader concurrence provided by Dr. Saber. I concur with their conclusions that Istodax may be approved and with the Post Marketing Recommendations.

Application  
Type/Number

Submission  
Type/Number

Submitter Name

Product Name

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NDA-22393

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ORIG-1

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GLOUCESTER  
PHARMACEUTICA  
LS INC

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ROMIDEPSIN FOR INFUSION

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/s/  
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JOHN K LEIGHTON  
10/20/2009

## MEMORANDUM

**Date:** October 20, 2009  
**From:** Haleh Saber, Ph.D.  
Pharmacology/Toxicology Acting Team Leader  
Division of Drug Oncology Products  
**To:** File for NDA #22,393  
Istodax® (romidepsin) for injection  
**Re:** Approvability for Pharmacology and Toxicology

Istodax® (Romidepsin) is a histone deacetylase (HDAC) inhibitor, indicated for the treatment of cutaneous T-cell lymphoma (CTCL) in patients who have received at least one prior systemic therapy. In pharmacology studies, romidepsin modulated the expression pattern of several genes involved in tumor progression or suppression. Modulation of gene expression was associated with an increased incidence of histone acetylation on the promoter region of the genes studied, further indicating HDAC inhibition. The anti-cancer activity of romidepsin was demonstrated *in vitro* in several human tumor cell lines including leukemia cells and in *in vivo* using mouse xenograft models of murine and human tumors.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. Based on the general toxicology studies, toxicities associated with romidepsin include effects in the hematopoietic system, liver, heart, and male and female reproductive systems. In addition, decreased calcium, potassium, iron, and phosphate, and hyperglycemia were observed in some studies. Radioactivity detected in the CNS indicates that romidepsin or its metabolites can cross the blood-brain barrier. A \_\_\_\_\_ was likely present in some or all of the non-clinical batches of romidepsin and could have contributed to CNS-related toxicities observed in some nonclinical studies. CNS effects are observed after inhalation of T

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Based on the findings in the general toxicology studies, male and female fertility may be compromised by romidepsin treatment. Female-specific findings in toxicology studies (pituitary hyperplasia; elevated cholesterol, and atrophy of mammary gland, uterus, ovary and vagina, and ovarian maturation arrest) may be secondary to modulation of the estrogen pathway. Romidepsin was shown to compete with  $\beta$ -estradiol for binding to estrogen receptors *in vitro*. Considering that modulation of the estrogen pathway may be of risk to patients, the applicant has been asked to assess estrogenic and anti-estrogenic effects of romidepsin, as a post-marketing requirement (PMR). Drug-related effects in the male reproductive system included testicular degeneration, and decreased seminal vesicle and prostate weights. Of note, the 26-week toxicology study, simulating the recommended clinical schedule of dosing and in which effects on male and female reproductive organs were reported, did not contain recovery groups. The effects in both female (maturation arrest of ovarian follicles and decreased weight of ovaries) and male

(reduced weight of seminal vesicles and prostate) reproductive organs were reversible after a 2 week drug-free period, when romidepsin was administered daily to rats for a total of 28 days.

Embryofetal toxicities associated with the use of romidepsin were not adequately assessed in the definitive reproductive toxicology study; the highest dose tested resulted in approximately 5% reduction in fetal weight; reduction was not statistically significant. No maternal toxicity was observed at this dose and toxicokinetic data were not available to assess adequacy of exposure in pregnant animals. Based on the mechanism of action of romidepsin and its effects on reproductive organs described in toxicology studies, possibly related to the modulation of the estrogen pathway, romidepsin is potentially embryo-fetal toxic. Therefore, a Category D has been proposed for romidepsin. The applicant is asked to conduct embryo-fetal toxicities post-marketing, to assess the potential risk to a developing fetus.

A chronic toxicology study was conducted in one species only. Because, toxicities in short-term studies in conjunction with effects observed in the chronic study were predictive of toxicities observed in patients, the lack of the chronic toxicology study in a second species is not of an issue at this time. If post-marketing data indicate new or unexpected adverse effects in patients, additional toxicology studies with romidepsin may be needed.

Romidepsin was not genotoxic *in vitro* or *in vivo* under conditions tested.

The drug product contains the  $\Gamma$  \_\_\_\_\_ at a relatively high level. The applicant had originally proposed a specification level of \_\_\_\_\_ /10 mg romidepsin vial with a maximum allowed dose of 40 mg romidepsin (4 vials) or up to \_\_\_\_\_ per patient. This specification was not supported by clinical or nonclinical data. The applicant agreed to reduce the specification to \_\_\_\_\_ / 10 mg vial based on the highest dose of romidepsin and \_\_\_\_\_ administered to patients in the GPI-04-0001 study and the recommended romidepsin dose of 14 mg/m<sup>2</sup>. The specification of \_\_\_\_\_ / 10 mg romidepsin vial is acceptable for the marketing of the drug based on available clinical data and the benefit:risk consideration. Since high amount of this \_\_\_\_\_ will be delivered to patients and the toxicity profile of I.V. administered \_\_\_\_\_ has not been fully established, the applicant is asked to conduct toxicology study(ies) post-marketing to assess the safety of I.V. administered \_\_\_\_\_ and to propose a safe dose of the \_\_\_\_\_. Based on the results obtained additional regulatory action(s) may be taken.

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The following comments were communicated to the applicant and are the basis of studies for PMR:

- 1) 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.

2) 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

3) 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 [ ] 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.

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The applicant indicated that they would assess the estrogenic/anti-estrogenic effects of romidepsin in nonclinical studies.

The nonclinical studies were reviewed in detail by Dr. Alexander Putman and Dr. Todd Palmby. The nonclinical findings are summarized in the "Executive Summary", "Discussion and Conclusions", and "Overall Conclusions and Recommendations" of the review, and reflected in the product label.

**Recommendation:** I concur with Drs. Putman and Palmby that Istodax® (romidepsin) may be approved for the proposed indication. There are no outstanding pharmacology/toxicology issues to preclude approval of the drug. The applicant needs to conduct additional nonclinical studies as Post Marketing Requirements (PMR). The studies consist of a further assessment of potential embryofetal toxicity and estrogenic/anti-estrogenic effects of romidepsin and toxicity of I.V. administered [ ] Additional regulatory actions may be taken after review of the study data from the Post Marketing Requirements.

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/s/

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HALEH SABER  
10/20/2009



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

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22,393  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 1/12/09  
PRODUCT: **ISTODAX® (Romidepsin)**  
INTENDED CLINICAL POPULATION: **Cutaneous T-cell lymphoma in patients  
who have received at least one prior  
systemic therapy**  
SPONSOR: **Gloucester Pharmaceuticals, Inc.**  
DOCUMENTS REVIEWED: **Electronic Submission, Module 4**  
REVIEW DIVISION: **Division of Drug Oncology Products**  
PHARM/TOX REVIEWER: **Alexander Putman, Ph.D.  
Todd Palmby, Ph.D.**  
PHARM/TOX SUPERVISOR: **Haleh Saber, Ph.D.**  
DIVISION DIRECTOR: **Robert Justice, MD**  
PROJECT MANAGER: **Lisa Skarupa**  
  
Date of review submission to DARRTS: 19 October 2009

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## EXECUTIVE SUMMARY

### I. Recommendations

#### A. Recommendation on approvability

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

#### B. Recommendation for nonclinical studies

Additional non-clinical studies of romidepsin are required. The following were communicated to the applicant of this NDA and will be the basis of post-marketing requirement (PMR) studies.

- 1) 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.
- 2) 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.
- 3) The final ISTODAX drug product contains  $\text{F}$  <sup>1</sup>  
\_\_\_\_\_. 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.

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C. Recommendations on labeling

The recommendations to the sponsor's proposed labeling were discussed within team meetings and communicated to the sponsor. Information in the nonclinical sections of the label reflects findings of studies reviewed within this document.

**II. Summary of nonclinical findings**

A. Brief overview of nonclinical findings

Romidepsin is a histone deacetylase (HDAC) inhibitor. The ability of romidepsin to modulate gene expression was demonstrated by the suppression of VEGF mRNA and the induction of p21/WAF1/Cip1 mRNA levels in cancer cells. In addition, anticancer activity of romidepsin was demonstrated in xenograft models of human tumors.

Romidepsin and its metabolites were widely distributed to many tissues in rats following intravenous administration. Distribution occurred rapidly, with a  $T_{max}$  of  $\leq 5$  minutes for most tissues. Radioactivity detected in the CNS indicates that romidepsin or its metabolites can cross the blood brain barrier (BBB). Romidepsin was highly metabolized by rat, dog and human liver microsomes *in vitro* and in rats following intravenous administration, resulting in over 20 metabolites, with most present at less than 5% of total radioactive material in the plasma, bile, feces or urine. The majority of romidepsin hepatic metabolism was accomplished by CYP3A4. The biliary (66%)/fecal (75%) excretion was the major route of elimination, with a smaller amount excreted through the urine (20%).

Safety pharmacology studies showed effects on the central nervous and respiratory systems. These included increased heart rate and respiration rate, effects on equilibrium and gait, and central nervous system excitation after a single dose of 6 mg/m<sup>2</sup> romidepsin in rats. Dogs had an increase in spontaneous motor activity at 2-6 mg/m<sup>2</sup>, increased body temperature at  $\geq 6$  mg/m<sup>2</sup>, and increase in respiration rate ( $\leq 83\%$ ) after a single romidepsin dose of 20 mg/m<sup>2</sup>. In acute toxicology studies, when comparable or higher doses of romidepsin were used (up to 2.2-fold the human dose based on the body surfaced area), results were similar. Toxicities in the acute studies included tonic convulsion, tremor, irregular breathing, irregular heart rhythm, and staggering gait in rats and/or dogs. Cardiovascular effects were seen in the safety pharmacology and toxicology studies; see below and Section II C, for detailed information.

The nonclinical findings have shown the target sites of toxicity with romidepsin to be the hematopoietic system (leucopenia, lymphocytopenia, thrombocytopenia, thymic atrophy, degenerative necrosis of lymphocytes in spleen, lymph nodes, and bone marrow, and bone marrow hypocellularity), liver (increased liver enzymes), heart (irregular rhythm, QTc prolongation, dark/red spots on the heart, thickening of epicardium and pericardium, retention of dark/red fluid in the pericardium cavity), GI tract, and reproductive systems. In addition, decreased calcium, potassium, iron, phosphate, and hyperglycemia were observed in some studies. Female-specific findings in toxicology studies (e.g. pituitary hyperplasia; elevated cholesterol, and atrophy of mammary gland, uterus, ovary and

vagina) may be secondary to modulation of the estrogen pathway. Romidepsin was shown to bind to estrogen receptors *in vitro*. Clinical signs of neurotoxicity were observed in some studies. The potential for drug-induced neurotoxicity was further confirmed by the distribution study (drug or its metabolites crossed the blood brain barrier) and by the *in vitro* binding assay indicating binding of romidepsin to neurokinin<sub>2</sub> receptor.

Romidepsin was not mutagenic *in vitro* in the bacterial reverse mutation assay (Ames test) or the mouse lymphoma assay. Romidepsin was not clastogenic in an *in vivo* rat bone marrow micronucleus assay when tested to the maximum tolerated dose of 1 mg/kg (6 mg/m<sup>2</sup>) in males and 3 mg/kg (18 mg/m<sup>2</sup>) in females. These doses were up to 1.3-fold the recommended human dose, based on body surface area.

Male and female fertility may be compromised by romidepsin treatment. Administration of romidepsin in toxicology studies resulted in testicular degeneration in male rats and mice, and decreased seminal vesicle and prostate weights at sub-therapeutic exposures of romidepsin. In the 26-week rat toxicology study, effects in the male reproductive system were observed at 0.33 mg/kg of romidepsin. The AUC<sub>0-inf</sub> in animals at this dose was 1.9% the exposure in patients who received the recommended dose of romidepsin. Rat 26-week toxicology study resulted in atrophy in the ovary, uterus, vagina and mammary gland of female rats, starting at the lowest dose tested (0.1 mg/kg). This dose had AUC<sub>0-inf</sub> values that were 0.3% of those in patients receiving the recommended dose of 14 mg/m<sup>2</sup>/dose. Maturation arrest of ovarian follicles and decreased weight of ovaries were observed in a 4-week daily study in rats at doses that were approximately 30% the estimated human daily dose based on body surface area.

Embryofetal toxicities associated with the use of romidepsin were not adequately assessed in the dose range finding or the definitive reproductive toxicology studies; no maternal or embryo-fetal toxicities were observed. Based on the mechanism of action and the potential interference with the estrogen pathway, romidepsin may cause fetal harm when administered to a pregnant woman. In an animal reproductive toxicology study, pregnant rats received daily intravenous romidepsin during the period of organogenesis up to a dose of 0.06 mg/kg/day (0.36 mg/m<sup>2</sup>/day). This dose in rats is approximately equivalent to 18% the estimated human daily dose based on body surface area and resulted in 5% reduction in fetal weight.

#### B. Pharmacologic activity

Romidepsin is an inhibitor of histone deacetylase (HDAC). HDACs catalyze the removal of acetyl groups from acetylated lysine residues in histone and non-histone proteins, resulting in the modulation of gene expression. The ability of romidepsin to modulate gene expression was demonstrated by the suppression of VEGF mRNA and the induction of p21/WAF1/Cip1 mRNA levels in cancer cells. These genes are potentially important in cancer pathophysiology, considering their involvement in angiogenesis and cell cycle progression, respectively. Romidepsin-induced modulation of gene expression was associated with an increased incidence of histone acetylation on the promoter region of

these genes, further indicating HDAC inhibition. Additional effects of romidepsin included a time-dependent increase in the apoptotic population, G1 and G2/M cell cycle arrest, and romidepsin-induced differentiation to CD1b+ and CD14+ cells in human lymphoma U-937 cells. Experiments in NIH3T3 cells transfected with the Ha-ras oncogene demonstrated that romidepsin did not form interstrand DNA-DNA cross-links, break single strand DNA, or inhibit DNA synthesis in isolated nuclei. Romidepsin did however cause G0/G1 cell cycle arrest and decreased c-myc mRNA expression in these cells.

The anti-cancer activity of romidepsin was demonstrated *in vitro* in several human tumor cell lines including leukemia, renal, prostate, colon, lung, stomach, and breast cancer. Multiple experiments conducted *in vivo* using mouse xenograft models demonstrated that romidepsin inhibited the growth of tumors derived from murine (leukemia, lymphoma, melanoma, and colon) and human (colon, lung, breast, prostate, and renal) cancer.

#### C. Nonclinical safety issues relevant to clinical use

Clinically, toxicities associated with romidepsin included nausea, vomiting, anorexia, infection, anemia, thrombocytopenia, hypocalcemia, hyperglycemia, hypophosphatemia, hypokalemia, neutropenia, lymphopenia, hepatotoxicity (increased liver enzymes), and cardiovascular effects, e.g. arrhythmia, and ECG T-wave and ST-segment changes. Most of these toxicities were seen in nonclinical studies.

Cardiovascular toxicity is a recent concern with HDAC inhibitors and was seen clinically and in animal studies. In non-clinical toxicology studies in dogs, QTc prolongation was observed following 1 mg/kg or 2 mg/kg (20 or 40 mg/m<sup>2</sup>, respectively) romidepsin, when dosing was twice weekly, for 4 weeks. The QTc interval increase was an average of 33 ± 25 ms (10%) or 28 ± 24 ms (12%) above baseline following 1 or 2 mg/kg romidepsin, respectively. Additional findings following 2 mg/kg dosing in dogs included an increased ST segment after dosing-day 20 and dark red spots, white coloration, and thickening of the epicardium and pericardium with retention of dark red fluid in the pericardial cavity. In a separate safety pharmacology study in dogs, cardiovascular changes included a decrease in systolic (≤ 12%), diastolic (≤ 16%) and mean blood pressures (≤ 13%) following doses ≥ 0.1 mg/kg (2 mg/m<sup>2</sup>). Concurrent increases in heart rate (≤ 52%) and shortening of the RR interval (≤ 34%) were noted. QTc prolongation following 1 mg/kg (20 mg/m<sup>2</sup>) was also confirmed in this single-dose study; the increase was 6-8% at 6 hrs post-dose. Together with *in vitro* data (i.e. the action potential assessment), these animal studies provide evidence that romidepsin has the potential to cause a clinically relevant impact on the cardiovascular system. Of note, romidepsin-related cardiomyocyte injury was detected in a special toxicology study, which indicated ↑LDH isozymes correlating with microscopic damage, e.g. focal necrosis and inflammation in the heart.

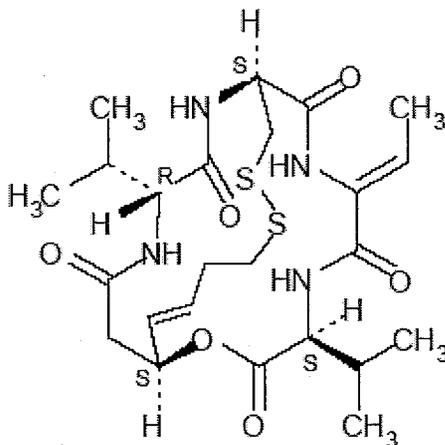
An *in vitro* binding assay determined that romidepsin competes with β-estradiol for binding to estrogen receptors. This is likely related to toxicities observed specifically in female rats, such as atrophy in the ovary, uterus, vagina and mammary gland, pituitary

hyperplasia, and elevated cholesterol. No further characterization of estrogenic or anti-estrogenic effects of romidepsin has been conducted. Therefore, women of childbearing potential need to be advised that romidepsin may reduce the effectiveness of estrogen-containing contraceptives. Additionally, romidepsin may cause fetal harm. Women should be advised to avoid becoming pregnant while being treated with this drug.

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

<b>NDA #:</b>	22,393
<b>Review #:</b>	1
<b>Sequence #/date/type of submission:</b>	000/January 12, 2009/Electronic
<b>Information to applicant:</b>	Yes; deficiency and PMRs have been communicated to the applicant
<b>Sponsor and/or agent:</b>	Gloucester Pharmaceuticals, Inc. One Broadway, 14th Floor Cambridge, MA 02142 USA
<b>Manufacturer for drug substance:</b>	Ben Venue Laboratories, Inc. 300 Northfield Road Bedford, Ohio 44146
<b>Reviewer name:</b>	Alexander Putman, Ph.D. Todd Palmby, Ph.D.
<b>Division name:</b>	Division Drug Oncology Products
<b>Review completion date:</b>	October 16, 2009
<b>Drug:</b>	
Trade name:	ISTODAX®
Generic name:	Romidepsin
Code name:	FK228, FR901228, NSC-630176(D)
Chemical name:	(1 <i>S</i> ,4 <i>S</i> ,7 <i>Z</i> ,10 <i>S</i> ,16 <i>E</i> ,21 <i>R</i> )-7-ethylidene-4,21-bis(1- methylethyl)-2-oxa-12,13-dithia-5,8,20,23- tetraazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22- pentone
CAS registry number:	128517-07-7
Molecular formula:	C <sub>24</sub> H <sub>36</sub> N <sub>4</sub> O <sub>6</sub> S <sub>2</sub>
Molecular weight:	540.71 g/mol
Structure:	



**Relevant INDs/NDAs/DMFs:** IND 63573

**Pharmacologic class:** Histone deacetylase (HDAC) inhibitor

**Intended clinical population:** Cutaneous T-cell lymphoma (CTCL) in patients who have received at least one prior systemic therapy.

**Clinical formulation:** white crystalline powder, see composition below.

**Drug Product Composition**

Component	Function	Quantity per Vial	Reference to Quality Standard
Romidepsin	Active ingredient	10 mg	In-house specification
Povidone	Bulking agent	20 mg	USP
		—	NF/EP
		N/A	NF/EP
		N/A	USP/EP
		N/A	ACS

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**Diluent Composition**

Component	Function	Quantity per Vial <sup>1</sup>	Reference to Quality Standard
Propylene glycol	—	—	USP
Dehydrated alcohol	—	—	USP

<sup>1</sup>Based upon a minimum fill volume of 2.0 mL per vial  
 Tables excerpted from sponsor's package

**Route of administration:** Intravenous (I.V.)

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:**

**Pharmacology:**

Study #	Study title
CRE010130	Cytotoxic activity of FK228 to human cell lines
CRR910025	Anti-tumor Effects and Toxicity of FR901228 in animals
CRE040013	Effects of FK228, a novel histone deacetylase inhibitor, on human lymphoma U-937 in vitro and in vivo
CRE040012	Anti-tumor efficacy of FK228, a novel histone deacetylase inhibitor, depends on the effect on expression of angiogenesis factors
CRE010131	Anti-tumor activity of FK228 against human tumor xenografts in nude mice

**Safety Pharmacology:**

Study #	Study title
GLR030774	Safety pharmacology study of FR901228 – effects on the central nervous system in rats
GLR030533	Safety pharmacology study of FR901228 - hERG assay
GLR030460	Safety pharmacology study of FR901228 (2): Action potential measurement by microelectrode techniques
DVRS-003	In vitro investigation of the interaction potential of dithiothreitol reduced romidepsin for potassium channels
GLR030775	Safety pharmacology study of FR901228 – Effects on the central nervous, cardiovascular and respiratory systems in dogs
GLR030404	Investigation of binding of FR901228 for several receptors

**Pharmacokinetics:**

Study #	Study title
CRD040009	Non-clinical pharmacokinetics: Distribution and excretion of radioactivity after single intravenous administration of [ <sup>14</sup> C]-FR901228 in rats
CRD040011	Non-clinical pharmacokinetics: <i>in vitro</i> protein binding of FR901228 in rats, dogs, humans, and human serum proteins
CRD040012	Non-clinical pharmacokinetics: <i>in vitro</i> transfer of FR901228 to blood cells in rats, dogs and humans
CRD040010	Drug metabolism: Metabolism in rats after intravenous administration of [ <sup>14</sup> C]-FR901228
CRD030200	Drug metabolism: Species differences in hepatic metabolism of FR901228 between rats, dogs, and humans
CRD040013	Drug metabolism: Identification of <i>in vitro</i> and <i>in vivo</i> metabolites of FR901228 by liquid chromatography-tandem mass spectrometry
CRD030201	Drug metabolism: Identification of human P450 enzymes involved in the metabolism of FR901228

**Single-dose Toxicity:**

Study #	Study title
GLR910291	Acute toxicity study of FR901228 in rats after intravenous dosing
GLR910294	Acute toxicity study of FR901228 in dogs after intravenous dosing

**Repeat-dose Toxicity:**

Study #	Study title
SRI-Chm-92-653-6464-LXXXIX	Acute multiple dose toxicity study of cyclic peptide (NSC-630176D) in male mice
GLR910293	Four-week intravenous toxicity study of FR901228 in rats with 4-week recovery study
501650	FK228: A 26-week intravenous injection toxicity study in the albino rat
GLR910296	Four-week intravenous toxicity study of FR901228 in dogs with 4-week recovery study
GLR910298	Four-week intermittent intravenous toxicity study of FR901228 in dogs

**Genetic Toxicology:**

Study #	Study title
IRN25107	FK228 testing for mutagenic activity with <i>Salmonella typhimurium</i> TA 1535, TA 100, TA 1537 and TA 98 and <i>Escherichia coli</i> WP2uvrA
IRN25590	FK228 mouse lymphoma cell mutation assay
IRN25561	FK228 micronucleus test in bone marrow

**Reproductive and Developmental Toxicology:**

Study #	Study title
IRN25115	FK 228 preliminary developmental toxicity study in rats
IRN25501	FK 228 developmental toxicity in rats

**Local Tolerance:**

Study #	Study title
IRN25565	FK228 local lymph node assay
IRN25564	FK228 acute dermal irritation test in rabbits

**Special Toxicology:**

Study #	Study title
Hipple CRC	Myelotoxicity of cyclic peptide (NSC-630176) to human, canine, and murine CFU-GM progenitor cells
SRI-CBE- 93-362- 8000-XLI	<i>In Vitro</i> 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- 91-1145- 6464- LXXX	Cardiotoxicity study of NSC-630176D in female nude mice

**Studies not reviewed within this submission:**

**Pharmacology:**

study #	study title
/	

b(4)

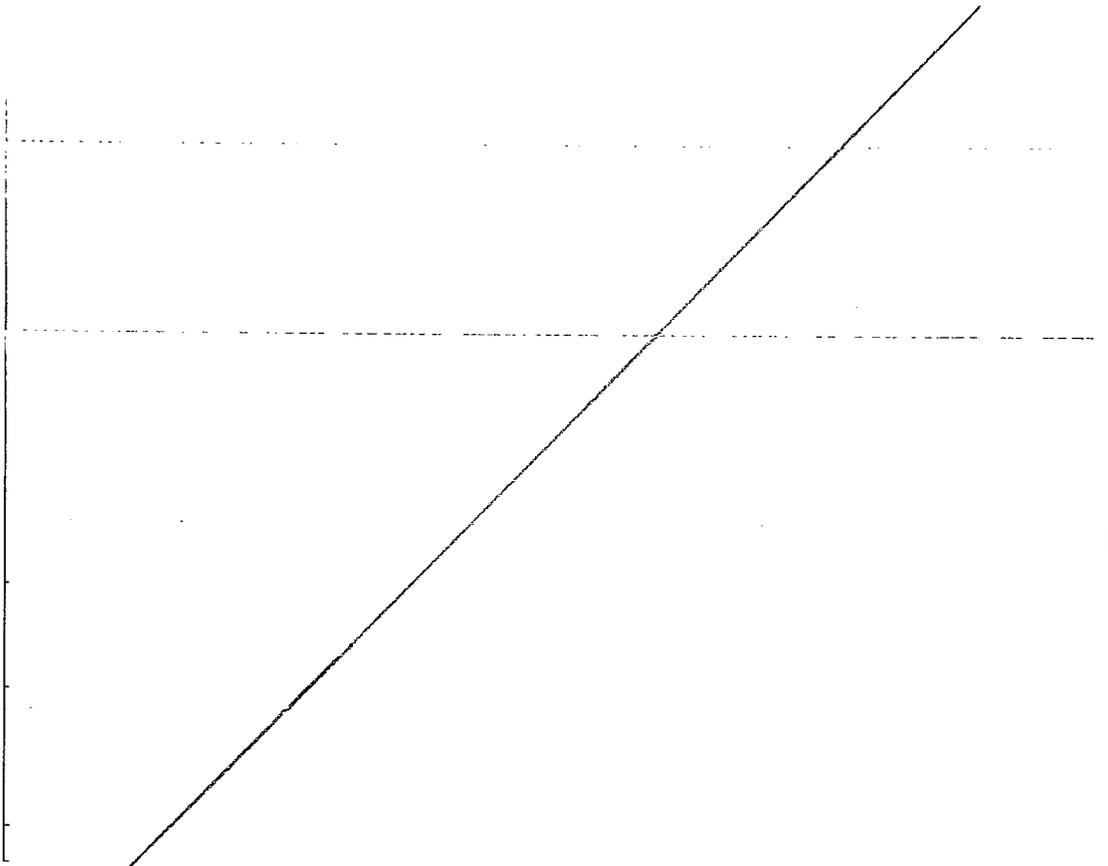
**Pharmacokinetics:**

study #	study title
/	

b(4)

**Repeat-dose Toxicity:**

study #	study title
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**Local Tolerance:**

study #	study title

**b(4)**

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

Romidepsin is a histone deacetylase (HDAC) inhibitor. HDACs catalyze the removal of acetyl groups from acetylated lysine residues in histone and non-histone proteins, resulting in the modulation of gene expression. The ability of romidepsin to modulate gene expression was demonstrated by the suppression of VEGF mRNA expression and the induction of p21/WAF1/Cip1 mRNA expression levels in cancer cells. These genes are potentially important in cancer pharmacology considering their involvement in angiogenesis and cell cycle progression, respectively. This romidepsin-induced modulation of gene expression was associated with an increased incidence of histone acetylation on the promoter region of these genes and therefore likely a result of HDAC inhibition. Additional effects of romidepsin included a time-dependent increase in the apoptotic population, G1 and G2/M cell cycle arrest, and romidepsin-induced differentiation to CD1b+ and CD14+ cells, in human lymphoma U-937 cells. Experiments in NIH3T3 cells transfected with the Ha-ras oncogene demonstrated that romidepsin did not form interstrand DNA-DNA cross-links, break single strand DNA, or inhibit DNA synthesis in isolated nuclei. Romidepsin did however cause G0/G1 cell cycle arrest and decreased c-myc mRNA expression in these cells.

The anti-cancer activity of romidepsin was demonstrated *in vitro* in several human tumor cell lines including leukemia, renal, prostate, colon, and lung, stomach, and breast cancer. Multiple experiments were also conducted *in vivo* using mouse xenograft models to demonstrate that romidepsin inhibited the growth of tumors derived from murine (leukemia, lymphoma, melanoma, and colon) and human (colon, lung, breast, prostate, renal) cancers.

A battery of safety pharmacology studies addressed the effects of romidepsin on the central nervous system (CNS), the respiratory system and the cardiovascular system and the potential for secondary effects through binding extracellular receptors. A study of neurological effects in rats identified decreased activity and limb tone and grip strength at a dose of 1.8 mg/m<sup>2</sup>. Significant neurological effects were observed at 6 mg/m<sup>2</sup> including increased heart and respiration rates, decreased activity, decline in muscle tone, effects on equilibrium and gait, CNS excitation, and depressed autonomic response. These effects were seen 24 hours after dosing, which indicated a delay in CNS effects that did not correlate with romidepsin plasma concentration based on pharmacokinetic studies in rats. In another study, dogs received ascending doses of 2, 6 and 20 mg/m<sup>2</sup> with 6 days between doses, which lead to an increase in spontaneous motor activity at 2 and 6 mg/m<sup>2</sup> and increased body temperature ( $\leq 1.6$  °C) at  $\geq 6$  mg/m<sup>2</sup>.

The CNS dog study described above, also included respiratory and cardiovascular effects in telemetered dogs. Respiration rate was increased by a maximum of 83% of predose value at 20 mg/m<sup>2</sup> by 16 hours postdose. The most significant effects in this study were on the cardiovascular system. A decrease in systolic ( $\leq 12\%$ ), diastolic ( $\leq 16\%$ ) and mean blood pressures ( $\leq 13\%$ ) were observed at  $\geq 2$  mg/m<sup>2</sup>, with maximum changes from predose values occurring from 8 to 12 hours postdose. A concurrent increase in heart rate ( $\leq 52\%$ ) and shortening of the RR interval ( $\leq 34\%$ ) were seen at  $\geq 2$

mg/m<sup>2</sup> with the greatest effects occurring at 10 hours postdose. A prolongation of the QTc (8%) was observed at 20 mg/m<sup>2</sup> after 6 hours.

In addition to the *in vivo* assessment of cardiovascular risk, *in vitro* studies included assessment of the potential of romidepsin to inhibit the human Ether-a-go-go Related Gene (hERG) potassium channel, normally expressed in cardiomyocytes. Romidepsin was found to be a low potency blocker of the hERG channel (IC<sub>50</sub> > 18.49 μM) under the conditions of the assay performed, and therefore is not predicted to have effects on ventricular repolarization of the heart. An additional study with the metabolite identified in non-clinical pharmacokinetic analyses, reduced romidepsin (metabolite M1), found no significant binding to potassium channels, including hERG (IC<sub>50</sub> > 10 μM). Finally, the potential for romidepsin to impact the action potential in an isolated ventricle slice from a guinea pig was assessed. This study determined that romidepsin shortened the action potential duration at 90% repolarization (APD<sub>90</sub>) and decreased the action potential amplitude (APA) at a high concentration of 10 μg/mL. Together, the safety pharmacology studies provide evidence that romidepsin has the potential to cause a clinically relevant impact on the cardiovascular system.

Another study included in the safety pharmacology assessment of romidepsin was an *in vitro* receptor binding assay. The results of this assay suggest that romidepsin has the potential to bind to estrogen receptors and neurokinin<sub>2</sub> receptors under the *in vitro* conditions tested. In this assay, β-estradiol binding to estrogen receptors was inhibited by 97.76% at 1x10<sup>4</sup> ng/mL and by 26.62% at 1x10<sup>3</sup> ng/mL romidepsin. Neurokinin A binding to neurokinin<sub>2</sub> receptors was inhibited by 71.36% at 1x10<sup>4</sup> ng/mL romidepsin. Estrogen receptor binding has implications in the results of non-clinical toxicology studies performed with romidepsin. In a 26 week study in rats, administration of romidepsin led to atrophy of female reproductive organs, including mammary glands, uterus, ovary and vagina. In addition, female rats in this study had pituitary hyperplasia, and elevated cholesterol and triglyceride levels, all of which can be effects of estrogen signaling modulation. Neurokinin<sub>2</sub> receptor binding may be related to CNS effects that were seen in the rat and dog safety pharmacology studies described above.

#### 2.6.2.2 Primary pharmacodynamics

**CRE010130:** Cytotoxic activity of FK228 to human cell lines.

This *in vitro* study examined the anti-tumor activity of FK228 against 18 human tumor cells lines including leukemia (lymphoma), renal, prostate, colon, and lung cancer. FK228-induced cell growth inhibition was determined by measuring the number of viable cells following a 72-hour incubation using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-dipenyltetrazolium (MTT) assay. As shown below, the mean IC<sub>50</sub> values against leukemia (lymphoma), renal, prostate, colon, and lung cancer were 3.23, 4.97, 1.61, 1.61, and 3.83 ng/mL, respectively.

**Cytotoxicity of FK228 against cultured cells:**

	Cell lines	IC <sub>50</sub> (ng/mL)	mean IC <sub>50</sub> (ng/mL)
Leukemia (Lymphoma)	CCRF-CEM	3.76	
	U-937	3.20	
	THP-1	4.09	
	ML-3	3.31	
	HL-60	0.60	3.23
	JOSK-1	3.75	
	K562	4.52	
	JOK-1	2.97	
Renal	OUR10	4.68	
	ACHN	4.25	4.97
	A-498	5.98	
Prostate	PC-3	1.21	1.61
	DU-145	2.01	
Colon	SW-480	1.50	
	Colo201	1.11	1.61
	HT-29	2.23	
Lung	PC-10	4.53	3.83
	NCI-H69	3.12	
	Mean	3.16	

IC<sub>50</sub> values were an average of three determinations.  
 Table excerpted from package

**CRE040012:** Anti-tumor efficacy of FK228, a novel deacetylase inhibitor, depends on the effect on expression of angiogenesis factors.

To determine the effect of FK228 on the expression of angiogenesis factors *in vitro*, PC-3 prostate cancer (FK228 sensitive) and ACHN renal cancer (FK228 resistant) cells were treated with 5 ng/mL FK228 for 1, 3, 12, and 24 hours. Expression levels of VEGF and bFGF mRNA were estimated using real-time quantitative PCR. As shown below, in the presence of FK228 for 12 hours, a 51% decrease in the expression level of VEGF mRNA was seen in PC-3 prostate cancer cells. FK228 did not modulate VEGF mRNA expression levels in ACHN renal cancer cells. The expression level of bFGF mRNA was decreased in both PC-3 prostate cancer (94%) and ACHN renal cancer (81%) cells following a 12 hour exposure to FK228.

**Changes in expression of VEGF and bFGF mRNA in cultured PC-3 and ACHN cells after treatment with FK228**

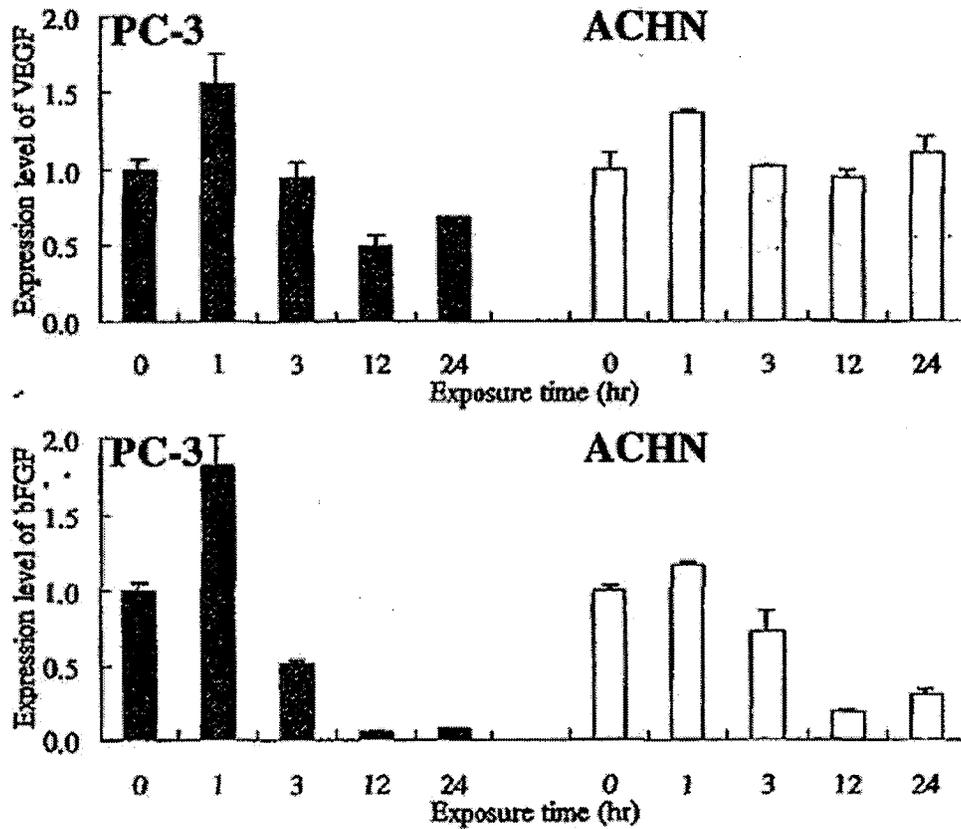


Figure excerpted from package

Since the transcription of VEGF is induced by hypoxia and tumor angiogenesis extends under conditions of hypoxia, the following experiment evaluated the effect of FK228 on the expression of VEGF mRNA in PC-3 prostate cancer cells under hypoxic conditions. In addition to estimating expression levels of VEGF mRNA, the expression level of HIF-1 $\alpha$ , a subunit of HIF-1, was also estimated to determine if FK228-induced decreases in VEGF mRNA expression were caused by FK228-induced decreases in HIF-1 $\alpha$ . PC-3 prostate cancer cells were collected without treatment at 0 hour and the remaining were cultured with or without 5 ng/mL FK228 for 2, 4, 8, and 16 hours from the onset of hypoxic conditions (under 2% O<sub>2</sub> in low glucose medium). Expression levels of VEGF and HIF-1 $\alpha$  mRNA were estimated using real-time quantitative PCR. The time-dependent increase in the expression of VEGF mRNA seen in the control group was inhibited by FK228 without any effect on HIF-1 $\alpha$  mRNA expression (data not shown in this review). FK228 also inhibited high expression of VEGF mRNA, induced by 4 hours of hypoxic conditions, at 8 and 16 hours without any effect on HIF-1 $\alpha$  mRNA expression (data not shown in this review).

To determine the effect of FK228 on the expression of angiogenesis factors *in vivo*, PC-3 prostate cancer (FK228 sensitive) and ACHN renal cancer (FK228 resistant) xenografts in BALB/c *nu/nu* mice were utilized. Once the tumor weight reached 100-300 mg, animals were administered a single 3.2 mg/kg intravenous injection of FK228. Using RNA samples extracted at 1, 3, 6, and 24 hours post-FK228 administration, real-time quantitative PCR was used to estimate expression levels of VEGF and bFGF mRNA. As shown below, twenty-four hours following FK228 administration, a 68% and 88% decrease in the expression levels of VEGF and bFGF mRNA, respectively, was seen in the PC-3 prostate cancer xenograft. FK228 failed to modulate VEGF and bFGF expression levels in the ACHN renal cancer xenograft.

Changes in the expression of VEGF and bFGF mRNA in PC-3 and ACHN xenografts after FK228 administration

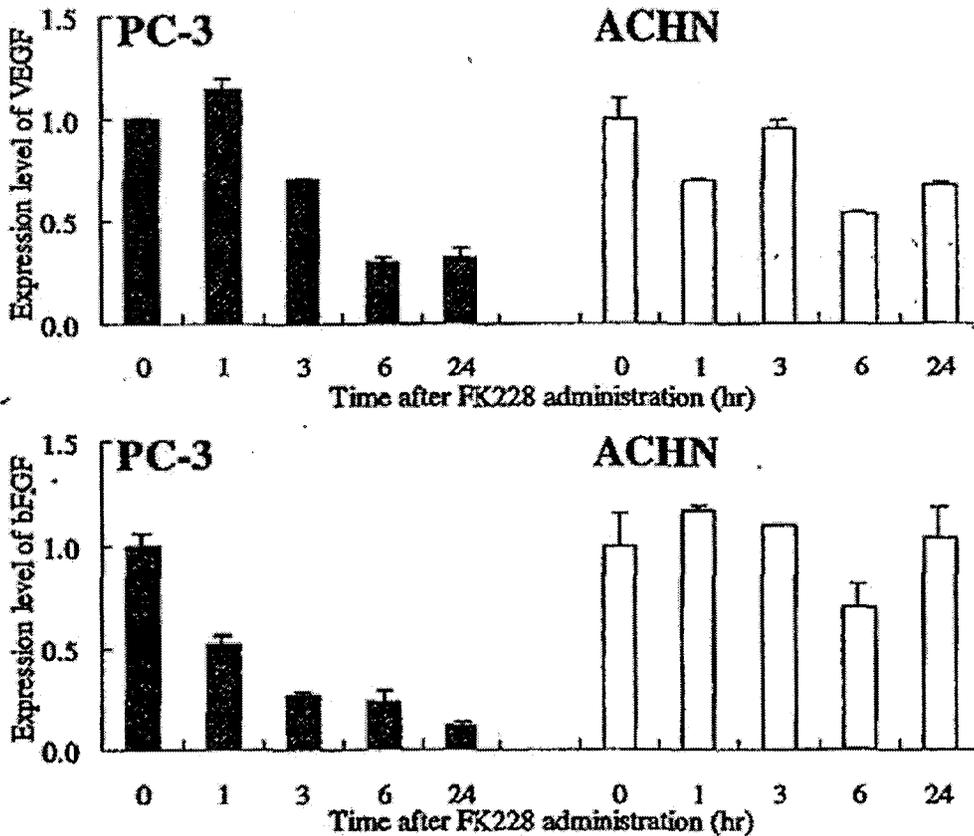


Figure excerpted from package

This next experiment examined the effect of FK228 on acetylation of histone H4 or H3 associated with the VEGF promoter in PC-3 prostate cancer cells using the chromatin immunoprecipitation (ChIP) assay. Cells were cultured with or without 5 ng/mL of FK228 for 2 hours under hypoxic conditions and chromatin fragments were extracted from cells and immunoprecipitated with antibodies to acetylated histone H3 or H4. Isolated DNA was amplified using primers to the VEGF promoter, as shown below.

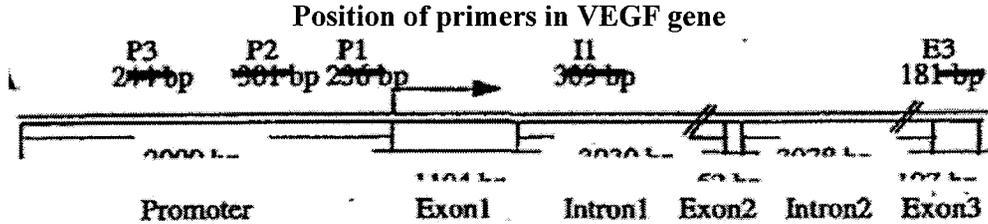
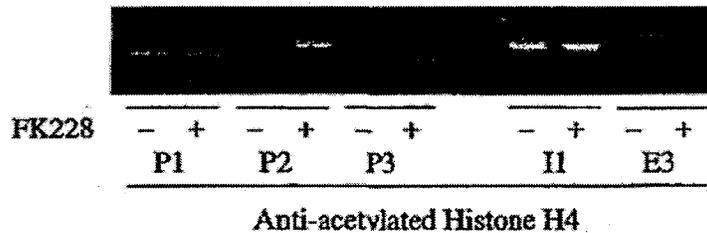
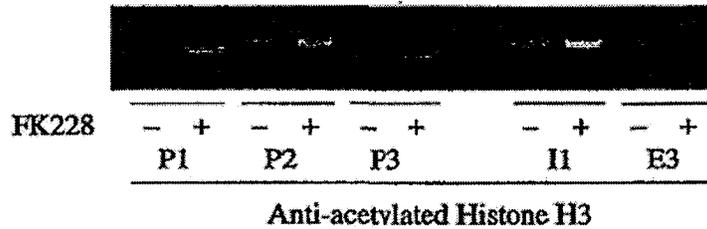
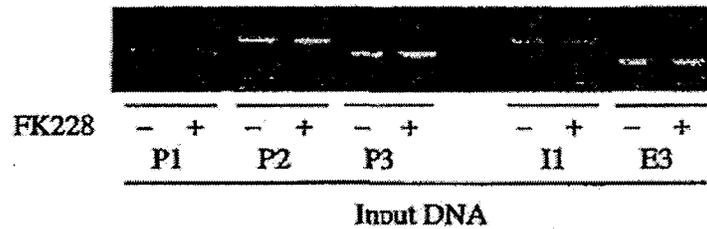


Figure excerpted from package

The results, as shown below, demonstrate that treatment with FK228 increased acetylation of histone H3 and H4 for all regions of the VEGF promoter, with the highest level of FK228-induced acetylation occurring in the P2 region. In the II region, an increase in acetylation was only seen in histone H3. FK228 did not appear to have any effect on histone acetylation of the E3 region of the VEGF promoter.

**FK228 acetylated histones associated with the VEGF promoter**



II – Intron 1; E3 – Exon 3; P1, P2, P3 – Promoter regions

Figure excerpted from package

**CRE040013:** Effects of FK228, a novel histone deacetylase inhibitor, on human lymphoma U-937 *in vitro* and *in vivo*.

The following study examined *in vitro* and *in vivo* effects of FK228 on human lymphoma U-937 cells. The first experiment utilized the histiocytic lymphoma cell line U-937, the chronic myelogenous leukemia cell line K562, and the acute lymphoblastic leukemia cell line CCRF-CEM for growth inhibition assays. Following 72 hour incubation with FK228, the number of viable cells was determined using a MTT assay. FK228 inhibited the growth of U-937, K562, and CCRF-CEM cells with IC<sub>50</sub> values of 5.92, 8.36, and 6.95 nM, respectively. The second experiment used a scid mouse model to test the anti-tumor activity of FK228 on the lymphoma cell line U-937 *in vivo*. Male mice were inoculated with tumor cells and 24 hours later, administered FK228 intraperitoneally, once or twice a week, at doses ranging from 0.1 to 1 mg/kg. As shown below, the results suggest that FK228 prolonged survival time of scid mice inoculated with lymphoma U-937 cells. While the median survival time (MST) of control animals was 20 days, the MST of 0.1-1 mg/kg FK228 treated animals was 22.5-30.5 days when treated once per week and 25-33 days when treated twice per week.

**Effect of FK228 on survival time of scid mice inoculated with lymphoma U-937**

The observation period was 60 days after tumor cell inoculation and the antitumor effect was evaluated by survival time. Survival was analyzed for statistical significance by Peto's test. <sup>a</sup> *p* < 0.01 vs control. 6 and 12 mice were used for FK228-treated and control groups, respectively.

Treatment	MST (days)	% of Control
Control	20.0	100
FK228 once a week	0.1 mg/kg	113 <sup>a</sup>
	0.18 mg/kg	135 <sup>a</sup>
	0.32 mg/kg	140 <sup>a</sup>
	0.56 mg/kg	153 <sup>a</sup>
	1 mg/kg	140 <sup>a</sup>
FK228 twice a week	0.1 mg/kg	125 <sup>a</sup>
	0.18 mg/kg	130 <sup>a</sup>
	0.32 mg/kg	165 <sup>a</sup>
	0.56 mg/kg	135 <sup>a</sup>
	1 mg/kg	128 <sup>a</sup>

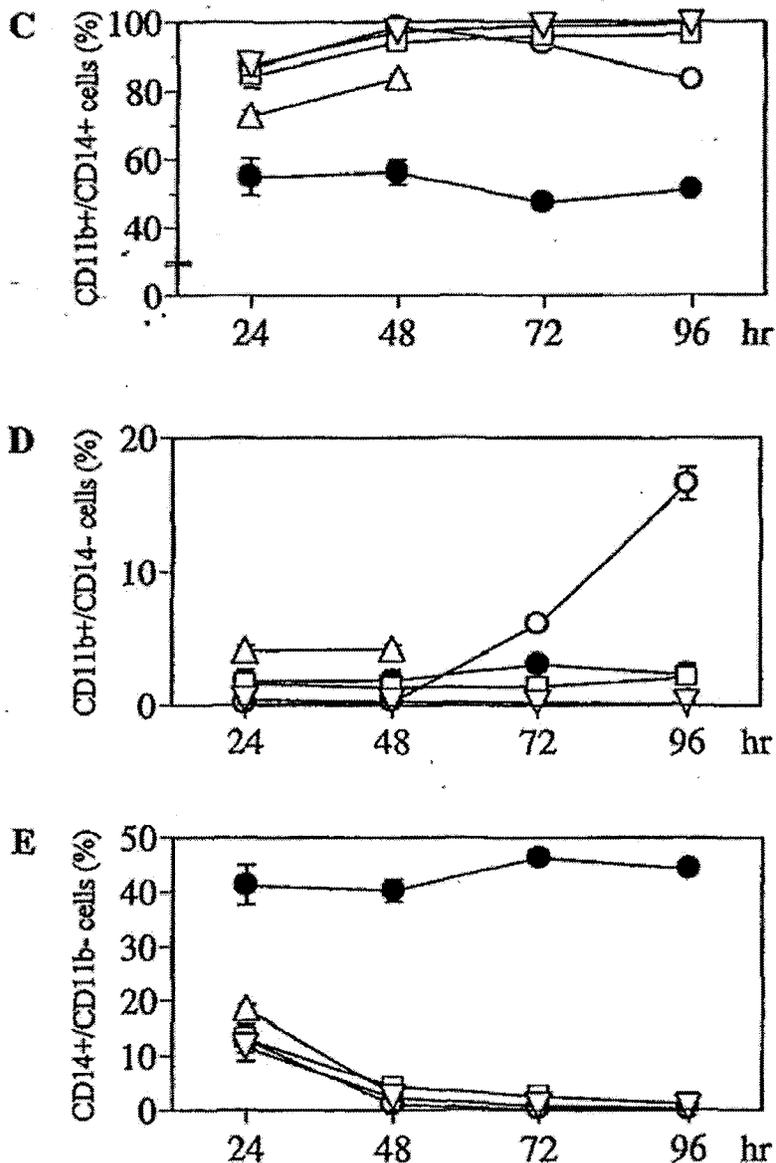
MST = mean survival time  
Table excerpted from package

To characterize the anti-tumor mechanism of FK228 on U-937 lymphoma cells, additional studies were conducted to investigate the effect of FK228 on apoptosis, cell cycle arrest, and differentiation. Using 5 ng/mL FK228, as determined from the IC<sub>50</sub> value in the first experiment discussed above, cells were treated with FK228 for up to 72 hours and analyzed for the presence of apoptosis using flow cytometry. The rate of apoptosis in FK228 treated cells appeared to increase in a time-dependent manner up to 48 hours (data not shown in this review).

To evaluate the effect of FK228 on the U-937 lymphoma cell cycle, cells were exposed to 5 ng/mL FK228 for up to 24 hours. An additional experiment consisted of incubating U-937 cells with 0.5 ng/mL FK228 for 48 hours followed by 24 hour incubation in drug free medium. Cell cycles were evaluated by propidium iodide staining and the relative DNA content was measured by flow cytometry. According to the sponsor, incubating U-937 cells with 5 ng/mL FK228 for 12 hours caused G2/M arrest (data not shown in this review). In addition, exposing U-937 cells to 0.5 ng/mL FK228 for 48 hours and culturing these cells in drug free medium for 24 hours appeared to cause G1 arrest (data not shown in this review).

To assess whether FK228 has an effect on the differentiation of U-937 cells, cells were seeded with and without 5 and 0.5 ng/mL FK228 for up to 96 hours and the expression of surface antigens CD11b and CD14 was measured by flow cytometry. ATRA and Vitamin D3 were used as positive controls at concentrations of 1 and .1 uM, respectively. Cells treated with 5 ng/mL FK228 for 24 hours showed an increase in differentiation (increased CD11b+/CD14+). A decrease in the total number of stained cells was also noted and according to the sponsor, is likely due to apoptosis occurring after 20 hours, as previously mentioned above. Treating cells with 0.5 ng/mL FK228 for 24 hours also showed an increase in differentiation and at levels similar to both positive controls. However, following 96 hours of 0.5 ng/mL FK228 treatment, a decrease in CD11b+/CD14+ cells and an increase in CD22b+/CD14- cells was seen. This did not occur in the positive controls at 96 hours. According to the sponsor, while the viability of cells treated with 0.5 ng/mL FK228, ATRA, or Vitamin D3 remained at the same level as controls cells throughout the experiment (data not shown), these agents caused growth inhibition beginning at 72 hours (inhibition rates at 96 hours were 62.1, 85.1, and 54.6%, respectively). Thus, the sponsor believes that FK228 induced differentiation of U-937 cells and this may be related growth inhibition.

Expression of CD11b and CD14 antigens in U-937 cells treated with FK228, ATRA and Vitamin D (Continued)



Closed circles = control  
 Open circles = 0.5 ng/mL FK228  
 Pointed up triangles = 5 ng/mL FK228  
 Pointed down triangles = Vitamin D3  
 Open squares = ATRA  
 Figure excerpted from package

An additional experiment consisted of evaluating the effect of 5 ng/mL FK228 on p21/WAF1/Cip1 and gelsolin mRNA expression in U-937 lymphoma cells. Using real-time quantitative PCR, FK228 was shown to induce a 9-fold increase in the expression of p21/WAF1/Cip1 mRNA at 1 hour and up to a 654-fold increase at 24 hours. Similarly,

FK228 caused a 2-fold induction of gelsolin mRNA at 1 hour and up to a 152-fold increase at 24 hours. The results from each time point are shown below.

**Changes in the expression of p21/WAF1/Cip1 and gelsolin mRNA in U-937 cells induced by FK228**

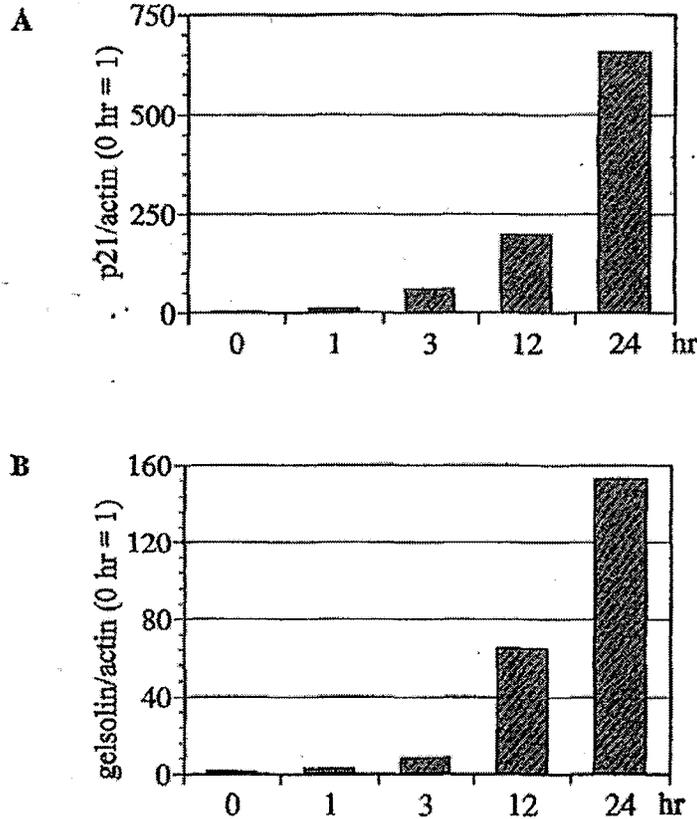


Figure excerpted from package

The purpose of the final experiment in this study was to test the hypothesis that FK228 modulation of p21/WAF1/Cip1 mRNA was induced by the acetylation of histones associated with target gene promoter regions. Thus, utilizing a chromatin immunoprecipitation (ChIP) assay, the acetylation of histone H3 and H4 associated with p21/WAF1/Cip1 promoter was examined. Lymphoma U-937 cells were treated with or without 5 ng/mL FK228 for 2 hours and chromatin fragments were extracted from cells and immunoprecipitated with antibodies to acetylated histone H3 or H4. Isolated DNA was amplified using primers to the p21/WAF1/Cip1 promoter, as shown below.

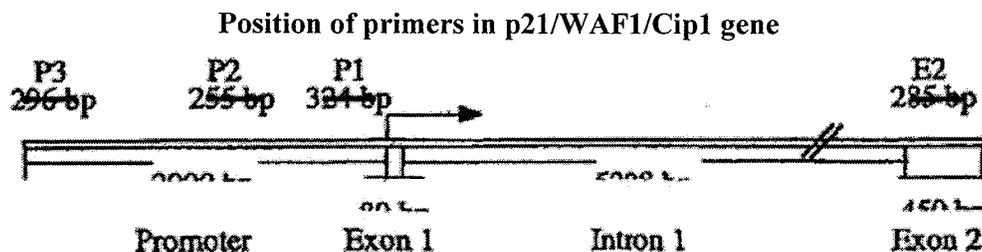


Figure excerpted from package

The results demonstrate that treatment with FK228 increased acetylation of histone H3, specifically at the P1 and P2 regions while an increased acetylation of H4 was seen at all regions of the p21/WAF1/Cip1 promoter and exon 2 (data not shown in this review).

**CRR910025:** Anti-tumor effects and toxicity of FR901228 in animals.

The following study included *in vitro* and *in vivo* pharmacology studies to assess the anti-tumor effect of FR901228 and additional *in vitro* studies to ascertain the mechanism(s) of action of FK228. Although the results of toxicology studies in rats and dogs were also included in this study, toxicology results will not be discussed here.

The first experiment measured the anti-tumor activity of FR901228 against *in vitro* human tumor cells, normal human cells, and murine embryo cells. As shown below, among the cell lines tested, FR901228 showed stronger anti-tumor activity on human lung, stomach, breast, and colon cancer cells than on normal human fibroblast and endothelium cells, and murine embryo cells.

**Cytotoxic effects of FR901228 against *in vitro* cultured cells**

Cell line		IC <sub>50</sub> (ng/mL)	
		FR 901228	Adriamycin
Lung	A549	0.7	13.8
	PC-9	0.3	
Lung	PC-1	1.2	
	PC-10	2.4	
Lung (SCLC)	ADH	1.0	
	LX-1	1.2	
Stomach	MKN28	0.9	
	" 74	0.9	
Breast	MCF-7	0.6	13.0
	ZR-75-1	0.7	
Colon	Colo-201	0.3	
	SW 480	1.0	
Normal fibroblast (human)	HNFF	>1,000	129.9
Normal endothelium (human)	HE-9	7.0	
Normal (mouse)	Balb/C3T3	9.2	
Normal (mouse)	NIH/3T3	3.2	

Table excerpted from package

The second experiment measured the anti-tumor effects of FR901228 on 3 types of ascitic tumors (P388 and L1210 leukemia, and B16 melanoma) in mice, compared to mitomycin C and cisplatin. Animals were inoculated with tumor cells and given 0.032-1.8mg/kg FR901228 intraperitoneally once a day for 5 days (P388) or 9 days (L1210 and B16). Anti-tumor activity results were expressed as the percent mean tumor size of the treated groups (T) to that of the control group (C). As shown below, FK228 showed anti-tumor activity against all three cancer xenografts.

**Anti-tumor effects of FR901228 on murine ascitic tumors in mice**

Drug	Dose (mg/kg)	P388 (ip-ip) D1-5		L1210 (ip-ip) D1-9		B16 (ip-ip) D1-9	
		T/C %	activity	T/C %	activity	T/C %	activity
FR901228	0.032	108	—	111	—	116	—
	0.056			113	—	129	+
	0.1	121	+	118	—	126	+
	0.18			125	+	152	++
	0.32	125	+	125	+	165	++
	0.56	138	+	129	+	174	++
	1.0	83	Tox	97	—	78	Tox
	1.8	50	Tox	—	Tox	32	Tox
MMC	0.1	117	—	109	—	116	—
	0.32	138	+	118	—	139	+
	0.56	142	+	130	+	163	++
	1.0	154	+	151	++	176(1)	++
	1.8	138(1)	+	149	+	61	Tox
	3.2	79	Tox	103	—	39	Tox
CDDP	0.32	121	+	116	—	115	—
	1.0	142	+	139	+	185	++
	3.2(4)	>250	++	195	++ (Tox)	65	Tox
	5.6	83	Tox	116	— (Tox)	35	Tox
	10	50	Tox	77	Tox	29	Tox

P388 ; +, ≥120% ++, ≥175%

( ): survival animals

L1210, B16 ; +, ≥125% ++, ≥150%

Tox : survival rate <86%

(Tox): Tox caused by B.W. decrease

T/C% = Mean of change in tumor size (T) / Mean of change in tumor size (C) x 100

Table excerpted from package

The anti-tumor effect of FR901228 was then tested on 6 types of murine solid tumors in mice, compared to mitomycin C, cisplatin and 5-FU. Following subcutaneous or intradermal tumor implantation, animals received 0.56-5.6 mg/kg FR901228 intravenously every 3 days, for a total of 3 or 4 doses. Anti-tumor activity results were expressed as the percent mean tumor size of the treated groups (T) to that of the control group (C). As shown below, administering FR901228 twenty-four hours post tumor implantation resulted in growth inhibition in Colon 38 (colon), B16 (melanoma), and M5076 (reticulum sarcoma) derived tumors. When FR901228 was administered 4 to 7 days post tumor implantation, growth inhibition was seen in Colon 38 (colon), M5076 (reticulum sarcoma) and Meth A (fibrosarcoma) derived tumors.

Anti-tumor effects of FR901228 on murine solid tumors in mice

Drug	Dose (mg/kg)	Colon38(sc-iv)Q3D		LLC(sc-iv)Q3D		B16(sc-iv)Q3D		B16(sc-iv)Q3D		Colon26(sc-iv)Q3D		H 5076(sc-iv)Q3D		Meth A(sc-iv)Q3D			
		Days 1,4,7,10	I-T/C% Activity	Days 1,4,7,10	I-T/C% Activity	Days 1,4,7,10	I-T/C% Activity	Days 1,4,7,10	I-T/C% Activity	Days 1,4,7,10	I-T/C% Activity	Days 1,4,7,10	I-T/C% Activity	Days 1,4,7,10	I-T/C% Activity	Days 1,4,7,10	I-T/C% Activity
FR901228	0.56	-24	-	-6	-	24	-	23(9)	-	23	-	23	-	23	-	23	
	1.0	2	-	-3	-	38(8)	-	19	-	-2	-	33	-	33	-	33	
	1.8	25	-	-20(9)	-	16	4	30	-	28	-	39	-	39	-	39	
	3.2	60	+	Tox	Tox	21	12	43(9)	-	61	-	73(6)	-	73(6)	-	Tox	
	5.6	94	++	Tox	Tox	63(3)	65	-	Tox	78	+	-	-	-	-	Tox	
	10	NT	NT	Tox	Tox	-	82	-	Tox	90(3)	+	-	-	-	-	Tox	
Hydrocortin-C	1.0	49	-	23	-	47	-	34	-	52	-	35	-	35	-	35	
	1.8	86	+	34	-	66	+	45	-	82	+	42	-	42	-	42	
	3.2	97	++	51	-	82	+	83	-	93	+	71	-	71	-	71	
	5.6	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox
	1.0	17	-	1	-	39	-	39	-	45	-	45	-	45	-	45	
	1.8	32	-	11	-	29	-	39	-	39	-	23	-	23	-	23	
5-FU	3.2	41	-	22	-	75	+	74	-	86	+	46	-	46	-	46	
	5.6	64	+	66	+	93	++	98(7)	++	94	++	72(6)	Tox	72(6)	Tox	72(6)	
	10	-	Tox	-	Tox	-	Tox	-	Tox	100(8)	++	-	-	-	-	Tox	
	18	83	+	-	-	-	-	-	-	2	-	24	-	24	-	24	
	32	99	++	-	-	-	-	-	-	-3	-	57	-	57	-	57	
	56	100	+++	-	-	-	-	-	-	6	-	76	-	76	-	76	
100	-	Tox	-	Tox	-	Tox	-	Tox	-	-	-	-	-	-	Tox		
FR901228	0.56	32	-	32	-	41	-	46	-	46	-	46	-	46	-	46	
	1.0	NT	NT	19	-	3	-	77	+	77	+	77	+	77	+	77	
	1.8	NT	NT	4	-	11	-	84	+	84	+	84	+	84	+	84	
	3.2	63	+	21	-	59	+	92	++	92	++	92	++	92	++	92	
	5.6	76	+	39(9)	-	85	+	95	++	95	++	95	++	95	++	95	
	10	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox
Hydrocortin-C	1.0	34	-	34	-	41	-	22	-	22	-	22	-	22	-	22	
	1.8	45	-	83	-	91	++	41	-	41	-	41	-	41	-	41	
	3.2	83	+	-	Tox	-	Tox	-	58	-	58	-	58	-	58	-	58
	5.6	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox	-	Tox
	1.0	39	-	34	-	34	-	12	-	12	-	12	-	12	-	12	
	1.8	74	+	73	+	73	+	40	-	40	-	40	-	40	-	40	
3.2	98(7)	++	-	Tox	-	Tox	-	42(9)	-	42(9)	-	42(9)	-	42(9)	-	42(9)	
5.6	-	Tox	-	Tox	-	Tox	-	-	-	-	-	-	-	-	-	-	
10	-	Tox	-	Tox	-	Tox	-	-	-	-	-	-	-	-	-	-	

Criteria : +, ≥58%, ++, ≥90%  
 Tox : Survival rate < 65%  
 ( ) : No. of survivors  
 N = 10

T/C% = Mean of change in tumor size (T) / Mean of change in tumor size (C) x 100  
 Table excerpted from package

The next xenograft assay examined the anti-tumor effect of FR901228 on 9 types of human tumors in BALB/c *nu/nu* mice, compared to mitomycin C and cisplatin. Following subcutaneous tumor implantation, animals received 1.8-5.6 mg/kg FR901228, intravenously once every 4 days, for a total of 3 doses. Anti-tumor activity results were expressed as the percent mean tumor size of the treated groups (T) to that of the control group (C). As shown below, growth inhibition in response to FR901228 was seen in SC-6 (stomach), LU-65 (lung giant cell), LC-6 (non-small cell lung), and MX-1 (breast) xenografts.

**Anti-tumor effects of FR901228 on human xenograft tumors in nude mice**

Drug	Dose (mg/kg)	LX-1(sc-iv) Q4D, 3times (day 25)		LU-65(sc-iv) Q4D, 3times (day 28)		A549(sc-iv) Q4D, 3times (day 28)		LC-6(sc-iv) Q4D, 3times (day 18)		PC-9(sc-iv) Q4D, 3times (day 28)	
		1-T/C%	Activity	1-T/C%	Activity	1-T/C%	Activity	1-T/C%	Activity	1-T/C%	Activity
FR901228	1.8	44	-	63	-	51	-	64	-	54	-
	3.2	66	-	74	-	48	-	74	-	61	-
	5.6	72(3)	Tox	83(4)	+	70	-	82	+	66	-
	10	-	Tox								
Mitomycin-C	1.0	45	-	63	-	19	-	52	-	49	-
	1.8	72	-			33	-			60	-
	3.2	92	++	92	++	51	-	97	++		-
	5.6	-	Tox					-	Tox		Tox
CDDP	3.2	38	-	51	-	15	-	65	-	17	-
	5.6	42	-	86	+	13	-	87	+	43	-
	10	86	+			41	-	98	++	68(5)	-

Drug	Dose (mg/kg)	SC-6(sc-iv) Q4D, 3times (day 21)		Colo201(sc-iv) Q4D, 3times (day 25)		MX-1(sc-iv) Q4D, 3times (day 28)		MX-1(sc-iv) Q4D, 3times (day 21)		MCF-7(sc-iv) Q4D, 3times (day 48)	
		1-T/C%	Activity	1-T/C%	Activity	1-T/C%	Activity	1-T/C%	Activity	1-T/C%	Activity
FR901228	1.8	77	-	12	-	44	-	70	-	31	-
	3.2	84	+	26	-	51	-	75	-	47	-
	5.6	92(5)	++	69	-	63	-	81	+	56	-
	10					-	Tox			-	Tox
Mitomycin-C	1.0					98	++				
	1.8					99	++				
	3.2					99	++				
	5.6					-	Tox				
CDDP	3.2	64	-	4	-	98	++				
	5.6	78	-			99	++				
	10	95	++	61	-	99(5)	++				

Criteria : +, ≥80%, ++, ≥90% ( ) : No. of survivors  
 Tox : Survival rate < 65% N = 6

T/C% = Mean of change in tumor size (T) / Mean of change in tumor size (C) x 100  
 Table excerpted from package

The final xenograft assay used tumor cells cross-resistant to other chemotherapeutic drugs including adriamycin, mitomycin C, cisplatin, vincristine, and 5-FU to assess the anti-tumor effect of FR901228. Once mice were inoculated with tumors, animals received 0.18-1 mg/kg FR901228 intraperitoneally once per day for 4 days. Anti-tumor activity results were expressed as the percent mean tumor size of the treated groups (T) to that of the control group (C). As shown below, FR901228 was effective against each drug-resistant tumor in mice, except the adriamycin-resistant tumor.

**Anti-tumor effects of FR901228 on drug-resistant P338 in mice**

Drug	Dose [mg/kg]	P388		P388/ADR		P388/MNC		P388/CPH		P388/VCR		P388/5FU	
		T/C(%)	Activity	T/C(%)	Activity	T/C(%)	Activity	T/C(%)	Activity	T/C(%)	Activity	T/C(%)	Activity
FR901228	0.18	116	-	110	-	136	+	122	+	125	+	167	+
	0.32	116	-	115	-	150	+	133	+	121	+	167	+
	0.56	121	+	110	-	155	+	156	+	125	+	170	+
	1.0	126	+	70	Tox	164	+	161	+	138	+	183	++
ADR	0.32	122	+	100	-								
	1.0	133	+	100	-								
	3.2	178	++	95	-								
MNC	1.0	122	+			109	-						
	1.8	122	+			118	-						
CPH	10	111	-					95	-				
	32	144	+					95	-				
VCR	0.1	122	+							117	-		
	0.32	133	+							113	-		
5FU	3.2	122	+									106	-
	10	144	+									94	-
	32	183	++									100	-

N=6, +; ≥120, ++: ≥175

T/C% = Mean of change in tumor size (T) / Mean of change in tumor size (C) x 100

Table excerpted from package

Of the various *in vitro* studies conducted to investigate the mechanism(s) of action of FR01228, the first study exposed murine L1210 lymphocytic leukemia cells to FR01228 for 24 hours to measure the effect of FR01228 on DNA, RNA, and protein synthesis. As shown below, FR901228 caused a dose-dependent inhibition of the incorporation of radiolabeled thymidine, uridine, and leucine into DNA, RNA and protein, respectively. RNA synthesis appeared the most sensitive to FR901228-induced inhibition (data not shown in this review).

The next experiment studied the effect of FR901228 on interstrand DNA-DNA cross-links and single-strand DNA breaks in murine L1210 lymphocytic leukemia cells using an alkaline elution method. Following a 1 hour exposure to 15-50  $\mu\text{M}$  FR901228, L1210 cells did not form interstrand DNA-DNA cross-links. Additionally, using adriamycin as a positive control, 50  $\mu\text{M}$  FR901228 did not appear to induce single-strand DNA breaks (data not shown in this review).

Using isolated nuclei of Ras1 cells derived from NIH3T3 cells transfected with the Ha-ras oncogene, FR901228 was investigated for effects on DNA synthesis. Following a 40 min exposure, 10 nM – 1  $\mu\text{M}$  FR902228 did not inhibit DNA synthesis in the isolated nuclei of Ras1 cells, as shown below. Aphidicolin and Ara-C were used as positive controls.

**Effects of FR901228 on DNA synthesis in isolated nuclei**

addition		DNA synthesis (%)
Buffer control *		100.0 $\pm$ 1.8
no incubation		6.1 $\pm$ 1.1
FR901228	10 nM (5ng/ml)	92.3 $\pm$ 3.3
	100 nM	100.6 $\pm$ 2.8
	1 $\mu\text{M}$	104.9 $\pm$ 0.3
Aphidicolin	1 $\mu\text{M}$ (0.3 $\mu\text{g/ml}$ )	82.7 $\pm$ 2.4
	10 $\mu\text{M}$	63.3 $\pm$ 2.7
	100 $\mu\text{M}$	22.9 $\pm$ 2.6
Ara-CTP	10 $\mu\text{M}$ (5 $\mu\text{g/ml}$ )	92.9 $\pm$ 2.1
	100 $\mu\text{M}$	85.6 $\pm$ 5.4
	1 mM	35.2 $\pm$ 12.5

\* reactions were incubated at 37  $^{\circ}\text{C}$  for 40 min

*Table excerpted from package*

Utilizing Ras1 cells again, the effect of FR901228 on cell cycle and mRNA expression of c-myc and Ha-ras was examined. Using flow cytometry and incubating cells in

FR901228 for up to 48 hours, FR901228 appeared to arrest the cells in G0/G1 phase, as shown below. This effect was reversible in less than 12 hours following removal of FR901228 from the medium. Within the same figure below, mRNA expression results from northern dot hybridization showed a decrease in c-myc mRNA expression in the presence of FR901228. This expression decrease was also reversible following removal of FR901228.

**Effects of FR901228 mRNA expression of c-myc and Ha-ras (left) and the effects of FR901228 on cell cycle (right)**

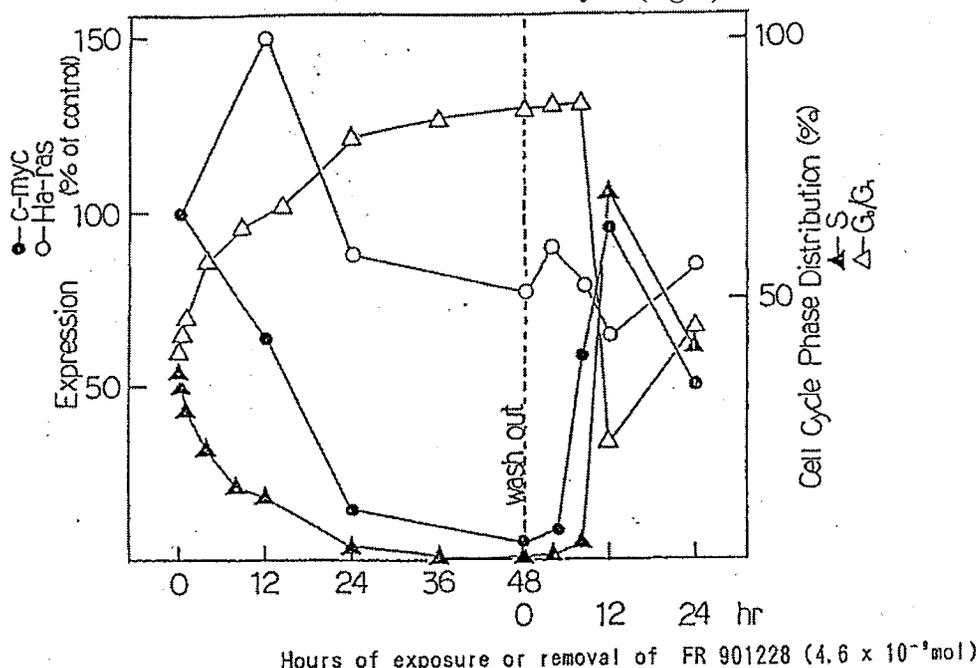


Figure excerpted from package

**CRE010131:** Anti-tumor activity of FK228 against human tumor xenografts in nude mice.

This study assessed the anti-tumor activity of FK228 using renal (ACHN and RXF-3IL), prostate (PC-3 and DU-145), and colon cancer (HT-29, HCC2998, and HCT-15) human tumor xenografts in BALB/c *nu/nu* mice (n=6 per group). After the tumor weight reached ~100-300 mg, 1.8 or 3.2 mg/kg FK228 was intravenously administered once every 4 days for a total of 3 doses. Approximately 10 days following the last injection, tumor sizes were compared to those prior to drug treatment. Taxol, administered daily for 5 days as a 24 mg/kg intravenous injection, was used as a comparator for anti-tumor activity. Anti-tumor activity results were expressed as the percent mean tumor size of the treated groups (T) to that of the control group (C). As shown below, FK228 exhibited anti-tumor activity against the PC-3 prostate cancer and RXF63IL renal cancer xenografts.

**Anti-tumor effect of FK228 against human tumor xenografts in nude mice**

Tumor	Drug	Dose (mg/kg)	T/C%	Evaluation	Rate of Max. Body weight change (%)	Mortality
ACHN	FK228	3.2	80	-	13	0/6
	FK228	1.8	82	-	12	0/6
	Taxol	24	69	-	19	0/6
RXF-631L	FK228	3.2	17	+	15	0/6
	FK228	1.8	24	+	3	0/6
	Taxol	24	24	+	15	0/6
PC-3	FK228	3.2	8	+	15	0/6
	FK228	1.8	22	+	14	0/6
	Taxol	24	23	+	14	0/6
DU-145	FK228	3.2	42	-	11	0/6
	FK228	1.8	41	-	4	0/6
	Taxol	24	2	+	16	0/6
HT-29	FK228	3.2	52	-	19	0/6
	FK228	1.8	60	-	11	0/6
	Taxol	24	4	+	17	0/6
HCT-15	FK228	3.2	65	-	13	0/6
	FK228	1.8	85	-	5	0/6
	Taxol	24	24	-	17	0/6
HCC2998	FK228	3.2	66	-	16	0/6
	FK228	1.8	71	-	9	0/6
	Taxol	24	0	+	13	0/6

T/C% = Mean of change in tumor size (T) / Mean of change in tumor size (C) x 100  
 Table excerpted from package

**2.6.2.3 Secondary pharmacodynamics**

No studies conducted.

**2.6.2.4 Safety pharmacology**

Neurological effects:

**Study title:** Safety pharmacology study of FR901228 – Effects on the central nervous system in rats

**Key study findings:**

- There were slight effects on activity, limb tone and grip strength at 0.3 mg/kg romidepsin.

- At 1 mg/kg, increased heart and respiration rates, decreased activity, decline in muscle tone, effects on equilibrium and gait, CNS excitation, and depressed autonomic response were observed, most of which were noted at 24 hours postdose.
- Three animals died at 1 mg/kg by 24 hours postdose.

**Study no.:** GLR030774

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** March 6, 2003

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** FR901228, lot # GLP-201102L

#### Methods

Doses: 0 (vehicle), 0.1, 0.3, 1 mg/kg (0.6, 1.8, 6 mg/m<sup>2</sup>)  
Formulation/vehicle: IV; 0.2 v/v% ethanol – 0.8 v/v% propylene glycol – physiological saline; 4 hour infusion (2 mL/kg/hr)  
Species: — :CD (SD) IGS / rat b(4)  
Number of animals: 6 males/group  
Age/weight: 5 weeks / 180.6 – 268.8 g  
Dose justification: An IV infusion toxicity study in rats resulted in death or morbid sacrifice at 1.67 mg/kg. A dose of 1 mg/kg was considered to be a maximum tolerated dose in this study.  
Study design: Intravenous infusion was through a cannula inserted into the external jugular vein of anesthetized rats. Each rat received a single dose of vehicle or FR901228 and was observed for 24 hours.  
Endpoints: General symptom and behavior (including locomotor activity, motor affective responses, equilibrium and gait, CNS excitation, sensory-motor responses, muscle tone, autonomic responses) was observed approximately hourly until 8 hours postdose, then at 24 hours postdose.

Statistical analysis: no statistical test was performed

#### Results

There were no effects on general symptom or behavior observed in rats that received 0.1 mg/kg FR901228.

At 0.3 mg/kg, one animal had a decrease in locomotor activity at 2 hours postdose. The same animal had a decline in limb tone and grip strength at 1, 2 and 4 hours postdose.

The most significant effects of FR901228 administration were observed at 1 mg/kg, which included increased heart and respiration rates, decreased activity, decline in muscle (limb) tone, effects on equilibrium and gait (ataxic and abnormal gait), CNS excitation (twitches), and depressed autonomic response (decreased startle reflex). The startle reflex was decreased in one animal from 1 through 24 hours postdose. Another animal had a decline in limb tone and grip strength at 2 and 4 hours postdose, and a decrease in activity at 2 hours postdose. Effects of FR901228 administration were more prevalent at 24 hours postdose. Three of 6 animals in the 1 mg/kg group died by 24 hours postdose. Of the three surviving animals, 2 had a decrease in activity and an ataxic and abnormal gait. One of the surviving animals had twitches, decline in limb tone and grip strength and an incomplete eyelid opening. In the second surviving animal, convulsions, decline in pain response and startle reflex and an increase in heart rate and respiration rate were observed. The third surviving 1 mg/kg animal at 24 hours postdose was noted to have twitches. The most significant effects observed in this study from administration of 1 mg/kg FR901228 were seen at 24 hours postdose, indicating a delay in CNS effects. Based on pharmacokinetic studies in male rats after a single romidepsin injection of 0.67 mg/kg, the time ( $t_{max}$ ) at which the maximum plasma concentration of romidepsin ( $C_{max}$ ) was reached was estimated to be < 10 minutes, and the half life ( $t_{1/2}$ ) was 0.867 hours. Therefore, the effects observed in this study occurred long after the peak romidepsin concentration was achieved and after the last time point of measurable plasma romidepsin of 4 hours postdose.

Cardiovascular effects:

**Study title:** Safety pharmacology study of FR901228 – hERG assay

**Key study findings:**

- Romidepsin was a low potency hERG blocker ( $IC_{50} > 18.49 \mu M$ ) under the conditions of this assay.

**Study no.:** GLR030533

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** October 25, 2002

**GLP compliance:** Yes

**QA reports:** Yes **Drug, lot #:** FR901228, lot # GLP-201102L

**Methods**

Cells: HEK293 cell line stably transfected with hERG cDNA

Reference compound (positive control): E-4031

Treatment Groups:

Vehicle (extracellular superfusing solution: NaCl 137, KCl 4, CaCl<sub>2</sub> 1.8,

MgCl<sub>2</sub> 1, glucose 10, and HEPES 10 (in mmol/L), pH 7.4); n=5 cells  
Positive control (100 nM E-4031); n=5 cells  
FR901228: 0.3, 1, 10 µg/mL; n=5 cells/concentration  
Time: cells were treated for 10 minutes

#### Study design and results

The effects of FR901228 on the hERG channel current were studied in HEK293 cells expressing the hERG channel with a patch-clamp technique. The dose justification of FR901228 used in this assay was based on the highest concentration of  $1 \times 10^{-5}$  g/mL, which is approximately 40-fold the  $C_{max}$  value (235.29 ng/mL) that was estimated from clinical administration of 9.1 mg/m<sup>2</sup>/dose romidepsin in Phase 1 clinical studies, or 25-fold the  $C_{max}$  value (377 ng/mL) achieved in patients receiving the recommended clinical dose of 14 mg/m<sup>2</sup>/dose. The positive control for this assay was E-4031 applied at 100 nM, which resulted in 82.5% mean inhibition of the hERG potassium current, within the expected range of above 80%. FR901228 inhibited hERG current by a mean 7.9% at 0.3 µg/mL (0.56 µM), 18.0% at 1 µg/mL (1.85 µM) and 37.3% at 10 µg/mL (18.49 µM). hERG inhibition at 1 and 10 µg/mL FR901228 was statistically significant ( $p < 0.001$ ) compared to vehicle. The  $IC_{50}$  could not be determined from the concentrations tested, but was greater than 18.49 µM making FR901228 a potential low potency blocker under the conditions of this assay.

**Study title:** *In vitro* investigation of the interaction potential of dithiothreitol reduced romidepsin for potassium channels

#### Key study findings:

- Reduced romidepsin (M1) at 10 µM did not significantly bind to potassium channels (hERG, rat SK<sub>CA</sub> and hamster K<sub>ATP</sub>).

**Study no.:** DVRS-003

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

**Conducting laboratory and location:**

b(4)

**GLP compliance:** No

**QA reports:** No

**Drug, lot #:** DTT-reduced FR901228 (metabolite M1) from romidepsin lot # 49800203

**Methods:** Dithiothreitol (DTT) reduced romidepsin was produced, which was purified by HPLC, followed by stability studies (48 hours). Radioligand competition binding assays were performed for three ion channels to assess the potential for binding. Reduced romidepsin (M1) (vehicle: 1% DMSO) was used at 10 µM to assess binding with the following channel/ligand combinations:

- hamster K<sub>ATP</sub> potassium channel:



**Dose justification:** The concentrations of FR901228 used in this assay were chosen based on the highest concentration of 10 µg/mL, which is approximately 40-fold the  $C_{max}$  value (235.29 ng/mL) that was estimated from clinical administration of 9.1 mg/m<sup>2</sup>/dose romidepsin in Phase 1 clinical studies, or 25-fold the  $C_{max}$  value (377 ng/mL) that was achieved from administration of the recommended clinical dose of 14 mg/m<sup>2</sup>/dose.

**Study design:**

One papillary muscle per animal was removed from the right ventricle of the heart. The isolated muscle preparation was fixed with a pin in an organ bath, which was perfused with Tyrode solution. A microelectrode was inserted into the papillary muscle cells, and action potentials were obtained by a field electric stimulation and monitored on oscilloscope via an amplifier for microelectrode and recorded with action potential analysis software. One preparation was used for one series of superfusion, which consisted of three ascending concentrations of FR901228 followed by the positive control, sotalol, for the treatment group, or four sessions of superfusion of Tyrode solution for the vehicle group. Each session of FR901228, positive control or vehicle superfusion was 30 minutes in duration.

**Endpoints:**

Action potential duration at 90% repolarization (APD<sub>90</sub>), resting membrane potential (RP), action potential amplitude (APA) and maximum rate of depolarization (dV/dt) were measured before and during each superfusion session. The value obtained after 30-minute treatment with FR901228 was used as the baseline for evaluation of the positive control article effect. For each timepoint of measurement, 10 tracings were analyzed to calculate a mean value.

**Statistical analysis:**

- homogeneity of variances analyzed by F-test ( $p < 0.1$ )
- Student's t-test or Aspin-Welch's t-test ( $p < 0.05$ ) was used to compare vehicle and FR901228 groups

**Results**

The treatment of guinea pig papillary muscle preparations with 0.3 and 1 µg/mL FR901228 resulted in no significant changes in APD<sub>90</sub>. However, treatment with 10 µg/mL FR901228 significantly shortened the APD<sub>90</sub> to 85.1% of baseline, as compared to 98.4% of baseline in the vehicle control group (statistically significant [ $p < 0.01$ ]). Treatment with 0.3 and 1 µg/mL FR901228 resulted in unremarkable effects on APA, but 10 µg/mL decreased APA to 96.6% of baseline as compared to 99.7% of baseline for vehicle control group (statistically significant [ $p < 0.01$ ]). There were no effects on RP or dV/dt observed as a result of FR901228 treatment at 0.3, 1 or 10 µg/mL. The positive control, sotalol, produced significant effects on all endpoints measured, indicating a valid assay.

The results of this assay suggest that FR901228 did not affect action potentials in isolated guinea pig papillary muscle, but that it shortened APD<sub>90</sub> and decreased APA at a

high concentration of 10 µg/mL. It is possible that FR901228 may lead to inactivation of calcium channels and/or the activation of potassium channels.

**Study title:** Safety pharmacology study of FR901228 – Effects on the central nervous, cardiovascular and respiratory systems in dogs.

**Key study findings:**

- Respiratory effects: There was an increased respiration rate of  $\leq 83\%$  at 16 hours postdose at 1 mg/kg romidepsin
- Cardiovascular effects: A decrease in systolic (maximum 12%), diastolic (maximum 16%) and mean blood pressures (maximum 13%) resulted from  $\geq 0.1$  mg/kg romidepsin compared to predose, with the largest change occurring from 8-12 hours postdose. A concurrent increase in heart rate resulted from  $\geq 0.1$  mg/kg romidepsin (maximum 52% at 0.3 mg/kg) compared to predose, with the largest change occurring at 10 hours postdose.
- ECG changes: Romidepsin administered at  $\geq 0.1$  mg/kg resulted in a shortened RR interval with a maximum decrease of 34% at 0.3 mg/kg occurring at 10 hours postdose. A QTc prolongation was observed at 1 mg/kg, with a maximum change of 8% at 6 hours postdose.
- Increased body temperature was observed at  $\geq 6$  mg/m<sup>2</sup>

**Study no.:** GLR030775

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** March 6, 2003

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** FR901228, lot # GLP-201102L

**Methods**

Doses: 0 (vehicle), 0.1, 0.3, 1 mg/kg (2, 6, 20 mg/m<sup>2</sup>); positive control – Astemizole 20 mg/kg (400 mg/m<sup>2</sup>)

Formulation/vehicle: IV; 0.2 v/v% ethanol – 0.8 v/v% propylene glycol – physiological saline; 4 hour infusion (2 mL/kg/hr); (positive control administered via oral gavage)

Species: Beagle dog

Number of animals: 4 males/group

Age/weight: 12 months / 10.25 – 11.35 kg

Dose justification: A repeat dose toxicity study with 4-hour intravenous infusion of FR901228 in dogs resulted in death or morbid sacrifice at a dose of 2.0 mg/kg. The maximum tolerated dose (MTD) in this study was

found to be 1 mg/kg. This was the basis for setting the highest dose in the current safety pharmacology study at 1 mg/kg.

Study design:

A telemetry transmitter was fixed in the abdominal cavity and a blood pressure sensor was inserted and fixed into a femoral artery. Electrocardiogram electrodes were subcutaneously fixed on the right and left flanks, and an access port for measurement of hemoglobin oxygen saturation was fixed on the dorsal part with the sensor inserted into the femoral artery. Access ports for FR901228 administration and for the hematological examination were fixed dorsally with the tube inserted into the femoral vein. Administration of FR901228 began when the animals had recovered from surgery and stable values for endpoints were obtained (17-20 days after surgery). Each animal received doses of vehicle, each dose of FR901228 in ascending order, and Astemizole with 6 days between each administration.

Endpoints:

Clinical signs: digital recordings of animals from 2 hr before infusion to 24 hr after completing infusion

\*Spontaneous motor activity: counted for 15 min at each determination as changes in intensity of signals from transmitter fixed on animal

\*Body temperature: sensor in abdominal cavity

\*Respiration rate: respiration variations counted based on blood pressure waveform

Hemoglobin oxygen saturation: hemoglobin saturation (sO<sub>2</sub>), pH, pO<sub>2</sub> and pCO<sub>2</sub> were determined with blood gas meter from 2 mL blood (plus heparin) drawn from femoral artery tube at times before infusion and 2, 4 and 24 hr after completing infusion

\*Blood pressure: recorded with femoral artery sensor

\*Heart rate: counted based on blood pressure waveform

\*Electrocardiogram: RR interval, PR interval, QRS duration, QT interval and QTc (Bazett's formula:  $QTc = QT/RR^{1/2}$ ), Fridericia's formula:  $QTc = QT/RR^{1/3}$ ) and Matsunaga's formula:  $QTc = \log 600 \times QT / \log RR$ )

Hematological examination: the following parameters were measured from 2 mL of blood (plus EDTA-2K) drawn from femoral vein at times before infusion and 24 hr after completing infusion:

- White blood cell count (WBC)
- Lymphocytes
- Red blood cells (RBC)
- Hemoglobin (HGB)
- Hematocrit (HCT)
- Mean corpuscular volume (MCV)

- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelets (PLT)

\* results from 0.5, 1, 2, 4, 4.5, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hr after completing infusion were used

**Statistical analysis:**

- One-way ANOVA between negative control and each test group for metric values
- Dunnett’s multiple test was performed when differences between groups were significant
- Student’s t-test was performed between negative control and positive control
- Statistical significance defined as 5%

**Results**

Mortality: None

Clinical signs: ↓ food consumption at 1 mg/kg

Spontaneous motor activity: ↑ at 0.1 and 0.3 mg/kg

Body temperature: ↑ ≤ 1.3°C at 0.3 mg/kg; ↑ ≤ 1.6°C at 1 mg/kg

Respiration rate:

- ↑ respiration rate (breaths/minute) at 1 mg/kg (maximum 83% at 16 hours postdose) when compared to predose

Hemoglobin oxygen saturation: unremarkable

Blood pressure:

- ↓ systolic (maximum 12%), diastolic (maximum 16%) and mean blood pressures (maximum 13%) at ≥ 0.1 mg/kg compared to predose, with the largest change from 8-12 hours postdose

Heart rate:

- ↑ heart rate at ≥ 0.1 mg/kg as compared to predose values
- Maximum increase at 0.3 and 1 mg/kg occurred 10 hours postdose

**Mean percent change in heart rate compared to predose**

Hours relative to start date	0.1 mg/kg	0.3 mg/kg	1 mg/kg	Astemizole 20 mg/kg
0.5	↓6	↑17	↓13	↑32 <sup>#</sup>
2	↓6	↑3	↑10	↑35
4	↓6	↑10	↑16	↑34
5	↑6	↑18	↑19 <sup>#</sup>	↑25 <sup>#</sup>
6	↑1	↑14	↑31 <sup>#</sup>	↑29 <sup>*</sup>
10	↑17	↑52	↑34	↑33
14	↑16	↑34	↑13	↑23

<sup>#</sup> statistically significant value (p<0.01)

<sup>\*</sup> statistically significant value (p<0.05)

Electrocardiogram:

- No changes in PR interval or QRS duration
- Shortening of RR interval at  $\geq 0.1$  mg/kg
  - $\downarrow$  RR interval (maximum 21%; 16% at 10 hours postdose)
  - $\downarrow$  RR interval (maximum 34% at 10 hours postdose) at 0.3 mg/kg compared to predose
  - $\downarrow$  RR interval (maximum 26% at 10 hours postdose) at 1 mg/kg compared to predose
- Shortening of QT interval at 0.1 and 0.3 mg/kg, but no change at 1 mg/kg
- Prolongation of QTc at 1 mg/kg
  - Maximum of 8% (Bazett's) and 5% (Fridericia's) at 6 hours postdose when compared to predose (statistically significant difference from control values [p<0.01])

Hematological examination:

**Hematological effects of FR901228 (% difference from control values)**

24 hours postdose	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Lymphocytes	-	$\downarrow 35^{\#}$	$\downarrow 61^{\#}$
WBC	-	$\uparrow 11$	$\uparrow 29$
RBC	$\uparrow 15$	$\uparrow 47$	$\uparrow 67^*$
HCT	$\uparrow 13$	$\uparrow 42$	$\uparrow 51$
HGB	$\uparrow 16$	$\uparrow 49$	$\uparrow 67^{\#}$

<sup>#</sup> statistically significant value (p<0.01)

\* statistically significant value (p<0.05)

**Summary and conclusions**

This study examined the effects of FR901228 on the cardiovascular and respiratory systems of dogs, with a lesser observation of the central nervous system. FR901228 was administered via intravenous infusion over four hours at 0.1, 0.3 and 1 mg/kg (2, 6 and 20 mg/m<sup>2</sup>). Animals received vehicle first, then ascending doses of FR901228, followed by a positive control, with 6 days between each administration. Minor effects were noted on the CNS as evident by slight increases in spontaneous motor activity at doses of 0.1 and 0.3 mg/kg FR901228, and by increased body temperature at 0.3 mg/kg (max 1.3°C) and 1 mg/kg (max 1.6°C) FR901228. Respiration rate was increased by a maximum of 83% of predose mean value at 1 mg/kg FR901228 by 16 hours postdose.

The most significant effects of FR901228 in this study were on the cardiovascular system. There was a decrease in the systolic (maximum 12%), diastolic (maximum 16%) and mean blood pressures (maximum 13%) at  $\geq 0.1$  mg/kg FR901228 when compared to predose, with the largest maximum decrease occurring from 8-12 hours postdose. There was a concurrent increase in heart rate at  $\geq 0.1$  mg/kg FR901228 with the maximum changes from predose values occurring 10 hours postdose (max 52% at 0.3 mg/kg). There were no effects of FR901228 on the PR or QRS duration or QT intervals. However, a shortening of the RR interval was observed at  $\geq 0.1$  mg/kg with the



Data was expressed as percent inhibition, which was calculated from 100 minus the binding ratio, defined as:  $[(B-N)/(B_0-N)] \times 100$  (%), where B is bound radioactivity in the presence of FR901228 or positive control, B<sub>0</sub> is bound radioactivity in the absence of FR901228 or positive control and N is non-specific bound radioactivity. Data was expressed as the mean values of duplicate samples for each assay. The criteria for a positive result included a binding inhibition ratio greater than 20%.

**Results**

The inhibition ratios of FR901228 to rat estrogen receptors were 97.76% at  $1 \times 10^4$  g/mL and 26.61% at  $1 \times 10^3$  ng/mL for  $\beta$ -estradiol binding. The inhibition ratio to the human recombinant neurokinin<sub>2</sub> receptors was 71.36% at  $1 \times 10^4$  ng/mL for neurokinin A binding. These ratios indicate FR901228 inhibition of ligand binding to these receptors at the concentrations tested. Inhibition ratios at other concentrations and to other receptors at all the concentrations were less than 20%, indicating no binding affinity. The inhibition ratios of the positive controls were more than 50%, indicating a valid assay.

While data suggest that inhibition of the estrogen pathway is the likely consequence of romidepsin binding to estrogen receptors, an agonistic effect cannot be excluded at this time. Modulation of estrogen pathway could have potential effects on the female reproductive organs, as well as numerous additional effects (e.g. effects on the cardiovascular system and bone). In addition, inhibition of estrogen binding to estrogen receptors may impact the effectiveness of oral contraceptive hormone therapy in patients receiving ISTODAX.

Neurokinin<sub>2</sub> receptors may be inhibited by romidepsin at the therapeutic exposure levels. The mean C<sub>max</sub> achieved in patients receiving romidepsin (377 ng/mL) was approximately 3.7% of the concentration of romidepsin that inhibited neurokinin A binding to neurokinin<sub>2</sub> receptors by 71.36% *in vitro*. This could potentially impact the function of the GI tract, upper respiratory system and the central nervous system.

**2.6.2.5 Pharmacodynamic drug interactions**

Not reviewed.

**2.6.3 PHARMACOLOGY TABULATED SUMMARY**

<i>Primary Pharmacokinetic Studies</i>		
Study	Test system	Significant findings
Cytotoxic activity to human cell lines	18 human tumor cells lines	-Mean IC <sub>50</sub> values against leukemia (lymphoma), renal, prostate, colon, and lung cancer were 3.23, 4.97, 1.61, 1.61, and 3.83 ng/mL, respectively.
Effect on expression of angiogenesis factors	PC-3 prostate cancer cells, ACHN renal cancer cells, BALB/c nu/nu mouse xenografts	- <i>In vitro</i> , the presence of romidepsin, a 51% decrease in the expression level of VEGF mRNA was seen in PC-3 prostate cancer cells (romidepsin sensitive). - <i>In vitro</i> , romidepsin did not alter the expression of VEGF mRNA in ACHN renal cancer cells (romidepsin resistant). - <i>In vivo</i> , romidepsin decreased (68%) the expression level of VEGF in the PC-3 prostate cancer xenograft but not in the ACHN renal cancer xenograft. - <i>In vitro</i> , romidepsin increased acetylation of histone H3 and H4 for all regions of the VEGF promoter in PC-3 prostate cancer cells.

Effect on human lymphoma U-937 cells	Human lymphoma U-937 cells, chronic myelogenous leukemia cell line K562, and the acute lymphoblastic leukemia cell line CCRF-CEM, scid mouse xenografts	<p><i>-In vitro</i>, romidepsin inhibited the growth of U-937, K562, and CCRF-CEM cells with IC<sub>50</sub> values of 5.92, 8.36, and 6.95 nM, respectively.</p> <p><i>-In vivo</i>, using a scid mouse model, romidepsin prolonged survival time of scid mice inoculated with lymphoma U-937 cells.</p> <p>-5 ng/mL romidepsin appeared to increase the rate of apoptosis, cause G2/M arrest, increase differentiation (increased CD11b+/CD14+), induce the expression of p21/WAF1/Cip1 and geloslin mRNA, and increase acetylation of the P1 and P2 regions of histone H3 and all regions of histone H4, in U-937 lymphoma cells.</p> <p>-0.5 ng/mL romidepsin appeared to cause G1 arrest and increase differentiation (increased CD11b+/CD14+) in U-937 lymphoma cells.</p>
Anti-tumor effects in animals	Human tumor cells, normal human cells, and murine embryo cells, P388 and L1210 leukemia mouse xenograft, B16 melanoma mouse xenograft, 6 murine solid tumors, BALB/c <i>nu/nu</i> mouse xenografts, drug resistant tumor mouse xenografts, isolated nuclei of Ras1 cells derived from NIH3T3 cells	<p>-Romidepsin exhibited stronger anti-tumor activity <i>in vitro</i> on human lung, stomach, breast, and colon cancer cells than on normal human fibroblast and endothelium cells, and murine embryo cells.</p> <p>-Romidepsin showed anti-tumor activity against P388 and L1210 leukemia and B16 melanoma mouse xenografts.</p> <p><i>-In vivo</i>, romidepsin administration resulted in growth inhibition in murine solid tumors derived from murine colon, melanoma, reticulum sarcoma, and fibrosarcoma cancer cells.</p> <p>-Using 9 types of human tumors in BALB/c <i>nu/nu</i> mouse xenografts, romidepsin caused growth inhibition in stomach, lung giant cell, non-small cell lung, and breast cancer cells.</p> <p>-Romidepsin also caused growth inhibition in a number of drug-resistant tumors in mice, except adriamycin-resistant tumors.</p> <p>-Exposing L1210 lymphocytic leukemia cells to romidepsin, <i>in vitro</i>, caused a dose-dependent inhibition of the incorporation of radiolabeled thymidine, uridine, and leucine into DNA, RNA, and protein, respectively. RNA synthesis appeared the most sensitive. Also, in the same cell line, romidepsin did not appear to induce single-strand DNA breaks.</p> <p>-In isolated nuclei of Ras1 cells derived from NIH3T3 cells, romidepsin appeared to arrest cells in G0/G1 phase, decrease c-myc mRNA expression, and did not inhibit DNA synthesis.</p>
Anti-tumor activity against human tumor xenografts in nude mice	Renal (ACHN and RXF-3IL), prostate (PC-3 and DU-145), and colon (HT-29, HCC2998, and HCT-15) human tumor xenografts in BALB/c <i>nu/nu</i> mice	-Romidepsin exhibited anti-tumor activity against human PC-3 prostate cancer and human RXF63IL renal cancer in BALB/c <i>nu/nu</i> mice xenografts.

**Safety Pharmacology Studies**

Species / Test system	Route / Duration	N/sex/dose	Dose (mg/kg)/ Concentration (µg/mL)	Dose (mg/m <sup>2</sup> )	Significant findings
Rat Neurological effects	IV Single dose	6 males	1 0.3 0.1	6 1.8 0.6	6 mg/m <sup>2</sup> : 50% mortality; ↑ heart rate, respiration rate; ↓ activity; effects on equilibrium and gait; CNS excitation; ↓ muscle tone, autonomic response (24h) 1.8 mg/m <sup>2</sup> : ↓ activity, muscle tone
hERG assay in HEK293 cells	<i>in vitro</i>	5 cells / conc.	10 1 0.3	-	low potency hERG blocker (IC <sub>50</sub> > 18.49 µM)

Potassium channel binding assay with reduced romidepsin (M1)	<i>in vitro</i>	-	10 $\mu$ M	-	no significant binding to potassium channels (hERG, rat SK <sub>CA</sub> , hamster K <sub>ATP</sub> )
Action potential measurement in isolated guinea pig ventricular muscle	<i>in vitro</i>	5 males	10 1 0.3	-	10 $\mu$ g/mL: shortened action potential duration at 90% repolarization (APD <sub>90</sub> ); $\downarrow$ action potential amplitude (APA)
Dog CNS, Cardiovascular, Respiratory effects	IV Single dose	4 males	1 0.3 0.1	20 6 2	Respiratory effects: $\uparrow$ respiration rate ( $\leq$ 83%) at 20 mg/m <sup>2</sup> after 16 hours CNS effects: $\uparrow$ spontaneous motor activity at $\leq$ 6 mg/m <sup>2</sup> , body temperature at $\geq$ 6 mg/m <sup>2</sup> Cardiovascular effects: $\downarrow$ systolic ( $\leq$ 12%), diastolic ( $\leq$ 16%) and mean blood pressures ( $\leq$ 13%) at $\geq$ 2 mg/m <sup>2</sup> with maximum change from 8-12 hours postdose; $\uparrow$ heart rate at $\geq$ 2 mg/m <sup>2</sup> (maximum 52% at 0.3 mg/kg) with maximum change at 10 hours postdose; ECG changes: shortened RR interval (maximum $\downarrow$ of 34% at 0.3 mg/kg) at 10 hours postdose; QTc prolongation (8%) at 1 mg/kg 6 hours postdose
Receptor binding assay	<i>in vitro</i>	-	10 1 0.3	-	10 $\mu$ g/mL: inhibited $\beta$ -estradiol binding to estrogen receptors by 97.76%; inhibited neurokinin A binding to neurokinin <sub>2</sub> receptors by 71.36% 1 $\mu$ g/mL: inhibited $\beta$ -estradiol binding to estrogen receptors by 26.61%

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

Based on the summary data excerpted from the package, a single intravenous infusion of romidepsin at 0.3 mg/kg (6 mg/m<sup>2</sup>) and 1.0 mg/kg (20 mg/m<sup>2</sup>) in Beagle dogs resulted in a T<sub>max</sub> of 1-2 hours. By 10 hours postdose, plasma concentrations were below the limits of quantitation (0.5 ng/mL) at both doses. The C<sub>max</sub> and AUC values were approximately dose-proportional.

In a 26-week toxicity study in rats, romidepsin was administered intravenously once a week for three weeks out of every four weeks at 0.1, 0.33 and 0.67 mg/kg (0.6, 1.98 and 4.02 mg/m<sup>2</sup>). Plasma AUC<sub>0-tlast</sub> and C<sub>max</sub> values, as measured by LC/MS/MS, increased with increasing doses. There were no relevant differences in exposures between males and females. Exposures and t<sub>1/2</sub> increased on day 176 compared to day 1, suggesting the potential for drug accumulation upon repeated administration: t<sub>1/2</sub> = 0.2-0.9 hr on day 1

and 0.2-2.0 hr on day 176;  $AUC_{0-t} = 17-18$  ng\*hr/mL at high dose on day 1 and 73-88 ng\*hr/mL at high dose on day 176.

#### Distribution:

$^{14}C$ -romidepsin was widely distributed in rats following a single dose. Distribution to most tissues was very rapid, as the highest concentrations achieved in all tissues except ileum occurred at 5 minutes postdose. The tissue distribution occurred in the following order from highest concentration ( $C_{max}$  with units of ng eq/mL) to the lowest: kidney (2187.3), urinary bladder (791.0), jejunum (684.3), liver (663.7), adrenal gland (586.7), lung (496.1), heart (489.6), submaxillary gland (450.2), spleen (443.8), thyroid gland (426.5), pancreas (422.8), blood (412.0), bone marrow (313.0), pituitary gland (301.2), stomach (297.0), brown fat (292.3), plasma (276.5), colon (236.5), mesentery lymph node (229.2), prostate gland (215.0), muscle (200.2), ileum (198.6), skin (128.2), thymus (125.6), thigh bone (79.4), eye ball (60.1), white fat (34.1), testis (25.8), cerebellum (10.2), cerebrum (9.0) and brain stem (6.9). In all tissues except cerebellum, cerebrum, brain stem, white fat and prostate gland, radioactivity was reduced, but measurable at 168 hours postdose. The amount of romidepsin and/or its metabolites that was in blood was almost 2-fold higher than in plasma.

Binding to serum proteins and cells in the blood was assessed in two studies. Romidepsin was highly protein bound, but binding was independent of concentration from 50 ng/mL – 500 ng/mL in rat and dog and from 50 ng/mL – 1000 ng/mL in human. Binding was higher in serum from dogs (> 73%) and humans (> 82%) than in rats (37%). There was no significant difference of protein binding in human plasma compared to human serum. Romidepsin bound better to  $\alpha_1$ -AGP (93%) than to albumin (20%). The blood:plasma ratio of romidepsin was less than 1 and was nearly constant in rats and humans from 50 to 5000 ng/mL. The percent of  $^{14}C$ -romidepsin that was transferred from plasma to blood cells was  $\leq 25\%$  in rats,  $\leq 12\%$  in dogs and  $\leq 9\%$  in humans. In an *in vivo* study in rats, romidepsin concentrations,  $AUC_{0-inf}$  and  $t_{1/2}$  were higher in blood than plasma.

#### Metabolism:

Romidepsin was found to be extensively metabolized in *in vitro* and *in vivo* rat studies. Identification and/or quantification of romidepsin metabolites found in human plasma after clinical administration was not performed. In one metabolism study including both *in vitro* and *in vivo* analysis of romidepsin metabolism, 28 metabolites were identified. Metabolites M1-M14 were produced *in vitro* by rat and human liver microsomes. Metabolites M15-M17 were produced *in vivo* and isolated from rat bile following intravenous romidepsin injection. Romidepsin contains a disulfide bond, which has the potential to be reduced by glutathione in cells. Romidepsin was shown to form glutathione conjugates *in vitro*, identified as metabolites M18-M20. Dithiothreitol (DTT) was used to artificially reproduce the glutathione reduction of romidepsin in metabolism studies. Finally, metabolites M21-M28 were produced by human liver microsomes at a shorter incubation time point than that which resulted in the formation of M1-M14. Together, there were 6 mono-oxidation metabolites of romidepsin: M3, M7, M13, M14 M17 and M21. Five mono-oxidation and reduction of disulfide metabolites

were identified: M4, M5, M6, M22 and M23. Two new di-oxidation metabolites were described: M24 and M25, and three new di-oxidation and reduction of disulfide metabolites were described: M26, M27 and M28.

In another *in vitro* study designed to compare hepatic metabolism of romidepsin by liver microsomes and liver S9 fractions from rats, dogs and humans, 20 metabolites were identified from all species. No comparison was made between this study and the one described above, so there was no direct correlation of metabolites identified in each study. The twelve major metabolites were: M1, M3, M6, M7, M8, M10, M11, M12, VMH-1, VMH-2, VMH-3 and VMH-4. VMH-1, VMH-2, VMH-3 and VMH-4 were unidentified metabolites. The formation of VMH-3 and VMH-4 were only produced from rat microsomes. Conversely, the formation of M10 was not produced from rat, but was produced from dog and human microsomes, suggesting M10 may not be present in non-clinical reproductive and developmental toxicology or genetic toxicology studies. Considering the proposed indication and the low abundance of this metabolite produced by the human microsomes (7.6% of the total radioactivity recovered), additional nonclinical studies of the metabolite is not deemed necessary. In addition, it is unclear whether this metabolite was present in rats in other metabolism studies as metabolites seemed to be coded independently in different studies. The metabolite M1 was produced non-enzymatically, was the most abundant metabolite, and was created by DTT reduction. M1 is the reduced form of romidepsin and was metabolized further similarly by rat, dog and human liver microsomes. The major metabolites produced when romidepsin was reduced to M1 by DTT before incubation with liver microsomes were M2, M7, M8, M9 and VMH-16 in all species, indicating these are produced from M1. DTT reduction of romidepsin after incubation with liver microsomes did not result in any species differences, but did increase the recovery of radiolabeled material from all species. This indicates that romidepsin and/or its metabolites are protein bound through disulfide bonds as DTT reduction released bound radioactive material. The recovery of radioactivity was 76.4%, 89.9% and 75.9% in rats, dogs and humans.

In an additional *in vivo* study in rats, romidepsin was found to be extensively metabolized, resulting in a total of 31 metabolites (MH-1 through MH-31) detected in plasma, urine, bile and feces. Again, no comparison was made between the metabolites identified in this study and in the two metabolism studies described above, and metabolites identified in this study were named following a different naming system than was used in other studies. This study was designed to combine analysis of the metabolism and excretion of romidepsin and its metabolites. DTT reduction of romidepsin resulted in the production of MH-28, which may correspond to the reduced form of romidepsin, M1, described in other studies, but this information was not conveyed. Metabolism of romidepsin was not influenced by biliary glucuronidation. Most of the metabolites in plasma were protein bound, as reduction with DTT led to an increase in recovered radioactivity. Most metabolites were present at less than 5% of total radioactive material in the plasma, bile, feces or urine (in the absence of DTT). The metabolite MH-31 was present at 9% in the plasma up to 4 hours postdose, and MH-21 was present at 7% in the bile after 48 hour postdose collection.

Metabolism of romidepsin by specific recombinant P450 enzymes was assayed *in vitro*. Romidepsin was also incubated with liver microsomes and ketaconazole, a CYP3A inhibitor, or an anti-CYP3A4 monoclonal antibody. Romidepsin metabolic

activity was highest by CYP3A4 enzyme. Ketaconazole and anti-CYP3A4 antibody both inhibited romidepsin disappearance by  $\geq 90\%$  after incubation with human liver microsomes. This indicates that the majority of romidepsin hepatic metabolism is accomplished by CYP3A4.

**Excretion:**

The mode and rate of elimination of romidepsin and/or its metabolites from rats after a single injection of  $1.8 \text{ mg/m}^2$  ( $28.6 \text{ } \mu\text{Ci/kg}$ )  $^{14}\text{C}$ -romidepsin were assessed in studies of distribution and metabolism. The major route of excretion of radioactive material was found to be the bile/feces, accounting for 66.1% (biliary) and 75.1% (fecal) of the total radioactivity administered, with a more minor elimination of 20% through urine after 48 hours in rats with cannulated bile ducts. High levels of radioactivity found in liver, kidney, bladder and jejunum after content removal supports these routes of excretion. Metabolites identified in this study of metabolism as MH-1 and MH-11 were only found in rat feces, suggesting they may be products of rat gut bacteria.

**2.6.4.2 Methods of Analysis**

Separate methods of analysis reports were not reviewed; see under each study report.

**2.6.4.3 Absorption**

Not applicable for intravenously administered drugs.

**2.6.4.4 Distribution**

**Study title:** Non-clinical pharmacokinetics: *in vitro* protein binding of FR901228 in rats, dogs, humans, and human serum proteins

**Key study findings:**

- Protein binding was independent of romidepsin concentration from 50 ng/mL – 500 ng/mL in rat and dog serum and from 50 ng/mL – 1000 ng/mL in human serum.
- Protein binding decreased at 5000 ng/mL romidepsin in human and dog serum.
- Protein binding was higher in serum from dogs (86%) and humans (94%) than in rats (37%) at 500 ng/mL romidepsin.
- Romidepsin bound better to  $\alpha_1$ -AGP (93%) than to albumin (20%).

**Study no.:** CRD040011

**Volume #, and page #:** electronic submission

**Conducting laboratory and location:**

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**b(4)**

**Date of study initiation:** May 8, 2003

**GLP compliance:** No

**QA reports:** No

**Drug and % purity:**  $^{14}\text{C}$ -FR901228, code no. 6710, 98% radiochemical purity

**Methods:** Binding of <sup>14</sup>C-FR901228 to protein was determined by ultrafiltration.

**Results:**

**Serum and plasma protein binding of FR901228 *in vitro* (% bound)**

Species		Concentration of FR901228 (ng/mL)			
		50	500	1000	5000
Rat	Serum	40.81 ± 0.89	37.75 ± 0.28	-	37.98 ± 0.27
		(59.19 ± 0.89)	(62.25 ± 0.28)	-	(62.02 ± 0.27)
Dog	Serum	87.70 ± 0.40	86.48 ± 0.70	-	73.14 ± 0.12
		(12.30 ± 0.40)	(13.52 ± 0.70)	-	(26.86 ± 0.12)
Human	Serum	94.53 ± 0.25	94.18 ± 0.37	93.55 ± 0.12	82.16 ± 1.18
		(5.47 ± 0.25)	(5.82 ± 0.37)	(6.45 ± 0.12)	(17.84 ± 1.18)
Human	Plasma	92.04 ± 1.04	92.49 ± 0.84	93.80 ± 0.29	82.28 ± 0.16
		(7.96 ± 1.04)	(7.51 ± 0.84)	(6.20 ± 0.29)	(17.72 ± 0.16)

Mean ± S.E., n=3

Figures in parentheses represent free fraction (%).

***In vitro* binding of FR901228 to human serum proteins at 500 ng/mL (% bound)**

Protein	Conc.	Protein binding of FR901228 (%)
Alubmin	40mg/mL	19.91±0.41 (80.09±0.41)
α <sub>1</sub> -AGP	1mg/mL	93.51±0.51 (6.49±0.51)

Mean ± S.E., n=3

Figures in parentheses represent free fraction (%).

*Tables excerpted from sponsor's package*

**Study title:** Non-clinical pharmacokinetics: *in vitro* transfer of FR901228 to blood cells in rats, dogs and humans

**Key study findings:**

- Blood to plasma concentration ratios were less than 1 and nearly constant in rats, dogs and humans from 50 to 5000 ng/mL romidepsin.
- The percent of romidepsin that was transferred from plasma to blood cells was ≤25% in rats, ≤12% in dogs and ≤9% in humans.

**Study no.:** CRD040012

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

**Conducting laboratory and location:**

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**Date of study initiation:** May 8, 2003

**GLP compliance:** No

**QA reports:** No

**Drug and % purity:** <sup>14</sup>C-FR901228, code no. 6710, 98% radiochemical purity

**Methods:** <sup>14</sup>C-FR901228 was incubated with blood collected from male Sprague-Dawley rats, male Beagle dogs and human male volunteers. Solutions of 50, 500 and 5000 ng/mL <sup>14</sup>C-FR901228 final concentration were added to rat, dog and human blood sample, with a fourth concentration of 1000 ng/mL prepared in human blood. After plasma collection, radioactivity was measured by a liquid scintillation counter for all blood and plasma samples. The blood/plasma ratio was calculated by dividing the radioactivity in whole blood by the radioactivity in plasma. The percentage transfer to blood cells (%) was calculated by the following equation: (%) = [1 – radioactivity in plasma x (1 – hematocrit) / radioactivity in whole blood] x 100.

**Results:**

***In vitro* blood to plasma concentration ratio and percentage transfer to blood cells**

Species	Hematocrit value	Mean blood to plasma ratio			
		FR901228 (ng/mL)			
		50	500	1000	5000
Rat	0.43	0.75 (22.8)*	0.75 (25.2)	-	0.68 (15.6)
Dog	0.43	0.59 (2.3)	0.58 (1.5)	-	0.65 (12.3)
Human	0.44	0.59 (4.7)	0.61 (8.7)	0.56 (1.4)	0.60 (4.8)

\* Values in parentheses are percentage transfer to blood cells (%)

**Study title:** Non-clinical pharmacokinetics: distribution and excretion of radioactivity after single intravenous administration of [<sup>14</sup>C]-FR901228 in rats

**Key study findings:**

- Romidepsin and/or its metabolites had a high degree of tissue distribution.
- Tissue C<sub>max</sub> was highest in kidney, urinary bladder, jejunum, liver and adrenal glands.
- T<sub>max</sub> was within 5 minutes postdose in all tissues examined except ileum.
- The primary route of elimination occurred through bile (66% at 48 h postdose), with less occurring through urine (20% at 48 h postdose) after IV administration.

**Study no.:** CRD040009

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

**Conducting laboratory and location:**

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**Date of study initiation:** January 23, 2003

**GLP compliance:** No

**QA reports:** No

**Drug and % purity:**  $^{14}\text{C}$ -FR901228, code no. 6710, 96.4% chemical purity, 98% radiochemical purity

**Vehicle:** propylene glycol/ethanol (8:2, v/v) in saline

**Methods:**

The tissue distribution of total  $^{14}\text{C}$ -FR901228 was determined after a single intravenous injection via tail vein of 0.3 mg/kg (28.6  $\mu\text{Ci/kg}$ ). The tissues in the following table were collected from rats sacrificed after blood collection at 5 minutes and 4, 24 and 168 hours postdose. The organs were weighed and then homogenized and aliquots placed in liquid scintillation counter (LSC) vials for measurement of radioactivity by LSC. Aliquots of whole blood and plasma were also added to LSC vials for counting.

Excreta were collected to measure routes of excretion. Urine was sampled at 4, 8, 24, 48, 72, 96, 144 and 168 hours, bile at 4, 8, 24 and 48 hours, and feces at 24, 48, 96, 144 and 168 hours postdose. Expired air was collected at 24, 48 and 72 hours post dose into methanol/monoethanolamine solution (3:1). The GI tract was removed after sacrifice at 168 hours postdose and the contents removed, at which time the carcass was lysed in hydrochloric acid solution. Cage washings were also collected at 168 hours postdose. Aliquots of urine, bile, fecal homogenates, cage washings, carcass lysates and expired air solution were added to LSC vials for counting to measure relative amounts of radioactivity per sample.

Species: male Sprague-Dawley rat  
 Number: 3/group  
 Age/weight: not provided / 254.1-312.0 g  
 Dose: 0.3 mg/kg (28.6  $\mu\text{Ci/kg}$ )  
 Frequency: single dose  
 Route: intravenous injection in tail vein

**Radioactivity in plasma and blood in rats**

Time (h)	Radioactivity concentration ( ng eq./mL of FR901228 )		B/P ratio
	Plasma	Blood	
0.083	276.5 ± 18.5	412.0 ± 27.5	( 1.48 )
0.25	159.6 ± 9.3	190.1 ± 6.8	( 1.19 )
0.5	117.9 ± 2.5	146.0 ± 1.3	( 1.23 )
1	76.5 ± 5.6	110.8 ± 6.4	( 1.44 )
2	46.0 ± 3.5	87.4 ± 4.1	( 1.90 )
4	23.1 ± 0.7	71.0 ± 3.7	( 3.06 )
6	12.5 ± 1.3	52.7 ± 1.9	( 4.27 )
8	8.6 ± 0.2	45.7 ± 1.2	( 5.28 )
24	2.6 ± 0.0	25.7 ± 1.8	( 9.49 )
48	1.0 ± 0.0	11.1 ± 1.0	( 10.60 )
72	0.0 ± 0.0	6.5 ± 0.0	( n.c. )
144	0.0 ± 0.0	3.1 ± 0.0	( n.c. )
168	0.0 ± 0.0	2.4 ± 0.1	( n.c. )
$t_{1/2}$ (h) 8→48 h	13.2	19.6	
$t_{1/2}$ (h) 72→168h	-	66.9	
AUC <sub>(0-48)</sub> (ng eq. · h/mL)	466.1	-	
AUC <sub>(0-168)</sub> (ng eq. · h/mL)	-	2313.9	
AUC <sub>(0-∞)</sub> (ng eq. · h/mL)	483.5	2546.4	

Each value represents the mean ± S.E. of three rats.

Figures in parentheses represent the ratio of concentration in blood relative to plasma.

(Table excerpted from sponsor's package)

**Concentration of radioactivity in tissues**

Tissue	T <sub>max</sub> (hr)	C <sub>max</sub> (ng eq/g)
Plasma	0.083	276.5
Blood	0.083	412.0
Cerebrum	0.083	9.0
Cerebellum	0.083	10.2
Brain stem	0.083	6.9
Pituitary gland	0.083	301.2
Eye ball	0.083	60.1
Submaxillary gland	0.083	450.2
Thyroid gland	0.083	426.5
Thymus	0.083	125.6
Heart	0.083	489.6
Lung	0.083	496.1
Liver	0.083	663.7
Kidney	0.083	2187.3
Adrenal gland	0.083	586.7
Spleen	0.083	443.8
Pancreas	0.083	422.8
White fat	0.083	34.1
Brown fat	0.083	292.3
Muscle	0.083	200.2
Skin	0.083	128.2
Prostate gland	0.083	215.0
Urinary bladder	0.083	791.0
Testis	0.083	25.8
Thigh bone	0.083	79.4
Bone marrow	0.083	313.0
Mesentery lymph node	0.083	229.2
Stomach	0.083	297.0
Jejunum	0.083	684.3
Ileum	4	198.6
Colon	0.083	236.5

**Excretion of radioactivity after injection of <sup>14</sup>C-FR901228**

Sample	T <sub>max</sub> (hr)	Cumulative excretion of radioactivity 168 h postdose (% of dose)
Bile	0-4	66.1 (48 h)
Urine (w/ cannulated bile duct)	0-24 0-4	16.5 20.0 (48 h)
Feces (w/ cannulated bile duct)	0-24 0-24	79.4 5.2 (48 h)
Expired air	0-24	0.1

Sample	T <sub>max</sub> (hr)	Cumulative excretion of radioactivity 168 h postdose (% of dose)
GI contents (w/ cannulated bile duct)	NC*	0.1
	NC	0.8 (48 h)
Carcass (w/ cannulated bile duct)	NC	1.8
	NC	7.5
Cage washing	NC	0.0
Recovery (w/ cannulated bile duct)	NC	97.8
	NC	99.6

\* NC, not calculated

### Summary and conclusions

FR901228 (romidepsin) and/or its metabolites were widely distributed. Radioactivity was low in the brain, but moderate in the pituitary gland, however, this indicates that FR901228 (romidepsin) and/or its metabolites can cross the blood-brain barrier. The tissue to blood ratios of radioactivity was  $\geq 1$  for multiple tissues, indicating relatively extensive distribution. There were higher levels of radiolabeled material in blood than in plasma by almost 2-fold. The primary route of elimination of FR901228 after IV injection is through the bile, with less in the urine. The high levels of radioactivity found in the liver, kidney, bladder and jejunum support these routes of excretion.

### 2.6.4.5 Metabolism

**Study title:** Drug metabolism: identification of *in vitro* and *in vivo* metabolites of FR901228 by liquid chromatography-tandem mass spectrometry

#### Key study findings:

- The chemical structures were determined of 28 romidepsin metabolites.
- Metabolites M1-M14 were produced *in vitro* by rat and human liver microsomes, and following DTT reduction after incubation with human liver microsomes.
- Metabolites M15-M17 were isolated from rat bile as *in vivo* produced metabolites.
- Incubation of FR901228 with glutathione produced conjugate metabolites M18-M20.
- Metabolites M21-M28 were produced *in vitro* by human liver microsomes.

**Study no.:** CRD040013

**Volume #, and page #:** Electronic submission, Module 4; 4.2.2.4.1

**Conducting laboratory and location:**

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b(4)

**Date of study initiation:** January 6, 2003

**GLP compliance:** No

**QA reports:** No

**Drug and % purity:** <sup>14</sup>C-FR901228, code no. 6710, 98% radiochemical purity

**Vehicle:** *in vitro* - methanol/acetonitrile (1:1, v/v); *in vivo* – propylene glycol/ethanol (8:2, v/v) in saline

**Methods:** Metabolites of  $^{14}\text{C}$ -FR901228 were formed *in vitro* and *in vivo* in rats. The parent compound and metabolites were isolated by HPLC and identified by LC/ESI/ion trap MS<sup>n</sup>.

*In vitro* metabolites formed by human liver microsomes:

Pooled human liver microsomes prepared from 46 individuals were obtained and used for *in vitro* metabolism of  $^{14}\text{C}$ -FR901228. Human liver microsomes (2 mg protein/mL) were incubated for 40 minutes with  $^{14}\text{C}$ -FR901228 (60 $\mu\text{M}$ ) and the NADPH-generating system. This procedure was repeated with a 10 minute incubation.

Preparation of glutathione conjugates of FR901228:

FR901228 (200 $\mu\text{M}$ ) was incubated at room temperature for 15 minutes with glutathione (5 mM) with potassium phosphate (100 mM, pH 7.4). This was then separated and analyzed as *in vitro* liver microsome samples.

*In vitro* metabolites formed by human liver microsomes followed by DTT treatment:

After incubation of  $^{14}\text{C}$ -FR901228, the NADPH-generating system and human liver microsomes for 40 minutes, DTT (20 mM) was added for 10 minutes.

*In vitro* metabolites formed by rat liver microsomes:

Livers from five seven-week old rats [— :CD(SD)IGS] were pooled to isolate microsomes (used at 1 mg protein/mL) for *in vitro* metabolism of  $^{14}\text{C}$ -FR901228. Rat liver microsomes were incubated with  $^{14}\text{C}$ -FR901228 (60  $\mu\text{M}$ ) and the NADPH-generating system for 40 minutes. b(4)

*In vivo* metabolites formed in rat bile:

Seventeen seven-week old male rats [— :CD(SD)IGS] were administered a single dose of  $^{14}\text{C}$ -FR901228 intravenously into the thigh vein at 0.3 mg/kg (1.8 mg/m<sup>2</sup>) at 5 mL/kg. Bile was collected through a cannulated bile duct for 8 hours after dosing. After preparation, the solution containing  $^{14}\text{C}$ -FR901228 and metabolites isolated from rat bile was separated and analyzed as in the *in vitro* samples. b(4)

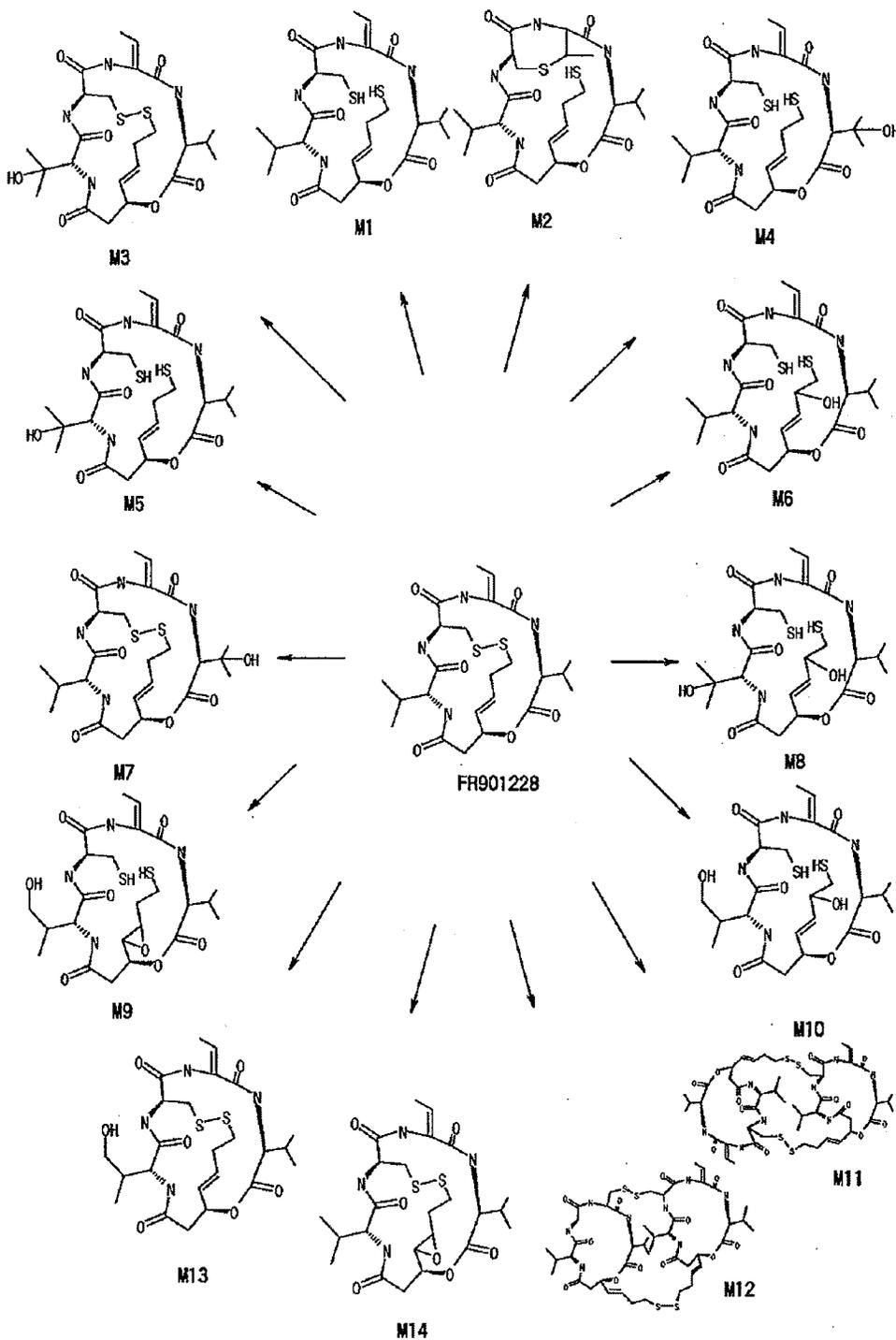
## Results

Metabolites M1-M14 were produced by incubation of  $^{14}\text{C}$ -FR901228 with both rat and human liver microsomes, and with DTT treatment after incubation with human liver microsomes. Metabolites M15-M17 were isolated from rat bile as *in vivo* produced metabolites. Incubation of FR901228 with glutathione produced conjugate metabolites M18-M20. In addition to M1-M14, a shorter *in vitro* incubation of  $^{14}\text{C}$ -FR901228 and human liver microsomes also produced M21-M28.

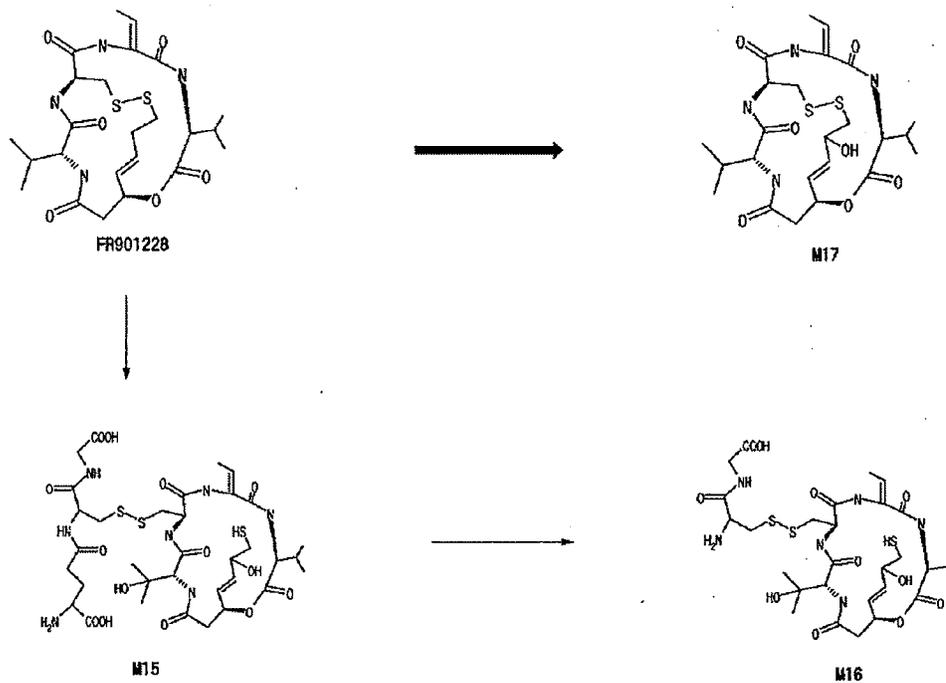
There were 6 mono-oxidation metabolites of FR901228: M3, M7, M13, M14 M17 and M21. Five mono-oxidation and reduction of disulfide metabolites were identified: M4, M5, M6, M22 and M23. Two new di-oxidation metabolites were described: M24

and M25, and three new di-oxidation and reduction of disulfide metabolites were described: M26, M27 and M28.

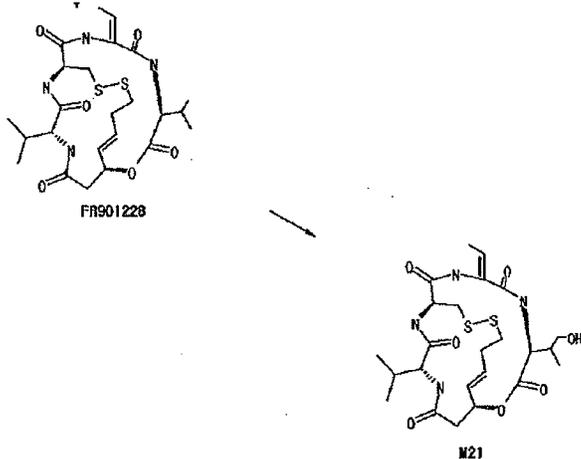
***In vitro* metabolism of FR901228**



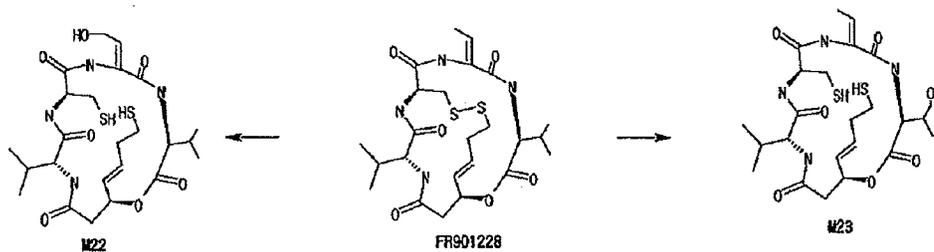
***In vivo* metabolism of FR901228 in rats**



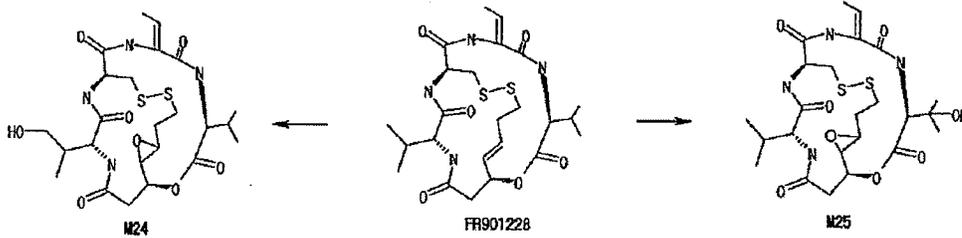
**Additional mono-oxidized FR901228 metabolites**



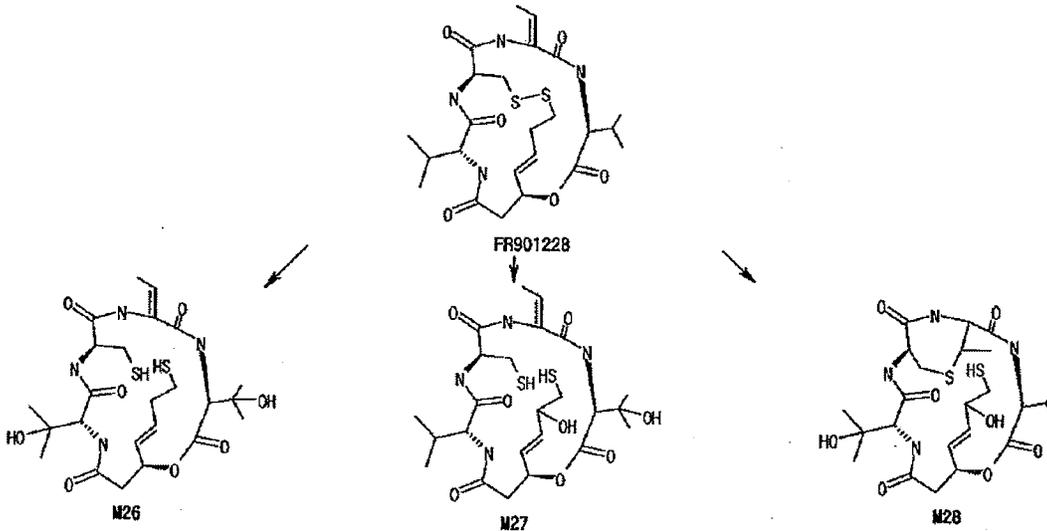
**Additional mono-oxidized and reduced metabolites of FR901228**



**Di-oxidized FR901228 metabolites**



**Di-oxidized and reduced FR901228 metabolites**



(Figures modified from sponsor's package)

**Study title:** Drug metabolism: species differences in hepatic metabolism of FR901228 between rats, dogs, and humans

**Key study findings:**

- At least 20 metabolites were produced by liver microsomes.
- An abundant metabolite (M1) was produced rapidly and non-enzymatically, and is metabolized similarly by microsomes from rats, dogs and humans.
- All metabolites detected in humans were detected in rats or dogs.
- One metabolite (M10) produced from human and dog microsomes was not produced from rat.

**Study no.:** CRD030200

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** February 24, 2003

**GLP compliance:** No

**QA reports:** No

**Drug and % purity:**  $^{14}\text{C}$ -FR901228, code no. 6710, 98% radiochemical purity

**Vehicle:** methanol/acetonitrile (1:1, v/v)

**Methods:** Liver S9 and microsomal fractions (1 mg protein/mL) were prepared from male Sprague-Dawley rats, male Beagle dogs and humans. Parent compound ( $^{14}\text{C}$ -FR901228) was used at a concentration of 10.  $\mu\text{M}$ .

FR901228 metabolic activity in liver microsomes:

The metabolic activity of FR901228 in liver microsomes was analyzed by measuring the radioactivity of the metabolites formed and the remaining parent compound after HPLC separation. The  $^{14}\text{C}$ -FR901228 and liver microsomes were incubated with an NADPH-generating system (2 mM NADP, 10 mM G-6-P, 1 unit/mL G-6-P DH, 5 mM  $\text{MgCl}_2$ ) and 0.1 M potassium phosphate buffer (pH7.4). The reaction was incubated for 0, 5, 10, 20 and 40 minutes, then separated by HPLC, and radioactivity was measured in eluates. Controls were reactions containing denatured rat liver microsomes, no NADPH-generating system or no incubation period. Dithiothreitol (DTT), was also added in some samples to reduce disulfide bonds of FR901228 and its metabolites, which were then measured in the same way.

Measurement of FR901228 reduced-form (metabolite M1) activity in liver microsomes:  $^{14}\text{C}$ -FR901228 was reduced to M1 by incubation with 60 mM DTT before adding liver microsomes and NADPH-generating system to the incubation.

FR901228 metabolic activity in liver S9 fraction with glutathione (GSH):

Incubation mixtures contained  $^{14}\text{C}$ -FR901228 (10  $\mu\text{M}$ ), liver S9 fraction (1 mg protein/mL), 5 mM GSH, 10 mM G-6-P, and 0.1 M potassium phosphate buffer (pH 7.4) and proceeded for 10 minutes. To assess any non-enzymatic reaction between FR901228 and GSH, 10  $\mu\text{M}$   $^{14}\text{C}$ -FR901228, 5 mM GSH, and 0.1 M potassium phosphate buffer (pH 7.4) were incubated for 0, 1, 2.5 and 5 hours. All samples were assayed by HPLC.

## Results

Species differences in liver microsomes:

The recovery of radioactivity in the supernatant from the incubation mixtures decreased over time. After 40 minutes, the recovery was 76.4%, 89.9% and 75.9% in rats, dogs, and humans, respectively. The same trend was seen with denatured rat liver microsomes in the incubation. When  $^{14}\text{C}$ -FR901228 was reduced with DTT prior to incubation with liver microsomes, recovery of radioactivity at 40 minutes was > 98% in all species. When  $^{14}\text{C}$ -FR901228 and its metabolites were reduced with 2 mM DTT after 40 minutes of incubation with liver microsomes, no increase in recovery was observed as compared to no DTT. However, reduction of  $^{14}\text{C}$ -FR901228 and metabolites with 20 mM DTT after 40 minutes of incubation with liver microsomes increased the recovery of radioactivity in rat, dog and human by 78.3-87.7%, 90.6-99.2% and 72.4-88.6%,

respectively, suggesting the parent compound, <sup>14</sup>C-FR901228 may form a reversible disulfide bond with protein thiols, which may be reduced by high concentrations of DTT.

**Metabolism in the absence of DTT:**

Incubation of <sup>14</sup>C-FR901228 with liver microsomes and a NADPH-generating system produced at least 20 metabolites. The 12 major metabolites were M1, M3, M6, M7, M8, M10, M11, M12, VMH-1, VMH-2, VMH-3 and VMH-4. VMH-1, VMH-2, VMH-3 and VMH-4 are unidentified metabolites (see table below). The production of M1 did not require NADPH and was produced in greater quantities in the presence of denatured microsomes compared to native microsomes. This suggests that M1 was non-enzymatically produced. M11 and M12 also are likely to be non-enzymatically produced. However, all other metabolites were created in the presence of NADPH-dependent enzymatic reactions in all species. Levels of M1 recovered peaked at only 5 minutes of incubation, and were higher in the absence of a NADPH-generating system and in the presence of denatured compared to native microsomes, indicating M1 may be further metabolized by liver microsomes. The following table shows FR901228 metabolism after 10 minutes incubation:

**Species differences in FR901228 metabolism by liver microsomes from male rats, male dogs, and humans**

Species	FR901228 metabolic activity (pmol/min/mg protein)									
	FR901228 disappearance	Metabolite formation								
		M3	M6	M7	M8	M10	VMH-1	VMH-2	VMH-3	VMH-4
Rat	116.7	15.1	8.0	0.0	19.2	0.0	9.1	4.6	17.0	21.0
Dog	49.7	14.7	0.0	5.4	13.5	24.0	2.7	0.4	0.0	0.0
Human	192.0	35.3	3.1	16.4	22.8	29.5	16.6	17.7	0.0	0.0

*Table excerpted from sponsor's package*

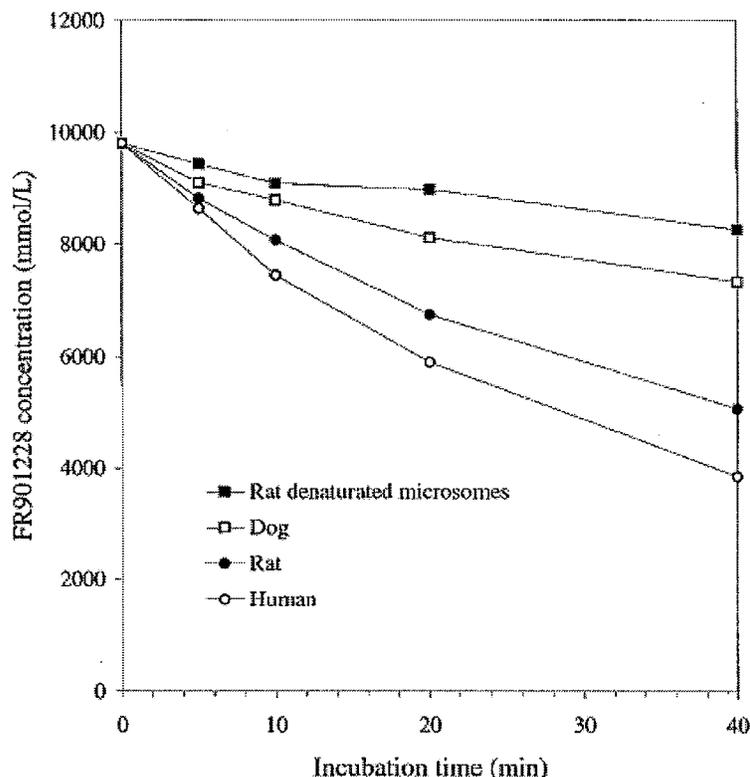
The major metabolites in rats were M3, M8, VMH-3 and VMH-4, in dogs were M3, M8 and M10 and in humans were M3, M7, M8, M10, VMH-1 and VMH-2. The formation of VMH-3 and VMH-4 was not observed in dogs and humans. Conversely, the formation of M10 was not observed in rats, but was present in humans and dogs. Metabolite M10 was 7.6% of the total recovered radioactive material produced from human liver microsomes, as seen in the table below.

**FR901228 and metabolite M1 and M10 concentrations in reaction medium after 10 minute incubations with rat, dog or human liver microsomes in the presence of a NADPH-generating system**

Species	Concentration in reaction medium (nmol/L)			
	FR901228	M1	M10	Total radioactive material
Rat	5068	32	0	8639
Dog	7326	114	345	9098
Human	3855	68	583	7593

The figure below demonstrates that the disappearance rate of FR901228 was highest in humans, followed by rats and dogs, with a difference of up to 3.9-fold between species.

**Time course of FR901228 disappearance by liver microsomes from male rats, male dogs and humans, and denatured rat liver microsomes in the presence of the NADPH-generating system**



(Table excerpted from sponsor's package)

After 20 mM DTT reduction of <sup>14</sup>C-FR901228 and metabolites formed from incubation with liver microsomes, M8 was the major metabolite in all species. Other major metabolites were M2, M4, M5 and M9. Minor metabolites present after incubation with liver microsomes, but before reduction with DTT were M2, M4, M5 and M9. There were no species differences in profiles of metabolites reduced with DTT after incubation with liver microsomes.

**FR901228 metabolism in the presence of DTT:**

The major metabolites produced from DTT reduction of <sup>14</sup>C-FR901228 followed by incubation with liver microsomes were M2, M7, M8, M9 and VMH-16 in all species. There were no species differences between rats, dogs and humans in profiles of metabolites produced after reducing <sup>14</sup>C-FR901228 with DTT. This data suggests the M2, M7, M8, M9 and VMH-16 are produced from M1.

FR901228 metabolism by liver S9 fraction in the presence of glutathione (GSH):  
The recovery of radioactivity was higher when GSH was present than when absent in all species. The major metabolite was M1 in the presence of GSH, but only a small amount of M1 was produced in the absence of GSH. M1 was produced in the absence of S9 fraction, suggesting M1 is produced non-enzymatically. The majority of substrate disappeared within 10 minutes of incubation with liver S9 fraction. M1 is potentially produced by exchanging the disulfide bond of FR901228 with the thiol of GSH. GSH is abundant in many cells so the production of M1 is potentially one of the most prevalent metabolic reactions after FR901228 enters cells.

**Study title:** Drug metabolism: identification of human P450 enzymes involved in the metabolism of FR901228

**Key study findings:**

- Romidepsin was metabolized by P450 CYP3A4.

**Study no.:** CRD030201

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** February 26, 2003

**GLP compliance:** No

**QA reports:** No

**Drug and % purity:** <sup>14</sup>C-FR901228, code no. 6710 and 6713, 98% radiochemical purity

**Methods:** Pooled human liver microsomes were used in inhibition studies with ketaconazole to inhibit CYP3A or with an anti-CYP3A4 monoclonal antibody. Microsomes were prepared from insect cells co-expressing recombinant human P450s (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C981 (wild-type), 2C18, 2C19, 2D681 (wild-type), 2E1 + cytochrome b<sub>5</sub> (b<sub>5</sub>), 3A4 + b<sub>5</sub>, 3A5 and 4A11) and human NADPH-P450 reductase.

**Results:** After a 10 minute incubation of <sup>14</sup>C-FR901228 with the NADPH-generating system and microsomes from cell lines expressing recombinant human P450s, the FR901228 metabolic activity of CYP3A4 was the highest, followed by 3A5, 1A1, 2B6 and 2C19, which had values of 2098, 352, 109, 30 and 28 pmol/50 pmol P450 in 10 minutes, respectively. All other P450 incubations showed no activity. The addition of 0.1, 1 and 10 μM ketaconazole to human liver microsomes inhibited the FR901228 activities to 41.2%, <10% and <10% of control values, respectively. In addition, anti-CYP3A4 antibody inhibited FR901228 disappearance by ≥ 90%. CYP3A4/5 enzymes are major catalysts involved in FR901228 metabolism, and are estimated to have a relative contribution ≥ 90% in human liver microsomes.

**Study title:** Drug metabolism: metabolism in rats after intravenous administration of [<sup>14</sup>C]-FR901228

**Key study findings:**

- There was no comparison made between metabolites identified in this study to those identified in other metabolism studies.
- Parent compound and identified metabolites accounted for 85%, 81%, 85% and 80% of recovered radiolabeled material in plasma, urine, bile and feces, respectively, indicating the majority of metabolites were identified.
- Parent drug accounted for 15% of the total radiolabel in plasma, indicating extensive metabolism.
- Biliary glucuronidation was not a mechanism of romidepsin metabolism.
- Many metabolites were plasma protein bound since DTT reduction resulted in a significant increase in recovered radioactivity.
- The amount of radiolabeled material recovered in bile, urine and feces as a percentage of the administered radioactivity (mass balance) was 66.1%, 15.9% and 75.1%, respectively. Of note, the 75.1% fecal excretion includes the biliary excretion.

**Study no.:** CRD040010

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** February 20, 2003

**GLP compliance:** No

**QA reports:** No

**Drug, lot, % purity:** <sup>14</sup>C-FR901228, code no. 6710, 96.4% chemical purity, ≥99%  
radiochemical purity

FR901228, lot no. 100118G

**Vehicle:** propylene glycol/ethanol (8:2, v/v) in saline

**Methods:**

Excreta material and plasma used in this study was collected from rats dosed in study CRD040009. The parent compound and metabolites were identified by HPLC and were quantified as the percent of radioactivity eluted from the column compared to the entire sample. Urine, bile and feces were collected from 3 rats for 48 hours postdose following a single intravenous injection of 0.3 mg/kg (28.6 μCi/kg) <sup>14</sup>C-FR901228. Plasma was collected from three rats at 5 min., 1 and 4 hours postdose, and pooled as one sample for each timepoint. The plasma concentration of radioactivity at 24 hours postdose was below the limits of quantification, so this data was not included in the results of the study. HPLC spectra for unchanged parent FR901228 and dithiothreitol (DTT) reduced <sup>14</sup>C-FR901228 were compared. Plasma and fecal homogenate was pretreated with DTT, or

water as control, before preparation of a supernatant for radioactivity recovery and HPLC analysis.

Species: male Sprague-Dawley rats  
 Number: 3  
 Age/weight: not provided / 254.1-312.0 g  
 Dose: 0.3 mg/kg (28.6 µCi/kg)  
 Frequency: single dose  
 Route: intravenous injection in tail vein

**Results:**

A total of 31 metabolites (MH-1 – MH-31) were detected in bile, urine, feces and plasma of rats administered <sup>14</sup>C-FR901228 intravenously. Treatment of <sup>14</sup>C-FR901228 with DTT produced a product (MH-28) that is potentially the reduced form of FR901228. This would suggest MH-28 corresponds to metabolite M1 described in previously reviewed *in vitro* studies, although no direct comparison was ever made between metabolites identified in different studies.

**Composition of FR901228 and its metabolites in plasma supernatant**

Metabolite	% of the sample					
	5 min		1 h		4 h	
	Non	DTT	Non	DTT	Non	DTT
Recovery (%)	19.1	84.0	43.0	83.7	47.2	77.5
FR901228	5.2	8.2	8.9	8.0	14.8	8.1
MH-14	0.2	0.0	0.4	0.0	0.0	0.0
MH-19	0.2	1.1	0.3	2.1	0.0	1.4
MH-20	0.2	0.5	0.6	0.3	0.0	0.0
MH-21	0.2	0.7	0.2	0.5	0.0	1.2
MH-23	1.0	1.0	1.7	1.9	1.8	0.7
MH-24	0.6	0.4	1.1	0.9	2.1	0.0
MH-26	0.5	0.6	1.0	0.8	0.0	0.9
MH-27	0.3	0.8	0.6	1.0	0.0	1.6
MH-28	1.7	13.8	4.2	13.7	4.0	21.2
MH-29	1.1	7.1	3.1	6.8	1.4	5.4
MH-30	0.6	25.6	1.8	15.6	0.0	9.2
MH-31	3.8	15.3	8.6	21.2	9.1	16.5

Recovery (%) is expressed as % of extracted from the sample.

Non : Non-reacted                      DTT : Reacted with Dithiothreitol

(Table excerpted from sponsor's package)

The amount of radioactivity detected in the pellet fraction of plasma protein was decreased after treatment with DTT.

**Composition of FR901228 and its metabolites in bile, urine and feces**

Metabolite	% of the sample			
	Bile (0-48 h)	Urine (0-48 h)	Feces (0-48 h)	
			Non	DTT
Recovery (%)	93.4	99.0	61.0	70.3
FR901228	4.5	26.7	2.1	2.7
MH-1			0.6	1.0
MH-2	3.7		2.3	2.4
MH-3	5.4		1.6	1.7
MH-4	2.6		0.6	0.8
MH-5	7.0		0.7	0.7
MH-6	3.8		0.8	0.6
MH-7	1.1		0.7	0.4
MH-8	1.5	0.5	0.4	0.4
MH-9	2.2	1.0	0.5	0.4
MH-10	7.5	0.5	0.6	0.5
MH-11			0.4	1.2
MH-12	4.0	0.9	0.3	0.3
MH-13	5.9	2.4	0.8	0.5
MH-14	1.5	1.7	0.7	0.5
MH-15	5.5	4.4	2.8	4.5
MH-16	2.2	2.1	3.4	2.7
MH-17	1.4	1.2	1.7	1.8
MH-18	2.6	1.7	2.2	2.1
MH-19	2.2	2.2	2.0	5.2
MH-20	1.9	2.7	1.6	1.5
MH-21	6.9	5.5	5.2	6.2
MH-22	1.0	2.0	2.1	2.0
MH-23	1.3	4.6	2.4	1.9
MH-25		1.5	1.3	1.1
MH-26		2.3	2.1	1.6
MH-27		1.4	1.3	1.0
MH-28	0.6	1.3	1.1	1.5
MH-29			0.8	2.9
MH-30	1.5	8.5	2.8	3.1
MH-31	2.0	4.8	1.6	2.8

Recovery (%) is expressed as % of extracted from the sample.

Non : Non-reacted                      DTT : Reacted with Dithiothreitol

*Table excerpted from sponsor's package)*

Hydroxylation of bile with  $\beta$ -glucuronidase or  $\beta$ -glucuronidase/arylsulfatase did not result in a change in the type or amount of metabolites recovered. Therefore, biliary glucuronidation is not expected to be a major mechanism of FR901228 metabolism. The amount of radiolabeled material recovered in bile, urine and feces as a percentage of the administered radioactivity was 66.1%, 15.9% and 75.1%, respectively.

### Summary and Conclusions

The majority of radiolabeled FR901228 or metabolites collected in plasma, urine, bile and feces were identified. A small proportion of radioactivity was identified as parent drug, which indicates FR901228 is extensively metabolized. Metabolites MH-1 and MH-11 were only found in rat feces, suggesting they may be products of rat gut bacteria. Most of the metabolites in plasma were bound to plasma protein, as reduction of a disulfide bond within FR901228 increased the amount of radioactivity recovered in plasma. The majority of metabolites were present at less than 5% in the plasma, bile, feces or urine (in the absence of DTT). Metabolite MH-31 was present at approximately 9% in the plasma from 1-4 hours postdose (4 hours postdose being the last timepoint of measurement). Metabolite MH-21 was present at approximately 7% in the bile (after 0-48 hour postdose collection). The majority of parent compound and metabolites were excreted through the bile (66%) and in the feces (75%) with a smaller amount excreted through the urine (16%).

#### 2.6.4.6 Excretion

The major route of elimination of romidepsin and its metabolites was through biliary excretion (66%) into the feces (75-79%). A smaller amount of romidepsin excretion occurred through the urine (16-20%). Approximately 90% of administered romidepsin and its metabolites were recovered from the feces and urine.

See reviews of distribution and metabolism studies, sections 2.6.4.4 and 2.6.4.5.

#### 2.6.4.7 Pharmacokinetic drug interactions

Not reviewed

#### 2.6.4.8 Other Pharmacokinetic Studies

Not reviewed.

#### 2.6.4.9 Discussion and Conclusions

See the PK/TK summary, section 2.6.4.1.

#### 2.6.4.10 Tables and figures to include comparative TK summary

<i>Mean Pharmacokinetic Parameters following a single romidepsin dose (not reviewed)</i>			
Species	Rat	Dog	Human
Route	IV	IV	IV
Dose (mg/m <sup>2</sup> )	4.02	20	14
T <sub>max</sub> (h)	0	1.15	NC
C <sub>max</sub> (ng/mL)	76.85	182	376.90
C <sub>max</sub> /dose (ng/mL)/(mg/m <sup>2</sup> )	19.12	9.1	26.92

AUC <sub>0-inf</sub> (h*ng/mL)	18.10	562.65	1548.53
AUC <sub>0-inf</sub> /dose (h*ng/mL)/(mg/m <sup>2</sup> )	4.50	28.13	110.61
t <sub>1/2</sub> (h)	0.67	NC	2.92
V <sub>z</sub> (L/m <sup>2</sup> )	NC	NC	38.16
CL (L/hr)/(m <sup>2</sup> )	NC	NC	9.04

t<sub>1/2</sub> – time at which maximum concentration is reached, C<sub>max</sub> – maximum plasma concentration, AUC<sub>0-inf</sub> – area under the curve from time zero to infinity, t<sub>1/2</sub> – terminal half life, V<sub>z</sub> – volume of distribution, CL – clearance  
 NC – not calculated

### 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

<i>Distribution Studies</i>				
Route	Species	Dose (mg/kg)/ Concentration (ng/mL)	Design	Significant findings
<i>in vitro</i>	rat, dog, human	50, 500, 1000, 5000	protein binding study in serum and with human serum proteins	-protein binding was independent of romidepsin concentration up to 500 ng/mL -protein binding was higher in serum from dogs (86%) and humans (94%) than in rats (37%) at 500 ng/mL romidepsin -romidepsin bound better to α <sub>1</sub> -AGP (93%) than to albumin (20%)
<i>in vitro</i>	rat, dog, human	50, 500, 1000, 5000	plasma to blood cell transfer	-blood:plasma ratios < 1 in rats, dogs and humans at ≥ 5000 ng/mL -transfer from plasma to blood cells was ≤25% in rats, ≤12% in dogs and ≤9% in humans
IV	Rat	0.3	organ distribution and excretion of <sup>14</sup> C- romidepsin following IV injection	-romidepsin and/or metabolites were widely distribution -C <sub>max</sub> was highest in kidney, urinary bladder, jejunum, liver and adrenal glands -T <sub>max</sub> within 5 minutes postdose in all tissues except ileum -primary route of elimination occurred through bile (66% at 48 h postdose), with less occurring through urine (20% at 48 h postdose) after IV administration.
<i>Metabolism Studies</i>				
Route	Species	Dose (mg/kg)/ Concentration (ng/mL)	Design	Significant findings
<i>in vitro</i> / IV	rat, human	60 μM (microsome incubations);	metabolism of <sup>14</sup> C- romidepsin <i>in vitro</i> by liver microsomes and <i>in vivo</i> following IV	-28 metabolites were identified -M1-M14 were produced by rat and human microsomes <i>in vitro</i> -M15-M17 were isolated from rat bile <i>in</i>

		200 µM (glutathione conjugation); 0.3 mg/kg ( <i>in vivo</i> )	injection, and <i>in vitro</i> glutathione conjugation	<i>vivo</i> -M18-M20 were formed by glutathione conjugation <i>in vitro</i> -M21-M28 were produced by human microsomes <i>in vitro</i>
<i>in vitro</i>	rat, dog, human	10 µM	metabolism of <sup>14</sup> C-romidepsin <i>in vitro</i> by liver microsomes or liver S9 fraction, and reduction with dithiothrietol (DTT) or glutathione	-20 metabolites were produced by liver microsomes -metabolite (M1) was the reduced form of romidepsin, which was metabolized similarly by microsomes from rats, dogs and humans -all metabolites detected in humans were detected in rats or dogs. -one metabolite (M10) produced from human and dog microsomes was not produced from rat
<i>in vitro</i>	human	10 µM	inhibition studies of romidepsin metabolism <i>in vitro</i>	-romidepsin was metabolized by CYP3A4
IV	rat	0.3 mg/kg	metabolism and excretion of <sup>14</sup> C-romidepsin <i>in vivo</i> following IV injection in rats	-no comparison between metabolites identified in this study to those identified in other metabolism studies -the majority of romidepsin metabolites were identified -parent drug accounted for 15% of the total radiolabel in plasma, indicating extensive metabolism -romidepsin did not undergo biliary glucuronidation -many metabolites were plasma protein bound -romidepsin was mostly excreted in bile (66.1%), urine (15.9%) and feces (75.1%) (including biliary excretion), as a percentage of the administered radioactivity (mass balance)

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

Single-dose toxicology: Acute toxic effects of romidepsin consisted of respiratory, cardiac, and CNS/neuro toxicities, in addition to leucopenia, lymphocytopenia, hypocalcemia, hyperglycemia, and hypokalemia.

Acute toxicology studies were conducted in rats and dogs to test the effect of a single intravenous (I.V.) injection of romidepsin. In the rat study, animals received 0, 4.2, 6.0, 8.4, 11.4, 15.6, 21.6, or 30.6 mg/m<sup>2</sup> of romidepsin. The observed clinical signs included decreased motility, staggering gait, prone position, tachypnea, hypopnea, tonic convulsion, and hematuria among romidepsin treated animals. However, the vehicle control group also had decreased motility, staggering gait, prone position, tachypnea and hematuria, which suggests that many of the clinical signs observed in the romidepsin groups were due to the vehicle. Animals died during the study as early as one day after dosing in the highest dose groups. The lack of histopathologic examination of tissues and of toxicokinetic analysis of exposures makes the results of this study difficult to interpret. The high mortality rate at the highest two doses of romidepsin indicate significant toxicity, but the absence of a detailed toxicological analysis of the animals failed to provide significant information about toxicities resulting from romidepsin administration.

An acute toxicity study was also conducted in dogs, in which animals received 0, 0.2, 2.0, and 20 mg/m<sup>2</sup> of romidepsin. Immediate clinical signs (0-6 hours postdose) included an irregular heart beat, shallow and erratic breathing, decreased body temperature and tremor at the high dose. Observations made between day 1 and 14 postdose included decreased movement, emesis, irregular respiration and congestion of the visible mucosa at the high dose. Body weight decreased on days 1-2 when compared to predose (< 10%) and began to recover by day 4-5 postdose in high dose groups. Body weight changes correlated with decreased food consumption during the same days. Hematologic effects were limited to a decrease in white blood cells and lymphocytes in high dose animals. There was a significant increase in AST levels in the high dose group, but no corresponding pathological findings were observed. There were also increases in glucose and decreases in calcium and potassium in the high dose group within 1 day postdose. The major target organ of romidepsin in this study was the thymus resulting in atrophy and decreased cortical lymphocytes in high and mid dose animals. Overall, a single dose of romidepsin had effects on the cardiovascular and respiratory systems, evident as immediate functional alterations, and the thymus. The persistence of the observed electrolyte imbalance could exacerbate any cardiotoxic effects.

Repeat-dose toxicology: Toxic effects of romidepsin in repeat-dose studies included effects in the hematopoietic system (occasional anemia; ↓WBCs and differentials, except for occasional ↑neutrophils which may be secondary to inflammation/ bleeding; thrombocytopenia; thymic atrophy; degenerative necrosis of lymphocytes in spleen, lymph nodes, and bone marrow; and bone marrow hypocellularity), liver (↑AST, ↑ALT, ↑bilirubin), heart (irregular rhythm, QT prolongation, ↑CK, dark/red spots on the heart,

thickening of epicardium and pericardium, retention of dark/red fluid in the pericardium cavity), GI tract (emesis and abnormal feces), and ♂ and ♀ reproductive systems. In addition, decreased calcium, potassium, iron, and phosphate were observed in some studies. Female-specific findings in toxicology studies (e.g. maturation arrest of ovarian follicles, atrophy of mammary gland, uterus, ovary and vagina; pituitary hyperplasia; elevated cholesterol) may be secondary to modulation of the estrogen pathway. Male-specific reproductive effects included testicular degeneration.

Four-week standard GLP studies were conducted in mice and rats. In mice, 24 mg/m<sup>2</sup>/day of romidepsin (I.V.) caused 20% mortality when administered biwx4 and 40% mortality when treated q7dx4. All drug treated animals showed signs of swelling, necrosis or both at the injection site. There was a dose-dependent decrease in weight or weight gain. Dosing caused anemia in animals receiving 15.9 or 24 mg/m<sup>2</sup> irrespective of schedule. On the biwx4 schedule, RBC decreased by about 20% following 15.9 or 24 mg/m<sup>2</sup> with reticulocytosis suggestive of marrow toxicity and a regenerative response. Red blood cell parameters had returned to control values by Day 50. Dosing caused a neutrophilic leukocytosis by day 28 of between 58-69% above baseline in mice given 15.9 and 24 mg/m<sup>2</sup> (biwx4) without a clear shift to the left suggestive of inflammation. Dosing also caused thrombocytosis (150%) that progressed in the recovery period (168%) for mice in the 24 mg/m<sup>2</sup> biwx4 group again suggestive of chronic inflammation. AST concentrations were elevated on Day 23 (q7dx4) or 28 (biwx4) but were normal by Day 50. All animals given 24 mg/m<sup>2</sup> q7dx4 showed microscopic signs of injection site inflammation, splenic cellular proliferation and necrosis, bone marrow cellular hyperplasia and depletion, hepatic hematopoietic foci and fatty degeneration, testicular degeneration, and thymic depletion. These lesions, except hepatic fatty degeneration, were also seen in mice given 24 mg/m<sup>2</sup> biwx4. The lowest non-lethal dose on either schedule was 15.9 mg/m<sup>2</sup>.

In the rat study, animals received romidepsin intravenously at doses of 0, 0.0192, 0.06, 0.192, and 0.6 mg/m<sup>2</sup>/day for 4 weeks. A single mortality occurred following exposure to 0.192 mg/m<sup>2</sup>. Due to copious foam in the right auricle of the heart and posterior cardinal vein, the sponsor believes the cause of death of this animal was an intubation error. Target organs in this study were the thymus, spleen, bone marrow, and ovaries. Clouding of the thymic parenchyma was observed in nearly all high-dose males and approximately 50% of females. Decreased thymus weight coincided with histopathological degeneration and necrosis of lymphocytes. Degeneration and necrosis of lymphocytes was also noted in the spleen, lymph nodes, and bone marrow. Additional histopathological findings included an increased number of tingible body macrophages in the thymus, spleen, and lymph nodes, and maturation arrest of ovarian follicles. Other noted toxicities included a dose-responsive decrease in white blood cells, triglycerides, an increase in urine volume, and a decrease in salivary and adrenal gland weight. In the recovery period, a sex-specific increase in female pituitary weight was noted.

In a 26-week GLP study in rats, animals were administered romidepsin I.V. at doses of 0, 0.6, 1.98, and 6 mg/m<sup>2</sup> weekly for 3 out of every 4 weeks, for a total duration of 26 weeks. One female died following 1.98 mg/m<sup>2</sup> due to a blood clot in the ventral cervical region extending into the ventral thoracic region with the presence of hemorrhage in the surrounding skeletal muscle. One male also died following 0.6 mg/m<sup>2</sup> due to unknown reasons. A control male was sacrificed due to limited usage of its hindlimbs. Major

toxicities associated with romidepsin were seen in bone marrow, liver, and reproductive organs. 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 noted. Liver toxicity was seen with a dose-responsive increase in 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. Similar to the 4-week rat study, a female-specific and dose-responsive increase in pituitary size and weight was noted with histopathological evidence of hyperplasia in the pars distalis. Females also showed elevated cholesterol levels.

Completion of a standard 4-week GLP study in dogs showed that daily I.V. doses of romidepsin as high as 0.64 mg/m<sup>2</sup>/day for 28 days caused no mortality or overt clinical signs. Body weight decreased slightly in these high dose dogs and there was a slight decrease in white cell count. Liver enzymes decreased slightly suggestive of decreased protein synthesis. There were no changes in heart rate. There were frequent incidences of mild degenerative changes or necrosis in the thymus, spleen and lymph nodes. In the bone marrow, there was slight to moderate decrease of erythroblasts and cellular phagocytosis in all high dose animals. This study is not informative of the safety of romidepsin considering the doses were too small to cause significant toxicity.

Thus, an additional non-GLP study in dogs was reviewed. Utilizing a twice weekly for four weeks schedule (~ twice as dose intense as the proposed clinical schedule), dogs (only one per dose group) tolerated I.V. doses as high as 40 mg/m<sup>2</sup>. This dose caused emesis, abnormal feces and injection site damage. The high dose dogs body weight decreased 26% and that of the low dose dog (20 mg/m<sup>2</sup>) decreased 15%. Dosing caused lymphopenia with decreased monocyte counts and increased neutrophils. Both dogs had decreased iron, PO<sub>4</sub><sup>+++</sup>, Ca<sup>++</sup>, K<sup>+</sup>, fibrinogen and increased SGOT (AST). In addition, LDH and BUN increased in dogs in the high dose dog. In the bone marrow there was a marked decrease in erythroblastic cells. Electrocardiography demonstrated QT prolongation after each dose at both dose levels (as high as 90 ms, average about 30 ms). The ST segment increased in the high dose dog after dosing. At necropsy, both dogs had a small thymus. 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. The presence of red spots on the heart suggests that the pericardial effusion is hemorrhagic. The epicardial thickening is probably an adaptive response to increased pericardial pressure. There were dark red foci on the corticomedullary zone of the kidney, nodes of splenic abscess, dark red to yellowish white foci in the left anterior lobe of the lung, and hepatization in part of the lung.

#### **Genetic toxicology:**

An assessment of genetic toxicology was conducted using an *in vitro* Ames assay, *in vitro* mouse lymphoma cell mutation assay, and an *in vivo* rat bone marrow micronucleus assay. Romidepsin was not mutagenic in any of these genetic toxicology assays. The highest doses tested in the *in vivo* micronucleus test were 1 mg/kg in males and 3 mg/kg

in females; these doses were defined to be the MTD in ♂s and ♀s, respectively. In the mouse lymphoma cell mutation assay, the 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. Therefore, the FDA/CDER quantitative structure activity relationship (QSAR) analysis was utilized to assess if FK228 had structural alerts for genotoxicity. QSAR analysis indicated that romidepsin was not mutagenic in an *in vitro* mammalian genetic mutation test, the Chinese Hamster Ovary (CHO) / Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT) mutation assay.

**Carcinogenicity:**

No carcinogenicity studies were conducted.

**Reproductive toxicology:**

Two embryofetal reproductive toxicology studies were completed in rats. The first study was a preliminary dose-range finding study that evaluated the effects of romidepsin on embryofetal development by administering daily intravenous injections of vehicle, 0.0192, 0.06, 0.192, or 0.6 mg/m<sup>2</sup> romidepsin into pregnant females from gestation day 6 through 16. There were no significant maternal or embryofetal toxicities in this study; reduced fetal weight of 9% was noted at high-dose. Considering that only 6 animals/group were used in this study, a definitive conclusion on drug-related toxicity cannot be made.

The second embryofetal reproductive toxicology study was a GLP study to assess the potential for romidepsin-induced developmental toxicity in rats by daily intravenous injections of vehicle, 0.036, 0.12, or 0.36 mg/m<sup>2</sup> romidepsin into pregnant females from gestation day 6 through 16. Throughout the dosing period, there was a decrease in uncorrected body weight gain (statistically significant – 13%) and food consumption (16%) in 0.36 mg/m<sup>2</sup>/day females. The 13% reduction in the uncorrected body weight was secondary to the reduced uterine weight; body weight gains were comparable among all groups when corrected for uterine weight. There were no other maternal effects that could be attributed to romidepsin. Likewise, there were no observed uterine effects of romidepsin. Mean fetal weights were slightly decreased (5%) in the ≥ 0.12 mg/m<sup>2</sup>/day groups, but were not statistically significant. Romidepsin related embryofetal effects were limited to a decreased incidence of diaphragm thinning with liver protrusion at 0.36 mg/m<sup>2</sup>/day and a decreased incidence of incomplete ossification of cervical and sacral vertebral arches and skull bones at ≥ 0.036 mg/m<sup>2</sup>/day. Values for the incidence of the skeletal observations in ≥ 0.12 mg/m<sup>2</sup>/day groups were lower than the historical control ranges provided. However, the concurrent control values for these parameters were higher than the historical control ranges, which impacts the validity of this observation. No other romidepsin related embryofetal effects were observed. In conclusion, there were no significant toxicological effects of romidepsin on maternal health or embryofetal development in rats under the conditions for this study. However, the lack of a toxicokinetic analysis precludes the determination that adequate maternal, and hence embryofetal, exposure was achieved.

**Local tolerance:**

A local lymph node assay was conducted in female mice to assess the potential of romidepsin to cause delayed contact hypersensitivity. Initially, this assay was completed with 0, 1, 2.5, and 5% romidepsin. However, since these dose levels led to a severity in clinical signs (hunched and thin appearance, shallow and labored respiration, etc.) resulting in sacrifice, the experiment was repeated using lower dose levels. Administering romidepsin at dose levels of 0, 0.025, 0.05, 0.1, or 0.25% resulted in a dose-responsive increase in lymph node stimulation. Thus, romidepsin was considered a sensitizer in mice and has the potential to cause delayed contact hypersensitivity.

An additional local tolerance test completed was an acute dermal irritation test in rabbits. Two male New Zealand White rabbits were subject to a single 4 hour dermal exposure to 0.5g romidepsin on the upper and lower region of the dorsal trunk. 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.

**Special toxicology:**

*In vitro* studies were conducted to assess differences in romidepsin-induced myelotoxicity and cardiac myocyte toxicity among rodents, non-rodents, and humans. In the myelotoxicity study, mouse, dog, and human bone marrow cells (CFU-GM) were treated with 0, 0.001, 0.01, 1, 1.0, 3.0, or 10 nM concentrations of romidepsin for 12 to 14 days. The applicant states that no significant differences existed across species since there was not a 10-fold difference in IC<sub>50</sub>, IC<sub>75</sub>, and IC<sub>90</sub> values across species. However, this conclusion is not established statistically.

In regard to the cardiac myocyte toxicity study, cardiac myocytes derived from neonatal rats, Beagle dogs, and immortalized human fetal SV<sub>40</sub> transformed cells (W1) were exposed to romidepsin (0.1-100 pM), doxorubicin (0.001-10 pM), or minoxidil (0.1-100 pM) for 6 hours. Cell culture viability was determined by the MTT (3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) dye exclusion assay and by measuring extracellular LDH levels. Both assays were completed at 24, 48, and 72 hours after the initiation of treatment (30, 50, and 70 hr. for dog cell cultures). Romidepsin was cytotoxic and caused a concentration dependent release of LDH in all three myocyte models. Romidepsin was more cytotoxic to myocytes derived from all three species than minoxidil or doxorubicin, the positive controls. 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 are likely inter-species differences in the response to the assays. Nevertheless, the assays both demonstrated that romidepsin is toxic to cardiomyocytes and even more so than doxorubicin and minoxidil.

An *in vivo* study of cardiac toxicity showed increased LDH-1 and LDH-2 in mice receiving 4.8 mg/m<sup>2</sup>/day (I.P., q4dx3) or greater and elevated LDH-3 in mice receiving 15.9 mg/m<sup>2</sup>/day. In mice given romidepsin I.V. daily for five days, LDH-3 increased at doses of 2.88 mg/m<sup>2</sup>/day or greater. This toxicity correlated with microscopic damage in the heart. Damage included focal mineralization, chronic inflammation and focal necrosis. Damage increased in frequency and severity with increasing dose and varied with schedule. This study also demonstrated dose and schedule dependant mild anemia,

leukopenia, lymphopenia and mild thrombocytopenia with an increased myeloid/erythroid ratio in the marrow.

#### 2.6.6.2 Single-dose toxicity

**Study title:** Acute toxicity study of FR901228 in rats after intravenous dosing.

**Key study findings:**

- Romidepsin administration resulted in a high mortality rate at 3.6 (60%) and 5.1 mg/kg (90%).
- Tonic convulsions were observed at multiple doses, up to 1 day postdose.
- Gross pathological observations resulting from romidepsin administration included foci on the thymus, red coloration of the lung and petechiae in the glandular stomach.
- There were no clear toxicities identified due to a lack of clinical pathological and histopathological examination.

**Study no.:** GLR910291

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** August 24, 1989

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, and % purity:** FR901228, Lot # 004196L, 98.1%

**Methods:**

Doses: Saline (negative control), 80% propylene glycol in sterile water (vehicle control), 0.7, 1.0, 1.4, 1.9, 2.6, 3.6 and 5.1 mg/kg (4.2, 6.0, 8.4, 11.4, 15.6, 21.6 and 30.6 mg/m<sup>2</sup>)

Species/strain: Rat — CD(SD)

b(4)

Number/sex/group or time point (main study): 5

Route, formulation, volume, and infusion rate: IV infusion, 80% propylene glycol, 5 mL/kg, 2 mL/min.

Satellite groups used for toxicokinetics or recovery: None

Age: 6 weeks

Weight: 206-236 g – males; 150-174 g – females

Sampling times: FR901228 concentrations were measured in each dosing solution before dosing

**Observations and times:**

Mortality: Twice daily up to 14 days post dose

Clinical signs: Twice daily up to 14 days post dose

Body weights: Approximately every other day post dose

Food consumption: Not conducted

Ophthalmoscopy: Not conducted

Hematology: Not conducted

Clinical chemistry: Not conducted

Urinalysis: Not conducted

Gross pathology: Day 14 postdose

Organ weights: Not conducted

Histopathology: No histopathologic investigation was performed on any tissues during this study. Only gross pathologic observations were made.

**Results:**

Mortality:

- 6/10 (60%) rats died at 3.6 mg/kg
- 9/10 (90%) rats died at 5.1 mg/kg

Group	Number	Sex	Time Relative to Dose	Symptom
0.7 mg/kg	1	Male	6-7 minutes	Found dead – tonic convulsion, ↓ spontaneous motility, prone position
3.6 mg/kg	1	Male	6-7 minutes	Found dead – tonic convulsion, hypopnea, absence of right reflex
3.6 mg/kg	4	Male	1 day	Found dead - ↓ spontaneous motility, staggering gait, hematuria, tonic convulsion, tremor (5.1 mg/kg males)
3.6 mg/kg	1	Female	1 day	
5.1 mg/kg	5	Male	1 day	
5.1 mg/kg	4	Female	1 day	

Clinical signs:

0-6 hour post dose:

- Males –
  - ↓ spontaneous motility, tachypnea, staggering gait, hematuria at  $\geq 0.7$  mg/kg (+ vehicle control)
  - Prone position in all groups except 1.0 mg/kg
  - Tonic convulsion in 0.7 and 1.4 mg/kg groups
- Females –
  - Staggering gait, hematuria at  $\geq 0.7$  mg/kg (+ vehicle control)
  - ↓ spontaneous motility in all groups except 1.9 mg/kg
  - Tachypnea, prone position at 0.7 mg/kg
  - Tonic convulsion in all groups except 1.9 and 2.6 mg/kg

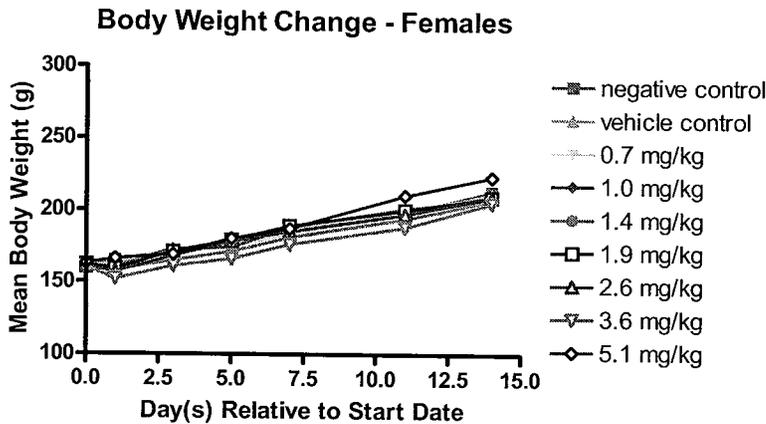
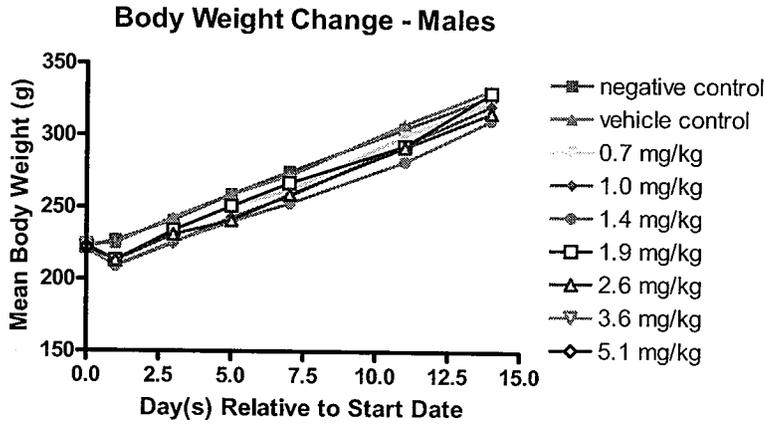
1-2 days post dose:

- Females –
  - ↓ spontaneous motility, staggering gait, piloerection in 1 females at 3.6 and 5.1 mg/kg
  - Tonic convulsion, tremor at 3.6 mg/kg
  - Irregular respiration in 1 female at 5.1 mg/kg

≥ 4 days post dose:

- Females –
  - Loss of hair at 3.6 and 5.1 mg/kg

Body weights: Unremarkable



Food consumption: Not conducted

Ophthalmoscopy: Not conducted

Hematology: Not conducted

Clinical chemistry: Not conducted

Urinalysis: Not conducted

Gross pathology:

- No changes in animals that died on dosing day
- Changes in animals that died 1 day after dosing
- 14 days post dose:
  - many minute white foci in thymus, discoloration of splenic surface, blackish coloration of tail in 1 female at 5.1 mg/kg
  - clouding of thymic parenchyma, many minute white foci in thymus, diffuse petechiae in glandular stomach in females
  - no changes in any males

**Abnormal gross pathology of animals that died 1 day post dose**

Observation	Number examined	Dose group			
		Males		Females	
		3.6 mg/kg	5.1 mg/kg	3.6 mg/kg	5.1 mg/kg
		4	5	1	4
Thymus					
Clouding of parenchyma		3	5	1	4
Dark red foci		1	1	0	2
Lung					
Dark red coloration		4	5	1	4
Stomach					
Diffuse petechiae in glandular stomach		1	0	0	0

Organ weights: Not conducted

Histopathology: No histopathologic investigation was performed on any tissues during this study. Only gross pathologic observations were made.

Toxicokinetics: Not conducted

**Study title:** Acute toxicity study of FR901228 in dogs after intravenous dosing.

**Key study findings:**

- Clinical signs after administration of romidepsin included decreased motility, irregular rhythm of heart, irregular respiration, emesis, tremor, decreased body temperature and mucosal congestion.
- A decreased mean body weight occurred 1-2 days postdose at  $\geq 0.1$  mg/kg.
- Decreased relative lymphocytes (maximum 89%) were observed at 1 mg/kg compared to control.
- Clinical chemistry changes included increased AST (maximum 296%) and glucose (maximum 41%) and decreased calcium (maximum 27%) and potassium (maximum 28%) at 1 mg/kg.
- Effects of romidepsin on the thymus included thymic atrophy, decreased thymic weight and decreased cortical lymphocytes of 1 mg/kg males and 0.1 mg/kg females.

**Study no.:** GLR910294

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

**Conducting laboratory and location:**

b(4)

**Date of study initiation:** November 20, 1989

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, and % purity:** FR901228, Lot # 004196L, 98.1%

**Methods:**

Doses: Vehicle control, 0.01, 0.1, 1 mg/kg (0.2, 2, 20 mg/m<sup>2</sup>)

Species/strain: Dog / Beagle

Number/sex/group or time point (main study): 1

Route, formulation, volume, and infusion rate: IV infusion (cephalic vein), 80% propylene glycol-20% ethanol diluted in physiological saline, 4 mL/kg, 10 mL/min.

Satellite groups used for toxicokinetics or recovery: None

Age: 5 months

Weight: 8.30-9.95 kg – males; 7.75-8.70 kg – females

Sampling times: FR901228 concentrations were measured in each dosing solution before dosing

**Observations and times:**

Mortality: Twice daily up to 14 days post dose

Clinical signs: Twice daily up to 14 days post dose

Body weights: Daily x 7, then day 9, 11, 14

Food consumption: Daily

Ophthalmoscopy: Not conducted

EKG: Not completed; heart rate counted 6 times postdose

Hematology: 4 times postdose

Clinical chemistry: 4 times postdose

Urinalysis: Not conducted

Gross pathology: Day 14 postdose

Organ weights: See histopathology inventory for this NDA

Histopathology: Adequate Battery: Yes

Peer review: No

Histopathology examination included organs and tissues with abnormalities.

See histopathology inventory for this NDA for list of organs and tissue examined.

**Results:**

Mortality: None

Clinical signs:

0-6 hour post dose:

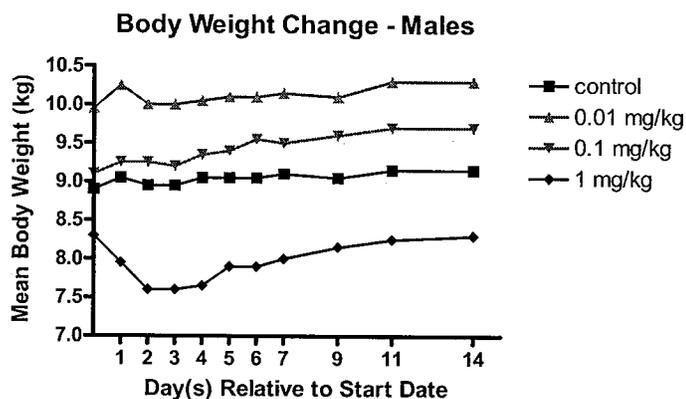
- Males –
  - Licking at 0.1 mg/kg
  - Irregular rhythm of heart, shallow and jerky breathing, tremor, ↓ temperature at 1 mg/kg
- Females –
  - Tremor, ↓ temperature at 1 mg/kg

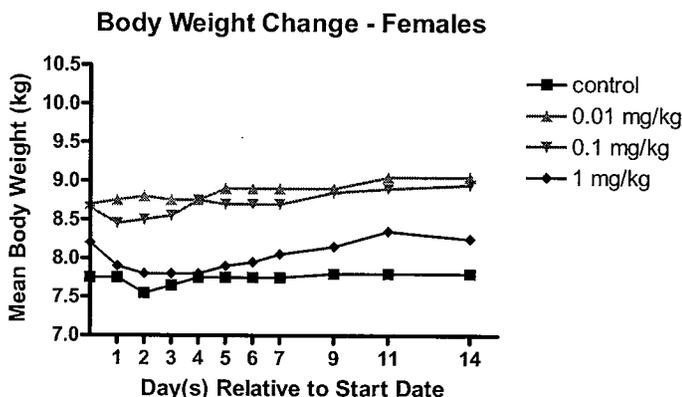
1-14 days post dose:

- Males –
  - ↓ movement, wet eyeballs, dry nose, emesis at 0.1 mg/kg
  - ↓ movement, irregular respiration, cough, emesis, congestion of visible mucosa, loose stool at 1 mg/kg
- Females –
  - Wet eyeballs, ↓ movement, salivation, emesis, congestion of visible mucosa at 1 mg/kg

Body weights:

- ↓ slightly on days 1-2 in 1 mg/kg males, then ↑ for rest of dosing period
- ↓ slightly on days 1-2 in ≥ 0.1 mg/kg females, then ↑ for rest of dosing period





Food consumption:

- ↓ food consumption on days 1-4 in 1 mg/kg animals
- ↓ food consumption on days 1-2 in 0.1 mg/kg females

Ophthalmoscopy: Not conducted

EKG: EKG not conducted

- Unremarkable changes in heart rate

Hematology:

- ↓ (≤ 69%) white blood cells ( $10^2/\text{mm}^3$ ) at 1, 4, 7 days postdose in 1 mg/kg animals compared to control
- ↓ (≤ 89%) relative lymphocytes (%) at 1 day postdose in 1 mg/kg animals compared to control

Clinical chemistry:

1 mg/kg, 1 day postdose:

Males –

- ↑ (290%) AST, (27%) glucose and ↓ (56%) urea nitrogen, (27%) calcium, (21%) potassium

Females –

- ↑ (296%) AST, (41%) glucose, (75%) (90% - day 4 postdose) cholesterol, (57%) phospholipids and ↓ (26%) calcium, (28%) potassium

0.1 mg/kg, 1 day postdose:

Females –

- ↑ (54%) phospholipids

→ No changes from 7 days post dose or in 0.01 mg/kg group

Urinalysis: Not conducted

Gross pathology:

- Thymic atrophy in 1 mg/kg males and 0.1 mg/kg females, not found in control or 0.01 mg/kg animals

Organ weights:

- Study report claims ↓ thymus weights in 1 mg/kg males and 0.1 mg/kg females, corresponding to gross pathological changes
- \* Note: table of organ weights contains only thymus weights for 1 mg/kg males and 0.1 mg/kg females, and denotes all other animals as “not measured” for thymus weights

Histopathology: Adequate Battery: Yes

Peer Review: No

Slightly ↓ cortical lymphocytes in the thymus of 1 mg/kg male and 0.1 mg/kg female with gross pathological thymic atrophy

Toxicokinetics: Not conducted

**2.6.6.3 Repeat-dose toxicity**

**Study title:** Acute Multiple Dose Toxicity Study of Cyclic Peptide (NSC-630176) in Male Mice (SRI Study A92-TXM-2; May 25, 1994). Reviewed by W. David McGuinn, Ph.D. and slightly modified for this NDA review.

**Key study findings:**

- Hematologic toxicities included anemia, neutrophilic leukocytosis, and thrombocytosis.
- Histopathological changes noted were splenic cellular proliferation and necrosis, thymic depletion, bone marrow cellular hyperplasia and depletion, hepatic hematopoietic foci and fatty degeneration, and testicular degeneration.
- AST levels were elevated.

**Methods:**

Species: Male CD2F1 mice

Drug: Depsipeptide, sample I/D2

Dose/Schedule: 0, 3.6, 5.3, or 8.0 mg/kg/day IV once weekly for a total of 4 doses; or twice a week for a total of 8 doses

Route, formulation, volume: I.V. / absolute ethanol/propylene glycol (1:4) in 0.9% sterile sodium chloride for injection, USP at a concentration of 0.36, 0.53, or 0.80 mg/mL / 10 ml/kg

Group designation and dose levels: 10/dose group

GLP: Yes

**Observations and times:**

Clinical signs: Daily

Body weights: Days 1, 4, 5, 8, 12, 15, 19, 22, 23, 26, 28, 29, 36, 43, 50 and before death  
Clinical chemistry: Day 23 and 50  
Hematology: Day 23 and 50  
Necropsy: 5 animals/group on Day 23 for the q7dx4 schedule  
5 animals/group on Day 28 for the biwx4 schedule  
All surviving animals on day 50  
Histopathology: On vehicle control group (biwx4) and the 8.0 mg/kg/dose group (both schedules)

**Results:**

Mortality: 4 of 10 mice died in the 8.0 mg/kg/dose group on a q7dx4 dosing schedule. Two mice were found dead on Day 4, and 2 were sacrificed moribund on Day 15. 2 of 10 mice given 8.0 mg/kg/dose biwx4 were sacrificed moribund on Day 20.

Clinical Signs: Injection site toxicity (swelling and/or necrosis) all drug-treatment groups.

Body Weight: 7% decrease in body weight occurred on Days 36-43 in mice in the 5.3 mg/kg/dose on the q7dx4 dosing schedule. Mice given 8.0 mg/kg/dose on the q7dx4 dosing schedule did not gain weight between Days 29 and 50. On the biwx4 schedule, mice given 8.0 mg/kg/dose had a 17% decrease in body weight on Days 36-50.

Hematology: Anemia in groups given 5.3 or 8.0 mg/kg/dose on either dosing schedule. On the biwx4 schedule, mean RBCs were ~80% of control values on Day 28 for the 5.3 and 8.0 mg/kg/dose groups. Red blood cell parameters had returned to control values by Day 50. A neutrophilic leukocytosis was seen on Day 28 (58-69% above baseline in mice given 5.3 and 8.0 mg/kg/dose on the biwx4 dosing schedule). A mild thrombocytosis was seen on Day 28 (150%) and Day 50 (168%) for mice in the 8.0 mg/kg/dose biwx4 group.

Clinical Chemistry: AST levels were elevated on Day 23 (q7dx4) or 28 (biwx4) but were normal by Day 50.

Histopathology: Changes seen in all animals given 8.0 mg/kg/dose q7dx4, these included injection site inflammation, splenic cellular proliferation and necrosis, bone marrow cellular hyperplasia and depletion, hepatic hematopoietic foci and fatty degeneration, testicular degeneration and thymic depletion. These lesions, except hepatic fatty degeneration, were also seen in mice given 8.0 mg/kg/dose biwx4.