

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**22-110**

**PHARMACOLOGY REVIEW(S)**

Memo to the Division File

NDA 22-110

Televancin (Vibativ)

From: Wendelyn Schmidt, Pharmacology/Toxicology Supervisor

Through: Chris Davi, Project Manager

Date: September 3, 2009

Background:

In the original review dated 12/6/06, the pharmacologist, Zhou Chen, recommended a Pregnancy Category X designation. After subsequent discussions with the CDER Reproductive Toxicity Committee, the sponsor, and other groups within CDER, Dr. Chen concluded that the Pregnancy Category (C or X) would be contingent on the clinical benefit (see memo dated 9/7/07). After the input from the Advisory Committee on 11/18/08, where Dr. Chen presented the reproductive data from rats, rabbits and minipigs, a Category C recommendation was made.

After negotiations with both the sponsor and the Maternal Health Group, the label now reads as follows:

### **1.1 Pregnancy**

Teratogenic effects: Pregnancy Category C

#### *Pregnancy Exposure Registry*

There is a pregnancy registry that monitors pregnancy outcomes in women exposed to VIBATIV during pregnancy. Physicians are encouraged to register pregnant patients, or pregnant women may enroll themselves in the VIBATIV pregnancy registry by calling 1-888-658-4228.

#### *Fetal Risk Summary*

All pregnancies have a background risk of birth defects (about 3%), pregnancy loss (about 15%), or other adverse outcomes regardless of drug exposure.

There are no data on VIBATIV use in pregnant women. In 3 animal species, VIBATIV exposure during pregnancy at clinically relevant doses caused reduced fetal weights and

increased rates of digit and limb malformations in offspring. These data raise concern about potential adverse developmental outcomes in humans (see *Data*).

#### *Clinical Considerations*

Given the lack of human data and the risks suggested by animal data, avoid using VIBATIV in pregnant women unless the benefits to the patient outweigh the potential risks to the fetus.

#### *Data*

##### Human Data

There are no data on human pregnancies exposed to VIBATIV.

##### Animal Data

In embryo-fetal development studies in rats, rabbits, and minipigs, telavancin demonstrated the potential to cause limb and skeletal malformations when given intravenously during the period of organogenesis at doses up to 150, 45 or 75 mg/kg/day, respectively. These doses resulted in exposure levels approximately 1- to 2-fold the human exposure (AUC) at the maximum clinical recommended dose. Malformations observed at <1% (but absent or at lower rates in historical or concurrent controls), included brachymelia (rats and rabbits), syndactyly (rats, minipigs), adactyly (rabbits), and polydactyly (minipigs). Additional findings in rabbits included flexed front paw and absent ulna, and in the minipigs included misshapen digits and deformed front leg. Fetal body weights were decreased in rats.

In a prenatal/perinatal development study, pregnant rats received intravenous telavancin at up to 150 mg/kg/day (approximately the same AUC as observed at the maximum clinical dose) from the start of organogenesis through lactation. Offspring showed decreases in fetal body weight and an increase in the number of stillborn pups. Brachymelia was also observed. Developmental milestones and fertility of the pups were unaffected.

There are no recommendations for further non-clinical studies at this time. There are no pending pharmacology/toxicology issues to be resolved.

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/s/

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WENDELYN J SCHMIDT  
09/03/2009

Comments on N22-110 telavancin  
From A Jacobs 8/27/09

1. The pharm/tox review of 2006 suggests a pregnancy category X. However, subsequent discussions led to a decision that C would be the appropriate pregnancy category.
2. The pharm/tox team leader should write a memo documenting the decision to recommend a pregnancy C category.
3. I concur that the pregnancy category should be a C and that there are no other outstanding pharm/tox issues.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22110	ORIG 1	THERAVANCE INC	TELAVANCIN

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/s/

ABIGAIL ABBY C C JACOBS  
08/27/2009

Memo to the Division File

NDA 22-110, Televancin, submissions dated 1/21/08 and 3/13/09

From: Wendelyn Schmidt, Pharmacology/Toxicology Supervisor

Through: Chris Davi, Project Manager

Date: August 12, 2009

**Background:**

The pharmacology/toxicology information for the televancin NDA was submitted in the initial filing. No new pharm/tox data for review was included in the filings for cycles 2 and 3 of this initial NDA review.

**Recommendation:**

There are no actions based on pharmacology or toxicology data to be taken at this time. From the pharmacology/toxicology perspective, televancin can be approved.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22110	ORIG 1		TELAVANCIN
NDA 22110	ORIG 1		TELAVANCIN
NDA 22110	ORIG 1		TELAVANCIN

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/s/

WENDELYN J SCHMIDT  
08/12/2009

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY  
PTCC SUBCOMMITTEE CONSULT  
FOR INTERNAL USE WITHIN FDA ONLY**

**Date:** August 1, 2007

**From:** Lynnda Reid, PhD  
RDTs Co-Chair

**To:** Zhou Chen, PhD  
Acting Pharmacology Team Leader, DAIOP

J. Christopher Davi, RPM, MS  
Regulatory Health Project Manager, DAIOP

**Date of Consultation:** July 2, 2007

**RE:** NDA 22-110: The sponsor and reviewer have different interpretations regarding the positive findings from reproductive studies in three species. Therefore, there is a difference in Pregnancy Category determination. The division would like RDTs members to have an unbiased review and evaluation for the three pivotal Segment 2 studies and make a labeling suggestion.

**Background information:** Telavancin is a glycopeptide antibiotic indicated for the treatment of complicated skin and skin-structure infections (cSSSI). Administration of telavancin is via intravenous injection at a proposed maximum recommended human dose (MRHD) of 10 mg/kg. To support potential exposures in pregnant women, three embryo/fetal developmental (Segment 2) studies were conducted in rats, rabbits and minipigs.

Following review of these studies, the primary nonclinical reviewer, Dr. Zhou Chen, concluded that telavancin is a multi-species teratogen with external/skeletal (limb) malformations. Findings across species involving limb development consisted of brachymelia, syndactyly, adactyly, and polydactyly. These effects were observed at doses comparable to human doses based on plasma AUC levels.

**Comparison of Systemic Exposure to Telavancin at the Lowest Dose with Toxicity between Animals and Humans**

Species	General toxicity		Segment 2 studies			Clinical studies
	Rat	Dea	Rabbit	Rat	Adalate	Human
Dose (mg/kg)	30*	33*	75	100	25	10
AUC <sub>0-24h</sub> (µg-h/ml)	1012-1227	600-624	1357	829	780	666-760
Animal/human	1.5-3.5	0.77-0.83	1.75	1.05	1	

\* This is the dose with clear liver and renal findings. Other positive findings (e.g., macrophage hypertrophy/hyperplasia affecting the bone marrow, spleen, thymus, and duodenum) were seen at the doses as low as 6.25 mg/kg with AUC of 88-126 µg-h/ml.

Incidence per litter of limb related external malformations (number of affected fetuses in parentheses):

**Rats:**

	<i>Diluent</i>	<i>Placebo</i>	<i>50 mg/kg/day</i>	<i>100 mg/kg/day</i>	<i>150 mg/kg/day</i>
Litters Evaluated:	25	24	25	24	25
Fetuses evaluated:	319	322	312	332	322
Brachymelia	0	0	0	1 (1) 4.2%	1 (1) 4.0%
Syndactyly	0	0	0	1 (1) 4.2%	0
<b>Total Litter Incidence*</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4.2%</b>	<b>4.0%</b>

\* Incidence for Brachymelia, micromelia or syndactyly were not in the historical data base submitted.

**Rabbits:**

	<i>Placebo</i>	<i>60 mg/kg/day</i>	<i>75 mg/kg/day</i>
Litters Evaluated:	18	20	19
Fetuses evaluated:	138	172	156
Flexed Front Paws, brachymelia, and adactyly	0	0	1 (1) 5.3%
Absent ulna	0	0	1 (1) (5.3%)
<b>Total Litter Incidence</b>	<b>0</b>	<b>0</b>	<b>10.6%</b>

\* Historical Control Incidence for Malrotated Hindlimbs = 0.8%; Flexed front paws - 0.8%; adactyly - 0.3%; no incidence rate given for brachymelia or absent ulna.

**Gottingen Minipigs:**

	<i>Diluent</i>	<i>Placebo</i>	<i>25 mg/kg/day</i>	<i>50 mg/kg/day</i>	<i>75 mg/kg/day</i>
Litters Evaluated:	7	5	9	8	5
Fetuses evaluated:	34	24	31	36	17
Syndactyly	0	0	0	1 (1) 12.5%	0
Polydactyly: Single Limb	0	1 (1) 20%	2 (2) 22.2%	2 (4) 25%	0
Polydactyly: Multiple limbs	0	0	2 (2) 22.2%	1 (1) 12.5%	0
Misshapen digits & deformed leg	0	0	0	1 (1) 12.5%	0
<b>Total Litter Incidence*</b>	<b>0%</b>	<b>20%</b>	<b>33.3%</b>	<b>50%</b>	<b>0%</b>

\* Historical Control Incidence for Polydactyly = 5.71%; Syndactyly = 2.86%

**Discussion and Conclusions:** It was the consensus of the committee that the limb defects observed in these studies were related to the drug. While the evidence of drug-induced limb malformations in each species is weak, the weight of evidence across all three species strongly supports that the findings are drug-related. Furthermore, although the incidence rates were low, they occurred in a dose-dependent manner and at rates higher than in the historical control databases reported by the Sponsor. Of greatest concern is that these malformations occurred at clinically relevant maternal exposures based on AUC.

Species		Dose (mg/kg/day)	Cmax (µg/ml)	AUC0-24 (µg.h/ml)
Rat	Maternal Plasma	50	420	829
		100	760	1236
		150	914	1726
	Amniotic Fluid	50	NA	NA
		100	0.250	NA
		150	0.450	5.97
Rabbit	Maternal Plasma	60	541	1027
		75	716	1387
	Amniotic Fluid	Drug was detected in the amniotic fluid from only one dam indicative of limited placental transfer in rabbits at 75 mg/kg.		
	Minipig	Maternal Plasma	25	347
50			545	1206
75			871	1781
	Amniotic Fluid	Amniotic fluid from Minipigs was not analyzed.		

In the final rabbit study report from Covance, the contract laboratory responsible for conducting both the rat and rabbit studies, they concluded that *"the limb malformations noted (brachymelia, adactyly and absent ulna) mimic or are similar to the malformations of brachymelia and syndactyly observed in rats... These findings further support a direct effect of AMI-6524 [telavancin] on the developing fetus."* The total litter incidence rates for skeletal malformations in rabbits were 5.6, 5.0, and 26% in the placebo, 60 mg/kg/day and 75 mg/kg/day groups, respectively. Five fetuses in separate high-dose litters exhibited skeletal malformations. Of note is the lack of significant maternal toxicity at the high-dose in this study.

The diluent control was 5% dextrose and the composition of the placebo and test agents were as follows:

	Placebo (250 mg/vial)	Telavancin (250 mg/vial)
AMI-6424 (telavancin)	0	250 mg
Hydroxypropyl-β-Cyclodextrin	2500 mg	2500 mg
Mannitol	312.5 mg	312.5 mg
1 N NaOH	QS to pH 4.5	
1 N HCl		

Although high concentrations of hydroxypropyl- $\beta$ -cyclodextrin were present in the test articles used in the studies, there was only one occurrence of polydactyly in a single limb in the minipig study placebo control group, and no limb malformations in placebo controlled rats or rabbits. This is also not a reported finding associated with cyclodextrin exposures. Therefore, the presence of hydroxypropyl- $\beta$ -cyclodextrin alone cannot account for the increased rates of limb malformations.

Maternal toxicity was observed in high-dose rats and rabbits as reductions in weight gain compared to controls. However, we do not think that the limb malformations were a result of maternal toxicity. Observations typically associated with maternal toxicity as evinced by decreased weight gain include increased early and/or late resorptions, decreased fetal weights and delayed ossification. None of these typical findings associated with decreased maternal weight gain were significantly increased in the high-dose litters of rats and rabbits. In minipigs, there were drug or dose-related effects on weight gain or clinical signs. Therefore, it is doubtful that the teratogenic effects observed in these studies can be attributed to maternal toxicity.

#### **RDTS Conclusions and Recommendations:**

The RDTS agrees with the primary reviewer that the limb defects are drug-related. As such we recommend that the findings be detailed in labeling. As to the appropriate Pregnancy Category, we could not come to a consensus on whether telavancin should be labeled under category C or X. Either category could be appropriate based on the risk/benefit profile of the drug. The category should be based on the risk/benefit potential of the product in pregnant women. Factors which should be considered include the following:

- Seriousness of the indication and the potential for serious complications in pregnancy associated with the indication
- Availability of alternative treatments
- Teratogenic effects occurring at or near the proposed human dose

In order to label the product under Category C, the potential benefit to the mother and/or the fetus should clearly exceed any potential risk to the fetus otherwise we recommend that this product should be labeled under Category X.

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Lynnda Reid  
8/3/2007 09:27:19 AM  
PHARMACOLOGIST

Comments on NDA 22-110 telavancin HCl  
From A. Jacobs 7/13/07

**1. Approvability**

Approvability would be based on the clinical determination of the risk-benefit ratio for patients. Although the primary reviewer/TL concluded that the P/T findings did not support approval of this product (see review in DFS), the decision regarding product approval would be based on clinical data. There are no approvability issues with this NDA from a pharm/tox perspective.

**2. Pregnancy category:**

I would recommend Pregnancy Category C as appropriate for telavancin, rather than the Category X recommended by the reviewer. Category X is unprecedented for an antimicrobial and indicates that the risk benefit ratio would never be appropriate for a pregnant woman. Findings from the animal studies may be described in the labeling.

3. Perhaps an addendum could be written to the pharm/tox review, if the reviewer agrees.

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/s/

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Abby Jacobs  
7/13/2007 11:39:42 AM  
PHARMACOLOGIST



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: 22-110  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 12/6/2006  
DRUG NAME: Telavancin  
INDICATION: Complicated skin and skin-structure infections (cSSSI)  
SPONSOR: Theravance, Inc., 901 Gateway Boulevard, South San Francisco, CA  
94080  
Tel: 650-808-6076; Fax: 650-808-3786  
DOCUMENTS REVIEWED: Module 4  
REVIEW DIVISION: Division of Anti-Infective and Ophthalmology Products  
PHARM/TOX REVIEWER: Zhou Chen, MD, PhD  
PHARM/TOX SUPERVISOR: Terry Peters, DVM  
DIVISION DIRECTOR: Janice Soreth  
PROJECT MANAGER: Davi Christopher

Date of review submission to Division File System (DFS): June 1, 2007

## TABLE OF CONTENTS

Executive Summary .....	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW .....	6
2.6.1 INTRODUCTION AND DRUG HISTORY .....	6
2.6.2 PHARMACOLOGY .....	12
2.6.2.1 Brief summary .....	12
2.6.2.2 Primary pharmacodynamics .....	12
2.6.2.3 Safety pharmacology .....	12
2.6.2.5 Pharmacodynamic drug interactions .....	16
2.6.3 PHARMACOLOGY TABULATED SUMMARY .....	16
2.6.4 PHARMACOKINETICS/TOXICOKINETICS .....	16
2.6.4.1 Brief summary .....	16
2.6.4.2 Methods of Analysis .....	16
2.6.4.3 Absorption .....	17
2.6.4.4 Distribution .....	22
2.6.4.5 Metabolism .....	28
2.6.4.6 Excretion .....	30
2.6.4.7 Pharmacokinetic drug interactions .....	32
2.6.4.8 Other pharmacokinetic studies .....	33
2.6.4.9 Discussion and Conclusions .....	36
2.6.5 PHARMACOKINETICS TABULATED SUMMARY .....	37
2.6.6 TOXICOLOGY .....	37
2.6.6.1 Overall toxicology summary .....	37
2.6.6.2 Single-dose toxicity .....	38
2.6.6.3 Repeated-dose toxicity .....	43
2.6.6.4 Genetic toxicology .....	72
2.6.6.5 Carcinogenicity .....	77
2.6.6.6 Reproductive and developmental toxicology .....	77
2.6.6.7 Local tolerance .....	101
2.6.6.8 Special toxicology studies .....	103
2.6.6.9 Discussion and Conclusions .....	111
2.6.7 TOXICOLOGY TABULATED SUMMARY .....	112
OVERALL CONCLUSIONS AND RECOMMENDATIONS .....	113
APPENDIX/ATTACHMENTS .....	114

***EXECUTIVE SUMMARY***

**I. Recommendations**

**A. Recommendation on approvability**

Safety concerns were raised in the nonclinical studies for renal, hepatic, and reproductive toxicity findings at plasma exposure levels similar to those seen in clinical studies. The approvability is not supported by these positive findings from a pharmacology/toxicology standpoint. The findings should be considered by other reviewing disciplines.

**B. Recommendation for nonclinical studies**

No additional studies are necessary.

**C. Recommendations on labeling**

Several modifications of labeling in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section are recommended.

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[Redacted]

**b(4)**

**Carcinogenesis, Mutagenesis, Impairment of Fertility**

[Redacted]

[Redacted]

**b(4)**

**Pregnancy**

[Redacted]

[Redacted]

**b(4)**

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## II. Summary of nonclinical findings

### A. Brief overview of nonclinical findings

Telavancin is a glycopeptide antibiotic indicated for complicated skin and skin-structure infections (cSSSI). In safety pharmacology studies, TD-6424 inhibited *hERG* channels and elicited a prolongation of action potential in isolated canine cardiac Purkinje fibers. No treatment-related cardiovascular effects were seen in *in vivo* studies in dogs. No drug-induced respiratory or neurological effects were noted.

In mice, rats and rabbits, plasma C<sub>max</sub> and AUC demonstrated dose-dependent increases following single IV dosing. PK studies showed significant differences in t<sub>1/2</sub> between the various species (mice- 1.2- 9.5 hours; rats- 1.3- 14.8 hours; dogs- 1.4-12.8 hours; monkeys- 2.3 hours; rabbit- 1.33-2.33 hours). In tissue distribution studies conducted in dogs, rats and mice, high concentrations of radioactivity were seen in the bone, liver and kidney. TD-6424 is highly protein bound (approximately 90%) in mouse, rat, dog, bovine, rabbit and human plasma as well as human skin blister fluids. Following IV dosing, AMI-11352 (7-OH metabolite), AMI-999 (N<sup>3</sup>'-[2-(decylamino)ethyl] vancomycin, or other hydroxylated metabolites were observed in serum samples of rats, dogs and monkeys. The hydroxylated metabolites are the major metabolites in rat, dog and monkey urine. Urine excretion is the major route in dogs, mice, rats.

b(4)

Several toxicological studies were conducted with durations of up to 6-months in rats and 3 months in dogs. The organs of toxicity identified in these studies include the kidney and liver in both species. Multiple organ macrophage accumulation/hypertrophy/hyperplasia was also noted. The drug is a multi-species teratogen. Although some of the findings (e.g., increased BUN, creatinine, AST, and ALT levels) were seen in the placebo (hydroxypropyl-β-cyclodextrin) control animals, the findings were more significant and more frequent in the drug-treated animals, leading to the conclusion that the active compound contributed significantly to the alterations.

### B. Pharmacologic activity

Telavancin, a synthetic derivative of vancomycin, is a lipoglycopeptide antimicrobial indicated for the treatment of patients with complicated skin and skin structure infections (cSSSI) caused by susceptible strains of the following Gram-positive microorganisms: *Staphylococcus aureus* [methicillin-resistant and -susceptible strains (MRSA and MSSA)], *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* group (including *S. anginosus*, *S. intermedius* and *S. constellatus*), and *Enterococcus faecalis* (vancomycin-susceptible isolates only).

Telavancin exerts rapid, concentration-dependent, bactericidal activity against Gram-positive organisms *in vitro*. The mechanism includes inhibition of bacterial cell wall synthesis, and disruption of the functional integrity of the bacterial membrane. Telavancin inhibits cell wall biosynthesis by binding to late-stage peptidoglycan precursors, which prevents both the polymerization of precursor into peptidoglycan and subsequent cross-linking events. Telavancin also binds to bacterial membranes and causes membrane

depolarization and increased membrane permeability. Collectively, these actions of telavancin result in inhibition of peptidoglycan, protein, RNA, and lipid syntheses, and lead to rapid bacterial cell death.

C. Nonclinical safety issues relevant to clinical use

The significant nonclinical toxicity findings (renal, hepatic and reproductive toxicities) observed with systemic exposure levels to the drug similar to those seen in clinical studies suggest that clinical use of the drug may not be safe.

**2.6 PHARMACOLOGY/TOXICOLOGY REVIEW**

**2.6.1 INTRODUCTION AND DRUG HISTORY**

**NDA number:** NDA 22-110

**Review number:** 001

**Sequence number/date/type of submission:** 000/December 6, 2006/Commercial

**Information to sponsor:** Yes ( X ) No ( )

**Sponsor and/or agent:** Theravance, Inc., 901 Gateway Boulevard, South San Francisco, CA 94080

**Manufacturer for drug substance:** ScinoPharm Taiwan, Ltd., 1 Nan-Ke 8th Road, Tainan Science-Based Industrial Park, Shan-Hua, Tainan County 74144, Taiwan

**Reviewer name:** Zhou Chen, MD, PhD

**Division name:** Division of Anti-Infective and Ophthalmology Products

**Review completion date:** June 1, 2007

**Drug:**

Trade name: Vibativ™ (proposed)

Generic name: Telavancin hydrochloride

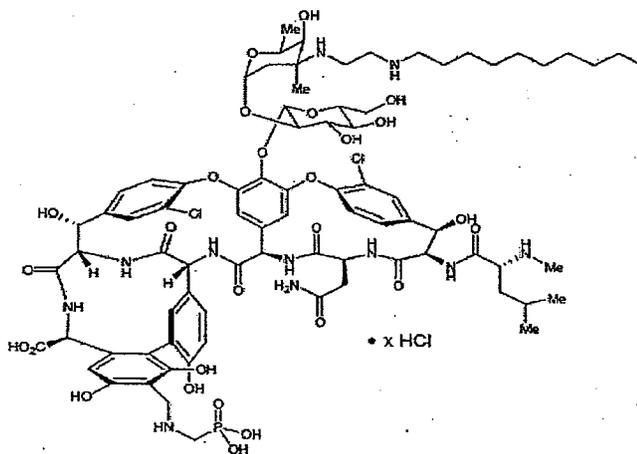
Code name: TD-6424, SPT3104, CD4, AMI-6424

Chemical name: Vancomycin, N3''-[2-(decylamino)ethyl-29-[(phosphonomethyl) amino] methyl]-hydrochloride

CAS registry number: 380636-75-9

Molecular formula/molecular weight: C<sub>30</sub>H<sub>106</sub>Cl<sub>2</sub>N<sub>11</sub>O<sub>27</sub>P.xHCl (x = 1-3), MW= 1755.63 (free base)

Structure:



**Relevant INDs/NDAs/DMFs:** IND 60,237 (Theravance), DMF \_\_\_\_\_ DMF (Ben Venue), DMF \_\_\_\_\_ DMF \_\_\_\_\_ DMF \_\_\_\_\_ DMF \_\_\_\_\_

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**Drug class:** Glycopeptide antibiotic

**Indication:** Complicated skin and skin-structure infections (cSSSI)

**Clinical formulation:**

Components	Quality standard	Function	Amount per 250 mg vial (mg/ml)	Amount per 750 mg vial (mg/ml)
Telavancin HCl (free base equi.)		Drug substance		
Hydroxypropyl-β-cyclodextrin	Ph.Eur	Solubilizing agent		
Mannitol	USP			
Sodium hydroxide	NF	pH adjustment		pH 4.5
Hydrochloric acid	NF	pH adjustment		pH 4.5
Diluent* (ml)	USP	Reconstitution agent		
Total volume (ml)				

\* 5% Dextrose Injection, Sterile Water for Injection, or 0.9% Sodium Chloride Injection. After reconstitution, the product is further diluted in 5% Dextrose Injection (D5W), 0.9% Sodium Chloride Injection, or Lactated Ringer's Injection before administration by intravenous infusion.

**Route of administration:** Intravenous injection

**Proposed use:** 10 mg/kg administered over a 60-minute period by intravenous infusion once every 24 hours for 7 to 14 days

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

**Studies reviewed within this submission:**

**Pharmacology:**

**Primary pharmacodynamics**

The studies in this section are being reviewed by the Microbiology team.

**Safety pharmacology**

02-006-01 SPH02-007: Effects of AMI-6424 on *hERG* Tail Current Recorded from Stably Transfected HEK293 Cells

02-006-02 SPP02-001: Effects of AMI-6424 on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers

02-006-06 Dure1000: Effects of AMI-6424 on Action Potential Parameters in Sheep Isolated Cardiac Purkinje Fibers

02-003-23 93431: A Cardiovascular Profile Study Following an Intravenous Infusion of AMI-6424 in the Conscious Unrestrained Beagle Dog

01-001-15 2039/001: AMI 6424: Cardiovascular and Respiratory Effects in the Anesthetized Dog Following Intravenous Administration

01-001-13 2039/002: AMI 6424: Effects on General Activity and Behavior in the Rat Following Intravenous Administration

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**PK:**

**Absorption:**

01-6424-PK-03: Pharmacokinetics of AMI-6424 in Mice  
01-6424-PK-04: Pharmacokinetics of AMI-6424 in Rats  
01-6424-PK-05: Pharmacokinetics of AMI-6424 in Beagle Dogs  
01-6424-PK-06: Pharmacokinetics of AMI-6424 in Monkeys  
04-6424-PK-24: Pharmacokinetics of TD-6424 in New Zealand White Rabbits  
04-6424-PK-25: Pharmacokinetics and Bioavailability of TD-6424 Following Intravenous and Subcutaneous Administration to Normal or Neutropenic Female Mice  
05-6424-PK-36: Absorption, Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C TD-6424 in Dogs Following Intravenous Administration of TD-6424 for Injection and Alternate Formulations

**Distribution:**

01-6424-PK-08: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 in Mice  
01-6424-PK-09: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 in Rats  
01-6424-PK-10: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 with Various Formulations  
01-6424-PK-11: Tissue Distribution and Excretion of AMI-6424 in Rats Following Single or Repeated Dosing  
01-6424-PK-12: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 Following Intravenous Infusion to Dogs  
01-6424-PK-15: Quantitative Whole-Body Autoradiography of <sup>3</sup>H-AMI 6424 in Rats  
02-6424-PK-22: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 in Rats  
02-6424-PK-23: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 Following Intravenous Infusion to Dogs  
04-6424-PK-26: Tissue Concentration of TD-6424 in Female Rats Following Fourteen-Day Dosing with a 7- and 14-Day Recovery Period  
05-6424-PK-30: Tissue Distribution of Total Radioactivity in the Pigmented Rat Following Intravenous Administration of <sup>14</sup>C-TD-6424 (Quantitative Whole Body Autoradiography)  
05-6424-PK-35: Tissue Distribution of Total Radioactivity in the Pigmented Rat Following Intravenous Administration of <sup>14</sup>C-TD-6424 in Alternate Formulations (Quantitative Whole Body Autoradiography)

**Metabolism**

01-6424-PK-13: *In vitro* Metabolism of AMI-6424  
01-6424-PK-16: Metabolite Analysis of Dog Urine Samples for <sup>3</sup>H-AMI-6424  
01-6424-PK-19: Pharmacokinetics and Metabolite Profiles of AMI-6424 in Rats, Dogs and Monkeys  
06-6424-PK-29: *In Vitro* Metabolism of TD-6424 in Liver and Renal S9 Fractions  
05-6424-PK-37: Metabolite Identification for TD-6424 in Dog and Human Urine Samples Following Intravenous Administration of <sup>14</sup>C-TD-6424

**Excretion**

01-6424-PK-08: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 in Mice  
01-6424-PK-09: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 in Rats  
01-6424-PK-12: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 Following Intravenous Infusion to Dogs  
02-6424-PK-22: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 in Rats  
02-6424-PK-23: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 Following Intravenous Infusion to Dogs

#### **Drug interactions**

01-6424-PK-20: Pharmacokinetic Drug Interaction Study of AMI-6424 with Concomitant Administrations of Azetronam and Metronidazole in Female Rats  
05-6424-PK-31: *In Vitro* Evaluation of Telavancin as Potential Inhibitor of Human Cytochrome P450 Enzymes

#### **Other PK studies**

01-6424-PK-07: Interspecies Scaling of Pharmacokinetics of AMI-6424  
01-6424-PK-14: Plasma Protein Binding of AMI- 6424 in Rat, Mouse, Dog and Human  
01-6424-PK-18: Pharmacokinetics of <sup>3</sup>H-AMI-6424 in Mice Following Intraperitoneal and Subcutaneous Administration  
01-6424-PK-21: Analysis of <sup>3</sup>H-AMI-6424  
04-6424-PK-27: Pharmacokinetics of Hydroxypropyl-Beta-Cyclodextrin in Rats and Dogs Following Single Intravenous Administration of Telavancin for Injection or Hydroxypropyl-beta-Cyclodextrin  
05-6424-PK-32: Pharmacokinetics of TD-6424 in Rats Following Single Intravenous Administration of TD-6424 for Injection and Alternate Formulations  
05-6424-PK-33: Pharmacokinetics of TD-6424 in Dogs Following Single Intravenous Administration of TD-6424 for Injection and Alternate Formulations  
05-6424-PK-34: Tissue Concentration of TD-6424 in Female Rats Following Seven-Day Dosing with TD-6424 for Injection and Alternate Formulations  
06-6424-PK-38: Pharmacokinetic and Pharmacodynamic Studies of TD-6424 in Various Rabbit Efficacy Models  
06-6424-PK-39: Protein binding of <sup>14</sup>C-TD 6424 in Plasma from Mouse, Rat, Dog, Bovine and Human and Human Skin Blister Fluid

#### **Toxicology:**

##### **Single dose studies**

00-036-020: Exploratory Single-Dose Intravenous Nephrotoxicity Study with AMI-6424 in Rats  
00-036-028: Exploratory Single-Dose Intravenous Nephrotoxicity Study with AMI-6424 in Rats: Effect of Varying the Concentration of Hydroxypropyl-β-Cyclodextrin (HP-β-CD) on Nephrotoxicity  
00-036-033: Evaluation of the Potential Effects of Pretreatment with 25% Hydroxypropyl-β-Cyclodextrin on the Single Dose Toxicity of AMI-6424 in Rats  
01-001-11 7507-131: Acute Toxicity Study of AMI-6424 When Administered Intravenously in Mice  
01-001-12 7507-130: Acute Toxicity Study of AMI-6424 When Administered Intravenously to the Rat

**Repeated dose studies**

00-001-09 7507-109: 7-Day Exploratory Intravenous Infusion Nephrotoxicity Study with AMI-6424 in Dogs with a 14-Day Recovery  
00-036-022: Exploratory Seven-Day Intravenous Nephrotoxicity Study with AMI-6424 Administered in 5% Hydroxypropyl- $\beta$ -Cyclodextrin in Rats  
00-036-032: Exploratory Seven-Day Intravenous Nephrotoxicity Study with AMI-6424 Administered in 1% Hydroxypropyl- $\beta$ -Cyclodextrin in Rats  
00-036-037: Exploratory Seven-Day Intravenous Nephrotoxicity Study in Female Rats: Effects of Varying the Ratio of Hydroxypropyl- $\beta$ -Cyclodextrin to AMI-6424 on the No-Effect Dosage Level for Nephrotoxicity  
00-036-038: Exploratory Seven-Day Intravenous Nephrotoxicity Study in Female Rats: Effects of Varying the Ratio of Sulfobutylether (SBE)- $\beta$ -Cyclodextrin to AMI-6424 on the No-Effect Dosage Level for Nephrotoxicity  
01-001-01 7057-110: Pilot Intravenous (Infusion) Toxicity Study with AMI-6424 in Male Dogs  
01-001-09 7057-111: 2-Week Intravenous Toxicity Study with AMI-6424 in Rats with a 2-Week Recovery  
01-001-10 7057-112: Two Week Intravenous Toxicity Study with AMI-6424 in Beagle Dogs with a 2-Week Recovery  
02-001-01 7057-144: 4-Week Intravenous Infusion Toxicity Study with AMI-6424 in Rats with a 4-Week Recovery  
02-003-05 57328: A 13 Week Intravenous Infusion Toxicity Study (with a 28 day Recovery Period) of AMI-6424 in the Beagle Dog  
02-001-06 0757-148: 13 Week Intravenous Infusion Toxicity Study with AMI-6424 in Rats with a 4-Week Recovery  
02-001-14 7057-168: Exploratory 7-Day Intravenous Toxicity Study with AMI-6424 in Male Rats  
02-003-01 57387: A 28-Day Intravenous Infusion Toxicity Study (with a 28-Day Recovery Period) of AMI-6424 in the Beagle Dog  
03-001-07 7057-199: 26-Week Intravenous Infusion Toxicity and Toxicokinetic Study with AMI-6424 in Rats with a 4-Week Recovery Period

**Genetic toxicology**

01-001-10 22005-0-409: *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with AMI-6424 (Trifluoroacetic Acid Salt)  
01-001-03 22005-1-409OECD: *Salmonella-Escherichia coli*/Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with AMI-6424  
01-001-04 22005-0-449OECD: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes  
02-001-08 23353-0-409OECD: *Salmonella-Escherichia coli*/Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with AMI-6424  
01-001-05 22005-0-455OECD: *In Vivo* Mouse Micronucleus Assay with AMI-6424

**Reproductive and developmental toxicology**

01-001-24 7507-125: Range-finding Intravenous Injection Toxicity Study of AMI-6424 in Pregnant Female Rats

01-001-19 7507-115: Range-finding Intravenous Injection Toxicity Study of AMI-6424 in Non-pregnant Female Rabbits  
01-001-23 7507-123: Range-finding Intravenous Injection Toxicity Study of AMI-6424 in Pregnant Female Rabbits  
02-001-05 7057-128: Intravenous Injection Study of Fertility and Early Embryonic Development to Implantation with AMI-6424 in Rats: Amended Final Report  
03-001-04 7057-197: 6-Week Intravenous Injection Study of Potential Gonadal Effects and Reversibility with AMI-6424 in Male Rats with an 8 Week Recovery Period  
02-001-15 7057-175: Intravenous Injection Rabbit Developmental Toxicity Study with AMI-6424  
02-001-03 7057-124: Intravenous Injection Rabbit Developmental Toxicity Study with AMI-6424  
02-001-04 7057-126: Intravenous Injection Rat Developmental Toxicity Study with AMI-6424  
04-013-01 57155: Range Finding Toxicity Study in Non-Pregnant Minipigs  
05-013-01 58564: Telavancin: Range Finding Toxicity Study in Pregnant Minipigs  
02-001-07 7057-129: Intravenous Injection Study for Effects on Pre- and Postnatal Development, Including Maternal Function, with AMI-6424 in the Rat

**Local tolerance**

05-013-06 60876: Telavancin (API and Drug Product): Acute Dermal Irritation Study in the Rabbit (the Sequential Approach)  
05-013-05 60877: Telavancin (API and Drug Product): Acute Eye Irritation/Corrosion Study in the Rabbit

**Special toxicology**

04-001-04 7057-218: 6-Week Intravenous Infusion Immunotoxicity Study with TD-6424 in Rats with a 4-Week Recovery Period  
99-007-59: AMI-999: Exploratory Single Dose Intravenous Nephrotoxicity Study with AMI-999 in Rats  
99-007-78: AMI-999: Exploratory Single Dose Intravenous Nephrotoxicity Study with AMI-999 in Rats  
00-036-018: AMI-999: Exploratory Single Dose Intravenous Nephrotoxicity Study in Rats  
00-001-02 7057-114: Hemolytic Potential Testing with AMI-6424 in Rat, Dog, and Human Whole Blood  
00-001-25 7057-145: Hemolytic Potential Testing with Lyophilized Formulation of AMI-6424 in Rat, Dog, and Human Whole Blood  
05-016-01 EOD00001: Neutral Red Uptake Phototoxicity Assay of Telavancin in Balb/c3T3 Mouse Fibroblasts  
05-036-008: Exploratory Single-Dose Intravenous toxicity Study in Male Rats  
05-001-58 7057-400: Exploratory 4-Week Intravenous Infusion toxicity Study with Various Telavancin Formulations in Rats  
9808-TX-001 7668-135: Local Tolerance Study with Telavancin via Perivenous, Intraarterial, and Intravenous Injections in Rabbits

**Studies not reviewed within this submission:**

Pharmacokinetics  
Analytical methods and validation reports

b(4)

b(4)

b(4)

b(4)

**Reviewer's comments:** Most of the studies in this NDA package were reviewed by Dr. Terry Peters.

## **2.6.2 PHARMACOLOGY**

### **2.6.2.1 Brief summary**

The PD studies are reviewed by the microbiology reviewer. In safety pharmacology studies, TD-6424 inhibited *hERG* channels and elicited a prolongation of action potential in isolated canine cardiac Purkinje fibers. No treatment-related cardiovascular effects were seen in *in vivo* studies in dogs. No drug-induced respiratory or neurological effects were noted.

### **2.6.2.2 Primary pharmacodynamics**

The studies are being reviewed by the microbiology reviewer.

### **2.6.2.3 Safety pharmacology**

Neurological effects:

01-001-13 2039/002: AMI 6424: Effects on General Activity and Behavior in the Rat Following Intravenous Administration

This study was conducted by Covance Laboratories Ltd., North Yorkshire, England under UK GLP and OECD GLP conditions.

Groups of 6 young adult male Wistar rats/group were given diluent (5% dextrose), vehicle or 12.5, 25 or 50 mg/kg AMI- 6424 i.v. Behavior as well as autonomic and motor effects (Irwin methodology) on the rats were evaluated at 5, 15, 30, 60 and 120 minutes post-dosing. The animals were observed for an additional 7 days.

Results: No significant treatment-related signs or differences from controls were noted.

Cardiovascular and pulmonary effects:

**01-001-15 2039/001: AMI 6424: Cardiovascular and Respiratory Effects in the Anesthetized Dog Following Intravenous Administration**

This study was conducted by Covance Laboratories Ltd., North Yorkshire, England and Covance Laboratories, Inc., Madison, WI, under UK GLP and OECD GLP conditions.

In this study, 12 beagle dogs (males, 10-11 months of age) were anesthetized, divided into 3 groups (vehicle, 25 mg/kg and 50 mg/kg groups by i.v. infusion over 2 hrs.) and instrumented for evaluation of: systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, dP/dt (derivative of left ventricular pressure), heart rate, EKG wave-form heights, EKG intervals (corrected and uncorrected), mean femoral blood flow, tidal volume, minute volume and respiratory rate. No analyses were performed on the test article as administered doses. The animals were anesthetized using propofol anesthetic and also given alfentanil as needed.

Two baseline recordings were made for each animal prior to the first dose and again at 5, 10, 20, 30, 60, 90, 120, 150, 180 and 210 minutes post-initiation of dosing. Blood samples for PK analysis were taken at baseline, 10, 20, 40, 60, 90, 120, 150, 180 and 210 minutes post-initiation of dosing.

Results: Increases in R-R interval, decrease in heart rate, decreases in peak inspiratory flow, peak expiratory flow and minute volume but increased respiratory rate were noted when comparing the 50 mg/kg dose animals to controls. The magnitude of the differences was generally <10% so the changes are not considered biologically significant.

No significant differences from controls were noted in other respiratory or cardiovascular parameters.

Concentrations ( $\mu\text{g/ml}$ ) of TD-6424 in dog plasma

Animal #	Group (mg/kg)	Time (minutes) post-initiation of infusion								
		0	10	20	60	90	120	150	180	210
1	25 mg/kg	<0.250	18.3	35.3	90.6	126	157	NS	NS	
2	25 mg/kg	39.4*	18.1	<0.250*	108	158	190	162	145	126
3	25 mg/kg	0.604**	24.2	46.8	117	170	199	172	162	145
4	25 mg/kg	0.341**	17.5	39.2	112	146	186	153	116	132
9	50 mg/kg	0.589**	44.4	80.9	192	257 <sup>^</sup>	324 <sup>^</sup>	288 <sup>^</sup>	255 <sup>^</sup>	224 <sup>^</sup>
10	50 mg/kg	1.15**	37.2	81.2	199	278 <sup>^</sup>	347 <sup>^</sup>	302 <sup>^</sup>	295 <sup>^</sup>	224 <sup>^</sup>
11	50 mg/kg	0.888**	32.7	76.5	189	224 <sup>^</sup>	282 <sup>^</sup>	237 <sup>^</sup>	193	179
12	50 mg/kg	<0.250	40.3	79.9	196	269 <sup>^</sup>	329 <sup>^</sup>	308 <sup>^</sup>	245 <sup>^</sup>	243 <sup>^</sup>

\* Reassayed in duplicate- "Possibly mislabeled"

\*\* "Low level AMI 6424 values due to possible carryover"

<sup>^</sup> Reassayed at partial volume- exceeded curve range

There are several problems with this portion of the study:

- 1) At time 0, there should be <0.250  $\mu\text{g/ml}$  in all animals as they should have been naïve with respect to this compound.
- 2) The samples that exceeded the curve range should have been diluted and reassayed with an explanation of the dilution and reason(s) for it.
- 3) The plasma samples were not obtained for a period of time appropriate to estimate the necessary pharmacokinetic parameters. The dogs should have been followed for at least 24 hours post-initiation of dosing in order to estimate the elimination rate constant,  $t_{1/2}$ , AUC,  $C_{\text{max}}$ , etc. Obtaining plasma samples for only 1.5 hours after the end of the infusion severely limits the utility of the study with respect to characterization of the pharmacokinetic parameters.

#### 02-006-01 SPH02-007: Effects of AMI-6424 on *hERG* Tail Current Recorded from Stably Transfected HEK293 Cells

This study was conducted by  and was conducted in compliance with the GLP regulations.

 and was conducted in compliance with the

b(4)

Stably transfected human embryonic kidney 293 (HEK293) cells were tested in final perfusion concentrations of 1.5, 15, 150, 300 and 600  $\mu\text{g/mL}$  of AMI-6424 (lot #AME004) or the same concentrations of "Placebo for AMI-6424 for Injection, 250 mg/vial" (previously described as the hydroxypropyl- $\beta$ -cyclodextrin and mannitol solution). The diluent was D5W. The positive control was E-4031. Once the whole cell stable patch-clamp was established, recordings were made at -80 mV, +20 mV and -50 mV (tail current). The test was performed in 4-5 cells and the reference E-4031 was applied to 3 placebo and one test article-treated cell.

The E4031 inhibited the *hERG* tail current after 10 min exposure, produced a residual tail current of 32.7 +/- 3.0% when compared to the values obtained prior to the addition of E4031. This equates to a decrease in tail current of 67.3%. Thus, the positive control performed as expected. Placebo treatment produced residual tail currents of 88.4% to 62.7% (for the respective low to high dose volumes) when compared to controls. The test article inhibited the tail currents at all doses  $\geq 15 \mu\text{g/ml}$  (44.6% at the 600 nM concentration). When correction for the placebo effects were done, an  $\text{IC}_{50}$  could not be calculated as it would be > the maximal 600  $\mu\text{g/ml}$  tested.

**02-006-02 SPP02-001: Effects of AMI-6424 on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers**

This study was conducted by (C ) and was conducted in compliance with the GLP regulations. The experiment was initiated on 5/28/02. b(4)

Isolated male canine cardiac Purkinje fibers were electrically paced at 0.5 and 1 Hz and challenged with AMI-6424 at free-base concentrations of 5, 50 and 150 µg/mL. Test article and "Placebo for AMI-6424 for Injection, 250 mg/vial" were reconstituted with D5W. The positive control was *dl*-sotalol at 50 µM.

Drug or placebo was placed into the system for 30 minutes onto 8 fibers. Effects of high dose AMI-6424 and placebo were also evaluated at a frequency of 3 Hz to evaluate any effects on the sodium channel at high dose. The placebo fibers were finally treated with the positive control agent to validate the assay.

No effects on action potential parameters were found at 5 µg/ml. At 50 µg/ml, prolongation of the APD<sub>60</sub> (11%) and APD<sub>90</sub> (7.1%) was reported at 1 Hz when compared to placebo and 13.1% and 9.3%, respectively, at 0.5 Hz. Thus, it was concluded that AMI-6424 elicits an increase in action potential duration at both frequencies. At 150 µg/ml, the increases were slightly larger. The positive controls performed as expected, thus validating the assay.

**02-003-23 ( ) 93431: A Cardiovascular Profile Study Following an Intravenous Infusion of AMI-6424 in the Conscious Unrestrained Beagle Dog**

This study was conducted by (C ) and was initiated on 4/22/02. It was conducted in compliance with the GLP regulations. b(4)

Catheterized male beagle dogs were given escalating doses of AMI-6424 (lot #AME003) as 2 hour infusions of 0, 25, 50 or 100 mg/kg/d as a single dose and 100 mg/kg/d as repeat doses for up to 4 days. The drug/placebo was administered at 5 ml/kg/hr. In addition to the standard evaluations (body weight, clinical signs, etc.), EKGs were taken intermittently (3x during the pre-dosing period, ~1 hr following initiation of dosing, at the end of the dosing period and at 2 and 22 hrs) and heart rate and blood pressure were measured continuously for 90 minutes prior to dosing and up to 24 hours post-dosing during the escalation phase and on the last day of the repeat dose phase. Samples for PK were taken at the same times. Neither gross necropsies nor histologic evaluations was performed.

Signs of histaminic reactions (reddened pinnae, scratching) were reported at 100 mg/kg/d as a single or repeat dose. No treatment-related effects were reported on blood pressure, heart rate or EKG parameters. Plasma drug levels increased with dose and no accumulation was appreciated.

**02-006-06 Dure1000: Effects of AMI-6424 on Action Potential Parameters in Sheep Isolated Cardiac Purkinje Fibers**

This study was conducted by (C ) and was initiated on 6/25/02. It was conducted in compliance with OECD GLP regulations. b(4)

Isolated sheep cardiac Purkinje fibers were electrically paced at 1 Hz and challenged with AMI-6424 at free-base concentrations of 5, 50 and 150 µg/mL. Test article and "Placebo for AMI-6424 for Injection, 250 mg/vial" were reconstituted with D5W. The positive control was *dl*-sotalol at 50 µM.

Drug or placebo was placed into the system for 30 minutes onto 8 fibers. Effects of high dose AMI-6424 and placebo were also evaluated at a frequency of 3 Hz to evaluate any effects on the sodium channel at high dose. The placebo fibers were finally treated with the positive control agent to validate the assay.

No effects on action potential parameters were found at any dose of AMI-6424 or placebo except for a slight decrease in maximal rate of depolarization with the increase in stimulation frequency. However, when comparison between groups (treated and placebo) was made, no significant differences were appreciated. The positive controls performed as expected (at a lesser increase than in the canine assay), thus validating the assay.

#### **Safety pharmacology conclusions:**

TD-6424 inhibited *hERG* channels and elicited a prolongation of action potential in isolated canine cardiac Purkinje fibers. No treatment-related cardiovascular effects were seen in *in vivo* studies in dogs. No drug-induced respiratory or neurological effects were noted.

#### **2.6.2.5 Pharmacodynamic drug interactions**

There is no significant drug-drug interaction for AMI-6424 when co-administered with aztreonam or metronidazole.

#### **2.6.3 PHARMACOLOGY TABULATED SUMMARY**

Not applicable.

#### **2.6.4 PHARMACOKINETICS/TOXICOKINETICS**

##### **2.6.4.1 Brief summary**

In mice, rats and rabbits, plasma C<sub>max</sub> and AUC demonstrated dose-dependent increases following a single IV dosing. PK studies showed significant differences of t<sub>1/2</sub> between the various species (mice- 1.2- 9.5 hours; rats- 1.3- 14.8 hours; dogs- 1.4 or 12.8 hours; monkeys- 2.3 hours; rabbit- 1.33-2.33 hours). In tissue distribution studies conducted in dogs, rats and mice, high concentrations of radioactivity were seen in the bone, liver and kidney. TD-6424 is highly protein bound (approximately 90%) in mouse, rat, dog, bovine, rabbit and human plasma as well as human skin blister fluids. Following IV dosing, AMI-11352 (7-OH metabolite), AMI-999 or other hydroxylated metabolites were observed in serum samples of rats, dogs and monkeys. The hydroxylated metabolites are the major metabolites in rat, dog and monkey urine. Urine excretion is the major route in dogs, mice, and rats.

##### **2.6.4.2 Methods of Analysis**

See descriptions under individual study reviews.

**2.6.4.3 Absorption****01-6424-PK-03: Pharmacokinetics of AMI-6424 in Mice**

This is a summary of three studies: In all studies, blood samples were collected at pre-dose, and 2, 5, 15, 30 minutes and 1, 2, 4, 8, and 24 hours post-dose.

a) 2K-007-070- Three male CD-1 mice/timepoint were treated at 10 mg/kg i.v. Drug was diluted in 5% dextrose containing 5% HP- $\beta$ -CD.

Blood levels were below the level of quantitation by 8 hours post-dosing.

Parameter	AMI-6424
C <sub>max</sub> ( $\mu$ g/ml)	155
AUC <sub>0-<math>\infty</math></sub> ( $\mu$ g-hr/ml)	99
T <sub>1/2</sub> (hr)	1.2
Cl (L/hr/kg)	0.101
V <sub>ss</sub> (L/kg)	0.146

b) 2K-007-087- Dose escalation in female NSA mice (3/dose/timepoint) at 1, 3, 10 or 25 mg/kg i.v. using <sup>3</sup>H-AMI-6424. Drug was diluted in 5% HP- $\beta$ -CD/D5W. The neutropenic mice were created by giving 2 doses of cyclophosphamide 2 and 4 days prior to dosing with the tritiated AMI 6424. Results are shown in the table below.

Dose (mg/kg)	1	3	3 (neutropenic)	10	25
C <sub>max</sub> ( $\mu$ g/ml)	21.2	40.6	31.6	232	334
AUC <sub>0-<math>\infty</math></sub> ( $\mu$ g-hr/ml)	10.9	32.6	31.6	126	420
T <sub>1/2</sub> (hr)	7.5	9.5	7.3	5.7	4.8
Cl (L/hr/kg)	0.092	0.092	0.095	0.079	0.060
V <sub>ss</sub> (L/kg)	0.286	0.470	0.381	0.277	0.186
C <sub>max</sub> /dose	21.2	13.5	10.5	23.2	13.4
AUC/dose	10.9	10.9	10.5	12.6	16.8

c) 01-007-014- Three CD-1 mice/sex/timepoint received 10 mg/kg <sup>3</sup>H-AMI-6424 i.v. Drug was diluted in 2% HP- $\beta$ -CD. PK results are summarized in the table below.

Parameter	Male	Female
C <sub>max</sub> ( $\mu$ g/ml)	114	102
AUC <sub>0-<math>\infty</math></sub> ( $\mu$ g-hr/ml)	98.9	91.2
T <sub>1/2</sub> (hr)	5.8	6.5
Cl (L/hr/kg)	0.101	0.110
V <sub>ss</sub> (L/kg)	0.304	0.328

Serum concentrations were measured by LC/MS and scintillation.

Results: The terminal t<sub>1/2</sub> for AMI-6424 (1.2 hr) was longer than that observed for vancomycin (0.26 hr).

PK in neutropenic mice was comparable to that in normal animals.

The C<sub>max</sub> was dose-proportional at 1-25 mg/kg and the AUC<sub>0- $\infty$</sub>  increased proportionally with dose up to 10 mg/kg. The 25 mg/kg dose showed a greater than dose-proportional increase. There were no apparent gender differences with respect to metabolism.

**01-6424-PK-04: Pharmacokinetics of AMI-6424 in Rats**

This is a summary of three studies: In all studies, blood samples were collected at pre-dose, and 2, 5, 15, 30 minutes and 1, 2, 4, 6, 8, and 24 hours post-dose.

a) 2K-007-059- Three fasted Sprague-Dawley male rats were given 10 mg/kg i.v. AMI 6424. Drug was diluted in 5% dextrose containing 5% HP- $\beta$ -CD. Last samples were taken at 8 hours postdosing.

Parameter	AMI-6424
C <sub>max</sub> ( $\mu$ g/ml)	145
AUC <sub>0-8</sub> ( $\mu$ g-hr/ml)	198
T <sub>1/2</sub> (hr)	1.3
Cl (L/hr/kg)	0.051
V <sub>ss</sub> (L/kg)	0.10

b) 2K-007-088- IV dose escalation study in male Sprague-Dawley rats (N=3/dose) using <sup>3</sup>H-AMI- 6424 at 1-25 mg/kg. Drug was diluted in 5% dextrose containing 5% HP- $\beta$ -CD.

Dose (mg/kg)	1	3	10	25
C <sub>max</sub> ( $\mu$ g/ml)	8.79	23.5	96.2	254
AUC <sub>0-24</sub> ( $\mu$ g-hr/ml)	12.2	38.6	140	367
T <sub>1/2</sub> (hr)	14.3	14.8	7.2	6.9
Cl (L/hr/kg)	0.082	0.078	0.071	0.068
V <sub>ss</sub> (L/kg)	0.71	0.75	0.26	0.29
C <sub>max</sub> /dose	8.79	7.83	9.62	10.2
AUC/dose	12.2	12.9	14.0	14.7

C<sub>max</sub> and AUC increased dose-proportionately but a decrease in V<sub>ss</sub> was noted at >10 mg/kg. This caused a decrease in the t<sub>1/2</sub>. The sponsor attributed the increased t<sub>1/2</sub> in this study (when compared to the study above) to the increased sensitivity over the methodology used above. However, it is probably due to the 24 hour timepoint in this study allowing a more thorough evaluation.

c) 01-007-015- Three Sprague-Dawley rats/sex/timepoint using <sup>3</sup>H-AMI-6424 at 10 mg/kg. Drug was prepared in 2% HP- $\beta$ -CD in a 10:1 ratio (CD: AMI 6424).

Parameter	Male	Female
C <sub>max</sub> ( $\mu$ g/ml)	88.3	98.6
AUC <sub>0-8</sub> ( $\mu$ g-hr/ml)	164	151
T <sub>1/2</sub> (hr)	5.8	6.8
Cl (L/hr/kg)	0.061	0.066
V <sub>ss</sub> (L/kg)	0.26	0.30

Results: The terminal t<sub>1/2</sub> for AMI-6424 (1.3 hr) was longer than that observed for vancomycin (0.72 hr). The C<sub>max</sub> and AUC demonstrated dose-proportional increases. There were no apparent gender differences with respect to metabolism.

Sera were also evaluated for metabolites in the radioactive studies. Results showed >80% of the radioactivity as unchanged parent compound.

**01-6424-PK-05: Pharmacokinetics of AMI-6424 in Beagle Dogs**

This study was intended to demonstrate the pharmacokinetics in male beagle dogs (N=3) after a 2 hour i.v. infusion at 10 mg/kg. Urine and plasma samples were collected pre-dose, 1 hour into the infusion, end of infusion, 2.083, 2.25, 2.5, 3, 4, 6, 8, 12 and 24 hours post-dosing. Drug levels were determined using HPLC/UV.

Parameter	AMI-6424
C <sub>max</sub> (µg/ml)	51.5
T <sub>max</sub> (Hr)	2.0
AUC <sub>0-∞</sub> (µg-hr/ml)	154
T <sub>1/2</sub> (hr)	1.4
Cl (L/hr/kg)	0.065
V <sub>ss</sub> (L/kg)	0.14

Results: The terminal t<sub>1/2</sub> (1.4 hr) of AMI-6424 was similar to that of vancomycin (2.2 hr) (unlike in mice and rats), but the C<sub>max</sub> and AUC were higher for AMI-6424 at the same dose. AMI-6424 was below the level of detection by 8 hours post-dosing for all of the animals. Urinary recovery of unchanged parent drug was ~25%.

#### **01-6424-PK-06: Pharmacokinetics of AMI-6424 in Monkeys**

Two naïve cynomolgus monkeys/sex were administered a single dose of 10 mg/kg AMI-6424 via a 2 hour i.v. infusion. The AMI-6424 was formulated in 6.25% HP-β-CD/3.75% dextrose as a 2 mg/ml solution. Blood samples were collected at pre-dose, mid-infusion, end of infusion, 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post-end of infusion. Serum chemistries were taken at the same time, as well as 7 days post-infusion. Urine was collected from the end of the infusion through 24 hours post-infusion. Samples were analyzed using a HPLC/UV system.

Results: Serum concentrations were below the level of quantitation for all animals by 12 hours post-end of infusion. The t<sub>1/2</sub> was comparable to vancomycin but the AUC was much greater for AMI-6424 (177 vs. 80 µg.hr/ml), and the peak levels were also much higher (48.5 vs 25 µg/ml) at the same dose.

Urinary recovery overall of unchanged parent compound was approximately 20% (mean 29.3% in males, 10.6% in females).

Parameter	AMI-6424
C <sub>max</sub> (µg/ml)	48.5
T <sub>max</sub> (Hr)	2.0
AUC <sub>0-∞</sub> (µg-hr/ml)	176
T <sub>1/2</sub> (hr)	2.3
Cl (L/hr/kg)	0.058
V <sub>ss</sub> (L/kg)	0.166

#### **04-6424-PK-24: Pharmacokinetics of TD-6424 in New Zealand White Rabbits**

This study was intended to demonstrate the pharmacokinetics in male NZW rabbits (N = 3/dose) after a 10-min i.v. infusion at 10 and 50 mg/kg. The AMI-6424 was formulated in 5% dextrose as a 5 and 20 mg/mL solution for 10 and 50 mg/kg dose levels. Plasma samples were collected pre-dose, 5-min into the infusion, end of infusion, 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post-dosing. Drug levels were determined using LC-MS/MS. AMI-11352 (a metabolite of AMI-6424) and AMI-999 were also measured.

Results: Following IV dosing, the Cmax and AUC levels of AMI-6424 were approximately linear.

Parameter	AMI-6424 10 mg/kg	50 mg/kg
Cmax (µg/ml)	85.0	408
Tmax (hr)	0.167	0.167
AUC <sub>0-1</sub> (µg-hr/ml)	101	627
AUC <sub>0-∞</sub> (µg-hr/ml)	107	634
T1/2 (hr)	1.33	2.32
Cl (L/hr/kg)	0.0965	0.0793
Vss (L/kg)	0.169	0.227
Cmax/dose	8.50	8.16
AUC/dose	10.1	12.5
AUC ratio (11352/6424)	0.0693	0.0298
AUC ratio (999/6424)	0.00230	0.0125

**04-6424-PK-25: Pharmacokinetics and Bioavailability of TD-6424 Following Intravenous and Subcutaneous Administration to Normal or Neutropenic Female Mice**

This is a summary of four studies: In all studies, blood samples were collected at pre-dose, and 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose. The studies were intended to demonstrate the pharmacokinetics and systemic bioavailability of TD-6424 after intravenous bolus (IV) or subcutaneous (SC) administration at a dose of 40 mg/kg in normal or neutropenic female mice (3 mice/time-point/route). In study 04-067-041, TD-6424 was administered to normal female mice via IV or SC administration. Studies 04-067-005 and 04-067-019 used the murine pneumonia model. TD-6424 was administered to infected mice via IV (study 04-067-005) and SC (study 04-067-019) administration. In study 04-067-095, the murine septic peritonitis model was used. TD-6424 was administered to infected mice via SC administration. Plasma concentrations were determined by LC-MS/MS with a limit of quantitation (LOQ) of 0.250-1.00 µg/ml.

Results: There was no significant difference in PK parameters in normal and neutropenic mice following IV administration. Following SC administration of TD-6424 in neutropenic mice, a slightly higher Cmax and higher AUC was observed in comparison to that observed in normal mice. The systemic bioavailability of TD-6424 following SC administration was 74% in normal mice, 104% in neutropenic female mice infected intranasally with MRSA, and 64% in neutropenic female mice infected intraperitoneally with MRSA. However, the T<sub>last</sub> following SC administration was shorter (8 hour vs. 24 hour) in normal mice and neutropenic mice infected intraperitoneally with MRSA. Therefore, the AUC and bioavailability may be underestimated.

Model	Normal		Pneumonia (neutropenic)		Peritonitis (neutropenic)
	04-067-041		04-067-005	04-067-019	04-067-095
Study #	IV	SC	IV	SC	SC
C <sub>0</sub> or Cmax (µg/ml)	595	86.8	353	113	102
Tmax (hr)		1.00		2.00	2.00
Tlast (hr)	8.00	8.00	24.0	24.0	8.00
AUC <sub>0-1</sub> (µg-hr/ml)	484	358	755	788	483
AUC <sub>0-∞</sub> (µg-hr/ml)	521	Not determined	759	792	574
T1/2 (hr)	2.29	4.15	3.25	2.82	2.74
Cl (L/hr/kg)	0.0767	Not determined	0.0527	0.0505	0.0696
Vss (L/kg)	0.204		0.202		
F(%)		74.1		104	64.0

**05-6424-PK-36: Absorption, Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C TD-6424 in Dogs Following Intravenous Administration of TD-6424 for Injection and Alternate Formulations**

The objective of these studies was to examine the tissue distribution, excretion, metabolism and mass balance of <sup>14</sup>C-radiolabeled TD-6424 (25 µCi/mg) in male and female dogs (2/sex/formulation) following intravenous administration via a 60 min infusion at a dose of 25 mg-eq/kg. Three formulations were evaluated: Formulation A: TD-6424 for Injection (containing TD-6424/ hydroxypropyl-β-cyclodextrin (HP-β-CD) with a molar ratio of 1 to 10, Study PK05-108); Formulation B: TD-6424/ HP-β-CD with molar ratio of 1 to 2 (Study PK05-100) and Formulation C: TD-6424/ sulfobutylether-β-cyclodextrin (SBE-CD) with molar ratio of 1 to 1 (Study PK05-100). Excretion of radioactivity was assessed by analysis of urine and feces collected for 0-12 and 12-24 hr in one dog/sex/formulation and every 24 hr for 168 hr in one dog/sex/formulation. One dog/sex/formulation was euthanized at 24 hr and 168 hr post-dose and selected tissues were collected and processed. Plasma samples were also collected from all the dogs for PK profiles at pre-dose, mid-infusion, 0, 2, 5, 15 and 30 minutes, and 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hr post-infusion. Total radioactivity in plasma was determined by liquid scintillation counting. Concentration of TD-6424, AMI-11352 (main metabolite) and AMI-999 (C) in plasma was determined using a validated bioanalytical method by LC-MS/MS at Covance Laboratories with a limit of quantitation (LOQ) of 0.250 µg/mL for all three analytes. b(4)

**Results:** The tissue distribution profiles of <sup>14</sup>C-TD-6424 in dogs was similar for all three formulations. Higher tissue concentrations were observed for the two alternate formulations TD-6424/ HP-β-CD (1:2) and TD-6424/SBE-CD (1:1) compared to that observed for clinical formulation TD-6424/ HP-β-CD (1:10). There was no significant gender-related difference observed for all three formulations.

At 24 hr post-dose, radioactivity was detected in all tissues examined. The highest concentrations of radioactivity in organs and tissues occurred in the kidney and liver. Radioactivity concentrations in brain, cerebrospinal fluid (CSF), and spinal cord were very low indicating low CNS penetration or retention of <sup>14</sup>C-TD-6424 in dogs following intravenous administration. Renal excretion was the main route of elimination of <sup>14</sup>C-TD-6424 in dogs. The total radioactivity recovered in selected organs, tissues, and excreta at 24 hr post-dose was 90.9%, 97.9% and 89.6% for formulations containing TD-6424/ HP-β-CD (1:10), TD-6424/ HP-β-CD (1:2) and TD-6424/SBE-CD (1:1), respectively.

At 168 hr post-dosing, radioactivity was detected in all tissues examined for all three formulations. The highest concentrations of radioactivity occurred in the liver and kidney. The half-life based on the total radioactivity in the liver was greater than 168 hr for all three formulations, and 95 hr, 101 hr, and 88 hr in the kidneys for Formulations A, B and C, respectively.

At 168 hr post-dose, radioactivity recoveries in excreta as percent dose in Formulations A, B, and C were 91.1%, 80.8% and 79.3% in urine, respectively and 2.61%, 2.73% and 4.12% in feces, respectively. The total radioactivity recovered in selected organs, tissues, and excreta at 168 hr post-dose was 100%, 93.2% and 91.9% for formulations A, B, and C, respectively.

In the urine samples collected over 0-12 hr, the major radioactive peak was TD-6424 that accounted for 73.5-81.8% of the total radioactivity. In the urine samples collected over 12-24 hr, TD-6424 accounted for 14.9-17.8% of the total radioactivity. At least two radioactive peaks present in the urine samples had similar retention time as the metabolite, AMI-11352. The percent dose recovered in urine as TD-6424 over 0-24 hr

period was 61% for TD-6424 for Injection and was slightly higher than that observed for two alternate formulations (51%).

Higher systemic exposures ( $C_{max}$  and AUC values) were observed in animals dosed with two alternate formulations. TD-6424 was below the limit of quantitation (0.250  $\mu\text{g/ml}$ ) in the plasma beyond 12 hr post-dose for all three formulations.

For AMI-11352 and AMI-999, slightly higher AUC values were observed for animals dosed with two alternate formulations but the mean AUC ratio of metabolites to TD-6424 were similar for all three formulations ranging (0.025 to 0.032 for AMI-11352 and 0.017 for AMI-999).

In summary, higher tissue concentrations were observed for the two alternate formulations TD-6424/ HP- $\beta$ -CD (1:2) and TD-6424/SBE-CD (1:1) compared to that observed for clinical formulation at 24 and 168 hr post-dose. Higher systemic exposures ( $C_{max}$  and AUC values) of TD-6424, AMI-11352 and AMI-999 were observed in animals dosed with the two alternate formulations. However, the AUC ratios of AMI-11352 or AMI-999 vs TD-6424 were similar for all three formulations.

#### **2.6.4.4 Distribution**

##### **01-6424-PK-08: Tissue Distribution, Excretion and Metabolism of $^3\text{H}$ -AMI-6424 in Mice**

Five male CD-1 mice were administered i.v.  $^3\text{H}$ -AMI-6424 in 5% HP- $\beta$ -CD. Urine and feces were collected for each animal and collections were combined for analysis. At 24 hours post-dosing, animals were euthanized and tissues (liver, kidneys, lung, heart, skin, serum and spleen) were harvested. Sample analysis was performed by HPLC. Radioactivity was evaluated by liquid scintillation counting.

Total recovery approximated  $72\% \pm 23.6\%$ . The highest tissue concentrations were in liver (4.4% of dose) and kidneys (1% of dose). Serum concentration at 24 hours was 0.06% of dose. Urine excretion appeared to be the major route in these mice, with urinary recovery being 61% of the total radioactivity recovered with 48% of that being unchanged parent compound. Metabolites accounted for ~21% of the total recovered radioactivity.

##### **01-6424-PK-09: Tissue Distribution, Excretion and Metabolism of $^3\text{H}$ -AMI-6424 in Rats**

Three male Sprague-Dawley rats were administered 10 mg-equiv/kg i.v.  $^3\text{H}$ -AMI-6424 in 5% HP- $\beta$ -CD. Urine and feces were collected for each animal and collections were combined for analysis. At 24 hours post-dosing, animals were euthanized and tissues (liver, kidneys, lung, heart, skin, spleen and serum) were harvested. Sample analysis was performed by HPLC. Radioactivity was evaluated by liquid scintillation counting.

Total recovery approximated  $72\% \pm 23.6\%$ . The highest tissue concentrations were in liver (6.8% of dose) and kidneys (1.3% of dose). Serum concentration at 24 hours was 0.1% of dose. Urine excretion appeared to be the major route in these rats, with urinary recovery being 57.8% of the total radioactivity recovered and fecal recovery was 3.8%. Parent compound accounted for 76% of the urinary radioactivity. Metabolites accounted for ~24% of the total urinary recovered radioactivity.

**01-6424-PK-10: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 with Various Formulations**

Two Sprague-Dawley rats/dose were administered <sup>3</sup>H-AMI-6424 (25 mg-equiv./kg i.v.) in HP-β-CD or SBE-β-CD with a 1:1 or 5:1 molar ratio. At 24 hours post-dose, all animals were sacrificed and tissues (liver, kidneys, lung, heart, spleen, skin, submaxillary gland, bladder and serum) were harvested. Urine and feces were collected and pooled separately for analysis.

No apparent differences in distribution and excretion were reported between any of the dosing formulations. Mean total radioactivity recovered ranged from 81.5- 90.9% for the formulations. The highest tissue concentrations were in liver (6-9.5%) and kidneys (1-2.6%). Serum concentration at 24 hours was 0.09-0.15% of recovered radioactivity. Urinary excretion was the major route in the rats with 61-80% of the total radioactive dose while fecal radioactivity was 3-8%. Metabolites accounted for 3.5-16% of the urinary radioactivity.

**01-6424-PK-11: Tissue Distribution and Excretion of AMI-6424 in Rats Following Single or Repeated Dosing**

This report is a summary of Studies 00-036-020, 00-036-028, 00-036-033, 00-036-022, 00-036-032, 00-036-037, and 00-036-038.

AMI-6424 was administered intravenously to 3-5 female rats. The dosing regimens were as follows:  
Single AMI-6424 dose: 25, 5 or 1% HP-β-CD or 5% D5W.  
Repeated AMI-6424 dose: 5 or 1% HP-β-CD with fixed molar ratio of 1:1, 2.5:1, 5:1 or 10:1 or in SBE-β-CD with fixed weight ratio of 1.3:1, 3.3:1, 6.7:1, or 13.3:1.  
Some animals were pretreated with excipient (15 minutes prior to drug dosing or simultaneously with active drug).

At 24 hours post-single dosing or 7 doses of post-repeated dosing, the animals were euthanized. Serum, liver and kidneys were retained for analysis. Urine samples were collected for 0-24 hours in the single dose animals and 168-192 hours in the repeat dose animals.

Results: In the single dose animals, clearance was markedly affected by the concentration of HP-β-CD. Urinary recoveries significantly decreased with decreased amounts of excipient. At lower amounts of excipient or D5W, higher drug concentrations were detected in liver, kidney and serum.

When the animals were pre-treated with excipient (simultaneously or 15 minutes prior to dosing), drug concentrations in tissues were markedly reduced in liver and kidney.

In the repeat dose animals, urinary recoveries were high for 1% and 5% HP-β-CD. Neither significant hepatic nor renal accumulation of AMI-6424 was found. The type of excipient did not seem to affect the above mentioned parameters.

In summary, the total % recovered from liver and kidneys was lower in the repeated dose animals than the single dose animals with a given formulation.

**% Unchanged parent drug recovered**

Study number	% HP- $\beta$ -CD	Serum ( $\mu$ g/ml)	Urine	Liver	Kidney
00-036-020	25%	<1.32	50.4	3.89	0.93
00-036-028	25%	0.86	90.9	1.9	0.62
	5%	1.66	40.5	4.89	2.08
	1%	17.1	17.4	8.47	5.68
	0(D5W)	59.8	12.6	14.2	17.8
00-036-033 (pre-treatment with HP- $\beta$ -CD)	24 hours	4.88	47.2	13.2	7.89
	15 minutes	1.65	33.1	3.75	0.82
	Co-administration	0.72	44.1	2.29	0.56
	0	6.61	20.5	13.5	12.3
00-036-022 7 day dosing 25 mg/kg	5%	1.44	57.7	1.3	0.16
50 mg/kg	5%	3.8	67.5	1.33	0.47
00-036-032 7 day dosing 12.5 mg/kg	1%	<0.5	36.0	3.06	0.76
25 mg/kg	1%	1.02	50.5	3.86	0.97
00-036-037 7 day dosing 6.25 mg/kg	1:1	<5.0	55.0	3.75	1.95
	2.5:1	<5.0	31.8	3.63	1.34
	5:1	<5.0	38.5	3.78	1.47
	10:1	<5.0	41.1	3.16	0.97
12.5 mg/kg	1:1	<5.0	41.3	4.3	1.32
	2.5:1	<5.0	35.4	3.54	1.24
	5:1	<5.0	39.5	3.85	1.28
	10:1	<5.0	32.2	3.24	1.01
25 mg/kg	1:1	<5.0	32.9	5.10	1.40
	2.5:1	<5.0	37.4	4.13	0.99
	5:1	<5.0	20.1	4.25	1.02
	10:1	<5.0	32.4	3.04	0.78

The standard deviations were quite wide for most of the parameters evaluated.

#### **01-6424-PK-12: Tissue Distribution, Excretion and Metabolism of $^3$ H-AMI-6424 Following Intravenous Infusion to Dogs**

In this study (conducted by Covance Laboratories, Madison, WI), one beagle dog/sex was treated with 10 mg-eq/kg  $^3$ H-AMI-6424 in 2% HP- $\beta$ -CD via a 2-hour infusion. Serum samples were taken at 1 and 2 hours (end of infusion) and at 2, 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12 and 24 hours post-end of infusion. Urine and feces were collected for 24 hours. At 24 hours post-dose, both animals were euthanized and tissues (essentially the entire standard histology tissue list) were removed for analysis. One kidney and a section of liver were frozen for autoradiography.

Results: Total radioactivity recovered was >100% of total dose. Urinary excretion was the major route of elimination with urinary recovery ~85% of total dose. Metabolites accounted for 53% (male) and 35% (female) of the total radioactivity recovered. Fecal excretion was <2% of the total dose. There are no apparent gender related differences in the excretion and tissue distribution of radioactivity in dogs. Levels of radioactivity were highest in liver (6%), muscle (2%) and kidney (1.2%). However, tissue concentrations were lower in the autoradiography samples. The sponsor suggests that this difference is due to loss of tritiated water during the desiccation or less homogenous distribution of radioactive material. When comparing the results for this study with the LC-MS analysis, lower levels were shown with the LC-MS analysis.  $T_{1/2}$ s were longer [ $\sim$ 13 hours (N= 1/sex) in this study] and higher AUC and lower CI were found. This uncertainty reinforces the concern about the  $^3$ H-label and the FDA preference for a  $^{14}$ C-label. Additionally, it appears that there was a limited partition of drug into the red blood cells of these dogs.

**01-6424-PK-15: Quantitative Whole-Body Autoradiography of <sup>3</sup>H-AMI 6424 in Rats**

One Sprague-Dawley rat/timepoint was treated with 10 mg-eq/kg <sup>3</sup>H-AMI-6424 in 2% HP-β-CD/D5W in a molar 10:1 ratio to AMI-6424. Animals were sacrificed at 0.25, 24, 72, 168, and 336 hours post dosing and whole carcasses were processed for autoradiography. Urine and feces were collected from 1 animal/sex/time point (0-24, 0-72, 0-168 or 0-336 hours).

Results: Urinary recoveries ranged from 65-82% while fecal recoveries were 2.8-26%. Radioactivity was detected in all tissues at the 0.25 and 24 hour timepoints. T<sub>1/2s</sub> terminal were ~46 hours in urine. T<sub>1/2s</sub> terminal were ~200 hours in epiphysis, kidney and liver. Highest tissue levels were detected in epiphysis (51 µg/g), kidney (48 µg/g) and lung (29 µg/g) at 0.25 hours post-dose and epiphysis (27.5 µg/g), liver (14 µg/g) and kidney (10 µg/g) at 24 hours post-dosing. Radioactivity was detected in the epiphysis, kidney (especially the renal medulla) and spleen at all timepoints, but below the level of detection for most other tissues by 72 hours.

**02-6424-PK-22: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 in Rats**

AMI-6424 (Lot AME002) and <sup>14</sup>C-TD-6424 were reconstituted in D5W and administered at 25 mg-eq IV to 3 Sprague Dawley rats/sex. The infusion rate was 0.75 ml/min and took approximately 2 minutes/infusion. After 24 hours, the animals were euthanized and appropriate tissues were collected, as were urine and feces.

Total recovery for males was 99.3%, for females was 90.8%. The highest concentrations were found in bone>liver>skin>muscle and were at least 60%> plasma levels. Urinary recovery was 62%, fecal recovery was 4.5%. The majority of the radioactivity in urine was parent compound (>80%) but M1, M2 and M3 accounted for 11% of the total radioactivity. M3 may also be present in the liver samples. This differs from the prior rat tritiated thymidine ADME study where no M2 or M3 were detected. (

) . Further study is planned by the sponsor.

**02-6424-PK-23: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 Following Intravenous Infusion to Dogs**

AMI-6424 (Lot #AME002) and <sup>14</sup>C-TD-6424 were reconstituted in D5W and administered at 25 mg-eq. i.v. to one beagle/sex. The infusion took 2 hours. After 24 hours, the animals were euthanized and appropriate tissues were collected, as were urine and feces.

Total recovery for males was 90.8%, for females was 97.3%. The highest concentrations were found in liver>bone>kidney and were at least 15x> plasma levels at 24 hours post-dose. Urinary recovery was ~77%. Tissue radioactivity accounted for ~12% of the administered dose. Metabolites (primarily M1, with small amounts of M3) accounted for ~19% of the recovered urinary radioactivity. (

) The sponsor plans to further define this possibility.

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b(4)

**04-6424-PK-26: Tissue Concentration of TD-6424 in Female Rats Following Fourteen-Day Dosing With a 7- And 14-Day Recovery Period**

The purpose of the study was to determine the concentrations of TD-6424 in liver and kidney and to assess the potential of TD-6424 to accumulate in these tissues following intravenous administrations of AMI-6424 (100 mg/kg/day, a slow bolus of 1 or 3-5 minutes) to female rats for 1, 3, 7, 10 or 14 days (10 animals/group). A 7- and 14-day recovery period was included in the study design. Blood, liver and kidney samples were collected 24 hours after the last dose, or 7 and 14 days post dose for recovery animals. Samples were analyzed using HPLC assay. The in-life phase of this study was conducted with Study 02-036-019.

Liver and kidney tissue concentrations of TD-6424 increased with the number of doses administered. No steady-state after 14 doses was reached. The percent of total dose recovered from liver and kidneys following 14 doses administration of TD-6424 were 5.26% and 1.04%, respectively, which were lower than that observed following a single dose administration. Therefore, no significant accumulation of TD-6424 in liver and kidneys was observed following 14 days of repeated dosing. The half-life of TD-6424 in liver and kidneys was 10.5 days and 14 days, respectively.

**Tissue concentration and % dose recovered in plasma, liver and kidneys**

Tissue	Concentration (µg/g)			Dose recovered (%)	
	Plasma	Liver	Kidney	Liver	Kidney
Day 1	0.722	166	265	7.57	2.66
Day 3	0.878	531	524	8.38	1.75
Day 7	1.31	864	837	5.74	1.24
Day 10	1.57	1048	969	5.01	1.04
Day 14	1.78	1548	1303	5.26	1.04
Recovery Day 21	0.624	1193	920	4.28	0.76
Recovery Day 28	0.353	616	661	2.30	0.58

**05-6424-PK-30: Tissue Distribution of Total Radioactivity in the Pigmented Rat Following Intravenous Administration of <sup>14</sup>C-TD-6424 (Quantitative Whole Body Autoradiography)**

The purpose of this study was to examine the tissue distribution of radiolabeled TD-6424 in male pigmented rats using quantitative whole body autoradiography (QWBA) following intravenous administration at a nominal dose of 25 mg-eq/kg. AMI-6424 (Lot 2213-10-645043) and <sup>14</sup>C-TD-6424 were reconstituted in D5W and administered at 25 mg-eq IV to 6 male pigmented rats. One animal per timepoint was sacrificed at 5 minutes, 2, 24, 72, 168 and 336 hr post-dose. Each animal was analyzed using QWBA techniques.

Radioactivity was distributed throughout the tissues following intravenous administration of <sup>14</sup>C-TD-6424, and was eliminated slowly from the majority of the tissues examined. High levels of radioactivity were found associated with the bone at all timepoints. This radioactivity appears to be mainly located in the growth plates but was also found in the bone marrow with an estimated half-life of 332 hr (~14 day). High levels of radioactivity were observed in the liver and kidney with estimated half-life of 96 and 118 hr, respectively.

Radioactivity was poorly distributed to CNS tissues, indicating minimal penetration of <sup>14</sup>C-TD-6424 through the blood-brain barrier. There was no evidence for high affinity melanin-specific binding of radioactivity in the pigmented tissues (pigmented skin and uveal tract).

**05-6424-PK-35: Tissue Distribution of Total Radioactivity in the Pigmented Rat Following Intravenous Administration of <sup>14</sup>C-TD-6424 in Alternate Formulations (Quantitative Whole Body Autoradiography)**

This study, conducted in ( ) was to examine the tissue distribution of radiolabeled TD-6424 in pigmented rats using QWBA following intravenous administration at a nominal dose of 25 mg-eq/kg. Two alternative formulations, with different molar ratios of TD-6424 to HP-β-CD or SBE-CD (1:2 and 1:1, respectively), were evaluated and compared to the clinical formulation (TD-6424/HP-β-CD of 1:10). <sup>14</sup>C-TD-6424 of the two formulations was administered at 25 mg-eq IV to three pigmented rats/sex/formulation. One animal/sex/formulation/timepoint was sacrificed at 5 minutes, 24 hr and 336 hr post-dose. Each animal was analyzed using QWBA techniques. Urine and feces samples were collected at specified intervals for up to 168 hr post-dose from one male and one female animals (assigned to the 336 hr timepoint) per formulation.

b(4)

The results showed that the two alternate formulations had similar distribution and elimination patterns, and had no significant effect on the disposition of TD-6424 compared to that observed for the clinical formulation. Radioactivity was eliminated slowly from the majority of the tissues. High levels of radioactivity were found in the bone, liver and kidney. Radioactivity was poorly distributed to CNS tissues for both formulations, indicating minimal penetration of <sup>14</sup>C-TD-6424 through the blood brain barrier. There was no evidence for high affinity melanin-specific binding of radioactivity in the pigmented tissues (pigmented skin and uveal tract).

Following intravenous dosing of <sup>14</sup>C-TD-6424 formulated in TD-6424/HP-β-CD with a molar ratio of 1 to 2, total radioactivity recoveries at 24 hr post-dose ranged from 47% to 54% in urine, and 10% to 17% in feces. For SBE-CD formulation, total radioactivity recoveries at 24 hr post-dose ranged from 52% to 54% in urine, and 12% to 13% in feces. The fecal recoveries for the two alternate formulations in rats were higher than that observed for the clinical formulation (TD-6424 for injection). The sponsor indicated that higher fecal recoveries might be the result of cross-contamination of urine samples during sample collection. The reviewer considers that it was possible since similar radioactivity concentrations in liver were observed for the two alternate formulations and the clinical formulation at all time points examined.

For the metabolite profiles, in urine samples collect over 0-6 hr and 6-24 hr, the major radioactive peak was TD-6424 and accounted for greater than 90% and 54-85% of the total radioactivity for both formulations, respectively. At least two radioactive peaks were present in the urine samples collected beyond 6 hr timepoint. The major radioactive peak had similar retention time as the metabolite, AMI-11352. Metabolite profiling was not examined in the feces due to low amount of radioactivity recovered.

**06-6424-PK-14: Plasma Protein Binding of AMI-6424 in Rat, Mouse, Dog, and Human**

The purpose of this study is to evaluate the *in vitro* binding of AMI-6424 (0.1 to 100 µg/ml) to human, rat, dog, and mouse plasma proteins. The binding was evaluated using equilibrium dialysis. The results indicated that AMI-6424 was highly protein-bound in the plasma of all species. AMI-6424 was slightly more protein bound at 100 µg/ml and this was observed consistently in all species examined. In summary, AMI-6424 was 93.1-94.3%, 93.4-95.6%, 91.5-94.3%, and 93.8-96.2% bound in human, rat, dog, and mouse plasma, respectively.

**06-6424-PK-39: Protein Binding of <sup>14</sup>C-TD-6424 in Plasma from Mouse, Rat, Dog, Bovine, Rabbit and Human and Human Skin Blister Fluid**

The *in vitro* binding of 1.0 to 100 µg/mL of TD-6424 to mouse, rat, dog, bovine, rabbit and human plasma proteins as well as human skin blister fluids was evaluated in this study using equilibrium dialysis. The *in vitro* binding of 1.0 to 500 µg/mL of TD-6424 was also determined for human serum, human serum albumin and human alpha-1 acid glycoprotein.

TD-6424 was highly protein bound (~90%) and exhibited species-independent ranges in protein binding (see table below). TD-6424 was highly protein-bound in human serum and mainly bound to human serum albumin. Over the concentration range studied (1 to 500 µg/mL), TD-6424 was approximately 88-92% protein bound in pooled human serum. However, only 32-60% protein-bound was observed with purified human alpha-1 acid glycoprotein. At a concentration of 100 µg/ml, the protein binding of <sup>14</sup>C-TD-6424 in pooled human skin blister fluid was 85.8% and similar to that observed in the pooled human plasma (90.3%) from the same subjects.

**Mean protein binding of TD-6424 in mouse, rat, dog, bovine, rabbit, and human plasma (%)**

TD-6424 (µg/ml)	Mouse	Rat	Dog	Bovine	Rabbit	Human
1	91.0	92.3	89.3	83.3	88.9	87.0
10	91.1	92.0	88.7	85.4	88.3	86.3
100	92.7	92.9	89.3	88.9	90.3	89.7

**2.6.4.5 Metabolism**

**01-6424-PK-13: In Vitro Metabolism of AMI-6424**

The *in vitro* stability of AMI-6424 was tested in liver microsomes (male rat, dog, human), plasma (same species) and with selected cDNA-expressed CYP enzymes.

Results: Following incubation with liver microsomes the results showed >99% AMI-6424 remaining after 60 minute at 37°C and >131% after incubation in plasma. After incubation with cDNA-expressed human CYPs (2D6, 3A4 and 4A11), >94% AMI-6424 remained. Index reactions were dextromethorphan O-demethylation and testosterone 6-β-hydroxylation. The results show that AMI-6424 has weak inhibitory effects on CYP3A and 2D6 but stronger effects than vancomycin.

Inhibitor	% inhibition of CYP2D6	% inhibition of CYP3A4
10 µg/ml AMI-6424	-1.3	1.1
100 µg/ml AMI-6424	21.1	31.4
100 µg/ml vancomycin	-1.3	17.4
1 µM Quinidine (positive control)	78	Not tested
1 µM Ketoconazole (positive control)	Not tested	97.3

**01-6424-PK-16: Metabolite Analysis of Dog Urine Samples for <sup>3</sup>H-AMI-6424**

The metabolite profiles of <sup>3</sup>H-AMI-6424 in dog urine were evaluated with LC/MS/MS technology. The urines came from the *in vivo* portion of Study 01-6424-PK-012.

Results: LC/MS/MS analysis showed at least two metabolites. The previous analysis with HPLC and flow detection showed metabolite(s) as 53% and 35% of total urinary radioactivity for males and females,

respectively. In analysis with full scan mass spectra with 50 volts voltage, the metabolites appeared to be the oxidative metabolite with hydroxylation on the alkyl side chain. Further co-injection study with standards for various hydroxyl groups showed the metabolites to be the 7-OH and 8-OH hydroxylated metabolites.

At the end of the study report, the sponsor made the following statement: '(

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#### **01-6424-PK-19: Pharmacokinetic and Metabolite Profile of AMI-6424 in Rats, Dogs, and Monkeys**

This report is a summary of the following studies: 01-067-107 (rats given 10 or 25 mg/kg AMI-6424 i.v.), Covance Study 7057-147 (beagle dogs given 10 mg/kg AMI-6424 via a 30 min IV infusion), and 01-6424-PK-06 (monkey study). In all studies, blood samples were collected for up to 24 or 26 hours and urine samples were collected in dogs and monkeys for 0 to 24 or 26 hours. Concentrations of AMI-6424 and metabolites, AMI-999 (des phosphonate), AMI-11352 (7-OH metabolite) and two other hydroxylated metabolites in serum and urine were determined by LC/MS.

Results: Results are summarized in the table below. AMI-11352 (7-OH metabolite), AMI-999 (des phosphonate) or other hydroxylated metabolites were observed in serum or urine samples following intravenous administrations of AMI-6424 to rats, dogs and monkeys. The metabolites concentrations in serum were low and accounted for 0.3% (rats) to 12% (dogs) of the total AUC values. In urine, AMI-6424 accounted for more than 60-89% of the total amount recovered. The hydroxylated metabolites (AMI-11352 and two other metabolites) are the major metabolites in dog and monkey urine.

##### **Metabolite profiles**

Species and treatment	Serum (% of total AUC, m/f)				Urine (% of total urinary recovery, m/f)	
	Rats (10 mg/kg)	Rats (25 mg/kg)	Dogs (10 mg/kg)	Monkeys (10 mg/kg)	Dogs (10 mg/kg)	Monkeys (10 mg/kg)
AMI-6424	99.7/100	98.4/99.3	88.0/91.0	93.8/94.8	60.0/67.8	88.7/86.1
AMI-11352	0.33/0	0.17/0.23	5.6/4.1	2.26/2.06	19.5/15.8	4.6/5.9
AMI-999	0/ND	1.47/0.27	0.62/0.6	1.92/1.47	1.66/0.89	1.85/1.7
OH-metabolites	ND (not determined)		5.8/4.3	2.04/1.71	19.35/15.5	4.89/6.3

#### **06-6424-PK-29: In Vitro Metabolism of TD-6424 in Liver and Renal S9 Fractions, Tissue Slices and Human Recombinant CYP450s**

The *in vitro* stability of AMI-6424 was tested in liver S9 fractions from rat and renal S9 fraction from rat, dog and human as well as liver and kidney slices from dog and liver slices from rat and human. The metabolic stability of TD-6424 in human recombinant CYP450s (CYP450s-1A2, 2C9, 2C19; 2D6, 3A4, 3A5 and 4A11) was also evaluated.

Results: TD-6424 was stable in liver and renal S9 fractions from rat, dog and human as well as in liver and kidney slices from rat, dog and human. TD-6424 was also stable in the presence of human recombinant CYP450s-1A2, 2C9, 2C19, 2D6, 3A4, 3A5 and 4A11. There were no degradation products or metabolites detected in any of the biological matrices examined.

***In vitro* stability of 14C-TD-6424 in liver and renal S9 fractions**

Time (min)	% remaining following incubation at 37 °C			T1/2 (min)	
	0	60 or 120			
Rat liver S9	100	92		> 60	
Dog liver microsomes	100	91		> 60	
Rat renal S9	100	93		> 60	
Dog renal S9	100	95		> 60	
Human renal S9	100	98		> 60	
Time (hr)	0	2	4	6	> 60
Rat liver slices	100	121	89.7	110	
Dog liver slices	100	88.7	107	79.4	
Dog kidney slices	100	100	70.8	84.6	
Human liver slices	100	95.2	95.6	89.6	

**05-6424-PK-37: Isolation and Structure Elucidation of Urinary Metabolites of TD-6424 in Human**

The purpose of this study was to isolate and propose the chemical structures of human urinary metabolites of TD-6424 using LC-MS/MS and NMR. The metabolic profile of urinary metabolites of TD-6424 in rats and dogs was also evaluated. Human urine samples were obtained from a Phase I clinical study (Protocol 0027). Urine samples were collected from six healthy subjects for up to nine days following a one hour IV infusion of <sup>14</sup>C-TD-6424 at a dose of 10 mg-eq/kg. Urine samples collected over 0-48 hours post-dose from one subject were used for metabolites isolation. The metabolites were eluted with 5% trifluoroacetic acid (TFA) in 50% acetonitrile then further purified by HPLC. Individual fractions containing each metabolite were pooled for LC-MS/MS and NMR analysis.

A total of three metabolites (M1, M2 and M3) were identified from human urine and confirmed by LC-MS/MS as hydroxylated metabolites of TD-6424. In the human urine samples, AMI-11352 (M3), the 7-OH metabolite, was the major metabolite of TD-6424 and accounted for 50.5% of total peak areas of the three hydroxylated metabolites. AMI-11355 (8-OH metabolite, M2) and AMI-11353 (9-OH metabolite, M1) were the minor metabolites and accounted for 24.2% and 25.3% of the total peak areas of the three hydroxylated metabolites, respectively.

**Metabolite profile of TD-6424 in human urine**

Analyte	% peak area based on UV absorbance of the three metabolites
AMI-11352 (7-OH), M3	50.5±1.2
AMI-11355 (8-OH), M2	24.2±1.0
AMI-11353 (9-OH), M1	25.3±0.5

Following intravenous administration of TD-6424 to the rat, AMI-11352 was the major metabolite in the urine and two minor metabolites; one had the same retention time as M2 (AMI-11355) and another unknown OH metabolite. Following intravenous administration of TD-6424 to the dog, at least three major urinary metabolites with similar retention time as AMI-11352 (7-OH), AMI-11355 (8-OH) and AMI-11354 (10-OH) were observed. AMI-11353 (9-OH) was a minor metabolite in the dog urine.

**2.6.4.6 Excretion****01-6424-PK-08: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 in Mice**

Five male CD-1 mice were administered (IV) <sup>3</sup>H-AMI-6424 in 5% HP-β-CD. Urine and feces were collected for each animal and collections were combined for analysis. At 24 hours post-dosing, animals

were euthanized and tissues (liver, kidneys, lung, heart, skin, serum and spleen) were harvested. Sample analysis was performed by HPLC. Radioactivity was evaluated by liquid scintillation counting.

Total recovery was 72% ± 23.6%. The highest tissue concentrations were in liver (4.4% of dose) and kidneys (1% of dose). Serum concentration at 24 hours was 0.06% of dose. Urine excretion appeared to be the major route in these mice, with urinary recovery being 61% of the total radioactivity recovered with 48% of that being unchanged parent compound. Metabolites accounted for ~21% of the total recovered radioactivity.

#### **01-6424-PK-09: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 in Rats**

Three male Sprague-Dawley rats were administered 10 mg-equiv/kg i.v. <sup>3</sup>H-AMI-6424 in 5% HP-β-CD. Urine and feces were collected for each animal and collections were combined for analysis. At 24 hours post-dosing, animals were euthanized and tissues (liver, kidneys, lung, heart, skin, spleen and serum) were harvested. Sample analysis was performed by HPLC. Radioactivity was evaluated by liquid scintillation counting.

Total radioactivity recovery was 72% ± 23.6%. The highest tissue concentrations were in liver (6.8% of dose) and kidneys (1.3% of dose). Serum concentration at 24 hours was 0.1% of dose. Urine excretion appeared to be the major route in these rats, with urinary recovery being 57.8% of the total radioactivity recovered and fecal recovery was 3.8%. Parent compound accounted for 76% of the urinary radioactivity. Metabolites accounted for ~24% of the total urinary recovered radioactivity.

#### **01-6424-PK-12: Tissue Distribution, Excretion and Metabolism of <sup>3</sup>H-AMI-6424 Following Intravenous Infusion to Dogs**

In this study (conducted by Covance Laboratories, Madison, WI), one beagle dog/sex was treated with 10 mg-eq/kg <sup>3</sup>H-AMI-6424 in 2% HP-β-CD via a 2 hour infusion. Serum samples were taken at 1 and 2 hours (end of infusion) and at 2, 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12 and 24 hours post-end of infusion. Urine and feces were collected for 24 hours. At 24 hours post-dose, both animals were euthanized and tissues (essentially the entire standard histology tissue list) were removed for analysis. One kidney and a section of liver were frozen for autoradiography.

Results: Total radioactivity recovered was >100% of total dose. Urinary excretion was the major route of elimination with urinary recovery ~85% of total dose. Metabolites accounted for 53% (male) and 35% (female) of the total radioactivity recovered. Fecal excretion was <2% of the total dose. There are no apparent gender related differences in the excretion and tissue distribution of radioactivity in dogs. Levels of radioactivity were highest in liver (6%), muscle (2%) and kidney (1.2%). However, tissue concentrations were lower in the autoradiography samples. The sponsor suggests that this difference is due to loss of tritiated water during the desiccation or less homogenous distribution of radioactive material.

#### **02-6424-PK-22: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 in Rats**

AMI-6424 (Lot AME002) and <sup>14</sup>C-TD-6424 were reconstituted in D5W and administered at 25 mg-eq. i.v. to 3 Sprague Dawley rats/sex. After 24 hours, the animals were euthanized and appropriate tissues were collected, as were urine and feces.

Total recovery for males was 99.3%, for females was 90.8%. The highest concentrations were found in bone>liver>skin>muscle and were at least 60%> plasma levels. Urinary recovery was 62%, fecal recovery was 4.5%. The majority of the radioactivity in urine was parent compound (>80%) but three metabolites (M1, M2 and M3) accounted for 11% of the total radioactivity.

**02-6424-PK-23: Tissue Distribution, Excretion and Metabolism of <sup>14</sup>C-TD-6424 Following Intravenous Infusion to Dogs**

AMI-6424 (Lot #AME002) and <sup>14</sup>C-TD-6424 were reconstituted in D5W and administered at 25 mg-equiv. i.v. to one beagle/sex. The infusion took 2 hours. After 24 hours, the animals were euthanized and appropriate tissues were collected, as were urine and feces.

Total recovery for males was 90.8%, for females was 97.3%. The highest concentrations were found in liver>bone>kidney and were at least 15x> plasma levels at 24 hours post-dose. Urinary recovery was ~77%. Tissue radioactivity accounted for ~12% of the administered dose. Metabolites (primarily M1, with small amounts of M3) accounted for ~19% of the recovered urinary radioactivity. {

⤵ The sponsor plans to further define this possibility.

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**2.6.4.7 Pharmacokinetic drug interactions**

**01-6424-PK-20: Pharmacokinetic Drug Interaction Study of AMI-6424 with Concomitant Administration of Aztreonam and Metronidazole in Female Rats**

This report is a summary of the following studies: 01-6424-PK-19 (Rats given 10 or 25 mg/kg AMI-6424 i.v.), 01-067-097 (PK of aztreonam at 10 mg/kg in 3 female rats), 02-067-003 and -004 (PK of aztreonam or metronidazole with 10 or 25 mg/kg AMI-6424, respectively, n=3/ concomitant medication group) and 02-067-003 (PK of metronidazole orally in female rats). In all of these studies, blood samples were collected at pre-dose, 2, 5, 15, 30 minutes and 1, 2, 4, 8 and 24 hours post-dosing. Concentrations of AMI-6424, aztreonam and metronidazole in serum or plasma were determined by LC/MS/MS.

Results: The Cmaxs and AUCs were significantly higher for AMI-6424-treated animals at 10 mg/kg when given concomitant medications. No PK effect was noted for AMI-6424 treatment at 25 mg/kg.

Parameter	AMI-6424 (10 mg/kg)			AMI-6424 (25 mg/kg)		
	Alone	Aztreonam	Metronidazole	Alone	Aztreonam	Metronidazole
Co or Cmax (µg/ml)	74.4	109.5	119.3	184.1	188.0	174.1
Tmax (hr)	NA	NA	0.03	NA	NA	NA
AUC <sub>0-24</sub> (µg-hr/ml)	86.8	133.5	121.8	344.8	288.6	282.7
T1/2 (hr)	1.5	1.2	1.4	2.5	2.7	2.7
Cl (L/hr/kg)	0.12	0.08	0.08	0.12	0.09	0.09
Vss (L/kg)	0.17	0.13	0.15	0.19	0.21	0.21

Higher AUCs for aztreonam were found when co-administered with AMI-6424 at both doses. No significant effect was found for co-administration of AMI-6424 and metronidazole except that the t<sub>1/2</sub> doubled for AMI-6424 at 25 mg/kg.

The sponsor concluded that there is no significant drug-drug interaction for AMI-6424 when co-administered with aztreonam or metronidazole. The differences in AUC and Cmax values for AMI-6424 observed at 10 mg/kg dose level with co-administration may be due to the inconsistency in the exposure

data for AMI-6424 at 10 mg/kg. The reviewer agrees because the differences are not large, and no differences were seen at 25 mg/kg.

#### **05-6424-PK-31: The Inhibitory Potential of TD-6424 on the Metabolic Activity of Five Major CYP450 Enzymes**

This *in vitro* study was to evaluate the effect of TD-6424 on inhibiting the major CYP enzymes in human liver microsomes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5). The purpose of this study was to determine the potential of TD-6424 to inhibit the metabolism of concomitantly administered drugs. Human liver microsomes from a pool of nine individuals were incubated with marker substrates in the presence or absence of TD-6424 (0.1 to 100  $\mu\text{M}$ ). TD-6424 was also evaluated for its ability to function as a metabolism-dependent inhibitor at the same concentrations mentioned above, in which case TD-6424 was pre-incubated with human liver microsomes and NADPH-generating system for 30 min to allow for the generation of metabolites that might inhibit CYP activity.

Positive controls used in the study significantly inhibited the enzyme activity in all cases. TD-6424 was a direct inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 with  $\text{IC}_{50}$  values of 40  $\mu\text{M}$ , 89  $\mu\text{M}$ , 54  $\mu\text{M}$ , 35  $\mu\text{M}$ , 25  $\mu\text{M}$ , and 14  $\mu\text{M}$ , respectively. The inhibition assay was conducted in the absence of serum albumin and therefore the concentrations of TD-6424 tested (0.1-100  $\mu\text{M}$ ) represent free drug. The highest concentration of TD-6424 (100  $\mu\text{M}$ ; 176  $\mu\text{g/mL}$ ) examined in this study is approximately fifteen-fold higher than the unbound peak plasma concentration ( $\text{C}_{\text{max}}$ ) in human at a dose of 10 mg/kg (11.4  $\mu\text{g/mL}$ , 6.5  $\mu\text{M}$ ). At 10  $\mu\text{M}$ , no significant inhibition was observed for TD-6424 on the activity of CYP1A2, CYP2C9, CYP2C19 and CYP2D6. Ten  $\mu\text{M}$  TD-6424 had an effect on the activity of CYP3A4/5 with approximately 20-40% decrease in enzyme activity. TD-6424 did not cause metabolism-dependent inhibition of any of the CYP enzymes tested.

**Effects of TD-6424 on the activity of CYP450 enzymes**

CYP enzymes	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4/5	
					Testosterone	Midazolam
$\text{IC}_{50}$ ( $\mu\text{M}$ )	40	89	54	35	25	14
$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )	70.2	156	94.8	61.4	43.9	24.6
% inhibition at 10 $\mu\text{M}$	7.2	-18	8.7	12.9	21.8	41.4

#### **2.6.4.8 Other pharmacokinetic studies**

##### **01-6424-PK-07: Interspecies Scaling of Pharmacokinetics of AMI-6424**

The purpose of this report is to predict the clearance and volume of distribution at steady state for AMI-6424 in human based on the pharmacokinetic parameters obtained from previous studies in mice, rats, dogs, and monkeys (IV, 10 mg/kg). Interspecies scaling was performed by using the allometric scaling method. Pharmacokinetic profiles of AMI-6424 in mice, rats, dogs, and monkeys were described by non-compartmental model. The extrapolation was based on the power function, as the body weight from preclinical species is plotted against the clearance or steady-state volume of distribution ( $\text{V}_{\text{ss}}$ ) on a log-log scale.

Results: Good correlations were observed between the clearance and body weight and between  $\text{V}_{\text{ss}}$  and body weight. The clearance, area under curve (AUC) and steady-state volume of distribution of AMI-6424 in human are expected to be 0.055 L/hr/kg, 182  $\mu\text{g}\cdot\text{hr/mL}$  and 0.164 L/kg, respectively.

**01-6424-PK-18: Pharmacokinetics of <sup>3</sup>H-AMI-6424 in Mice Following Intraperitoneal and Subcutaneous Administration**

The pharmacokinetics of <sup>3</sup>H-AMI-6424 following intraperitoneal (IP) or subcutaneous (SC) administration to female mice (n = 3 per route per time point) at 10 mg/kg was evaluated. Blood samples were collected for up to 24 hours. Serum concentrations of <sup>3</sup>H-AMI-6424 were determined using liquid scintillation counting.

Results: Results are summarized in the table below. The terminal half-life of AMI-6424 in mice was longer following IP injection (42 hour) than following SC or IV (9 and 6 hour respectively). Similar C<sub>max</sub> values were observed following IP and SC administration (approximately 1/10 of that observed following IV administration). The T<sub>max</sub> values were 0.5 and 1 hour for IP and SC respectively, indicating rapid absorption to systemic circulation. Bioavailabilities following IP and SC administration of AMI-6424 were high at 92 and 89%, respectively.

**Non-compartment PK parameters following IP and SC injections of <sup>3</sup>H-AMI-6424 at 10 mg/kg**

Parameter	IP	SC	IV (Report 01-6424-PK-03)
Co/C <sub>max</sub> (µg/ml)	27.35	25.71	232
T <sub>max</sub> (hr)	0.5	1	NA (Not applicable)
AUC <sub>0-42</sub> (µg-hr/ml)	113.19	110.1	123
AUC <sub>0-∞</sub> (µg-hr/ml)	176.34	122.1	126
T <sub>1/2</sub> (hr)	41.69	9.26	5.7
Cl (L/hr/kg)	0.06	0.08	0.08
%F	92%	89.4%	NA

**04-6424-PK-27: Pharmacokinetics of Hydroxypropyl-Beta-Cyclodextrin in Rats and Dogs Following Single Intravenous Administration of Telavancin for Injection or Hydroxypropyl-Beta-Cyclodextrin**

The pharmacokinetics of HP-β-CD was evaluated in rat PK studies 03-067-141, 03-067-143 and 03-067-150, and in the dog PK study PK-968. In these studies, TD-6424 for Injection (Lot # AME001) was formulated in 5% dextrose for injection (D5W) and given to male rats (3/group) and female dogs (3/group) *via* intravenous infusion at a dose of 250 mg/kg of HP-β-CD and 25 mg/kg of TD-6424. HP-β-CD (Lot # AMD002) was formulated in D5W and given to male rats and female dogs *via* intravenous infusion at a dose of 250 mg/kg. Blood samples were collected for up to 24 hours post-dosing. Plasma concentrations of HP-β-CD were determined using HPLC with fluorescent detection. Plasma concentrations of TD-6424 were determined using LC-MS/MS.

Results: The PK parameters of HP-β-CD in dogs and rats were independent of TD-6424 when administered together at the molar ratio of 10:1. The TD-6424/HP-β-CD complex was not observed in the plasma following IV administration to rats and dogs.

**05-6424-PK-32: Pharmacokinetics of TD-6424 in Rats Following Single Intravenous Administration of TD-6424 for Injection and Alternate Formulations**

**05-6424-PK-33: Pharmacokinetics of TD-6424 in Dogs Following Single Intravenous Administration of TD-6424 for Injection and Alternate Formulations**

The pharmacokinetics of TD-6424 was evaluated in rat PK study 05-067-021 and in dog PK study PK968-004. TD-6424 for Injection (Lot # 2213-10-645048, containing TD-6424/HP-β-CD with a molar ratio of 1 to 10) and five alternate formulations, containing TD-6424/HP-β-CD with molar ratios of 1:4, 1:2

and 1:1 and TD-6424/SBE-CD with molar ratios of 1: 2 and 1:1, were formulated in 5% dextrose for injection (D5W) and given to female rats (3/formulation) or six male beagle dogs (crossover design) via intravenous infusion at the dose of 25-29 mg/kg. Blood samples were collected for up to 24 hours post-dosing. Plasma concentrations of TD-6424, metabolite (AMI-11352) and  $\epsilon$  (AMI-999) were determined using LC-MS/MS. b(4)

Results: In both species, there was a slight increase in systemic exposure to TD-6424 with decreased cyclodextrin, suggesting that PK parameters of TD-6424 were affected by HP- $\beta$ -CD and SBE-CD. The AUC ratios of the metabolites were similar for all six formulations ranged from 0.00140 to 0.00238 for AMI-11352 and 0.0213 to 0.0439 for AMI-999.

**05-6424-PK-34: Tissue Concentration of TD-6424 in Female Rats Following Seven-Day Dosing with TD-6424 for Injection and Alternate Formulations**

The pharmacokinetics of TD-6424 was evaluated in rat ADME study 05-118-012 to determine the concentrations of TD-6424 in liver and kidney following intravenous administration of TD-6424 for Injection and five alternate formulations, containing TD-6424/HP- $\beta$ -CD with molar ratios of 1:4, 1:2 and 1:1 and TD-6424/SBE-CD with molar ratios of 1: 2 and 1:1, to female rats for 7 consecutive days. Blood, liver and kidney samples were collected from animals (n = 3 per formulation) at 24 hours following the last dose. Plasma, liver and kidney concentrations of TD-6424, metabolite (AMI-11352) and  $\epsilon$  (AMI-999) were determined using LC-MS/MS. b(4)

Results: Following 7-day repeated dosing in rats, higher plasma, liver and kidney concentrations of TD-6424 were observed in groups with lower ratios of TD-6424/HP- $\beta$ -CD or TD-6424/SBE-CD (see table below). The ratios of the metabolite, AMI-11352 or AMI-999 vs TD-6424 in kidney were similar for all six formulations.

**TD-6424 concentrations in plasma, liver and kidneys (mean  $\pm$  SD)**

Formulation (molar ratio of TD-6424:CD)	Concentration ( $\mu$ g/mL)		
	Plasma	Liver	Kidney
TD-6424 for injection (TD-6424:HP- $\beta$ -CD = 1:10)	0.216 $\pm$ 0.034	194 $\pm$ 35	173 $\pm$ 12
TD-6424:HP- $\beta$ -CD = 1:4	0.340 $\pm$ 0.143	307 $\pm$ 39	153 $\pm$ 42
TD-6424:HP- $\beta$ -CD = 1:2	0.317 $\pm$ 0.131	269 $\pm$ 114	243 $\pm$ 18
TD-6424:HP- $\beta$ -CD = 1:1	0.816 $\pm$ 0.471	325 $\pm$ 34	397 $\pm$ 16
TD-6424:SBE-CD = 1:2	0.339 $\pm$ 0.159	212 $\pm$ 72	165 $\pm$ 32
TD-6424:SBE-CD = 1:1	0.343 $\pm$ 0.010	239 $\pm$ 38	237 $\pm$ 48

**06-6424-PK-38: Pharmacokinetic and Pharmacodynamic Studies of TD-6424 in Various Rabbit Efficacy Models**

Concentrations of TD-6424 in plasma, serum, homogenates of vegetation from aortic valves, CSF and bone or bone marrow were determined in three experimental efficacy models with rabbits infected with gram-positive pathogens.

Following 15 mg/kg IV bolus administration in the rabbit endocarditis model, TD-6424 had a mean AUC of 197  $\mu$ g.hr/mL. The half-life of TD-6424 in the experimental rabbit endocarditis model was 1.52 hr. In a separate study with the experimental rabbit endocarditis model, the mean serum concentration of TD-6424

was 1.33 µg/mL at 8 hours post-dose following IV administered for two days. However, nine out of twelve rabbits in the same study had no measurable concentration in the homogenates of vegetation recovered from aortic valves.

Pharmacokinetics and penetration across the blood-brain barrier of TD-6424 was evaluated in rabbits with normal or inflamed meninges in the rabbit pneumococcal meningitis model. Similar pharmacokinetic parameters in serum were observed in normal and infected rabbits following intravenous injection of TD-6424. Much higher concentrations of TD-6424 in CSF were observed in rabbits with inflamed meninges (C<sub>max</sub>: 0.593 µg/mL vs. 5.07 µg/mL; AUC: 1.69 µg-hr/mL vs. 16.2 µg-hr/mL). The penetration of TD-6424 into CSF averaged 0.16% for rabbits with normal meninges and 1.85% in rabbits with inflamed meninges.

Pharmacokinetics and concentrations of TD-6424 in bone and bone marrow were evaluated in rabbits following SC administration in support of *in vivo* efficacy studies in the rabbit osteomyelitis model. Following 10 and 30 mg/kg SC administration, TD-6424 had a mean plasma AUC of 127 and 438 µg-hr/mL, respectively. Concentrations of TD-6424 in bone marrow to plasma at one hr after the last dose administered had a mean ratio of 0.00798 and 0.0289 following 10 and 30 mg/kg SC administration, respectively.

#### 2.6.4.9 Discussion and Conclusions

In mice, rats and rabbits, plasma C<sub>max</sub> and AUC demonstrated dose-dependent increases following single IV dosing. There were no apparent gender differences with respect to PK parameters. Studies showed significant differences of t<sub>1/2</sub> between the various species (mice- 1.2-9.5 hours; rats- 1.3-14.8 hours; dogs- 1.4-12.8 hours; monkeys- 2.3 hours; rabbit-1.33-2.33 hours). In rats and mice, the terminal t<sub>1/2</sub> for AMI-6424 was longer than that observed for vancomycin. In dogs and monkeys, the terminal t<sub>1/2</sub> of AMI-6424 was similar to that of vancomycin but the C<sub>max</sub> and AUC were higher for AMI-6424 at the same dose. PK results in neutropenic mice were comparable to that in normal animals.

In tissue distribution studies conducted in dogs, rats and mice, high concentrations of radioactivity were seen in the bone, liver and kidney. In rats, no significant accumulation of the drug in liver and kidneys was observed following 14 days of repeated dosing. At lower amounts of HP-β-CD (e.g., increased drug/cyclodextrin ratios), higher drug concentrations were detected in liver, kidney and serum. Urinary recoveries significantly decreased with decreased amounts of HP-β-CD.

In studies conducted in dogs and rats, radioactivity was poorly distributed to CNS tissues, indicating minimal penetration of <sup>14</sup>C-TD-6424 through the blood-brain barrier. In a rabbit PD/PK study, higher concentrations of TD-6424 in CSF were observed in rabbits with inflamed meninges. There was no evidence for high affinity melanin-specific binding of radioactivity in the pigmented tissues.

TD-6424 is highly protein bound (approximately 90%) in mouse, rat, dog, bovine, rabbit and human plasma as well as human skin blister fluids. In human plasma, TD-6424 is mainly bound to human serum albumin.

AMI-11352 (7-OH metabolite), AMI-999 or other hydroxylated metabolites were observed in serum samples following intravenous administrations of AMI-6424 to rats, dogs and monkeys. The hydroxylated metabolites are the major metabolites in rat, dog and monkey urine. In rat urine, AMI-11352 was the major

metabolite. In dog urine, at least three major urinary metabolites were observed: AMI-11352 (7-OH), AMI-11355 (8-OH) and AMI-11354 (10-OH).

Urine excretion is the major route of elimination in dogs, mice, and rats.

## 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

In single dose rat studies using intravenous injection (50 mg/kg), increased urinary protein was found with the semiquantitative Multistix<sup>®</sup> method, but not with a more quantitative method. The increased levels are considered false positives related to the presence of drug in the urine. Tubular dilatation and tubular epithelial cell degeneration were found with the increased incidence and severity as the concentration of HP- $\beta$ -CD decreased. This finding is consistent with the BUN and creatinine changes. The results support the conclusion that the biodistribution of AMI-6424 is altered by adding HP- $\beta$ -CD to the vehicle. In rat studies, the maximal tolerated dose is 25 mg/kg in males and 50 mg/kg in females. In mouse studies, the maximal tolerated dose is 35 mg/kg in females and 50 mg/kg in males.

Several repeated dose toxicological studies were conducted with durations of up to 6-months in rats and 3 months in dogs. The organs of toxicity identified in these studies include the renal and hepatic systems in both species. Multiple organ macrophage accumulation/hypertrophy/hyperplasia was also noted. Although some of the findings (e.g., increased BUN, creatinine, AST, and ALT levels) were seen in the placebo (hydroxypropyl- $\beta$ -cyclodextrin) control animals, the findings were more significant and more frequent in the drug-treated animals, leading to the conclusion that the active compound contributed significantly to the alterations.

#### Genetic toxicology:

AMI-6424 was negative in a battery of genotoxicity studies.

#### Reproductive toxicology:

In a fertility and early embryonic development study in rats, female rats treated with AMI-6424 at 150 mg/kg showed no changes in fertility indices. In male rats, AMI-6424 at  $\geq 50$  mg/kg caused decreased sperm motility, decreased sperm counts, and abnormal sperm morphology.

Based on data from Segment 2 studies in rats, rabbits, and minipigs, it is concluded that telavancin is a multi-species teratogen with external/skeletal (limb) malformations being the primary terata. The incidences of syndactyly and polydactyly are significantly higher in animals dosed with telavancin than in the

conducting laboratories' historical databases. Additional effects of telavancin dosing were found in pre- and post-implantation parameters.

AMI-6424 was detected in several amniotic fluid samples in rats and rabbits, indicating that AMI-6424 crosses the placental barrier. This increases the concern for fetal exposure.

In the Segment 3 study in rats, due to the increased incidence of stillborn pups and clinical findings at 150 mg/kg/d in the F1 generation, the fetal/pup NOAEL is determined to be 100 mg/kg/d. Brachymelia, seen in one F1 pup in the 150 mg/kg/d group, was consistent with a previous Segment 2 study with AMI-6424 (7057-126) from this laboratory thus confirming a treatment-related aspect.

#### Special toxicology:

AMI-6424 at dose levels of 50 and 100 mg/kg/day produced reversible immunomodulatory effects as evidenced by changes in a T-cell dependent antigen (AFC) response and macrophage function. AMI-6424 did not cause hemolysis of rat, dog, or human whole blood. AMI-6424 at concentrations of up to 272 mg/L did not exhibit phototoxic potential. The drug has potential to induce local/vascular irritation. When telavancin was administered as an intravenous bolus with either HP- $\beta$ -CD or SBE- $\beta$ -CD, the severity of renal injury and ALT and AST changes decreased as the ratio of cyclodextrin to telavancin increased. AMI-999 (a minor metabolite ( ), when given to rats as a single IV dose at 50 mg/kg, was nephrotoxic evidenced by elevated serum concentrations of urea nitrogen and creatinine as well as tubular necrosis.

#### **2.6.6.2 Single-dose toxicity**

##### **00-036-020: Exploratory Single-Dose Intravenous Nephrotoxicity Study with AMI-6424 in Rats**

This study was conducted under non-GLP conditions at ( ) to evaluate the potential nephrotoxic effects of AMI-6424 on rats at 50 mg/kg as a single IV dose in 25% HP- $\beta$ -CD.

In the study, 3 female CrI:CD<sup>®</sup>(SD)IGS rats received 50 mg/kg AMI-6424. Three additional female rats received vehicle alone. They were observed prior to dosing and at 1 and 24 hours post-dosing. Urine samples were collected for 24 hours post-dosing in metabolism cages. After the collection period, all animals were euthanized, weighed and serum samples were taken for drug concentrations. Liver and both kidneys were collected and examined grossly. Sections were taken for histopathology. One kidney and a section of liver were frozen and evaluated for drug levels from the AMI-6424-treated rats.

Results: No treatment-related clinical signs were reported. No significant changes from baseline in clinical chemistries or urinalysis were found. When using the semiquantitative Multistix<sup>®</sup>, an increase in urinary proteins was detected but this was not found using the more quantitative methods. Neither gross nor histologic differences from controls were noted in the kidney or the liver.

Of the % unchanged parent compound recovered, 50% was in urine, 4% in liver, 1% in kidney and 4% in serum.

**00-036-028: Exploratory Single-Dose Intravenous Nephrotoxicity Study with AMI-6424 in Rats: Effect of Varying the Concentration of Hydroxypropyl- $\beta$ -Cyclodextrin (HP- $\beta$ -CD) on Nephrotoxicity**

b(4)

This study was conducted under non-GLP conditions at C to evaluate the potential nephrotoxicity of AMI-6424 when administered once i.v. at 50 mg/kg in 0-25% HP- $\beta$ -CD.

In this study, 3 female CrI:CD<sup>®</sup>(SD)IGS BR rats were dosed with 50 mg/kg AMI-6424 i.v. in 5% dextrose or 1, 5 or 25% HP- $\beta$ -CD. Controls for each of these animals (N=3/group) were dosed with dextrose or one of the concentrations of HP- $\beta$ -CD. The conduct and parameters evaluated were comparable to the study above (Report- 00-036-020).

Results: Increases in BUN and creatinine were detected in the AMI-6424 groups.

Increased urinary protein was also found in the AMI-6424 in 25% HP- $\beta$ -CD group, using both the Microstix<sup>®</sup> and Biotrol methods. However, as the increased protein was associated with increased levels of AMI-6424 in the urine, and no microscopic lesions were found in the kidneys of these animals, the increased levels are considered to be related to the presence of drug in the urine.

In the kidneys of all other dose groups, tubular dilatation and tubular epithelial cell degeneration were found. The incidence and severity of the tubular necrosis increased as the concentration of HP- $\beta$ -CD decreased. That finding is consistent with the BUN and creatinine changes. Thus, the biodistribution of AMI-6424 is altered by adding HP- $\beta$ -CD to the vehicle.

**00-036-033: Evaluation of the Potential Effects of Pretreatment with 25% Hydroxypropyl- $\beta$ -Cyclodextrin on the Single Dose Toxicity of AMI-6424 in Rats**

b(4)

This study was conducted under non-GLP conditions at C to evaluate the potential effects of varying the time of pretreatment of 25% HP- $\beta$ -CD on the nephrotoxicity of AMI-6424 when administered to rats as a single IV 50 mg/kg dose.

Five female CrI:CD<sup>®</sup>(SD) IGS BR rats/group were treated with 50 mg/kg of AMI-6424. The HP- $\beta$ -CD was administered either 24 or 0.25 hours prior to or concomitantly with the AMI-6424 dosing. One group received 5% dextrose as the vehicle for the AMI-6424 without HP- $\beta$ -CD.

Four control groups were used with HP- $\beta$ -CD at the various time-points or 5% dextrose alone. All animals were given treatment i.v. at 0.75 ml/min with a dosing volume of 15 ml/kg.

Morbidity and mortality checks were performed prior to dosing, at 1 hour post-dosing and at 24 hours post-dosing. Urine collections were made for 24 hours post-dosing for complete urinalysis and test compound concentration. At 24 hours post-injection, the rats were anesthetized and blood samples were taken via cardiac puncture for clinical chemistries and test compound concentration. After euthanasia, liver and kidneys were examined grossly and their weights were taken. One kidney and a section of liver were frozen for measurement of drug concentration.

Results: Neither premature decedents nor clinical signs were reported.

Increases in BUN and creatinine were found in the dextrose-AMI 6424-treated and in the 24 hour pretreatment group with 25% HP- $\beta$ -CD-AMI-6424-treated animals.

Group	BUN (mg/dl)	Creatinine (mg/dl)
<b>Vehicle</b>		
5% dextrose	12 $\pm$ 2	0.21 $\pm$ 0.01
25% HP- $\beta$ -CD co-admin. in 5% dextrose	16 $\pm$ 7	0.25 $\pm$ 0.14
25% HP- $\beta$ -CD 0.25 hrs. prior to 5% dextrose	12 $\pm$ 2	0.19 $\pm$ 0.04
25% HP- $\beta$ -CD 24 hrs. prior to 5% dextrose	13 $\pm$ 2	0.18 $\pm$ 0.02
<b>AMI-6424 +</b>		
5% dextrose	35 $\pm$ 8	0.38 $\pm$ 0.03
25% HP- $\beta$ -CD co-admin. with 5% dextrose	13 $\pm$ 2	0.18 $\pm$ 0.05
25% HP- $\beta$ -CD 0.25 hrs. prior to 5% dextrose	14 $\pm$ 1	0.23 $\pm$ 0.05
25% HP- $\beta$ -CD 24 hrs. prior to 5% dextrose	32 $\pm$ 8	0.37 $\pm$ 0.06

As with previous studies, increased urinary proteins were detected in the AMI-6424-treated rats with the Multistix<sup>®</sup> method but not with the Biotrol method. Thus at least part of the increase in protein was probably due to the high concentration of test article in the urine of these animals.

No microscopic differences from controls were appreciated in the livers of the AMI-6424-treated animals. Renal lesions were found in AMI-6424 in 5% dextrose and pre-treated 25% HP- $\beta$ -CD-treated rats. They included dilated tubules with attenuated epithelium, necrosis of epithelial cells and focal tubular mineralization.

#### % of administered dose

Group	Urine	Liver	Kidney	Serum concentration ( $\mu$ g/ml)
AMI-6424 in 5% dextrose	20.47 $\pm$ 5.48	13.48 $\pm$ 3.07	12.34 $\pm$ 3.96	6.61 $\pm$ 2.16
25% HP- $\beta$ -CD co-admin. With AMI-6424 in 5% dextrose	29.68 $\pm$ 7.58	1.54 $\pm$ 0.22	0.38 $\pm$ 0.07	0.72 $\pm$ 0.18
25% HP- $\beta$ -CD 0.25 hrs prior to AMI-6424 in 5% dextrose	33.06 $\pm$ 6.03	3.75 $\pm$ 0.94	0.82 $\pm$ 0.27	1.65 $\pm$ 1.08
25% HP- $\beta$ -CD 24 hrs prior to AMI-6424 in 5% dextrose	47.22 $\pm$ 7.43	13.20 $\pm$ 1.37	7.89 $\pm$ 2.08	4.88 $\pm$ 1.97

The sponsor stated that pre-treatment with 25% HP- $\beta$ -CD altered the biodistribution of AMI-6424 and the nephrotoxicity of the drug. These changes were evident in the animals with co-administration and those with pre-treatment at 0.25 hrs prior to the AMI-6424 dosing. Thus the protective effects of the HP- $\beta$ -CD treatment is seen only when administration is proximal to the AMI-6424 dose.

#### **01-001-11 7507-131: Acute Toxicity Study of AMI-6424 When Administered Intravenously in Mice**

**Key study findings:** 100 mg/kg appears to be the lethal dose while the MTD appears to be 35 mg/kg in males and 50 mg/kg in females

**Study no:** AMI CSN: 01-001-11 or Covance 7057-131

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 5/3/01

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, and % purity:** AMI-6424, Lot AMB001 presumed 100% pure

**Formulation/vehicle:** Vehicle- 100 mg/ml HP- $\beta$ -CD, 40 mg/ml dextrose monohydrate, pH adjustment to 4.8

**Methods:**

**Dosing:**

Species/strain: Crl:CD-1®(ICR)BR mice  
#/sex/group or time point (main study): 5/sex/group except the 100 mg/kg group with 3 males and 2 females  
Satellite groups used for toxicokinetics or recovery: None  
Age: 4 weeks of age  
Weight: 19.5-26.4 g  
Doses in administered units: 0 (5% dextrose), 0 (vehicle), 25 mg/kg, 50 mg/kg, 100 mg/kg, or 35 mg/kg.  
Route, form, volume, and infusion rate: I.V. at 10 mg/kg and 0.75 mL/min into the lateral tail vein.

**Observations and times:**

Clinical signs: Twice/day for mortality and immediately post-dosing, and 0.5, 1 and 4 hours later.  
Body weights: Prior to study initiation, prior to dosing on Day 1, and on Days 8 and 15.  
Food consumption: Not performed  
Ophthalmoscopy: Not performed  
EKG: Not performed  
Hematology: Not performed  
Clinical chemistry: Not performed  
Urinalysis: Not performed  
Gross pathology: Examination of the viscera in situ, and removal of heart, lungs, spleen, liver, pancreas, stomach, intestines and kidneys for more thorough examination.  
Organs weighed: Not performed  
Histopathology: Not performed

**Results:**

Mortality: All of the 100 mg/kg animals died within 2 minutes- 1 hour post-dosing.  
Clinical signs: Hypoactivity was reported in females dosed at 35 mg/kg and in males at 50 mg/kg (noted at dosing). Before the end of the day of dosing, females at 50 mg/kg and all 100 mg/kg animals showed hypoactivity, ataxia, prostration, dyspnea, and urogenital staining/erect tail. All animals were considered normal by Day 2.  
Body weights: No treatment-related findings were reported.  
Gross pathology: No treatment-related findings were reported.

**Summary of individual study findings:** The minimum lethal dose was 100 mg/kg in this study in CD-1 mice. The maximal tolerated dose in this single dose i.v. study appears to be 35 mg/kg in females and 50 mg/kg in males.

**01-001-12 7507-130: Acute Toxicity Study of AMI-6424 When Administered Intravenously to the Rat**

**Key study findings:** The lethal dose for rats in this single dose study was 100 mg/kg for males and >150 mg/kg in females; the maximal tolerated dose was 50 mg/kg for males and 150 mg/kg in females (per sponsor). However, it appears that the maximal tolerated dose is 25 mg/kg in males and 50 mg/kg in females on the basis of clinical signs.

**Study no:** AMI CSN: 01-001-12, Covance 7057-130

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 5/3/01

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, and % purity:** Lot AMB001 presumed 100% pure

**Formulation/vehicle:** 100 mg/mL HP- $\beta$ -CD, 40 mg/mL dextrose monohydrate with pH adjustment to 4.8.

**Dosing:**

Species/strain: Crl:CD<sup>®</sup> (SD)IGS rats

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

Satellite groups used for toxicokinetics or recovery: None

Age: 8-10 weeks of age

Weight: 220- 270 gms.

Doses in administered units: 0 (5% dextrose), 0 (vehicle), 25, 50, 100 or 150 mg/kg

Route, form, volume, and infusion rate: I.V. as a solution at 0.75 mL/min

**Observations and times:**

Clinical signs: Twice/day for mortality and immediately post-dosing, and 0.5, 1 and 4 hours later.

Body weights: Prior to study initiation, prior to dosing on Days 1, 8 and 15/death.

Food consumption: Not evaluated

Ophthalmoscopy: Not evaluated

EKG: Not evaluated

Hematology: Not evaluated

Clinical chemistry: Not evaluated

Urinalysis: Not evaluated

Gross pathology: Examination of the viscera in situ and removal of heart, lungs, spleen, liver, pancreas, stomach, intestines, and kidneys for more thorough examination.

Organs weighed: Not evaluated

Histopathology: Not evaluated

Toxicokinetics: Not evaluated

**Results:**

Mortality: One 100 mg/kg male and two 150 mg/kg males died on Day 1. All other animals survived to study termination.

Clinical signs: Males (N=3) at 50 mg/kg and most other animals at higher doses showed hypoactivity and/or dyspnea on Day 1 and even the high dose animals appeared normal on Day 2.

Body weights: No treatment-related findings were reported except one 50 mg/kg female and one 150 mg/kg female that showed slight weight loss during the first week.

Gross pathology: No treatment-related findings were determined.

**Summary of individual study findings:** The sponsor concluded that the minimum lethal dose for rats in this single dose study was 100 mg/kg for males and >150 mg/kg in females and the maximal tolerated dose was 50 mg/kg for males and 150 mg/kg in females. It appears that the maximal tolerated dose is 25 mg/kg in males and 50 mg/kg in females on the basis of clinical signs.

### 2.6.6.3 Repeated-dose toxicity

#### 00-001-09 7507-109: 7-Day Exploratory Intravenous Infusion Nephrotoxicity Study with AMI-6424 in Dogs with a 14-Day Recovery

**Key study findings:** The NOEL was 15 mg/kg/d.

**Study no:** Covance 7057-109; AMI CSN: 00-001-09

**Conducting laboratory and location:** Covance Laboratories, Vienna, VA

**Date of study initiation:** 10/20/00

**GLP compliance:** Yes

**QA report:** No as the sponsor considered this an exploratory study.

**Drug, lot #, % purity:** AMI-6424, Lot DO-176-87, presumed 100% pure

**Formulation/vehicle:** 5% HP- $\beta$ -CD

#### **Methods:**

##### **Dosing:**

Species/strain: Beagle dogs

#/sex/group or time point (main study): 3/sex/group with 1/group as a 14-day recovery animal

Age: 8-9 months of age

Weight: 7.3-11.6 kg for males and 6.5-9.9 kg for females

Doses in administered units: 0 (vehicle), 5, 10 or 15 mg/kg/d as a 2 hour infusion

Route, form, volume, and infusion rate: I.V. through a jugular venous catheter with a vascular access port at 2.5 mL/kg/hr. The animals were implanted 6 weeks before dosing initiation and were treated with Baytril® post-surgically. The report states that no antibiotics were given within 1 week of initiation of treatment or throughout the duration of the study.

##### **Observations and times:**

Clinical signs: Twice/day for mortality and morbidity

Body weights: Prior to dosing on Days 1 and 7 and on Days 8 (terminal sacrifice), 15 and 22 for recovery animals

Food consumption: Daily

Ophthalmoscopy: Not performed

EKG: Not performed

Hematology: Prior to catheter implantation, prior to dosing on Days -20 and -7 and on Days 7 and 21 (recovery dogs only). APTT and PT were also evaluated.

Clinical chemistry: Same as hematology

Urinalysis: Same as hematology. Dipstick methods were also used to determine if false positives occurred with that methodology.

Gross pathology: Each dog at terminal sacrifice or premature decedence.

Organs weighed: Adrenals, brain, heart, kidney, liver with gallbladder, lung, ovary, pituitary, prostate, salivary gland, spleen, testes, thymus, thyroid with parathyroid, uterus

Histopathology: Only liver, kidney and gross lesions were evaluated.

Toxicokinetics: Samples were taken at 0, 1, 2, 4, 8, 12 and 22 hours post-dosing on Days 1 and 7 and at Day 21 (single timepoint).

Other: Samples of liver and kidney from each animal were collected for tissue drug concentration analysis.

**Results:**

Mortality: One Group 3 female's catheter 'plugged up' on Day 2 so was not dosed.

Clinical signs: No significant treatment-related findings were reported.

Body weights: Mean body weights decreased (0.3 kg) for all males and for the low and high dose females. The changes in the males were attributed to dosing but the decreased body weight gains were slight and of questionable toxicologic insignificance in this 7 day study.

Food consumption: No significant treatment-related findings were reported.

Hematology: No significant treatment-related findings were reported.

Clinical chemistry: No significant treatment-related findings were reported.

Urinalysis: No significant treatment-related findings were reported. With the dipstick method, all AMI 6424-treated dogs had a + to ++ reading. When analyzed by a turbidometric method, all urines were within normal range, therefore, validating the sponsor's conclusion that the drug in urine causes false positive readings with the dipstick method.

Organ weights: No significant treatment-related findings were reported.

Gross pathology: No significant treatment-related findings were reported.

Histopathology: Focal hemorrhages were reported in the bladder walls of 3 control and 3 treated dogs. This is a not uncommon finding in laboratory beagles. The only toxicologically significant findings were those related to chronic catheterization (minimal to mild chronic inflammation of the liver and kidneys, small thrombi in small diameter vessels).

Toxicokinetics:

Males Parameter	5 mg/kg/d		10 mg/kg/d		15 mg/kg/d	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Cmax (µg/ml)	19.3	18.4	41.8	46.2	68.6	69.1
Tmax (hr)	2.0	2.0	2.0	2.0	2.0	2.0
AUC <sub>0-∞</sub> (µg.hr/ml)	43.9 *	88.4**	129.0	146.8	234.6	253.7
T 1/2 (hr)	1.88 *	3.61**	1.71	1.47	2.38	1.94
Cl (L/hr/kg)	0.13 *	0.06**	0.08	0.07	0.07	0.06
Vss (L/kg)	0.26 *	0.30**	0.19	0.16	0.21	0.18
<b>Females</b>						
Cmax (µg/ml)	18.8	18.7	36.2	38.3	63.2	62.8
Tmax (hr)	2	2	2	2	2	2
AUC <sub>0-∞</sub> (µg.hr/ml)	52.7*	68.5**	108.8	112.3*	190.8*	207.4
T 1/2 (hr)	1.41*	9.80**	2.45	1.71*	1.61*	1.82
Cl (L/hr/kg)	0.10*	0.08**	0.10	0.09*	0.09*	0.07
Vss (L/kg)	0.23*	0.66**	0.29	0.22*	0.21*	0.19

\*N=2, \*\*N=1; otherwise N=3

**Summary of individual study findings:** The sponsor determined the NOAEL to be 15 mg/kg/d in this 7-day beagle 2-hour infusion study.

**00-036-022: Exploratory Seven-Day Intravenous Nephrotoxicity Study with AMI-6424 Administered in 5% Hydroxypropyl-β-Cyclodextrin in Rats**

This non-GLP compliant exploratory study was conducted to evaluate the nephrotoxic potential of the intended excipient for AMI-6424. Fifteen female Crl:CD<sup>®</sup>(SD)IGS BR rats were divided into groups of three. Two groups were given 25 or 50 mg/kg/d of AMI-6424 once/day for 7 days by i.v. injection into the

lateral tail vein at a dosing volume of 5 ml/kg. Five additional females were given 5% HP- $\beta$ -CD in 4% dextrose once/day via the same route and volume.

Observations: Mortality and clinical signs, body weights, urine collection for 24 hours after the last dose, terminal body weights, serum chemistries at terminal sacrifice, liver and kidney from the AMI-6424-treated animals for tissue levels of drug and histopathology on kidney and liver only.

Results: No rats died prematurely. No treatment-related effects were noted in body weights, clinical signs, or serum chemistries. Slightly increased urinary protein levels were detected in AMI-6424-treated animals when compared to controls when measured by the Multistix<sup>®</sup> methodology. Given that no increases were detected with the Biotrol method and there was no dose response, it is difficult to determine the significance of this finding. However, at 50 mg/kg/d, there was slight renal tubular injury (dilated tubules with attenuated epithelium). Thus it is not possible to completely discount this finding.

Group	Multistix <sup>®</sup> Result	Biotrol Results
Vehicle	0- <30 mg/dl	11.0 mg/dl
25 mg/kg/d AMI-6424	30- 100 mg/dl	17.8 mg/dl
50 mg/kg/d AMI-6424	100 mg/dl	15.0 mg/dl

BUNs were slightly increased in the 50 mg/kg/d AMI-6424 groups compared to vehicle controls. Low concentrations of AMI-6424 were found in liver (<10  $\mu$ g/50 mg tissue) and kidney (<2  $\mu$ g in 25 mg/kg/d and <14  $\mu$ g in 50 mg/kg/d per 50 mg tissue) and serum at 24 hours after the last dose. The NOEL for this study in rats is set at 25 mg/kg/d.

**00-036-032: Exploratory Seven-Day Intravenous Nephrotoxicity Study with AMI-6424 Administered in 1% Hydroxypropyl- $\beta$ -Cyclodextrin in Rats**

This non-GLP compliant exploratory study was conducted to evaluate the nephrotoxic potential of AMI-6424 in a vehicle containing 1% HP- $\beta$ -CD in 5% dextrose. Fifteen female CrI:CD<sup>®</sup>(SD)IGS BR rats were divided into groups of three. Two groups were given 12.5 or 25 mg/kg/d of AMI-6424 once/day for 7 days by i.v. injection into the lateral tail vein at a dosing volume of 10 ml/kg (0.75 ml/min). Five additional females were given 1% HP- $\beta$ -CD in 5% dextrose once/day via the same route and volume.

Observations: Mortality and clinical signs (once daily), body weights (Days 4 and 8), urine collection for 24 hours after the last dose, terminal body weights, serum chemistries at terminal sacrifice (Day 8), liver and kidney from the AMI-6424-treated animals for tissue levels of drug and histopathology on kidney and liver only.

Results: No rats died prematurely. No treatment-related effects were noted in body weights, clinical signs, serum chemistries, or urinalysis. At 25 mg/kg/d, there was very slight to slight renal tubular injury (dilated tubules with attenuated epithelium) in two rats.

AMI-6424 was present at low concentrations in tissues (kidney and liver) and serum at 24 hours after the last dose, but exhibited a relatively high urinary recovery (see table below).

**% of administered dose**

Group	Urine	Liver	Kidney	Serum concentration (µg/ml)
AMI-6424 12.5 mg/kg/day	35.95 ± 12.49	3.06 ± 0.69	0.76 ± 0.07	< 0.5 (BLQ)
AMI-6424 50 mg/kg/day	50.49 ± 9.89	3.86 ± 0.77	0.93 ± 0.19	1.02 ± 0.15

In conclusion, when administered intravenously for seven consecutive days at 12.5 and 25 mg/kg/day in 1% HP-β-CD, AMI-6424 was not associated with mortality, clinical signs, organ weight, body weight, serum biochemical parameter or urinary parameter changes. Microscopically, very slight to slight renal tubular injury was noted in two rats given AMI-6424 at 25 mg/kg/day. Under the conditions of the study, the NOEL of AMI-6424 in 1% HP-β-CD for renal injury was 12.5 mg/kg/day.

**00-036-037: Exploratory Seven-Day Intravenous Nephrotoxicity Study in Female Rats: Effects of Varying the Ratio of Hydroxypropyl-β-Cyclodextrin to AMI-6424 on the No-Effect Dosage Level for Nephrotoxicity**

This non-GLP compliant exploratory study was conducted to evaluate the effects of varying the ratio of hydroxypropyl-β-cyclodextrin (HP-β-CD) to AMI-6424 on the no-effect dosage level for nephrotoxicity. Twelve groups of female Crl:CD<sup>®</sup>(SD)IGS BR rats (4/group) were given 6.25, 12.5 or 25 mg/kg/d of AMI-6424 once/day for 7 days by i.v. injection into the lateral tail vein at a dosing volume of 5 ml/kg (0.75 ml/min). At each dosage level, four different ratios of vehicle (HP-β-CD) to AMI-6424 were used: 1:1, 2.5:1, 5:1, or 10:1. In addition, five groups of four females each received one of the following vehicles: 5% dextrose solution, or HP-β-CD at a concentration of 5, 12.5, 25, or 50 mg/ml.

When administered intravenously for seven consecutive days at 6.25, 12.5 and 25 mg/kg/day with HP-β-CD to AMI-6424 ratios of 1:1 to 10:1, AMI-6424 was not associated with mortality, clinical signs, and treatment-related changes in organ weight, body weight, or serum biochemical parameters. There were no treatment-related necropsy and microscopic changes in the liver and kidneys. The urinary protein levels were a little high (mostly 1+) at 12.5 mg/kg and 25 mg/kg doses in one method but were comparable with the more quantitative methods. Twenty-four hours after the last dose, concentrations of AMI-6424 in the serum, liver and kidney were very low, but urinary recovery was relatively high.

**00-036-038: Exploratory Seven-Day Intravenous Nephrotoxicity Study in Female Rats: Effects of Varying the Ratio of Sulfobutylether (SBE)-β-Cyclodextrin to AMI-6424 on the No-Effect Dosage Level for Nephrotoxicity**

This non-GLP compliant exploratory study was conducted to evaluate the effects of varying the ratio of sulfobutylether-β-cyclodextrin (SBE-CD) to AMI-6424 on the no-effect dosage level for nephrotoxicity. Twelve groups of female Crl:CD<sup>®</sup>(SD)IGS BR rats (4/group) were given 6.25, 12.5 or 25 mg/kg/d of AMI-6424 once/day for 7 days by i.v. injection into the lateral tail vein at a dosing volume of 5 ml/kg (0.75 ml/min). At each dosage level, four different ratios of vehicle (SBE-CD) to AMI-6424 were used: 1.3:1, 3.3:1, 6.7:1, or 13.3:1. In addition, five groups of four females each received one of the following vehicles: 5% dextrose solution, or SBE-CD at a concentration of 6.6, 16.7, 33.3, or 66.6 mg/ml.

When administered intravenously for seven consecutive days at 6.25, 12.5 and 25 mg/kg/day with SBE-CD to AMI-6424 ratios of 1.3:1 to 13.3:1, AMI-6424 was not associated with mortality, clinical signs, and treatment-related changes in organ weight, body weight, or serum biochemical parameters. There were no treatment-related necropsy and microscopical changes in the liver and kidneys. The urinary protein levels

were a little high (mostly 1+) at 12.5 mg/kg and 25 mg/kg doses in one method but were comparable with the more quantitative methods. Twenty-four hours after the last dose, concentrations of AMI-6424 in the serum, liver and kidney were very low, but urinary recovery was relatively high.

Based on these exploratory studies, it is clear that the increased urinary protein levels are not related to the ratios of SBE-CD or HP- $\beta$ -CD to AMI-6424.

**01-001-01 7057-110: Pilot Intravenous (Infusion) Toxicity Study with AMI-6424 in Male Dogs**

**Key study findings:** Slow infusion was less toxic than a rapid bolus.

**Study no:** AMI CSN: 01-001-01, Covance 7057-110

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 2/19-23/01

**GLP compliance:** Not necessary

**QA report:** No

**Drug, lot #, radiolabel, and % purity:** Not specified

**Formulation/vehicle:** Not specified

Two male beagles/group were given i.v. AMI-6424 on Days 1 (13.5 mg/kg) and 4 (26.5 mg/kg). Doses were given as 5 minute bolus, 0.5 hour, 1 hour or 2 hour infusions via an implanted jugular catheter. The dogs were observed twice/day for morbidity and mortality. Blood samples for clinical chemistries and hematology were taken pre-dosing and approximately 24 hours after each dose. After the samples were taken, the dogs were returned to the stock colony.

**Results:** All dogs survived the dosing. The 13.5 mg/kg dose as a 5 minute bolus injection elicited signs of hypersensitivity (swollen muzzle, mandible and periorbital region, reddened skin, scratching, hives). These animals were only dosed on Day 1 due to the severity of these findings.

In the other dose groups, the signs were less severe (ears and body warm to the touch and reddened skin inside the ears), regardless of the length of the infusion. Two animals developed "limited use of the front limb" but the sponsor considered this to be an incidental finding unrelated to treatment.

No treatment effects on body weight were appreciated.

One animal in the 0.5 hour dose group showed significantly elevated white blood cell counts, higher at the Day 4 sampling. The sponsor proposed that this animal had an underlying/predisposing condition.

**Summary of individual study findings:** The sponsor determined that slow infusion was preferable so plan to do the definitive studies with a slow infusion.

**01-001-09 7057-111: 2-Week Intravenous Toxicity Study with AMI-6424 in Rats with a 2-Week Recovery**

**Key study findings:** Diffuse renal tubular vacuolation was reported in vehicle controls and high dose animals with male dosed animals were slightly more affected than their vehicle controls.

**Study no:** AMI CSN: 01-001-09, Covance 7057-111

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 6/21/01

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, and % purity:** AMI-6424, Lot #AMB001, assumed 100% pure

**Formulation/vehicle:** 5% dextrose or compound vehicle (100 mg/ml HP- $\beta$ -CD, 39 mg/ml dextrose monohydrate, pH adjusted to 4.7 and q.s. with water for injection)

**Methods:**

**Dosing:**

Species/strain: Crl:CD®(SD)IGS BR rats

#/sex/group or time point (main study): 10/sex/group for 2 week sacrifice and 5/sex/group for 2 week recovery sacrifice.

Satellite groups used for toxicokinetics or recovery: 9/sex/group

Age: 52-59 days of age

Weight: 183- 294 gms

Doses in administered units: 0 (diluent control), 0 (vehicle control), 6.25, 12.5 or 25 mg/kg/d

Route, form, volume, and infusion rate: I.V. at a volume of 5 ml/kg as a 2 hour infusion into the femoral vein. Sterile saline was infused through the catheter when drug was not being infused.

**Observations and times:**

Clinical signs: Twice/day

Body weights: Once prior to dosing, on Day 1 and weekly thereafter

Food consumption: Weekly

Ophthalmoscopy: Pre-dosing and Weeks 2 and 4. Evaluations were done by a laboratory veterinarian, not an ophthalmologist as stated in the protocol.

EKG: Not performed

Hematology: At terminal sacrifice

Clinical chemistry: At terminal sacrifice

Urinalysis: At terminal sacrifice

Gross pathology: All animals underwent macroscopic examination

Organs weighed: Adrenals, brain, heart, liver, ovaries, pituitary, kidney, testes, thymus, and thyroid/parathyroids

Histopathology: Tissues from each animal in the control groups and the high dose group. All premature decedents and animals that were terminated early were also examined histologically, as well as all gross lesions from all dose groups. Peer review was performed by *f* *p*

Toxicokinetics: Samples were taken on Days 1 and 14 at 0, 1, 2, 4, 8, 12, and 22 hours after the infusion was completed in the TK groups.

Other: Frozen sections were made of liver and kidney for possible analysis of tissue concentrations.

**Results:**

Mortality: 5 TK animals died/were sacrificed prematurely. Most were due to catheter-related events.

Clinical signs: No significant treatment-related effects were reported.

Body weights: No significant treatment-related effects were reported.

b(6)

Food consumption: No significant treatment-related effects were reported.  
Ophthalmoscopy: No significant treatment-related effects were reported.  
Hematology: No significant treatment-related effects were reported.  
Clinical chemistry: No significant treatment-related effects were reported.  
Urinalysis: No significant treatment-related effects were reported except for increased granular casts and positive occult urine bloods in the high dose males after 2 weeks of dosing.  
Organ weights: No significant treatment-related effects were reported.  
Gross pathology: No significant treatment-related effects were reported.  
Histopathology: Diffuse renal tubular vacuolation was reported in vehicle controls and high dose animals with male dosed animals were slightly more affected than their vehicle controls. The recovery animals were comparable across vehicle and dosed animals to the 14 day sacrifice animals.

Minimal to moderately severe perivascular eosinophilic infiltrates, with some macrophages, were found in all study groups and were attributed to the catheters or another commonality between the groups. As eosinophils generally reflect an immunologically mediated phenomenon, it is difficult to understand how the presence of an inert catheter could elicit this finding.

Toxicokinetics: Unfortunately, for the males, the samples were not taken at the end of the infusion but were taken 16-21 minutes after the end. While questions may arise as to the possibility of sampling that many animals (3 males/group) in that time period (5 minutes) without affecting the quality of the samples, the assumptions about C<sub>max</sub> and therefore all of the other PK parameters for the Day 1 samples in males are questionable. The AUC and C<sub>max</sub> for males on the Day 1 sample were lower by ~33% than females. At the Day 14 sample, males and females had approximately equal mean blood levels. The sponsor stated that the t<sub>1/2</sub> was <2 hours on Day 1 and <3 hours on Day 14, especially notable in the high dose group. Increases in C<sub>max</sub> and AUC were generally proportional to dose.

**Summary of individual study findings:** The sponsor assigned a NOEL of 12.5 mg/kg/d on the basis of the urinalysis and histopathologic findings in the 25 mg/kg/d animals.

**01-001-10 7057-112: Two Week Intravenous Toxicity Study with AMI-6424 in Beagle Dogs with a 2-Week Recovery**

**Key study findings:** Degeneration of the femoral growth plate, and chronic inflammation at the catheter site were noted in all groups.

**Study no:** Covance: 7057-112; AMI CSN: 01-001-10

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 5/23/01

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, radiolabel, and % purity:** AMI-6424, Lot AMB001 at presumed 100% purity

**Formulation/vehicle:** Hydroxypropyl-β-Cyclodextrin at 100 mg/ml, Dextrose monohydrate at 40 mg/ml, pH adjustment to 4.8 and q.s. to 1 ml with water for injection; the diluent control was 5% dextrose injection

**Dosing:** The original protocol called for animals to be dosed with lactated Ringer's solution whenever dosing was not occurring. However, due to procedural problems, not all animals received this solution during acclimation or when not being administered test solutions.

Species/strain: Beagle dogs  
 #/sex/group or time point (main study):

Group	Males	Females	Dose (mg/kg/d)	Dose concentration (mg/ml)
1 - Diluent control	4	4	0	0
2- Vehicle control	6- 2 recovery	6- 2 recovery	0	0
3- Low dose	4	4	6.25	1.25
4- Mid dose	6- 2 recovery	6- 2 recovery	12.5	2.5
5- High dose	6- 2 recovery	6- 2 recovery	25	5.0

Satellite groups used for toxicokinetics or recovery: 2/sex/vehicle, mid and high dose animals  
 Age: 4-6 months of age at study initiation. Animals were implanted with jugular vein catheters at least one week prior to study initiation.

Weight: 6.3- 9.4 kg

Doses in administered units: 0 (diluent and vehicle controls), 6.25, 12.5 or 25 mg/kg/d

Route, form, volume, and infusion rate: I.V. through implanted catheter once/day at a volume of 5 ml/kg over a 2 hour period using an infusion pump and tether jacket system.

#### Observations and times:

Clinical signs: Twice/day for mortality and morbidity, continuously during dosing on Days 1 and 2, and immediately post-dosing and 1 hour later for the remainder of the study.

Body weights: Weekly prior to dosing, on Day 1 and weekly thereafter

Food consumption: Weekly

Ophthalmoscopy: Prior to dosing and during Weeks 2 and 4.

EKG: Prior to dosing and during Weeks 2 (after dosing) and 4.

Hematology: Twice pre-dosing and at terminal sacrifice. Animals were fasted prior to sample collection.

Clinical chemistry: Twice pre-dosing and at terminal sacrifice. Animals were fasted prior to sample collection.

Urinalysis: Twice pre-dosing and at terminal sacrifice

Gross pathology: On Day 15, 4 animals/sex/group were necropsied.

Organs weighed: Adrenals, brain, heart, liver, ovaries, pituitary, kidney, testes, thymus, and thyroid/parathyroid

Histopathology: Samples of tissues from each animal (see Histopathology Inventory) were preserved and stained with H&E and read by ( ) and peer reviewed by ( )

Toxicokinetics: Samples were collected on Days 1 and 14 at 0 (defined as immediately following the end of the infusion) and 1, 2, 4, 8, 12, and 22 hours post-end of infusion. Unfortunately, without a sample at the beginning of the infusion, the only valuable information to be derived from these data is the elimination constant, the  $t_{1/2}$  and  $C_{max}$ . Samples from controls were prepared from Day 1 only. All other samples from these animals were discarded. Assays were performed using HPCL with LC/MS/MS detection.

Other: Representative samples of the liver and left kidney were removed and flash frozen for possible evaluation of AMI-6424 levels.

#### Results:

Mortality: No animals died prematurely.

Clinical signs: The signs noted were related to the catheter site with limited limb use, swollen catheter sites, red skin (limbs, shoulder, ventral cervical area) and discharge from the catheter site. Three animals (2 control males, 1 mid dose female) were lame and were treated with Etogesic on Day 14.

Body weights: Slight body weight decreases were noted in all animals during Weeks 2 and 3. These decreases were attributed to the fasting for clinical pathology sampling and terminal necropsy.

Food consumption: No treatment-related effects were reported on mean food consumption.

Ophthalmoscopy: No treatment-related ophthalmic lesions were reported.

Electrocardiography: No significant treatment-related abnormalities in cardiac rhythm or conduction were reported.

Hematology: Markedly decreased platelet counts were noted in all groups, including controls. The decreases were approximately 45% of the original counts in all but the low dose males (~1/3 of original count). The coagulation parameters (PT, PTT) were not significantly affected. The sponsor and consultant clinical pathologist attributed this finding to "complications associated with chronic catheterization." This appears to be a reasonable conclusion.

Clinical chemistry: No treatment-related effects were reported.

Urinalysis: Of questionable relation to treatment were higher urine volume (~2x) and lower specific gravity in high dose males on Day 15. Similar differences from controls were noted in the females. No correlative gross necropsy or histopathologic findings were found.

Organ weights: No significant treatment-related findings were reported.

Gross pathology: No significant treatment-related findings were reported.

Histopathology: All findings (inflammation, thrombosis and hemorrhage at the catheter site, inflammation of heart valves, within liver, kidneys and lungs) were comparable across dose groups and were attributed to chronic catheterization or spontaneous lesions common in laboratory beagles on studies. Of interest is the degeneration of the proximal femoral growth plate (2/4 animals/group) with changes in the metaphyseal bone and bone marrow, given the finding of drug in epiphyses in the PK studies. The growth plate changes were characterized as degeneration. Localized depletion of marrow hematopoietic cells, paratrabecular fibroplasia, marrow hemorrhage and inflammation were changes reported in metaphyseal bone and marrow. The study pathologist attributed these changes to "a combination of spontaneous background changes common to beagles as well as complications to the indwelling jugular catheters." They stated that since similar incidences and severities were found across groups, that there is no relationship to treatment.

#### Toxicokinetics:

##### Mean Values for Dogs Treated with AMI-6424

Dose Group	Gender	Cmax (µg/ml)	Tmax (hrs)	AUC <sub>0-∞</sub> (µg.hr/ml)	T 1/2 (hrs)
Day 1					
3 (N=4)	M	27.1	2	73.7	1.14
(N=4)	F	29.8	2	97.4	2.96
4 (N=6)	M	62.2	2	209	1.42
(N=6)	F	62.1	2	228	1.9
5 (N=6)	M	130	2	507	1.86
(N=6)	F	128	2	504	1.72

Day 14					
3 (N=4)	M	26.7	2	77.4*	1.17
(N=4)	F	30.8	2	117*	5.37
4 (N=6)	M	57.6	2	216*	3.05
(N=6)	F	59.9	2	233*	1.95
5 (N=6)	M	131	2	560*	2.68
(N=6)	F	127	2	555*	2.63

\* The sponsor assumed that  $AUC_{0-24} = AUC_{0-\infty}$  after multiple dosing. Samples were collected at the end of the 2 hour infusion. Although no significant gender differences were found, it is interesting that the AUCs, except for the high dose animals, were higher in females and the  $t_{1/2}$ s were longer and detectable levels were found at 24 hours in females more frequently than in males.

Exposure increased proportionately with increasing dose. A mean increase/prolongation in  $t_{1/2}$  was noted as well as a slight increase in AUC after repeated dosing.

**02-001-01 7057-144: 4-Week Intravenous Infusion Toxicity Study with AMI-6424 in Rats with a 4-Week Recovery**

**Key study findings:** Renal tubular degeneration (mid and high doses) and tubular vacuolation (placebo and all doses) were found.

**Study no:** Theravance Reference: AMI CSN: 02-001-01; Covance #7057-1444

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 1/11/02

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot #, and % purity:** AMI-6424, Lot No. AMB001 at 94% purity

**Formulation/vehicle:** "Placebo for AMI-6424 for Injection, 250 mg/vial", lot #AMD001 with 2500 mg hydroxypropyl- $\beta$ -cyclodextrin, 312.5 mg mannitol and ~1% water. The diluent was D5W.

**Methods:**

**Dosing:**

Species/strain: Crl:CD<sup>®</sup>(SD)IGS BR rats. The animals were catheterized prior to receipt at the testing laboratory.

#/sex/group or time point (main study): 15

Satellite groups used for toxicokinetics or recovery: 9/sex/dose of active compound

Age: 62-69 days of age at study initiation

Weight: 210- 256 gms

Doses in administered units: 0 (D5W), 0 ("Placebo for AMI-6424 for Injection"), 12.5, 25 or 50 mg/kg/d.

Route, form, volume, and infusion rate: I.V. at 5 ml/kg as a bolus injection through a femoral vein catheter. Whenever test article was not being administered, 0.9% sodium chloride was given at 0.15 ml (females) or 0.31 ml (males)/hr through the catheter.

**Observations and times:**

Clinical signs: Twice/day

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Prior to initiation, at Weeks 4 and 8

EKG: Not performed

Hematology: At scheduled sacrifice

Clinical chemistry: At scheduled sacrifice

Urinalysis: At scheduled sacrifice

Gross pathology: On Day 29, 10/sex/dose were sacrificed and necropsied. The remaining animals were sacrificed and necropsied on Day 58.

Organs weighed: Adrenals, brain, heart, liver, ovaries, pituitary, kidneys, testes, thymus and thyroid/parathyroids.

Histopathology: All tissues listed in Histopathology Inventory for all placebo controls, diluent controls and high dose animals, as well as all premature decedents. Gross lesions and kidneys from all remaining animals were examined histologically. The histologic slides were evaluated by (

). Peer review was performed.

Toxicokinetics: Samples were taken from the PK animals on Days 1 and 28 at 0 (immediately following the infusion to include the 'saline flush'), 1, 2, 4, 8, 12 and 23.5 hrs post-infusion. Three animals/timepoint were bled. At the end of the collection period, the animals were euthanized and discarded without examination.

Other: The length of the femur was measured using a caliper.

**Results:**

Mortality: None of the premature decedents was attributed to treatment with AMI-6424. All unscheduled deaths (2 placebo males, 2 control females, low dose female, 3 high dose males) were attributed to catheter-related problems.

Clinical signs: No compound-related effects were reported.

Body weights: No compound-related effects were reported.

Food consumption: No compound-related effects were reported.

Ophthalmoscopy: No treatment-related effects were noted.

Hematology: No treatment-related effects were noted.

Clinical chemistry: BUN and creatinine were slightly increased in the high dose animals. These findings were not found at the end of the recovery period. Urinary protein was increased with the dipstick methodology but not with a quantitative methodology.

Urinalysis: Urine occult blood was positive for the males given placebo, 25 mg/kg/d or 50 mg/kg/d of active compound. This finding was attributed to the hydroxypropyl- $\beta$ -cyclodextrin excipient.

Organ weights: No compound-related effects were reported.

Gross pathology: No compound-related effects were reported.

Histopathology: Minimal focal or multifocal renal tubular degeneration was reported in mid and high dose animals at the end of the dosing period but not at the end of the recovery period. Diffuse renal tubular vacuolation was noted in all placebo- and active compound-treated animals and was attributed to the

b(4)

hydroxypropyl- $\beta$ -cyclodextrin excipient. This finding was not completely reversed by the end of the recovery period.

Alveolar histiocytosis was increased in the placebo and high dose animals but the pathologist attributed this finding, as with the renal tubular vacuolar change, to the hydroxypropyl- $\beta$ -cyclodextrin.

Urinary bladder urothelium had vacuolation, similar to that in the kidney, especially in the placebo and high dose animals. This was increased at the end of the recovery period and attributed to "clearance of placebo material from the kidneys".

Toxicokinetics: Exposures increased proportionally with dose. Concentrations of active compound in plasma were higher on Day 28 than on Day 1 indicating accumulation.

**Mean PK Parameters from Rats Treated for 29 Days with AMI-6424**

Dose (mg/kg/d)		Gender	Cmax ( $\mu$ g/ml)	AUC <sub>0-24</sub> ( $\mu$ g.hr/ml)	T 1/2 (hrs)
12.5	Day 1	M	78.1	185	1.51
		F	62.3	164	1.85
25		M	142	393	4.56
		F	120	332	1.62
50		M	275	631	2.47
		F	255	661	2.84
12.5	Day 28	M	67.2	NA	2.48
		F	80.5	NA	1.80
25		M	173	NA	2.98
		F	126	NA	3.06
50		M	324	NA	3.20
		F	301	NA	3.17

Other: No compound-related effects on bone measurement were reported.

**Summary of individual study findings:** The sponsor concluded that the NOAEL for this 4 week rat study was 12.5 mg/kg/d on the basis of reversible increased BUN and creatinine and minimal focal to multifocal renal tubular degeneration at 25 and/or 50 mg/kg/d.

**02-003-05 57328: A 13 Week Intravenous Infusion Toxicity Study (with a 28 day Recovery Period) of AMI-6424 in the Beagle Dog**

**Key study findings:** No NOAEL can be set for this study due to the persistence of macrophage accumulation/vacuolation at all doses throughout the recovery period and the increased BUN/creatinine/urine volumes at the mid and high doses. As the findings were comparable to those found in the 13 week rat study, it is difficult to support the proposed 6 week study in man.

**Study no:** AMI CSN: 02-003-05 or 57328

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** 4/16/02

**GLP compliance:** Yes

**QA report:** yes

**Drug, lot # and % purity:** AME003 at 99.0% purity

**Formulation/vehicle:**

**Methods (unique aspects):** Topical Polymyxin B, Bacitracin and Neomycin were applied to the catheter exteriorization sites daily until termination. This is an unusual addition to standard toxicology studies with catheterized animals.

b(4)

**Dosing:**

Species/strain: Catheterized beagle dogs  
#/sex/group or time point (main study): Initially 4/sex  
Satellite groups used for toxicokinetics or recovery: Initially 2/sex but due to losses of main study animals, some groups (diluent control males, placebo control females, and mid dose males) only had 1 animal for recovery.  
Age: 5-6 months of age  
Weight: Males: 8.5- 10.3 kg; females: 5.9-8.5 kg  
Doses in administered units: 0 (diluent, 5% dextrose), 0 (placebo emulsion), 12.5, 25 or 100 mg/kg/d in "free base equivalents"  
Route, form, volume, and infusion rate: I.V. at 10 mL/kg over 1 hr/day for 13 weeks

**Observations and times:**

Clinical signs: Twice daily  
Body weights: Weekly  
Food consumption: Daily  
Ophthalmoscopy: Prior to study initiation, at Weeks 6, 13 and 17  
EKG: As for Ophthalmoscopy. EKGs were taken within 30 minutes of dosing termination.  
Hematology: Prior to initiation, and at Weeks 6, 13 and 17  
Clinical chemistry: Prior to initiation, and at Weeks 6, 13 and 17  
Urinalysis: Prior to initiation, and at Weeks 6, 13 and 17  
Gross pathology: All animals at study termination  
Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, ovaries/testes, pituitary, spleen, thymus, thyroids and parathyroids, and uterus.  
Histopathology: All tissues listed in Histopathology Inventory from all animals at study termination  
Toxicokinetics: All animals were sampled on Days 1, 28 and 90 at 0 (following end of infusion), 1, 2, 4, 8, 12 and 23.5 hours after the end of the infusion. Samples from diluent and emulsion controls were discarded without analysis.

**Results:**

Mortality: No treatment-related mortality was reported. However, catheter-related problems were common in this study. Eleven animals had  $\geq 2$  'repair' surgeries. Between Weeks 4-11, catheters could not be repaired or replaced for 9 animals (2 diluent controls, 2 emulsion controls, 1 low dose, 3 mid dose and 1 high dose animal). These animals were euthanized. No significant clinical signs were attributed to treatment of these animals other than inability to infuse the drug. After Week 11, animals without patent catheters (N=8) were infused via peripheral veins in a sling restraint. One Group 4 animal was euthanized from the mid dose group during week 9 due to "severe subcutaneous inflammation".

Clinical signs: Apparently histamine-related reactions (excessive shaking/scratching, swollen limbs and faces, erythema and papules) were noted in the high dose group. For the first 20 days of this study, the laboratory felt the need to administer diphenhydramine i.v. prior to dosing to ameliorate the reactions.

Body weights: No treatment-related effect on body weight was appreciated.

Food consumption: No treatment-related effects on feed consumption were found.

Ophthalmoscopy: No treatment-related differences from controls were discovered.

Electrocardiography: No treatment-related differences from controls were determined.

Hematology: No significant treatment-related differences from placebo controls were described. Changes related to the inflammatory process in these animals (reduced RBC counts, hemoglobin concentration and hematocrit) were described in Week 6 only. White cell counts increased over the course of the study for all placebo and AMI-6424-treated animals but remained within the diluent control values (1-3x pre-dosing counts). Reticulocyte counts were elevated in treated groups during Week 13.

Clinical chemistry: Increased ALT (3x) and AST (~2x) levels were revealed by Week 6 in the 100 mg/kg/d groups. Levels were markedly higher by Week 13 [3x (ALP), 4x (AST) and 28x (ALT) increases]. These increases were partially reversed by the end of the recovery period (AST at 1.8x and ALT at 3-7x when compared to diluent controls). Correlative histopathology of hepatocellular degeneration/necrosis was discovered.

Approximately 2x increases in BUN and creatinine and urinary output (up to 5x increases) were noted in the high dose males and mid and high dose females at Weeks 6 and 13. Correlative histopathology of renal tubular vacuolation and/or degeneration/necrosis were discussed and persisted throughout the recovery period.

Organ weights: Mean liver weights (relative and absolute) were significantly increased (up to 47%) in the high dose males at the end of the dosing period when compared to the diluent controls and ~20% when compared to the placebo emulsion controls. Similar increases were not found in the 100 mg/kg/d females. Liver weights were comparable across the small numbers of animals at the end of the recovery period. The sponsor attributed the increased weights to macrophage accumulations and reactive sinusoidal lining cells in the livers.

Gross pathology: Pale kidneys and pale areas of the lungs were noted in some animals from all but the diluent control group. Gross enlargement of lymph nodes throughout the body was reported.

Histopathology: Peer review was performed by another staff pathologist. Lesions discovered in the placebo emulsion group included kidney lesions (renal tubular vacuolation, dilatation, necrosis and eosinophilic cytoplasmic inclusions, and the urethra, urinary bladder) and vacuolation of the epithelium of the epididymides as well as macrophage accumulation and/or vacuolation in essentially all tissues. However, all of these changes were exacerbated by AMI-6424 and increased in incidence and severity with increasing AMI-6424 dose. In the 12.5 and 25 mg/kg/d animals, the tubular vacuolation and necrosis were reported but at a lesser severity than in the placebo control or high dose animals. This finding is probably attributed to the lesser amount of hydroxypropyl- $\beta$ -cyclodextrin administered to these animals.

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Histiocytic infiltrates were noted in lymph nodes from mid dose females and high dose animals of both sexes. No improvement in the degree of involvement was noted by the end of the recovery period.

Eosinophilic cytoplasmic inclusions were described in the animals given the hydroxypropyl- $\beta$ -cyclodextrin with an exacerbation at the high dose in males. These inclusions may represent altered lysosomes due to the hydroxypropyl- $\beta$ -cyclodextrin administration but no further analysis to prove this

hypothesis was performed. The lesions were essentially unchanged at the end of the recovery period in the kidneys, liver and lymph nodes.

Within the liver, an increased incidence and severity of reactive sinusoidal lining cells was appreciated in the high dose animals when compared to the placebo controls. Increased centrilobular macrophages were also significant in the high dose animals. Hepatocellular degeneration/necrosis (graded as slight) was found in all 100 mg/kg/d animals, including the premature decedents. One of the 4 high dose recovery animals showed this lesion. This is considered a treatment effect.

Vacuolation of the epididymal tubular epithelium was noted in the placebo controls and all 25 and 100 mg/kg/d males, during the main study and the recovery period. The severity increased with increasing dose.

Infusion site reactions (inflammation and fibrosis within the vena cava and/or surgical site) were considered associated with the indwelling catheters. However, the AMI-6424-treated animals had more macrophage accumulations in the associated inflammation than did the placebo-treated animals. The lesions in the vena cavae were not resolved by the end of the recovery period but the peripheral vein lesions (in animals where catheters did not remain patent) were resolved. Other findings that the sponsor attributed to chronic catheterization included: hypercellularity in bone marrow, lymph nodes (to include plasmacytosis) and inflammatory changes in lymph nodes, lung and spleen. Additionally, sciatic nerve fiber degeneration was noted in all groups receiving placebo or AMI-6424, with the highest incidence in 100 mg/kg/d males. The sponsor considered this finding "associated with pronounced infusion and surgical site lesions seen in this study" and that it was a fortuitous event rather than a direct effect of treatment.

Dose Main Study	Dil. M	Dil. F	Placebo M	Placebo F	Low M	Low F	Mid M	Mid F	High M	High F
Slight hep. Degen/necro.	0	0	0	0	0	0	0	0	4	4
Macrophage accumul										
-Slight	0	0	0	0	0	0	0	0	3	3
-Moderate	0	0	0	0	0	0	0	0	0	1
Reactive sinusoidal cells, -Minimal	0	1	2	2	0	0	1	0	0	0
-Slight	0	0	2	2	0	0	0	0	3	3
-Moderate	0	0	0	0	0	0	0	0	0	1
<b>Recovery</b>										
Min. hep. degen./necr.	0	0	0	0	0	0	0	0	1	0
Macrophage accumul										
-Min/Slight	0	0	0	0	0	0	0	0	2	1
-Moderate	0	0	0	0	0	0	0	0	0	1
Reactive sinusoidal cells, -Minimal	0	0	1	1	0	0	0	0	0	1
-Slight	0	0	0	0	0	0	0	0	0	1
<b>Main study</b>										
Renal tubular dilatation										
-Minimal/ Slight	0	0	1	0	0	0	0	0	3	4
-Moderate	0	0	0	0	0	0	0	0	1	0
Vacuolation										
-Slight/Min	0	0	1	3	1	0	1	1	3	4
-Moderate	0	0	1	0	0	0	0	0	1	0
Tubular necrosis										
-Min/slight	0	0	1	3	1	0	0	1	4	4
-Moderate	0	0	1	0	0	0	0	0	0	0
Inclusions										
-Min/slight	0	1	1	3	2	2	2	4	0	4
-Moderate	0	0	1	1	0	0	0	0	2	0

-Marked	0	0	0	0	0	0	0	0	1	0
<b>Recovery</b>										
Vacuolation										
-Slight/Min	0	0	1	1	0	1	1	0	2	2
Tubular necrosis, -min	0	0	1	0	0	1	1	0	2	2
Inclusions										
Min/slight	0	0	1	0	0	1	0	0	0	1
<b>Main study</b>										
Alveolar histocytes										
-Min/slight	0	2	3	4	1	2	2	1	1	2
-Moderate	0	0	1	0	0	0	0	0	3	2
<b>Recovery</b>										
Alveolar histocytes										
-Min/slight	1	0	0	1	2	0	1	1	2	2
<b>Main study</b>										
Epididymides, tubular vacuolation										
-Min/slight	0		4		0		1		2	
-Moderate	0		0		0		0		2	
<b>Recovery</b>										
Epididymides, tubular vacuolation										
-Min/slight	0		2		0		1		2	
-Moderate	0		0		0		0		2	
<b>Main study</b>										
Histiocytosis in lymph nodes -Min/slight	0	0	2	2	1	0	2	2	2	3
-Moderate	0	0	0	0	0	0	0	0	1	2
<b>Recovery</b>										
Histiocytosis in lymph nodes -Min/slight	0	0	1	1	0	0	0	1	2	2

Toxicokinetics: Exposure increased with increasing dose in approximately a dose-dependent fashion between 12.5 and 25 mg/kg/d but at slightly less than dose-proportional from 25 to 100 mg/kg/d. However, the AUC<sub>0-24</sub> values for AMI-999 (a minor metabolite of AMI-6424) were increased in a greater than dose-proportional manner between Days 1 and 90. No drug accumulation of this metabolite was apparent over time.

Accumulation of AMI-11352 (another minor metabolite) increased over the dosing period. The increases were not generally dose proportional to the increased AMI-6424 dose. It is possible that the accumulation was due to saturation at the highest dose level.

**Mean PK Parameters for Dogs Treated for 13 Weeks with AMI-6424**

Dose (mg/kg/d)	Gender	Cmax (µg/ml)	Tmax (Hrs)	AUC <sub>0-24</sub> (µg.hr/ml)	T <sub>1/2</sub> (hrs)
Day 1 12.5	M	80.3	1	229	1.34
	F	77.5	1	209	1.18
25	M	157	1	514	1.52
	F	141	1	489	1.68
100	M	477	1	1775	2.84
	F	507	1	1672	2.38
Day 28 12.5	M	71.3	1.17	230	1.85
	F	86.0	1	270	1.55
25	M	137	1	533	2.15
	F	134	1	532	1.87
100	M	460	1.0	1877	2.68
	F	427	1.33	1804	2.37
Day 90 12.5	M	74.1	1	237	1.8
	F	75.8	1	252	1.77
25	M	105	1	533	2.04
	F	164	1	639	2.15
100	M	480	1.2	1992	3.12
	F	327	1.5	2056	2.59

**Summary of individual study findings:** The sponsor considered the NOAEL for this study to be 25 mg/kg/d on the basis of "generally well tolerated" at that dose. However, due to the changes in BUN and creatinine seen in the 25 mg/kg/d females and the macrophage accumulations/vacuolations that persisted throughout the recovery period, a NOAEL for this study cannot be set.

**02-001-06\_0757-148: 13 Week Intravenous Infusion Toxicity Study with AMI-6424 in Rats with a 4-Week Recovery**

**Key study findings:** The sponsor considered the 12.5 mg/kg/d group to provide a NOEL except for the non-reversible finding of systemic and multiorgan macrophage hypertrophy/hyperplasia for which no NOEL could be determined in this study. As the incidence and severity of this finding increased with increasing dose, it is of concern that no NOEL could be identified.

**Study no:** Theravance #CSN: 02-001-06, Covance #7057-148

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 3/25/02

**GLP compliance:** Yes

**QA report:** yes

**Drug, lot #, and % purity:** AMI-6424, Lot AME003 at 90% purity

**Formulation/vehicle:** AMI-6424 placebo consisting of 2500 mg hydroxypropyl-β-cyclodextrin, 312.5 mg mannitol and pH adjustment to pH 4.5 as needed. The diluent was 5% dextrose.

**Methods:**

**Dosing:**

Species/strain: CrI(SD) IGS rats

#/sex/group or time point (main study): 18 with 5/group having at least 4 weeks of recovery

Satellite groups used for toxicokinetics or recovery: 9/sex/dose of AMI-6424

Age: ~8 weeks of age at study initiation

Weight: Males: 276-342 gms; females: 190-254 gms.

Doses in administered units: 0 (diluent) [Group 1], 0 (placebo control) [Group 2], 12.5 [Group 3], 50 [Group 4] or 100 mg/kg/d [Group 5]

Route, form, volume, and infusion rate: I.V. through implanted femoral vein catheter at 10 ml/kg as a once/day 30 minute infusion. During non-dosing periods, 0.9% sodium chloride was infused at 0.15 (females) and 0.2 (males) ml/hr.

**Observations and times:**

Clinical signs: Twice daily for morbidity and mortality. Detailed observations were made prior to initiation, once/week during the dosing period and recovery period.

Body weights: Prior to dosing, and weekly thereafter

Food consumption: Measured weekly for main study animals only

Ophthalmoscopy: All animals prior to study initiation, and during Weeks 13 and 17 (recovery animals only)

EKG: Not performed

Hematology: Blood and urine were collected on Day 27 from main study and recovery animals.

Clinical chemistry: Blood and urine were collected on Day 27 from main study and recovery animals.

Urinalysis: Blood and urine were collected on Day 27 from main study and recovery animals.

Gross pathology: All main study and recovery animals at termination.

Organs weighed: Adrenals, brain, heart, liver, ovaries, pituitary, kidneys, testes, thymus, and thyroids with parathyroids.

Histopathology: Tissues listed in the Histopathology Inventory were evaluated for both control groups and the 100 mg/kg/d group and premature decedents only. Later, the thymus, lymph node, spleen, liver, kidney, duodenum, jejunum, ileum (males), testes, epididymides, femoral bone marrow, sternal bone marrow and the ovaries were examined from the low and mid dose animals. A peer review was performed on the evaluations.

Toxicokinetics: Samples were taken on Days 1 and 90, immediately post-dosing, and 1, 2, 4, 8, 12 and 23.5 hours post-dosing from the TK animals (N=3/timepoint). Blood was collected from the jugular vein and each animal was bled at least twice.

Other: Bone measurements were taken at necropsy.

**Results:**

Mortality: Two 5% dextrose females, 1 placebo female and 1 high dose female, as well as 2 placebo control males and 1 50 mg/kg/d male, were found dead or euthanized in extremis. They were debilitated. These animals had severe bacterial infections and/or marked inflammation at the catheter site.

Clinical signs: No treatment-related clinical signs were reported. Scabbing and "sores" appeared throughout the study and were considered to be related to the catheters/jacket tethers used on the animals.

Body weights: Significant effects on body weights were related directly to treatment. Mean body weights for all dosed males (up to 15% compared to placebo controls and 17% for diluent controls) and for mid dose and high dose females (up to 12%) were lower than controls. These findings were dose related. The decreases were maintained at the same levels for 50 mg/kg/d females (Days 99-113) and 100 mg/kg/d females (throughout the recovery period). Mean body weight gains were similarly affected in males at 50 (14%) and 100 (35%) mg/kg/d and in females (38% and 42% respectively) at the same doses. There were no significant differences from controls during the recovery period.

Food consumption: Feed consumption was decreased for mid and high dose males and females. This decrease continued through the recovery period for the high dose animals of both sexes.

Ophthalmoscopy: No treatment-related effects were observed.

Hematology: Anemia (characterized by decreased RBC counts, hemoglobin and hematocrit) was reported in the mid and high dose animals. White cell counts were elevated (up to 30%) in the high dose animals (increased neutrophils, lymphocytes and monocytes). The sponsor claims that the placebo "was at least partially responsible" for these effects. Indeed, the counts for the 5% dextrose animals were not changed over time and the placebo-treated animals showed similar but lesser changes to the AMI-6424 animals. Reversibility was not found for red cell counts, hemoglobin or hematocrit for mid or high dose animals. The MCHC values did not significantly differ between controls and treated animals so it appears that the effects on red cell parameters did not elicit a regenerative anemia.

Clinical chemistry: Increased BUN (20-40%) and creatinine (up to 33%) were reported at Week 4 and Week 14 (>2x for BUN, 1.5x for creatinine) for all of the treated animals. Increased AST and ALT were found in the mid and high dose animals (33-45% at Week 4 and >4x at Week 14). ALP was also increased in the high dose animals. The sponsor contends that the placebo was at least partially responsible for the increased BUN, creatinine, AST and ALT (males only), and ALP (females only). By the end of the recovery period, BUNs were still increased up to 2x diluent control levels. ALT and AST values were still elevated above control values.

Urinalysis: Urinary occult blood was increased during Week 14 at all doses. Granular casts were increased in high dose males and all dosed females as were amorphous crystals in all dosed males and high dose females. Renal ability to concentrate was not apparently affected by these effects. The sponsor suggests that the placebo was at least partially responsible for all but the increased casts. They state that the effects on clinical chemistry parameters "indicative of kidney and liver injury demonstrated variable degrees of recovery following 4 weeks without treatment." However, the increased creatinine levels did not reverse in mid or high dose males or high dose females. The other dosed animals showed some but not complete recovery. Urinary occult blood remained high for the high dose males throughout the recovery period. Urinary protein was elevated in males and decreased in females at the end of the recovery period.

Organ weights: (All % compared to diluent control values) Increased mean absolute and relative kidney weights were found in the placebo-treated animals (45% for males, 44% for females) as well as treated animals (40% for mid dose males and females, 80% for high dose males and females). By the end of the recovery period, the increased relative weights were 26% for the placebo controls, 18% for the mid dose animals and 53% for the high dose animals. Mean relative liver weights were increased in AMI 6424-treated animals (9% for placebo controls, 13-24% for treated animals in a dose-related fashion) and a significant dose-related decrease in thymic weights (~1/2 the controls) was appreciated.

Gross pathology: Placebo administration elicited mottled kidneys and lungs and enlarged lymph nodes (primarily lumbar and iliac). AMI 6424 treatment showed significant exacerbation of these findings to include enlarged renal lymph nodes).

Histopathology: Renal lesions including increased incidence/severity of proximal tubular degeneration, tubular casts (probably secondary to the tubular damage), cortical tubular vacuolation and

inflammatory cell infiltrates were noted in all treated animals, including the placebo controls. The incidence and/or severity of the lesions increased with increasing dose. These lesions persisted throughout the recovery period. Additional lesions included hepatocellular degeneration (especially mid and high dose animals), vacuolation in the urothelium, epididymides and prostate, endothelial vacuolation of post-capillary venules (lymph nodes, Peyer's patches and G.I. tract) and macrophage hypertrophy/hyperplasia (including major tissue systems and bone marrow and the testicular interstitium in males). Pulmonary alveolar macrophage hypertrophy/hyperplasia was characterized by partial to complete filling of alveolar spaces with foamy macrophages. The sponsor considered these to be "exacerbated placebo effects" even though they were not reported in diluent or placebo treated animals. The bone marrow hyperplasia/hypertrophy was characterized by increased numbers of macrophages with eosinophilic material in the cytoplasm. The sponsor considered this a placebo effect "based upon the presence of similar macrophage effects in other tissues in the placebo control group through animals given 100 mg/kg/d." However, the pathologist noted that "similar macrophages were not noted in sternal bone marrow from diluent or placebo control groups. The NOEL for AMI-6424-related marrow effects (sternum/femur) was 12.5 mg/kg/d in females. There was no no-observed-effect level for this effect in males." This finding persisted throughout the recovery period.

**Histologic Lesions in Rats Treated for 13 Weeks with AMI-6424 (Main Study)**

Group	1♂*	2♂	3♂	4♂	5♂	1♀	2♀	3♀	4♀	5♀
N	13	11	13	12	13	11	12	13	13	12
Marrow (femur) M. hyperpl/trophy	0 0	0 0	4 0.3	12 1.6	13 2.0	0 0	1 0.1	0 0	11 0.8	12 1.6
Marrow (sternum) Macroph. hyperpl/trophy	0 0	0 0	0 0	12 1.1	13 1.0	0 0	0 0	0 0	2 0.2	9 0.8
Kidney-tubular Degeneration	0 0	10 0.9	5 0.4	11 1.2	13 1.7	0 0	9 0.8	0 0	12 1.0	12 1.3
Inflammation	4	9	8	12	12	2	0	0	0	0
Liver- Macroph. hyperpl/trophy	0 0.0	9 0.8	8 0.8	12 1.7	13 3.0	2 0.2	7 0.6	3 0.2	13 1.5	12 2.9
Lung- Macroph. hyperpl/trophy	0 0.0	11 3.0	0 0.0	2 3.0	13 3.1	1 0.1	12 2.8	0 0.0	1 2.0	12 2.9
Thymus- Macroph. hyperpl/trophy	0 0.0	0 0.0	1 0.1	12 1.0	12 1.7	0 0.0	2 0.2	2 0.2	13 1.0	12 1.6
Testes- Macroph. hyperpl/trophy	0 0.0	11 1.0	8 0.6	11 1.3	13 1.8					
Infusion site- chr. inflamm.	13 10	10 1.1	3 4.7	0 0	11 1.5	11 1.1	12 2.8	2 4.0	2 3.5	12 1.4

\*Second # in each box is mean severity score.

At the recovery sacrifice, no organ weight differences from controls were reported. However, the sponsor attributed the increased mean absolute and relative kidney and liver weights to placebo administration. There was a significant decrease in terminal body weights in the AMI-6424-treated animals when compared to controls. Similarly, the sponsor attributed the mottled lungs found at recovery sacrifice to be due to placebo administration but the "diffusely light" kidneys in high dose males to be due to AMI-6424. Reversibility (partial) was reported for placebo and treated animals for the vacuolar change in multiple epithelia and the hepatic degeneration but not for the macrophage hypertrophy/hyperplasia.

Toxicokinetics: One high dose TK animal had no detectable drug levels at both the 2 and 12 hour collection times on Day 1. It is unclear why this happened. Reassay confirmed the low levels.

**Mean TK Parameters of AMI-6424 in Rat Plasma**

Dose (mg/kg/d)	Gender	Cmax (mg/ml)	Tmax (hrs)	AUC 0-24 (µg·hr/ml)	AUC 0-4 (µg·hr/ml)	T1/2 (hrs)
Day 1	Male	77.8	0.5	226	224	1.68
12.5	Female	75.1	0.5	206	205	1.5
50	Male	308	0.5	748	750	2.7
	Female	281	0.5	696	697	2.58
100	Male	476	0.5	1180	1182	2.55
	Female	429	0.5	1078	1079	2.55
Day 90						
12.5	Male	98.5	0.5	366	NA	2.67
	Female	66.1	0.5	347	NA	3.25
50	Male	403	0.5	1375	NA	4.19
	Female	347	0.5	1426	NA	3.44
100	Male	610	0.5	2295	NA	5.28
	Female	503	0.5	2465	NA	4.03

Thus, the plasma levels increased as the dose increased (dose proportional between 12.5 and 50 mg/kg/d and slightly less than dose proportional between 50 and 100 mg/kg/d) and levels were slightly higher on Day 90 than on Day 1. The levels of AUC<sub>0-24</sub> were higher on Day 90 than on Day 1 (60- 130%), indicating accumulation over time or changes in the disposition of the drug after subchronic administration. Similar ratios were found for the AMI-999 metabolite (not the major metabolite) but the increase over time was greater than for parent compound (166-247%).

Other: No significant compound-related differences from controls were noted in bone measurements.

**Summary of individual study findings:** The sponsor considered the 12.5 mg/kg/d group to provide a NOEL except for the non-reversible finding of systemic macrophage hypertrophy/hyperplasia for which no NOAEL could be determined in this study. As the incidence and severity of this finding increased with increasing dose, it is of concern that no NOAEL could be identified.

**02-001-14 7057-168: Exploratory 7-Day Intravenous Toxicity Study with AMI-6424 in Male Rats**

**Study no:** Theravance Reference: AMI CSN: 02-001-14; Covance #7057-168

**Conducting laboratory and location:** Covance Laboratories, Inc., Vienna, VA

**GLP compliance:** "In the spirit of the GLPs"

**QA report:** No

**Methods:** This study was used as the dose range finding study for the fertility studies.

**Dosing:**

Species/strain: Male Crl:CD<sup>®</sup>(SD)IGS BR rats

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

Satellite groups used for toxicokinetics or recovery: None

Doses in administered units: 0 ("Placebo for AMI-6424 for Injection"), or 150 mg/kg/d

Route, form, volume, and infusion rate: I.V. at 15 ml/kg/d as a bolus injection

**Results:**

Mortality: No rats died prematurely.

Clinical signs: Some compound treated animals appeared thin and had scant feces/diarrhea.

Body weights: Decreased mean body weights (-8.4%) and feed consumption (-45%) were reported for the treated animals when compared to the controls.

The sponsor concluded that the doses for the fertility study should be 50, 75 and 100 mg/kg/d. This seems to be a reasonable conclusion although it might be possible to 'push' the dose a bit higher as an 8.4% decrease in body weight may not be biologically significant.

**02-003-01 57387: A 28-Day Intravenous Infusion Toxicity Study (with a 28-Day Recovery Period) of AMI-6424 in the Beagle Dog**

**Key study findings:** Renal tubular effects at mid (25 mg/kg) and high dose (50 mg/kg)

**Study no:** ANU CSN: 02-003-01; 57387

**Conducting laboratory and location:** \_\_\_\_\_ except for the PK, dose analysis and blood drug analyses that were done by Covance Laboratories, Inc., Madison, WI.

**Date of study initiation:** 1/3/02

**GLP compliance:** Yes

**QA report:** Yes

**Drug, lot, and % purity:** AMI-6424, Lot AME001 at 94% purity. Dosing solutions were prepared daily.

**Formulation/vehicle:** The diluent was 5% dextrose; the placebo was "Placebo for AMI-6424 for Injection, 250 mg vial" previously described as the 2500 mg hydroxypropyl- $\beta$ -cyclodextrin with 312.5 mg mannitol with pH adjustment solution.

**Methods:**

**Dosing:**

Species/strain: Beagle dogs. The animals were implanted with vena caval intravenous catheters.

#/sex/group or time point (main study): 4

Satellite groups used for toxicokinetics or recovery: 2

Age: 6-7 months of age at study initiation.

Weight: Males: 7.2- 10 kg; females: 7.0- 9.3 kg

Doses in administered units: 0 (diluent), 0 (placebo), 12.5, 25 or 50 mg/kg/d

Route, form, volume, and infusion rate: I.V. at 5 mL/kg/d and at 10 mL/kg/h over 30 minutes each day

**Observations and times:**

Clinical signs: Twice/day

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Prior to dosing (post-catheter implantation) and during Weeks 4 and 8

EKG: As for ophthalmoscopy. Tracings were interpreted by a board certified veterinary cardiologist.

Hematology: Once pre-dosing and during Weeks 2, 4, 6 and 8

Clinical chemistry: As for hematology

Urinalysis: As for hematology

Gross pathology: All animals at study termination

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Organs weighed: Adrenals, brain, heart, kidneys, liver, lungs, ovaries/testes, pituitary, spleen, thymus, thyroids/parathyroids, and uterus.

Histopathology: All tissues listed in the Histopathology Inventory were examined from all animals and the results were subjected to peer review.

Toxicokinetics: Samples were taken on Days 1 and 28 at the following times: immediately at the end of dosing, 1, 2, 4, 8, 12, and 23.5 hours after the end of the infusion.

**Results:**

Mortality: There were no premature decedents.

Clinical signs: Clinical signs related (possibly) to histaminic reactions were reported, especially prominent at the 50 mg/kg/d dose. None of these effects were noted in the 12.5 mg/kg/d group. The reactions usually started within 20 minutes of dosing initiation and included excessive shaking and/or scratching, swollen limbs and face, erythema and papules. These signs resolved without treatment and by the second week of dosing, tolerance to the drug appeared to develop. Injection site reactions were common across dose groups.

Body weights: There were no treatment-related effects noted.

Food consumption: No significant treatment-related effects were reported.

Ophthalmoscopy: No treatment-related effects were found.

Electrocardiography: "All interval measurements recorded during Weeks 4 and 8 remained comparable with the pretreatment and/or the concurrent control group ranges."

Hematology: One high dose female had a 3x increase in APTT during the fourth week of dosing. No other animals had any significant treatment-related effects.

Clinical chemistry: The high dose males had increased mean BUNs by Week 2, high dose females by Week 4. Placebo females also had elevated BUNs during Week 4. The increases were 25-45% over the controls. One high dose male and one high dose female remained with elevated BUNs throughout the recovery period. Tubular dilatation was also reported in the animals with elevated BUNs.

Urinalysis: Urine volumes were increased in high dose animals (males by Week 2, females by Week 4). A decreased urine specific gravity accompanied the increased volume. This change was prominent in high dose males during Week 2, where their values were less than the lowest control values. The sponsor suggests that this finding is not biologically significant as the females were not affected and males were not affected at later time-points.

Organ weights: No significant treatment-related differences from controls were appreciated.

Gross pathology: No gross treatment-related effects were noted.

Histopathology: Renal tubular vacuolation and degeneration/necrosis (urothelium of the renal pelvis and urinary bladder) were present at comparable incidence across placebo (slight, multifocal, bilateral) and

high dose animals. Similar changes were reported in 1/sex from the mid dose group. This leads to the conclusion that these changes are potentially due to the hydroxypropyl- $\beta$ -cyclodextrin. However, the changes were more significant and more frequent in the treated animals, leading to the conclusion that the active compound contributed to the alteration. All other findings were considered incidental and/or related to the catheterization.

In the recovery animals, the incidence and severity of the renal changes were decreased except in one high dose female (#5662) where there was an exacerbation of the earlier findings (moderate, multifocal, renal tubular dilatation and degeneration/necrosis). The etiology of this exacerbation is not identified. Therefore, it seems appropriate to attribute it to drug until proven otherwise.

Toxicokinetics: Exposure to active compound appeared to increase proportionately with dose. There were no significant gender differences at either timepoint. No accumulation was found after 28 days of dosing.

**Mean PK Parameters from Dogs Treated with AMI-6424**

Dose (mg/kg/d)	Gender	C <sub>max</sub> ( $\mu$ g/ml)	T <sub>max</sub> (hrs)	AUC <sub>0-<math>\infty</math></sub> ( $\mu$ g.hr/ml)	T <sub>1/2</sub> (hrs)
Day 1 12.5	M	102	0.5	263	1.6
	F	119	0.5	290	1.36
25	M	203	0.5	624	1.96
	F	211	0.5	610	1.91
50	M	350	0.5	1147	2.36
	F	337	0.5	1036	2.10
Day 28 12.5	M	98.0	0.5	253	1.7
	F	112	0.5	302	1.75
25	M	177	0.5	600	2.02
	F	201	0.5	602	1.58
50	M	365	0.5	1287	2.79
	F	374	0.5	1190	2.39

The sponsor concluded that the NOAEL for this study was 25 mg/kg/d on the basis of the renal effects noted in the 50 mg/kg/d group. As 1 animal/sex from the mid dose group showed similar effects, the NOAEL for this study should be set at 12.5 mg/kg/d, providing an HED of 6.76 mg/kg in the human.

**03-001-07 7057-199: 26-Week Intravenous Infusion Toxicity and Toxicokinetic Study with AMI-6424 in Rats with a 4-Week Recovery Period**

**Key study findings:** Lesions seen only in the AMI-6424-treated animals included renal tubular degeneration and macrophage accumulation/hypertrophy/hyperplasia in bone marrow, spleen, thymus and duodenum. While these accumulations were similar to those seen in other tissues, they were only found in these tissues in AMI-6424-treated animals. The sponsor assigned the NOEL of 12.5 mg/kg/d for all effects except the bone marrow and mesenteric lymph node macrophage accumulation/hypertrophy/hyperplasia and the epididymal epithelial vacuolation. For these effects, they assign a NOEL of 6.25 mg/kg/d. For the thymic and splenic macrophage accumulation/hypertrophy/hyperplasia, they did not assign a NOEL. For the recovery animals, the sponsor assigned "placebo-associated alterations" a much slower recovery. It is unreasonable to assign different NOELs for different tissues and it appears that the macrophage

accumulation is part of a continuum across tissues. Thus, the conclusion for this study is that it is not possible to assign a NOAEL for the macrophage changes or for the overall study.

**Study no.: Covance 7057-199**

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 8/18/03

**GLP compliance:** Yes

**QA report:** yes

**Drug, lot #, and % purity:** AMI-6424, Lot #AME002 at 94.0% purity with fresh dose preparations made weekly

Ingredients	AMI-6424, 250 mg/vial	Placebo for AMI-6424
AMI-6424 (freebase equiv.)	250 mg	0
Hydroxypropyl- $\beta$ -cyclodextrin	2500 mg	2500 mg
Mannitol, USP	312.5 mg	312.5 mg
IN NaOH, NF	As needed	As needed
IN HCl, NF	As needed	As needed
Water for injection	<1%	~1%

**Methods**

Doses: 0 (diluent control- D5W), 0 (placebo control), 6.25, 12.5 or 50 mg/kg/d

Species/strain: Crl:CD<sup>®</sup>(SD)IGS BR rats

Number/sex/group or time point (main study): 18

Route, formulation, volume, and infusion rate: I.V. over 30 minutes via implanted femoral vein catheters at 5 ml/kg. Sterile saline was infused through the catheters at all times except the daily dosing period.

Satellite groups used for toxicokinetics or recovery: 9/sex/group for PK, 5/sex/group from main study for recovery

Age: 61-69 days of age at study initiation

Weight: Males- 252-322 g; females- 191-236 g

Sampling times: TK samples were taken on Days 1, 90 and during Week 26 from TK animals at the end of the infusion, and 1, 2, 4, 8, 12 and 23.5 hours later. Samples were taken from 3 animals/sex/group at staggered time points so that each animal was bled twice.

**Observation and Times:**

Clinical signs: Twice daily

Body weights: Prior to dosing, weekly for the first 14 weeks and thereafter every 4 weeks until termination.

Food consumption: Daily

Ophthalmoscopy: Prior to dosing and during Week 26 on main study animals only

EKG: Not performed

Hematology: Samples were taken during Week 13, at study termination and the end of the recovery period. Bone marrow smears were made for each animal at termination.

Clinical chemistry: As for hematology

Urinalysis: As for hematology

Gross pathology: All animals at study termination

Organ weights: Adrenals, brain, heart, kidney, liver, lung, ovaries, pituitary, testis, thymus and thyroid with parathyroid

**Histopathology:** Complete tissues were examined from both control groups and the high dose animals only (see Histopathology Inventory). Tissues examined histologically from the low and mid dose animals included gross lesions, liver, kidney, spleen, duodenum, jejunum, ileum, thymus, lungs, lymph nodes, testes, epididymides, femoral bone marrow, sternal bone marrow and ovaries. Lung sections (20/animal) were taken from 2 main study animals/sex from both controls and high dose groups for immunohistochemical analyses.

Peer review: yes (x)

**Results:**

**Mortality:** None of the premature decedents were considered to be treatment-related deaths. There were 4 males and 5 females from the D5W group, 4 males and 6 females from the placebo group, 3/sex from the 6.25 mg/kg/d group, 1 MD male and 2 HD females that died on study or were euthanized in extremis. All but the 12.5 mg/kg/d male were considered to be catheter-related compromise (occlusion, infections, 'retracted'). The mid dose male was considered an "accidental death" due to aggressive behavior.

**Clinical signs:** No treatment-related clinical signs were reported.

**Body weights:** No treatment-related effects on body weights were seen.

**Food consumption:** No treatment-related effects on feed consumption were appreciated.

**Ophthalmoscopy:** No treatment-related ocular effects were noted.

**Hematology:** Decreased red cell counts, hemoglobin and hematocrit were noted in the high dose animals at study termination. APTT was decreased for females (-15%, -24%, -18%, -22%, -16% for the respective groups). The sponsor suggested that these changes were due, at least in part, to the placebo. Mean corpuscular hemoglobin and mean corpuscular volume were decreased in high dose animals at Week 27 only. Absolute lymphocyte numbers were decreased in treated animals with the most pronounced change at the Week 27 test point (-16%, -8%, -15%, -12%, -29% for the respective male groups, and -13%, -34%, -29%, -28%, -35% for the respective female groups).

**Clinical chemistry:** Increased BUN and creatinine (~2x control values) were found in the 50 mg/kg/d animals. The sponsor considered the placebo to be "at least partially responsible for these effects in males." AST and ALT were increased ~2 x in the high dose animals (females > males) when compared to diluent or placebo controls. The sponsor considered the placebo to be "at least partially responsible for these effects in males." No histologic correlates were found.

**Urinalysis:** Increased granular casts were found in the urine of both sexes at 50 mg/kg/d and females only at 12.5 mg/kg/d. No casts were reported in control animals' urine. The sponsor considered the placebo to be "at least partially responsible for these effects in males." Urine occult blood and white cells were increased in treated animals (males all dose levels, females high dose only) in Week 27. The increase was not appreciably decreased during the recovery period.

Of interest is the increased numbers of bacteria in the urine of all treated males during Week 13 and for all high dose males during Week 27.

Gross pathology: No significant gross lesions were found in the most of the premature decedents. In the others, lesions included thickening at the catheter site, enlarged spleens and lymph nodes, and masses and/or "cysts" associated with the catheter as well as distal limb edema.

Gross lesions attributable (according to the sponsor) to placebo included pale kidneys, "light foci/areas affecting the lungs", enlarged renal lymph nodes and infusion site "masses". However, the sponsor also attributed the "diffusely light kidneys" to treatment with AMI-6424.

Organ weights: Main study animals: Kidney weights (mean relative and absolute) were significantly increased in placebo controls when compared to diluent controls. Mean absolute and relative kidney weights were significantly increased in the high dose animals when compared to diluent controls and slightly higher than placebo values. Mean relative and absolute lung weights were increased in high dose animals when compared to either control group. These organ weight differences from male controls persisted through the recovery period but the numbers were increased only when compared to diluent controls.

Treatment-related differences from controls were appreciated in the recovery animals. In males, increased absolute and relative kidney and lung weights were increased in the placebo control animals when compared to the diluent controls. These weights were even greater in the high dose animals. In females, placebo-associated differences included increased liver and kidney weights. These weights were even greater in the high dose animals.

#### Histopathology:

Catheter related abscesses increased in incidence and severity with increasing dose of AMI-6424.

Urinary tract lesions noted in placebo controls and treated animals included vacuolar change in renal tubular epithelium, increased interstitial lymphocytic inflammatory cell infiltrates, tubular dilatation and/or cast formation, and urothelial vacuolation in the urinary bladder and renal pelvices. While most of these findings were also seen in the placebo controls, the incidence and severity increased with increasing dose of AMI-6424. Additionally, the urinalyses showed occult blood and white cells that were increased in treated animals. Lesions seen only in the AMI-6424-treated animals included renal tubular degeneration and macrophage accumulation/hypertrophy/hyperplasia in bone marrow, spleen, thymus and duodenum. While these accumulations were similar to those seen in other tissues, they were only found in these tissues in AMI-6424-treated animals.

Lung lesions included increased perivascular inflammation that the sponsor attributed to placebo administration. Mean relative and absolute lung weights were increased when compared to either of the control groups.

Vacuolation was reported in epididymal epithelial cells and lymphoid venules (within lymph nodes and Peyer's Patches).

Systemic macrophage accumulation/hypertrophy/hyperplasia was found in many tissues that the sponsor attributed to placebo administration. However, the incidence and severity increased with increasing dose, especially in the high dose animals (Grade 3 vs. Grade 1 in placebo controls). Hematopoietic hyperplasia in femoral and sternal marrow also increased in both incidence and severity with increasing dose.

**Microscopic Lesions with AMI-6424 (Terminal Sacrifice + Recovery Animals)**

Lesion	Dil		Plac		6.25 mg/kg/d		12.5 mg/kg/d		50 mg/kg/d	
	M	F	M	F	M	F	M	F	M	F
No. of animals	14	14	14	14	13	13	13	13	13	13
Kidney-tubular vacuolation	0	0	14 (2.8)	14 (3.1)	13 (1.1)	13 (1.1)	12 (2.1)	13 (2.4)	13 (4.0)	13 (4.0)
Kidney-tubular dilatation/casts	0	0	5	5	0	0	6	0	18	15
Kidney-proximal tubular degeneration	0	0	0	0	0	0	0	0	9 0.7	1 0.1
Liver-Macrophage accumulation	0	0	7 (0.5)	0	0	0	0	0	13 (3.0)	13 (2.0)
Lung-Macrophage accumulation	1 (0.1)	2 (0.1)	14 (1.7)	13 (2.1)	6 (0.5)	7 (0.5)	10 (0.8)	10 (0.8)	13 (2.9)	13 (2.9)
Lung-chronic Perivascular inflam.	3 (0.4)	4 (0.3)	10 (0.8)	8 (0.6)	4 (0.6)	6 (0.5)	5 (0.4)	9 (0.7)	10 (0.8)	9 (0.9)
Infusion site abscess	3 (0.8)	3 (0.8)	6 (2.1)	11 (3.2)	6 (3.0)	7 (2.8)	3 (2.4)	10 (3.3)	11 (3.5)	7 (2.1)
Marrow-hyperplasia/hematopoietic (femur/stern)	6/ 11	13/ 13	10/ 13	12/ 12	9/ 12	14/ 15	9/ 14	18/ 18	17/ 16	16/ 16
Marrow Macro. accumulation	0	0	0	0	0	0	5	1	13	10

( ) = degree of severity from 0-4 = mean severity score

Toxicokinetics: The AUC and Cmax increased essentially proportionally to dose. Plasma levels were higher after multiple dosing than on Day 1. The T 1/2 was longer after multiple dosing than on Day 1. The AUC values were higher on the 2 later test points than on Day 1. Similar conclusions can be drawn for the metabolite, AMI-999 but the T 1/2 increased ~3x after multiple dosing and the AUC and Cmax were increased in a greater than dose proportional fashion.

**Mean Toxicokinetic Parameters for AMI-6424 in Rat Plasma**

Group	Dose (mg/kg/day)	Sex	Cmax (µg/ml)	AUC0-t (µg-hr/ml)	AUC0-∞ (µg-hr/ml)	T1/2 (hr)
Day 1	6.25	M	44.4	125	127	1.24
		F	36.4	116	118	1.29
	12.5	M	82.5	244	244	1.22
		F	79.0	248	249	1.48
	50	M	282	891	893	2.83
		F	315	942	944	2.66
Day 90	6.25	M	40.5	135	135	NC
		F	51.1	164	164	NC
	12.5	M	132	428	428	NC
		F	105	353	353	2.33
	50	M	435	1357	1357	3.73
		F	431	1542	1542	3.64
Week 26	6.25	M	26.4	88.1	88.1	NC
		F	32.9	126	126	NC
	12.5	M	63.6	255	255	NC
		F	76.2	334	334	3.32
	50	M	191	1012	1012	4.48
		F	157	1227	1227	4.58

It is interesting that the sponsor assigned the NOEL of 12.5 mg/kg/d for all effects except the bone marrow and mesenteric lymph node macrophage accumulation/hypertrophy/hyperplasia and the epididymal epithelial vacuolation. For these effects, they assign a NOEL of 6.25 mg/kg/d. For the thymic and splenic macrophage accumulation/hypertrophy/hyperplasia, they did not assign a NOEL. For the recovery animals, the sponsor assigned "placebo-associated alterations" a much slower recovery. It is unreasonable to assign

different NOELs for different tissues. Thus the conclusion for this study is that it is not possible to assign a NOAEL for the macrophage changes or the overall study.

No NOEL has been set in any of the preclinical studies performed with AMI-6424. This is a significant concern when coupled with the increased incidence of catheter-related abscesses with increasing dose of AMI-6424. As it is impossible to separate out vehicle-induced toxic effects from drug-induced toxic effects, the level of concern for the overall safety of this product is in question, especially when coupled with the teratologic effects seen in the reproductive toxicity studies in more than one species.

#### Histopathology inventory

Study	7057-199	7057-112	7057-111	7057-119	02-003-01	7057-144	7057-148	02-003-05
Species	Rat	Dog	Rat	Dog	Dog	Rat	Rat	Dog
Adrenals	X	X	X	X	X	X	X	X
Aorta	X	X	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X	X	
Bone (femur)	X	X	X	X		X	X	X
Brain	X	X	X	X	X	X	X	X
Cecum	X	X	X	X	X	X	X	X
Cervix	X	X	X	X	X	X	X	X
Colon	X	X	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X	X	X
Esophagus	X	X	X	X	X	X	X	X
Eye	X	X	X	X	X	X	X	X
Fallopian tube								
Gall bladder		X		X	X			X
Gross lesions	X	X	X	X	X	X	X	X
Harderian gland	X		X			X	X	
Heart	X	X	X	X	X	X	X	X
Ileum	X	X	X	X	X	X	X	X
Injection site	X	X	X	X	X	X	X	X
Jejunum	X	X	X	X	X	X	X	X
Kidneys	X	X	X	X	X	X	X	X
Lachrymal gland	X	X	X	X		X	X	
Larynx								
Liver	X	X	X	X	X	X	X	X
Lungs	X	X	X	X	X	X	X	X
Lymph nodes, cervical								
Lymph nodes mandibular					X			X
Lymph nodes, mesenteric	X	X	X	X	X	X	X	X
Mammary Gland	X	X	X	X		X	X	X
Nasal cavity								
Optic nerves	X	X	X	X	X	X	X	X
Ovaries	X	X	X	X	X	X	X	X
Pancreas	X	X	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X	X	X
Peripheral nerve	X	X	X	X	X	X	X	X
Pharynx								
Pituitary	X	X	X	X	X	X	X	X
Prostate	X	X	X	X	X	X	X	X
Rectum	X	X	X	X		X	X	

Salivary gland	X	X	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X	X	X
Seminal vesicles	X		X			X	X	
Skeletal muscle	X	X	X	X	X	X	X	X
Skin	X	X	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X	X	X
Spleen	X	X	X	X	X	X	X	X
Sternum	X	X	X	X	X	X	X	X
Stomach	X	X	X	X	X	X	X	X
Testes	X	X	X	X	X	X	X	X
Thymus	X	X	X	X	X	X	X	X
Thyroid	X	X	X	X	X	X	X	X
Tongue	X			X	X			X
Trachea	X	X	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X	X	X
Uterus	X	X	X	X	X	X	X	X
Vagina	X	X	X	X	X	X	X	X

X, histopathology performed

#### 6.6.6.4 Genetic toxicology

##### **01-001-10 22005-0-409: Salmonella - Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with AMI-6424 (Trifluoroacetic Acid Salt)**

**Key findings:** No increases in the mean number of revertants were reported. The drug substance is not mutagenic under the conditions of this study.

**Study no:** Covance 22005-0-409; AMI CSN: 00-001-10

**Conducting laboratory and location:** Covance Laboratories, Vienna, VA

**Date of study initiation:** 12/11/00

**GLP compliance:** Yes but stability and dosing solution concentrations were not evaluated.

**QA reports:** Yes

**Drug, lot #:** AMI-6424 (trifluoroacetic acid salt), Lot 26

#### **Methods:**

Strains/species/cell line: *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA.

Dose selection criteria:

Basis of dose selection: Not specified

Range finding studies: Not specified

Test agent stability: Not evaluated

Metabolic activation system: S9 homogenate from Aroclor 1254-induced rat liver

Vehicle: Water

Positive controls: Benzo[a]pyrene (TA98), 2-aminoanthracene (TA100, TA1535, TA1537, WP2uvrA) with S9; 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), ICR-191 (TA1537), 4-nitroquinoline-N-oxide (WP2uvrA). These were plated in triplicate

**Exposure conditions:**

Doses used in definitive study: 5000, 3300, 1000, 333, 100, 33.3, 10, 3.33, 1 and 0.333 µg/plate with and without S9.

**Analysis:**

No. of replicates: Each test plate was a duplicate, and each control plate was a triplicate.

Counting method: Not specified but presumed standard microscopic evaluation.

Criteria for positive results: Not specified but presumed standard Ames assay counting of revertant colonies.

**Summary of individual study findings:**

Study validity: The positive and negative controls performed as expected so the study was considered valid.

Study outcome: No increase in the number of revertants was found in any of the tester strains with or without S9. Thus, AMI-6424 is considered non-mutagenic in this assay.

**01-001-03 22005-1-409OECD: *Salmonella-Escherichia coli*/Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with AMI-6424**

**Key findings:** No increases in the mean number of revertants were reported. The drug substance is not mutagenic under the conditions of this study.

**Study no:** Covance 22005-1-409OECD; AMI CSN: 01-001-03

**Conducting laboratory and location:** Covance Laboratories Inc, Vienna, VA

**Date of study initiation:** 4/11/01

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, % purity:** AMI-6424, Lot # 1 AB03016 presumed 100% purity

b(4)

**Formulation/vehicle:** DMSO or water

**Methods:**

Strains/species/cell line: *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2uvrA

Dose selection criteria:

Basis of dose selection: Range finding study

Range finding study: Using TA100 and WP2uvrA strains with and without S9 and 10 doses of test article at one plate per dose at a maximum of 5000 µg/plate. Cytotoxicity was found in TA100 at ≥333 µg/plate with and without S9, in WP2uvrA at ≥33.3 µg/plate with S9 and at ≥1000 µg/plate without S9.

Test agent stability: The sponsor was responsible for this information

Metabolic activation system: S9 liver microsomal enzymes from male Sprague-Dawley Aroclor 1254-treated rats

Controls:

Vehicle: Water

Positive controls: Benzo[a]pyrene (TA98), 2-aminoanthracene (TA100, TA1535, TA1537, WP2uvrA) with S9; 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), ICR-191 (TA1537), 4-nitroquinoline-N-oxide (WP2uvrA). These were plated in triplicate.

Exposure conditions: Standard Ames assay protocol.

Study design: Standard OECD Guideline 471 for standard Ames assay. Doses were 1.0, 3.33, 10.0, 33.3, 100, 333 and 1000 µg/plate for the *Salmonella* strains and 10.0-5000 µg/plate for the *E. coli*. The results from these plates were used to determine the doses for the confirmatory study.

**Analysis:**

No. of replicates: All plates were done in triplicate.

Counting method: Macroscopically and microscopically and revertant colonies by automated counter.

Criteria for positive results: A 2x increase (TA98, TA100 and WP2uvrA) and 3x increase (TA1535 and TA1537) compared to controls with a dose response to increasing concentrations of test article.

**Summary of individual study findings:**

Study validity: Cultures of *Salmonella* retaining sensitivity to crystal violet, TA98 and TA100 retained resistance to ampicillin, tester strains retained a 'characteristic' number of spontaneous revertants, and the positive controls achieved at least a 3x increase in revertants compared to controls.

All of these conditions were met.

**Study outcome:**

None of the doses of AMI-6424 elicited an increase in the mean number of revertants in any of the tester strains with or without S9 metabolic activation.

**01-001-04 22005-0-449OECD: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes**

**Key findings:** No induction of chromosomal aberrations was reported.

**Study no:** Covance 22005-0-449OECD; AMI CSN: 01-001-04

**Conducting laboratory and location:** Covance Laboratory Inc., Vienna, VA

**Date of study initiation:** 4/11/01

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, % purity:** AMI-6424, Lot: / AB03016. Each gram of AMI-6424 (hydrochloric acid salt) contains 781.3 mg of the free base. A correction factor of 1.28 was used as a divisor to convert test article weight to the free base weight.

b(4)

**Methods:**

Strains/species/cell line: Cultured human lymphocytes from a healthy adult donor

Dose selection criteria:

Basis of dose selection: Precipitation (At 2470 µg/mL, the test article precipitated forming an opaque, light red, homogeneous suspension.)

Metabolic activation system: Post-mitochondrial fraction (S9) from the livers of male rats treated with Aroclor 1254.

Controls:

Vehicle: In the initial assay, water.

Negative controls: Cells in culture medium alone

Positive controls: Mitomycin C for the cultures without S9 activation and Cyclophosphamide with S9 activation.

**Exposure conditions:**

Incubation and sampling times: 3 hours with and without S9, and 19 hours without metabolic activation with harvest 22 hours later.

Doses used in definitive study: 17.2, 24.5, 35.0, 50.0, 71.4, 102, 145, 207, 295, 422, 603, 861, 1230, 1750, 2500 µg free base/mL with or without S9.

Study design: Cultures treated with 207, 422, 861 and 1750 µg free base/mL without S9, and 207, 295, 422 and 603 µg free base/mL with S9 were analyzed for chromosomal aberrations.

**Analysis:**

No. of replicates: Duplicates were made for all cultures.

Counting method: 100 cells from each duplicate culture from 4 concentrations of test article were analyzed with negative, vehicle and one dose level from the positive controls.

Criteria for positive results: Significant increase ( $p < 0.01$ ) in the number of cells with chromosomal aberrations at one or more concentrations. A dose-response should accompany this change.

**Summary of individual study findings:**

Study validity: Acceptable controls, acceptable high dose and acceptable number of doses.

Study outcome: In the cultures without metabolic activation, a precipitate was seen in the 1750 and 2500 µg/mL plates and a slight precipitate at  $\geq 861$  µg/mL. Reductions in the mitotic indices were found in these cultures when compared to the controls. No significant increases in aberrations, polyploidy or endoreduplication were reported.

In the cultures with metabolic activation, a precipitate was seen in the cultures with  $\geq 603$  µg/mL. Reductions were noted in the mitotic indices of cultures at  $\geq 50$  µg/mL. Chromosomal aberrations were evaluated in cultures treated with 207-861 µg/mL. No significant increases in aberrations, polyploidy or endoreduplication were reported.

In the confirmatory assay concentrations were 26-1750 µg/mL for cultures without S9 and 52-1750 µg/mL with metabolic activation. No significant increases in aberrations, polyploidy or endoreduplication were reported in either assay.

**02-001-08 23353-0-409OECD: Salmonella-Escherichia coli/Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with AMI-6424**

**Key findings:** No increases in the mean number of revertants were reported. The drug substance is not mutagenic under the conditions of this study.

**Study no:** Covance 23353-0-409OECD; AMI CSN: 02-001-08

**Conducting laboratory and location:** Covance Laboratories Inc, Vienna, VA

**Date of study initiation:** 1/30/2002

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, % purity:** AMI-6424, Lot: A0 4630101D

**Methods:**

Strains/species/cell line: *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2uvrA

Range finding studies: Test article at 1, 3.33, 10.0, 33.3, 100, 333, 1000 and 5000 µg/plate with/without S-9 in all tester strains. No positive increases were noted.

Test agent stability: Appropriate samples were taken from both assays for analysis.

Metabolic activation system: S-9 from male Sprague-Dawley rats injected with Aroclor 1254

Controls:

Vehicle: Water

Positive controls: For TA98- benzo[a]pyrene (with S-9) and 2-nitrofluorene (without S-9); for TA100, TA1537 and TA1535- 2-aminoanthracene (with S-9) and sodium azide (without S-9); for TA1537-ICR-191 (without S-9); for WP2uvrA- 2-aminoanthracene (with S-9) and 4-nitroquinoline-N-oxide (without S-9)

Exposure conditions:

Incubation and sampling times: As per Ames methodology

Doses used in definitive study: 1.00, 3.33, 10.0, 33.3, 100, 333 and 1000 µg/plate for the *Salmonella* strains (except TA 1535 where the doses added were 3330 and 5000 µg/plate). For the E. coli strain, doses were 10.0, 33.3, 100, 330, 1000 and 5000 µg/plate.

Analysis:

No. of replicates: All plates were done in triplicate.

Counting method: Automated colony counter

Criteria for positive results: A 2x increase (TA98, TA100 and WP2uvrA) and 3x increase (TA1535 and TA1537) compared to controls with a dose response to increasing concentrations of test article.

**Summary of individual study findings:**

Study validity: The positive controls performed as expected. This study is considered valid.

Study outcome: No increases in the mean number of revertants/plate were found in any of the strains, with/without S-9 added.

**01-001-05 22005-0-455OECD: In Vivo Mouse Micronucleus Assay with AMI-6424**

**Key findings:** No increase in micronuclei were noted at any dose

**Study no:** Covance 22005-0-455OECD; AMI CSN: 01-001-05

**Conducting laboratory and location:** Covance Laboratories, Inc., Vienna, VA

**Date of study initiation:** 5/22/01

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** AMI-6424, AMB001 assumed 100% purity

**Methods:**

Strains/species/cell line: Crl:CD-1(ICR)BR mice, 5/sex/group

Range finding studies: Three male Crl:CD-1(ICR)BR mice were dosed with 75 mg/kg i.v. Due to excessive mortality, the definitive doses were 12.5, 25 and 50 mg/kg in males and females. Due to the potential of systemic toxicity, a second group of 3 mice/sex was dosed at 50 mg/kg.

Test agent stability: Not tested for this study.

Metabolic activation system: None

Controls:

Vehicle: AMI-6424 vehicle

Negative controls: 5% dextrose

Positive controls: 80 mg/kg cyclophosphamide

Doses used in definitive study: the definitive doses were 12.5, 25 and 50 mg/kg in males and females. Signs of toxicity were found in 50 mg/kg animals (hypoactivity, labored breathing, hyperactivity, cold, prostration, tremors).

Study design: Animals (5/sex/ 12.5 or 25 mg/kg or positive controls) were dosed and sacrificed 24 hours after dosing. The remaining animals were sacrificed 24 or 48 hours post-dosing. Bone marrow was extracted and at least 2000 polychromatic erythrocytes/animal were evaluated.

Analysis: Cytotoxicity was evaluated by scoring the number of PCEs and NCEs in at least 500 erythrocytes/animal.

**Summary of individual study findings:**

Study validity: The controls (positive and negative) performed as predicted and were consistent with historical controls from this laboratory.

Study outcome: Neither increases in the number of micronucleus PCEs nor decreases in NCE/NCE were reported under the conditions of this study.

**Genetic toxicology summary:** No indication of genetic toxicity was found in any of the assays performed.

**2.6.6.5 Carcinogenicity**

No studies were performed.

**2.6.6.6 Reproductive and developmental toxicology**

**01-001-24 7507-125: Range-finding Intravenous Injection Toxicity Study of AMI-6424 in Pregnant Female Rats**

**Key study findings:** Decreases in maternal body weight gain and food consumption, and pale kidneys in necropsy were noted in animals treated at  $\geq 150$  mg/kg. Doses of 50, 100, and 150 mg/kg/day were recommended for the definitive study.

**Study no:** Theravance Reference: AMI CSN: 01-001-24; Covance #7057-125

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 9/13/01

**GLP compliance:** No

**QA report:** No

**Drug, lot #, and % purity:** AMI-6424, Lot No. AMB001 and AME001 at 90% purity

**Vehicle:** Vehicle for AMI-6424 Injectable Solution,

**Methods:**

**Dosing:**

Species/strain: Crl:CD<sup>®</sup>(SD)IGS BR rats.

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

Satellite groups used for toxicokinetics or recovery: No

Age: 12-week old

Weight: 205-246 g

Doses in administered units: See table below

Route, form, volume, and infusion rate: I.V.

The females were mated at the supplier using males of the same strain. The day of confirmation was designated as GD 0. The study design is listed in the table below.

Group	N of Females	Dose (mg/kg/day)	Concentration (mg/mL)	Dosing volume (mL/kg)	Dosing schedule (days of gestation)
1 D5W	5	0	0	5	6-17
2 Vehicle for AMI-6424 injectable solution	5	0	0	5	6-17
3 Low	5	6.25	1.25	5	6-17
4 Mid-low	5	12.5	2.5	5	6-17
5 Mid-high	5	25.0	5.0	5	6-17
6 High	5	50.0	10.0	5	6-17
7 Vehicle for AMI-6424 injectable solution	5	0	0	7.5	6-17
8 High	5	75.0	10.0	7.5	6-17
9 Placebo Control	5	0	0	20	6-17
10 Low	5	100	10.0	10	6-17
11 Mid	5	150.0	10.0	15	6-17
12 High	5	200.0	10.0	20	6-17

Parameters evaluated included clinical signs, body weights, food consumption, clinical chemistry, sacrifice at Cesarean section, and fetal examination.

**Results:** The following positive findings were noted.

**Body weights:** Treatment with AMI-6424 at doses  $\geq 150$  mg/kg (Groups 11 and 12) during the treatment period (GD 6 to 17) resulted in decreases in mean maternal body weight gain of 27 and 32%, respectively, when compared to placebo controls (Group 9).

**Food consumption:** Decreases in food consumption data were observed in animals treated with AMI-6424 at doses of 150 and 200 mg/kg/day (11 and 14%, respectively) when compared to placebo control (Group 9).

**Parental necropsy:** Treatment-related necropsy findings were limited to an increased incidence of pale kidneys (4/5 and 3/5 vs. 1/5 in placebo treated animals) at doses  $\geq 150$  mg/kg/day.

Decreases in mean fetal weights were observed in rats treated at doses of 150 and 200 mg/kg/day (3.55 g and 3.37 g vs. Group 9 control's 3.62 g). However, as these weights were similar to those observed in Group 7 (vehicle for AMI-6424, 3.36 g), they are equivocal and are not attributed to AMI-6424.

**Summary:** Treatment with AMI-6424 at doses of 150 and 200 mg/kg/day caused decreases in mean body weight gain and food consumption. An increased incidence of pale kidneys was also noted at necropsy in rats administered doses  $\geq 150$  mg/kg/day. Cesarean section data indicated that AMI-6424 had no effect on embryo/fetal viability. Mean fetal weight data were equivocal and decreases could not be attributed to AMI-6424. Based on these results, the sponsor chose the doses of 50, 100, and 150 mg/kg/day for the rat developmental study.

**01-001-19 7507-115: Range-finding Intravenous Injection Toxicity Study of AMI-6424 in Nonpregnant Female Rabbits**

**Key study findings:** Decreases in body weight gain and food consumption were noted in animals treated at 25 and 50 mg/kg. Doses of 6.25, 12.5, 25 and 40 mg/kg/day were recommended for a subsequent dose range-finding study in pregnant rabbits.

**Study no:** Theravance Reference: AMI CSN: 01-001-19; Covance #7057-115  
**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI  
**Date of study initiation:** 9/4/01  
**GLP compliance:** No  
**QA report:** No  
**Drug, lot #, and % purity:** AMI-6424, Lot No. AMB001 at 90% purity  
**Vehicle:** Vehicle for AMI-6424 Injectable Solution,

**Methods:****Dosing:**

Species/strain: Premated female Hra:(NZW)SPF rabbits  
 #/sex/group or time point (main study): 5/group  
 Satellite groups used for toxicokinetics or recovery: No  
 Age: 6 months old  
 Weight: 3500 g  
 Doses in administered units: See table below  
 Route, form, volume, and infusion rate: I.V. at 5 ml/kg, once daily for 14 days

The study design is listed in the table below.

Group	N of Females	Dose (mg/kg/day)	Concentration (mg/mL)	Dosing volume (ml/kg)
1 D5W (5% dextrose injection)	5	0	0	5
2 Vehicle for AMI-6424	5	0	0	5
3 Low	5	6.25	1.25	5
4 Mid-low	5	12.5	2.5	5
5 Mid-high	5	25.0	5.0	5
6 High	5	50.0	10.0	5

Parameters evaluated in this study included clinical signs, body weights, food consumption, clinical pathology, and necropsy.

**Results:**

**Clinical signs:** Compound-related clinical observations were limited to findings of few feces and thin appearance in the 50 mg/kg/day group.

**Body weights:** Treatment with AMI-6424 resulted in decreases in body weight gain of 26% in Group 5 (25 mg/kg) and body weight loss in Group 6 (50 mg/kg) animals.

**Food consumption:** Decreases in food consumption data were observed in animals treated with AMI-6424 at doses of 25 and 50 mg/kg/day (846-866 g for Group 5 and 432-509 g for Group 6) when compared to control animals (1148-1284 g for Group 1 and 1032-1306 g for Group 2).

**Summary:** Compound-related changes in this study were limited to clinical observations (thin appearance and few feces), decreased food consumption, and associated body weight loss at 50 mg/kg/day. At a dose of

25 mg/kg/day, a decrease in mean body weight gain was observed. Mean food consumption values was also lower. Based on the results of this study, the sponsor chose the doses of 6.25, 12.5, 25, and 40 mg/kg/day for a subsequent dose range-finding study in pregnant rabbits.

**01-001-23 7507-123: Range-finding Intravenous Injection Toxicity Study of AMI-6424 in Pregnant Female Rabbits**

**Key study findings:** Decreases in maternal body weight gain and food consumption were noted in animals treated at 25 and 40 mg/kg. Doses of 12.5, 25 and 45 mg/kg/day were recommended for the definitive study.

**Study no:** Theravance Reference: AMI CSN: 01-001-23; Covance #7057-123

**Conducting laboratory and location:** Covance Laboratories, Inc., Madison, WI

**Date of study initiation:** 10/15/01

**GLP compliance:** No

**QA report:** No

**Drug, lot #, and % purity:** AMI-6424, Lot No. AMB001 at 90% purity

**Vehicle:** Vehicle for AMI-6424 Injectable Solution,

**Methods:**

**Dosing:**

Species/strain: Female Hra:(NZW)SPF rabbits

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

Satellite groups used for toxicokinetics or recovery: No

Age: 3-4 months old

Weight: 2816-3715 g

Doses in administered units: See table below

Route, form, volume, and infusion rate: I.V. at 5 ml/kg, once daily for GD7 to GD 20

The study design is listed in the table below.

Group	N of Females	Dose (mg/kg/day)	Concentration (mg/mL)	Dosing volume (ml/kg)
1 D5W (5% dextrose injection)	5	0	0	5
2 Vehicle for AMI-6424	5	0	0	5
3 Low	5	6.25	1.25	5
4 Mid-low	5	12.5	2.5	5
5 Mid-high	5	25.0	5.0	5
6 High	5	40.0	8.0	5

Parameters evaluated in this study included clinical signs, body weights, food consumption, clinical pathology, Cesarean section examination, and fetal examination.

**Results:** The following positive findings were noted.

**Clinical signs:** Compound-related clinical observations were limited to few or no feces in four of five 40 mg/kg/day rabbits.

**Body weights:** Treatment with AMI-6424 during the treatment period (GD 7 to 20) resulted in decreases in maternal body weight gain (162.8 g for Group 5 and 5.4 g for Group 6) when compared to controls (212.8 g for Group 1 and 278.8 g for Group 2). Body weight was unaffected at doses  $\leq$  12.5 mg/kg/day.

Food consumption: Decreases in food consumption were observed in animals treated with AMI-6424 at 25 and 40 mg/kg/day (10 and 29%, respectively) when compared to control groups. Food consumption was unaffected at doses  $\leq$  12.5 mg/kg/day.

Caesarean section: Although the mean number of live fetuses was similar across groups, mean fetal weight for the 40 mg/kg/day group (36.57 g vs. control's 42.23 and 42.39 g) was slightly lower. However, fetal weights in the 40 mg/kg/day group were similar to mean fetal weights for the group receiving AMI-6424 at the lowest dose (6.25 mg/kg/day, 38.55 g) and there was no dose response. In light of these observations, the relationship between AMI-6424 and fetal weights could not be established.

Fetal external evaluations: No external fetal variations were observed. The malformation of protruding tongue was observed in one fetus from the 40 mg/kg/day group. As this finding was observed in only one fetus, a definitive relationship to treatment can not be established.

Summary: Compound-related changes in this study were limited to clinical observations (few or no feces), decreased food consumption, and decreased body weight gain at 25 and 40 mg/kg/day. Of uncertain relationship to treatment were lower fetal weights and a malformation of protruding tongue in one fetus at 40 mg/kg/day. The sponsor chose the doses of 12.5, 25, and 45 mg/kg/day for the definitive rabbit study.

**02-001-05 7057-128: Intravenous Injection Study of Fertility and Early Embryonic Development to Implantation with AMI-6424 in Rats: Amended Final Report**

**Key study findings:** No NOAEL was set for the male reproductive effects. Sperm motility and epididymal counts were decreased and abnormal morphology spermatozoa were increased in placebo control and AMI-6424-treated males. The placebo elicited similar, yet less extensive, findings but the effects were more significant in the AMI-6424-treated groups. The NOAEL for effects on fertility indices (embryo/fetal viability) after 28 days of dosing is 150 mg/kg/day for females.

**Study no.:** Covance 7057-128 or AMI CSN:02-001-05

**Conducting laboratory and location:** Covance Laboratories, Vienna, VA

**Date of study initiation:** 5/16/02

**GLP compliance:** Yes

**QA reports:** yes

**Drug, lot #, and % purity:** AMI-6424, Lot A04630101D and A04630201D with purities of ~88%.

**Formulation/vehicle:** Placebo for AMI-6424 per vial consisting of 2500 mg hydroxypropyl- $\beta$ -cyclodextrin (Kleptose HPBJ), 312.5 mg mannitol, with pH adjustment to 4.5

**Methods:**

Species/strain: Crl:CD<sup>®</sup>(SD)IGS rats, aged 10 weeks at study initiation

Doses employed: 0 [diluent control, 5% dextrose injection (Group 1)], 0 [placebo control (Group 2)], 50, 75 or 100 mg/kg/day (males) or 50, 100 or 150 mg/kg/day (females) in dose volumes of 5 mL/kg/d, 7.5 mL/kg/d, 10 mL/kg/d, respectively (Groups 3, 4, and 5).

Route of administration: I.V. once daily by slow bolus injection (over ~ 3 minutes) into lateral tail vein

Study design: The males were dosed for at least 28 days prior to mating and females for at least

14 days prior to mating. Both were dosed throughout the mating period and females were dosed until Gestation Day 7 (GD 7).

Number/sex/group: 20

Parameters and endpoints evaluated: Morbidity and mortality, body weights, feed consumption, breeding parameters, estrous cycle determination, confirmation of breeding, gross necropsy, Caesarian section parameters (GD 13, number and placement of implantation sites, number of live:dead feti, resorptions, numbers of corpora lutea), weights of male reproductive organs, sperm analysis (first 10 males/group) and histopathologic evaluation of male reproductive organs with qualitative spermatogenic staging.

**Results:**

Mortality: No treatment-related deaths were reported. One premature mid-dose male death was noted but not considered related to treatment.

Clinical signs: "Scaling" of the tail was noted on Days 56 and 63 in placebo, mid and high dose males and was related to treatment but not necessarily active ingredient in the formulation. Females had "sores" on Day 3 in low and high dose groups but this was a transient finding.

Body weight: Mean body weights in all males were significantly reduced at all doses when compared to the diluent controls. Absolute body weights for placebo-treated males were less than diluent controls beginning on Day 49. Decreased body weight gain was seen at MD (15%) and HD (27%) animals relative to the placebo control animals.

In females, mean body weight changes for placebo controls and all treated groups were significantly less than the diluent controls with weight loss in the mid and high dose females on Days 0-3. After that point in time, no weight effects were noted in any females that were not confirmed pregnant. In the pregnant females, mean maternal weight gains was significantly lower for placebo controls and all dosed females when compared to the diluent control females.

Food consumption: Food consumption was significantly decreased for mid and high dose males during the pre-mating period (up to 10%) and correlated with the decreased body weights. During the pre-mating period, high dose females had significantly decreased feed consumption. During gestation, the maternal feed consumption was decreased (9-12%) when compared to diluent controls in all dose groups.

Toxicokinetics: Not performed

*For fertility studies:*

In-life observations: No significant treatment-related effects were noted on estrous cycle patterns.

Terminal and necropsic evaluations:

Males: No significant treatment-related effects were reported from the necropsies.

Mean final body weights were reduced for placebo and AMI-6424-treated males. Epididymal weights were decreased in these same groups but relative weight was similar among groups. Mean testis-to-body weight percentages were increased in AMI-6424-treated animals but not absolute testicular weights.

Sperm motility (decreased 76%- 41% of control values in respective dose groups) and epididymal sperm counts (up to 35% but not in a dose-related fashion) were decreased and abnormal morphology spermatozoa were increased in placebo control and AMI-6424-treated males. The percentage of abnormal spermatozoa increased in a dose-related fashion and was comparable in the placebo controls and the low dose animals but was primarily due to one placebo animal with an extremely high level of abnormalities. In

MD and HD animals, the sperm motility was lower than the placebo control and the abnormal sperm morphology was higher than the placebo control, suggesting AMI-6424 was responsible for the abnormal changes.

It seems reasonable to conclude that while the placebo may have elicited some alterations in male fertility parameters, the addition of the active compound, AMI-6424, had even more significantly abnormal findings which occurred in a dose-related fashion. While the abnormalities appear real, there was no significant effect on fertility indices. It is questionable whether this was due to short term administration prior to mating (28 days) instead of a more standard dosing for at least 2 spermatogenic cycles.

Females: Pale kidneys were found in placebo and AMI-6424-treated animals (6/20 in placebos, 6/20 in high dose).

*For embryofetal development studies:*

Terminal and necroscopic evaluations:

Dams: No compound-related effects were noted on pregnancy rate, live:dead feti, number of corpora lutea, implantation sites, implantation losses or resorptions.

**03-001-04 7057-197: 6 Week Intravenous Injection Study of Potential Gonadal Effects and Reversibility with AMI-6424 in Male Rats with an 8 Week Recovery Period**

**Key study findings:** Based upon clinical signs (increased touch sensitivity, tail effects), body weights (~10% decrease in the 100 mg/kg/d group) and feed consumption, the NOAEL for this study would be 50 mg/kg/d. However, given the effects on the epididymides (sloughed epithelium) and sperm counts (decreased numbers) and morphology (increased abnormal sperm), the NOAEL for functional effects in males is set at 25 mg/kg/d. All findings except epithelial vacuolation in the epididymides were reversible within the 8 week recovery period.

**Study no.:** Covance #7057-197/ Theravance #03-001-04

**Conducting laboratory and location:** Covance Laboratories, Inc., Vienna, VA

**Date of study initiation:** 6/3/03

**GLP compliance:** Yes

**QA reports:** yes

**Drug, lot #, and % purity:** AMI-6424, Lot AME004 with 90% purity

**Methods**

Doses: 5% dextrose for injection (diluent control), placebo for AMI-6424 for Injection, 12.5, 25, 50 or 100 mg/kg/d

Species/strain: CrI:CD<sup>®</sup>(SD)IGS BR rats

Number/sex/group: 20 males/group

Route, formulation, volume, and infusion rate: i.v. at 10 mL/kg/d

Satellite groups used for toxicokinetics: None

Study design: Males were treated daily for 6 weeks and then underwent an 8 week recovery to assess reversibility, persistence and potential delayed effects.

Parameters and endpoints evaluated: Animals were evaluated for clinical signs, body weights and feed consumption. Ten rats/group were euthanized at the end of the dosing period and 10/groups 3 and 4

and 9/groups 5 and 6 were terminated at the end of the 8-week recovery period. Brain, testes, prostate with seminal vesicle, and epididymides were weighed. Sperm parameters were evaluated from all animals at sacrifice. Histopathology on epididymides, prostate, seminal vesicles and testes was performed by ( Sperm/spermatid analyses were done by )

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### Results

**Mortality:** Two premature decedents were reported. One premature decedent was from the 50 mg/kg/d group and the other from the 100 mg/kg/d group. There were no gross findings to enable the sponsor to attribute cause of death.

**Clinical signs:** Treatment-related signs included increased touch sensitivity (high dose only), cloudy genital discharge (some animals from all AMI-6424 groups) and swollen tails with superficial darkening and scabs in the placebo, 50 and 100 mg/kg/d animals. The sponsor attributed the tail lesions to "components of the placebo".

**Body weight:** During the treatment period, 100 mg/kg/d treated animals weighed less (~10%) than the controls. By the end of the recovery period, weights were still less (~7%) but partially recovered.

**Food consumption:** During the treatment period, controls ate more than the high dose animals. No effects were noted during the recovery period.

**Toxicokinetics:** None performed

**Necropsy:** No gross findings were attributed to treatment.

On histologic evaluation, the primary finding was sloughed testicular germ cells in the epididymides of the high dose and a few of the mid dose animals. This finding was only reported at the end of the dosing period. No animals showed this lesion at the end of the recovery period. The lesion is associated with germ cell defoliation from the seminiferous tubules.

Vacuolated macrophages were found in the testes of placebo and 50 and 100 mg/kg/d males at the end of the dosing period. These were identified as interstitial macrophages by PAS-H staining. These cells were not found at the end of the recovery period.

Epithelial vacuolation was noted in the epididymides of placebo and 100 mg/kg/d animals. This finding was considered of minimal severity at the end of the dosing period but increased in severity (to slight) and was noted in the lower dosed animals at the end of the recovery period. The sponsor attributed this effect to components of the placebo. It becomes difficult to assign a NOAEL when the severity and incidence increased through the recovery period.

**Fertility parameters:** Decreased mean epididymal weights (absolute and relative) were found in the high dose animals when compared to controls but this finding was probably secondary to the lower body weights.

Sperm motility and counts were significantly decreased and abnormal sperm morphology increased at 50 and 100 mg/kg/d when compared to diluent controls at the end of the dosing period. As the epididymal sperm counts were affected in placebo controls and treated animals, the excipient may be responsible for some of the changes. Substantial effects in the high dose animals indicate a contribution by the test article.

Placebo controls had decreased sperm counts when compared to diluent controls. No significant effects were reported on spermatid counts at doses of up to 100 mg/kg/d. At the end of the recovery period, all parameters were comparable between groups.

Based upon clinical signs, body weights and feed consumption, the NOAEL for this study would be 50 mg/kg/d. However, given the effects on the epididymides and sperm counts and morphology, the NOAEL for functional effects in males is set at 25 mg/kg/d.

This would provide an HED= 4.15 mg/kg in man. Since the epithelial vacuolation within the epididymides increased in incidence and severity during the recovery period, it is difficult to assign a NOAEL to this study for the excipient effects.

### **Embryofetal development**

#### **02-001-15 7057-175: Intravenous Injection Rabbit Developmental Toxicity Study with AMI-6424**

**Key study findings:** In the high dose group (75 mg/kg), there were malformations to include absent ulna, fusion of sternbrae, adactyly and vertebral anomalies. These were noted in single animals from 5 litters and were not appreciated in the 60 mg/kg/d group. Many of these findings were comparable to those found in Study 7057-126, including the brachymelia, adactyly and absent ulna, and are all considered to be treatment-related. The exposure increased proportionally with increasing dose. Only one animal had detectable levels in amniotic fluid. This increases the concern for the fetal abnormalities as fetal exposure appears to have been limited. The NOAEL for developmental toxicity in this rabbit study is 60 mg/kg/d but the concerns for fetal developmental effects are quite high given the enormity of the effects.

**Study no.:** AMI CSN: 02-001-15 or 7057-175

**Conducting laboratory and location:** Covance Laboratories, Vienna, VA

**Date of study initiation:** 11/13/02

**GLP compliance:** Yes

**QA reports:** yes

**Drug, lot #, and % purity:** AMI-6424, Lots AME003 and AME004.

**Formulation/vehicle:** 5% dextrose and placebo emulsion as described above.

**Methods:**

Species/strain: Female Hra(NZW)SPF rabbits, aged 5 months and weighted 3400 g at study initiation

Doses employed: 0 (placebo, 7.5 mL/kg/d), 60 (in 3 mL/kg/d) or 75 mg/kg/d (in 6 mL/kg/d) on GD 7-20.

Route of administration: I.V. as a slow bolus over 5-7 minutes of administration

Study design:

Number/sex/group: 20/group for main study, 4/group for TK sampling (added as 50% of the first TK animals were not pregnant) and 10/group for amniotic fluid sampling

Parameters and endpoints evaluated: Mortality, clinical signs, uterine parameters, pregnancy parameters, amniotic fluid analysis and toxicokinetics.

Toxicokinetics: Samples were taken from the medial ear vein at 0 (defined as within 3 minutes after the end of the infusion), 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose.

**Results:**

Mortality and clinical signs: No premature decedents were reported. Excessive salivation was noted in one animal in each of the AMI-6424-treated groups.

Body weight: Weight loss was noted in the treated animals from GD 7 when compared to the placebo treated animals. Mean weight changes showed some "recovery". By the end of the study, no effects on maternal weights were found with AMI-6424.

Food consumption: Decreased feed consumption was noted in AMI-6424-treated animals which correlated with the weight losses.

*For embryofetal development studies:*

Terminal and necroscopic evaluations:

Dams: No significant treatment-related findings were reported grossly. C-section data were comparable across groups as were mean fetal weights.

Offspring: All fetuses were examined for external, soft tissue and skeletal abnormalities. In the 75 mg/kg/d group, one fetus had flexed front paw, brachymelia, adactyly, gastroschisis and another had an umbilical hernia.

Soft tissue findings (diaphragmatic hernia and gallbladder agenesis) in one high dose fetus were reported but have been found in the historical controls for this laboratory. All other findings were within historical limits (intermediate lung lobe missing, atria reduced, irregularly shaped liver) and found in all groups.

Skeletal variations included an increased incidence of unilateral 13th ribs and presacral vertebrae in treated animals when compared to controls. In the high dose group, there were malformations to include absent ulna, fusion of sternbrae, adactyly and vertebral anomalies. These were noted in single animals from 5 litters and were not appreciated in the 60 mg/kg/d group.

Of these findings, many were comparable to those found in Study 7057-126, including the brachymelia, adactyly and absent ulna. They are all considered to be treatment-related.

The exposure increased proportionally with increasing dose. Only one animal had detectable levels in amniotic fluid. This increases the concern for the fetal abnormalities as fetal exposure appears to have been limited or the compound rapidly metabolized. From the data submitted, we are unable to determine which scenario is the correct one.

In the study report, it is indicated that "the number of malformations noted at 75 mg/kg/day suggests that the external, visceral, and skeletal malformations noted (flexed front paws, brachymelia, adactyly, gastroschisis, umbilical hernia, diaphragmatic hernia, gallbladder agenesis, absent ulna, major fusion of the sternbrae, and vertebral anomaly with/without associated rib anomaly) are related to treatment with AMI-6424. Even though the majority of these findings (with the exception of brachymelia and the umbilical hernia) have been observed in the Covance historic controls, the fact that the majority of these malformations occurred in one 75 mg/kg/day fetus (Fetus 1, Litter F60427) and five fetuses in separate high-dose litters exhibited skeletal malformations indicate a possible compound-related effect. In addition, the limb malformations noted (brachymelia, adactyly, and absent ulna) mimic or are similar to the malformations of brachymelia

and syndactyly observed in rats at doses of 150 and 100 mg/kg/day, respectively, in Covance Study 7057-126. These findings further support a direct effect of AMI-6424 on the developing fetus." The reviewer agrees.

Maternal effects observed in this study were a transient increase in the magnitude of body weight loss, associated with the beginning of the dosing period (GD 7-9) and a corresponding decrease in food consumption at 75 mg/kg/day. The NOAEL for developmental toxicity in this rabbit study is 60 mg/kg/d but the concerns for fetal developmental effects are quite high given the enormity of the effects.

**02-001-03 7057-124: Intravenous Injection Rabbit Developmental Toxicity Study with AMI-6424**

**Key study findings:** Maternal effects observed in this study were a transient increase in the magnitude of body weight loss and decreased body weight gain, and a corresponding decrease in food consumption at all doses. The NOAEL for developmental toxicity in this rabbit study was 45 mg/kg/d.

**Study no.:** AMI CSN: 02-001-03 or 7057-124

**Conducting laboratory and location:** Covance Laboratories, Vienna, VA

**Date of study initiation:** 3/12/02

**GLP compliance:** Yes

**QA reports:** yes

**Drug, lot #, and % purity:** AMI-6424, Lots AME003, purity = 90%

**Formulation/vehicle:** 5% dextrose and placebo emulsion consisting of 2500 mg hydroxypropyl- $\beta$ -cyclodextrin (Kleptose HPBJ), 312.5 mg mannitol, with pH adjustment to 4.5

**Methods:**

**Species/strain:** Female Hra(NZW)SPF rabbits, aged 5 to 6 months and weighted 2877-4388 g at study initiation

**Doses employed:** 0 (Group 1, diluent control, 5% dextrose), 0 (Group 2, placebo), 12.5, 25, or 45 mg/kg/d (Group 3, 4, and 5) in 5 mL/kg/d once daily on GDs 7-20.

**Route of administration:** I.V. as a slow bolus over 5 minutes of administration

**Study design:**

**Number/sex/group:** 20/group for main study for Phase I, 4/group for TK sampling and 10/group for amniotic fluid sampling for Phase II

**Parameters and endpoints evaluated:** Mortality, clinical signs, body weight and food consumption, necropsy (sacrifice at C-section on GD 29), uterine parameters, fetal examination, pregnancy parameters, amniotic fluid analysis and toxicokinetics.

**Toxicokinetics:** Samples were taken from the medial ear vein at 0 (defined as within 3 minutes after the end of the infusion), 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose.

**Results:**

**Mortality:** There were no deaths attributed to treatment with AMI-6424. One LD female was euthanatized in a moribund condition on GD 23. One MD rabbit was found dead on GD 21. Two MD females that aborted on GD 26 and 21, respectively, were removed from the study.

**Clinical signs:** An increased incidence of few or no feces occurred in all treatment groups when compared to diluent and placebo controls. This finding was considered compound-related, although there was no direct dose response as the highest incidence occurred in the 12.5 and 25 mg/kg/day groups.

Body weight: Treatment-related changes in body weights were limited to a greater body weight loss on GDs 7 to 9 and decreased body weight gain during GDs 7 to 21 at 12.5 mg/kg/day and higher. The decreases in body weight gains (weight loss on Gestation Days 7 to 9) correlated with decreased food consumption during the same interval. By the end of the study, no effects on maternal weights were found with AMI-6424.

Food consumption: Decreased feed consumption was noted in all AMI-6424-treated groups which correlated with the weight losses.

*For embryofetal development studies:*

Terminal and necroscopic evaluations:

Dams: No significant treatment-related findings were reported grossly. Mean gravid uterine weights and mean corrected body weights were similar across treatment groups. There were no compound-related changes in pregnancy rate, number of dams with no live fetuses, or postimplantation loss. Two 25 mg/kg/day females aborted on GD 26 and GD 21 and one was found dead on GD 21. Dams with no viable fetuses were observed in all treatment groups (3, 2, and 1 dam at 12.5, 25, and 45 mg/kg/day, respectively). This finding was not observed in any dose group for the range-finding study in rabbits conducted in this laboratory (Covance Study 7057-123). Historical controls have a range of 0-2 dams with no viable fetuses. The incidence observed at 12.5 mg/kg/day is only slightly outside of this range and there was no dose response. Therefore, this finding may not be treatment related. A compound-related increase in postimplantation loss was observed in all treated groups when compared to both diluent and placebo controls; however, this finding was not dose-related. When dams with no viable fetuses were excluded, postimplantation loss was similar across treatment groups.

Offspring: All fetuses were examined for external, soft tissue and skeletal abnormalities. There were no drug-related fetal external variations and malformations. Malformations were limited to a single incidence of malrotated hindlimbs in one LD fetus, which was not related to treatment with AMI-6424, as it has been observed in historical controls for this laboratory and MARTA (the Mid-Atlantic Reproduction and Teratology Association's historical database), and was not observed at doses  $\geq 25$  mg/kg/day.

Fetal soft tissue variation attributed to AMI-6424 included an increase in the incidence of dilatation of lateral ventricles of the brain observed in groups that received AMI-6424 (4.4 to 5.2% versus 0.6 to 3.2% in control groups). However, the incidence did not change with dose. Other noted variations included an increase in the variation of small/missing intermediate lobe of the lung (observed at 45 mg/kg/day; 10% versus 1.7 to 5.8% in control groups), fluid-filled thoracic and abdominal cavities and small lung lobes (at 12.5 mg/kg/day), variations of the major vessels (seen in all groups), and increased renal pelvic cavitation (in diluent and placebo controls and at 45 mg/kg/day). None of these could be attributed to treatment with AMI-6424.

Soft tissue malformations were limited to a single incidence of hydrocephaly at 45 mg/kg/day; heart and/or great vessel malformations in the placebo control (one fetus) and at 12.5 mg/kg/day (two fetuses in two litters); and malpositioned kidneys at 12.5 mg/kg/day (three fetuses in two litters). Since all of the aforementioned findings have been observed in the historical controls at this laboratory and/or MARTA, and there was no dose-dependence, there findings were not considered related to treatment.

Increased incidences of incomplete ossification of the 5th and 6th sternbrae, unossified 5th sternbrae, and unossified 6th sternbrae, were observed in the 45 mg/kg/day group. The findings were also seen in other groups including control groups and were within the historical control range. The other fetal skeletal variations were not dose-dependent and were not related to treatment with AMI-6424. A malformed skull bone, vertebral anomaly with/without associated rib anomaly, and forked/fused ribs were noted in the 45 mg/kg/day group. These malformations were observed in only one fetus. Since these malformations (with the exception of the malformed skull bone) have been observed in the historical database at this laboratory and/or the MARTA database, they are not considered to be treatment-related. Other findings were sporadic and are unrelated to treatment.

**TK:**

Following the end of dosing, the mean  $T_{1/2}$ , ranged from 1.51 to 3.24 hours, slightly increased with the dose. The exposure increased proportionally with increasing dose. Concentrations of AMI-6424 in amniotic fluid were all below the limit of quantitation (0.25 µg/ml) at all collection times, indicating that AMI-6424 did not cross the placenta.

Maternal effects observed in this study were a transient increase in the magnitude of body weight loss, associated with the beginning of the dosing period (GD 7-9) and a corresponding decrease in food consumption at all doses. The NOAEL for developmental toxicity in this rabbit study was 45 mg/kg/d. [Reviewer's comments: No significant skeletal malformations were noted as in other Segment II studies. This may be due to the low doses used in this study.]

**02-001-04 7057-126: Intravenous Injection Rat Developmental Toxicity Study with AMI-6424**

**Key study findings:** Maternal effects observed in this study were a decrease in body weight gain and food consumption at  $\geq 100$  mg/kg/day. At doses  $\geq 100$  mg/kg/day, an effect on fetal growth and embryo/fetal development, as evidenced by decreases in fetal weights and external malformations of syndactyly and brachymelia was observed. The NOAEL is 50 mg/kg/day for maternal toxicity, and 50 mg/kg/day for developmental toxicity.

**Study no.:** AMI CSN: 02-001-04 or 7057-126

**Conducting laboratory and location:** Covance Laboratories, Vienna, VA

**Date of study initiation:** 4/22/02

**GLP compliance:** Yes

**QA reports:** yes

**Drug, lot #, and % purity:** AMI-6424, Lots AME004, purity = 90%

**Formulation/vehicle:** 5% dextrose and placebo emulsion consisting of 2500 mg hydroxypropyl- $\beta$ -cyclodextrin (Kleptose HPBJ), 312.5 mg mannitol, with pH adjustment to 4.5

**Methods:**

**Species/strain:** Female Crl:CD<sup>®</sup>(SD)IGS BR rats, aged 10 to 12 weeks old at study initiation

**Doses employed:** 0 (Group 1, diluent control, 5% dextrose), 0 (Group 2, placebo), 50, 100, and 150 mg/kg/d (Group 3, 4, and 5) at a dose volume of 5 mL/kg/day (Group 3), 10 mL/kg/day (Group 4), and 15 mL/kg/day (Groups 1, 2, and 5) once daily on GD 6-17.

Route of administration: I.V. as a slow manual push over 3 or 5 minutes for 5/10 mL/kg and 15 mL/kg groups, respectively

Study design:

Number/sex/group: 25/group for main study for Phase I, 16/group for TK sampling and 13/group for amniotic fluid sampling for Phase II

Parameters and endpoints evaluated: Mortality, clinical signs, body weight and food consumption, necropsy (sacrifice at C-section on GD 20), uterine parameters, pregnancy parameters, fetal examination (Each fetus was sexed, weighed, examined for external abnormalities. Approximately one-half of all fetuses were processed for visceral examination. The remaining fetuses were processed for skeletal examination.)

Toxicokinetics: Samples were collected at 0 (within 3 minutes after the end of the infusion), 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose on GD 17. Amniotic samples were collected at 0.5, 2, 8 and 24 hr post dose on GD 17.

#### Results:

**Mortality:** All rats survived to scheduled sacrifice, with the exception of one 150 mg/kg/day rat that died overnight after being dosed over a 3-minute instead of a 5-minute interval. The death of this animal may possibly be attributed to the rate of dose delivery.

**Clinical signs:** Compound-related clinical observations were few and limited to a thin appearance in HD group and in one MD animal.

**Body weight:** Decreased body weight (GDs 6-8) and body weight gain were noted in MD and HD animals (see table below). The decreases correlated with decreased food consumption during the same interval. By the end of the study, body weight in the HD animals was still low.

#### Body weight changes (g)

Group	1 (DSW)	2 (Placebo)	3 (50 mg/kg)	4 (100 mg/kg)	5 (150 mg/kg)
GD 6	248.4	249.8	248.8	249.0	247.8
GD 8	257.3	254.9	255.5	248.6	233.0
GD 18	328.1	326.6	327.4	319.0	298.2
<b>Body weight gain</b>					
GDs 6-8	8.9	5.1	6.7	-0.3	-14.8
GDs 6-18	79.8	76.8	78.7	70.1	50.3
% Group 1 control	100	96.2	98.6	87.8	63.0

**Food consumption:** Decreased feed consumption was noted in MD and HD groups which correlated with the weight losses. For the GD 6 to 18 treatment period, mean food consumption was 88.5 and 77.4% of the diluent control and 90.9 and 79.5% of the placebo control for the MD and HD groups, respectively.

#### Food consumption changes (g/group/day)

Group	1 (DSW)	2 (Placebo)	3 (50 mg/kg)	4 (100 mg/kg)	5 (150 mg/kg)
GD 6-8	24.4	22.8	22.9	18.7	10.8
GDs 6-18	27.0	26.3	26.6	23.9	20.9

#### For embryofetal development studies:

Terminal and necroscopic evaluations:

**Dams:** Positive necropsy findings included one liver with dark areas in the 150 mg/kg/day group, pale kidneys in one animal for the placebo control and one animal each for all drug-treated groups, and kidneys with dilated pelvises at 50 (one animal) and 150 mg/kg/day (two animals).

Mean gravid uterine weights were similar across treatment groups and unaffected by AMI-6424. Corrected body weights (268.29 g vs. controls' 285.56g and 282.92 g) and net change from Day 0 (47.53 g vs. controls' 65.12 g and 61.59 g) was significantly decreased at 150 mg/kg/day.

AMI-6424 had no effect on embryo/fetal viability and pregnancy rates. All dams had viable fetuses. There were no early deliveries or abortions. Pre- and postimplantation loss was similar across treatment groups. Other cesarean section parameters were similar to controls.

Covariate-adjusted mean fetal weights were significantly decreased when compared to the diluent control at doses of 100 and 150 mg/kg/day of AMI-6424. Male mean fetal weights were also significantly decreased at 150 mg/kg/day when compared to the placebo control.

#### Offspring:

Fetal external malformations, seen in 4 fetuses (2 diluent control, one 100 mg/kg/day and one 150 mg/kg/day), consisted of macroglossia, protruding tongue, malformed nose, anophthalmia, brachymelia, syndactyly, micrognathia, and astomia (see table below). Of these, the sponsor stated that brachymelia was considered treatment-related and syndactyly was of uncertain relationship to treatment. One fetus in the 100 mg/kg/day group had multiple findings of protruding tongue, brachymelia (left hind limb), syndactyly (left hind limb, middle three digits), and anophthalmia. The brachymelia observed at doses of 150 mg/kg/day was limited to one fetus in one litter and was not associated with any other external findings. Brachymelia observed in this study may possibly be attributed to treatment with AMI-6424 at doses  $\geq$ 100 mg/kg/day, because this finding is not observed in historical control databases (both Covance and MARTA) for this strain of rat. Because syndactyly is not observed in historical control databases (both Covance and MARTA), and it also occurred in reproductive studies in other species, it was considered drug-related by the reviewer. Fetal external malformations not considered compound-related included macroglossia, micrognathia, anophthalmia, and a malformed nose seen in two diluent control fetuses. There were no drug-related fetal external variations.

**Summary of fetal external malformation (fetal incidence/litter incidence)**

Group	1 (DSW)	2 (Placebo)	3 (50 mg/kg)	4 (100 mg/kg)	5 (150 mg/kg)
Litters evaluated	25	24	25	24	25
Fetuses evaluated	319	322	312	332	322
Protruding tongue	0	0	0	1/1	0
Brachymelia	0	0	0	1/1	1/1
Syndactyly	0	0	0	1/1	0
Astomia	1/1	0	0	0	0
Macroglossia	1/1	0	0	0	0
Micrognathia	1/1	0	0	0	0
Anophthalmia	2/2	0	0	1/1	0
Nose malformed	1/1	0	0	0	0
Total	2/2	0	0	1/1	1/1

Soft tissue malformations were not noted. Compound-related fetal soft tissue variations were limited to the fetal incidence of dilatation of the lateral ventricles of the brain. This finding was compound-related and the fetal incidence (12%) was above the upper limit of the historical controls of this laboratory (fetal incidence range of 0 to 7.5%). Findings of increased renal pelvic cavitation (in the placebo control and at doses  $\geq$ 50 mg/kg/day) and dilated ureters (at doses  $\geq$ 50 mg/kg/day) occurred at incidences within the range of historical controls.

**Summary of fetal soft tissue variations (fetal incidence/litter incidence)**

Group	1 (D5W)	2 (Placebo)	3 (50 mg/kg)	4 (100 mg/kg)	5 (150 mg/kg)	Historical control
Litters evaluated	25	24	25	24	25	468
Fetuses evaluated	160	161	155	165	164	3254
Dilatation of lateral ventricle	2/2	3/3	6/5	6/3	19/7	0-7.5/0-39
Increased renal pelvic cavitation	0	2/2	3/2	4/3	4/3	0.7-8.1/5.6-35
Dilated treter	0	0	3/3	2/2	2/2	0-8.3/0-29
Total	2/2	4/4	11/9	11/6	24/11	

There were no skeletal malformations noted in fetuses of the groups treated with AMI-6424. Significant changes in the incidence of skeletal variations in all or most of the dose groups treated with AMI-6424 when compared to the diluent control included an increase in unossified vertebral arches (100 and 150 mg/kg/day), a decrease in incomplete ossification of 5th and 6th sternebra (50 and 150 mg/kg/day), an increase in unossified 5th and 6th sternebra (150 mg/kg/day), and an increase in wavy/bent ribs (100 and 150 mg/kg/day). The incidence of unossified hyoid body was increased at 150 mg/kg/day but there was no evidence of a dose-response. These variations occurred at incidences well within the range of the historical controls. In addition, the incidence for the finding of less than four caudal vertebrae ossified was increased above diluent and placebo control values, but within the historical range. These fetal skeletal variations are indicative of a delay in development (*i.e.*, decreased fetal weight), suggesting that treatment with AMI-6424 may contribute to a delay in fetal development, either by directly affecting the fetus, or more probably, as a result of the decreases in maternal food consumption and body weight attributed to AMI-6424.

**Summary of fetal skeletal variations (fetal incidence/litter incidence)**

Group	1 (D5W)	2 (Placebo)	3 (50 mg/kg)	4 (100 mg/kg)	5 (150 mg/kg)	Historical control
Litters evaluated	25	24	25	24	25	469
Fetuses evaluated	159	161	157	167	158	3273
Unossified hyoid body	23/12	46/19	30/15	26/16	39/21	3.6-54/9.1-96
Less than 4 caudal vertebrae ossified	1/1	1/1	0	3/2	5/3	13-64/28-100
Unossified vertebral arch	0	1/1	0	8/4	7/3	0-11/0-25
5 <sup>th</sup> /6 <sup>th</sup> sternebrae incomplete ossification	81/24	84/22	55/17	76/22	62/17	16-84/74-100
5 <sup>th</sup> sternebrae unossified	10/6	21/9	18/8	15/6	31/12	11-70/46-100
6 <sup>th</sup> sternebrae unossified	12/6	27/9	18/7	22/11	38/12	0-46/0-95
14 <sup>th</sup> rudimentary ribs	3/3	2/2	1/1	0	4/2	0-13/0-56
Wavy/bent ribs	0	8/4	2/1	5/5	5/4	0-4.8/0-28
Total	113/24	137/24	93/24	119/23	125/25	

TK:

Following the end of dosing, the mean  $T_{1/2}$ , ranged from 2.75-3.38 hours. The exposure increased slightly less proportionally with increasing dose. Concentrations of AMI-6424 in amniotic fluid were detectable in four samples in Group 4, and in almost all samples in Group 5. However, values for  $C_{max}$  and  $AUC_{0-24}$  (0.420  $\mu\text{g/mL}$  and 5.97  $\mu\text{g-hr/mL}$ , respectively) were approximately 2000 and 300 fold lower than those observed in plasma, indicating that AMI-6424 crosses the placental barrier to a very small extent.

Maternal effects observed in this study were a decrease in body weight gain and food consumption at  $\geq 100$  mg/kg/day. There was no effect on embryo/fetal viability. At doses  $\geq 100$  mg/kg/day, an effect on fetal growth and embryo/fetal development, as evidenced by decreases in fetal weights and external malformations of syndactyly and brachymelia was observed. Based on these results, the NOAEL for AMI-6424 is 50 mg/kg/day for maternal toxicity, and the NOAEL is 50 mg/kg/day for developmental toxicity.

**04-013-01\_57155: Range Finding Toxicity Study in Non-Pregnant Minipigs**

**Key study findings:** Treatment with telavancin at doses of up to 100 mg/kg/day to minipigs caused marked clinical signs in one animal at 100 mg/kg/d. Based on the results, a high dose of 100 mg/kg/day was recommended for subsequent studies in minipigs with Telavancin.

**Study no:** Theravance Reference: CRN: 04-013-01; Lab Scantox #7057-115

**Conducting laboratory and location:** Lab Scantox, Hestehavevej, 36A, Ejby, DK4623 Lille Skensved, Denmark

**Date of study initiation:** 10/20/04

**GLP compliance:** No

**QA report:** No

**Drug, lot #, and % purity:** Telavancin for injection, Lot No. 2213-10-645043

The purpose of this study was to determine the toxicity of telavancin administered daily by slow bolus IV injection to female minipigs for 25 days for selecting doses in the following reproductive toxicity studies in minipigs. In the 1<sup>st</sup> phase, two female minipigs were given an ascending series of single doses at 25, 50, and 100 mg/kg with a 3-day washout period. Clinical signs, body weight and food consumption were monitored. The only clinical sign was redness in the area around the neck during dose administration at 50 mg/kg in one animal. This redness disappeared one hr later. No other findings in clinical signs, body weight and food consumption were noted.

In the second phase, two minipigs per group were dosed at 0 (5% dextrose control), 0 (placebo control), 12.5, 50 or 100 mg/kg/day (groups 3, 4, and 5) for 25 days with a dose volume of 10 mL/kg. Clinical signs, body weight and food consumption were monitored. A macroscopic examination was performed on Day 26. One LD animal was passive with poor appetite between Days 2 and 4. One HD animal showed trembling during and after dosing, poor appetite, abnormal feces, retching, blue coloration of the extremities, dyspnea and elevated temperature from Day 1 to Day 18. Decreased food consumption was seen in this animal on Days 11 and 12. The signs in the HD animal were no longer observed following treatment with an anti-inflammatory agent (Clamoxyl<sup>®</sup>) and an anti-histamine agent (diphenhydramine). Therefore, the signs may be in part related to a histamine response. No other drug-related positive findings were noted.

Exposure to telavancin, AMI-11352 and AMI-999 increased with the increase in the dose levels. The increase was slightly less than dose-proportional.

In conclusion, treatment with telavancin at doses of up to 100 mg/kg/day to minipigs caused marked clinical signs in one animal at 100 mg/kg/d. Based on the results, a high dose of 100 mg/kg/day was recommended for subsequent studies in minipigs with telavancin.

**05-013-01\_58564: Range Finding Toxicity Study in Pregnant Minipigs**

**Key study findings:** Decreases in maternal body weight gain and food consumption were noted in animals treated at 25 and 40 mg/kg. Doses of 12.5, 25 and 45 mg/kg/day were recommended for the definitive study.

**Study no:** Theravance Reference: CRN: 05-013-01; Covance #58564

**Conducting laboratory and location:** Lab Scantox, Hestehavevej, 36A, Ejby, DK4623 Lille Skensved, Denmark

**Date of study initiation:** 10/20/04

**GLP compliance:** No

**QA report:** No

**Drug, lot #, and % purity:** Telavancin for injection, Lot No. 2213-99-731674

The purpose of this study was to assess the embryofetal toxicity of telavancin when administered daily by slow bolus intravenous injection (10 mL/minute) to pregnant minipigs from Gestation Day (GD) 11 to GD 35. Fifteen female primiparous Göttingen SPF minipigs were mated and subsequently allocated to five groups (3/group). The animals were treated once daily by slow bolus intravenous injection for 25 days (from GDs 11 to 35) with 5% dextrose (diluent, Group 1), placebo for telavancin for injection (placebo; Group 2), or telavancin at doses of 12.5, 50 or 100 mg/kg/day (Groups 3, 4 and 5, respectively). The dosing volume was 10 mL/kg.

Maternal clinical signs, body and food consumption were monitored. Blood TK samples were collected on GDs 11 and 35 for determination of telavancin and the metabolite, AMI-11352 (THR-651540) and the ( ) AMI-999 (THR-689909), in plasma. On GD 56, the females were sacrificed and macroscopic examination was performed. The uterus and ovaries were examined for the following: numbers of implantation sites, number of early resorptions, distribution and number of live and dead and resorption sites in the uterine horns, and numbers of corpora lutea in each ovary. Live fetuses and placentas were weighed; fetuses were examined for external abnormalities, and the sexes determined. b(4)

There were no treatment-related mortalities in the study. One LD dam was found dead on GD 37. Necropsy revealed a thrombus in the right atrium and right ventricle, suggesting the mortality was related to the heart thrombus which could be a consequence of administration of material through the vascular access port. One HD dam aborted 3 fetuses on GD 31 and was euthanized. Historical data indicate a spontaneous abortion frequency of 0.25%.

High incidences of clinical signs including subdued/passive behavior and reduced or no appetite during the dosing period were seen in HD animals. Clinical signs unique to Group 5 included red/purple spots on the skin and vomiting. Potential treatment-related effects on food consumption were noted in HD animals evidenced by food remains being occasionally seen in each of the three HD dams during the dosing period. There were no treatment related effects on maternal body weight or body weight gain. There were no treatment-related effects on reproductive parameters (number of live/dead fetuses, number of early or late resorptions, post-implantation loss). A dose-dependent increase in fetal body weights was noted (36.4-41.5 g vs. controls' 33.7-34.9 g). At necropsy, two fetuses in Group 3 and one fetus in Group 4 had extra toes (polydactylism).

Exposure to telavancin and AMI-999 increased with the increase in the dose level. AMI-11352 plasma levels were generally similar between dose levels and there was not an effect on AMI-11352 plasma concentration following increases in telavancin dose. Concentrations of telavancin, AMI-11352 and AMI-999 in plasma were generally similar on Day 1 compared with Day 25.

In conclusion, slow daily intravenous treatment of pregnant minipigs for 25 days with telavancin at 100 mg/kg/day resulted in treatment-related clinical signs including subdued/passive behavior, reduced or no appetite, red/purple spots on the skin and vomiting. A decrease in food consumption was also noted in HD

dams. Polydactylism was found in two fetuses in Group 3 (LD) and one fetus in Group 4 (MD). Based on the maternal clinical signs and possible food effects noted at 100 mg/kg/day, the sponsor recommended that the high dose for the subsequent definitive embryofetal minipig study be less than 100 mg/kg/day.

**05-013-04 58857: Telavancin: Study for Effects on Embryo-Fetal Development in the Minipig**

**Key study findings:** Increased preimplantation loss and postimplantation loss was seen in all dose groups. Increased external malformations evidenced by polydactyly, syndactyly and deformed foreleg were seen in LD and MD groups. Telavancin is a teratogen in the minipig as well as the rat and the rabbit with skeletal (limb) malformations being the primary terata.

**Study no.:** Scantox 58857; CRN: 05-013-04

**Conducting laboratory and location:** LAB Scantox, Lille Skensved, Denmark

**Date of study initiation:** 4/8/05

**GLP compliance:** Yes

**QA reports:** Yes except for ultrasound examinations

**Drug, lot #:** Telavancin, Lots 2213-10-645043 and 2213-10-645066

**Methods:** Of interest in this study is the significant number of non-telavancin antimicrobial treatments to include Formocibazol (topical ointment), Fucithalmic (topical ointment), Helosan ("mild antiseptic ointment" at the end of daily dosing on all animals), Tiamulin Vet (systemic antimicrobial agent), Clamoxyl Vet (systemic antimicrobial agent), Borgal Vet (systemic antimicrobial agent), Temgesic (analgesic), Metacam (analgesic), and Diphenhydramine (antihistamine).

Doses: Diluent (5% dextrose), placebo ("Placebo for Telavancin for Injection"; Lot 2213-99-732674), 25, 50 or 75 mg/kg/day, from GD 11 to GD 35

Species/strain: Female Ellegaard Göttingen minipigs. The average litter size for this strain of minipig is stated to be 5-6 feti (ref. supplier).

Number/sex/group: 14 females/group

Route, formulation, volume, and infusion rate: I.V. at 2.5- 7.5 mg/mL and 10 mL/kg and 10 mL/min

Satellite groups used for TK: 3 pregnant females/telavancin dose that were dosed from GD 11-16 only and then euthanized and discarded without further examination. Covance Laboratories, Madison, WI performed the serum analyses.

Study design: Estrous synchronicity was elicited by Regumate added to the diet. Vascular access ports were implanted at least 5 days prior to mating. Pregnant minipigs were injected i.v. daily by slow bolus injection from Days 11- 35 of gestation. Diphenhydramine HCl was administered as needed to "moderate signs potentially related to a histamine reaction."

Parameters and endpoints evaluated: Clinical signs, body weights, feed consumption, pharmacokinetics (from kinetic animals predose, immediately post-dose, at 0.5, 1, 2, 4, 8 and 24 hrs post-dose on GD 16), uterine weight, numbers of corpora lutea, implantation sites, resorptions, live: dead ratio, sex and weight of each fetus, weight of the placenta, external and internal fetal abnormalities and fetal jaw length, and fetal skeletal findings.

## **Results**

**Mortality (dams):** No dams died prematurely. However, some animals were sacrificed after severe clinical signs or after abortion. There were 4 animals in the 5% dextrose group (3 miscarriages, 1 "poor health"), 4

in the placebo group (3 miscarriages, 1 “poor health”), 2 in the 25 mg/kg/d telavancin group (1 miscarriage, 1 “poor health”), 3 in the 50 mg/kg/d telavancin group (2 miscarriages, 1 “poor health”) and 4 (main group) + 1 (kinetic animal) from the 75 mg/kg/d telavancin group (3 miscarriages, 1 “poor health”) that were sacrificed *in extremis*. Many of these animals were treated with antimicrobials in addition to the study drug. This is a very unusual happenstance in toxicology studies submitted for regulatory review. Such animals were evenly distributed across groups so no treatment relationship is assigned but the validity of the study is brought into question.

**Clinical signs (dams):** Diarrhea/loose stools were seen across groups as were shivering (attributed by the sponsor to administration of cold dosing formulations but not a reasonable conclusion as the dosing solutions were supposedly brought to room temperature as of the 2<sup>nd</sup> day of dosing) and limping. “Cream milk acidified with lactobacillus acidophilus was offered occasionally in the study in an attempt to alleviate the diarrhea/loose stools and/or stimulate food consumption.” Reddened ears and snouts were noted in all except the 5% dextrose controls. The sows showing such signs were treated with diphenhydramine.

**Clinical Signs in Minipigs Treated with Telavancin**

Group	1	2	3	4	5
Diarrhea/loose stools	3	3	1	1	4
Shivering	5	2	5	3	5
Limping	3	4	2	3	6
Erythema/reddening	0	1	5	3	1

The pregnancy rate seems rather unacceptably low, especially for the placebo and high dose groups. The historical control pregnancy rates were reported as 65%- 93% over 3 studies from the testing laboratory.

**Pregnancy Findings in Minipig Sows Treated with Telavancin**

Group	Confirmed mated	Early delivery (dead feti)	Not pregnant	Killed in extremis	Early farrowing (live feti)	Pregnant on GD 109-111	Pregnancy rate <sup>a</sup>
1	14	3	3	1 (4)**	0	7	50%
2	14	3	6	0 (4)	0	5	36%
3	14	1	3	1 (3)	0	9	64%
4	14	2	4	0 (3)	0	8	57%
5	14	3	5	0 (4)	1	5	36%

<sup>a</sup>Number pregnant to term/number mated

\*\* Numbers in ( ) are from individual animal data and were not in the original sponsor table

**Body weight (dams):** No treatment-related differences from controls were reported.

**Food consumption (dams):** Consumption was comparable across groups.

**Gross necropsy:** The primary lesion at sow necropsy was thrombi in the hearts (2, 1, 3, 3, and 3 for the respective groups) and lungs (0, 0, 5, 2, and 0 for the respective groups). These lesions were considered related to the long term catheter placement and not specifically to the test compound.

**Toxicokinetics:** Exposure to the parent compound increased in a less than dose proportional manner. Increases in AMI-11352 (metabolite) and AMI-999 ( ) also increased in a less than dose proportional manner. The ratios of drug were 1.0: 2.0: 3.0 with the C<sub>max</sub> increasing 1.0: 1.6: 2.5 and the AUC 0-24 increasing 1.0: 1.5: 2.3. AMI-11352 appears to be a minor metabolite in minipigs.

b(4)

**Mean TK Parameters in Minipig Sows (N=3/group) Treated with Telavancin**

Group (Dose mg/kg/d)	Cmax (µg/mL)	Tmax (hr)	AUC <sub>0-24</sub> (µg,h/mL)	T ½ (hr)
<b>TD-6424</b>				
3 (25)	347	0.367	780	3.34
4 (50)	545	0.300	1206	3.80
5 (75)	871	0.300	1568	3.29
<b>AMI-11352</b>				
3 (25)	2.85	3.03	30.6	NA
4 (50)	3.37	1.46	41.1	6.41
5 (75)	3.48	1.97	48.9	9.44
<b>AMI-999</b>				
3 (25)	2.59	0.367	20.2	7.75
4 (50)	4.25	0.300	31.4	8.70
5 (75)	6.04	0.300	47.3	6.32

Terminal and necropsic evaluations: An increased number of late resorptions (numbers of litters affected) were noted in the mid (mean= 0.6) and high dose (mean= 0.8 over 4/5 litters) groups but were higher than the historical control range (maximum mean= 0.4) for the testing laboratory. The sponsor indicated that the number of late absorption on an individual litter basis in Groups 4 and 5 was within historical control range (maximum of up to 2 late absorption per litter). Preimplantation loss was increased at all doses but the number was within historical range. Postimplantation loss was increased in all treated groups, and was over the historical control range.

**Mean Litter Data from Minipigs Treated with Telavancin**

Group	Live feti/litter	Dead feti/litter	Malformed	Early resorp.	Late resorp.	Total resorp
1	4.9±1.2	0	0.1± 0.4	1.1± 1.1	0	1.1± 1.1
2	4.8± 1.3	0	0.2± 0.4	1.0± 0.7	0.2± 0.4	1.2± 0.8
3	3.4± 1.9	0	0.4± 0.7	1.7± 1.5	0.2± 0.4	1.9± 1.8
4	4.5± 2.0	0	0.5± 0.8	1.3± 1.0	0.6± 0.7	1.9± 1.6
5	3.4± 1.5	0	0.4± 0.5	2.0± 1.6	0.8± 0.4	2.8± 1.5

**Group Mean Data from Minipig Sows Treated with Telavancin**

Group	Preimplant loss (%)	Postimplant loss (%)	Implantations
1	0	19.2± 17.87	6.0± 0.6
2	0	20.9± 15.94	6.0± 0.7
3	24.9± 31.09	31.5± 26.19	5.3± 2.6
4	15.0± 27.72	28.0± 21.05	6.4± 2.2
5	5.7± 12.79	45.6± 21.36	6.2± 1.3

**Historical Litter Data from Control Göttingen Minipigs**

Parameter	# of litters	Mean	Mean Minimum	Mean Maximum
Live feti/litter	35	6.4	6.0	7.0
Early resorptions	35	1.0	0.3	2.0
Late resorptions	35	0.2	0	0.4
Total # of resorptions	35	1.2	0.3	2.4
Implantations	35	7.0	5.4	8.5
Preimplant loss (%)	33	14.0	0	27.2
Postimplant loss (%)	35	15.5	6.6	26.7

Offspring: No terata were found according to the sponsor. In their assessment, all external, soft tissue and skeletal findings were comparable across groups. One litter in the 50 mg/kg/d group had a much lower mean weight than all others. As this was found in only one litter, it is not considered treatment-related.

**External and Soft Tissue Abnormalities in Minipig Feti (Sponsor's Table)**

Group	Total # litters exam	Dam #	Fetus #	Total Feti exam	Anomaly	Total % of litters with affected fet
1	7	6	1	34	Retained testis	14.3%
2	5	21	1	24	Retained testis; polydactyly	20%
3	9	35	3	31	Polydactyly on 2 limbs	33.3%
		39	2		Polydactyly	
		41	2		Polydactyly	
		41	3		Polydactyly on 2 limbs	
4	8	43	3	36	Polydactyly on 2 limbs	75.0%
		45	3		Retained testis	
		50	3		Discolored diaphragm	
		52	1		Polydactyly, misshapen digits, deformed front leg (radial agenesis)	
		54	3		Polydactyly	
		54	4		Polydactyly	
		54	6		Polydactyly	
		56	1		Syndactyly	
5	5	65	5	17	Diaphragmatic hernia	20.0%

Not included in the table above were the results from: A fetus from sow #40 (Group 3; killed in extremis) with a deformed head and misshapen digit, a fetus from sow #47 (Group 4; killed in extremis) with "legs turned inwards", fetus #3 from sow 51 (Group 4) that had multiple absent ossification sites, absent tarsal bones on both legs. The sponsor attributed these findings to the lower birth weight of this fetus (79 gms) and possible delay in development. While the absent ossification sites may be due to a delay in maturation, the absent tarsal bones on both legs could not be due to this delay. A fetus from another sow (#43) had absent ossification sites distal to the metacarpi. This fetus did not have a reduced birth weight. Sow #60 (Group 5; killed in extremis) had a fetus with a deformed head, forelegs and snout but it was autolytic so no conclusions were made about it, and sow #61 (Group 5; killed in extremis) had a fetus with a deformed hind leg. One mid dose fetus had exophthalmos and one had anencephaly, not seen in any other groups.

**Historical Incidence of Malformations/Abnormalities in Göttingen Minipigs (N=3764):** The sporadic limb diagnoses had a total incidence of 0.7% to include radial agenesis, ulna agenesis, tibial agenesis; hexadactyly, syndactyly, talipes and "abnormal flexure of a leg in a joint" (ref. provided by sponsor). Additional information showed that polydactyly in the historical database occurred in 1/35 litters and syndactyly also occurred in only 1/35 litters.

b(4)

In contrast, the producers of the Göttingen minipigs (Ellegaard Minipigs in Japan) have provided significantly different information. From the Danish C, an incidence of syndactyly was ≤0.4% for the past 5 years in control piglets and the incidence of pentadactyly was ≤2.93% for the past 5 years in control piglets. However, they have changed from a line breeding program to a population based program as of 11/04 and the incidence of such terata has markedly decreased (<0.2% and <0.7%, respectively). From the Japanese database, polydactyly was found in 7/505 (1.4%) of newborn piglets. From these databases, it appears that 5.6 is their usual fecundity index (number of feti/litter) with preimplantation losses of 11.7% and postimplantation losses of 15.6%. It is clear that values for all of these parameters were higher in this study than in the historical databases.

Given that telavancin has been found to be a teratogen in rabbits and rats and that the terata were syndactyly and misshapen forelimbs, including radial agenesis, as well as the increased pre- and post-implantation losses, it is concerning that the lesions found in this study include polydactyly (sometimes on 2 limbs), syndactyly and a deformed foreleg with absent radius (described as radial agenesis). It can be concluded that telavancin is a teratogen in the minipig with external/skeletal (limb) malformations being the primary terata. Additional effects of telavancin dosing were found in pre- and post-implantation parameters.

The sponsor considered the NOAEL for this minipig teratology study to be 75 mg/kg/d. They did not find any evidence of teratogenicity or other treatment-related fetal effects. This is not a reasonable conclusion. They also determined that sufficient feti were available from all groups to make a determination of potential for developmental toxicity in the minipig. This is not a reasonable conclusion.

**02-001-07 7057-129: Intravenous Injection Study for Effects on Pre- and Postnatal Development, Including Maternal Function, with AMI-6424 in the Rat**

**Key study findings:** The NOAEL for F0 maternal effects was 50 mg/kg/d on the basis of reduced body weight and decreased feed consumption. Due to the increased incidence of stillborn pups and clinical findings at 150 mg/kg/d in the F1 generation, the fetal/pup NOAEL is determined to be 100 mg/kg/d.

**Study no.:** AMI CSN: 02-001-07 or 7057-129

**Conducting laboratory and location:** Covance Laboratories, Inc., Vienna, VA

**Date of study initiation:** 6/26/02

**GLP compliance:** Yes

**QA reports:** yes

**Drug, lot #:** AMI-6424, AME001 and AME003

**Formulation/vehicle:** 5% dextrose or placebo emulsion (hydroxypropyl- $\beta$ -cyclodextrin 2500 mg, Mannitol 312.5 mg, pH 4.5)

**Methods:**

Species/strain: Premated CrI:CD<sup>®</sup>(SD)IGS BR rats, 10-12 weeks old, 200-242 g on GD 0

Doses employed: 0 (5% dextrose), 0 (placebo), 50, 100 or 150 mg/kg/d in 15 mL/kg/d for groups 1, 2 and 5 and 5 mL/kg/d for the low dose group and 10 mL/kg/d for the mid dose group.

Route of administration: I.V. into the lateral tail vein over approximately 3 minutes.

Study design: F0 pregnant females were dosed from GD 6 to lactation Day 20, inclusive.

Number/sex/group: 25 females

Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption (F0 dams). During lactation and weaning, the F1 litters were evaluated for growth and development. Necropsies were performed on F0 females, F1 adults and F1 and F2 offspring "as appropriate". At weaning, 1 pup/sex/litter was retained for breeding (total= 20/rats/sex/group) and five additional animals/sex were maintained until breeding began. The additional animals were euthanized and discarded without examination.

**Results:**

**Mortality:** No treatment-related mortality was reported. During lactation, total litter deaths were noted in 3 F0 dams- one in the placebo control and two in the high dose group. Two non-pregnant controls were euthanized when they failed to deliver pups.

Compound-related increases in the number of stillborn pups and the number of dams with stillborn pups increased with increasing dose.

*For peri- and postnatal development studies:*

*In-life observations:*

**Dams:** Decreased mean maternal body weights and decreased mean body weight changes were found in the 100 and 150 mg/kg/d F0 dams. Decreased feed consumption was also appreciated in these groups when compared to diluent and placebo controls.

For the F1 parents, no clinical signs attributable to treatment were determined and all pups survived to maturation. Mean body weights for F1 males were significantly (~10%) lower for the 100 and 150 mg/kg/d groups than for either control group throughout the dosing period. Mean body weights for F1 females were lower during Study Weeks 1-3 only and no treatment-related effects were noted during gestation and lactation. No effects of treatment were noted on any reproductive parameter.

**Offspring:** For the F1 pups, the 150 mg/kg/d dose, the pups were cyanotic (2 litters), swollen (2 litters), and anophthalmia (3 litters) and one pup had "limited use of a forelimb" (brachymelia). These findings were consistent with a previous study with AMI-6424 (7057-126) from this laboratory thus confirming a treatment-related aspect.

Mean F1 pup weights were decreased at  $\geq 50$  mg/kg/d when compared to controls. Delayed time to vaginal opening was found in the 150 mg/kg/d pups but was attributed to the maternal toxicity and decreased body weights of these pups. No differences from controls were found for other developmental landmarks.

Although locomotor activities were increased for the high dose pups, the increases were within historical control limits at the performing laboratory. Learning and memory of pups were not affected by AMI-6424 treatment.

In the F2 generation, no clinical observations were found related to AMI-6424 treatment.

*Terminal and necroscopic evaluations:*

**Dams:** Pale kidneys were reported for the placebo (4 animals) and all treated dams (F0) [4 low dose dams, 4 mid dose dams and 9 high dose dams]. For the F1 generation, dilated renal pelvices (1 control female and all AMI-6424-treated females as well as all males at 50 and 150 mg/kg/d) was the only significant gross finding.

**Offspring:** No significant treatment-related gross findings were appreciated in F0, F1 or F2 pups.

The NOAEL for F0 maternal effects was 50 mg/kg/d on the basis of reduced body weight and decreased food consumption. Due to the increased incidence of stillborn pups and clinical findings at 150 mg/kg/d in the F1 generation, the fetal/pup NOAEL is determined to be 100 mg/kg/d.

**Reproductive study summary:** In a fertility and early embryonic development study in rats, female rats treated with AMI-6424 at 150 mg/kg showed no changes in fertility indices. In male rats, AMI-6424 at  $\geq 50$  mg/kg caused decreased sperm motility, decreased sperm counts, and abnormal sperm morphology. The placebo elicited similar, yet less extensive, findings but the effects were more significant in the AMI-6424-treated groups.

In the segment 2 rat study (7057-126), at doses  $\geq 100$  mg/kg/day, an effect on fetal growth and embryo/fetal development, as evidenced by decreases in fetal weights, skeletal variations, and external malformations of syndactyly and brachymelia was observed. The NOAEL of 50 mg/kg/day was determined for developmental toxicity.

In the segment 2 rabbit study (7057-175), at 75 mg/kg (HD), there were skeletal malformations including absent ulna, fusion of sternbrae, adactyly and vertebral anomalies. These were noted in single animals from 5 litters and were not appreciated in the 60 mg/kg/d group. One HD fetus showed external abnormalities including flexed front paw, brachymelia, adactyly, gastroschisis. Many of these findings were comparable to those found in Study 7057-126, including the brachymelia, adactyly and absent ulna; and are all considered to be treatment-related. The NOAEL for developmental toxicity in this rabbit study is 60 mg/kg/d.

In the segment 2 minipig study, increased preimplantation loss and postimplantation loss was seen in all dose groups (25, 50 and 75 mg/kg). Increased external malformations evidenced by polydactyly, syndactyly and deformed foreleg with absent radius (described as radial agenesis) were seen in LD and MD groups.

Based on data from segment 2 studies in rats, rabbits, and minipigs, it is concluded that telavancin is a multi-species teratogen with external/skeletal (limb) malformations being the primary terata. The incidences of syndactyly and polydactyly are significantly higher in animals dosed with telavancin than in their databases. Additional effects of telavancin dosing were found in pre- and post-implantation parameters.

AMI-6424 was detected in amniotic fluid several samples in rats and rabbits, indicating that AMI-6424 crosses the placental barrier to a very small extent. This increases the concern for the fetal abnormalities.

In the Segment 3 study in rats, due to the increased incidence of stillborn pups and clinical findings at 150 mg/kg/d in the F1 generation, the fetal/pup NOAEL is determined to be 100 mg/kg/d. Brachymelia, seen in one F1 pup in the 150 mg/kg/d group, was consistent with the Segment 2 study with AMI-6424 (7057-126) from this laboratory thus confirming a treatment-related aspect.

#### **2.6.6.7 Local tolerance**

##### **05-013-06 60876: Telavancin (API and Drug Product): Acute Dermal Irritation Study in the Rabbit (the Sequential Approach)**

The study, conducted at LAB Scantox, Lille Skensved, Denmark, in September 2005, was performed to evaluate local irritant effects of telavancin on female NZW rabbit skin following a single topical exposure. The diluted drug product (10 mg/ml, 0.5 ml was applied) and API (active pharmaceutical ingredient, 0.5 g moistened with 0.5 ml of 0.9% NaCl to ensure good skin contact) were used in the study. Four test areas on the back of each animal were used for exposure times of 3 min, 60 min, 4 hr, and untreated control, respectively. The test articles were applied to the skin and covered with a surgical gauze patch (2.5 cm x 2.5

cm). All sites were recorded 1, 24, 48 and 72 hours after termination of exposure. The observations were scored according to the following scale:

<b>Erythema and Eschar Formation</b>	<b>Score</b>	<b>Edema Score</b>	<b>Score</b>
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising)	2
Moderate to severe erythema	3	Moderate oedema (raised approximately 1 mm)	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4	Severe oedema (raised more than 1 mm, extending beyond the area of exposure)	4

The study was initiated in two rabbits: one for the API and one for the drug product. No skin reactions were observed in these two initial animals; therefore the testing proceeded with four additional rabbits (two for the API and two for the drug product). Apart from very slight erythema observed at one test site in one animal treated with drug product only at one hour after end of the 4-hr treatment, no reactions were observed at the other sites at any times or at this site at 24 to 72 hrs after the end of treatment, and no skin reactions were observed in other animals. No treatment-related clinical signs were observed in the animals during the daily observations. After 4 hours of exposure to the API or drug product, the mean score for erythema was 0.0 and the mean score for edema was 0.0.

In conclusion, telavancin [API and diluted drug product (10 mg/ml)] was classified as a non-irritant.

**05-013-05 60877: Telavancin (API and Drug Product): Acute Eye Irritation/Corrosion Study in the Rabbit**

The study, conducted at LAB Scantox, Lille Skensved, Denmark, in September 2005, was performed to evaluate local irritant effects of telavancin on female NZW rabbit eye following a single topical ocular exposure. The drug product was diluted with 5% dextrose, and API (active pharmaceutical ingredient) was ground to minute fine dust in order to minimize the mechanical damage to the eye. The study was initiated in two rabbits. One animal was exposed to 0.1 g API in the left conjunctival sac and the second animal was exposed to 0.1 ml diluted drug product (10 mg/ml) in the left conjunctival sac. The right eye remained untreated and served as control. Both eyes of each rabbit were examined at 1, 24, 48 and 72 hours after treatment. Following the initial assessment, the study was continued with two additional animals each for API and drug product (total of four animals) treatment in a similar manner to the initially treated animals.

Similar results were seen following the initial and second treatments. Severe irritation (scores ranging between 2 and 4) of the conjunctiva evidenced by chemosis, discharge and redness was observed in the animals treated with API. However, the degree of irritation diminished quickly (by 24 hours post treatment), and was considered to be mainly due to the mechanical irritation of the powder rather than an indication of telavancin-specific irritancy. Reactions in the cornea and iris were not observed. No irritation of the conjunctiva was observed in animals treated with the diluted drug product, nor were there any reactions in the cornea and iris.

[Reviewer's comments: The sponsor should not use API powder in this irritation study since the irritation can be mainly due to the mechanical irritation of the powder. The drug product volume, 0.1 mL, was too much.]

**2.6.6.8 Special toxicology studies****04-001-04 7057-218: 6-Week Intravenous Infusion Immunotoxicity Study with TD-6424 in Rats with a 4-Week Recovery Period**

**Key study findings:** TD-6424 at dose levels of 50 and 100 mg/kg/day produced reversible immunomodulatory effects as evidenced by changes in a T-cell dependent antigen (AFC) response and macrophage function.

**Study no:** CRN: 04-001-06; Covance: 7057-218

**Conducting laboratory and location:** Covance Laboratories Inc., 3301 Kinsman Boulevard, Madison, Wisconsin 53704-2595

**Date of study initiation:** 4/27/2004

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** TD-6424, Lot AME008, purity = 93.0%

The purpose of this study was to evaluate the immunotoxicity potential of TD-6424 when administered daily by intravenous infusion (30-minute duration) to rats for at least 6 weeks followed by a 4-week recovery period. Endpoints in the study included immunophenotyping to examine potential shifts in lymphocyte subpopulations, an assay to examine the antibody response to a T-cell dependent antigen [sheep red blood cells (SRBC)], and macrophage function with focus on phagocytosis and cidal activity. Study design is shown in the table below.

Group	n/sex*	Dose level (mg/kg/day)	Dosing volume (mL/kg)
<b>Macrophage Function and Immunophenotyping Animals</b>			
1 Diluent Control: 5% dextrose for injection	15	0	10
2 Placebo Control	15	0	10
3 TD-6424	15	12.5	10
4 TD-6424	15	50	10
5 TD-6424	15	100	10
<b>Antibody-Forming Cell Assay (T-Cell Test) Animals</b>			
6 Diluent Control: 5% dextrose for injection	15	0	10
7 Placebo Control	15	0	10
8 TD-6424	15	12.5	10
9 TD-6424	15	50	10
10 TD-6424	15	100	10
11 Immunomodulation Control: cyclophosphamide, ip, once a day x 4 days	10	25	10

\* 5/sex/group were recovery animals.

**Results:**

The administration of TD-6424 at dose levels of 50 and 100 mg/kg/day produced immunomodulatory effects as evidenced by changes in a T-cell dependent antigen (AFC) response and macrophage function. Specifically, these effects included reversible decreases in the antibody response to SRBCs, reversible increases in macrophage phagocytosis, and decrease in macrophage respiratory burst activity. Regardless, of this effect on respiratory burst, macrophages from treated animals maintained the ability to increase respiratory burst in response to stimuli (PMA) as evidence by increased burst activity compared with non-stimulated cells. Although an increase in all lymphocyte subpopulations was noted for males given 50 or

100 mg/kg/day, no shifts in any of the examined phenotypes was noted; therefore this latter effect was not considered adverse. The no-observable-effect level with regard to immunomodulation is 12.5 mg/kg/day.

**99-007-59: AMI-999: Exploratory Single Dose Intravenous Nephrotoxicity Study with AMI-999 in Rats**

**Key study findings:** AMI-999 was nephrotoxic in rats evidenced by elevation in serum concentrations of urea nitrogen and creatinine and by tubular necrosis. The recovery of AMI-999 in the urine, liver and kidney were  $16.3\% \pm 4.79$ ,  $37.3\% \pm 2.39$  and  $22.5\% \pm 4.78$ , respectively.

The purpose of the non-GLP study was to evaluate the potential nephrotoxicity and to determine the urinary recovery and tissue distribution of AMI-999 after a single intravenous injection to male rats at 50 mg/kg. Study design is listed in the table below.

Species/strain/sex	Rat/ Crl:CD(SD)IGS BR/ male
No. animals per dose	4
Route of administration/dose	i.v./0 and 50 mg/kg
Vehicle	10% PEG 400
Dose volume	2 mL/kg
Dosing regimen	Single dose on Day 1
Study duration	2 Days
Observation/parameters	Mortality; clinical signs, body weight; serum biochemistry examinations; urinalysis, creatinine clearance, dose recovery in serum, urine, kidney and liver, organ weights, histopathology of kidney

There were no deaths in the study. Clinical observations including transient inactivity, ventral recumbency and an abnormal gait were seen in all treated rats. There was no effect on body weight. Serum BUN and creatinine in animals treated with AMI-999 were 4.2- and 3-fold higher, respectively, than controls. Serum glucose and AST were also higher (1.7 and 1.5-fold, respectively) when compared to concurrent controls. Changes in urine chemistry included lower urinary creatinine (by 41%) and higher urinary blood and protein for rats administered AMI-999. The lower urinary creatinine corresponded with a creatinine clearance that was 74% lower than controls. Approximately 76% of the administered AMI-999 was recovered in the tissues samples; the recovery of AMI-999 in the urine, liver and kidney was  $16.3\% \pm 4.79$ ,  $37.3\% \pm 2.39$  and  $22.5\% \pm 4.78$ , respectively.

Kidney and liver weights were 27% and 15% higher, respectively, in treated rats compared to controls. Pale and/or mottled, bruised, enlarged or patchy kidneys were observed in all rats given AMI-999. Tubular necrosis (grade 1-2) was seen in the kidneys of all animals treated with AMI-999.

In conclusion, AMI-999 was nephrotoxic when given to rats as a single IV injection at 50 mg/kg as indicated by elevation in serum concentrations of urea nitrogen and creatinine and by tubular necrosis. Approximately 76% of the administered AMI-999 was recovered; the recovery in the urine, liver and kidney were  $16.3\% \pm 4.79$ ,  $37.3\% \pm 2.39$  and  $22.5\% \pm 4.78$ , respectively.

**99-007-78: AMI-999: Exploratory Single Dose Intravenous Nephrotoxicity Study with AMI-999 in Rats**

**Key study findings:** AMI-999 was nephrotoxic in rats evidenced by elevation in serum concentrations of urea nitrogen and creatinine. The recovery of AMI-999 in the urine, liver and kidney was  $2.04\% \pm 0.361$ ,  $32.4\% \pm 4.28$  and  $15.8\% \pm 2.84$ , respectively.

The purpose of the non-GLP study was to evaluate the potential nephrotoxicity and to determine the urinary recovery and tissue distribution of AMI-999 after a single intravenous injection to male rats at 50 mg/kg. Study design is listed in the table below.

Species/strain/sex	Ra/ Cri:CD(SD)IGS BR/ male
No. animals per dose	2 for control and 4 for AMI-999
Route of administration/dose	i.v./0 and 50 mg/kg
Vehicle	10% PEG 400
Dose volume	2 mL/kg
Dosing regimen	Single dose on Day 1
Study duration	2 Days
Observation/parameters	Mortality; clinical signs, body weight; serum biochemistry examinations; urinalysis, creatinine clearance, dose, recovery in serum, urine, kidney and liver, organ weights

There were no deaths in the study. Clinical observations including transient inactivity, and ventral recumbency were seen in all treated rats. There was no effect on body weight. Serum BUN and creatinine in animals treated with AMI-999 were 4.2- and 5.3-fold higher, respectively, than controls. Serum glucose and AST were also higher (2.7 and 1.7-fold, respectively) when compared to concurrent controls. Changes in urine chemistry included lower urinary creatinine (by 33%) and higher urinary blood and glucose for rats administered AMI-999. The lower urinary creatinine corresponded with a creatinine clearance that was 82% lower than controls. The concentration of AMI-999 in the serum 24 hours post-dosing was  $9.05 \pm 0.948$  µg/mL. Approximately 50% of administered AMI-999 was recovered in the tissues sampled; the recovery of AMI-999 in the urine, liver and kidney was  $2.04\% \pm 0.361$ ,  $32.4\% \pm 4.28$  and  $15.8\% \pm 2.84$ , respectively.

Kidney weights were 25% higher than controls in treated rats. Mottled, pale and/or lobular kidneys were observed in all rats given AMI-999. The kidneys of one control rat were mottled.

In conclusion, AMI-999 was nephrotoxic when given to rats as a single IV administration at 50 mg/kg as indicated by elevated serum concentrations of urea nitrogen and creatinine. The recovery of AMI-999 in the urine, liver and kidney was  $2.04\% \pm 0.361$ ,  $32.4\% \pm 4.28$  and  $15.8\% \pm 2.84$ , respectively.

#### **00-036-018: AMI-999: Exploratory Single Dose Intravenous Nephrotoxicity Study in Rats**

**Key study findings:** AMI-999 was nephrotoxic following a single IV administration (50 mg/kg) to rats. The toxicity was evidenced by elevated serum concentrations of urea nitrogen and creatinine and by tubular necrosis. AMI-999 formulated in HP-β-CD induced severer effects than the D5W formulation.

The purpose of the non-GLP study was to evaluate the potential nephrotoxicity of AMI-999, formulated in 5% dextrose in water for injection (D5W) or HP-β-CD, when given once intravenously to male rat at 50 mg/kg. Study design is listed in the table below.

Species/strain/sex	Rat/ CrI:CD(SD)IGS BR/ male
No. animals per dose	3/group (12 rats in total)
Route of administration/dose	i.v./0 and 50 mg/kg
Vehicle	5% dextrose in water for injection or HP- $\beta$ -CD
Dose volume	5 mL/kg
Study duration	2 Days
Observation/parameters	Mortality; clinical signs, body weight; serum biochemistry examinations; urinalysis, creatinine clearance, dose recovery in serum, urine, kidney and liver, organ weights, histopathology of kidney

There were no deaths in the study. Transient inactivity, labored respiration and ventral recumbency were seen in all treated rats. There was no effect on body weight.

Administration of AMI-999 in both D5W and HP- $\beta$ -CD formulations caused an increase in serum BUN, creatinine, AST, ALT, phosphorous, and triglycerides levels. Except for triglycerides, the increase was higher in animals given AMI-999 formulated in HP- $\beta$ -CD than in D5W (see table below).

Treatment group	BUN (mg/dL)	CREAT (mg/dL)	GLUC (mg/dL)	AST (U/L)	ALT (U/L)	AP (U/L)	BILI-T (mg/dL)	Na (mEq/L)
D5W	12.0 $\pm$ 1.0	0.20 $\pm$ 0.00	123 $\pm$ 21.7	98.0 $\pm$ 6.00	32.3 $\pm$ 2.08	248 $\pm$ 30.1	0.10 $\pm$ 0.00	150 $\pm$ 1.40
HP- $\beta$ -CD	11.7 $\pm$ 0.58	0.20 $\pm$ 0.00	103 $\pm$ 8.96	263 $\pm$ 106	55.7 $\pm$ 18.8	182 $\pm$ 39.9	0.10 $\pm$ 0.00	152 $\pm$ 1.83
AMI-999 D5W	61.7 $\pm$ 12.4	1.10 $\pm$ 0.91	131 $\pm$ 24.3	286 $\pm$ 127	48.0 $\pm$ 16.1	189 $\pm$ 27.6	0.20 $\pm$ 0.120	146 $\pm$ 0.950
AMI-999 HP- $\beta$ -CD	92.7 $\pm$ 27.5	1.40 $\pm$ 0.82	183 $\pm$ 54.5	2800 $\pm$ 895	564 $\pm$ 155	225 $\pm$ 24.3	0.10 $\pm$ 0.060	137 $\pm$ 3.54
Treatment group	Cl (mEq/L)	TRIG (mg/dL)	P (mg/dL)	PROT-T (G/DL)	ALB (g/dL)	GLOB (g/dL)	A/G Ratio	CHOL (mg/dL)
D5W	98.7 $\pm$ 0.577	33.7 $\pm$ 4.16	11.0 $\pm$ 0.50	6.30 $\pm$ 0.12	4.50 $\pm$ 0.15	1.80 $\pm$ 0.10	2.50 $\pm$ 0.20	49.7 $\pm$ 1.53
HP- $\beta$ -CD	98.3 $\pm$ 0.577	49.3 $\pm$ 16.3	11.0 $\pm$ 0.80	5.90 $\pm$ 0.17	4.10 $\pm$ 0.15	1.80 $\pm$ 0.06	2.20 $\pm$ 0.10	67.3 $\pm$ 6.51
AMI-999 D5W	91.0 $\pm$ 1.00	89.0 $\pm$ 49.5	13.0 $\pm$ 0.66	6.2 $\pm$ 0.12	4.00 $\pm$ 0.10	2.20 $\pm$ 0.21	1.90 $\pm$ 0.22	73.0 $\pm$ 17.4
AMI-999 HP- $\beta$ -CD	86.3 $\pm$ 3.06	77.3 $\pm$ 25.4	14.1 $\pm$ 2.52	5.00 $\pm$ 0.36	3.2 $\pm$ 0.15	1.80 $\pm$ 0.21	1.80 $\pm$ 0.12	43.0 $\pm$ 15.6

AMI-999-induced changes in urine chemistry included a decrease in urinary creatinine level that was 66% and 48% lower than D5W or HP- $\beta$ -CD controls, respectively and an increase in urine volume (2.0-fold) for rats given AMI-999 formulated in D5W. The lower urinary creatinine corresponded with creatinine clearance that were 77 and 87% lower in rats given AMI-999 formulated in D5W and HP- $\beta$ -CD, respectively. In addition, urinary protein was higher and triple phosphate was lower in rats given AMI-999 in both formulations. Red and white blood cells and bacteria were more frequent and there were traces of glucose in the urine of rats given AMI-999 formulated in HP- $\beta$ -CD.

The serum concentration of AMI-999, 24-hours post-dosing, was 23.7 and 36.7  $\mu$ g/mL for rats given AMI-999 formulated in D5W and HP- $\beta$ -CD, respectively. The % recovery of AMI-999 in the urine (12.0% vs. 6.42%) and liver (31.9% vs. 15.6%) was approximately 1.9-fold and 2.0-fold greater in rats given AMI-999 formulated in HP- $\beta$ -CD as opposed to D5W. The recovery of AMI-999 in kidney was similar between groups, 13.7 and 13.0% for AMI-999 formulated in D5W and HP- $\beta$ -CD, respectively.

Kidney weights were increased 12 and 35% and liver weights were increased 9 and 15% in rats treated with AMI-999 formulated in D5W and HP- $\beta$ -CD, respectively. Pale and/or mottled kidneys were observed in rats

with both formulations. Tubular necrosis was seen in the kidneys of all animals treated with AMI-999 with greater severity and tubular vacuolation in the HP- $\beta$ -CD formulation group.

In conclusion, AMI-999 was nephrotoxic when given to rats as a single IV administration at 50 mg/kg evidenced by elevated serum concentrations of urea nitrogen and creatinine and by tubular necrosis. Both D5W and HP- $\beta$ -CD formulations resulted in similar amounts of AMI-999 in the kidney. The AMI-999-induced effects in rats were greater with HP- $\beta$ -CD formulation.

**00-001-02 7057-114: Hemolytic Potential Testing with AMI-6424 in Rat, Dog, and Human Whole Blood**

The purpose of this non-GLP study was to evaluate the hemolytic potential of AMI-6424 in rat, dog, and human whole blood. Hemolytic potential tests were performed with AMI-6424 at concentrations of 2.0, 4.01, 8.01, and 16.02 mg/mL. After equal dilution with rat, dog, and human whole blood, the final concentrations were 1.0, 2.0, 4.01, and 8.01 mg/mL. Identical testing was performed with HP- $\beta$ -CD 180 mg/mL in aqueous dextrose (vehicle); 5% dextrose for injection; rat, dog, and human plasma (negative control); and 1% Saponin (positive control). All whole blood/material tubes were gently mixed, incubated for 40 to 45 minutes at 37°C, and centrifuged. The concentration of hemoglobin was measured spectrophotometrically.

Neither AMI-6424, at concentrations of 2.0, 4.01, 8.01, and 16.02 mg/mL, nor vehicle control caused hemolysis of rat, dog, or human whole blood.

**00-001-25 7057-145: Hemolytic Potential Testing with Lyophilized Formulation of AMI-6424 in Rat, Dog, and Human Whole Blood**

Hemolytic potential tests were performed by mixing equal parts of rat, dog, or human whole blood with the reconstituted lyophilized formulation of AMI-6424 for Injection at concentrations of 10, 5, 2.5, or 1.25 mg/mL (after equal dilution with blood, the final concentrations were 5, 2.5, 1.25, or 0.625 mg/mL). In addition, rat, dog, or human whole blood was mixed with equal volumes of species-matched rat, dog, and human plasma (negative controls); 5% dextrose for injection [D5W, (diluent)]; the lyophilized formulation of placebo for AMI-6424 for Injection (placebo); or 1% Saponin (positive control). All whole blood/material tubes were gently mixed, incubated for 40 to 45 minutes at 37°C, and centrifuged. The concentration of hemoglobin was measured spectrophotometrically.

The lyophilized form of AMI-6424 at concentrations of 10, 5, 2.5, or 1.25 mg/mL; the diluent (5% dextrose for injection); and the reconstituted placebo did not cause lysis of rat, dog, or human erythrocytes.

**05-016-01 EOD00001: Neutral Red Uptake Phototoxicity Assay of Telavancin in Balb/c3T3 Mouse Fibroblasts**

The purpose of this study was to evaluate the phototoxicity potential of TD-6424 as measured by a relative reduction in viability of Balb/c 3T3 mouse fibroblasts exposed to the test article in the presence and absence of light. Two trials were performed in the definitive assay using the following test article concentrations: 3.2, 13.6, 32.0, 68.0, 136, 272.0 mg/L (Definitive Trial 1) and 3.2, 6.8, 27.2, 32.0, 136, 272.0 mg/L (Definitive Trial 2) with and without UVA exposure (5 J/cm<sup>2</sup>). The highest concentration in the definitive

assay was very similar to the highest concentration tested in the range-finding assay and was based upon the solubility determination. The positive control article included in this study was chlorpromazine (CPZ) with and without UVA exposure.

Telavancin did not elicit reductions in viability of Balb/c 3T3 mouse fibroblasts either with or without UVA exposure. Therefore, phototoxic potential was not indicated. The positive control article elicited concentration-dependent reduction in cell survival without UVA exposure and an enhancement with UVA exposure, clearly demonstrating phototoxicity in this *in vitro* assay. In conclusion, telavancin at concentrations up to 272 mg/L did not exhibit phototoxic potential.

#### **05-036-008: Exploratory Single-Dose Intravenous toxicity Study in Male Rats**

This study was conducted under non-GLP conditions to define the potential intravenous toxicity of telavancin, formulated with different ratios of hydroxypropyl- $\beta$ -cyclodextrin or sulfobutylether- $\beta$ -cyclodextrin, when given once to male Crl:CD<sup>®</sup>(SD)IGS BR rats at doses of 50 or 100 mg/kg. The study design is listed in the table below.

Type of cyclodextrin	Ratio of cyclodextrin to telavancin	Telavancin dose level (mg/kg)	Dose concentration (mg/mL)	Number of animals
HP- $\beta$ -CD	1:1	50	5	3
	2:1	50	5	3
	4:1	50	5	3
	10:1	50	5	3
	1:1	100	10	3
	2:1	100	10	3
	4:1	100	10	3
	10:1	100	10	3
SBE- $\beta$ -CD	1.6:1	50	5	3
	3.2:1	50	5	3
	1.6:1	100	10	3
	3.2:1	100	10	3

Parameters evaluated included mortality, clinical observations, hematology, clinical chemistry, necropsy, and histopathology (kidneys only).

No mortality and clinical signs were noted. No significant hematological changes were noted. An increase in serum BUN and creatinine levels was noted. The increase was related to the decrease in the ratio of cyclodextrin to telavancin (see table below). There were no macroscopic findings. Microscopic examination in the kidneys collected 24 hrs after dosing showed renal tubular injury. The severity of renal injury decreased as the ratio of cyclodextrin (either HP- $\beta$ -CD or SBE- $\beta$ -CD) to telavancin increased.

#### **Serum BUN and creatinine levels (mg/dL, mean $\pm$ SD)**

Telavancin (mg/kg)	Ratio of cyclodextrin to telavancin	BUN	Creatinine
50	HP- $\beta$ -CD 1:1	29 $\pm$ 11.4	0.20 $\pm$ 0.04
	HP- $\beta$ -CD 2:1	20 $\pm$ 3.5	0.17 $\pm$ 0.03
	HP- $\beta$ -CD 4:1	21 $\pm$ 2.3	0.17 $\pm$ 0.03
	HP- $\beta$ -CD 10:1	19 $\pm$ 3.5	0.15 $\pm$ 0.02
100	HP- $\beta$ -CD 1:1	27 $\pm$ 7.6	0.19 $\pm$ 0.03
	HP- $\beta$ -CD 2:1	21 $\pm$ 3.6	0.21 $\pm$ 0.06
	HP- $\beta$ -CD 4:1	23 $\pm$ 4.5	0.17 $\pm$ 0.01
	HP- $\beta$ -CD 10:1	22 $\pm$ 3.0	0.15 $\pm$ 0.01
50	SBE- $\beta$ -CD 1.6:1	18 $\pm$ 0.6	0.13 $\pm$ 0.02
	SBE- $\beta$ -CD 3.2:1	18 $\pm$ 1.7	0.10 $\pm$ 0.091
100	SBE- $\beta$ -CD 1.6:1	23 $\pm$ 8.0	0.16 $\pm$ 0.03

	SBE-β-CD 3.2:1	20 ± 4.6	0.15 ± 0.03
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The results of this study indicated that, when telavancin was administered as an intravenous bolus with either HP-β-CD or SBE-β-CD, the severity of renal injury, assessed 24 hours after dosing, decreased as the ratio of cyclodextrin to telavancin increased.

**05-001-58 7057-400: Exploratory 4-Week Intravenous Infusion toxicity Study with Various Telavancin Formulations in Rats**

This study, conducted at Covance Laboratories, Inc., was to evaluate the toxicity of telavancin in various formulations of SBE-β-CD and HP-β-CD when administered for 30 minutes/day via intravenous infusion to rats for at least 4 weeks. The study design is listed in the table below.

Group	Treatment	n/sex	Dose (mg/kg)	Dose volume (ml/kg)
1	Diluent control (DSW)	5	0	10
2	HP-β-CD placebo control	5	0	10
3	SBE-β-CD placebo control	5	0	10
4	HP-β-CD : telavancin 2:1	5	100	10
5	HP-β-CD : telavancin 4:1	5	100	10
6	HP-β-CD : telavancin 10:1	5	100	10
7	SBE-β-CD : telavancin 1.6:1	5	100	10
8	SBE-β-CD : telavancin 3.2:1	5	100	10

Parameters evaluated included mortality, clinical observations, body weights, food consumption, clinical pathology, necropsy, and histopathology (kidneys, liver, testes, lungs, and macroscopic lesions).

One female in Group 4 with the lowest ratio of HP-β-CD:telavancin died on Day 29. The sponsor stated that the death was possibly due to telavancin-related effects on the kidney that were most severe in this group. There were no clear treatment-related clinical signs. Mean body weights and body weight gains were decreased for rats in Groups 4, 5, and 7. Decreases in mean body weight parameters were associated with decreases in mean food consumption. In general, lower ratios of cyclodextrin to telavancin were associated with greater effects on body weight and food consumption.

Telavancin-related hematology findings included mildly lower RBC count, hemoglobin, and hematocrit in Group 7 animals and in Groups 4 and 5 females. Telavancin-related findings for clinical chemistry parameters included higher urea nitrogen (30-60 mg/dL) and creatinine (1.0-1.4 mg/dL) at all doses, and higher aspartate aminotransferase (255-304 U/L) and alanine aminotransferase (77-88 U/L) in Groups 4-6 animals. Urea nitrogen and creatinine levels appeared to be the most affected at the lower ratios of cyclodextrin to telavancin, and were associated with urinalysis findings and histopathologic findings in the kidney.

Increases in the absolute and relative kidney weight were noted in all treatment groups receiving telavancin. The magnitude was greatest in Group 4 and Group 7 animals.

Microscopic findings associated with both placebos included alveolar macrophages (HP-β-CD only) and renal tubule vacuolation. Microscopic changes attributed to telavancin were identified in the liver (hyperplasia/vacuolation of Kupffer cells) and kidney (basophilic tubules, lymphocyte/macrophage infiltrates, tubular dilation, necrosis of tubular epithelium, and granular/cellular casts). Liver and kidney effects were most severe in the group (Group 4) given the lowest ratio (2:1) of HP-β-CD: telavancin.

In conclusion, intravenous infusion of telavancin at 100 mg/kg/day with varying ratios of either HP- $\beta$ -CD or SBE- $\beta$ -CD to telavancin to rats as a once-daily, 30-minute infusion was associated with decreases in body weight parameters and food consumption, and liver and renal effects evidenced by clinical and anatomic pathology parameters. Lower ratios of cyclodextrin to telavancin were associated with more marked effects.

**9808-TX-001 7668-135: Local Tolerance Study with Telavancin via Perivenous, Intraarterial, and Intravenous Injections in Rabbits**

This study was conducted in Covance Laboratories, Inc., to assess the local tolerance of the test article when administered as a single dose by perivenous, intra-arterial, and intravenous injections to rabbits and the local tolerance when administered for 14 days by intravenous injection to rabbits.

Male Hra:(NZW)SPF rabbits were assigned to 4 groups (5/group). Each group received intra-arterial, (Group 1, 10 mg/kg, 1 ml/kg), perivenous (Group 2, 2 mg/site, 0.2 ml/site), or intravenous injections (Groups 3 and 4, 10 mg/kg, 1 ml/kg) of telavancin in the right ear and control (D5W) injections in the left ear. Groups 1 to 3 were administered a single dose, observed for 4 days, and necropsied on Day 5. Group 4 was dosed for 14 days and necropsy was performed on Day 15.

Toxicity was assessed based on mortality, clinical observations, local irritation at the injection site (redness and swelling), body weight, and food consumption. Assessment of local tolerance was based on local irritation, macroscopic, and microscopic examination of the injection sites. At termination, the injection sites from all animals were evaluated by necropsy and histopathological examinations.

There were no treatment-related mortality, clinical observations, body weight changes and food consumption changes.

Macroscopic findings that were considered related to treatment were limited to two Group 4 rabbits. The right ear (test article administration) in one Group 4 animal showed discoloration at the injection site. These findings correlated with a microscopic diagnosis of moderate hemorrhage. Crust on the right and/or left ear was noted in two Group 4 animals. The remaining animals in Group 4 and Groups 1-3 were normal at the time of necropsy.

In histopathological examinations, no test article associated effects were seen at either the perivenous or intraarterial sites. Repeat dose intravenous administration caused vascular changes both in the D5W and test article including minimal to moderate intimal/medial/adventitial proliferation and minimal to slight intimal necrosis of the vessel, minimal to slight chronic inflammation, minimal to moderate hemorrhage (only in telavancin treated right ear), and minimal to slight scab formation were observed at the D5W and telavancin injection sites. These findings tended to be more severe and of higher frequency in telavancin injection site (right ear) when compared to the D5W injection sites. Minimal edema was present at the mid-point site of the right ear in a single rabbit.

In summary, telavancin administered by perivenous (2 mg/site), intra-arterial (10 mg/kg), or intravenous injections (10 mg/kg) to rabbits did not induce local irritation reactions that differed from D5W following single injections. Repeat intravenous administration for 14 days showed vascular changes in both D5W (1

mL/kg) and telavancin (10 mg/kg) treated ears. These changes were slightly more severe at the test article injection site, indicating that telavancin may have potential to induce local/vascular irritation.

**Summary of special toxicity studies:** AMI-6424 at dose levels of 50 and 100 mg/kg/day produced reversible immunomodulatory effects as evidenced by changes in a T-cell dependent antigen (AFC) response and macrophage function. AMI-6424 did not cause hemolysis of rat, dog, or human whole blood. AMI-6424 at concentrations of up to 272 mg/L did not exhibit phototoxic potential. The drug has potential to induce local/vascular irritation. When telavancin was administered as an intravenous bolus with either HP- $\beta$ -CD or SBE- $\beta$ -CD, the severity of renal injury and ALT and AST changes decreased as the ratio of cyclodextrin to telavancin increased. AMI-999 ( ), when given to rats as a single IV dose at 50 mg/kg, was nephrotoxic evidenced by elevated serum concentrations of urea nitrogen and creatinine and by tubular necrosis.

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#### 2.6.6.9 Discussion and Conclusions

The sponsor conducted several toxicological studies with durations of up to 6-months in rats and 3 months in dogs. The organs of toxicity identified in these studies include the renal and hepatic systems in both species. Multiple organ macrophage accumulation/hypertrophy/hyperplasia was also noted. The drug is teratogenic. Some of the findings (e.g., increased BUN, creatinine, AST, and ALT levels) were also seen in placebo (hydroxypropyl- $\beta$ -cyclodextrin) control animals, suggesting that these changes were at least partially due to the hydroxypropyl- $\beta$ -cyclodextrin. However, the findings were more significant and more frequent in the drug-treated animals, leading to the conclusion that the active compound contributed to the alteration.

Renal toxicity in rats, seen in several studies, was evidenced by increased serum BUN and creatinine levels ( $\geq 50$  mg/kg), proximal tubular degeneration ( $\geq 50$  mg/kg), urinary occult blood ( $\geq 12.5$  mg/kg), granular cast and amorphous crystals ( $\geq 12.5$  mg/kg), increased kidney weight ( $\geq 50$  mg/kg), diffusely light or mottled kidneys ( $\geq 50$  mg/kg), enlarged renal lymph nodes, and increased incidence/severity of tubular casts and/or dilatation, cortical tubular vacuolation, and increased interstitial inflammatory cell infiltrates ( $\geq 25$  mg/kg). The incidence and/or severity of the lesions increased with increasing dose. Many lesions (e.g., increased creatinine levels) persisted throughout the recovery period although variable levels of reversibility were noted. Increased urinary protein was noted with the dipstick methodology but not with a quantitative methodology. The sponsor explained that it was due to the semiquantitative method of measurement (Multistix<sup>®</sup>) in which AMI-6424 might interfere with the determination of urinary protein levels. With the more quantitative Biotrol Urine Proteins Methods, comparable protein levels were seen in drug and vehicle groups.

In the 13-week dog study, approximately 2x increases in urinary BUN and creatinine and urinary output were noted at  $\geq 25$  mg/kg/day, which was consistent with the observation of the exacerbated severity of tubular vacuolation and/or degeneration/necrosis in the kidneys of animals in these groups. Lesions discovered in the placebo emulsion group included kidney lesions (renal tubular vacuolation, dilatation, necrosis and eosinophilic cytoplasmic inclusions, and urothelial vacuolation in the kidneys, urethra, and urinary bladder). However, all of these changes were exacerbated by AMI-6424 and increased in incidence and severity with increasing AMI-6424 doses.

Hepatotoxicity was evidenced by marked increases in ALT and AST levels [up to 4x (AST) and 28x (ALT) increases], and liver weights in dogs at 100 mg/kg/d. These increases were partially reversed by the end of the recovery period. Hepatocellular degeneration/necrosis (graded as slight) was also found in all 100 mg/kg/d animals, including the premature decedents. In 3- and 6-month rat studies, increases in ALT and AST levels as well as the increased liver weights were seen at  $\geq 50$  mg/kg/d, which was, similar to dogs, related to hepatocyte degeneration.

Systemic and multiorgan macrophage accumulation/hypertrophy/hyperplasia was found in many tissues (including major tissue systems liver, kidneys, lungs, lymph nodes, spleen, esophagus, heart, salivary gland, and bone marrow and the testicular interstitium in males) in rat and dog studies that the sponsor attributed to placebo administration. However, the incidence and severity increased with increasing dose, and macrophage accumulation/hypertrophy/hyperplasia in bone marrow, spleen, thymus and duodenum was only seen in AMI-6424-treated animals in the 6-month rat study.

No indication of genetic toxicity was found in any of the assays performed.

In a fertility and early embryonic development study in rats, female rats treated with AMI-6424 at 150 mg/kg showed no changes in fertility indices. In male rats, AMI-6424 at  $\geq 50$  mg/kg caused decreased sperm motility, decreased sperm counts, and abnormal sperm morphology. The placebo elicited similar, yet less extensive, findings but the effects were more significant in the AMI-6424-treated groups.

In the segment 2 rat study (7057-126), at doses  $\geq 100$  mg/kg/day, an effect on fetal growth and embryo/fetal development, as evidenced by decreases in fetal weights and external malformations of syndactyly and brachymelia was observed. The NOAEL of 50 mg/kg/day was determined for developmental toxicity.

In the segment 2 rabbit study (7057-175), at 75 mg/kg (HD), there were skeletal malformations including absent ulna, fusion of sternbrae, adactyly and vertebral anomalies. These were noted in single animals from 5 litters and were not appreciated in the 60 mg/kg/d group. One HD fetus showed external abnormalities including flexed front paw, brachymelia, adactyly, gastroschisis. Many of these findings were comparable to those found in Study 7057-126, including the brachymelia, adactyly and absent ulna, and are all considered to be treatment-related. The NOAEL for developmental toxicity in this rabbit study is 60 mg/kg/d.

In the segment 2 minipig study, increased preimplantation loss and postimplantation loss was seen in all dose groups (25, 50 and 75 mg/kg). Increased external malformations evidenced by polydactyly, syndactyly and deformed foreleg with absent radius (described as radial agenesis) were seen in LD and MD groups.

Based on data from segment 2 studies in rats, rabbits, and minipigs, it is concluded that telavancin is a multi-species teratogen with skeletal (limb) malformations being the primary terata. The incidences of syndactyly and polydactyly are significantly higher in animals dosed with telavancin than in their databases. Additional effects of telavancin dosing were found in pre- and post-implantation parameters. Telavancin may have a significant effect on post-implantation losses.

AMI-6424 was detected in several amniotic fluid samples in rats and rabbits, indicating that AMI-6424 crosses the placental barrier to a very small extent. This increases the concern for the fetal abnormalities.

In the Segment 3 study in rats, due to the increased incidence of stillborn pups and clinical findings at 150 mg/kg/d in the F1 generation, the fetal/pup NOAEL is determined to be 100 mg/kg/d. Brachymelia, seen in

one F1 pup in the 150 mg/kg/d group, was consistent with a previous study with AMI-6424 (7057-126) from this laboratory thus confirming a treatment-related aspect.

The AMI-999, was nephrotoxic following a single IV administration (50 mg/kg) to rats. The toxicity was evidenced by elevated serum concentrations of urea nitrogen and creatinine and by tubular necrosis. AMI-6424 did not cause hemolysis of rat, dog, or human whole blood. AMI-6424 at concentrations of up to 272 mg/L did not exhibit phototoxic potential.

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The potential for telavancin-related toxicity has been assessed in nonclinical studies with target organs identified. All major toxicities observed in animal studies, including renal, hepatic, and reproductive toxicities, occurred with the systemic exposure levels to the drug similar to those seen in clinical studies at the proposed clinical dose (10 mg/kg, see table below). The reviewing pharmacologist has concerns with the toxicities since there is no safety margin over the proposed clinical dose. From the pharmacology/toxicology perspective, the clinical use of the drug at 10 mg/kg does not appear to be safe.

**Comparison of Systemic Exposure to Telavancin at the Lowest Dose with Toxicity between Animals and Humans**

Species	General toxicity		Segment 2 studies			Clinical studies
	Rat	Dog	Rabbit	Rat	Minipig	Human
Dose (mg/kg)	50*	25*	75	100	25	10
AUC <sub>0-24h</sub> (µg-hr/ml)	1012-1227	600-624	1387	829	780	666-780
Animal/human	1.3-1.6	0.77-0.80	1.78	1.06	1	

\* This is the dose with clear liver and renal findings. Other positive findings (e.g., macrophage hypertrophy/hyperplasia affecting the bone marrow, spleen, thymus, and duodenum) were seen at the doses as low as 6.25 mg/kg with AUC of 88-126 µg-hr/ml.

In conclusion, telavancin is a multi-species teratogen with external/skeletal (limb) malformations being the primary teratogenic findings. The kidneys and liver are the target organs of toxicity identified in general toxicity studies. There is no safety margin regarding the toxicities over the clinical dose. Nonclinical data do not provide support for the safety of the drug in clinical use as well as the approval of the drug.

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

Not applicable.

**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

Conclusions: Pharmacology/toxicology-related safety issues were addressed in this application. AMI-6424 is a glycopeptide antibiotic. The drug is a multi-species teratogenic. Nonclinical PK studies showed that following intravenous administration, the plasma Cmax and AUC demonstrated dose-dependent increases. Nonclinical toxicity studies showed the target organs of toxicity including the renal and hepatic systems in dogs and rats. Multiple organ macrophage accumulation/hypertrophy/hyperplasia was also noted. There is no safety margin regarding the toxicities over the clinical dose. Nonclinical data do not support the safety of the drug in clinical use.

Unresolved toxicology issues: None

Recommendations:

Reviewer: Zhou Chen

NDA 22-110

The safety of clinical use of the drug is not supported by nonclinical study data. An approval cannot be recommended from a nonclinical perspective due to safety concerns.

Several modifications of labeling are recommended as revised in the "Carcinogenesis, Mutagenesis, Impairment of Fertility" section and "Pregnancy" section.

**Suggested labeling:**



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Signatures:

Reviewer Signature \_\_\_\_\_  
Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

**APPENDIX/ATTACHMENTS: NONE**

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/s/

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Zhou Chen  
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PHARMACOLOGIST

Terry Peters  
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**M E M O R A N D U M**

**Date:** May 31, 2007 **Date Consulted:** February 20, 2007

**From:** Karen B. Feibus, M.D.  
Team Leader, Pediatric and Maternal Health Staff

**Through:** Sandra Kweder, MD  
Deputy Director, Office of New Drugs

Lisa Mathis, MD  
Associate Director, Pediatric and Maternal Health Staff

**To:** Division of Anit-Infective and Ophthalmology Products (DAIOP)

**NDA:** 22-110

**Drug:** Telavancin hydrochloride

**Subject:** Determination of pregnancy category and need for pregnancy exposure registry and/or risk minimization action plan (RiskMAP)

**Materials Reviewed:** Pharmacology/Toxicology Review and other relevant documents submitted to IND 60,237 and NDA 22-110

**Consult Question:**

Telavancin has exhibited consistent teratogenic effects in rats, rabbits, and minipigs, which represents a significant safety concern. The Division is requesting a PMHS consultation in the determination of appropriate labeling (i.e. pregnancy category) and pregnancy exposure registry considerations. In addition, we would like PMHS's opinion on the necessity of any pregnancy prevention risk management programs, and an assessment of fetal safety following in-utero drug exposure. The following documents are attached for your review:

1. DAIOP Pharmacology/Toxicology review of related IND 60,237 serial submission 171
2. A copy of the (sponsor's) proposed labeling for telavancin (NDA 22-110).

## EXECUTIVE SUMMARY

Telavancin is a semi-synthetic, lipoglycopeptide antibiotic that exhibits bactericidal activity against most gram-positive bacteria. The telavancin molecule core is identical to vancomycin and its antimicrobial coverage is similar. The current NDA application is for marketing telavancin as an antimicrobial to treat complicated skin and skin structure infections (cSSSI). Based on data review by both the DAIOP microbiologist and medical officer, telavancin is equivalent to, but not superior to, vancomycin for this indication. In addition, reproductive toxicology studies show similar teratogenic effects and increased post-implantation pregnancy loss in rats, rabbits, and Göttingen minipigs at non-maternotoxic doses of drug. While the presence or absence of teratogenic effects in any one animal species does not necessarily predict teratogenicity in developing humans, the occurrence of increased post-implantation loss and skeletal (limb) malformations across all three species at 1 – 15 times the human therapeutic dose is highly concerning. In addition, the minipig study showed a lower fecundity ratio than that in either historical database, and male fertility studies in rats showed decreased sperm motility and increased abnormal sperm morphology.

Telavancin is a multi-species teratogen. Its classification with regard to use in pregnancy should be based on both its potential risk to mother and fetus as well as its potential clinical benefits above other available therapies. Currently, there are eight antimicrobial agents FDA approved for the treatment of cSSSI. Vancomycin remains first-line therapy for severe infections possible caused by MRSA. Based on current labeling for these approved cSSSI antimicrobial therapies, telavancin does not offer broader or better antimicrobial coverage and has a much larger, consistent, and concerning animal safety signal for teratogenic potential in humans.

For the proposed indication of cSSSI, telavancin, if approved, should be assigned pregnancy category X because of a consistent teratogenic signal in three animal species combined with a lack of evidence of clinical benefit over eight other approved therapies for this indication. It is possible that data submitted for a different clinical indication in the future could support pregnancy category C if some direct benefit to mother or fetus was demonstrated. However, for the indication of cSSSI, there is no data to support such a benefit.

A RiskMAP that includes education and reminders alone will not adequately safeguard against telavancin use in pregnant women, and a RiskMAP with a performance-linked access system is probably not feasible for this drug, which will be used in acute care situations to treat acute infections.

If telavancin hydrochloride is approved, the Maternal Health Team recommends the following:

1. Boxed warning informing prescribers (and patients) that telavancin caused congenital anomalies and increased pregnancy loss in rats, rabbits, and minipigs and is, therefore,

a suspected teratogen in humans that should not be used in women of childbearing potential.

2. Pregnancy category X (based on no increased benefit over current therapies and the potential for greater risk based on consistent teratogenic and pregnancy loss safety signals in three animal species)
3. Indicated populations should include adult men and adult women who are not of childbearing potential.
4. Restricted distribution at the pharmacy level that requires documentation of age and gender of the patient. If the patient is female, documentation of menopause or other evidence of non-childbearing potential should be required.

## **INTRODUCTION**

On December 19, 2007, Theravance, Inc. submitted NDA 22-110 for Telavancin, an antimicrobial indicated for the treatment of adults with complicated skin and skin structure infections. The Telavancin drug development program is ongoing and focuses on treatment of adult patients with the following infections caused by susceptible strains of gram positive microorganisms:

- Hospital-acquired pneumonia, including cases with concurrent bacteremia
- Complicated skin and skin structure infections (cSSSI), including cases with concurrent bacteremia.

Reproductive toxicology data submitted under IND 60,237 and reviewed by the Division of Anti-infective and Ophthalmology Products (DAIOP) demonstrate teratogenic effects in rats, rabbits, and minipigs. On February 20, 2007, DAIOP consulted the Maternal Health Team to obtain input on drug labeling for use in pregnant and nursing women and the need for a pregnancy registry and/or a risk management action plan.

## **BACKGROUND**

Telavancin is a semi-synthetic, lipoglycopeptide antibiotic that exhibits bactericidal activity against most gram-positive bacteria. The telavancin molecule core is identical to vancomycin. The addition of a N-decaaminoethyl group provides a functional lipid tail that improves microbiological activity, and a phosphonomethyl aminoethyl group improves the pharmacokinetic profile to allow once daily dosing. Telavancin's antimicrobial activity is concentration dependent, and the inhibition of cell wall synthesis and disruption of the bacterial cell wall phospholipids are the primary mechanisms of action.

To document the in-vitro activity of telavancin, the applicant conducted 19 studies with more than 12,000 bacterial isolates from 165 centers worldwide. Telavancin demonstrated in-vitro activity against: staphylococci and  $\beta$ -hemolytic streptococci (the principal species involved with cSSSI), and all other Gram-positive species considered human pathogens. Isolates resistant to

oxacillin/methicillin, linezolid, clindamycin, fluoroquinolones, or trimethoprim/sulfamethoxazole and staphylococci resistant to daptomycin or with reduced susceptibility to vancomycin were susceptible to telavancin. Telavancin has potent and consistent activity against methicillin-resistant staphylococcus aureus (MRSA).

The applicant states that telavancin exhibited rapid bactericidal action in time-kill studies and suggests that this rapid killing action reduces that potential development of antimicrobial resistance. In-vitro resistance emergence testing with three staphylococcal strains found no resistant isolates. Resistant variants did occur with vancomycin-resistant enterococci (*E. faecalis* and *E. faecium*). Bactericidal activity against staphylococci [including methicillin sensitive *S. aureus* (MSSA), MRSA, vancomycin-intermediate *S. aureus*, and coagulase negative staphylococci] was concentration-dependent and superior to most comparator antimicrobial agents. Telavancin was bactericidal at low concentrations against streptococci, including  $\beta$ -hemolytic streptococci and *Streptococcus pneumoniae* isolates. At low multiples of the MIC (minimum inhibitory concentration), telavancin did not achieve a 3-log reduction in colony forming units of vancomycin-susceptible and non-vanA-type vancomycin-resistant enterococci but was bactericidal at concentrations of 16 – 32  $\mu$ g/mL. The applicant states that in-vitro, telavancin was superior to vancomycin against the majority of enterococcal isolates studied. However, Fred Marsik, Ph.D., microbiology team leader for DAIOP, noted in his reviews dated 05/31/2006 and 06/26/2004 that telavancin activity against vancomycin-resistant enterococci (VRE) is dramatically decreased in the presence of human sera. It is not clear how these conflicting results predict telavancin's clinical activity in humans with VRE infection, but the current indication of sCCCI and the ongoing studies for hospital acquired pneumonia do not require the treatment of VRE.

Animal model studies show that telavancin is active in-vivo against MRSA in both immunocompetent and immunocompromised models. In the mouse subcutaneous abscess (MSA) model, telavancin was 3-fold more potent than vancomycin and linezolid against MRSA. For MRSA, the telavancin ED<sub>50</sub> in the MSA model and the mouse neutropenic thigh model were similar. In contrast, the vancomycin and linezolid ED<sub>50</sub>'s were 10 and 34 times higher in the immunocompromised model than in the immunocompetent MSA model. The applicant concluded that telavancin efficacy was comparable or superior to vancomycin and/or linezolid for the treatment of clinically relevant Gram-positive pathogens including: MSSA, MRSA, glycopeptide-intermediate *S. aureus*, methicillin-sensitive *S. epidermitis*, VRE, and penicillin-sensitive and penicillin-resistant *S. pneumoniae*.

Based on preliminary review of the NDA submission, the review team made the following observations (as presented at the mid-cycle meeting on May 08, 20007:

- Preclinical Pharmacology/Toxicology
  - There are signs of liver toxicity in animal studies conducted in rats and dogs at telavancin doses equivalent to 1 – 2 times the therapeutic human dose. These findings included elevations of liver transaminases and mild hepatocellular degenerative changes.

- There are signs of renal toxicity in rats and dogs including renal tubular degeneration. Elevations in blood urea nitrogen and creatinine occurred with occult blood and amorphous crystals in the urine.
- Reproductive toxicology studies have positive findings in three species: rat, rabbit, and minipig. In Segment I studies in rats, televancin reduced sperm motility and increased abnormal sperm morphology. In Segment II studies, skeletal malformations occurred in all three species at doses that did not cause maternal toxicity.
- **Microbiology**
  - In-vitro and in-vivo testing suggest that televancin is bactericidal against the organisms responsible for complicated skin and skin structure infections. However, there are no data to support superiority of this drug for treatment of cSSSI.
  - No treatment emergent resistance occurred in-vitro or in clinical studies. Microbiology reviewers are awaiting data from the sponsor that can address the potential for development of hetero-resistance to televancin and the effectiveness of televancin against organisms with hetero-resistance to vancomycin.
- **Clinical Pharmacology**
  - The applicant initially studied televancin at doses of 7.5 mg/kg. They increased the dose to 10 mg/kg early in Phase III of the drug development programs after Phase II study analysis demonstrated a 15% increase in microbiological cure rates at the higher dose. However, the increase in clinical cure rate was only 5%. It is not clear how this increase in dose affects the incidence or degree of renal toxicity.
  - Results of a skin blister study suggests that televancin achieves adequate tissue levels in the skin to treat complicated skin and skin structure infections.
- **Clinical**
  - Clinical trials show a renal safety signal consistent with preclinical findings, but a hepatic safety signal is not evident on initial review.
  - Using a non-inferiority margin of 10%, the Phase III trials demonstrated non-inferiority of televancin to vancomycin for the treatment of cSSSI caused by Gram positive organisms.
  - The Phase III pooled study data did not demonstrate statistical superiority of televancin over vancomycin for the treatment of cSSSI caused by MRSA. (The team statistician stated that it was not even close.)

## REVIEW OF DATA

The following materials were submitted for review with the DAIOP consult:

- Pharmacology/toxicology review of IND 60,237, N171 (03/10/2006) by Terry S. Peters, D.V.M (dated 04/06/2006)
- Four published resources supporting the use of the Göttingen minipig as an animal model in teratogenic studies
  - Earl FL, Miller E, Van Loon EJ. Teratogenic research in beagle dogs and miniature swine. (This research was conducted at the FDA laboratories at Beltsville, MD)
  - Jørgensen KD. Minipig in Reproduction Toxicology. Scand J Lab Anim Sci. 1998; 25, suppl 1: 63-75.
  - Misawa J, Kanda S, Kokue E, Hayama T, Teramoto S, Aoyama H, Kaneda M, Iwasaki T. Teratogenic activity of pyrimethamine in Göttingen minipig. Toxicol Letters 1982; 10: 51-54.
  - Palludan B. The Teratogenic effect of Thalidomide in Pigs. Limb Development and Deformity: Problems of Evaluation and Rehabilitation. 1969. Charles C. Thomas, publisher. pp 199-202.

Other materials reviewed include:

- Pharmacology/toxicology review of IND 60,237, N014 (04/19/2003) by Terry S. Peters, D.V.M (dated 08/21/2003)
- Pharmacology/toxicology review of IND 60,237, N025 (11/19/2003) by Terry S. Peters, D.V.M (dated 12/10/2003)
- Reproductive and developmental toxicity sections of the toxicology written summary submitted to NDA 22-110. Specific studies were reviewed when needed.

The Division used the submitted publications to support their request for the reproductive study in the Göttingen minipig. The historical information for this species provides baseline malformation rates against which to compare the incidences of various malformations among study animals in the televancin reproductive toxicology study. Dr. Terry Peters, the pharmacology/toxicology reviewer for IND 60,237 reviewed the study report on the minipig study upon its initial submission (see review of submission N171 dated 04/06/2006). Currently, Dr. Zhou Chen, the pharmacology/toxicology reviewer for NDA 22-110, is reviewing this study as part of the NDA submission.

Table 1 on the next page summarizes the outcomes from five reproductive toxicology studies of televancin in three species:

- Rabbit: two segment I and II studies of televancin doses of 12.5 – 75 mg/kg/d administered on gestational days 7-20

- \* Rat: one fertility and early embryonic (segment I) study and one pre-and post-natal development study (segment II and III) at televancin doses of 50-150 mg/kg/d
- \* Minipig: embryo-fetal development (segment II) at doses of 25-75 mg/kg/d

**Table 1: Summary of Reproductive Toxicology Study Results Submitted to NDA 22-110 for Televancin Hydrochloride**

Species	Study Type	Treatment Groups	Treatment Duration	Positive Findings
Rabbit	Developmental toxicity Phase I and II AMI CSN: 02-001-03	5% dextrose Placebo (diluent?) 12.5 mg/kg/d televancin 25 mg/kg/d televancin 45 mg/kg/d televancin By slow IV bolus daily  Phase I: 20 females per group Phase II: satellite groups Toxicokinetics N=4 Recovery: N=4 Amniotic fluid: N=10	Gestational days 7-20	Two doses in the 25 mg/kg/d group aborted and were removed from the study.  In all televancin-dosed groups, there was a drug-related increase in post-implantation losses. This did not appear to be dose-related.  An increase in dilated lateral ventricles of the brain and missing intermediate lung lobes occurred in feti from all three televancin dose groups. This increase was statistically significant for both anomalies at the highest dose and for dilated ventricles in the low and medium dose groups.  There was incomplete ossification of the 5 <sup>th</sup> and 6 <sup>th</sup> sternbrae in the high dose televancin feti but this was not a statistically significant finding.  Maternal NOAEL = 45 mg/kg/d Fetal NOAEL = not clear
Rabbit	Developmental toxicity AMI CSN: 02-001-015	Placebo (diluent?) 60 mg/kg/d televancin 75 mg/kg/d televancin By slow IV bolus daily  Main study: 20 females per group  Toxicokinetics: N=4 Amniotic fluid: N=10	Gestational days 7-20	Only one animal had televancin levels detected in amniotic fluid. This suggests limited fetal exposure to drug or that the drug was rapidly metabolized.  Overall, televancin treated animals had skeletal variations including an increased incidence of unilateral 13 <sup>th</sup> ribs and presacral vertebrae.  In the televancin 75 mg/kg/d group: <ul style="list-style-type: none"> <li>■ One fetus from each of five litters had various skeletal malformations including: absent ulna, fusion of sternbrae, adactyly, and vertebral anomalies.</li> <li>■ Additional abnormalities noted were: one fetus with brachymelia, adactyly, and gastroschisis; one fetus with umbilical hernia; and one fetus with diaphragmatic hernia and gall bladder agenesis (the latter two conditions have been seen in historical controls)</li> </ul> NOAEL for developmental toxicity – 60mg/kg/d. The Pharmtox reviewer stated that the level of concern is quite high given “enormity of the effects.”

**Table 1: Summary of Reproductive Toxicology Study Results Submitted to NDA 22-110 for Televancin Hydrochloride**

Species	Study Type	Treatment Group	Treatment Duration	Positive Findings
Rat	Fertility and Early embryonic development to implantation AMI CSN: 02-001-05	Diluent control Placebo 50 mg/kg/d televancin 75 mg.kg.d televancin 100 mg/kg/d televancin By slow IV bolus daily 20 males and 20 females in each group	Males dosed for at least 28 days before mating Females dosed from at least 14 days before mating until gestation day 7.	Males: Decreased sperm motility Increased abnormal morphology  These effects were also seen in the placebo group but less often and to a smaller degree. The effects were dose dependent in the televancin treated groups.
Rat	Pre- and post-natal development, Including maternal function AMI CSN: 02-001-07	5% dextrose Placebo 50 mg/kg/d televancin 100 mg/kg/d televancin 150 mg/kg/d televancin By slow IV bolus daily 25 females per group	Gestational day 6 to Lactation day 20	Televancin treated F <sub>0</sub> dams in the two higher dose groups had decreased mean maternal body weights, mean body weight changes, and food consumption.  Total litter death in 3 F <sub>0</sub> dams: 1 placebo, 2 high dose. There was a dose-related increase in the number of stillborn pups and the number of dams with stillborn pups.  F <sub>1</sub> pups in the high dose group were cyanotic (2 litters), swollen (2 litters), and anophthalmic (3 litters), and one pup had brachymelia (limited use of a forelimb). These findings were consistent with those a previous study. Compared to controls, mean F <sub>1</sub> pup weights were decreased at 50 mg/kg/d. On necropsy, all F <sub>1</sub> pups treated with televancin had dilated renal pelvices compared with 1 control female pup.  NOAEL for F <sub>0</sub> maternal effects = 50 mg/kg/d NOAEL for F <sub>1</sub> fetal/pup effects = 100 mg/kg.d

**Table 1: Summary of Reproductive Toxicology Study Results Submitted to NDA 22-110 for Televancin Hydrochloride**

Species	Study Type	Treatment Groups	Treatment Duration	Positive Findings
Minipig	Embryo-fetal development	Diluent (5% dextrose) Placebo 25 mg/kg/d televancin 50 mg/kg/d televancin 75 mg/kg/d televancin By slow IV bolus daily  14 females per treatment group	Gestational days 11-35  Toxicokinetic satellite groups (3 animals/group) dosed gestational days 11-16 only and then euthanized	<p>Number of and reasons for dams sacrificed <i>in extremis</i> were similar by treatment group.</p> <ul style="list-style-type: none"> <li>▪ Many of these animals were treated with other antimicrobial agents (3 topical ointments, 3 systemic agents)<sup>1</sup></li> <li>▪ Pregnancy rates seemed unacceptably low to the review pharmacologist, especially in the placebo (36%) and high dose televancin (36%) groups<sup>2</sup></li> <li>▪ There were an increased number of late resorptions noted in the mid and high dose groups compared to historical controls</li> <li>▪ There was a &gt; 100% increase in post-implantation loss in the high dose treatment group compared with placebo and diluent</li> <li>▪ 45% of televancin-treated litters had feti with external and soft tissue abnormalities compared to 14% of litters and 20% of litters in the diluent and placebo groups respectively.</li> <li>▪ Among 58 feti from the placebo and diluent treated groups, the sponsor noted 1 fetus with retained testes and 1 with retained testes and polydactyly<sup>3</sup></li> <li>▪ Among 84 feti from the televancin treated groups, the sponsor noted the following findings: 9 feti with polydactyly (3 on two limbs), 1 fetus with diaphragmatic hernia, one with discolored diaphragm, one with syndactyly, and one with retained testes</li> </ul> <p>In addition, the pharmacology reviewer noted: a low dose fetus with deformed head and a misshapen digit; a mid-dose fetus with "legs turned inward"; a mid-dose fetus with multiple absent ossification sites and bilateral absence of tarsal bones; a mid-dose fetus with absent ossification sites distal to the metacarp; a mid-dose fetus with exophthalmos; a mid-dose fetus with anencephaly; a high-dose fetus with deformed head, forelegs, and snout (very autolytic); and a high dose fetus with a deformed hind leg.</p>

<sup>1</sup> According to the pharmacologist, this is very unusual among toxicology studies submitted for regulatory review. These animals were evenly distributed among treatment groups but call the validity of the study into question.

<sup>2</sup> Historical control pregnancy rates for Göttingen minipigs are 65-93% over three studies.

<sup>3</sup> The historical Danish database shows that the incidence of syndactyly is ≤ 0.4% and the incidence of pentadactyly was ≤ 2.3% for the past five years. However, after a change from line breeding to a population based breeding program in November 2004, these rates declined to <0.2% and <0.7% respectively. The historical Japanese database shows a 1.4% incidence of polydactyly among newborn piglets, a preimplantation loss rate of 11.7%, and a post-implantation loss rate of 15.6%.

Both the sponsor and Dr. Terry Peters, FDA pharmacology reviewer, acknowledge potential confounding factors in the minipig study; however, they disagree about their impact on study result interpretation and application. Many of the minipigs were treated with other antimicrobial agents (3 topical ointments, 3 systemic agents)<sup>4</sup>, but these animals were evenly distributed among treatment groups. Dr. Peters found the minipig pregnancy rates unusually low, especially in the placebo (36%) and high dose televancin (36%) groups.<sup>5</sup> Pregnancy rates were 64% in the low-dose group and 57% in the mid-dose group. Historical control pregnancy rates for Göttingen minipigs are 65-93% over three studies. There were an increased number of litters with late resorptions noted in the mid dose (mean = 0.6) and high dose (mean = 0.8) groups compared to historical controls (maximum mean = 0.4).

*Reviewer comment:*

*While these aberrations in the minipig study should not be discounted, the study findings are still very worrisome. The post-implantation loss increased by more than 100% in the high dose treatment group compared with the placebo and diluent treatment groups. Increased pregnancy loss and skeletal anomalies occurred at increased rates in all three species of animal studied. These similarities should not be attributed to coincidence and confounding alone.*

Table 2 summarizes the main fetal findings from the reproductive toxicology studies conducted using televancin in rats, rabbits, and minipigs. In addition, it includes details about televancin's antimicrobial coverage for the proposed indication, complicated skin and skin structure infections (cSSSI).

Drug	Pregnancy category	Reproductive toxicology study findings	Antimicrobial coverage for cSSSI
Televancin	?	Reproductive studies in rats, rabbits, and minipigs showed increased post-implantation losses and increased skeletal malformations including limb abnormalities and absent or decreased ossification centers. These effects occurred at doses 1 – 15 times the human therapeutic dose. In rats, sperm motility was decreased and abnormal sperm morphology was increased.	cSSSI caused by susceptible strains of the following gram positive organisms: <i>Staphylococcus aureus</i> (including methicillin-susceptible and -resistant strains), <i>Streptococcus pyogenes</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus anginosus</i> group, and <i>Enterococcus faecalis</i> (vancomycin-susceptible isolates only)

This information can be compared and contrasted with the reproductive toxicology study information included in the pregnancy section of labeling for all anti-microbials approved by the FDA for the treatment of cSSSI. This information is shown in Table 3 on following pages.

<sup>4</sup> According to the pharmacologist, this is very unusual among toxicology studies submitted for regulatory review.  
<sup>5</sup> Historical control pregnancy rates for Göttingen minipigs are 65-93% over three studies.

**Table 3: Anti-microbial Drugs Approved For the Treatment of Complicated Skin and Skin Structure Infections**

Drug	Pregnancy category	Reproductive toxicology study findings	Antimicrobial coverage for cSSSI
Daptomycin	B	<p>Studies performed in rats and rabbits at doses up to 2 and 4 times the human dose showed no evidence of fetal harm.</p> <p>There are no adequate and well-controlled studies in women.</p>	<p>cSSSI caused by susceptible isolates of the following gram positive organisms:</p> <p><i>Staphylococcus aureus</i> (including methicillin resistant isolates), <i>Streptococcus pyogenes</i>, <i>Streptococcus agalactiae</i>, <i>Streptococcus dysgalactiae</i> subspecies <i>equisimilis</i>, and <i>Enterococcus faecalis</i> (vancomycin susceptible isolates only)</p>
Piperacillin/Tazobactam	B	<p>Piperacillin: Reproduction and teratology studies in mice and rats have not revealed impaired fertility or harm to the fetus at 0.5 to 1 times the maximum human dose.</p> <p>Tazobactam: Reproduction studies in rats revealed no evidence of impaired fertility at up to 3 times the maximum human dose.</p> <p>There are no adequate and well-controlled studies in women.</p> <p>Mice and rats given three times and 1.2 times the equivalent human dose respectively showed no evidence of developmental fetal toxicity. In mice, there was a slight decrease in mean fetal weight and an associated decrease in the average number of ossified sacrocaudal vertebrae.</p>	<p>Uncomplicated and complicated skin and skin structure infections, including cellulitis, cutaneous abscesses and ischemic/diabetic foot infections, caused by piperacillin-resistant <math>\beta</math>-lactamase producing strains of <i>Staphylococcus aureus</i></p>
Ertapenem	B	<p>There are no adequate and well-controlled studies in women.</p> <p>Mice and rats given three times and 1.2 times the equivalent human dose respectively showed no evidence of developmental fetal toxicity. In mice, there was a slight decrease in mean fetal weight and an associated decrease in the average number of ossified sacrocaudal vertebrae.</p>	<p>cSSSI, including diabetic foot infections without osteomyelitis, due to <i>Staphylococcus aureus</i> (methicillin susceptible isolates only), <i>Streptococcus agalactiae</i>, <i>Streptococcus pyogenes</i>, <i>Escherichia coli</i>, <i>Klebsiella pneumoniae</i>, <i>Proteus mirabilis</i>, <i>Bacteroides fragilis</i>, <i>Peptostreptococcus</i> species, <i>Porphyromonas asaccharolytica</i>, or <i>Prevotella bivia</i></p>
Meropenem	B	<p>There are no adequate and well-controlled studies in women.</p> <p>Reproductive studies in the rat (1.8 times the human dose) and cynomolgus monkeys (3.7 times the human dose) revealed no evidence of impaired fertility or harm to the fetus due to meropenem. There were slight changes in fetal body weight at 0.4 times the human dose.</p>	<p>cSSSI due to <i>Staphylococcus aureus</i> (methicillin susceptible isolates only), <i>Streptococcus pyogenes</i>, <i>Streptococcus agalactiae</i>, viridans group streptococci, <i>Enterococcus faecalis</i> (excluding vancomycin-resistant isolates), <i>Pseudomonas aeruginosa</i>, <i>Escherichia coli</i>, <i>Proteus mirabilis</i>, <i>Bacteroides fragilis</i>, and <i>Peptostreptococcus</i> species</p>
Levofloxacin	C	<p>There are no adequate and well-controlled studies in women.</p> <p>Not teratogenic in rats at doses up to 9.4 times the oral human dose and 1.9 times the IV dose. The higher doses caused reduced fetal weights and increased fetal mortality. No teratogenic effects were seen in rabbits at doses 0.5 to 1.1 times the human dose.</p>	<p>cSSSI due to <i>Staphylococcus aureus</i> (methicillin susceptible isolates only), <i>Enterococcus faecalis</i>, <i>Streptococcus pyogenes</i>, or <i>Proteus mirabilis</i></p>
Linezolid	C	<p>There are no adequate and well-controlled studies in women.</p>	<p>cSSSI, including diabetic foot infections, without concomitant osteomyelitis, caused by <i>Staphylococcus aureus</i> (methicillin susceptible and -resistant strains), <i>Streptococcus pyogenes</i>, or <i>Streptococcus agalactiae</i></p>

**Table 3: Anti-microbial Drugs Approved For the Treatment of Complicated Skin and Skin Structure Infections**

Drug	Pregnancy category	Reproductive toxicology study findings	Antimicrobial coverage for cSSSI
Vancomycin	C	<p>No reproductive animal studies were conducted.</p> <p>There are no adequate and well-controlled studies in women. One small study of pregnant women using vancomycin in the second and third trimesters was published. This study evaluated the potential ototoxic and nephrotoxic effects of vancomycin on infants following maternal exposure. No sensorineural hearing loss or nephrotoxicity was attributed to vancomycin. The number of patients studied was limited. No other fetal/neonatal effects were reported.</p>	<p>cSSSI caused by susceptible strains of methicillin-resistant staphylococci</p>
Tigecycline	D	<p>Not teratogenic in the rat or the rabbit. Slight reductions in fetal weight and an increased incidence of minor skeletal anomalies (delays in bone ossification) occurred at 5 times and 1 time the human daily dose. Doses equivalent to the human dose were materno-toxic in rabbits and resulted in an increased incidence of fetal loss in rats and rabbits.</p> <p>There are no adequate and well-controlled studies in women.</p>	<p>cSSSI caused by <i>Escherichia coli</i>, <i>Enterococcus faecalis</i> (vancomycin-susceptible isolates only), <i>Staphylococcus aureus</i> (including methicillin resistant isolates), <i>Streptococcus agalactiae</i>, <i>Streptococcus anginosus</i> group, <i>Streptococcus pyogenes</i>, and <i>Bacteroides fragilis</i></p>

There are no adequate and well-controlled studies in pregnant women for any of the eight antibiotics approved for the treatment of cSSSI. Four of these drugs have reproductive toxicology studies in two species negative for teratogenic effects (pregnancy category B drugs). Linezolid is pregnancy category C due to embryo-fetal toxicities (not clear if this refers to increased post-implantation loss) but no teratogenic effects were seen in mice, rats, or rabbits. Similarly, levofloxacin is pregnancy category C due to increased fetal mortality and reduced fetal weights, but no teratogenic effects were seen in rats or rabbits. Tigecycline has a pregnancy category D but this category does not appear to be supported by the reproductive toxicology data included in the label. There are no adequate and well-controlled studies in women. There was an increased incidence of minor skeletal anomalies (delays in bone ossification). An increased incidence of fetal loss in rabbits occurred at materno-toxic doses.

Compared to FDA-approved antimicrobial agents indicated for the treatment of cSSSI, televancin does not offer any unique antimicrobial coverage. Televancin is a drug for intravenous administration, so its once daily dosing, while convenient, would not offer substantial advantages in terms of patient compliance.

## DISCUSSION

Televancin is a semi-synthetic, lipoglycopeptide antibiotic that exhibits bactericidal activity against most gram-positive bacteria. The televancin molecule core is identical to vancomycin and its antimicrobial coverage is similar. The current NDA application is for marketing televancin as an antimicrobial to treat complicated skin and skin structure infections (cSSSI). Based on data review by both the DAIOP microbiologist and medical officer, televancin is equivalent to, but not superior to, vancomycin for this indication. In addition, reproductive toxicology studies show similar teratogenic effects and increased post-implantation pregnancy loss in rats, rabbits, and Göttingen minipigs at non-maternotoxic doses of drug. While the presence or absence of teratogenic effects in any one animal species does not necessarily predict teratogenicity in developing humans, the occurrence of increased post-implantation loss and skeletal (limb) malformations across all three species at 1 – 15 times the human therapeutic dose is highly concerning. In addition, the minipig study showed a lower fecundity ratio than that in either historical database, and male fertility studies in rats showed decreased sperm motility and increased abnormal sperm morphology.

Televancin is a multi-species teratogen. Its classification with regard to use in pregnancy should be based on both its potential risk to mother and fetus as well as its potential clinical benefits above other available therapies. Currently, there are eight antimicrobial agents FDA approved for the treatment of cSSSI. Vancomycin remains first-line therapy for severe infections possible caused by MRSA. Based on current labeling for these approved cSSSI antimicrobial therapies, televancin does not offer broader or better antimicrobial coverage and has a much larger, consistent, and concerning animal safety signal for teratogenic potential in humans.

If approved, televancin would require a risk management action plan (RiskMAP) that could prevent use by pregnant women and provide for responsible outcomes tracking for those who

do become pregnant. Televancin is administered intravenously. It would potentially be used with direct or indirect healthcare practitioner supervision in hospitals, chronic care facilities, physician offices, and homes with instruction or home care assistance. However, unlike a teratogen that is used to treat a chronic condition (like isotretinoin), televancin would be used to treat severe skin and skin structure infections in acute care situations. Use in acute care situations and settings makes it more difficult to ensure that a woman of reproductive age is not pregnant prior to drug exposure. One negative serum pregnancy test is not adequate. Prior to initiating drug therapy, the iPLEDGE program for isotretinoin requires documented use of two forms of contraception for one month and two serum or highly sensitive urine pregnancy tests performed 19 days apart. These results must be documented and reviewed in an electronic database system before the pharmacist will dispense drug. These sorts of safeguards are not feasible with an acute infection that requires immediate antimicrobial therapy.

## CONCLUSIONS

For the proposed indication of cSSSI, televancin, if approved, should be assigned pregnancy category X because of a consistent teratogenic signal in three animal species combined with a lack of evidence of clinical benefit over eight other approved therapies for this indication. It is possible that data submitted for a different clinical indication in the future could support pregnancy category C if some direct benefit to mother or fetus was demonstrated. However, for the indication of cSSSI, there is no increase in benefit to offset the increase in risk for a pregnant patient.

A RiskMAP that includes education and reminders alone will not adequately safeguard against televancin use in pregnant women, and a RiskMAP with a performance-linked access system is probably not feasible in acute care situations to treat acute infections.

## RECOMMENDATIONS

If televancin hydrochloride is approved, the Maternal Health Team recommends the following:

1. Boxed warning informing prescribers (and patients) that televancin caused congenital anomalies and increased pregnancy loss in rats, rabbits, and minipigs and is, therefore, a suspected teratogen in humans that should not be used in women of childbearing potential.
2. Pregnancy category X (based on no benefit over current therapies combined with a consistent teratogenic and pregnancy loss safety signal in three species).
3. Indicated populations should include adult men and adult women who are not of childbearing potential. The following definitions may be used:

**Females not of Child-Bearing Potential (non-FCBP)** - Female patients who are not physically capable of becoming pregnant. This includes pre-pubertal females (Tanner Stages 1 and 2) and females who have undergone surgical (i.e., removal of the ovaries and/or the uterus) or natural menopause (see definition of menopause below). The risk

management plan should require confirmation of menopausal status and detail the procedure(s) providers will use for documenting and verifying non-FCBP patient status (e.g. by obtaining copies of surgical records or conducting blood tests – see below).

**Menopause** is the permanent cessation of menstruation following the loss of ovarian activity. Women pass through a transition from the reproductive stage of life to the post menopausal years, a period marked by waning ovarian function. This commonly occurs over a few years. The median age of menopause in the United States is 51.5 years.<sup>6</sup> A provider may assume that a woman is in menopause when there is:

- Appropriate medical documentation of prior complete bilateral oophorectomy, which results in surgically-induced menopause at the time of the procedure, or
  - Permanent cessation of menses (no menses for 12 months or longer) as a result of ovarian failure. Hormonal changes consistent with ovarian failure should be properly documented in the case of suspected spontaneous menopause as follows<sup>7,8</sup>:
    - If age >54 years and normal menses are absent: Elevated serum FSH (Follicle Stimulating Hormone) level in the post-menopausal range based on the laboratory reference range where the hormonal assay is performed.
    - If age <54 years and normal menses are absent: Negative serum or urine  $\beta$ -HCG with concurrently elevated serum FSH (Follicle Stimulating Hormone) level in the post-menopausal range, depressed estradiol ( $E_2$ ) level in the post-menopausal range, and absent serum progesterone level, based on the laboratory reference ranges where the hormonal assays are performed.
4. Restricted distribution at the pharmacy level that requires documentation of age and gender of the patient. If the patient is female, documentation of menopause or other evidence of non-childbearing potential should be required.

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<sup>6</sup> Speroff L, Glass RH, Kase NG. Clinical Gynecologic Endocrinology and Infertility, Chapter 18, 5<sup>th</sup> ed. 1994. Williams & Wilkins.

<sup>7</sup> Midlife Transitions: A Guide to Approaching Menopause 2003 [AP013] ACOG Patient Education Pamphlet. Available at: [http://www.acog.org/publications/patient\\_education/ab013.cfm](http://www.acog.org/publications/patient_education/ab013.cfm), accessed April 22, 2005.

<sup>8</sup> <http://www.ipledgeprogram.com>

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