CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

211996Orig1s000 212161Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 212161
Submission date: November 2, 2018
Drug: tafamidis free acid
Applicant: FoldRx Pharmaceuticals, Inc, a subsidiary of Pfizer Inc.
Indication: Treatment of Transthyretin amyloidosis (b) (4) cardiac (b) (4)
Reviewing Division: Division of Cardiovascular and Renal Products

Introductory Comments:
The pharmacology/toxicology reviewer and supervisor have determined that the nonclinical information is adequate to support approval of this NDA for the (b) (4) noted above. As noted in the primary review, most of the supporting nonclinical information was submitted to NDAs 202737 and 211996 for tafamidis meglumine from the same applicant.

Discussion:
Some developmental toxicity was noted in animal studies with essentially no margin between exposures associated with the effects and exposures achieved in humans. Labeling should adequately describe the findings. Wording for labeling has been discussed separately.

A 6-month carcinogenicity study in a transgenic mouse model (Tg.rasH2) and a 2-year study in Sprague Dawley rats were considered adequate by the Executive Carcinogenicity Assessment Committee and no drug-related neoplasms were noted.

Pharmacology studies show that tafamidis binds to the tetramer of transthyretin. This stabilizes the tetramer and inhibits its dissociation into monomers. Transthyretin stabilizer can be appropriate as the Established Pharmacologic Class (EPC).

Conclusions:
I agree that tafamidis may be approved for the above (b) (4) from a nonclinical perspective.

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/s/

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 212161
Supporting document/s:	SDN 001
Applicant's letter date:	11/2/18
CDER stamp date:	11/2/18
Product:	Tafamidis (free acid)
Indication:	Treatment of TTR amyloidosis (b) (4) cardiac (b) (4)
Applicant:	FoldRx Pharmaceuticals (a Division of Pfizer, Inc.)
Review Division:	DCRP
Reviewer:	William T. Link, Ph.D.
Supervisor/Team Leader:	Jean Wu, M.D., Ph.D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Maryam Changi

Template Version: September 1, 2010

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1 Executive Summary

NDA 212161 was submitted concurrently with NDA 211996 (tafamidis meglumine) on 02 November 2018 and primarily contains the CMC information supporting the tafamidis 61 mg free acid capsule. The proposed clinical indication is same for both NDAs.

The bioequivalence/bioavailability studies demonstrating the equivalence of tafamidis 61 mg free acid with 80 mg (4x 20 mg) tafamidis meglumine dose were included in NDA 211996. Clinical PK Study B3461056 demonstrated tafamidis free acid 61 mg oral capsule formulation is therapeutically equivalent to tafamidis meglumine 80mg (AUC of 166 µg·hr/mL on Day 7, following multiple oral doses of 80 (4 x 20 mg) tafamidis meglumine and AUC of 170 µg·hr/mL for tafamidis free acid).

Most of the nonclinical studies supporting both NDAs were reviewed previously under NDA 202737. Additional nonclinical data was reviewed and nonclinical review of NDA 202737 was referenced in the review of NDA 211996. The nonclinical data supporting the approval of tafamidis free acids 61 mg (NDA 212161) can be cross-referenced to the nonclinical review of NDA 211996 (tafamidis meglumine).

NDA 212161 (tafamidis free acid) is considered approvable from the Pharmacology/ Toxicology perspective.

2 Drug Information

2.1 Drug

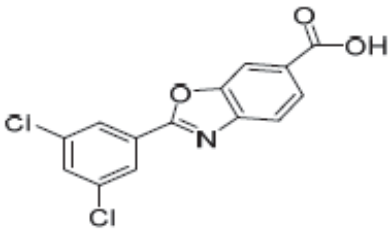
Generic Name: Tafamidis

Code Name: Fx-1006

Chemical Name: 2-(3, 5-dichloro-phenyl)-benzoxazole-6-carboxylic acid

Molecular Formula/Molecular Weight: $C_{14}H_7Cl_2NO_3$; 308.12

Structure or Biochemical Description



Pharmacologic Class: transthyretin stabilizer (new class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 71880, IND 74866, NDA 202737

2.3 Drug Formulation

Tafamidis will be provided as a size 9.5 oblong reddish brown soft gelatin capsule filled with a white to pink colored suspension. The capsule is printed with "VYN 61" in white. Each soft gelatin capsule contains 61 mg of tafamidis.

Name of Ingredient	Function	Reference to Standard	Unit formula	
			mg/ capsule	%
(b) (4)				
Tafamidis	Drug Substance	Pfizer	61.0	(b) (4)
Polyethylene Glycol 400	(b) (4)	Ph. Eur./NF		(b) (4)
Polysorbate 20		Ph. Eur./NF		
Povidone (K-value 90)		Ph. Eur./USP		
Butylated Hydroxytoluene		Ph. Eur./NF		
(b) (4)				
Gelatin (b) (4) Sorbitol (b) (4) (b) (4)	(b) (4)	Ph. Eur./NF NA USP/Ph. Eur.		(b) (4)
Iron Oxide, Red		NF, E172		
Purified Water [3]		USP, Ph. Eur.		
(b) (4)				

N/A = Not Applicable

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities or degradants of concern.

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/s/

WILLIAM T LINK
04/08/2019 02:48:14 PM

JEAN Q WU
04/08/2019 02:58:03 PM

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	211996
Supporting document/s:	SDN 005
Applicant's letter date:	11/2/2018
CDER stamp date:	11/2/2018
Product:	Vyndaqel (tafamidis meglumine)
Indication:	Treatment of amyloid cardiomyopathy
Applicant:	FoldRx Pharmaceuticals Inc., a subsidiary of Pfizer Inc.
Review Division:	DCRP
Reviewer:	William T. Link, Ph.D.
Supervisor/Team Leader:	Jean Wu, M.D., Ph.D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Maryam Changi

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Tafamidis has been developed as a stabilizer of transthyretin (TTR) tetramer for treatment of transthyretin amyloid cardiomyopathy (ATTR-CM), also referred to as TTR cardiomyopathy (TTR-CM), (b) (4) The recommended dose is 80 mg orally once daily.

Tafamidis meglumine [d-glucitol, 1-deoxy-1-(methylamino)-, 2-(3, 5-dichlorophenyl)-6-benzoxazolecarboxylate (1:1)], or PF-06291826-83 (also known as Fx-1006A), is the meglumine salt form of 2-(3, 5-dichloro-phenyl)-benzoxazole-6-carboxylic acid, or tafamidis free acid, or PF-06291826-00 (also known as Fx-1006), the only active ingredient contained in tafamidis meglumine. The dose of 80 mg tafamidis meglumine is considered bioequivalent to 61 mg tafamidis free acid.

ATTR-CM is a rare, fatal disorder, which is characterized by the deposition of misfolded transthyretin (TTR) amyloid fibrils in the ventricular walls (extra-myocardial), causing progressive disruption in the ability of the heart to effectively pump blood through the circulatory system. ATTR CM can be inherited as an autosomal dominant trait caused by mutation in the TTR gene (also known as familial amyloid cardiomyopathy), or by deposition of wild type transthyretin protein, previously called senile systemic or senile cardiac amyloidosis.

TTR is a transport protein for thyroxine (T4) and retinol-binding protein-retinol complex. It is secreted by the liver as homo-tetramers and is present in this form in plasma. By binding to both tetrameric wild-type (WT) and amyloidogenic variants of TTR, at the T4 binding site, tafamidis meglumine inhibits tetramer dissociation, the rate limiting step in the formation of TTR amyloid (Hammarström *et al., PNAS* 99 (S4):16427-16432, 2002), potentially disrupting the progression of TTR-FAP and TTR-CM.

The nonclinical data were originally submitted to NDA 202-737 (DNP, for FAP, 2/2011) and reviewed by Dr. David Hawver. The original NDA was considered approvable from the nonclinical perspective. Most summaries/discussions in this review were referred to Dr. Hawyer's NDA review except for some new studies submitted to the current NDA. Two concerns expressed in NDA 202-737 review, regarding the need for a rat carcinogenicity study and the adequacy of the chromosome aberration assay, have both been addressed and reconciled with the current NDA.

The human $AUC_{0\text{-}24hr}$ of 166.2 µg·hr/mL and Cmax of 9.1 µg /mL at the proposed 80 mg (4 x 20 mg) daily dose are used for comparison between animal and human exposures in this review unless indicated otherwise.

1.2 Brief Discussion of Nonclinical Findings

Primary pharmacology studies showed that tafamidis binds to up to two sites per TTR tetramer and slows the rate of dissociation into monomers. Substantial reduction in the rate of TTR tetramer dissociation was observed at a molar ratio of tafamidis: TTR in the range of 1:1 to 1.5:1. No other pharmacological activity was identified in an in vitro binding screen, except at delta-2 opioid receptors (IC_{50} = 8.3 μM); an in vitro functional assay confirmed that tafamidis acts as an agonist at the delta-opioid receptor in hamster vas deferens (EC_{50} >10 μM).

General toxicology

The pivotal repeat-dose toxicity studies with tafamidis were conducted in rats for up to 26 weeks of oral dosing and in dogs for up to 39 weeks of oral dosing.

In the pivotal 26-week oral toxicity study in rat (with a 13-week interim sacrifice), the NOAEL was determined at high dose of 30 mg/kg/day (Day 178 $AUC_{0\text{-}24hr}$ =2260 μg·hr/mL M, 3120 μg·hr/mL F). Despite the lack of toxicity, this dose was near the maximum tolerated dose, based on the mortality observed at 100 mg/kg/day in the 28-day study in rat and at 45 mg/kg/day in pregnant female rats in the embryofetal toxicity study.

In the pivotal 39-week oral toxicity study in dog (with a 13-week interim sacrifice), the incidence of soft/mucoid feces, emesis, and skin erythema was increased in treated groups, but the severity was not generally increased with dose. There were no test article-related macroscopic or microscopic changes. The NOAEL was identified at the high dose of 45 mg/kg/day (Day 271 $AUC_{0\text{-}24hr}$ =8.5 μg·hr/mL M, 10.6 μg·hr/mL F). The early deaths across all groups (including control) due to vomiting/aspiration were attributed to the 7.5% Vitamin E TPGS vehicle and not to the test article. Such aspiration-related mortality was not observed after the vehicle was changed on Day 48 from 7.5% (v/v) Vitamin E TPGS to 0.5% methylcellulose.

Despite the lack of drug-related toxicity observed in this study, the high dose of 45 mg/kg/day was considered adequate based on its being close to a maximum feasible dose, as aspiration-related death was observed at 100 mg/kg/day Fx-1006A in the 28-day study in dogs even with the more tolerable vehicle, 0.5% methylcellulose.

Genotoxicity

Fx-1006A was negative for genotoxicity in an *in vitro* bacterial reverse mutation assay, an *in vitro* chromosomal aberration assay in human lymphocytes, and an *in vivo* rat micronucleus assay.

Carcinogenicity

Carcinogenicity were evaluated in Tg.rasH2 transgenic mice dosed at 10, 30, or 90 mg/kg/day for 26 weeks and in Sprague Dawley rats dosed at 3, 10 or 30 mg/kg/day for 2 years. Both studies were considered adequate and found to be negative for increases in tumor findings with the Executive CAC's concurrence.

Reproductive Toxicology

Fertility and early embryonic development was evaluated in rats at doses of 5, 15 or 30 mg/kg. Males were dosed for 28 days prior to and throughout the 21-day mating period to the day before scheduled euthanasia, and females were dosed for 15 days before mating, throughout mating, and to GD 7. No effects on body weight were observed in males. Body weight loss and decreased food consumption was observed in females during the first week of dosing. Tafamidis meglumine did not affect mating, fertility, or early embryonic development at doses tested up to 30 mg/kg (approximately 4 times of the MRHD based on mg/m^2).

In a rat embryo-fetal development (EFD) study, pregnant rats were administered vehicle (7.5% Vitamin E TPGS) or tafamidis meglumine at 15, 30, or 45 mg/kg/day once daily by gavage from GD 7 through GD 17. Maternal toxicity including death was observed at 45 mg/kg/day, and fetal body weights were decreased at ≥30 mg/kg/day. There were no test article-related external, visceral, or skeletal variations or malformations at any dose. The NOAEL for maternal toxicity was 30 mg/kg/day, with an associated GD 17 AUC_{24} of 1610 µg•h/mL. The NOAEL for developmental toxicity was 15 mg/kg/day, which was associated with GD 17 AUC_{24} of 1090 µg•h/mL. Tafamidis meglumine was not teratogenic in rats.

In a rabbit EFD study, pregnant rabbits were administered vehicle (7.5% Vitamin E TPGS) or tafamidis meglumine at 0.5, 2, or 8 mg/kg/day once daily by oral gavage from GD 7 to GD 19. Mortality, body weight loss, and lower food consumption were observed at 8 mg/kg/day. Increased post-implantation loss, decreased fetal body weights, and malformations (depressed eye bulges, small eyes, and small eye sockets) in three fetuses from 2 litters were observed at 8 mg/kg/day. Increased incidence of alterations in the nasal bones (≥0.5 mg/kg/day) and supernumerary thoracic ribs and thoracic vertebrae (≥2 mg/kg/day) were observed. The NOAEL for development could not be identified.

In the pre/postnatal development study, rats were administered vehicle (7.5% Vitamin E TPGS) or tafamidis meglumine at 5, 15, or 30 mg/kg/day once daily by oral gavage from GD 7 to LD 20. Due to high mortality (no surviving pups in 20 of 25 litters by LD4), dosing at 30 mg/kg/day was terminated early in the lactation period and surviving rats and pups were euthanized on LD 1 to LD 4. At 15 mg/kg/day, 4 of 25 F0 females were euthanized on LD 2 or LD 3 as they had no surviving pups. Reduced survival, birth weight, postweaning body weight, delayed male sexual maturity, and impaired learning and memory were observed in the offspring of dams dosed at 15 mg/kg/day. The NOAEL for pre/postnatal development was 5 mg/kg/day.

Pregnant and lactating female rats were administered repeated daily oral doses of tafamidis meglumine (15 mg/kg/day) followed by a single oral gavage dose of [^{14}C]-tafamidis meglumine on Day 4 or 12 postpartum. Radioactivity in milk was observed by 1 hr post-dose on Day 4 and Day 12, increasing thereafter to peak levels at 8 and 24 hrs post-dose, respectively. The ratio of the highest radioactivity associated with [^{14}C]-tafamidis meglumine in milk (8 hours post-dose) vs. plasma (1 hour post-dose) was

approximately 1.6 on Day 12, indicating tafamidis meglumine is transferred to milk after oral administration

1.3 Recommendations

1.3.1 Approvability

The application is approvable from Pharmacology/Toxicology perspective with recommended labeling changes.

1.3.2 Additional Non-Clinical Recommendations

none

1.3.3 Labeling

Labeling recommendations for Sections 8.1, 8.2 and 13.1 are listed below (underline---insertion; crossline---deletion).

8.1 Pregnancy

Risk Summary

Based on findings from animal studies, VYNDAQEL may cause fetal harm when administered to a pregnant woman. (b) (4) In animal reproductive studies, oral administration of tafamidis meglumine to (b) (4) pregnant rabbits (b) (4) during organogenesis (b) (4) resulted in adverse effects on development (embryofetal mortality, fetal body weight reduction and fetal malformation) at a dosage providing approximately 9 (b) (4) times the (b) (4) human exposure (AUC) at the (b) (4) maximum recommended human dose (MRHD) of tafamidis meglumine (80 mg), and (b) (4) increased incidence of fetal skeletal variation at a dosage providing equivalent human exposure (AUC) at the MRHD. Postnatal mortality, growth retardation, and impaired learning and memory were observed in offspring of pregnant rats administered tafamidis meglumine during gestation and lactation at (b) (4) a dosage approximately (b) (4) 2 times the (b) (4) MRHD based on body surface area (mg (b) (4) /m^2) *(see Data)*.

Data

Animal Data

In (b) (4) pregnant rats, oral administration of tafamidis meglumine (0, 15, 30, and 45 mg/kg/day) (b) (4) throughout organogenesis resulted in decreased fetal body weights at ≥30 mg/kg/day (approximately (b) (4) 0 times the human (b) (4) exposure at the (b) (4) (b) (4) MRHD based on AUC). The no (b) (4) observed-adverse-effect-level (NOAEL) for embryofetal development in rats was 15 mg/kg/day (approximately 7 times the human exposure at the MRHD based on AUC).

(b) (4) In pregnant rabbits, oral administration of tafamidis meglumine (0, 0.5, 2, and 8 mg/kg/day) (b) (4) throughout organogenesis resulted in increased embryofetal mortality, reduced (b) (4) fetal body (b) (4) weights, and an increased incidence of fetal malformations (b) (4) (b) (4) at 8 (b) (4)

[redacted]-mg/kg/day (approximately [redacted]-9 times the human [redacted] at the [redacted]-MRHD based on AUC), which was also maternally toxic. Increased incidences of fetal skeletal variations were observed at doses ≥0.5 mg/kg/day (approximately equivalent to the human exposure at the MRHD based on AUC).

In [redacted] pre- and postnatal [redacted]-study, pregnant rats [redacted]-received oral administration of tafamidis meglumine at doses of 0, 5, 15, or 30 mg/kg/day [redacted]-throughout pregnancy and lactation (Gestation Day 7 [redacted]--Lactation Day 20.). Decreased [redacted] survival and body weights [redacted] delayed male sexual maturation [redacted]-and neurobehavioral effects (learning and memory [redacted]-impairment) were observed in the offspring of dams treated at 15 mg/kg/day (approximately 2 times the MRHD on a mg/m^2 basis). The NOAEL for pre- and postnatal development in rats was 5 mg/kg/day (approximately equivalent to the MRHD on a mg/m^2 basis).

8.2 Lactation

Data

Pregnant and lactating female rats were administered repeated daily oral doses of tafamidis meglumine (15 mg/kg/day) followed by a single oral gavage dose of ^{14}C-tafamidis meglumine on Lactation Day 4 or 12. Radioactivity was observed in milk by 1 hour post-dose and increased thereafter. The ratio of the highest radioactivity associated with ^{14}C tafamidis meglumine in milk (8 hours post-dose) vs. plasma (1 hour post-dose) was approximately 1.6 on Day 12, indicating tafamidis meglumine is transferred to milk after oral administration.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

There was no evidence of an increased incidence of neoplasia in the transgenic (Tg)-rasH2 mouse following repeated daily administration for 26 weeks at [redacted]-daily doses of 0, 10, 30 or 90 mg/kg. There was no evidence of increased incidence of neoplasia in a 2-year carcinogenicity study in rats at exposures up to 18 [redacted]-times the AUC at the [redacted] MRHD.

Impairment of Fertility

There were no effects of tafamidis meglumine on fertility, reproductive performance, or mating behavior in the rat at any dose. Rats were dosed daily (0, 5, 15, and 30 mg/kg/day) prior to cohabitation (for at least 15 days for females and 28 days for males), throughout the cohabitation period to the day prior to termination of males and through to implantation of females (Gestation Day 7). No adverse effects were noted on male and female rats in toxicity, fertility, and mating behavior at any dose [redacted] The paternal and maternal no observed adverse effect level for reproductive toxicity of tafamidis meglumine is [redacted]30 mg/kg/day [redacted] approximately 4 times the [redacted] MRHD on a mg/m^2 basis.

2 Drug Information

2.1 Drug

CAS Registry Number: 951395-08-7

Generic Name: Tafamidis meglumine

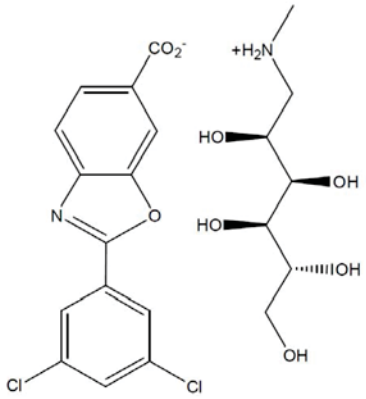
Code Names: Fx-1006A, PF-06291826

Chemical Name: d-glucitol,1-deoxy-1-(methylamino)-,2-(3,5-dichlorophenyl)-6-benzoxazolecarboxylate

Molecular Formula/Molecular Weight:

$C_{21}H_{24}Cl_2N_2O_8$; 503.34 (Tafamidis Meglumine)
$C_{14}H_7Cl_2NO_3$; 308.12 (Tafamidis Free Acid)

Structure:



Pharmacologic Class: Transthyretin stabilizer (new class)

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 202-737 Fx-1006A for Transthyretin Familial Amyloid Polyneuropathy
IND 74,866 Fx-1006A for Transthyretin Familial Amyloid Polyneuropathy
IND 71,880 Fx-1006A for TTR Familial Amyloid Cardiomyopathy

2.3 Drug Formulation

Tafamidis meglumine 20 mg will be provided as a size 9.5 oblong opaque yellow soft gelatin capsule filled with a white to pink colored suspension. The capsule is printed with “VYN 20” in red. Each soft gelatin capsule contains 20 mg of tafamidis meglumine.

Table 1 Composition of Tafamidis Me lumine 20 m Soft Gelatin Capsules

Name of Ingredient	Function	Reference to Standard	Unit formula	
			mg/ capsule	%
				(b) (4)
Tafamidis Meglumine	Drug Substance	Pfizer	20.0[1]	(b) (4)
Polyethylene Glycol 400	(b) (4)	Ph. Eur./NF/JP		(b) (4)
Polysorbate 80		Ph. Eur./NF/JP		
Sorbitan Monooleate		Ph. Eur./NF/JPE		
				(b) (4)
Gelatin (b) (4) Sorbitol (b) (4)	(b) (4)	Ph. Eur./NF/JP NA USP/Ph. Eur./JP		(b) (4)
Iron Oxide, Yellow		NF, E172, JPE		
Titanium Dioxide		USP, Ph. Eur./JP		
Purified Water[4]		USP, Ph. Eur./JP		
				(b) (4)

N/A = Not Applicable

[1] Tafamidis meglumine 20 mg is equivalent to 12.2 mg active of tafamidis as the free acid

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities or degradants of concern.

Additional assays (reverse mutation) for synthesis starting materials and potential impurities were submitted and reviewed herein (Section 7.4). There were no positive findings in these additional assays.

2.6 Proposed Clinical Population and Dosing Regimen

Tafamidis meglumine is indicated for the treatment of transthyretin amyloid cardiomyopathy (b) (4) in adults. The recommended dose is 80 mg orally once daily, (b) (4)

2.7 Regulatory Background

IND 71,880 was submitted on August 19, 2005 to the Division of CardioRenal Drug Products to support the development of Fx-1006A for the treatment of TTR Familial Amyloid Cardiomyopathy.

IND 74,866 was submitted on August 17, 2006 to the Division of Neurology Products to support the development of Fx-1006A for the treatment of TTR Familial Amyloid Polyneuropathy.

NDA 202-737 was submitted 2/24/2011 for treatment of TTR Familial Amyloid Polyneuropathy at the clinical dose of 20 mg. A Complete Response was sent 6/15/2012 based on clinical considerations. The NDA was considered approvable from a non-clinical perspective (see D. Hawver's NDA review in attachment).

3 Studies Submitted

3.1 Studies Reviewed

Pre-clinical review responsibility was shared between DCRP and DNP over the course of the development of tafamidis. The reviews for NDA 202-737 as well as the Executive CAC minutes are attached (see Appendices).

Studies reviewed in NDA 202-737 /IND 74866:
Reproductive toxicology studies, 13 and 26-week toxicology studies and the 26-week mouse carcinogenicity study were reviewed by David Hawver under IND 74866 and NDA 202-737 (attached).

Studies reviewed in IND 71880 and referenced in NDA 202-737:
Safety pharmacology, 4-week toxicology, genetic toxicology studies were reviewed by this reviewer.

Studies reviewed in the current NDA submission:

Study No. 805917 A 2-year Carcinogenicity Study of PF-06291826 by Oral Gavage Administration in Rats

Study No. 504677 Evaluation of The Mutagenic Activity of (b) (4) in the *Salmonella Typhimurium* Reverse Mutation Assay and the *Escherichia Coli* Reverse Mutation Assay

Study No. 507970 Evaluation of The Mutagenic Activity of (b) (4) in the *Salmonella Typhimurium* Reverse Mutation Assay

Study No. 504698 Evaluation of the Mutagenic Activity of (b) (4) in the *Salmonella Typhimurium* Reverse Mutation Assay and the *Escherichia Coli* Reverse Mutation Assay (With Independent Repeat)

Study No. 8000475: A 28-Day Oral Gavage Study to Assess the Effect of PF-06291826 on the T-cell Dependent Antibody Response (TDAR) in CByB6F1 Mice with a 4-Week Recovery

3.2 Studies Not Reviewed

A listing of all submitted studies is provided in David Hawver's NDA 202-737 review (page 10-15). Validation studies of methods used in analysis are not reviewed. Similarly, non-GLP studies are not all formally reviewed.

Additional studies which were not previously submitted to NDA202-737: none.

3.3 Previous Reviews Referenced

NDA 202-737 for Transthyretin Familial Amyloid Polyneuropathy, PharmTox Review by David B. Hawver, Ph.D., finalized on May 19, 2012.

The following IND reviews were referenced in the review of NDA 201-737:

IND 71,880 #000 Fx-1006A for TTR Familial Amyloid Cardiomyopathy, PharmTox Review by William T. Link, Ph.D., December 12, 2005.

IND 74,866 #001 Fx-1006A for Transthyretin Familial Amyloid Polyneuropathy, PharmTox Review by David B. Hawver, Ph.D., December 4, 2006.

IND 74,866 #017 & 018 Fx-1006A for Transthyretin Familial Amyloid Polyneuropathy, PharmTox Review by David B. Hawver, Ph.D., January 9, 2008.

4 Pharmacology

The pharmacological evaluation of tafamidis included studies to determine its binding characteristics to the thyroxine binding sites of TTR and the extent of stabilization of both the wild-type (WT) and amyloidogenic mutations of TTR, in physiological and non-physiological (acid and urea) conditions. The summary below is based on the discussion of the original NDA 202-737 (attached).

4.1 Primary Pharmacology

The affinity of tafamidis free acid for the TTR binding sites was measured during monomeric subunit exchange time course experiments and by isothermal titration calorimetry. Tafamidis free acid bound to TTR with negative cooperativity, with dissociation constants for the first binding site ($K_{d1}B$) of 2 to 3 nM, and dissociation constants for the second binding site (K_{d2}) of 154 to 278 nM. The binding stoichiometry of tafamidis free acid (7.2 μM) to TTR (3.6 μM, average human TTR conc.) was

determined to be 0.81 ± 0.02 in human plasma, indicating specificity of the binding of tafamidis free acid to TTR over all other plasma proteins.

Subsequent experiments investigated the tetrameric stabilization properties under physiologic (saline or plasma) or non-physiologic conditions such as acid or strong urea concentrations.

Under physiologic conditions, where subunits are free to dissociate and re-combine, tafamidis free acid decreased the rate of subunit exchange in a concentration-dependent manner, demonstrating kinetic stabilization of tetrameric TTR. At a tafadmidis free acid: TTR ratio of 1.5, negligible tetramer dissociation occurred.

TTR (3.6 μM, WT, Val30Met, or Val122Ile mutant) stabilization was also evaluated in an acidic medium (pH 5.4). Under these denaturing conditions, tetramers dissociate into monomers, which ultimately rearrange into amyloid fibrils, the amount of which can be evaluated by ultraviolet-visible (UV-vis) spectrometry. In all experiments, the concentration of TTR (WT or variant) was 3.6 μM. Amyloid fibril formation data were normalized to WT TTR amyloidogenesis in the absence of stabilizer, assigned to be 100% fibril formation. At 7.2 μM, tafamidis free acid inhibited fibril formation by 99% and 93% for the 2 amyloidogenic variants, Val30Met and Val122Ile, respectively.

TTR tetramers dissociate into unfolded monomers in the presence of high concentration of urea, thus preventing their re-association into a TTR tetramer. Therefore, in the presence of urea, the amount of TTR tetramer is inversely related to the rate of tetramer dissociation. Measuring the amount of tetrameric TTR under these urea conditions and in the presence of tafamidis free acid allows the estimation of the ability of tafamidis free acid to stabilize the TTR tetramer. TTR denaturation was induced by adding urea to a solution containing TTR (1.8 μM) and tafamidis free acid to yield a final urea concentration of 5.2 M. TTR was incubated with tafamidis free acid at different concentrations (tafamidis free acid: TTR concentration ratio of 0, 1, 2).

Tafamidis free acid strongly inhibited the formation of unfolded monomer, thus indicating the stabilization of TTR tetramer. Only 33% of TTR tetramer dissociated after incubation for 72 hours with equimolar quantity of tafamidis free acid. Negligible dissociation (<3%) was observed after 72 hours incubation when tafamidis free acid is present at 2-fold the concentration of TTR (3.6μM).

The sequence comparison between human TTR and rat, dog, or rabbit TTR revealed an 82%, 83%, and 84% identity between human and rat, dog, and rabbit, respectively. All amino acids forming the TTR central channel and all specific amino acids of the central channel involved in binding thyroxine have 100% homology in all these species. Additionally, dissociation constants for thyroid hormones T4 and T3 are similar between human and rat TTR, with K_d of 13.6 and 8.0 nM for T4, and 56.6 and 67.2 nM for T3, for human and rat, respectively. These findings add relevance to the rat, rabbit and dog toxicology studies as they can be expected to exhibit a similar pharmacologic response to tafamidis as in humans.

No *in vivo* primary pharmacodynamic studies were conducted with tafamidis.

4.2 Secondary Pharmacology

In secondary pharmacodynamic studies, tafamidis free acid was tested with more than 50 receptors (including the thyroxine receptor), enzymes, and ion channels. Tafamidis free acid was devoid of significant binding affinity to all except the δ_2-opioid receptor, where it showed a low binding affinity (50% inhibitory concentration [IC_{50}] = 8.3 µM; Ki = 4.9 µM). No significant affinity was demonstrated for the kappa and mu opioid receptors.

An *in vitro* functional test on hamster vas deferens confirmed the modest (6%, 37%, and 88% of control response at 3, 10, and 30 µM, respectively) agonistic activity of tafamidis free acid on the δ_2-opioid receptor (EC_{50} > 10 µM). Combined with high human plasma protein binding (>99%) of tafamidis, this suggests that effects mediated through an interaction with the δ_2-opioid receptor would not be expected at the human unbound steady-state C_{max} of 0.3 µM, at a clinical dose of 80 mg tafamidis meglumine and 0.28 µM at a clinical dose of 61 mg tafamidis free acid (Clinical study B3461056).

4.3 Safety Pharmacology

In the core GLP battery of safety pharmacology studies, tafamidis meglumine had no effects on the central nervous, cardiovascular, and pulmonary systems.

Emesis and salivation and clinical signs indicative of effects on the central nervous system (CNS), muscle leg twitching, were observed in the cardiovascular/pulmonary safety pharmacology study in dogs but were deemed secondary to toxicity at high exposures (~132,000 ng/mL; 14.5-fold human Cmax for 80 mg).

The no observed effect level (NOEL) for body temperature, reflex, and other behavioral measures of the CNS tests for male and female rats was >100 mg/kg.

In a definitive GLP hERG assay, tafamidis meglumine stimulated hERG current by (mean ± standard error of the mean) 0.5% at 1 µM, 4.7% at 10 µM, and 9.3% at 30 µM and inhibited hERG current by 5.4% at 3 µM, versus 0.1% in control. An IC_{50} could not be calculated, however no clinically relevant effect on hERG current was apparent.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The nonclinical pharmacokinetic (PK)/toxicokinetic (TK) and absorption, distribution, metabolism, and excretion (ADME) properties of tafamidis were studied *in vitro* and *in vivo* to support nonclinical safety evaluations and to assess the potential relevance to humans.

After single-dose intravenous (IV) administration of tafamidis to rats and dogs, tafamidis demonstrated low systemic clearance (CL) (0.4 mL/min/kg for both rats and dogs) and a

low volume of distribution (Vss) (271 mL/kg for rats and 317 mL/kg for dogs). Tafamidis is expected to cross the intestinal wall by passive diffusion and demonstrates complete absorption with high oral bioavailability (108% in rats and 136% in dogs), with some evidence of enterohepatic recirculation in the rat.

After repeat-dosing in mice (up to 26 weeks), rats (up to 2 years), rabbits (embryo-fetal development toxicity study), and dogs (up to 39 weeks), systemic exposure (as assessed by maximum concentration [Cmax] and area under the concentration-time curve from time 0 to 24 hours postdose [AUC_{0-24}]) to tafamidis generally increased with increasing dose in a less than dose proportional manner. There were no apparent sex-related differences in exposure observed across all nonclinical species. Accumulation was observed in mice, rats, and rabbits (AUC ratios between 1.4 and 2.0), while no accumulation was observed in dogs.

Tafamidis is highly bound (>97%) to plasma proteins. Tafamidis distributed preferentially into plasma relative to red blood cells, thus, drug concentrations measured in plasma are representative of systemic drug exposure. Tissue distribution of [^{14}C]-tafamidis in rats showed that drug-derived radioactivity was widely distributed throughout the body, especially in harderian glands, stomach, and liver. Elimination of radioactivity was slow with detection in most tissues at 168 hours postdose. In pregnant rats, [^{14}C]-tafamidis was transferred through the placenta and radioactivity was widespread in both maternal and fetal tissues.

5.1.1. Absorption and Pharmacokinetics

In vitro Permeability and Efflux
The *in vitro* permeability and efflux study in Caco-2 cells (Study 400483) suggests that tafamidis readily crossed the Caco-2 monolayer by passive transcellular diffusion and is not a substrate for P-gp and MRP2 efflux transporters.

Single-Dose Pharmacokinetics
The single-dose pharmacokinetics of tafamidis were characterized after oral and/or IV dosing of tafamidis and/or tafamidis meglumine in CByB6F1 hybrid mice, Sprague-Dawley rats, and Beagle dogs. During nonclinical development, different vehicles were used in rats and dogs: early studies used 0.5% methylcellulose and/or 0.5% carboxymethylcellulose (CMC) as a suspension and subsequent studies used 7.5% (v/v) Vitamin E d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) in deionized water to enable tafamidis to be administered in solution. Different capsule preparations of tafamidis were also evaluated in dogs to increase the delivery of tafamidis. These studies are summarized in the attached NDA 202737 review (page 35).

Repeat-Dose Pharmacokinetics and Toxicokinetics
The repeat-dose toxicokinetics and/or pharmacokinetics of tafamidis were characterized after oral dosing in CByB6F1 hybrid mice, Sprague-Dawley rats, New Zealand White rabbits, and Beagle dogs; and are discussed in their associated toxicology studies.

5.1.2. Distribution

Tissue Distribution
Tissue distribution of [^{14}C]-tafamidis-related radioactivity was similar in male and female rats. Radioactivity was well distributed with the highest mean concentrations of radioactivity in harderian glands, followed by stomach and liver. The T_{max} for most tissues occurred at 2 hours postdose, with radioactivity still detected in most tissues at 168 hours postdose. Low levels of radioactivity were observed in brain and cerebrospinal fluid (CSF) of both male and female rats, suggesting limited penetration of [^{14}C]-tafamidis-related radioactivity through the blood-brain barrier. Concentrations of dose-related material were generally similar in all brain regions.

In vitro Protein Binding

The extent of *in vitro* binding of tafamidis to rat, dog, and human plasma proteins was determined using equilibrium dialysis at a concentration of 10 μM [redacted] (b) (4)). The fraction unbound of tafamidis in rat, dog, and human plasma was determined to be 0.01, 0.009, and 0.008, respectively. Tafamidis binds to human albumin with a K_d of 2.1 to 2.5 µM or 0.65 to 0.77 µg/mL.

Red Blood Cell Distribution

Red blood distribution of [^{14}C]-tafamidis was similar in male and female rats. Concentrations of [^{14}C]-tafamidis were higher in plasma than in whole blood at all timepoints, and blood to plasma ratios ranged from 0.582 to 0.641.

Placental Transfer and Milk Transfer in Pregnant and Lactating Rats (Study 420613)
Pregnant and lactating female Sprague Dawley rats were administered repeated daily oral doses of tafamidis meglumine (15 mg/kg/day) from GD7, followed by a single oral gavage dose of [^{14}C]-tafamidis meglumine on GD15, GD19, or Days 4 and 12 postpartum. Following the radiolabeled-tafamidis dose, the content and concentration of radioactivity in milk and in maternal, fetal and neonatal blood, plasma and tissues, and the non-compartmental kinetics of total radioactivity in whole blood, plasma and tissues were determined.

Placental transfer of tafamidis as well as fetal exposure was observed. Radioactivity distribution was widespread and similar in both maternal and fetal tissues on GD15 and GD19. Fetuses were exposed to 1 to 4% of the administered dose and the highest concentrations were generally associated with the fetal liver and fetal heart.

Radioactivity in plasma and whole blood of dams peaked at 1-hour post-dose on PND4 and PND12. Radioactivity in milk was observed by 1 hr post-dose on PND4 and PND12, increasing thereafter to peak levels at 8 and 24 hrs post-dose, respectively. As shown in the table below (Dr. Hawver's review, page 44), The ratio of the highest radioactivity associated with ^{14}C tafamidis meglumine in milk (66.9 µg/g at 8 hours post-dose) vs. plasma (41.5 µg/mL at 1-hour post-dose) was approximately 1.6 on Day 12, indicating tafamidis meglumine is transferred to milk after oral administration.

Maternal Plasma

Group 6: At a Mean Dose of 15.5 mg/kg

T_{max} (h)	T_{last} (h)	K_{el} (1/h)	R^2	T½ (h)	C_{max} (µg eq/mL)	$AUC_{(0\text{-}tlast)}$ (µg eq•h/mL)	$AUC_{(0\text{-}\infty)}$ (µg eq•h/mL)	AUC% (tlast-∞)
1.25	24.0	0.0766	0.992	9.05	41.5	491	587	16.5

Maternal Blood

T_{max} (h)	T_{last} (h)	K_{el} (1/h)	R^2	T½ (h)	C_{max} (µg eq/mL)	$AUC_{(0\text{-}tlast)}$ (µg eq•h/mL)	$AUC_{(0\text{-}\infty)}$ (µg eq•h/mL)	AUC% (tlast-∞)
1.25	24.0	a	0.992	a	24.4	251	a	20.4

Maternal Milk

T_{max} (h)	T_{last} (h)	K_{el} (1/h)	R^2	T½ (h)	C_{max} (µg eq/g)	$AUC_{(0\text{-}tlast)}$ (µg eq•h/g)	$AUC_{(0\text{-}\infty)}$ (µg eq•h/g)	AUC% (tlast-∞)
8.00	24.0	b	b	b	66.9	c	b	b

a Values are not reported because the $AUC_{(0\text{-}\infty)}$ was extrapolated by more than 20% or R^2 was <0.8.
b No reportable results as the terminal phase could not be identified.
c Value not considered reportable because the number of quantifiable milk concentration time-points was less than 3.

Systemic radioactivity was observed in the pups indicating the pups ingested milk of the dosed lactating dams. During the lactation phase a minimum of 30% of the dose administered to the dams was transferred to the pups via the milk and subsequently absorbed. The ratio between the pups' plasma AUC_{last} and the dams' plasma AUC_{last} was approximately 0.16 on Day 4 postpartum and increased to approximately 0.5 on Day 12 postpartum.

5.1.3. Metabolism

Tafamidis demonstrated a high degree of metabolic stability in *in vivo* and *in vitro* studies. Overall, phase II metabolism (to form an acylglucuronide metabolite) represented the major route of biotransformation of tafamidis. The only phase I metabolite that was observed was a single oxidation metabolite which was found in mice and rabbits and in mouse microsomes.

There were no unique human metabolites observed. The acylglucuronide metabolite observed in human plasma was also the predominant metabolite in the nonclinical species except in rabbits.

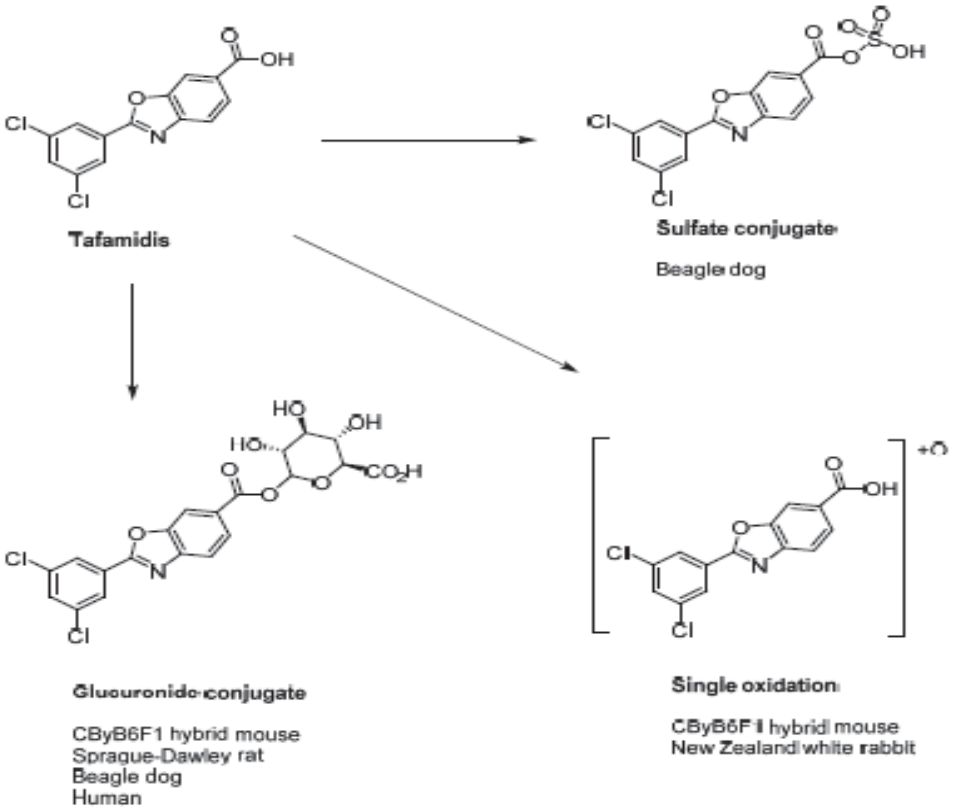
5.1.3.1. In vivo Metabolism

The *in vivo* metabolite profiles of tafamidis were evaluated in nonclinical species (mouse, rat, rabbit, and dog) and in humans using LC-MS/MS analyses of selected plasma samples. The metabolite profile of [^{14}C]-tafamidis was also conducted in rat plasma, urine, feces, and bile samples and in human plasma, feces, and urine using high performance liquid chromatography (HPLC) with radiometric detection and mass spectrometry.

A monoglucuronide (acylglucuronide) was the primary metabolite identified in human plasma and was also observed across all the nonclinical species tested except the rabbit. Mice demonstrated the additional presence of a single oxidation metabolite that was not detected in human plasma. Dogs exhibited a sulfate conjugate of tafamidis in addition to the acylglucuronide. Unique to the nonclinical species, rabbits demonstrated only the single oxidation metabolite. No apparent qualitative sex differences were noted in the metabolite profiles.

The proposed metabolic pathways for tafamidis in plasma are depicted as follows below.

Major Metabolic Pathways of Tafamidis



5.1.3.2. In Vitro Metabolism

Metabolite Identification in *In vitro* Incubations
In vitro metabolism of tafamidis (1 or 10 µM) was qualitatively assessed using subcellular (microsomes and S9) fractions of liver from the nonclinical species (mouse, rat, rabbit, and dog) and human (Studies 943005; 943008; 10400; 400477). Tafamidis was metabolically stable, with ≥95% of the parent molecule unchanged across all species following incubation for 1 hour. A small percentage of metabolic transformation was identified as a monoglucuronide (acylglucuronide) metabolite in mouse, rat, dog and human microsomal incubation mixture, and in the mouse, with additional formation of a single oxidation metabolite. There was no apparent *in vitro* metabolite formation in the rabbit incubation mixture and there were no metabolites unique to human.

UGT Enzymes Responsible for Glucuronidation
The UGT isoforms responsible for the glucuronidation of tafamidis was investigated using individual recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B4, UGT2B7 and UGT2B15) (Study 401242). UGT isoforms (0.5 mg/mL) were incubated with tafamidis (10 µM) for up to an hour and then analyzed using LC-MS/MS for tafamidis and its acylglucuronide metabolite. The major UGT isoforms responsible for the formation of the acylglucuronide are UGT1A9, UGT1A1, and UGT1A3, while minor activity was observed with isoforms UGT1A6, UGT1A7, UGT1A8, and UGT2B7.

5.1.4. Excretion

After oral administration of [^{14}C]-tafamidis meglumine, the predominant route of elimination of radioactivity in rats and humans was feces. In rats, there is evidence of enterohepatic recirculation of [^{14}C]-tafamidis.

Rats
Excretion of tafamidis-derived radioactivity was studied in intact and cannulated male and female Sprague-Dawley rats following oral administration of a single dose of 3 mg/kg [^{14}C]-tafamidis meglumine (Study 420372). Excretion was slow with 58% of the radioactive dose recovered in the excreta (urine and feces) at 48 hours postdose and 86% at 168 hours postdose. In rats, radioactivity in the feces accounted for 67% (males) and 79% (females) and radioactivity in the urine accounted for 19% (males) and 7.1% (females).

Biliary excretion of tafamidis was evaluated in male and female BDC rats following administration of a single dose of 3 mg/kg [^{14}C]-tafamidis. Bile was the major excretion route accounting for 48% of the dose over 72 hours, while feces and urine excretion represented 23% and 20% of the dose during the same period, respectively. Overall, 92 to 93% of the administered radioactivity was excreted up to 72 hours postdose.

In a study to investigate enterohepatic recycling in rats, bile was collected from one group of BDC animals dosed orally with 3 mg/kg [^{14}C]-tafamidis meglumine and infused

into a different group of BDC-duodenum cannulated animals via the duodenum. Metabolic profiling showed that the initially collected bile from the BDC rats consisted primarily of isomeric acylglucuronides, accounting for >82% of biliary radioactivity (parent compound was <2%).

Bile, urine and feces were collected for 72 hours from the BDC-duodenum cannulated rats, and radioactivity levels were measured.

Feces was the major excretion route of radioactivity in the BDC-duodenum cannulated male rats with 47% radioactivity excreted at 72 hours. Bile and urine excretion in males represented 25% and 17% of the dose at this time, respectively. Presence of radioactivity in urine and in bile suggests that tafamidis was reabsorbed after hydrolysis of the glucuronide metabolites, following duodenal-infusion in the BDC-duodenum cannulated rats, the biliary metabolite profile from these animals showed the same two acylglucuronide metabolites as the only detected radiochemical peaks. Additionally, the feces showed that parent compound was the sole radiochemical species detected. In the one BDC-duodenum cannulated female rat with a complete time-course, approximately 77% of the administered radioactivity was absorbed from the duodenum. These observations support the hypothesis that tafamidis undergoes enterohepatic recycling in the rat, with the glucuronide metabolites being eliminated in the bile, hydrolyzed to the parent compound in the GI tract and re-absorbed.

5.1.5. Pharmacokinetic Drug Interactions

Based on the *in vitro* results, the potential for tafamidis to cause DDI by inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5, UGT1A1, UGT1A4, UGT1A6, UGT1A9, and UGT2B7 or induction of CYP1A2, CYP2B6, and CYP3A4 is unlikely. Overall, based on the *in vitro* results, tafamidis showed a low potential to inhibit MDR1 (P-gp) (systemically and in the GI tract), OCT2, OATP1B1, OATP1B3, and MATE1, and MATE2K at clinically relevant concentrations. However, tafamidis has the potential to inhibit BCRP (systemically and in the GI tract), OAT1, and OAT3 at clinically relevant concentrations.

Tafamidis demonstrated weak inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (Ki >100 μM), while moderate inhibition was observed for CYP2C8 (Ki values of 19 and 24 μM).

5.2 Toxicokinetics

(included above under Absorption)

6 General Toxicology

All the studies summarized below were reviewed previously, and full details are available in the attached study reviews. Narratives may be copied directly from the previous reviews in numerous instances. (M=male, F=Female, Con=control; HD=high dose, MD=mid-dose, LD=low dose; HDM=high dose males; HDF=high dose females)

6.1 Single-Dose Toxicity

Single-dose toxicity was evaluated in dogs (Study SYI00028, Phase 1).

A Study to Determine the Potential Oral Toxicity of Both Single and Repeated Administrations of Fx-1006 to Beagle Dogs.

Beagle dogs (N=1M/group except for high dose [HD], which had 1M & 1F) were given a single dose of Fx-1006A at 0, 30, 100, 300, or 600 mg/kg vial oral gavage in 0.5% methylcellulose at 10 mL/kg. No treatment-related changes were observed in mortality, clinical signs, hematology, or clinical chemistry, except for a 3.8-fold increase in ALK at 24-hrs postdose in the M dog given 600 mg/kg Fx-1006A (from D Hawver review).

6.2 Repeat-Dose Toxicity

1 week – **Dog** (Study SYI00028, Phase 2)

Beagle dogs (the same ones described above, starting ~96 hrs after the Phase 1 dose) were given vehicle (0.5% methylcellulose; N=1M & 1F) or Fx-1006A (2/sex/group at 100 or 300 mg/kg/day) vial oral gavage at 10 mL/kg once daily for 7 days. Treatment-related changes were limited to increased incidence of soft stools and vomiting.

10-day – **Rat** (Study SYI00006; non-GLP)
A 10-Day Repeat Dose Toxicity Study in Female Sprague-Dawley Rats.

Female Sprague-Dawley rats (N=5/group) were treated with vehicle (0.5% methylcellulose), Fx-1003 (a drug candidate that was considered for development for ATTR-PN) at 10, 30, or 100 mg/kg/day, or Fx-1006 at 10, 30, or 100 mg/kg/day via oral gavage once daily at 10 mL/kg for 10 days.

No treatment-related changes were observed in mortality, clinical signs, body weights, hematology, urinalysis, or at necropsy. All Fx-1006 groups showed increased serum glucose, and HDF showed decreased serum globulin and increased serum A/G ratio. Absolute and relative liver weights were increased in Fx-1006 LDF and HDF groups. Mild bile duct hyperplasia was observed in one Fx-1003 HDF, and moderate hepatocellular vacuolization was observed in one Fx-1006 HDF.

The NOAELs for Fx-1003 and Fx-1006 in F SD rats in this study were both 100 mg/kg/day.

4-week - **Mouse** [redacted] (b) (4) Study AB44FV.2G3R.BTL Final Report) GLP
28-Day Repeated Dose Oral Toxicity and Toxicokinetic Study of Fx-1006A in CByB6F1 Hybrid Mice.

For additional details, see review by David B. Hawver, Ph.D., under IND 74,866 #017, January 9, 2008; GLP; QA). This report was used in the Executive CAC deliberations on dose selection for the 26-week Tg-rasH2 mouse carcinogenicity study.

Non-transgenic littermates of CByB6F1 hybrid Tg-rasH2 mice (10/sex/group + 20/sex/group TK) treated with 0, 10, 30, 45, or 60 mg/kg/day Fx-1006A via oral gavage in 7.5% (w/v) Vitamin E TPGS at 15 mL/kg once daily for 28 days showed no treatment-related changes, except minimal to mild single cell centrilobular necrosis at 30, 45, and 60 mg/kg/day.

A second, similar 28-day study was conducted at 0, 120, 240, and 480 mg/kg/day Fx-1006A. At the HD of 480 mg/kg/day excessive mortality was observed (9/10 M, D2-6; 5/10 F, D4-10), so dosing was stopped in M on Day 3 and in F on Day 6. At the MD of 240 mg/kg/day, mortality was also observed (6/10 M, D3-5; 1/10 M, D8), and dosing was stopped in M on Day 8 but was continued in F through Day 28. Similarly, due to high mortality in TK animals (16/20 HDM, 13/20 HDF, 7/10 MDM, and 1/20 MDF), the HDM, HDF, and MDM were sacrificed early on Days 6, 7, and 9, respectively.

Clinical signs observed in the MD and HD groups included decreased activity, impaired gait, seizures, labored respiration/dyspnea, and hunched appearance. No toxicologically important treatment-related changes were observed in body weight, food consumption, organ weights, or gross necropsy.

White material (likely test article) was observed in stomach of several MD and HD animals, with no associated microscopic findings. AST and ALT were increased ~2- to 3-fold in MDF and LDM, correlating with microscopic observations of centrilobular single cell necrosis (minimal to moderate). Total bilirubin was increased ~2-fold in MDF. Estrous cycle disruption and lymphoid depletion with lymphocytolysis in spleen, thymus, and/or lymph nodes observed in MD and HD were attributed to stress.

Liver centrilobular hypertrophy was observed in LDM, LDF, and MDF, and was considered an adaptive response. The LD of 120 mg/kg/day in the second study exceeded the maximum tolerated dose (MTD); therefore, the HD of 60 mg/kg/day in the first study was the NOAEL and the MTD for M and F mice.

4-week – **Rat** (Study SYI00010) GLP
A Study to Determine the Oral Toxicity of Fx-1006A Following 28 Consecutive Administrations with a 14-Day Recovery Period and BioAnalysis in Male and Female Sprague-Dawley Rats

Sprague-Dawley rats (10/sex/group Main Study; 5/sex/group Con & HD 14-day recovery; 6/sex/group TK) were given 0, 10, 30, 100, or 300 mg/kg/day Fx-1006A via

oral gavage in 10 mL/kg 0.5% methylcellulose once daily for 28 days (except HD, which were only dosed for 8 days [F] or 9 days [M], before termination of the group on Day 10, due to excessive toxicity; any rats at 100 mg/kg/day beyond the first 5 [M] or 4 [F] surviving to Day 29 became recovery animals, killed on Day 43).

The HD of 300 mg/kg/day resulted in death or moribund sacrifice in 10/21 HDM (D8-9) and 2/21 HDF (D9-10); scant feces, dry red material around nose and mouth, urine stains, hunched posture, and tremors; body weight loss (M: -5% D7, -9% D10; F: -2% D7, -11% D10); reduced food consumption; decreased white blood cells, lymphocytes, basophils, large unstained cells, and reticulocytes, and increases in neutrophils; increased ALT, ALK, BUN, CHOL, GGT, TBIL, and TRG; congestion in vasculature of abdominal organs and GI tract; distended stomach, due to gastric impaction with accumulated laminated test article material, that may have caused circulatory collapse, shock, vascular stasis, and death; mucosal necrosis in glandular stomach, renal tubular proteinosis, systemic thromboses; lymphoid depletion in thymus, spleen, lymph nodes, and/or bone marrow.

Dosing at 100 mg/kg/day resulted in death or moribund sacrifice in 1/16 M (D11) and 5/16 F (D10-23); hunched posture, lethargy, and rough hair coat; decreased body weight and food consumption; increased CRE, ALT (M), CHOL (M), and GLU (F); increased liver weights (reversible); and thymic lymphoid depletion (F).

The NOAEL for M and F rats in this study was 30 mg/kg/day Fx-1006A.

13 and 26-week **Rat** (Study SYI00012) GLP
A Study to Determine the Potential Oral Toxicity and Toxicokinetics of Fx-1006A When Administered to Male and Female Sprague-Dawley Rats for 13 or 26 Weeks with a 4-Week Recovery Period Following the 13-Week Arm.

Final Report dated October 13, 2006; for additional details, see review by David B. Hawver, Ph.D., under IND 74,866 #001, December 4, 2006.

Sprague-Dawley rats (N=10/sex/group Main Study Day 92 sacrifice; 10/sex/group Main Study Day 182 sacrifice; 5/sex Con & HD 14-day recovery after 92-Day dosing period; 6/sex/group TK dosed through Day 178) were given Fx-1006A at 0, 3, 10, or 30 mg/kg/day via oral gavage at 10 mL/kg in 7.5% v/v Vitamin E TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate) once daily for 92, 178, or 192 days.

No treatment-related findings were observed in mortality, clinical signs, body weights, food consumption, ophthalmoscopy, hematology, coagulation, urinalysis, gross pathology, or histopathology. Slight increases in total bilirubin in HDF (1.7X) and in BUN (M) and CRE (1.2X M, 1.4X F) were observed, but no histopathological correlates were found.

The NOAEL in this study was the HD of 30 mg/kg/day Fx-1006A for M and F, associated with AUC values of 2260 and 3120 µg•hr/mL, respectively.

Table 2 Mean TK Parameters of Tafamidis in Male and Female Rats Given Oral Repeat Doses of Tafamidis Meglumine

Study Type / Study Number	Day / Week	Dose (mg/kg/day)	T_{max} (h)	C_{max} (µg/mL)	AUC_{24} (µg•h/mL)
28-Day Toxicity/ SYI00010	Day 1	10	4.0	32.3	536
	Day 1	30	8.0	80.6	1450
	Day 1	100	4.0	164	3350
	Day 1	300	4.0	222	4430
	Day 28	10	3.0	68.3	1090
	Day 28	30	4.0	130	2230
	Day 28	100	4.0	231	4210
	Day 28	300	NS	NS	NS
26-Week Toxicity/ SYI00012	Day 1	3	1.5	9.49	137
	Day 1	10	2.0	37.6	535
	Day 1	30	3.0	89.8	1500
	Day 91	3	1	23.2	348
	Day 91	10	1	62.9	1014
	Day 91	30	6	131	2460
	Day 178	3	1.5	24.6	369
	Day 178	10	1.0	78.9	1200
	Day 178	30	4.5	159	2690
EFD/ SYI00039[a]	Day 1	15	0.5	44.8	757
	Day 1	30	4.0	83.4	1270
	Day 1	45	4.0	98.6	1990
	Day 17	15	2.0	72.8	1090
	Day 17	30	4.0	92.7	1610
	Day 17	45	4.0	133	2160
2-Year Carcinogenicity/ 805917	Week 26	3	1.0	30.1	570
	Week 26	10	1.0	89.9	1580
	Week 26	30	1.0	157	3020

AUC_{24} = Area under the concentration-time curve from zero to 24 hours; C_{max} = Maximum observed concentration; EFD = Embryofetal development; GD = Gestation day; NS = No samples were collected; T_{max} = Time at which C_{max} was observed.
a. Repeat oral dosages were administered to gravid rats from GD 7 to 17.

4-week – **Dog** (Study SYI00011) GLP
A Study to Determine the Oral Toxicity of Fx-I006A Following 28 Consecutive Administrations with a 14-Day Recovery Period and BioAnalysis in Male and Female Beagle Dogs

Beagle dogs (N=3/sex/group Con, LD, MD; 2/sex HD; + 2/sex Con & HD 14-day recovery) were given 0, 10, 100, or 300 mg/kg/day Fx-1006A via oral gavage in 0.5% methylcellulose at 10 mL/kg once daily for 28 days.

Due to toxicity, HDF (Day 7) and HDM (Day 8) received no dose for one day, then resumed at a dose level of 200 mg/kg for the remainder of the study.

Vomiting and aspiration of the test article immediately postdose and subsequent death or moribund sacrifice occurred in 1/3 MDM (D20), 1/4 HDM (D7), and 1/4 HDF (D7). One additional MDM was found dead on D11 without showing any clinical signs or decreases in body weight or food consumption, and one additional HDM was sacrificed moribund on D21 after showing mild swelling of face and salivation (D9-10); green discolored feces (D10); thin, cold to touch, lethargic, vomiting postdose periodically, body weight loss of 25%, scant food consumption (D12-21); decreased lymphocytes; and increased AST, ALT, BUN, GGT and TBIL compared to baseline; the cause of death in these two animals was unknown. Soft/mucoid feces and vomiting were increased in LD, MD, and HD groups compared to Con. MD and HD animals showed increased incidence of salivation and lethargy.

Clinical signs in HD animals also included ataxia, head bobs, and twitching. HDM showed increased ALT, ALK, BUN, GGT and TBIL compared to Con M, while HDF showed increased ALK compared to controls and baseline. Four of five early death animals showed red discoloration or thickening of some or all lobes of the lung, associated with pulmonary congestion.

Reduced food consumption observed in HD animals returned to normal during the 14-day recovery period. Serum chemistry changes observed in HD animals also normalized during the recovery period.

The NOAEL for oral administration of Fx-1006A in dogs for 28 days was 10 mg/kg/day.

13 and 39-week **Dog** (Study SYI00013) GLP
A Study to Determine the Potential Oral Toxicity and Toxicokinetics of Fx-1006A When Administered to Male and Female Dogs for 13 or 39 Weeks with A 4-Week Recovery Period Following the 13-Week Arm.

Final Report dated October 30, 2006; for additional details, see review by David B. Hawver, Ph.D., under IND 74,866 #001, December 4, 2006.

Beagle dogs (N=3/sex/group Day 92 sacrifice; 3/sex/group Day 272 sacrifice; 2/sex Con & HD recovery groups sacrificed 4 weeks after the 92-day dosing period) were given Fx-1006A at 0, 5, 15, or 45 mg/kg/day via oral gavage in 7.5% (v/v) Vitamin E TPGS [Days 1-47] or 0.5% methylcellulose [Day 48 onward] at 10 mL/kg once daily for 92 or 272 days.

No treatment-related findings were observed in mortality, body weights, food consumption, ophthalmoscopy, ECG, hematology, coagulation, urinalysis, organ weights, gross pathology, or histopathology. The incidence of soft/mucoid feces, emesis, and skin erythema was increased in a somewhat dose-dependent fashion, but severity was not generally increased.

Seven dogs (2/16 CON, 2/12 LD, 1/12 MD, and 2/16 HD) were euthanized prematurely due to sequelae from vomiting/aspiration and/or other causes not considered directly

related to the test substance. On Day 48, the formulation was changed from 7.5% Vitamin E TPGS to 0.5% methylcellulose to reduce the incidence of vomiting and aspiration thought to be vehicle-related (in the 28-day dog study vomiting and aspiration was observed only at Fx-1006A ≥ 100 mg/kg/day in 0.5% methylcellulose). No additional aspiration-related deaths were observed after the formulation change.

The NOAEL for dog in this 9-month gavage study was the HD, 45 mg/kg/day Fx-1006A, for males and females, associated with an AUC of 1440 and 1810 µg•hr/mL, respectively.

Table 3 Mean TK Parameters of Tafamidis in Male and Female Dogs Given Oral Repeat Doses of Tafamidis Meglumine

Study Type / Study Number	Sex /N	Day	Dose (mg/kg/day)	T_{max} (h)	C_{max} (µg/mL)	AUC_{24} (µg•h/mL)
28-Day Toxicity/SYI00011	M/3, F/3	1	10	2.0	37.0	383
	M/3, F/3	1	100	4.0	184	2270
	M/5, F/5	1	300	4.4	200	3020
	M/3, F/3	28	10	1.3	37.6	381
	M/1, F/3	28	100	2.0	157	1740
	M/3, F/4	28	200[a]	2.9	206	2860
13-Week Toxicity/SYI00013	M/3, F/3	1	5	0.8	24.0	243
	M/3, F/3	1	15	1.4	60.2	637
	M/5, F/5	1	45	2.6	126	1370
	M/3, F/3	48	5	1.6	31.5	337
	M/3, F/3	48	15	1.5	73.8	838
	M/5, F/5	48	45	1.9	109	1240
	M/3, F/3	91	5	0.8	29.6	315
	M/3, F/3	91	15	1.3	64.5	779
	M/5, F/5	91	45	1.8	131	1470
39-Week Toxicity/SYI00013	M/3, F/3	1	5	1.1	21.7	234
	M/3, F/3	1	15	1.2	55.8	531
	M/3, F/3	1	45	2.5	118	1400
	M/3, F/3	48	5	1.0	32.0	347
	M/3, F/3	48	15	2.3	48.3	577
	M/3, F/3	48	45	2.0	119	1540
	M/3, F/3	271	5	0.9	29.6	376
	M/3, F/3	271	15	1.8	54.3	633
	M/3, F/2	271	45	1.3	125	1620

AUC_{24} = Area under the concentration-time curve from zero to 24 hours; C_{max} = Maximum observed concentration; F = Females; M = Males; NA = Not applicable; T_{max} = Time at which C_{max} was observed.
a. A dosage of 200 mg/kg started on Day 9 for males and Day 8 for females.

7 Genetic Toxicology

This section is provided directly from Dr. Hawver's NDA 202-737 review as there were disagreements over study interpretation between our reviews that needed reconciliation (7.2, below). These are addressed, in italics at the point of commentary below, by this reviewer.

7.1 *In vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

(b) (4) Study 960707; Final Report dated February 1, 2006; GLP; QA; for additional details, see review by William T. Link, Ph.D., under IND 71,880 #000, December 12, 2005).

The test article (Fx-1006A, Lot 60106-05-001, 99.4% pure) was mixed into a homogeneous suspension with 0.5% (w/v) aqueous methylcellulose and tested in plate incorporation and pre-incubation bacterial mutation tests at up to 5000 µg/plate using *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of rat liver S9 metabolic activation.

Fx-1006A showed partial or complete loss of the background bacterial lawn at 500, 1581, and/or 5000 µg/plate and precipitate at 1581 and/or 5000 µg/plate in the presence or absence of S9. Positive and vehicle controls performed as expected. The study met criteria for validity and appeared to be adequately conducted.

Fx-1006A was considered negative in the *in vitro* bacterial reverse mutation assay under the conditions tested.

7.2 *In vitro* Assays in Mammalian Cells

Fx-1006A Chromosome Aberration Test

(b) (4) Study 960708; Final Report dated February 1, 2006; GLP; QA; for additional details, see review by William T. Link, Ph.D., under IND 71,880 #000, December 12, 2005).

The test article (Fx-1006A, Lot 60106-05-001, 99.4% pure) was mixed into a homogeneous suspension with 0.5% (w/v) aqueous methylcellulose (400 cps) and tested in a standard *in vitro* assay for chromosomal aberrations involving incubation with cultures of human lymphocytes for four hours in the presence and absence of rat liver S9 metabolic activation and for 21 hours in the absence of S9. The percentage of cells with chromosomal aberrations was evaluated in cultures incubated with the three highest concentrations of Fx-1006A that did not cause excessive toxicity (i.e., toxicity such that insufficient numbers of cells in metaphase were available for analysis).

No increases were observed in the frequency of cells with chromosomal aberrations at any Fx-1006A concentration in any of the three experiments. Vehicle and positive controls performed as expected. The sponsor considered this to be a valid negative study.

According to the Guideline for Industry: Specific Aspects of Regulatory Genotoxicity tests for Pharmaceuticals, ICH S2A, April 1996, the highest concentration of test article examined should be the lowest concentration that causes reduction in relative mitotic index (RMI) to below 50% of the mean Vehicle Control mitotic index. As shown in the tables below, however, in all three experiments in this study the lowest concentration studied that resulted in reduction of RMI to below 50% of the mean Vehicle Control value was not evaluable due to excessive toxicity. The sponsor should have conducted additional experiments to find evaluable Fx-1006A concentrations that met the criteria of reducing RMI to below 50% of the mean Vehicle Control value.

This study is not adequate. [Dr. Hawver's opinion]

[Reviewer response: While Dr. Hawver's interpretation is technically correct, there are no indications, at all, of any effect at the concentrations that approach the point where the 50% RMI is crossed (between concentration 200 and 400 µg/mL for the 4-hour incubations and between 100 and 200 µg/mL for the 24-hour incubation, see below). This reviewer did not see a high probability of a different outcome upon a re-test].

In addition, Dr. Hawver suggested that the study would not need repeating if the 2-year carcinogenicity study was negative (page 6, NDA 202-737 review).

Summary of results and statistical analysis

Treatment	Conc. (µg/mL)	MI	RMI (%)	No. cells examined	% Aberrant	No. of aberrations					Incidental observations †			
						b	e	B	E	other	(g	G	P	C)
4 hours treatment in the absence of S9 (0S9)														
0.5% MC	-	10.1	100	200	0.5	0	0	0	0	1	0	0	0	0
Fx-1006A	50.0	8.2	81	200	0.5	0	0	1	0	0	1	0	0	0
	100	7.9	78	200	0.5	0	0	1	0	0	0	0	0	0
	200	7.2	71	200	0.0	0	0	0	0	0	0	0	3	0
	400	0.5	5	6				N/A						
Mitomycin C	0.10	9.0	88	200	4.5*	0	6	3	0	0	2	1	1	0
4 hours treatment in the presence of S9 (+S9)														
0.5% MC	-	8.6	100	200	0.0	0	0	0	0	0	0	0	0	0
Fx-1006A	50.0	8.7	102	200	0.0	0	0	0	0	0	0	0	0	0
	100	7.5	87	200	0.0	0	0	0	0	0	0	0	4	0
	200	5.1	60	200	0.0	0	0	0	0	0	0	1	7	0
	400	0.1	1	0				N/A						
CP	12.0	5.4	63	200	23.0**	5	6	65	0	0	8	9	0	0
21 hours treatment in the absence of S9 (0S9)														
0.5% MC	-	6.5	100	200	0.0	0	0	0	0	0	0	0	0	0
Fx-1006A	25.0	7.9	121	200	0.0	0	0	0	0	0	0	0	0	0
	50.0	4.8	75	200	0.0	0	0	0	0	0	0	0	0	0
	100	5.1	78	200	0.0	0	0	0	0	0	0	0	0	0
	200	1.2	18	50				N/A						
Mitomycin C	0.10	4.8	73	200	5.5**	3	2	10	0	0	4	7	0	0

MI, RMI	Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g	Chromatid break, exchange, gap
B, E, G	Chromosome break, exchange, gap
other	Includes pulverized chromosomes and cells with > 8 aberrations
P	Polyploidy and endoreduplication
C	Centromeric disruption
†	g, G, P and C are excluded from the calculation of % aberrant cells
N/A	Not assessable (insufficient cells available due to toxicity)
MC	Methylcellulose
CP	Cyclophosphamide

Results of statistical analysis using one-tailed Fisher's exact test

*	$p \leq 0.01$ (significant)
**	$p \leq 0.001$ (highly significant)
otherwise	$p > 0.01$ (not significant)

7.3 *In vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Fx-1006A Rat Micronucleus Test

[redacted] (b) (4) Study 960709; Final Report dated March 6, 2006; GLP; QA; for additional details, see review by William T. Link, Ph.D., under IND 71,880 #000, December 12, 2005)

Sprague-Dawley rats were given a single dose of 0, 10, 30, or 100 mg/kg Fx-1006A (Lot 60106-05-001, 99.4% pure) via oral gavage at 10 mL/kg in 0.5% (w/v) aqueous methylcellulose (N=5/sex/group) or 20 mg/kg cyclophosphamide via oral gavage in sterile water for irrigation (N=3/sex). Clinical signs were reported hourly for the first four hours after dosing. All groups were analyzed for the frequency of micronuclei present in immature erythrocytes in bone marrow smears prepared from femurs of animals sacrificed at 24 hours after dosing; additional Con and HD groups were included for analysis at 48 hours after dosing.

The HD of 100 mg/kg was estimated (by the sponsor) to be a maximum tolerated dose based on findings in Study SYI00006, in which female Sprague-Dawley rats (N=5/group) given 0, 10, 30, or 100 mg/kg/day Fx-1006A via oral gavage at 10 mL/kg in 0.5% (w/v) methylcellulose once daily for 10 days showed increased serum glucose (all doses), decreased serum globulin and increased A/G ratio (HD), and increased liver weights (LD and HD); the NOAEL was considered to be 100 mg/kg/day Fx-1006A.

Results

No treatment-related changes in clinical signs were observed. No statistically significant increases in the number of micronucleated immature erythrocytes were observed at any dose of Fx-1006A compared to the Vehicle Control. No significant decreases in the proportion of immature erythrocytes were observed at any dose of Fx-1006A. Vehicle and Positive Controls performed as expected.

This study appears to have been adequately conducted. However, higher doses probably could have been tolerated, based on the absence of any mortality or clinical signs observed in male or female Sprague-Dawley rats after the first oral dose at 300 mg/kg/day Fx-1006A in Study SYI00010.

Fx-1006A was negative for genotoxicity in the *in vivo* rat micronucleus test under the conditions tested.

7.4 Other Genetic Toxicity Studies (impurities)

[redacted] (b) (4) **Bacterial Mutation Test**

[redacted] (b) (4) Study 963260; Final Report dated June 30, 2010; GLP; QA) (reviewed D. Hawver, NDA 202-737)

Two potentially genotoxic impurities [redacted] (b) (4) in the Fx-1006A drug substance were dissolved in methanol and tested in plate

incorporation and pre-incubation bacterial mutation tests at up to 5000 µg/plate using *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of rat liver S9 metabolic activation.

Positive and vehicle controls performed as expected. The studies met criteria for validity.

The test articles, (b) (4), were considered negative for genotoxicity in the *in vitro* bacterial reverse mutation assay under the conditions tested.

Study title: Evaluation of The Mutagenic Activity of (b) (4) in the *Salmonella Typhimurium* Reverse Mutation Assay and the *Escherichia Coli* Reverse Mutation Assay

Study no.:	504677
Study report location:	NDA 211996
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/8/2014
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch 20140101, 99.16% purity

Key Study Findings

(b) (4) (a starting material and potential impurity) was tested in the *Salmonella typhimurium* reverse mutation assay with four histidine-requiring strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and in the *Escherichia coli* reverse mutation assay with a tryptophan-requiring strain of *Escherichia coli* (WP2uvrA). The test was performed in three independent partial experiments in the presence and absence of S9-mix (rat liver S9-mix induced by Aroclor 1254).

Among the three assays performed, the largest increase in revertant was a 1.8 to 1.6-fold increase in the number of revertants was observed upon treatment with (b) (4) in tester strain TA100 in the absence and presence of S9-mix respectively. This does not reach the level of a two-fold increase in mutant frequency required for a positive response. The assay can be considered negative.

Methods

Strains:	TA1535, TA1537, TA98 and TA100, and *E. coli* (WP2uvrA)
Concentrations in definitive study:	1.7, 5.4, 17, 52, 164, 512, and 1600 µg/plate were tested in triplicate.
Basis of concentration selection:	toxicity
Negative control:	vehicle
Positive control:	See below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 ± 4 hrs

Positive and reference controls

Without metabolic activation (-S9-mix):

Strain	Chemical	Concentration/plate	Solvent
TA1535	sodium azide (SA)	5 µg	Saline
TA1537	ICR-191 (b) (4)	2.5 µg	DMSO
TA98	2-nitrofluorene (NF) (b) (4)	10 µg	DMSO
TA100	methylmethanesulfonate (MMS) (b) (4)	650 µg	DMSO
WP2uvrA	4-nitroquinoline N-oxide (4-NQO) (b) (4)	10 µg	DMSO

With metabolic activation (+S9-mix):

The positive control substance used for all tester strains was 2-aminoanthracene (2AA) (b) (4). The following concentrations were used:

Strain	Concentration/plate	Amount of S9-mix	Solvent
TA1535	2.5 µg	5 and 10%	DMSO
TA1537	2.5 µg	5%	DMSO
TA1537	5 µg	10%	DMSO
TA98	1 µg	5 and 10%	DMSO
TA100	1 µg	5%	DMSO
TA100	2 µg	10%	DMSO
WP2uvrA	15 µg	5 and 10%	DMSO

Solvents for reference substances

Saline = physiological saline (b) (4)

DMSO = dimethyl sulfoxide (b) (4)

Selection of an adequate range of doses was based on a dose range finding test with the strains TA100 and WP2uvrA, both with and without 5% (v/v) S9-mix. Eight concentrations, 1.7, 5.4, 17, 52, 164, 512, 1600 and 5000 µg/plate were tested in triplicate. The highest concentration of (b) (4) used in the subsequent mutation assay was 1600 µg/plate.

Mutation assay

At least five different doses (increasing with approximately half-log steps) of the test substance were tested in triplicate in each strain. In the first experiment (b) (4) was tested both in the absence and presence of 5% (v/v) S9-mix in tester strains TA1535, TA1537 and TA98.

In an independent repeat of the assay with additional parameters, the test substance was tested both in the absence and presence of 10% (v/v) S9-mix in all tester strains. The negative control (vehicle) and relevant positive controls were concurrently tested in each strain in the presence and absence of S9-mix.

Study Validity

The strain-specific positive control values were at least three times the concurrent vehicle control group mean indicating a valid assay.

Results

Dose range finding test/first mutation experiment

(b) (4) was tested in the tester strains TA100 and WP2uvrA with concentrations of 1.7, 5.4, 17, 52, 164, 512, 1600 and 5000 µg/plate in the absence and presence of S9-mix. Based on the results of the dose range finding test, the following dose range was selected for the mutation assay with the tester strains, TA1535, TA1537 and TA98 in the absence and presence of S9-mix: 5.4, 17, 52, 164, 512, and 1600 mg/plate. The results are shown in Table 1 and Table 2.

Precipitation of (b) (4) on the plates was only observed at the start of the incubation period at concentrations of 1600 and 5000 µg/plate.

Toxicity was observed in tester strain TA100 at concentrations of 512 µg/plate and above and TA98 at the concentration of 1600 µg/plate in the absence and presence of S9-mix. In tester strain WP2uvrA toxicity was observed at the concentration of 5000 µg/plate in the absence and presence of S9-mix. No toxicity was observed in tester strains TA1535 and TA1537 at the highest concentration tested (1600 µg/plate).

Mutagenicity

No increase in the number of revertants was observed upon treatment with (b) (4) under all conditions tested in tester strains TA1535, TA1537, TA98 and WP2uvrA. A 1.2-fold increase was observed in tester strain TA100 in the presence of S9-mix.

Table 1 **Dose range finding test: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with one strain of *Salmonella typhimurium* and one *Escherichia coli* strain.					
	TA100			WP2uvrA		
			Without S9-mix			
Positive control	868 ±	27		1361 ±	114	
DMSO	113 ±	10		31 ±	4	
1.7	118 ±	9		32 ±	5	
5.4	120 ±	13		38 ±	4	
17	119 ±	8		31 ±	8	
52	130 ±	8		29 ±	4	
164	127 ±	6	n	27 ±	6	
512	119 ±	51	s	33 ±	11	
1600			e MC	27 ±	3	n
5000	0 ±	0	e NP			e NP MC
			With S9-mix[1]			
Positive control	1719 ±	272		139 ±	19	
DMSO	129 ±	21		34 ±	8	
1.7	112 ±	16		35 ±	10	
5.4	120 ±	8		45 ±	12	
17	98 ±	12		34 ±	7	
52	110 ±	11		42 ±	13	
164	127 ±	21	n	38 ±	9	
512	156 ±	21	s	34 ±	9	
1600			e MC	31 ±	2	n
5000	0 ±	0	e NP			e NP MC

[1] Plate incorporation assay (5% S9)
MC Microcolonies
NP No precipitate
a Bacterial background lawn absent
e Bacterial background lawn extremely reduced
n Normal bacterial background lawn
s Bacterial background lawn slightly reduced

Table 2 **Experiment 1: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with different strains of *Salmonella typhimurium*.		
	TA1535	TA1537	TA98
	Without S9-mix		
Positive control	760 ± 70	703 ± 73	745 ± 59
DMSO	22 ± 9	5 ± 3	17 ± 9
5.4	19 ± 6	8 ± 2	11 ± 4
17	21 ± 15	7 ± 3	16 ± 9
52	19 ± 4	8 ± 5	12 ± 3
164	16 ± 2	4 ± 4	14 ± 4
512	15 ± 7	3 ± 2	17 ± 1 n
1600	18 ± 7 n NP	4 ± 2 n NP	5 ± 5 m NP
	With S9-mix[1]		
Positive control	238 ± 50	211 ± 17	642 ± 151
DMSO	14 ± 2	9 ± 4	24 ± 5
5.4	14 ± 2	8 ± 4	18 ± 2
17	11 ± 3	11 ± 5	20 ± 2
52	13 ± 1	9 ± 2	28 ± 5
164	14 ± 2	8 ± 4	22 ± 7
512	11 ± 1	7 ± 5	14 ± 4 n
1600	17 ± 5 n NP	7 ± 6 n NP	14 ± 9 m NP

[1] Plate incorporation assay (5% S9)
NP No precipitate
m Bacterial background lawn moderately reduced
n Normal bacterial background lawn

Experiment 2

A second mutation experiment was performed in the absence of S9-mix and in the presence of 10% (v/v) S9-mix. Based on the results of the first mutation assay, [redacted] (b) (4) was tested up to the dose level of 5000 µg/plate in strains TA1535, TA1537, TA98, TA100 and WP2uvrA. The results are shown in Table 3.

Table 3 **Experiment 2: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with different strains of *Salmonella typhimurium* and one *Escherichia coli* strain.									
	TA1535		TA1537		TA98		TA100		WP2uvrA	
Without S9-mix										
Positive control	637 ±	33	489 ±	67	768 ±	9	903 ±	81	1680 ±	140
DMSO	22 ±	8	6 ±	3	17 ±	1	123 ±	8	31 ±	3
5.4	18 ±	6	4 ±	1	18 ±	8	126 ±	3	33 ±	10
17	22 ±	7	9 ±	3	14 ±	8	138 ±	12	31 ±	3
52	23 ±	6	9 ±	5	13 ±	8	157 ±	13	41 ±	3
164	25 ±	13	6 ±	3	21 ±	9	226 ±	31	25 ±	5
512	24 ±	6 n	8 ±	4 n	21 ±	11 n	196 ±	31 n	30 ±	8
1600	0 ±	0 a NP	4 ±	3 s	0 ±	0 a	0 ±	0 a	23 ±	2 n
5000	0 ±	0 a NP	0 ±	0 a NP	0 ±	0 a NP	0 ±	0 a NP		e NP MC
With S9-mix[1]										
Positive control	180 ±	22	450 ±	73	564 ±	78	1298 ±	59	208 ±	30
DMSO	12 ±	5	10 ±	2	20 ±	4	132 ±	27	29 ±	9
5.4	15 ±	4	8 ±	5	25 ±	6	127 ±	16	26 ±	23 s
17	15 ±	4	6 ±	2	17 ±	3	145 ±	17	44 ±	6
52	24 ±	11	5 ±	3	21 ±	3	147 ±	12	45 ±	6
164	18 ±	6	5 ±	1	18 ±	2	215 ±	4	33 ±	4
512	16 ±	4 n	11 ±	5 n	29 ±	4 n	186 ±	17 n	26 ±	7
1600	15 ±	10 s	9 ±	3 s	19 ±	9 m	135 ±	16 m	30 ±	11 n
5000	0 ±	0 a NP	0 ±	0 a NP	0 ±	0 a NP	0 ±	0 a NP		e NP MC

[1] Plate incorporation assay (10% S9)
MC Microcolonies
NP No precipitate
a Bacterial background lawn absent
e Bacterial background lawn extremely reduced
m Bacterial background lawn moderately reduced
n Normal bacterial background lawn
s Bacterial background lawn slightly reduced

In the second mutation assay, toxicity was observed in tester strains TA1535, TA1537, TA100 and TA98 at the concentration of 1600 µg/plate and upwards. In tester strain WP2uvrA toxicity was observed at the concentration of 5000 µg/plate.

Mutagenicity

In the second mutation assay, a 1.8 to 1.6-fold increase in the number of revertants was observed upon treatment with [redacted] (b) (4) in tester strain TA100 in the absence and presence of S9-mix respectively. No increase in the number of revertants was observed upon treatment with [redacted] (b) (4) in all other tester strains.

Experiment 3

A third mutation experiment was performed with tester strain TA100 in the absence and in presence of 10% (v/v) S9-mix at the concentration range of 17, 52, 164, 512, 1600 and 5000 µg/plate. In the same experiment, tester strains TA1535 and TA1537 were tested in the absence of S9-mix and in the presence of 5% (v/v) S9-mix at the concentration range of 52, 164, 512, 1600 and 5000 µg/plate since in the first mutation assay no dose level was tested up to precipitate or toxicity. The results are shown in Table 4 and Table 5.

Table 4 **Experiment 3: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay (TA1535 and TA1537)**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with different strains of *Salmonella typhimurium*.			
	TA1535		TA1537	
	Without S9-mix			
Positive control	722 ± 31		433 ± 21	
DMSO	33 ± 0		7 ± 5	
52	25 ± 2		12 ± 4	
164	23 ± 3		6 ± 3	
512	21 ± 2	n	14 ± 5	n
1600		e MC		e MC
5000	0 ± 0	e NP	0 ± 0	e NP
	With S9-mix[1]			
Positive control	281 ± 19		513 ± 13	
DMSO	21 ± 8		9 ± 4	
52	20 ± 8		13 ± 4	
164	22 ± 10		8 ± 2	
512	21 ± 6	n	11 ± 1	n
1600		e MC	9 ± 3	m
5000	0 ± 0	e NP	0 ±	0

1 Plate incorporation assay (5% S9)
MC Microcolonies
NP No precipitate
a Bacterial background lawn absent
e Bacterial background lawn extremely reduced
m Bacterial background lawn moderately reduced
n Normal bacterial background lawn

Table 5 **Experiment 3: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay (TA100)**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with the *Salmonella typhimurium* tester strain TA100.	
	Without S9-mix	
Positive control	1040 ± 90	
DMSO	117 ± 11	
17	138 ± 12	
52	119 ± 7	
164	119 ± 19	n
512	111 ± 13	s
1600		e MC
5000	0 ± 0	a NP
	With S9-mix[1]	
Positive control	1885 ± 119	
DMSO	131 ± 6	
17	125 ± 8	
52	102 ± 4	
164	116 ± 8	
512	139 ± 5	n
1600	236 ± 13	m
5000	0 ± 0	a NP

[1] Plate incorporation assay (10% S9)
MC Microcolonies
NP No precipitate
a Bacterial background lawn absent
e Bacterial background lawn extremely reduced
m Bacterial background lawn moderately reduced
n Normal bacterial background lawn
s Bacterial background lawn slightly reduced

Study title: Evaluation of The Mutagenic Activity of (b) (4) in the *Salmonella Typhimurium* Reverse Mutation Assay

Study no.:	507970
Study report location:	NDA 211996
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/8/2015
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch 20140101

Key Study Findings

This study was conducted to verify if the borderline mutagenic response observed in Study 504677 in tester strain TA100 was related to the reactivity of the test substance with the solvent DMSO.

(b) (4) (dissolved in acetone) was tested up to concentrations of 5000 µg/plate in the absence and presence of S9-mix.

(b) (4) precipitated on the plates at dose levels of 512 µg/plate and upwards. Toxicity was observed at dose levels of 878 µg/plate and above in the absence of S9-mix and at 1600 µg/plate and above in the presence of S9-mix.

(b) (4) did not induce a significant dose-related increase in the number of revertant (His+) colonies in tester strain TA100 in the absence and presence of S9-metabolic activation.

Methods	
Strains:	TA100 only
Concentrations in definitive study:	see below
Basis of concentration selection:	Precipitation, toxicity
Negative control:	acetone
Positive control:	Without metabolic activation (-S9-mix): methylmethanesulfonate (MMS) 650 µg/plate, in DMSO With metabolic activation (+S9-mix): 2-aminoanthracene (2AA) 2 µg /plate, in DMSO
Formulation/Vehicle:	(b) (4) was dissolved in acetone rather than DMSO (used in above Study 504677)
Incubation & sampling time:	48 hr

Study Validity

Both the solvent and positive controls produced expected effects indicating a valid study.

Results

The reduction of the bacterial background lawn and the reduction in the number of revertants are presented below.

(Reduction of the bacterial background lawn and in the number of revertant colonies)

Strain	Without S9-mix			With S9-mix		
	Dose (µg/plate)	Bacterial background lawn	Revertant colonies	Dose (µg/plate)	Bacterial background lawn	Revertant colonies
TA100	878	slight	-[1]	1600	slight	slight
	1600	extreme	microcolonies	2800	moderate	moderate
	2800	extreme	microcolonies	5000	extreme	microcolonies
	5000	extreme	microcolonies			

-[1] No reduction in the number of revertant colonies less than the minimal value of the historical control data range.

Mutagenicity

There was no increase in the number of revertants observed upon treatment with (b) (4) under any of the conditions tested (results below).

APPEARS THIS WAY ON ORIGINAL

WITHOUT S9-MIX

plate	1		2		3		MEAN		SD
dose (µg/plate)									
positive control	310		588		765		554	±	229
solvent control	106		139		118		121	±	17
17	101		103		105		103	±	2
52	78		118		87		94	±	21
164	94		84		106		95	±	11
275	73	NP	79	NP	95	NP	82	±	11
512	87	n SP	97	n SP	95	n SP	93	±	5
878	84	s MP	73	s MP	57	s MP	71	±	14
1600		e MP MC		e MP MC		e MP MC			
2800		e MP MC		e MP MC		e MP MC			
5000		e MP MC		e MP MC		e MP MC			

WITH S9-MIX

plate	1		2		3		MEAN		SD
dose (µg/plate)									
positive control	883		1092		967		981	±	105
solvent control	105		99		116		107	±	9
17	82		110		71		88	±	20
52	88		102		102		97	±	8
164	105		101		114		107	±	7
275	114	NP	112	NP	103	NP	110	±	6
512	90	SP	103	SP	97	SP	97	±	7
878	79	n SP	83	n SP	94	n SP	85	±	8
1600	66	s MP	60	s MP	76	s MP	67	±	8
2800	35	m MP	63	m MP	42	m MP	47	±	15
5000		e MP MC		e MP MC		e MP MC			

MC	Microcolonies
MP	Moderate Precipitate
NP	No precipitate
SP	Slight Precipitate
e	Bacterial background lawn extremely reduced
m	Bacterial background lawn moderately reduced
n	Normal bacterial background lawn
s	Bacterial background lawn slightly reduced

Study title: Evaluation of the Mutagenic Activity of (b) (4) in the *Salmonella Typhimurium* Reverse Mutation Assay and the *Escherichia Coli* Reverse Mutation Assay (With Independent Repeat)

Study no.:	504698
Study report location:	NDA 211996
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/27/2014
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch E010014831

Key Study Findings

No increase in the number of revertants was observed upon treatment with (b) (4) under all conditions tested in two valid assays.

Methods

Strains:	*Salmonella typhimurium* strains: TA1535, TA1537, TA98 and TA100. *Escherichia coli* strain WP2uvrA.
Concentrations in definitive study:	See results
Basis of concentration selection:	Precipitation, toxicity
Negative control:	DMSO
Positive control:	see below
Incubation & sampling time:	48 hr

Without metabolic activation (-S9-mix):

Strain	Chemical	Concentration/plate	Solvent
TA1535	sodium azide (SA)	5 µg	Saline
TA1537	ICR-191 [redacted]	2.5 µg	DMSO
TA98	2-nitrofluorene (NF) [redacted]	10 µg	DMSO
TA100	methylmethanesulfonate (MMS) [redacted]	650 µg	DMSO
WP2uvrA	4-nitroquinoline N-oxide (4-NQO) [redacted]	15 µg	DMSO

With metabolic activation (+S9-mix):

The positive control substance used for all tester strains was 2-aminoanthracene (2AA) ([redacted] The following concentrations were used:

Strain	Concentration/plate	Amount of S9-mix	Solvent
TA1535	2.5 µg	5 and 10%	DMSO
TA1537	2.5 µg	5%	DMSO
TA1537	5 µg	10%	DMSO
TA98	1 µg	5 and 10%	DMSO
TA100	1 µg	5%	DMSO
TA100	2 µg	10%	DMSO
WP2uvrA	15 µg	5 and 10%	DMSO

Solvents for reference substances

Saline = physiological saline [redacted]

DMSO = dimethyl sulfoxide [redacted]

Dose range finding test

Selection of an adequate range of doses was based on a dose range finding test with the strains TA100 and WP2uvrA, both with and without 5% (v/v) S9-mix. Eight concentrations, 3, 10, 33, 100, 333, 1000, 3330 and 5000 µg/plate were tested in triplicate. Due to precipitate of the test substance on the plates, the highest concentration of [redacted] used in the subsequent mutation assay was 3330 µg/plate.

Mutation assay

At least five different doses (increasing with approximately half-log steps) of the test substance were tested in triplicate in each strain. In the first experiment [redacted] was tested both in the absence and presence of 5% (v/v) S9-mix in tester strains TA1535, TA1537 and TA98. In an independent repeat of the assay with additional parameters, the test substance was tested both in the absence and presence of 10% (v/v) S9-mix in all tester strains. The negative control (vehicle) and relevant positive controls were concurrently tested in each strain in the presence and absence of S9-mix.

Study Validity

Both the solvent and positive controls produced expected effects indicating a valid study.

Results

Dose-range and 1st Experiment

[redacted] was tested in the tester strains TA100 and WP2uvrA with concentrations of 3, 10, 33, 100, 333, 1000, 3330 and 5000 µg/plate in the absence and presence of S9-mix. Based on the results of the dose range finding test, the following dose range

was selected for the mutation assay with the tester strains, TA1535, TA1537 and TA98 in the absence and presence of S9-mix: 33, 100, 333, 1000 and 3330 µg/plate. The results are shown in Table 1 and Table 2.

Precipitate
Precipitation of (b) (4) on the plates was observed at the start and at the end of the incubation period at concentrations of 3330 µg/plate and upwards.

Toxicity
The bacterial background lawn was not reduced at any of the concentrations tested and no biologically relevant decrease in the number of revertants was observed up to the dose level of 3330 µg/plate. Since (b) (4) precipitated heavily on the plates at the test substance concentration of 5000 µg/plate the number of revertant colonies of this dose level could not be determined.

Mutagenicity
No increase in the number of revertants was observed upon treatment with (b) (4) under all conditions tested.

Experiment 2
A second mutation experiment was performed in the absence of S9-mix and in the presence of 10% (v/v) S9-mix. Based on the results of the first mutation assay, (b) (4) was tested up to the dose level of 3330 µg/plate in strains TA1535, TA1537, TA98, TA100 and WP2uvrA. The results are shown in Table 3.

Precipitate
Precipitation of (b) (4) on the plates was observed at the start and at the end of the incubation period at the concentration of 3330 µg/plate.

Toxicity
In the second mutation assay, there was no reduction of the bacterial background lawn and no biologically relevant decrease in the number of revertants at any of the concentrations tested in all tester strains in the absence and presence of S9-mix.

Mutagenicity
In the second mutation assay, no increase in the number of revertants was observed upon treatment with (b) (4) under all conditions tested.

Table 1 **Dose range finding test: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with one strain of *Salmonella typhimurium* and one *Escherichia coli* strain.			
	TA100		WP2uvrA	
Without S9-mix				
Positive control	976 ± 88		1318 ± 94	
Solvent control	122 ± 12		21 ± 5	
3	105 ± 9		30 ± 16	
10	126 ± 33		31 ± 11	
33	124 ± 28		28 ± 5	
100	101 ± 16		38 ± 7	
333	118 ± 9		34 ± 5	
1000	109 ± 8	NP	29 ± 8	NP
3330	113 ± 20	MP	30 ± 4	MP
5000		n HP		n HP
With S9-mix[1]				
Positive control	1321 ± 336		162 ± 107	
Solvent control	115 ± 14		39 ± 11	
3	101 ± 19		41 ± 11	
10	109 ± 13		37 ± 3	
33	113 ± 1		37 ± 8	
100	108 ± 9		51 ± 5	
333	101 ± 7		42 ± 6	
1000	110 ± 18	MP	38 ± 6	NP
3330	100 ± 3	MP	42 ± 3	MP
5000		n HP		n HP

1 Plate incorporation assay (5% S9)
HP Heavy Precipitate; The number of revertant colonies could not be determined
MP Moderate Precipitate
NP No precipitate
n Normal bacterial background lawn

Table 2 **Experiment 1: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with different strains of *Salmonella typhimurium*.		
	TA1535	TA1537	TA98
	Without S9-mix		
Positive control	697 ± 32	540 ± 60	929 ± 45
Solvent control	17 ± 4	12 ± 3	14 ± 0
33	13 ± 2	6 ± 4	19 ± 7
100	19 ± 8	5 ± 2	19 ± 7
333	19 ± 1	4 ± 2	17 ± 3
1000	16 ± 6 NP	8 ± 9 NP	18 ± 2 NP
3330	17 ± 2 n SP	4 ± 2 n SP	24 ± 6 n SP
	With S9-mix[1]		
Positive control	290 ± 8	234 ± 17	790 ± 116
Solvent control	13 ± 1	6 ± 2	15 ± 5
33	10 ± 2	6 ± 3	18 ± 2
100	13 ± 1	6 ± 1	21 ± 8
333	10 ± 4	6 ± 5	14 ± 0
1000	7 ± 3 NP	6 ± 2	16 ± 3 NP
3330	11 ± 4 n SP	4 ± 2 n MP	20 ± 5 n MP

[1] Plate incorporation assay (5% S9)
MP Moderate Precipitate
NP No precipitate
SP Slight Precipitate
n Normal bacterial background lawn

Table 3 **Experiment 2: Mutagenic response of [redacted] (b) (4) in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay**

Dose (µg/plate)	Mean number of revertant colonies/3 replicate plates (± S.D.) with different strains of *Salmonella typhimurium* and one *Escherichia coli* strain.									
	TA1535		TA1537		TA98		TA100		WP2uvrA	
				Without S9-mix						
Positive control	512 ±	42	463 ±	35	992 ±	43	1051 ±	33	1036 ±	24
Solvent control	18 ±	5	9 ±	1	15 ±	3	127 ±	37	28 ±	6
33	33 ±	12	6 ±	4	15 ±	1	120 ±	2	21 ±	2
100	18 ±	4	4 ±	3	13 ±	4	128 ±	4	37 ±	7
333	16 ±	7	5 ±	3	13 ±	4	111 ±	4	26 ±	3
1000	20 ±	5 NP	6 ±	1 NP	11 ±	5 NP	108 ±	9 NP	27 ±	13 NP
3330	13 ±	2 n SP	8 ±	6 n SP	23 ±	8 n SP	105 ±	9 n SP	30 ±	12 n SP
				With S9-mix[1]						
Positive control	182 ±	16	462 ±	31	444 ±	27	1583 ±	5	206 ±	20
Solvent control	10 ±	5	10 ±	4	18 ±	2	97 ±	13	39 ±	9
33	12 ±	2	8 ±	5	20 ±	10	106 ±	18	38 ±	11
100	10 ±	4	6 ±	4	17 ±	6	121 ±	18	36 ±	7
333	7 ±	3	6 ±	2	25 ±	3	106 ±	12	29 ±	9
1000	17 ±	8 NP	8 ±	2 NP	23 ±	4 NP	107 ±	20 NP	35 ±	7 NP
3330	9 ±	3 n SP	9 ±	5 n SP	19 ±	4 n SP	110 ±	14 n SP	39 ±	12 n SP

1 Plate incorporation assay (10% S9)
NP No precipitate
SP Slight Precipitate
n Normal bacterial background lawn

8 Carcinogenicity

Carcinogenicity studies were conducted in the mouse and rat. Both studies had prior Executive CAC approval for dose selection on 1/2/2008 (minutes was attached in D. Hawver review). Both these studies were found to be negative for increases in tumor findings. The Executive CAC concurred in this assessment (meeting minutes are attached in D. Hawver review for the mouse study and in Appendix for the rat study).

Mouse Study (from D. Hawver review)

26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice
Study no.: AB44FV.7G8R.BTL

Non-neoplastic findings: The HD of 90 mg/kg/day resulted in a tolerable level of toxicity. Treatment-related non-neoplastic changes were observed in kidney (increased incidence and severity of kidney nephrosis in HDM) and liver (increased incidence of hepatic centrilobular hypertrophy and scattered single cell necrosis in MDM, HDM, and HDF). The Vehicle Control (7.5% Vitamin E d-alpha Tocopheryl Poly-ethylene Glycol 1000 Succinate) did not result in any noticeable toxicity compared to the Water Control.

Evaluation of Tumor Findings
No treatment-related increases in neoplastic changes were observed, except in positive controls, which showed increased tumors in lungs (multiple adenoma and carcinoma in males and females, and hemangiosarcoma in females) and spleen (hemangiosarcoma in males and females) compared to Vehicle or Water Controls.

Study title: A 2-year Carcinogenicity Study of PF-06291826 by Oral Gavage Administration in Rats	
Study no.:	**805917**
Study report location:	IND 74866 SDN 127 (10/26/15)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/23/2012
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	GR05463/GR06390; 99.8%/99.9% purity
CAC concurrence:	Yes, 1/3/2008

Key Study Findings

Neoplastic findings: There was no statistically significant increases in any observed tumor type in any treated groups in male or female rats, when compared to the controls.

Non-neoplastic findings: Incidence and/or severity of the liver microscopic findings were higher (more prominent in females than males) in all treated groups, which included minimal to moderate hepatocellular hypertrophy (centrilobular) and minimal to moderate hepatocellular multinucleated giant cells in both sexes, and minimal to marked foci of clear cell cellular alteration; minimal to moderate hepatocellular necrosis (generally centrilobular); and minimal to moderate pigmentation of Kupffer cells in females.

Adequacy of Carcinogenicity Study

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, ophthalmology, clinical pathology parameters (hematology of health screen animals), toxicokinetic parameters, gross necropsy findings and histopathologic examinations. Statistical analyses were performed on the body weight, mortality and tumor data.

Based on the Exec CAC evaluations on dose selection, the highest dose level of 30 mg/kg was expected to produce over 25-fold the anticipated clinical human exposure (based on AUC) with a predicted human dose of 20 mg/day (b) (4) No significant toxicity was expected in this study. The low dose was expected to produce exposures near the anticipated human exposure.

The recommended maximum human dose (MRHD) is 80 mg (4 x 20mg) tafamidis meglumine or 61 mg tafamidis free acid (both are equivalent based on human exposure) in the current application. The actual exposure ratios determined from AUC at the high dose in this study were 19.2x and 16.3x for females and males, respectively, based on AUC at the MRHD of tafamidis.

The study design is considered adequate.

Evaluation of Tumor Findings

There were no statistically significant increases in any observed tumor type in any treated groups in male or female rats, when compared to the controls. Appropriate combinations of neoplastic observations within given tissues did not alter the statistical results or conclusions. Therefore, the study is considered negative.

Methods

Doses:	3, 10, or 30 mg/kg
Frequency of dosing:	daily
Dose volume:	10 mL/kg
Route of administration:	oral
Formulation/Vehicle:	Ultra-pure water or Vit. E TPGS
Basis of dose selection:	>25-fold AUC increase
Species/Strain:	Rat, Sprague Dawley
Number/Sex/Group:	see chart below
Age:	9 weeks
Animal housing:	3/cage (same sex/group)
Paradigm for dietary restriction:	Food provided *ad libitum*
Dual control employed:	Yes, water or Vit. E
Interim sacrifice:	no
Satellite groups:	see chart
Deviation from study protocol:	The procedures to be conducted on the Health Screen population was inadvertently omitted from the study plan: Ten male and ten female animals were selected from the total population using random numbers for the provision of blood samples and gross pathology examination for health screen purposes only. This deviation was considered to have had no impact on the study since all procedures were performed as appropriate.

The study design was as follows:

Group No.	No. of Carcinogenicity Animals		Identification	Dose Level[a] (mg/kg/day)	Concentration[a] (mg/mL)	Dose Volume (mL/kg)
	Male[b]	Female[b]				
1	70	70	UPW	0	0	10
2	70	70	Vit E TPGS	0	0	10
3	60	60	PF-06291826	3	0.3	10
4	60	60	PF-06291826	10	1	10
5	70	70	PF-06291826	30	3	10
6	10	10	Health Screen	-	-	-
7	-	24	Sentinels	-	-	-

UPW = Ultra Pure Water; Vit E TPGS = 7.5% Vitamin E TPGS, NF Grade.

[a] Dose level and concentration are expressed as free acid and were not corrected for purity.

[b] Males and Females were considered separately. Group 5 female reached 20 animals left on Day 711 and dosing of that group was stopped on Day 713. Group 2 female (control) reached 20 animals on study Day 714 and terminal necropsies of the entire female portion of the study was initiated on study Day 716.

Observations and Results

Mortality

Mortality/Survival Data

Group	Males		Females	
	Mortality	Survival	Mortality	Survival
1	48[a]/70	31%	45[a]/70	36%
2	49[a]/70	30%	50/70	29%
3	40/60	33%	43/60	28%
4	43/60	28%	44/60	27%
5	47/70	33%	50/70	29%

[a] Including rat found dead during the terminal necropsy period (Nos. 1037, 1522, 2043 and 2066).

The mortality rate was comparable between all groups for both sexes. For each preterminal decedent rat, the most probable cause of death was determined. The cause of death could not be determined for a small number of animals per group. No Vit E TPGS or PF-06291826-related effect was seen in the distribution of neoplastic or non-neoplastic lesions that contributed to unscheduled death or euthanasia of animals in this study. The 3 most frequent neoplastic causes of early death/euthanasia recorded in control animals (UPW or Vit E TPGS) and animals given PF-06291826 were pars distalis adenoma of the pituitary gland in both sexes followed by fibroadenoma and adenocarcinoma of the mammary gland in females.

Clinical Signs

There were no test item-related clinical observations noted during this study.

Clinically observed palpable masses, which were present during the dosing phase and at necropsy, were noted at a slightly higher proportion in animals from the control groups (UPW and Vit E TPGS), and more specifically for the females, but the onset, distribution and incidence of the palpable masses showed no indication of any difference that could be attributable to PF-06291826.

Incidence of Palpable Masses

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/dose)	0	0	3	10	30	0	0	3	10	30
No. Animals Examined	70	70	60	60	70	70	70	60	60	70
Number of animals	34	32	23	22	22	55	47	36	31	34
% of animals with mass	49	46	38	37	31	79	67	60	52	49

Body Weights

Test item-related effects on body weight were limited to males given 30 mg/kg/day, when compared to the vehicle control group (Group 2; Vit E TPGS). Minimal lower body weights were noted throughout the study in males given 30 mg/kg/day. The differences varied between -2.1% to -12.6% of the Vit E TPGS control mean and reached statistical significance on most occasions after Week 42.

Feed Consumption

There were no test item-related effects on food consumption noted during this study.

Gross Pathology

There were no macroscopic changes related to PF-06291826 in the study.

Histopathology

Peer Review

All findings were peer reviewed. An initial peer review, which included selected histopathologic findings, was conducted by:
Scott H Schelling, DVM, DACVP Associate Research Fellow
Pfizer Global Research & Development, Histology Laboratory, G3023
1 Burtt Road
Andover, MA. 01810 USA

A second pathology peer review, which included selected histopathologic findings, was conducted by:
Lindsay Tomlinson, DVM, DVSc, Dip. ACVP, Pfizer, Inc.
Building F, Office F1134
1 Burtt Road
Andover, MA. 01810
USA

Neoplastic

The statistical reviewer concluded “no tumor types had statistically significant dose response relationships in male or female rats. The pairwise comparisons did not show statistically significant increases in incidence in any observed tumor type in any treated groups in male or female rats.” Appropriate combinations of neoplastic observations within given tissues did not alter the statistical results or conclusions.

This reviewer concurs with the statistician’s conclusions.

Non-Neoplastic

Non-neoplastic microscopic changes related to PF-06291826 occurred in the liver of males and/or females given ≥ 3 mg/kg/day and are summarized below. There were no microscopic findings related to the Vit. E TPGS vehicle control.

Table 4 Summar of Microscopic Findin s – Scheduled and Unscheduled Euthanasia

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/dose)	0	0	3	10	30	0	0	3	10	30
No. Animals Examined	70	70	60	60	70	70	70	60	60	70
Liver (No. Examined)	70	70	60	60	70	70	70	60	60	70
Hypertrophy; hepatocellular	(7)[a]	(6)	(35)	(37)	(53)	(3)	(7)	(59)	(57)	(67)
Minimal	6	4	22	29	24	3	5	30	21	17
Mild	0	2	12	7	19	0	2	27	19	34
Moderate	1	0	1	1	10	0	0	2	16	16
Percent (%)[b]	10.0	8.6	58.3	61.7	75.7	4.3	10.0	98.3	95.0	95.7
Multinucleated giant cells; hepatocellular	(1)	(0)	(4)	(3)	(6)	(0)	(0)	(38)	(36)	(28)
Minimal	1	0	4	2	2	0	0	25	23	22
Mild	0	0	0	1	3	0	0	13	9	5
Moderate	0	0	0	0	1	0	0	0	4	1
Percent (%)	1.4	0.0	6.7	5.0	8.6	0.0	0.0	63.3	60.0	40.0
Focus of cellular alteration; clear cell	(20)	(20)	(21)	(14)	(17)	(15)	(17)	(29)	(30)	(26)
Minimal	12	16	13	8	15	10	12	11	12	8
Mild	5	0	4	3	2	5	4	7	9	9
Moderate	3	4	3	3	0	0	1	10	7	4
Marked	0	0	1	0	0	0	0	1	2	5
Percent (%)	28.6	28.6	35.0	23.3	24.3	21.4	24.3	48.3	50.0	37.1
Necrosis	(10)	(10)	(5)	(9)	(11)	(5)	(2)	(16)	(14)	(30)
Minimal	7	3	3	3	6	4	1	6	7	14
Mild	3	5	1	6	5	1	1	8	3	12
Moderate	0	2	0	0	0	0	0	2	4	4
Marked	0	0	1	0	0	0	0	0	0	0
Percent (%)	14.3	14.3	8.3	15.0	15.7	7.1	2.9	26.7	23.3	42.9
Pigmentation, Kupffer cell	(8)	(8)	(16)	(16)	(11)	(3)	(4)	(29)	(29)	(34)
Minimal	6	7	13	15	8	3	4	23	21	15
Mild	2	1	2	0	3	0	0	5	5	16
Moderate	0	0	1	1	0	0	0	1	3	3
Percent (%)	11.4	11.4	26.7	26.7	15.7	4.3	5.7	48.3	48.3	48.6

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Percent of the total number of animals with the finding.

Non-neoplastic microscopic changes in the liver were generally more prominent in females than males and seen in all dosed groups. They consisted of minimal to moderate hepatocellular hypertrophy (centrilobular) and minimal to moderate hepatocellular multinucleated giant cells in both sexes, and minimal to marked foci of clear cell cellular alteration; minimal to moderate hepatocellular necrosis (generally centrilobular); and minimal to moderate pigmentation of Kupffer cells in females.

These hepatic microscopic findings occurred generally in all treatment groups (including controls) with an increased incidence and/or severity in rats given 3, 10, and 30 mg/kg/day PF-06291826 compared to either control and with a comparable incidence and/or severity in rats administered 3, 10, and/or 30 mg/kg/day PF-06291826.

The hepatocellular multinucleated giant cells consisted of enlarged centrilobular hepatocytes containing three or more nuclei. The foci of clear cell cellular alteration

consisted of variably sized areas of hepatocytes with an enlarged microvesicular cytoplasm.

Toxicokinetics

Table 5 Summary Mean (±SE) PF-06291826 Toxicokinetic Parameters in Female and Male Sprague-Dawley Rat Plasma Following 3, 10 and 30 mg/kg/day Oral Administration of PF-06291826 on Week 26

Dose (mg/kg/day)	Sex	T_{max} (hour)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)
3	Female	1	36,500 ± 6770	698,000 ± 99,000
	Male	2	26,500 ± 2070	443,000 ± 15,800
	All	1	30,100 ± 4170	570,000 ± 59,900
10	Female	2	108,000 ± 4790	1,890,000 ± 136,000
	Male	1	78,000 ± 3760	1,260,000 ± 139,000
	All	1	89,900 ± 6080	1,580,000 ± 120,000
30	Female	2	179,000 ± 15,600	3,270,000 ± 277,000
	Male	1	152,000 ± 18,100	2,770,000 ± 294,000
	All	1	157,000 ± 12,100	3,020,000 ± 195,000

The exposure ratio (rat/human) based on AUC at the high dose was 19.2x and 16.3x for females and males, respectively.

Dosing Solution Analysis

All study samples analyzed had mean concentrations within or equal to the acceptance criteria of ± 10% (individual values within or equal to ± 15%) of their theoretical concentrations. For homogeneity, the RSD of concentrations for all samples in each group tested was within the acceptance criteria of ≤ 5%.

The difference between the mean results of the stored and fresh solutions of all stability samples were found to be within ± 10% of their theoretical concentrations. The dose formulations were within specification. Homogeneity testing showed that the formulation technique used produced homogeneous preparations.

9 Reproductive and Developmental Toxicology

All reproductive toxicology studies were reviewed (D. Hawver, NDA 202-737) and the summary below was based on the previous review. (Note: all Fx-1006A concentrations and doses are expressed according to the content of active moiety, Fx-1006 free acid; e.g., a nominal dose of 100 mg/kg/day was administered as 163 mg/kg/day meglumine salt, Fx-1006A).

9.1 Fertility and Early Embryonic Development

Fertility and Early Embryonic Development was evaluated in rats (Study SY100060). Doses of 5, 15 or 30 mg/kg (equivalent to 8.2, 24.5 or 49 mg/kg tafamidis meglumine) were used. Males were treated from 28 days prior to cohabitation through the day prior to sacrifice. Females were treated from 15 days prior to cohabitation through Gestation

Day (GD) 7. The maximum duration of cohabitation was 21 days. There was no toxicokinetic analysis.

No treatment-related deaths or clinical signs were observed. Body weight and food consumption were unaffected in males. In HD females, body weight loss was observed during the first week of dosing (-1.7% on Day 7 compared to Day 1), and body weight gain was reduced during the 15-day pre-cohabitation dosing period -43% compared to Con F). Maternal body weight was reduced in HDF on GD0 (-5.1%) to GD3 (-5.2%) compared to Con F.

No treatment-related changes were observed in estrous cycling or mating and fertility parameters (# of days in cohabitation, # of rats that mated, Fertility Index, # of rats with confirmed mating dates during the first week of cohabitation, or # of pregnancies per # of rats in cohabitation).

No treatment-related changes were observed in sperm parameters (# and % of motile sperm; # of nonmotile sperm; total sperm count from vas deferens; or cauda epididymal sperm count and density).

Caesarian-sectioning and litter observations were based on 21, 23, 23, and 21 pregnant F rats in the 0, 5, 15, and 30 mg/kg/day Fx-1006A dose groups, respectively. No treatment-related changes were observed in any of these parameters (litter averages for corpora lutea, implantations, viable and nonviable embryos, and % nonviable embryos per litter).

9.2 Embryonic Fetal Development

The effects of Fx-1006A on embryonic and fetal development were evaluated in rats and rabbits.

Rat

Pregnant female rats (Study (b) (4) SYI00039) were treated once daily with 15, 30, or 45 mg/kg/day (based on free acid) on GD 7-17 and sacrificed on GD21. Doses were selected based on dose-range Study SYI00037.

On GD13, 4/25 HDF rats were sacrificed due to adverse clinical signs and/or reduced body weights; these early deaths are believed treatment-related. Surviving rats showed reductions in mean body weight gain (GD7- 18 in LDF (-4.5%), MDF (-3.6%), and HDF (-16.3%) compared to Con F; and slight reductions in mean body weight on GD21 in LDF (-1.8%), MDF (-1.7%), and HDF (-4.2%) compared to Con F.

Absolute food consumption was reduced in HDF (-11.7%) during GD7-18 compared to Con F. Slight transient reductions were observed in LDF and MDF on GD7-10.

Treatment-related changes in fetal parameters were limited to reductions in live fetal body weights per litter in MD (-5.1%) and HD (-8.9%) compared to Control. There were

no treatment-related changes in gross external, soft tissue, or skeletal malformations or variations.

The NOAEL for embryo-fetal toxicity was determined at 15 mg/kg/day.

The results of toxicokinetic analysis conducted during the study are presented below.

Table 6 Toxicokinetics from Rat EFD study

Summary of Fx-1006 Plasma Toxicokinetic Parameters							
Group	Dosage (mg/kg)[a]	C_{max} (µg/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (µg·h/mL)	AUC (µg·h/mL)	$t_{1/2}$ (h)
Day 7							
II	15	44.8	0.5	24	757	1060	13.3
III	30	83.4	4	24	1270	NE	NE
IV	45	98.6	4	24	1990	NE	NE
Day 17							
II	15	72.8	2	24	1090	1470	12.6
III	30	92.7	4	24	1610	NE	NE
IV	45	133	4	24	2160	NE	NE

NE: Not estimated, due to insufficient characterization of the terminal phase
a: Daily dosage from Day 7 through Day 17 of presumed gestation

Rabbit

The study (b) (4) Study SYI00038) was conducted in two parts. The first (Part A) was for dose selection where non-mated rabbits were treated with 10, 30, or 90 mg/kg/day Fx-1006A for five days. Clinical signs, body weight and food consumption were evaluated.

Body weight gain was reduced 82.5% in LDF (+0.84%) compared to Con F (+4.8%), and body weight loss was observed in MDF (-10.1%) and HDF (-16.5%) from Day 1 to Day 6. Food consumption was reduced in MDF (-63.5%) and HDF (-92.5%).

Based on these findings, doses for Part B were set to 5, 10, or 20 mg/kg/day Fx-1006A using time-mated rabbits. Treatment-related clinical signs observed included soft/liquid/scant feces and ungroomed coat in LDF, MDF, and HDF; black feces in MDF and HDF; and no feces in HDF. Body weight losses were observed in MDF (-8.6%) and HDF (-17.9%), from GD7 to GD20, after correction for gravid uterine weight. Mean food consumption was reduced in LDF (-4.7%), MDF (- 44.5%), and HDF (-85.0%) compared to Con F.

Based on these results, doses of 0.5, 2, and 8 mg/kg/day Fx-1006A were chosen for the definitive embryofetal toxicity study in rabbit (b) (4) SYI00040). Pregnant female rabbits were treated once daily on GD 7-19 and sacrificed on GD29.

Maternal

Treatment-related reductions in body weight gain were observed in MDF (+0.3% vs. +1.2% in Con, GD13-16) and HDF (+0.9% vs. +2.8% in Con, GD7-10; +0.0% vs. +1.2% in Con, GD13-16; +1.8% vs. +6.8% in Con, GD7-20) during the dosing period, whereas increases in body weight gain were observed in MDF (+2.9% vs. +1.4% in Con, GD20-24) and HDF (+4.2% vs. +1.4% in Con, GD20-24; +8.4% vs. +4.6%, GD20-29) during the postdose period.

Food consumption was reduced in HDF (-23%) compared to Con during the dosing period.

Pregnancy

Statistically significant changes were observed, in pregnancy parameters at the HDF, in the number of late resorptions, live fetal body weights per litter, and percentage of resorbed conceptuses per litter. Also, the percentage of does with any resorptions was increased at all doses compared to Con, though not statistically significantly.

Fetal

In the offspring, statistically significant increases were observed in the number of fetuses with any alteration observed in MDF and HDF, and in the number of litters with fetuses with any alteration in HDF.

Specific alterations that were statistically significantly different from Con included increased depressed eye bulge, small eye socket and small eye in HDF only (in the same 3 fetuses); increased supernumerary thoracic ribs in MDF and HDF; decreased average number of ossified fore- and hind-limb phalanges in HDF; and irregular ossification of nasals in all treated dose groups. (b) (4)

The NOAEL for maternal toxicity was the MD of 2 mg/kg/day, due to observations of abortions, reduced body weight gain, and reduced food consumption in HDF.

The NOAEL for developmental toxicity was not determined in this study due to the adverse findings at the LD of 0.5 mg/kg/day, as well as in MDF and HDF, of increased incidence of irregular ossification of nasal bones.

The results of toxicokinetic analysis conducted during the study are presented below.

Table 7 Toxicokinetics from Rabbit EFD study

Summary of Mean[a] Fx-1006 Plasma Toxicokinetic Parameters							
Group	Dosage (mg/kg)[b]	C_{max} (µg/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (µg·h/mL)	AUC (µg·h/mL)	$t_{1/2}$ (h)
Day 7							
II	0.5	3.61	4	24	74.9	NE	NE
III	2	10.3	4	24	206	NE	NE
IV	8	41.8	8	24	795	803[c]	16.1[c]
Day 19							
II	0.5	8.14	4	24	157	429[c]	39.1[c]
III	2	18.5	4	24	357	NE	NE
IV	8	82.9	2	24	1540	4300[d]	28.7[d]

NE: Not estimated, due to insufficient characterization of the terminal phase
a: Median for t_{max} and t_{last}; n=3.
b: Daily dosage from Day 7 through Day 19 of gestation
c: n=1
d: n=2

9.3 Prenatal and Postnatal Development

Prenatal and postnatal development was evaluated in rats (Study # (b) (4) SYI00068). Pregnant female rats were treated once daily with 5, 15 or 30 mg/kg/day on GD7 through PND20, GD24 (rats that did not deliver a litter), or PND27 (if weaned on D28).

Maternal
No treatment-related maternal mortality was observed.

All HDF were terminated on PND1-4 because no surviving pups were found in 20/25 litters. 4/25 MDF were sacrificed on PND2 or PND3 as none of their pups survived either.

Body weight gain (BWG) was reduced compared to Con from GD7-20 (-7% LDF; -11% MDF; -16% HDF). Body weight (BW) was slightly reduced compared to controls on GD20 (-2% LDF; -4% MDF; -6% HDF). No differences in BWG or BW were observed during the lactation period.

Food consumption was reduced compared to Con in HDF GD7-10 (-8%) and GD7-20 (-7%), and in MDF PND1-14 (-15%).

F1 generation findings:

Pup weight per litter was reduced on PND1 in HD (-19%) and MD (-12.5%) compared to Con. Postweaning body weight gain was reduced compared to controls in MDM (-10% to -14%, PND22-50) and MDF (-7%, PND36-57).

The mean age of vaginal patency was not affected. Mean age of preputial separation was increased from PND45.7 in Con M to PND48.6 in MDM. Mean body weights on the day of preputial separation were comparable among all dose groups.

Neurological assessment: No treatment-related differences were observed in learning, short-term retention, long-term retention, or response inhibition as measured in the passive avoidance test.

Performance in the Morris water maze was impaired. The percentage of rats that failed to learn during session 1 was increased in MDM (20.0% vs. 8.7% in Con M) and MDF (35.0% vs. 0.0% in Con F), and the mean number of trials to criterion was slightly increased in MDF (10.8 vs. 8.6 in Con F) during session 1.

F2 generation:
Fetal body weights in female fetuses were reduced 4.6% in MD (15 mg/kg) compared to controls. This was not considered significant by either reviewer. No other adverse findings were observed.

The NOAEL for maternal and developmental toxicity was the LD of 5 mg/kg/day.

10 Special Toxicology Studies

Immunotoxicity

Study title: A 28-Day Oral Gavage Study to Assess the Effect of PF-06291826 on the T-cell Dependent Antibody Response (TDAR) in CByB6F1 Mice with a 4-Week Recovery

Study no.:	8000475
Study report location:	NDA 211996
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/19/2013
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	GR06390, 99.9% purity

Key Study Findings

There were no test item-related decreases in the anti-KLH IgM and IgG responses in males and females. Trends toward decreases in anti-KLH IgM antibody concentrations were observed in males and females on Day 22 when compared to the control (Group 1), but there were no statistically significant differences in dose group means.

Methods

Doses:	10, 30, 120 mg/kg
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	Each actual volume administered was based on the most recent body weight measurement of each animal.
Formulation/Vehicle:	7.5% (w/v) Vitamin E TPGS, NF Grade in Ultra Pure Water
Species/Strain:	Wildtype CByB6F1-Tg(HRAS)2Jic (CByB6F1) mice
Number/Sex/Group:	see Table below
Age:	6 weeks
Weight:	18.4 g to 29.8 g for males and 16.5 g to 23.4 g for females
Satellite groups:	For TK, see Table below

Experimental Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	No. of Animals			
				Main Study		Recovery Study	
				Males	Females	Males	Females
1	Vit E TPGS[a, c]	0	10	12	12	12	12
2	PF-06291826[c]	10	10	12	12	-	-
3	PF-06291826[c]	30	10	12	12	-	-
4	PF-06291826[c]	120	10	12	12	12	12
5	Cyclophosphamide[b, c]	100	20	12	12	12	12

[a] Animals received the reference Item: 7.5% (w/v) Vitamin E TPGS, NF Grade in Ultra Pure Water.
[b] Cyclophosphamide was administered by intraperitoneal injection on Days 11 to 14 for main study animals and on Days 39 to 42 for recovery animals.
[c] The keyhole limpet hemocyanin (KLH) solution was administered on Day 15 for main study animals and on Day 43 for recovery study animals, by intraperitoneal injection.

For toxicokinetic evaluation:

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	No. of Animals	
				Toxicokinetic Study	
				Males	Females
1	Vit E TPGS[a]	0	10	5	5
2	PF-06291826	10	10	20	20
3	PF-06291826	30	10	20	20
4	PF-06291826	120	10	20	20

[a] Animals received the reference Item: 7.5% (w/v) Vitamin E TPGS, NF Grade in Ultra Pure Water.

The high dose was selected because decreases in lymphocytes and slight decreases in thymus and spleen weights were observed at a dose of 120 mg/kg/day of PF-06291826 in a previous 28-day oral toxicity study in CByB6F1 mice (Study 8000474). The low dose is not anticipated to produce any observable toxicity. The mid dose was selected as an intermediate dose to characterize the dose-toxicity relationship of PF-06291826.

Cyclophosphamide was administered to the appropriate mice from Group 5 once daily on Days 11 to 14 for main study animals and on Days 39 to 42 for recovery animals by intraperitoneal injection into the animal's right side. The dosing volume was 20 mL/kg. Each actual volume administered was based on the most recent body weight measurement of each animal.

KLH solution (0.5 mL of a 0.2 mg/mL solution) was administered on Day 15 for main study animals and on Day 43 for recovery study animals, by intraperitoneal injection into the animal's right side.

Mortality/Moribundity
Throughout the study, animals were observed for general health/mortality and moribundity twice daily, once in the morning and once in the afternoon. Animals were not removed from their cage during observation, unless necessary for identification or confirmation of possible findings.

Clinical Observations
Cage side observations were performed once daily, beginning during Week -1 and lasting throughout the study (except on days of scheduled detailed examinations).

Body Weights
Animals were weighed individually once weekly, starting during Week -1. Terminal body weights were not collected from animals found dead or euthanized moribund.

Food Consumption
Food consumption was quantitatively measured weekly starting on Day -7 and continuing weekly throughout the dosing and recovery periods.

Blood was collected from the abdominal aorta after isoflurane anesthesia. Samples were collected according to the following Table.

Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology
1-5[a]	Day 29	X
1, 4 and 5[b]	Day 57	X

X = Sample collected.
[a] Samples were collected from those animals scheduled for euthanasia on Day 29 (main study animals).
[b] Recovery study animals.

Hematology
Blood samples were analyzed for the parameters specified below.

Hematology Parameters

Red blood cell count	Platelet count
Hemoglobin concentration	White blood cell count
Hematocrit	Neutrophil count (absolute)
Mean corpuscular volume	Lymphocyte count (absolute)
Red Blood Cell Distribution Width	Monocyte count (absolute)
Mean corpuscular hemoglobin concentration	Eosinophil count (absolute)
Mean corpuscular hemoglobin	Basophil count (absolute)
Reticulocyte count (absolute)	Large unstained cells

Main study and recovery animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Brain, adrenal gland, spleen and thymus were weighed and preserved along with a bone marrow smear. Histopathological evaluation was performed by a board-certified veterinary pathologist.

Results

The detection of mouse anti-KLH IgM antibodies was performed as per AP.8000475.IgM.01 for the CByB6F1 hybrid mouse serum. All analyses were performed using semi-quantitative ELISA methods previously validated at (b) (4) under Study Nos. 3000498, 27230, 27231, and 30170.

In all groups, as expected, there were no anti-KLH IgM antibodies detected on Day -1 prior to KLH administration (Day 15) except for the following animals: Nos. 1013, 1021, 1023, 1516, 2007, 4020, 4509, 4512, 5012, and 5508 which had low levels of anti-KLH IgM slightly above the LLOQ value.

Table 8 Mean Anti-KLH IgM Concentration

	Mean Anti-KLH IgM Concentration					
	Day -1* (µg/mL)		Day 22 (µg/mL)		Day 50 (µg/mL)	
	Males (Range)	Females (Range)	Males (Range)	Females (Range)	Males (Range)	Females (Range)
Group 1 (Vehicle)	0.251	0.214	1.848	1.845	1.892	2.919
	(0.196 to 0.75)	(0.196 to 0.61)	(1.12 to 2.44)	(1.26 to 2.87)	(0.98 to 2.99)	(1.54 to 6.07)
Group 2 (PF-06291826 10 mg/kg/day)	0.224	0.196	1.653	1.786	-	-
	(0.196 to 0.50)	(N/Ap)	(1.14 to 3.02)	(0.89 to 2.52)		
Group 3 (PF-06291826 30 mg/kg/day)	0.196	0.196	1.437	1.533	-	-
	(N/Ap)	(N/Ap)	(1.00 to 2.62)	(0.71 to 2.17)		
Group 4 (PF-06291826 120 mg/kg/day)	0.210	0.217	1.355	1.371	1.560	1.788
	(0.196 to 0.53)	(0.196 to 0.48)	(0.61 to 2.04)	(0.90 to 1.69)	(0.71 to 2.63)	(1.29 to 2.49)
Group 5 (Cyclophosphamide)	0.196	0.266	0.196**	0.229**	0.196**	0.196**
	(N/Ap)	(0.196 to 1.88)	(N/Ap)	(0.196 to 0.56)	(N/Ap)	(N/Ap)

* Main and recovery animals are included, where applicable.
** Statistically significant.

There were no test item-related decreases in the anti-KLH IgM and IgG responses in males and females. Trends toward decreases in anti-KLH IgM antibody concentrations were observed in males and females on Day 22 when compared to the control (Group 1), but there were no statistically significant differences in dose group means.

11 Integrated Summary and Safety Evaluation

Tafamidis has been developed as a specific stabilizer of transthyretin (TTR) tetramer for the treatment of ATTR-PN (TTR-FAP) and ATTR-CM(TTR-CM). The recommended human dose of tafamidis meglumine is 80 mg (4 x 20 mg) p.o. daily. The 80 mg tafamidis meglumine is bioequivalent to one capsule of tafamidis free acid 61 mg.

Pharmacology

In a series of pharmacology studies, the sponsor demonstrated that tafamidis bound the intended target, slowed the rate of dissociation of TTR tetramer into monomers, and inhibited TTR fibril formation. Binding of tafamidis to TTR was demonstrated using three techniques: 1) co-elution with TTR from a plasma sample run through a column with resin-bound anti-TTR antibodies; 2) isothermal calorimetry; and 3) X-ray crystallography, which showed tafamidis binding to the two symmetrical T_4 binding sites at the interface of the two TTR dimers.

Tafamidis free acid binds to TTR with negative cooperativity, at the thyroxine binding sites, with dissociation constants for the first binding site (K_{d1}) of 2 to 3 nM, and dissociation constants for the second binding site (K_{d2}) of 154 to 278 nM. The binding stoichiometry of tafamidis free acid to TTR was evaluated to be 0.81 ± 0.02 in human plasma, indicating specificity of the binding of tafamidis free acid to TTR over all other plasma proteins.

Under physiological (nondenaturing) conditions, tafamidis free acid decreased the exchange rate between WT and flag-tagged TTR tetramers. Under acidic conditions, tafamidis free acid decreased the amount of fibril formation of WT, substitution of methionine for valine at position 30 (Val30Met, also referred to as V30M), and substitution of isoleucine for valine at position 122 (Val122Ile, also referred to as V122I) amyloidogenic mutants of TTR. Under urea denaturing conditions and in human plasma, tafamidis free acid and tafamidis meglumine decreased TTR tetramer dissociation rate of WT, Val30Met, and Val122Ile amyloidogenic mutants of TTR and 26 other variants of TTR.

Although many TTR mutations are associated with both polyneuropathy and cardiac involvement, including Thr60Ala (Staunton et al, 1991) and Glu89Gly (Rapezzi et al, 2006), certain TTR mutations are associated with an almost exclusive cardiac phenotype, including Val122Ile (Jacobson et al, 1997) and Leu111Met (Svendsen et al, 1998).

No relevant animal disease models were available to evaluate the effect of TTR stabilizer during development of tafamidis.

In secondary pharmacodynamic studies, tafamidis free acid was tested with more than 50 receptors (including the thyroxine receptor), enzymes, and ion channels. Tafamidis free acid was devoid of significant binding affinity to all except the δ2-opioid receptor, where it showed a low binding affinity (50% inhibitory concentration [IC_{50}] = 8.3 µM; Ki = 4.9 µM). No significant affinity was demonstrated for the kappa and mu opioid receptors.

An *in vitro* functional test on hamster vas deferens confirmed the modest (6%, 37%, and 88% of control response at 3, 10, and 30 µM, respectively) agonistic activity of tafamidis free acid on the δ2-opioid receptor. Due to high human plasma protein binding (>99%) of tafamidis, effects mediated through an interaction with the δ2-opioid receptor would not be expected at the human unbound steady-state C_{max} of 0.3 µM at a clinical dose of 80 mg tafamidis meglumine and 0.277 µM at a clinical dose of 61 mg tafamidis free acid.

No inhibition of cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) was observed in *in vitro* assays conducted with recombinant human enzymes or human blood.

Safety Pharmacology
In a standard battery of safety pharmacology studies, tafamidis showed no biologically important changes in cardiovascular or respiratory parameters in telemetered female Beagle dogs given up to 300 mg/kg via oral gavage. Emesis and salivation and clinical signs indicative of effects on the central nervous system (CNS), muscle leg twitching, were observed in the cardiovascular/pulmonary safety pharmacology study in dogs but were deemed secondary to toxicity at high exposures.

No relevant effects on CNS parameters evaluated in male and female Sprague-Dawley rats given up to 100 mg/kg via oral gavage were apparent.

There were negligible effects on hERG currents through potassium channels expressed in human embryonic kidney cells measured under voltage clamp at concentrations up to 30 µM.

Toxicology

General toxicology
Rats and dogs were selected for use in general toxicology studies and rats and rabbits were selected for reproductive and developmental toxicity studies based on extensive background information available in these species and because of the high homology between TTR in humans, rats, rabbits, and dogs. Tafamidis meglumine was formulated in 0.5% methylcellulose or 7.5% Vitamin E d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS).

Rat
In a pivotal 28-day repeat-dose toxicity study in rats administered tafamidis meglumine at doses of 0, 10, 30, 100, or 300 mg/kg/day followed by a 14-day recovery phase. Test article-related deaths, adverse clinical signs, and lower body weight and food consumption were observed at ≥100 mg/kg/day. All surviving animals at 300 mg/kg/day were euthanized on Day 10.

Liver weights were higher at ≥10 mg/kg/day but there were no corresponding microscopic changes. Test article-related macroscopic findings were limited to impaction of the stomach with white, slightly firm, spongy material consistent with test

article in 1 female at 100 mg/kg/day and most rats at 300 mg/kg/day. This finding was adverse as it may have contributed to morbidity and mortality. Thymic lymphoid depletion was observed at ≥100 mg/kg/day, and superficial necrosis in glandular stomach, renal tubular proteinosis and red pigment in renal tubules, lymphoid depletion in spleen and mandibular lymph node, and bone marrow depletion were observed at 300 mg/kg/day. After the 14-day recovery phase, body weight remained lower in males, heart weight was lower in males, and ovary weight was lower and uterus weight was higher at 100 mg/kg/day; however, no test article-related macroscopic or microscopic findings were observed. Due to mortality and adverse clinical signs and gastric impaction at ≥100 mg/kg/day, the NOAEL in this 28-day study was 30 mg/kg/day (Day 28 AUC = 4190 µg·hr/mL M; 4220 µg·hr/mL F).

In the pivotal 13-/26-week repeat-dose toxicity study with a 4-week recovery phase in rats administered tafamidis meglumine at 0, 3, 10, or 30 mg/kg/day, effects after 13 weeks were limited to minor, reversible changes in lung, liver, thyroid, epididymides, testes, and uterus weights without microscopic correlates. After 26 weeks, minor non-adverse changes in bilirubin, urea nitrogen, creatinine, glucose, cholesterol, and chloride were observed. Liver weights were higher at ≥10 mg/kg/day and kidney weights were lower in females at all doses. There were no test article-related macroscopic or microscopic changes. None of the test article-related effects were considered adverse, and the NOAEL was 30 mg/kg/day (AUC Day 178 =2260 µg·hr/mL M, 3120 F, with exposure ratios of 13.3-fold M and 18.4-fold F).

Dog

In a pivotal 28-day repeat-dose study with a 14-day recovery phase in dogs administered tafamidis meglumine at 0, 10, 100, or 300/200 mg/kg/day, emesis and death at 300 mg/kg/day necessitated lowering the highest dose to 200 mg/kg/day on Day 7 (females) or Day 9 (males) following dosing suspension for 1 day. Three animals, 1 male and 1 moribund female at 300 mg/kg/day (Day 7) and 1 male at 100 mg/kg/day (Day 20), were euthanized in association with aspiration of test article and/or vomitus. One male at 100 mg/kg/day was found dead on Day 11.

At necropsy, findings among these animals included dark red lungs, white foamy accumulation (presumed to be the test article) in the trachea, stomach, and/or intestines, red spleen, pale discolored liver, increased ALT, ALP, urea nitrogen, gamma glutamyltransferase, and/or total bilirubin, decreased platelets, and/or right atrial thrombus. An additional male at 300/200 mg/kg/day was euthanized moribund on Day 21 in association with an approximate 25% body weight loss. This animal had vacuolation and necrosis of the myocardium and fibrinoid necrosis of myocardial blood vessels; however, the relationship of these findings to test article administration was uncertain as similar findings were not observed in other dogs in this study or in studies of longer duration (up to 39 weeks).

Fecal changes, vomiting, and salivation were observed at ≥100 mg/kg/day, and ataxia, head bobbing, thin, cool to touch, and twitching were observed at 300/200 mg/kg/day. Food consumption was lower at 300/200 mg/kg/day. ALP and ALT were higher at ≥100

mg/kg/day, and urea nitrogen, gamma glutamyltransferase, and total bilirubin were all higher in males at 300 mg/kg/day. Liver and kidney weights were higher and spleen weights were lower in males at 300/200 mg/kg/day. Other than pulmonary congestion, edema, and inflammation attributed to emesis and apparent aspiration of vomitus, there were no clear test article-related microscopic findings at termination. Based on mortality at ≥100 mg/kg/day, the NOAEL in this 28-day study was 10 mg/kg/day (Day 28 AUC_{24} of 327 µg•h/mL M and 435 µg•h/mL F).

Dogs were administered daily oral gavage doses of tafamidis meglumine at 0, 5, 15, or 45 mg/kg/day for 13 or 39 weeks in a pivotal subchronic/chronic toxicity study (Study SYI00013) that included a 4-week recovery phase after 13 weeks of dosing.

Early in the study, deaths attributed to the 7.5% Vitamin E TPGS vehicle occurred in all dose groups (2 control males, 1 male and 1 female at 5 mg/kg/day, 1 male at 15 mg/kg/day, and 2 males at 45 mg/kg/day were found dead on Days 5 to 36), and animals were replaced. Clinical observations in these animals included pale mucous membranes, lethargy, crackled sounds from the lungs, and/or vomiting immediately or shortly after dosing. At necropsy, findings among these animals were suggestive of aspiration/introduction of foreign material into the lungs via the airways and included severe, diffuse alveolar damage (acute respiratory distress syndrome) as characterized by diffuse alveolar edema, hemorrhage, inflammation, and fibrin deposition, acute inflammation involving bronchioles and surrounding alveoli consistent with chemical pneumonitis, and/or segmental necrosis suggestive of aspiration of foreign material and direct injury to the tracheal mucosa. Subsequently, the vehicle was changed to 0.5% methylcellulose from Day 48 to the end of the study. As the early deaths occurred across all dose groups and there was no evidence of technical errors with dosing, the early deaths were attributed to the 7.5% Vitamin E TPGS vehicle and not to the test article. The animals with early death associated with the 7.5% Vitamin E TPGS vehicle were not included in data analysis.

Test article-related effects were limited to a higher number of occurrences of soft/mucoid feces and emesis, and a higher number of occurrences and affected areas of skin erythema at 45 mg/kg/day. There were no test article-related macroscopic or microscopic changes. None of the effects were adverse, and therefore the NOAEL was 45 mg/kg/day, with associated Day 271 AUC_{24} of 1440 µg•h/mL in males and 1810 µg•h/mL in females. Exposure ratios, based on AUC for the study were 8.5-fold and 10.6-fold for M and F, respectively.

Genetic toxicology
Bacterial reverse mutation (Study 960707), in vitro clastogenicity (Study 960708) assay and in vivo rat bone marrow micronucleus assay (Study 960709) were conducted and the results indicated that tafamidis meglumine was not genotoxic in these assays. The adequacy of the chromosomal aberration assay, called into question by Dr. Hawver in NDA 202-737, was considered resolved with further discussion in this cycle and is superseded by the later, negative 2-year carcinogenicity study.

Carcinogenicity
26-week Transgenic mouse
In the carcinogenicity study in Tg.rasH2 mice, animals were administered tafamidis meglumine once daily via oral gavage at doses of 0 (Sterile Water for Injection, USP), 0 (7.5% Vitamin E TPGS), 10, 30, or 90 mg/kg/day for 26 weeks (Study AB44FV.7G8R.BTL). Animals in the positive control group were administered a single intraperitoneal (IP) injection of urethane (in saline) at 1000 mg/kg on Days 1, 3, and 5.

Tafamidis meglumine was not carcinogenic in Tg.rasH2 mice at doses up to 90 mg/kg/day.

2-year rat carcinogenicity study
In the 2-year carcinogenicity study in Sprague Dawley rats, tafamidis meglumine was administered once daily via oral gavage at doses of 0 (ultrapure water), 0 (7.5% Vitamin E TPGS), 3, 10, or 30 mg/kg/day for up to 104 weeks (Study 805917). The dose selection was approved with the Executive CAC previously in the nonclinical development for ATTR-PN. The high dose of 30 mg/kg/day selected was expected to produce exposure approximately 54x the predicted human steady state AUC of 50.34 µg•h/mL at a clinical dose of 20 mg tafamidis meglumine.

Tafamidis meglumine-related effects were limited to minimally lower body weights in males at 30 mg/kg/day and to nonneoplastic microscopic changes in the liver of males and/or females at ≥3 mg/kg/day. There was no evidence of any carcinogenic effect, and there were no statistically significant differences in the incidence of any neoplasms.

Plasma tafamidis free acid concentrations at Week 26 increased with increasing dose and were similar in males and females (see study review). The exposure ratio (rat/human) based on AUC at the high dose was 19.2x and 16.3x for females and males, respectively.

During the current NDA review of the study results, the Executive CAC agreed that the study design was adequate, and the study was negative.

Reproductive and Developmental Toxicity
Fertility and Early Embryonic Development
To assess potential effects on fertility and early embryonic development, rats were administered daily oral gavage doses of vehicle (7.5% Vitamin E TPGS) or tafamidis meglumine at 5, 15, or 30 mg/kg/day (Study SYI00060).

Males were dosed for 28 days prior to and throughout the 21-day mating period to the day before scheduled euthanasia, and females were dosed for 15 days before mating, throughout mating, and to GD 7. No effects were observed in males. Body weight loss and decreased food consumption was observed in females during the first week of dosing.

There were no treatment-related changes in estrous cycling or mating and fertility parameters (# of days in cohabitation, # of rats that mated, Fertility Index, # of rats with confirmed mating dates during the first week of cohabitation, or # of pregnancies per # of rats in cohabitation), sperm parameters (# and % of motile sperm; # of nonmotile sperm; total sperm count from vas deferens; or cauda epididymal sperm count and density) and any parameters (litter averages for corpora lutea, implantations, viable and nonviable embryos, and % nonviable embryos per litter) observed at Caesarian-section of the pregnant F rats.

Embryo-Fetal Development

Rat study

In a definitive embryo-fetal development (EFD) study, pregnant female rats were administered vehicle (7.5% Vitamin E TPGS) or tafamidis meglumine at 15, 30, or 45 mg/kg/day once daily by gavage from GD 7 through GD 17 (Study SYI00039). Doses were selected based on results of a dose range-finding study (Study SYI00037).

Maternal toxicity including death was observed at 45 mg/kg/day, and fetal body weights were lower at ≥30 mg/kg/day. There were no test article-related external, visceral, or skeletal variations or malformations at any dose. Based on these findings, the NOAEL for maternal toxicity in rats was 30 mg/kg/day, with an associated GD 17 AUC_{24} of 1610 µg•h/mL. The NOAEL for developmental toxicity was 15 mg/kg/day, with an associated GD 17 AUC_{24} of 1090 µg•h/mL (exposure 6.6-fold relative to human AUC). Tafamidis meglumine was not teratogenic in rats.

Rabbit study

In a definitive EFD study, time-mated female New Zealand White rabbits were administered vehicle (7.5% Vitamin E TPGS) or tafamidis meglumine at 0.5, 2, or 8 mg/kg/day once daily by oral gavage from GD 7 to GD 19 (Study SYI00040). Doses were selected based on results of a dose range-finding study (Study SYI00038).

Lower maternal body weight gain was observed at ≥2 mg/kg/day, and mortality, body weight loss, and lower food consumption were observed at 8 mg/kg/day. There were no effects on the number of corpora lutea, implantations, litter size, early resorptions, live fetuses, or percent live male fetuses. Post-implantation loss was higher and fetal body weights were lower at 8 mg/kg/day.

Three fetuses from 2 litters at 8 mg/kg/day had depressed eye bulges, small eyes, and small eye sockets. The increased incidences of supernumerary thoracic ribs and thoracic vertebrae and a decrease in the number of lumbar vertebrae were observed at ≥2 mg/kg/day. The average number of ossified fore- and hindlimb phalanges was lower at 8 mg/kg/day. Increased incidence of alterations in the nasal bones was observed at ≥0.5 mg/kg/day. Based on these findings, the NOAEL for developmental toxicity of tafamidis meglumine in rabbits was <0.5 mg/kg/day, the low dose. The NOAEL for maternal toxicity was the MD of 2 mg/kg/day, due to observations of abortions, reduced body weight gain, and reduced food consumption in HDF.

Prenatal and Postnatal Development, Including Maternal Function
In the prenatal/postnatal development study, rats were administered vehicle (7.5% Vitamin E TPGS) or tafamidis meglumine at 5, 15, or 30 mg/kg/day once daily by oral gavage from GD 7 to LD 20 (Study SYI00068). Due to high mortality (no surviving pups in 20 of 25 litters by LD4), dosing at 30 mg/kg/day was terminated early in the lactation period and surviving rats and pups were euthanized on LD 1 to LD 4. At 15 mg/kg/day, 4 of 25 F0 females were euthanized on LD 2 or LD 3 as they had no surviving pups.

Lower body weight gain and food consumption were observed at 30 mg/kg/day; thus, the NOAEL for maternal toxicity was 15 mg/kg/day. Preweaning, higher numbers of litters at 15 and 30 mg/kg/day had pups with no milk bands, were not nursing, or had purple or black discoloration of the whole body, tip of tail, lower midline, head, neck, and/or chest. There was no evidence of a lack of maternal care or nesting during lactation.

Postweaning, 1 F1 male at 15 mg/kg/day was found dead on PND 38 and another became moribund on PND 111 and was euthanized. All other F1 rats survived to scheduled termination. At 15 mg/kg/day, a higher number of F1 males and females were observed with domed head (transient, predominately between postweaning Weeks 4 to 9), microphthalmia (between postweaning Week 7 and termination), and/or chromodacryorrhea. The cause of the domed appearance of the head was unclear as there was no evidence of ventricular dilation or of a skeletal cause; there were no test article-related changes in absolute or relative brain weights in F1 male or female rats. Mean body weight and/or body weight gain and food consumption were lower at several intervals during the postweaning period at 15 mg/kg/day.

Preputial separation in F1 males at 15 mg/kg/day was delayed compared to control. Sexual maturation of F1 females was unaffected. At 15 mg/kg/day, a higher number of F1 animals failed to learn during session 1 of water maze testing, and the number of trials to criterion during session 1 was slightly higher in females. Performance in the second (retention) session was unaffected by tafamidis meglumine.

Neurohistopathologic examination of F1 offspring did not reveal any test article-related alterations. Based on these findings, the NOAEL for F1 viability and growth was 5 mg/kg/day. No adverse effects on mating and fertility parameters were noted in F1 animals. F2 female fetal body weights were decreased (-4.6% compared to the control) at 15 mg/kg/day which was not considered significant by either reviewer.

Exposure ratios relative to the clinical dose of 80 mg for the major toxicology studies are provided in the following Table.

Table 9 Exposure Ratios for Major Toxicology Studies

Study	Sex	NOAEL (mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (µg·hr/mL) @ NOAEL	Safety margin relative to Human exposure[1, 2]
26-week Rat Toxicology	M	30	1320 (Day 1) 2260 (Day 178)	7.9 13.6
26-week Rat Toxicology	F	30	1670 (Day 1) 3120 (Day 178)	10.1 18.8
39-week Dog Toxicology	M	45	1350 (Day 1) 1440 (Day 271)	8.1 8.7
39-week Dog Toxicology	F	45	1450 (Day 1) 1810 (Day 271)	8.7 10.9
Carcinogenicity				
Carcinogenicity - 26-week Mouse Tg.rasH2	M	90	1131 (Day 1) 1635 (Day 93)	6.8 9.8
Carcinogenicity - 26-week Mouse Tg.rasH2	F	90	1080 (Day 1) 1564 (Day 93)	6.5 9.4
Carcinogenicity - 2-year Rat	M	30	2770 (Week 26)	16.7
Carcinogenicity - 2-year Rat	F	30	3270 (Week 26)	19.7
Reproductive Toxicology				
Rat - Fertility and early development	F	30	1270* **HED 4.8 mg/kg** (mg/m^2 basis)	7.7 **3.7**
Rat - Embryo-fetal development	F	15	757 (GD 7) **1090 (GD 17)**	4.6 **6.6**
Rabbit - Embryo-fetal development	F	< 0.5	< 75 (GD 7) **< 157 (GD 19)**	0.5 **0.9**
Rat - Pre- and Post-natal development	F	5	363# **HED 0.8 mg/kg** (mg/m^2 basis)	2.2 **0.6**

1. Safety margins are based on AUC of 166 µg·hr/mL on Day 7, following multiple oral doses of 80 (4 x 20 mg) tafamidis meglumine. (Study B3461056)
2. Safety margins are estimated based on body surface area (mg/m^2) and human dose of 1.33 mg/kg (80 mg tafamidis meglumine for a 60-kg person) when animal AUC data is not available.

* - inferred from 30 mg/kg dose in rat EFD study

- estimated as 1/3 AUC at 15 mg/kg/day from rat EFD study

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