CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206038Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date:	June 17, 2015
From:	Timothy J. McGovern, PhD, ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA:	206038
Agency receipt date:	November 5, 2014
Drug:	ORKAMBI (lumacaftor/ivacaftor) Tablets
Sponsor:	Vertex Pharmaceuticals
Indication:	Treatment of cystic fibrosis (CF) in patients 12 years of age or older who are homozygous for the *F508del* mutation in the *cystic fibrosis transmembrane conductance regulator (CFTR)* gene
Reviewing Division:	Division of Pulmonary, Allergy, and Rheumatology Products

The primary pharmacology/toxicology reviewer and team leader concluded that the nonclinical data for ORKAMBI (lumacaftor/ivacaftor) Tablets support approval for the indication listed above.

ORKAMBI is a combination product containing the active pharmaceutical ingredients lumacaftor and ivacaftor in a fixed-dose combination tablet containing 200 mg lumacaftor (a new molecular entity) and 125 mg ivacaftor. The proposed daily dose is 800 mg lumacaftor and 500 mg ivacaftor achieved via two daily doses of two tablets each. Ivacaftor, a CFTR potentiator, was previously approved by FDA as a monoproduct (KALYDECO, NDA 2013188) at a recommended daily dose of 300 mg in tablet or granule formulations for treatment of patients as young as 2 years of age with CF defined by having one of ten mutations in the CFTR gene. While the nominal daily ivacaftor dose in ORKAMBI is higher than in KALYDECO, the ivacaftor exposure associated with ORKAMBI dosing is substantially lower due to drug interactions. Clinical exposure to ivacaftor is significantly reduced when administered in combination with lumacaftor since lumacaftor is a CYP 3A4 inducer and ivacaftor is a CYP 3A4 substrate.

The nonclinical program supporting clinical development of ORKAMBI included a complete program for ivacaftor (conducted in support of NDA 203188), a complete program for lumacaftor with the exception of a 2-year rat carcinogenicity study (to be completed as a Post-Marketing Requirement), and a program for the combination that included primary pharmacology and general toxicology studies up to 3 months in rats and 1 month in dogs. Ivacaftor was associated with hepatotoxicity in some studies in rats and mice and cardiac toxicity in dogs; the findings were considered to be clinically monitorable. Cataracts were observed in a study in juvenile rats; cataracts were also observed in pediatric patients and the finding is listed in the "Warnings and Precautions" section of the product label. Results of genetic toxicity, carcinogenicity, and developmental and reproductive toxicity studies are described in the approved labeling.

Lumacaftor was not associated with any target organs of toxicity in rats in a 6-month study. A 12-month study in dogs resulted in gastrointestinal effects and decreased red

blood cell parameters; these findings were considered clinically monitorable. A higher dose in 3-month study was associated with premature deaths and CNS effects; other target organs included the thymus, liver, and male reproductive organs. The no-observed-adverse-effect-level (NOAEL) doses were associated with > 2-fold exposure margins when compared to the anticipated clinical lumacaftor exposure. Lumacaftor was not associated with any findings of concern in genetic toxicity studies, a 6-month carcinogenicity study in Tg.RasH2 mice, and a battery of reproductive toxicity studies. The carcinogenicity study was evaluated by the Executive Carcinogenicity Assessment Committee; the Committee found that the study was acceptable and that there were no drug-related neoplasms.

The combination ivacaftor/lumacaftor program identified some novel findings including reversible necrosis, erosion, edema, and/or inflammation in the stomach and cataracts in rats and cardiac and male reproductive organ effects in dogs; the findings in dogs were observed with increased incidence and/or at lower doses compared to the results of studies with the monoproducts. The NOAEL doses provided at least a 1-fold exposure margin for lumacaftor and 30-fold exposure margin for ivacaftor.

The Established Pharmacologic Class (EPC) for ivacaftor is "CFTR potentiator". Extensive communications regarding the EPC for lumacaftor have occurred between the Applicant and the Division. The Applicant proposed (b) (4) Based on a review of the pharmacology studies and clinical data with lumacaftor, the proposed term was not considered justifiable. Rather, the Division concluded that the EPC be designated as "CFTR conformational stabilizer" based on what is known about its mechanism of action, to improve conformational stability of mutant F508del CFTR ion channel, resulting in increased cellular processing and trafficking of it to the cell surface. Of note, the Office of Prescription Drug promotion have also expressed concern regarding use of the term (b) (4) The EPC designation of "CFTR conformational stabilizer" seems appropriate based on the data provided.

Conclusion: I agree with the Division pharmacology/toxicology conclusion that this NDA can be approved from the pharmacology/toxicology perspective. I agree that the 2-year rat carcinogenicity study can be submitted as a Post-Marketing Requirement and note that the study is underway. I have discussed and am in agreement with labeling revisions proposed by the Division. The EPC for lumacaftor proposed by the Division seems appropriate. The potential for cataracts and hepatotoxicity are being addressed in the labeling.

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/s/

TIMOTHY J MCGOVERN
06/17/2015

NDA 206038 Comment to Sponsor regarding EPC

We have reviewed your communication dated June 11, 2015 regarding the determination of an Established Pharmacologic Class (EPC) for lumacaftor and have the following comments:

1. As previously communicated at the Late Cycle Meeting on April 29, 2015 and the teleconference on June 3, 2015, we are concerned with the use of the term (b) (4) The Division has determined that the proposed modifiers (b) (4) are insufficient to overcome these concerns; therefore, any EPC containing the term (b) (4) would be unacceptable.

2. We note that in consultation with the Office of Prescription Drug Promotion (OPDP), they have also expressed concern regarding the use of the term (b) (4) to describe lumacaftor. OPDP stated that (b) (4) to describe either lumacaftor or Orkambi could be considered misleading (b) (4) OPDP recommends that (b) (4) not be included in the proposed PI for Orkambi."

3. We continue to believe that the available data support the term "CFTR conformational stabilizer" for the following reasons:

a) The *F508del-CFTR* mutation leads to protein misfolding that affects the conformation, stability, and assembly of the domains of CFTR. This defective folding results in the processing and trafficking defect (e.g., retention in ER and degradation via ubiquitin-proteasome pathway) as well as impaired conformation, stability and function at the cell surface.[1]

b) Lumacaftor directly interacts with and stabilizes the MSD1 domain of F508del-CFTR, activity that is required for the "correction" effect. Vertex study report K124 and the associated publication[2] conclude that "*1) VX-809 biochemically stabilizes CFTR fragments that contain only MSD1. 2) VX-809 alters the conformation of MSD1 to protect it from proteolytic digestion with trypsin. 3) CFTR fragments fold to a conformation that is stabilized by VX-809 after biosynthesis of amino acid F374. F374 is located in the linker between MSD1 and NBD1 and helps stabilize MSD1 in conformation that is sensitive to VX-809…Data presented are consistent with the concept that* (b) (4) *such as VX-809 modulate the conformation of a specific region of CFTR to enhance global protein folding and assembly.*" These conclusions are supported by independent publications evaluating lumacaftor.[3]

c) Lumacaftor treatment increases the stability of cell surface F508del-CFTR to closer to wild-type levels, an effect which was attributed to "(b) (4) *of the defective protein conformation*" in Vertex study report D146. Additional independent studies support the conclusion that lumacaftor has a stabilizing effect on cell surface F508del-CFTR.[4]

The totality of the available in vitro data support the conclusion that lumacaftor acts directly on F508del-CFTR to partially reverse the folding defects, resulting in increased processing and trafficking to the cell surface as well as increased stability and channel function at the cell membrane. The assignment of "CFTR conformational stabilizer" as the EPC reflects the knowledge of the molecular mechanism of action of lumacaftor. In addition, this EPC term distinguishing lumacaftor from other classes of compounds which have also been referred to as "(b) (4) and result in increased processing and trafficking of F508el-CFTR to the cell surface, but act via completely distinct and in some cases, non-specific, mechanisms (e.g., osmolytes, PDE5 inhibition, transcriptional regulation, PARP inhibition, modulation of proteostasis). Finally, we note that literature references to the notion of (b) (4) in the context of *CFTR* gene therapy occurred well before publication of the initial temperature correction paper, and in the ensuing years the term has been used to refer to a wide variety of mechanisms by which the underlying CFTR defect could be targeted.

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[1] Boyle (2013) *Lancet Resp Med*; Chiaw (2011), *Essays Biochem*; Lukacs (2012) *Trends Mol Med*; Du (2005) *Nat Struct Mol Bio*; Okiyoneda (2013) *Nat Chem Bio*; Farinha (2013) *FEBS Journal*; Baroni (2014) *Cell Mol Life Sci*; Pasyk (2015) *Proteomics*.

[2] Ren (2013) *Molecular Biology of the Cell*.

(b) (4)

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/s/

ANDREW C GOODWIN
06/16/2015

TIMOTHY W ROBISON
06/16/2015
I concur

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:	206038
Supporting document/s:	Electronic Document Room (EDR) Supporting Documents (SD) #3, 13, 18, 20
Applicant's letter date:	November 5, 2014, March 3, 2015, May 4, 2015, June 1, 2015
CDER stamp date:	November 5, 2014, March 3, 2015, May 4, 2015, June 1, 2015
Product:	ORKAMBI (lumacaftor/ivacaftor)
Indication:	Treatment of cystic fibrosis (CF) in patients age 12 years and older who are homozygous for the *F508del* mutation in the *CFTR* gene.
Applicant:	Vertex Pharmaceuticals
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Leila Hann

Template Version: September 1, 2010

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described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 206038.

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1 Executive Summary

1.1 Introduction

Vertex Pharmaceuticals is seeking approval of ORKAMBI for the treatment of cystic fibrosis (CF) in patients age 12 years or older who are homozygous for the deletion of phenylalanine 508 (*F508del*) in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. A rolling submission under NDA 206038 was initiated on July 30, 2014 and the filing date of the complete application was November 5, 2014. The application was assigned priority review status with a target Prescription Drug User Fee Act (PDUFA) action date of July 5, 2015.

ORKAMBI is a combination product containing the active pharmaceutical ingredients (APIs), lumacaftor (VX-809) and ivacaftor (VX-770). The product consists of a fixed-dose combination tablet presentation containing 200 mg lumacaftor and 125 mg ivacaftor. The proposed daily dose of 800 mg lumacaftor and 500 mg ivacaftor is achieved via two daily doses of two tablets each.

Refer to the nonclinical review filed by Dr. Andrew Goodwin on May 5, 2015 for a thorough review of the pharmacology and toxicology studies supporting the lumacaftor-ivacaftor combination product. The conclusion of that review was that NDA 206038 was recommended for approval from the nonclinical perspective.

The present review addresses the following outstanding issues with regarding to the nonclinical review of NDA 206038 and associated product labeling for ORKAMBI:

- Determination of an Established Pharmacologic Class (EPC) for lumacaftor, a new molecular entity (NME)
- Labeling recommendations for Section 12.1 (Mechanism of Action)

1.3 Recommendations

1.3.1 Approvability

As documented in the review dated May 5, 2015, NDA 206038 is recommended for approval from the nonclinical perspective.

1.3.2 Additional Non Clinical Recommendations

This review recommends that the EPC for lumacaftor be designated as “cystic fibrosis transmembrane conductance regulator conformational stabilizer.”

1.3.3 Labeling

The reviewer’s recommended labeling is provided below. The sponsor’s proposed text is taken from NDA 206083 EDR SD #3 dated November 5, 2014. The reviewer’s

proposed insertions and deletions are indicated in blue font and ~~red strikethrough~~ text, respectively.

Reviewer's comments: Note that recommended labeling edits for sections 8.1, 8.3, 13.1 and 13.2 were provided in the nonclinical review dated May 5, 2015. References to the sponsor's proposed (b) (4) (b) (4) *will also need to be updated as listed below.*

INDICATIONS AND USAGE (HIGHLIGHTS OF PRESCRIBING INFORMATION)
ORKAMBI is a combination of lumacaftor, (b) (4) and ivacaftor, (b) (4) indicated for the treatment of cystic fibrosis in patients 12 years and older who are homozygous for the F508del mutation in the CFTR gene.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
The CFTR protein is a chloride channel present at the surface of epithelial cells in multiple organs. The *F508del* mutation (b) (4) causing a defect in cellular processing and trafficking that targets the protein for degradation and therefore reduces the quantity of CFTR at the cell surface. The small amount of F508del-CFTR that reaches the cell surface is less stable and has (b) (4) compared to wild-type protein. (b) (4) .

Lumacaftor (b) (4) improves the (b) (4) processing and trafficking of F508del-CFTR to the cell surface. (b) (4) Ivacaftor is a CFTR potentiator that facilitates increased chloride transport by potentiating the channel open probability (or gating) of the CFTR protein at the cell surface. In vitro studies have demonstrated that both lumacaftor and ivacaftor act directly on the CFTR protein in primary human bronchial epithelial (b) (4) cultures and other cell lines harboring the *F508del-CFTR* mutation to increase the quantity and function of F508del-CFTR at the cell surface, resulting in increased chloride ion transport. In vitro responses do not necessarily correspond to in vivo pharmacodynamic response or clinical benefit. (b) (4)

11 Integrated Summary and Safety Evaluation

NDA 206038 from Vertex Pharmaceuticals seeks approval of ORKAMBI, a fixed-dose combination product containing ivacaftor, an FDA-approved CFTR potentiator, and lumacaftor, a new molecular entity. As lumacaftor is a new active moiety, the nonclinical review process included the determination of an appropriate Established Pharmacologic Class (EPC) text phrase for inclusion in the product labeling.

The approach to the selection of an EPC is outlined in *Guidance for Industry and Review Staff*[1] and *Manual of Policies and Procedures*[2] documents published by FDA's Center for Drug Evaluation and Research (CDER). During the course of the review process for NDA 206038, the issue of an EPC for lumacaftor was discussed extensively by the reviewer, pharmacology-toxicology Team Leader Timothy Robison and Supervisor Marcie Wood, Medical Officer Robert Lim, Cross-Discipline Team Leader Anthony Durmowicz, Division Director Badrul Chowdhury and CDER Office of New Drugs Associate Directors for Pharmacology-Toxicology Paul Brown and Timothy McGovern. As summarized below, the issue of an EPC for lumacaftor has also been discussed with the sponsor. It is noted however, that the determination of an EPC (or the decision to not select an EPC) rests with the FDA.

KALYDECO, consisting of the ivacaftor monoproduct, is an FDA-approved product for cystic fibrosis patients with certain mutations in the *CFTR* gene. Ivacaftor represented the first approved product which directly targeted the underlying molecular cause of cystic fibrosis, the mutant CFTR protein. Based on its mechanism of action, as demonstrated in in vitro pharmacology studies and supported by clinical trial results, ivacaftor was classified as a ***CFTR potentiator*** (refer to nonclinical review filed to NDA 203188 by Dr. Marcie Wood, January 17, 2012).

The previous nonclinical review filed to NDA 206038 on May 5, 2015 summarized a number of in vitro pharmacology studies with lumacaftor conducted by the sponsor and/or published in the scientific literature. The totality of the data support the conclusion that lumacaftor also directly acts on the mutant CFTR protein, but via a different molecular mechanism of action than ivacaftor. Therefore, lumacaftor was considered as a first-in-class active moiety and was not considered to be a member of any existing EPC, including that of ivacaftor (CFTR potentiator).

Vertex proposes to classify lumacaftor as a (b) (4) In the NDA 206038 Late Cycle Meeting (LCM) background package sent to the sponsor on April 17, 2015, the interdisciplinary review team expressed several specific concerns with the proposed EPC. Following discussion of the issue at the LCM on April 29, 2015, the sponsor was invited to submit any additional justification. Vertex addressed each concern in a response dated May 29, 2015. The comments conveyed to the sponsor are reproduced below, and this review will discuss each identified concern and evaluate the sponsor's justification.

FDA's comments, LCM briefing package dated April 17, 2015
Note that according to the Guidance for Industry and Review Staff: Labeling for Human Prescription Drug and Biological Products- Determining Established Pharmacologic

[1] *Guidance for Industry and Review Staff: Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information*. October 2009.
[2] *Determining the Established Pharmacologic Class for Use in the Highlights of Prescribing Information*. MAPP 7400.13, July 2013.

Class for Use in the Highlights of Prescribing Information (October 2009), the EPC should indicate to health care professionals "what to expect from a drug and how it relates to other therapeutic options." The EPC should be scientifically valid, clinically meaningful. It should be based on either the drug's mechanism of action, physiologic effects, or chemical structure.

While acknowledging the (b) (4) *to refer to lumacaftor and related compounds, based on review of the nonclinical and clinical data included in NDA 206038, our determination is that your proposal to classify lumacaftor as a* (b) (4) *not acceptable for the following reasons:*

(b) (4)

1. The term (b) (4) **is too broad to be meaningfully interpreted, as it could refer to any number of mechanisms of action which specifically affect the CFTR protein.**

Vertex response

(b) (4)

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/s/

ANDREW C GOODWIN
06/04/2015

TIMOTHY W ROBISON
06/04/2015
I concur

Pharmacology and Toxicology Secondary Review for NDA 206038

TO: NDA 206038 (ORKAMBI; Combination of lumacaftor and ivacaftor)

FROM: Timothy W. Robison, Ph.D., D.A.B.T.
Pharmacology and Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: June 4, 2015

ORKAMBI is a combination product containing the active pharmaceutical ingredients (APIs), lumacaftor and ivacaftor. The product consists of a fixed-dose combination tablet presentation containing 200 mg lumacaftor and 125 mg ivacaftor. The proposed daily dose of 800 mg lumacaftor and 500 mg ivacaftor is achieved via two daily doses of two tablets each.

Dr. Goodwin's reviews dated May 5, 2015 and June 4, 2015 focused on the nonclinical safety assessment of the lumacaftor monoproduct and the combination of lumacaftor and ivacaftor. The Sponsor previously received FDA approval of the ivacaftor monoproduct, KALYDECO (NDA 203188).

I concur with the recommendations of Dr. Andrew Goodwin's reviews dated May 5, 2015 and June 4, 2015 that the nonclinical pharmacology and toxicology of the lumacaftor monoproduct and the combination of lumacaftor and ivacaftor have been adequately studied and ORKAMBI should be approved from the nonclinical perspective.

The Sponsor has complete nonclinical development programs for the lumacaftor and ivacaftor monoproducts as well as the combination of lumacaftor and ivacaftor. The one exception is a 2-year carcinogenicity study with lumacaftor in rats, which will be completed as a post-marketing requirement.

Pharmacology:
The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein is a chloride channel present at the surface of epithelial cells in multiple organs. The *F508del* mutation impacts the CFTR protein in multiple ways, primarily by causing a defect in cellular processing and trafficking that targets the protein for degradation and therefore reduces the quantity of CFTR at the cell surface. The small amount of F508del-CFTR that reaches the cell surface is less stable and has diminished gating activity compared to wild-type protein.

Lumacaftor is a CFTR conformational stabilizer that improves the conformational stability of F508del-CFTR, resulting in increased cellular processing and trafficking of mutant protein to the cell surface. Ivacaftor is a CFTR potentiator that facilitates increased chloride transport by potentiating the channel-open probability (or gating) of the CFTR protein at the cell surface. *In vitro* studies have demonstrated that both lumacaftor and ivacaftor act directly on the CFTR protein in primary human bronchial

epithelial cultures and other cell lines harboring the *F508del-CFTR* mutation to increase the quantity and function of F508del-CFTR at the cell surface, resulting in increased chloride ion transport. However, *in vitro* responses do not necessarily correspond to in vivo pharmacodynamic response or clinical benefit.

Lumacaftor Monoproduct:
The pivotal general toxicology studies with lumacaftor were conducted in rats and dogs. There were no relevant target organs of toxicity identified in rats. In the 12-month dog study, lumacaftor treatment led to gastrointestinal effects (emesis and abnormal stools) and decreased red blood cell parameters that were considered clinically monitorable. No target organs of toxicity were identified in the chronic dog study. In a previous 3-month dog study, a higher lumacaftor dose was associated with premature deaths, irregular gait, jerky movements and muscle rigidity. Histopathological findings at the higher doses were noted in the thymus (lymphoid depletion), liver (extramedullary hematopoiesis), and male reproductive organs (delayed maturation of testes and prostate, sloughed germ cells in epididymides). These findings in the 3-month study were not evident in the 12-month study.

Lumacaftor exhibited no potential for genotoxicity in a bacterial reverse mutation assay, an *in vitro* mammalian chromosome aberration assay, and an *in vivo* micronucleus assay. There was no evidence of test article-related tumorigenicity in a 6-month study in Tg.RasH2 mice.

Lumacaftor was not associated with any adverse effects in developmental and reproductive toxicology studies, including male and female fertility, embryofetal development, and pre- and post-natal development.

Lumacaftor-Ivacaftor Combination:
Toxicology studies evaluating the lumacaftor-ivacaftor combination were conducted in rats (1- and 3-month) and dogs (1-month). Novel findings identified in the combination studies are noted below.

In the 3-month rat study, reversible focal necrosis, erosion, edema and/or inflammatory infiltrate was noted in the stomach and was reversible after a 28-day recovery period. These findings were considered monitorable in a clinical setting. Bilateral, subcapsular cataracts were observed for one rat (2%) treated with the high dose of the combination.

In the 1-month dog study, electrocardiography (ECG) findings included prolonged PR interval and supraventricular premature complexes (SVPCs; early depolarization); these changes were considered clinically monitorable. At the highest dose level, adverse histopathological findings consistent with delayed male sexual maturation were noted in the testes (increased multinucleated degenerate germ cells), epididymides (sloughed germ cells) and prostate (acinar contraction, lower organ weights and decreased secretion). Test article-related effects were reversible after a 14-day recovery period. The cardiac and male reproductive findings were observed with increased incidence

and/or at lower doses compared to the ivacaftor and lumacaftor monoproduct studies, respectively.

Ivacaftor Monoproduct:
A complete nonclinical program for ivacaftor was completed and reviewed under NDA 203188 for the KALYDECO monotherapy product, and also supports the safety of ivacaftor in the proposed ORKAMBI combination product.

Ivacaftor is primarily metabolized by CYP 3A4, a profile that was significant for the clinical development program in combination with lumacaftor, a CYP 3A4 inducer. Two disproportionate human metabolites (M1 and M6) of ivacaftor were identified and qualified in nonclinical studies.

Pivotal toxicology studies with ivacaftor were conducted in rats for up to 6 months and dogs for up to 12 months. Test article-related deaths were noted at higher doses in the rat study and increased liver alanine aminotransferase (ALT) levels were observed. Key findings in the dog study include gastrointestinal effects (emesis and abnormal stools) and detection of SVPCs by ECG. The hepatic, gastrointestinal and cardiac findings were considered monitorable in the clinical setting.

Ivacaftor was negative in a standard battery of genetic toxicology assays. Two-year carcinogenicity studies were conducted in mice and rats and there was no evidence of test article-related tumorigenicity. Impaired male and female fertility was observed at high doses.

Ivacaftor was not teratogenic in rats or rabbits and had no effects on peri- or post-natal development in rats.

Cataracts were seen in juvenile rats dosed with ivacaftor from postnatal day 7-35 at dose levels of 10 mg/kg/day and higher.

Labeling:
Dr. Goodwin's reviews dated May 5, 2015 and June 4, 2015 recommend changes to product labeling in Indications and Usage under Highlights of Prescribing Information, Section 8.1 (Pregnancy), Section 8.3 (Nursing Mothers), Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility). I concur with Dr. Goodwin's recommendations for changes to the product label. See Dr. Goodwin's reviews for additional details of changes to the product labeling.

The Established Pharmacological Class (EPC) for Lumacaftor was designated as CFTR conformational stabilizer. See Dr. Andrew Goodwin's review dated June 4, 2015 for the details of this decision.

Recommendation: From the nonclinical perspective, approval of the application is recommended.

There is one Post-Marketing Requirement for the submission of the 2-year carcinogenicity study with lumacaftor in rats.

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/s/

TIMOTHY W ROBISON
06/04/2015
See Dr. Goodwin's reviews dated May 5, 2015 and June 4, 2015

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206038
Supporting document/s:	Electronic Document Room (EDR) Supporting Documents (SD) #1, 3, 8, 13, 15, 18
Applicant's letter date:	July 30, 2014; November 5, 2014; January 5, 2014; March 3, 2015, March 18, 2015, May 4, 2015
CDER stamp date:	July 30, 2014; November 5, 2014; January 5, 2014; March 3, 2015, March 18, 2015, May 4, 2015
Product:	ORKAMBI (lumacaftor/ivacaftor)
Indication:	Treatment of cystic fibrosis (CF) in patients age 12 years and older who are homozygous for the *F508del* mutation in the *CFTR* gene.
Applicant:	Vertex Pharmaceuticals
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Leila Hann

Template Version: September 1, 2010

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aa	Amino acid
ABC	ATP Binding Casette
ADME	Absorption, Distribution, Metabolism, Excretion
ALT	Alanine aminotransferase
API	Active Pharmaceutical Ingredient
ASL	Airway Surface Liquid
AUC	Area Under the Curve
BALF	Bronchoalveolar Lavage Fluid
C	Celsius
CBF	Ciliary Beat Frequency
CF	Cystic Fibrosis
CFTR	CF transmembrane conductance regulator
CHO	Chinese Hamster Ovary
cm	centimeter
CYP	Cytochrome P450
DMSO	Dimethyl Sulfoxide
DPARP	Division of Pulmonary, Allergy and Rheumatology Products
ECAC	Executive Carcinogenicity Assessment Committee
ECG	Electrocardiography
EDR	Electronic Document Room
ELF	Epithelial Lining Fluid
EPC	Established Pharmacologic Class
ER	Endoplasmic Reticulum
F508del	Deletion of phenylalanine at position 508 of CFTR
FDA	Food and Drug Administration
FRT	Fischer Rat Thyroid
g	gram
GD	Gestation Day
GLP	Good Laboratory Practices
HBE	Human Bronchial Epithelial
HEK	Human Embryonic Kidney
hERG	human Ether-à-go-go-Related Gene
ICL	Intracellular loop
IND	Investigational New Drug application
IV	intravenous
kAE1	Kidney anion exchanger 1
kg	kilogram
mcA	microampere
mcCi	microCurie
mcg	microgram
mcM	micromolar
mg	milligram
mL	milliliter

mol	mole
MRHD	Maximum Recommended Human Dose
MSD	Membrane Spanning Domain
NDA	New Drug Application
NF	National Formulary
NHERF1	Na^+/H^+ exchanger regulatory factor 1
NME	New Molecular Entity
nM	nanomolar
NOAEL	No Observed Adverse Effect Level
PDUFA	Prescription Drug User Fee Act
PMR	Post-Marketing Requirement
QWBA	Quantitative whole-body autoradiography
SD	Supporting Document
(b) (4)	(b) (4)
SPA	Special Protocol Assessment
SVPC	Supraventricular Premature Complex
TMD	Transmembrane Domain
USP	United States Pharmacopeia
WT	Wild-type

1 Executive Summary

1.1 Introduction

Vertex Pharmaceuticals is seeking approval of ORKAMBI for the treatment of cystic fibrosis (CF) in patients age 12 years or older who are homozygous for the deletion of phenylalanine 508 (*F508del*) in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. A rolling submission under NDA 206038 was initiated on July 30, 2014 and the filing date of the complete application was November 5, 2014. The application was assigned priority review status with a target Prescription Drug User Fee Act (PDUFA) action date of July 5, 2015.

ORKAMBI is a combination product containing the active pharmaceutical ingredients (APIs), lumacaftor and ivacaftor. The product consists of a fixed-dose combination tablet presentation containing 200 mg lumacaftor and 125 mg ivacaftor. The proposed daily dose of 800 mg lumacaftor and 500 mg ivacaftor is achieved via two daily doses of two tablets each.

Ivacaftor is a CFTR potentiator that is FDA-approved for the treatment of certain CF patients under the trade name KALYDECO. Ivacaftor was developed as a monoproduct under IND 74633 and NDA 203188. The code names VX-770 and VRT-813077 have been used in certain study reports and previous reviews, though this memo will consistently use ivacaftor for clarity.

Lumacaftor is a first-in-class new molecular entity (NME) that has been developed in combination with ivacaftor under IND 79521. The sponsor proposes to classify lumacaftor as a (b) (4), but this determination remains under review. Any reference to the term '(b) (4) in this review should be not be interpreted to represent a decision to assign this term as the EPC for lumacaftor. The code names VX-809 and VRT-826809 have been used in certain study reports, previous reviews and publications, but this memo will consistently use lumacaftor for clarity.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical evaluation of NDA 206038 relied on data from studies conducted with ivacaftor, lumacaftor, and the combination of the two APIs.

Lumacaftor
The nonclinical development program for lumacaftor conducted in support of NDA 206038 included pharmacology, safety pharmacology, general toxicology, genetic toxicology, carcinogenicity and developmental / reproductive toxicology studies.

The pharmacological effects of lumacaftor on F508del CFTR were evaluated in vitro using recombinant cell lines and primary culture of human bronchial epithelial (HBE) cells. F508del CFTR exhibits defective folding, domain assembly and stability, resulting

in retention in the endoplasmic reticulum (ER) and degradation while the wild-type (WT) protein undergoes continued processing and trafficking to the cell surface.

Lumacaftor treatment induces a protein conformation that is more WT-like based on resistance to proteolytic degradation and results in a 6-fold increase in F508del CFTR maturation. This apparent increase in the quantity of F508del CFTR at the cell surface is manifested as improved chloride transport, channel open probability and protein half-life. In HBE cells, lumacaftor treatment restored F508del CFTR channel function to approximately ~14% of WT levels. While the binding site has not been fully elucidated, the available data provide evidence that lumacaftor directly and specifically interacts with F508del CFTR. Lumacaftor is a first-in-class product, which the sponsor refers to as a (b) (4) As noted above, the review team has not made a final determination as to a potential Established Pharmacologic Class (EPC) for lumacaftor.

The absorption, distribution, metabolism and excretion (ADME) of lumacaftor was evaluated in mice, rats, dogs, monkeys and in vitro systems. Lumacaftor is rapidly absorbed and exhibited moderate (dogs) to high (rats) oral bioavailability. Distribution of lumacaftor was observed across the placental barrier in rats and rabbits and into the milk of lactating rats. Lumacaftor is a cytochrome P450 (CYP) 3A4 inducer, resulting in decreased exposure of the CYP 3A4 substrate ivacaftor in the combination product as compared to KALYDECO, despite a higher nominal ivacaftor dose level. One key human metabolite (M28) of lumacaftor was identified and qualified in nonclinical studies, but represented just 7% of total exposure at the intended clinical dose level. The primary route of elimination in rats was via the feces.

Safety pharmacology studies were conducted to assess potential respiratory, neurological, cardiovascular and gastrointestinal effects of lumacaftor. The only test article-related effect was increased motor activity in rats.

The pivotal general toxicology studies with lumacaftor were conducted in rats and dogs. There were no relevant target organs of toxicity identified in rats. The No Observed Adverse Effect Level (NOAEL) in the 6-month rat study provided a 3- or 7-fold safety margin compared to the proposed clinical dose on an exposure basis in males and females, respectively. In the 12-month dog study, lumacaftor treatment led to gastrointestinal effects (emesis and abnormal stools) and decreased red blood cell parameters that were considered clinically monitorable. No target organs of toxicity were identified in the chronic dog study. The NOAEL represented a 2-fold safety margin compared to the proposed clinical dose on an exposure basis in males and females. In a previous 3-month dog study, a higher lumacaftor dose was associated with premature deaths, irregular gait, jerky movements and muscle rigidity. Histopathological findings at the higher doses were noted in the thymus (lymphoid depletion), liver (extramedullary hematopoiesis), and male reproductive organs (delayed maturation of testes and prostate, sloughed germ cells in epididymides). These findings in the 3-month study were not evident in the 12-month study.

Lumacaftor exhibited no potential for genotoxicity in a bacterial reverse mutation assay, an in vitro mammalian chromosome aberration assay, and an in vivo micronucleus assay. There was no evidence of test article-related tumorigenicity in a 6-month study in Tg.RasH2 mice. A two-year carcinogenicity study in rats is ongoing and will be completed as a Post-Marketing Requirement (PMR).

Lumacaftor was not associated with any adverse effects in developmental and reproductive toxicology studies, including male / female fertility, embryofetal survival, teratogenicity, and post-natal development and sexual maturation.

Lumacaftor-Ivacaftor Combination
The sponsor conducted in vitro pharmacology studies to assess the effects of the lumacaftor-ivacaftor combination on F508del CFTR. Combined treatment resulted in greater effects on chloride transport, airway surface liquid height and ciliary beat frequency compared to either individual test article.

Toxicology studies evaluating the lumacaftor-ivacaftor combination were conducted in rats (1- and 3-month) and dogs (1-month). Novel findings identified in the combination studies are noted below.

In the 3-month rat study, reversible focal necrosis, erosion, edema and/or inflammatory infiltrate was noted in the stomach and was reversible after a 28-day recovery period. Bilateral, subcapsular cataracts were observed for one rat (2%) treated with the high dose of the combination. Given the proposed patient population, these findings were considered clinically monitorable and were excluded for the purposes of safety margin calculation. The NOAEL in the rat study represents at least 6- and 100-fold safety margins on an exposure compared to the clinical dose for lumacaftor and ivacaftor, respectively.

In the 1-month dog study, electrocardiography (ECG) findings included prolonged PR interval and supraventricular premature complexes (SVPCs; early depolarization); these changes were considered clinically monitorable. At the highest dose level, adverse histopathological findings consistent with delayed male sexual maturation were noted in the testes (increased multinucleated degenerate germ cells), epididymides (sloughed germ cells) and prostate (acinar contraction, lower organ weights and decreased secretion). Test article-related effects were reversible after a 14-day recovery period. The cardiac and male reproductive findings were observed with increased incidence and/or at lower doses compared to the ivacaftor and lumacaftor monoproduct studies, respectively. On an exposure basis, the NOAEL in the dog study provides a 1- or 2-fold safety margin (for males and females, respectively) for lumacaftor and at least a 30-fold safety margin for ivacaftor.

Combination genotoxicity, carcinogenicity, or reproductive and developmental toxicity studies were not performed.

Ivacaftor
A complete nonclinical program for ivacaftor was completed and reviewed under NDA 203188 for the KALYDECO monotherapy product, and also supports the safety of ivacaftor in the proposed ORKAMBI combination product.

In vitro pharmacology studies demonstrated that ivacaftor acts as a potentiator to increase chloride transport and channel open probability of mutant CFTR. These effects are most notable on gating-deficient CFTR mutants such as G551D, but ivacaftor also potentiates F508del CFTR.

Ivacaftor is primarily metabolized by CYP 3A4, a profile that was significant for the clinical development program in combination with lumacaftor, a CYP 3A4 inducer. Two disproportionate human metabolites (M1 and M6) of ivacaftor were identified and qualified in nonclinical studies.

Pivotal toxicology studies with ivacaftor were conducted in rats for up to 6 months and dogs for up to 12 months. Test article-related deaths were noted at higher doses in the rat study and increased liver alanine aminotransferase (ALT) levels were observed. Key findings in the dog study include gastrointestinal effects (emesis and abnormal stools) and detection of SVPCs by ECG. The hepatic, gastrointestinal and cardiac findings were considered monitorable in the clinical setting and were excluded for safety margin determination. The NOAELs in the rat and dog studies provide a minimum 75-fold safety margin as compared to the ivacaftor exposure in patients taking ORKAMBI. The nonclinical studies conducted with ivacaftor also provide adequate safety margins with respect to the M1 and M6 metabolites.

Ivacaftor was negative in a standard battery of genetic toxicology assays. Two-year carcinogenicity studies were conducted in mice and rats and there was no evidence of test article-related tumorigenicity. Impaired male and female fertility and reproductive performance was observed in rats at doses at least 20 times higher than the exposure in patients taking ORKAMBI. Ivacaftor was not teratogenic in rats or rabbits and had no effects on peri- or post-natal development in rats.

Cataracts were seen in juvenile rats dosed with ivacaftor from postnatal day 7-35 at dose levels of 10 mg/kg/day and higher.

1.3 Recommendations

1.3.1 Approvability

ORKAMBI (lumacaftor-ivacaftor tablets) is recommended for approval from the nonclinical perspective.

1.3.2 Additional Non Clinical Recommendations

The nonclinical evaluation of lumacaftor will be completed via a Post-Marketing Requirement (PMR) for the submission of the results of a two-year carcinogenicity study in rats. Labeling recommendations are provided below.

1.3.3 Labeling

The reviewer's recommended labeling is provided below. The sponsor's proposed text is taken from NDA 206083 EDR SD #3 dated November 5, 2014. Note that the updated label provided in SD #12 dated February 6, 2015 did not contain any edits to the proposed nonclinical labeling. The reviewer's proposed insertions and deletions are indicated in blue font and ~~red strikethrough~~ text, respectively.

Note that at this time, the determination of a potential Established Pharmacologic Class (EPC) for lumacaftor is still under review. A forthcoming supplemental labeling review will provide the reviewer's recommended language for the HIGHLIGHTS OF PRESCRIBING INFORMATION: INDICATIONS AND USAGE and 12.1: MECHANISM OF ACTION sections.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Teratogenic effects: Pregnancy Category B. There are ~~N~~no adequate and well-controlled (b) (4) trials of ORKAMBI or its individual components, lumacaftor or ivacaftor, in pregnant women (b) (4). Embryofetal development studies in rats and rabbits were conducted with the individual components of ORKAMBI, lumacaftor and ivacaftor. Because animal reproduction studies are not always predictive of human response, ORKAMBI should be used during pregnancy only if clearly needed.

Lumacaftor was not teratogenic in rats at approximately 8 times the maximum recommended human dose (MRHD) (b) (4) (on a lumacaftor AUC basis at a maternal oral dose of 2000 mg/kg/day). Lumacaftor was not teratogenic in rabbits at approximately 5 times the MRHD (b) (4) (on a lumacaftor AUC basis at a maternal oral dose of 200 mg/kg/day). Placental transfer of lumacaftor was observed in pregnant rats and rabbits.

Ivacaftor was not teratogenic in rats at approximately (b) (4) times the MRHD (b) (4) (based on summed AUCs for ivacaftor and its metabolites at a maternal oral dose of 200 mg/kg/day- (b) (4). Ivacaftor was not teratogenic in rabbits at approximately (b) (4) times the MRHD (b) (4) (on an ivacaftor AUC basis at a maternal oral dose of 100 mg/kg/day). Placental transfer of ivacaftor was observed in pregnant rats and rabbits.

(b) (4)

8.3 Nursing Mothers

Both lumacaftor and ivacaftor are excreted into the milk of lactating female rats. Excretion of ORKAMBI into human milk is probable (b) (4) There are no human studies that have investigated the effects of lumacaftor and ivacaftor on breast-fed infants. Caution should be exercised when ORKAMBI is administered to a nursing woman.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with ORKAMBI; however, studies are available for individual components, lumacaftor and ivacaftor, as described below.

Lumacaftor:
A 26-week study was conducted in transgenic Tg.rasH2 mice to assess carcinogenic potential of lumacaftor. No evidence of tumorigenicity was observed in Tg.rasH2 mice at lumacaftor oral doses up to 1500 and 2000 mg/kg/day in female and male mice, respectively (b) (4)

Lumacaftor was negative for genotoxicity in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, and *in vivo* mouse micronucleus test.

Lumacaftor had no effects on fertility and reproductive performance indices in male and female rats at 1000 mg/kg/day (approximately 3 and (b) (4) times, respectively, the MRHD (b) (4) on a lumacaftor AUC basis (b) (4)

Ivacaftor:
Two-year studies were conducted in mice and rats to assess carcinogenic potential of ivacaftor. No evidence of tumorigenicity was observed in mice and rats at ivacaftor oral doses up to 200 mg/kg/day and 50 mg/kg/day, respectively (approximately equivalent to (b) (4) and (b) (4) times the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites).

Ivacaftor was negative for genotoxicity in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, and *in vivo* mouse micronucleus test.

Ivacaftor impaired fertility and reproductive performance indices in male and female rats at 200 mg/kg/day (approximately (b) (4) the MRHD (b) (4)

(b) (4) based on summed AUCs of ivacaftor and its metabolites (b) (4) Increases in prolonged diestrus were observed in females at 200 mg/kg/day. Ivacaftor also increased the number of females with all nonviable embryos and decreased corpora lutea, implantations, and viable embryos in rats at 200 mg/kg/day (approximately (b) (4) times the MRHD (b) (4) (b) (4) based on summed AUCs of ivacaftor and its metabolites (b) (4) when dams were dosed prior to and during early pregnancy. These impairments of fertility and reproductive performance in male and female rats at 200 mg/kg/day were attributed to severe toxicity. No effects on male or female fertility and reproductive performance indices were observed at ≤100 mg/kg/day (approximately (b) (4) 8 times the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites (b) (4)

13.2 Animal Toxicology and/or Pharmacology

Cataracts were seen in juvenile rats dosed with ivacaftor from postnatal day 7-35 at dose levels of 10 mg/kg/day and higher (approximately (b) (4) times the MRHD for the ivacaftor component of ORKAMBI based on summed AUCs of ivacaftor and metabolites). This finding has not been observed in older animals.

2 Drug Information

2.1 Drug

Generic Name: Lumacaftor

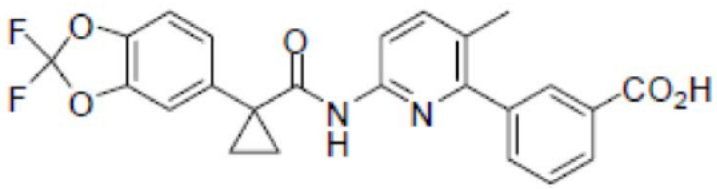
Code Name: VX-809

Chemical Name: 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid

Molecular Formula/Molecular Weight: $C_{24}H_{18}F_2N_2O_5$ / 452 g/mol

Structure or Biochemical Description

Figure 1. Chemical Structure of Lumacaftor (VX-809)



Pharmacologic Class: To be determined

Generic Name: Ivacaftor

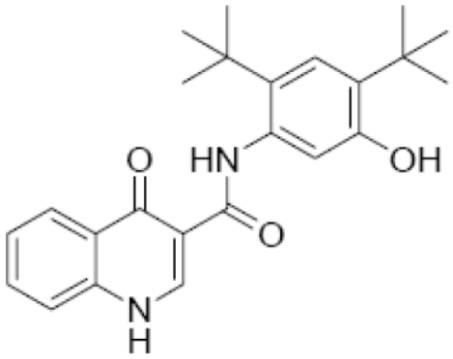
Code Name: VX-770, VRT-813077

Chemical Name: (N-(2,4-Di-tert-butyl-5-hydroxyphenyl)-1,4-dihydro-4-oxoquinoline-3-carboxamide)

Molecular Formula/Molecular Weight: $C_{24}H_{28}N_2O_3$ / 392.49 g/mol

Structure or Biochemical Description

Figure 2. Chemical Structure of Ivacaftor (VX-770)



Pharmacologic Class: Cystic fibrosis transmembrane conductance regulator (CFTR) potentiator

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 74633 and NDA 203188 (ivacaftor / VX-770 / KALYDECO)
- IND 79521 (lumacaftor-ivacaftor combination development)

2.3 Drug Formulation

ORKAMBI is formulated as a fixed-dose combination, immediate-release tablet containing 200 mg lumacaftor and 125 mg ivacaftor. The proposed dosing regimen consists of four tablets (two doses of two tablets each separated by 12 hours) for a total daily dose of 800 mg lumacaftor and 500 mg ivacaftor. The quantitative formulation and total exposure to each excipient are summarized in the table below.

Table 1. Quantitative Formulation of ORKAMBI Tablets

Component	Quality Standard	Function	Amount per tablet (mg)	Total daily exposure (mg)
(b) (4)				
Lumacaftor drug substance	In-house	API	200	800
(b) (4)	NDA 203188	DP		(b) (4)
Ivacaftor drug substance		API	125	500
(b) (4)				(b) (4)
Microcrystalline cellulose	USP/NF			
Croscarmellose sodium	USP/NF			
Sodium lauryl sulfate	USP/NF			
Povidone (b) (4)	USP/NF			
(b) (4)	USP			
	USP/NF			
	USP/NF			
Magnesium stearate	USP/NF			
Film coat				
(b) (4)	DMF (b) (4)			
	USP			
Print ink				
(b) (4) Black	BMF (b) (4)			
Total Tablet Weight			**582.5**	**4 tablets**

Table generated by reviewer from sponsor material in NDA 206038 3.2.P.1

(b) (4)

2.4 Comments on Novel Excipients

ORKAMBI does not include any novel excipients. All excipients are present at equal of greater levels in FDA-approved, orally administered products and are considered qualified from the nonclinical perspective.

2.5 Comments on Impurities/Degradants of Concern

Refer to nonclinical review filed to NDA 206038 by Dr. Andrew Goodwin on February 2, 2015.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is CF patients age 12 years or older who are homozygous for the *F508del CFTR* mutation. The dosing regimen is two tablets taken orally every 12 hours, for a total daily dose of 800 mg lumacaftor and 500 mg ivacaftor.

2.7 Regulatory Background

Ivacaftor was developed as a monoproduct under IND 74633, opened March 14, 2006. NDA 203188 was submitted on October 18, 2011 and was first approved with tradename KALYDECO on January 31, 2012.

No pre-IND meeting was held regarding the lumacaftor development program. IND 79521 was submitted on October 18, 2007. A partial clinical hold was placed on the IND on April 28, 2009 based on lack of nonclinical support for the proposed lumacaftor-ivacaftor combination study. The partial hold was removed on February 3, 2010 following review of 28-day combination toxicology studies in rats and dogs.

A Type B End of Phase 2 meeting was held on November 2, 2012 (see minutes dated November 30, 2012). The minutes included advice on the nonclinical program that would be required to support Phase 3 development of the lumacaftor-ivacaftor combination product. In addition, the Division indicated that a complete nonclinical program should be included in the NDA at the time of filing.

On December 5, 2012, the lumacaftor-ivacaftor combination was granted Breakthrough Therapy designation for the treatment of CF patients homozygous for the *F508del CFTR* mutation. A Type B meeting was held on February 12, 2013 (see minutes dated March 20, 2013), at which the sponsor proposed that the 6-month mouse carcinogenicity study would be included in the NDA submission and that the 2-year rat carcinogenicity study could be completed post-approval. After reviewing the sponsor's justification, the Division agreed that the rat study could be completed as a PMR (communication dated August 5, 2013).

A Type B Pre-NDA meeting was held on August 12, 2014 (see minutes dated September 2, 2014). The minutes reflect the Division's guidance regarding the designation of EPCs for lumacaftor and the lumacaftor-ivacaftor combination product. The Division clarified that EPCs are associated with individual active moieties and therefore no new EPC would be assigned for the combination product. Further, the Division noted that the sponsor's proposed classification of lumacaftor as a (b) (4) would be a review issue. In addition, the sponsor agreed to a request to submit the results of the 6-month mouse carcinogenicity study to the IND prior to the NDA submission in order to expedite review.

3 Studies Submitted

3.1 Studies Reviewed

The studies listed below were submitted to or cross-referenced in NDA 206038 and have been previously reviewed by DPARP under IND 74633 (ivacaftor), IND 79521 (lumacaftor-ivacaftor combination), or NDA 203188 (ivacaftor).

Table 2. List of Studies Reviewed in This Memo

Study #	Test Article	Study Title
4.2.1.1 Primary Pharmacodynamics		
D143	L	Effects of VRT-826809 on CFTR-Mediated Chloride Secretion in Human Bronchial Epithelia Isolated From Cystic Fibrosis Subjects (Versions 2, 3)
D146	L	Effects of VRT-826809 on Protein Conformation, Trafficking and Channel Gating of ΔF508-CFTR (Version 2)
K124	L	VX-809 directly and specifically interacts with MSD1 domain of CFTR
K248	L, I	Effect of VX-809 and VX-770 on airway surface liquid height and cilia beat frequency in HBE cells isolated from people who are F508 del homozygous
4.2.2.2 Absorption		
6536-451	L	Absorption, Distribution, Metabolism, and Excretion of 14C-VX-809 Following Oral and Intravenous Administration to Rats 4/29/2008
4.2.2.3 Distribution		
I353	L	Bronchoalveolar Lavage Fluid and Epithelial Lining Fluid Concentrations of VX-809 Following Oral Administration to Male Sprague Dawley Rats
K045	L	Placental Transfer and Lacteal Excretion of 14C-VX-809 Following Administration of a Single Oral Dose to Pregnant and Lactating Rats
K046	L	Placental Transfer of VX-809 Following Administration of a Single Oral Dose to Pregnant Rabbits

I = ivacaftor / VX-770 / VRT-813077
L = lumacaftor / VX-809 / VRT-826809

3.2 Studies Not Reviewed

The studies listed below were submitted to or cross-referenced in NDA 206038 but were not reviewed, as they were not pivotal to the nonclinical assessment of ORKAMBI.

Table 3. List of Studies Not Reviewed Under NDA 206038

Study #	Test Article	Study Title
4.2.1.1 Primary Pharmacodynamics		
D153	L	Validation of Primary Human Bronchial Epithelia Cultures to Evaluate the Pharmacological Action of CFTR Modulators
4.2.1.2 Secondary Pharmacodynamics		
VRT-0995096 -TX-004	M28	Spectrum Screen Data Report: VRT-0995096
4.2.1.3 Safety Pharmacology		
H063	M28	In vitro assessment of hERG inhibition potential by the major human metabolite of VX-809, M28 (also known as hydroxypyrrolidone-VX-809, VRT-0995096)

Study #	Test Article	Study Title
4.2.2.1 Analytical Methods and Validation Reports		
D154	L	Validation of a Method for the Quantitative Determination of VRT-826809 in Mouse Plasma by HPLC with MS/MS Detection
D120	L	Validation of the Bioanalytical Method for the Determination of VRT-826809 in Rat and Dog Plasma using LC-MS/MS Detection
D160	L	Validation of a Method for the Quantitative Determination of VRT-826809 in Rat Plasma by HPLC with MS/MS Detection
G099	L, M28	Validation of a Method for the Quantitative Determination of VRT-0995096 and VX-809 in Rat Plasma by LC/MS/MS
H172	L, M28	Validation of the Analytical Procedure for the Quantitative Determination of VX-809 and VRT-0995096 with 13C-d4-VX-809 and 13C3-d2-VRT-0995096 as Internal Standards in K3EDTA Rat Plasma
E143	L	Validation of Analytical Procedure for the Quantitative Determination of VX-809, with 13C-d4-VX-809 as an Internal Standard, in Rabbit Plasma
K009	L	The LC/MS/MS Quantification of VX-809 in New Zealand White Rabbit Plasma Between 2.00 and 2000 ng/mL
D151	L	Validation of Analytical Procedure for the Quantitative Determination of VRT-826809, with 13C-d4-VRT-826809 as an Internal Standard, in K3 EDTA Dog Plasma
G231	I	Non-GLP Bioanalytical Method for Determination of VX-770 Concentrations in Mouse, Rat, Dog and Monkey Plasma Samples and Dosing Solutions
8232055	I, M1, M6	Sample Collection Stability of VX-770, VRT-837018, and VRT-842917 in Rat, Rabbit, Dog, and Mouse Whole Blood
6536-383	I	Abbreviated Validation of a Method for the Determination of VRT-813077 in Mouse Plasma by HPLC with MS/MS Detection
E139	I, M1, M6	Validation of a Method for the Quantitative Determination of VRT-842917, VRT-837018, and VX-770 in Mouse Plasma by HPLC with MS/MS Detection
6536-382	I	Validation of a Method for the Determination of VRT-813077 in Rat Plasma by HPLC with MS/MS Detection
C141	I	Validation of a Method for the Quantitative Determination of VX-770 in Rat Plasma by HPLC with MS/MS Detection
E061	I, M1, M6	Validation of an Analytical Procedure for the Quantitative Determination of VX-770, VRT-837018, and VRT-842917 with d4-VX-770 or VRT-101793 as an Internal Standard in K3 EDTA Rat Plasma
06-8917	I	Validation of a Liquid Chromatographic Method with MS/MX Detection for the Determination of VX-770 in Rat and Dog Liver
J101	I, M1, M6	The LC/MS/MS Quantification of VX-770 and its Metabolites VRT-837018 and VRT-842917 in K2-EDTA Sprague Dawley Rat Plasma Between 2.00 and 2000 ng/mL
D052	I	Validation of a Method for the Quantitative Determination of VX-770 in Female Rabbit Plasma by HPLC with MS/MS Detection

Study #	Test Article	Study Title
6536-384	I	Abbreviated Validation of a Method for the Determination of VRT-813077 in Dog Plasma by HPLC with MS/MS Detection
C140	I	Validation of a Method for the Quantitative Determination of VX-770 in Dog Plasma by HPLC with MS/MS Detection
E056	I, M1, M6	Validation of an Analytical Procedure for the Quantitative Determination of VX-770, VRT-837018 and VRT-842917 with d4-VX-770 or VRT-101793 as an Internal Standard in K3 EDTA Dog Plasma
K222	I, M1, M6	The LC/MS/MS Quantification of VX-770, VRT-837018 and VRT-842917 in Dog Plasma Between 2.00 and 2000 ng/mL
K242	I, M1, M6	The LC/MS/MS Quantification of VX-770, VRT-837018 and VRT-842917 in Mouse Plasma Between 2.00 and 2000 ng/mL
K285	L, M28	The LC/MS/MS Quantification of VX-809 and VRT-0995096 in Rat Plasma Between 2.00 and 2000 ng/mL
K286	L	The LC/MS/MS Quantification of VX-809 in Dog Plasma Between 2.00 and 2000 ng/mL
K287	L	The LC/MS/MS Quantification of VX-809 in CBYB6F1 Mouse Plasma Between 2.00 and 2000 ng/mL
K288	L	The LC/MS/MS Quantification of VX-809 in Mouse Plasma Between 2.00 and 2000 ng/mL
I094	L, M28	Sample Collection Stability of VX-809 and VRT-0995096 in Mouse, Rat, Rabbit, and Dog Whole Blood by HPLC with MS/MS Detection
4.2.2.2 Absorption		
B268	I	The In Vitro Permeability and Efflux Characteristics of VRT-813077 in the Human Colon Carcinoma Cell Line (Caco-2)
E015	I	Pharmacokinetics and Dose-Proportionality of VX-770 in Male New Zealand White Rabbits Following Single Dose Oral Administration of VX-770 (b) (4) Suspended in 0.5 % MC/ 0.5% SLS/ 0.01 % Simethicone
H061	M28	Pharmacokinetics of VRT-0995096 (M28 Metabolite of VX- 809) Following Intravenous and Oral Administration in Male Sprague-Dawley Rats
H184	M28	Pharmacokinetics of VRT-0995096 (M28 Metabolite of VX- 809) Following Intravenous and Oral Administration in Male Beagle Dogs
MDCR- 09188	L	Bi-directional permeability of VX-809 (VRT-0826809) across Caco-2 cell monolayer
VX-809-DMPK -DM-020	L	BCS Potential, P-gp Substrate, and Inhibitor Assessment of VX-809
4.2.2.3 Distribution		
B251	I	Tissue Distribution of VRT-813077 in Male Sprague Dawley Rats Following Single Oral Administration
E166	I	Dosimetry Calculations for 14C-VX-770 Based on Tissue Concentration Data Following a Single Oral Administration to Rats
G085	M28	In Vitro Binding of VRT-0995096 to Plasma Proteins in Mouse, Rat, Dog, Monkey, and Human Plasma

Study #	Test Article	Study Title
G168	I	Bronchoalveolar Lavage Fluid and Epithelial Lining Fluid Concentrations of VX-770 Following Oral Administration to Male Sprague Dawley Rats
I204	L, M28	Vertex Compound (VX-809 and VRT-995096) Protein Binding and Competition studies
K100	M28	Evaluation of Placental Transfer of VRT-0995096 in Sprague-Dawley Rats by Assessing Maternal and Fetal Plasma Exposures Following Single Oral Dose of 25 mg/kg
VX-770-DMPK-DM-040	I, M1, M6	In Vitro Protein Binding of 14C-VX-770, 14C-VRT-837018, and 14C-VRT-842917 to Mouse, Rat, Dog, and Human Plasma, HSA, AAG, and HGG, and Protein Binding Displacement Interactions between VX-770, VRT-837018, and VRT-842917 and Warfarin
4.2.2.4 Metabolism		
CBDM304464	I, M1, M6	Evaluation of the Substrate and Inhibitor Potential of VX-770, VRT-842917, and VRT-837018 of Organic Anion Transporting Polypeptide 1B1 and 1B3
G140	M28	Assessment of VRT-995096 Reversible Inhibition Potential of Human Cytochrome P450 Isozymes
H060	M28	The In Vitro Stability of VRT-0995096 in Human Recombinant CYP Isozymes
J174	L	Evaluation of VRT-0826809 as an Inducer of CYP3A4 using Primary Cryopreserved Human Hepatocytes
K020	L	Evaluation of VX-809 as an Inducer of CYP2C8, CYP2C9 and CYP2C19 using Primary Cryopreserved Human Hepatocytes
K028	L	Evaluation of VRT-0826809 as an Inducer of CYP1A2 and CYP2B6 using Primary Cryopreserved Human Hepatocytes
K111	L	Evaluation of the Inhibitor Potential of VX-809 of Uptake Transporters
K112	L	Evaluation of the Substrate Potential of VX-809 of Organic Anion Transporting Polypeptide 1B1 and 1B3
VX-809-DMPK-DM-019	L, M28	Induction Potential of VX-809 on Cytochromes P450, Assessment of the Cytotoxicity of VX-809, and Induction Potential of VX-661 and VRT-0995096 (M28) on Cytochrome P450 3A4/5 in Human Hepatocyte Cultures
VX-809-DMPK-DM-028b	L, I	Evaluation of the Induction Potential of VX-809 and VX-661 on Cytochromes P450 in Human Hepatocyte Cultures and Associated Effects on the Metabolism of VX-770
4.2.2.6 Pharmacokinetic Drug Interactions		
F114	L, I	The Effect of VX-770 on the Pharmacokinetics Parameters of VX-809 after IV Co-administration and after Single or Repeated Oral Co-administration to Male Sprague-Dawley Rats
K027	L	Screening Report: Human PXR/SXR Activation on Compounds VX-809, VX-661, VXC-874, VRT-0926930, VRT-0928580 and VRT-0922563
F099	L, I	The Effect of VX-770 on the Pharmacokinetics Parameters of VX-809 after IV Co-administration and after Single or Repeated Oral Co-administration to Male Beagle Dogs

Study #	Test Article	Study Title
4.2.2.7 Other Pharmacokinetic Studies		
F131	L	Pharmacokinetics of VRT-826809 in Male Beagle Dogs Following Oral Administration of VRT-826809 in Different Formulations
F129	L	Pharmacokinetics of VX-809 in Male Beagle Dogs Following Single Oral Administration of VX-809 in Different Formulations
F130	L	Pharmacokinetics of VX-809 in Male New Zealand White rabbits Following Oral Administration of VX-809 at Different Doses
F132	L	Pharmacokinetics of VX-809 in Male Beagle Dogs Following Oral Administration of VX-809 at Different Doses
G133	L	Pharmacokinetics of VX-809 in Male Beagle Dogs Following a Single Oral Administration of VX-809 in Different Formulations
G150	L	Pharmacokinetics of Two Dose Formulations of VX-809 after a Single Oral Dose to Dogs
G115	L	Determination of the Pharmacokinetics of Three Dose Formulations of VX-809 after Oral Administration to Dogs
J060	L	Pharmacokinetics of VX-809 in Male Beagle Dogs Following Administration of a Single Nominal 10 mg/kg Oral Dose in Capsule versus Suspension
K074	L	Pharmacokinetics of VX-809 Following Single Dose Administration at Nominal Dose of 100 mg to Male Beagle Dogs in Capsule formulation with Different Drug Substance Lots
K099	L	Pharmacokinetics of VX-809 Following Single Dose Administration at Nominal Dose of 100 mg to Male Beagle Dogs with Two Different Solid Forms in Tablet Formulation
D022	I	Pharmacokinetics of VX-770 in Male Sprague-Dawley Rats Following a Single Nominal Oral Dose of 35 mg/kg of VX-770 as Capsule Formulations
E039	I	Pharmacokinetic Characteristics of VX-770 in Male Sprague-Dawley Rats Following Oral Administration of VX-770 in Different Formulations at Different Doses
F122	I	Pharmacokinetics of VX-770 in Male Sprague-Dawley Rats Following Oral Administration of VX-770 in Different Formulations at Different Doses
D142	I	Pharmacokinetics of VX-770 in Male Beagle Dogs Following Oral Administration of VX-770 in Different Tablet Formulations at 10 mg/kg dose
G132	M1, M6	Intravenous and Oral Pharmacokinetics of VRT-0837018 or VRT-0842917 in Male Sprague Dawley Rats
D241	I	Pharmacokinetic Characteristics of VX-770 in Male Beagle Dogs Following Oral Administration of VX-770 in Different Formulations at Different Doses
D248	I	Pharmacokinetic Characteristics of VX770 in Male Beagle Dogs Following Oral Administration of VX770 at 60 and 120 mg/kg as a single dose or divided dose administered together or 10 hour apart

Study #	Test Article	Study Title
E026	I	Pharmacokinetics and Dose-Proportionality of VX-770 in Male Beagle Dogs Following Single Dose Oral Administration of VX-770
E041	I	Effect of Food on the Pharmacokinetics of VX-770 Following Oral Gavage Administration to Male Beagle Dogs.
E042	I	Pharmacokinetics of VX-770 in Male Beagle Dogs Following Oral Administration of VX-770 in Suspension, Capsule and Tablet formulations at Different Doses
F120	I	Pharmacokinetics of VX-770 in Male Beagle Dogs Following Oral Administration of VX-770 in Different Formulations at Different Doses
F121	I	Pharmacokinetics of VX-770 following oral administration of VX-770 immediate release (IR) and sustained release (SR) tablet formulations to male Beagle dogs
F127	I	Pharmacokinetics of VX-770 in Male Beagle Dogs Following Oral Administration of VX-770 in Different Formulations at a Nominal Dose of 15 mg/kg
G130	I	Pharmacokinetic Characteristics of VX-770 in Male Beagle Dogs Following Oral Administration of VX-770 in Different Formulations at Different Doses
K071	L	Pharmacokinetics of VX-809 Following Single Dose Administration at Nominal Dose of 100 mg to Male Beagle Dogs in different formulations
K257	L	Pharmacokinetic Characteristics of Suspension and Nanosuspension Formulations of VX-809 Following Oral Administration of 250 mg/kg Dose to Male Beagle Dogs
4.2.3.2 Repeat-dose Toxicity		
VX-770-TX-004	I	VX-770: Maximum Tolerated Dosage (MTD) Oral (Stomach Tube) Toxicity and Toxicokinetics Study in Female Rabbits (non-GLP)
4.2.3.3 Genotoxicity		
VRT-826809 - TX-001	L	VRT-826809: *Salmonella* Plate Incorporation Mutagenicity Assay
AB05LA-LB 501.BTL	I	VRT-811899 and VRT-813077: *Salmonella* Plate Incorporation Mutagenicity Assay
AB07YE 501.BTL	I	VRT-813077 Lot 11: *Salmonella* Plate Incorporation Mutagenicity Assay
VRT-826809 - TX-003	L	VRT-826809: Mammalian Erythrocyte Micronucleus Screening Test
VRT-813077 - TX-002	I	VRT-813077: Mammalian Erythrocyte Micronucleus Screening Assay
4.2.3.6 Local Tolerance		
VX-809-TX-020	L	VX-809: Episkin Skin Irritation Test 15 minutes - 42 hours
VX-809-TX-021	L	VX-809: The Bovine Corneal Opacity and Permeability Assay (BCOP)
VX-770-TX-019	I	VX-770: Skin Irritation to the Rabbit
VX-770-TX-020	I	VX-770: The Bovine Corneal Opacity and Permeability Assay (BCOP)
4.2.3.7 Other Toxicity Studies		

Study #	Test Article	Study Title
VX-809-TX-022	L	VX-809: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)
VX-770-TX-021	I	VX-770: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
VRT-0995096-TX-007	M28	VRT-0995096: An Oral (Gavage) Dose Range-Finding Study of the Effects on Embryo/Fetal Development in Rats
PDD-1326	M1, M6	VX-770 M1 & M6 Metabolite Synthesis Summary
VRT (b) (4) -TX-001	L IMP	VRT (b) (4): Bacterial Reverse Mutation Assay
VRT- (b) (4) -TX-005	L IMP	VRT (b) (4): Bacterial Reverse Mutation Assay
VRT- (b) (4)	L IMP	VRT (b) (4): Bacterial Reverse Mutation Assay
VRT- (b) (4) TX-005	L IMP	VRT- (b) (4): Bacterial Reverse Mutation Assay
(b) (4)		
VX-809-TX-011	L	VX-809: Partition Coefficient (b) (4)
VX-809-TX-025	L	VX-809: Activated Sludge Respiration Inhibition Test
VX-809-TX-026	L	VX-809: Algal Growth Inhibition Assay

I = ivacaftor / VX-770 / VRT-813077
L = lumacaftor / VX-809 / VRT-826809
M28 = lumacaftor metabolite VRT-0995096
M1 = ivacaftor metabolite VRT-837018
M6 = ivacaftor metabolite VRT-842917

3.3 Previous Reviews Referenced

The reviews listed in the table below were filed to IND 79521 and have been referenced during the review of NDA 206038.

Table 4. Summary of Previously Reviewed Nonclinical Studies

Study #	Test Article	Study Title	Review
4.2.1.1 Primary Pharmacodynamics			
B227	I	VRT-813077: Activation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Mutants Expressed in NIH3T3 and FRT Cells and electivity for Ion Channels	IND 74633 5/19/2006
B228	I	Interactions of VRT-813077 with the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) and its Signaling Pathways in NIH3T3 and FRT Cells	IND 74633 5/19/2006

Study #	Test Article	Study Title	Review
B229	I	In Vitro Activity of VRT-813077 in Primary Bronchial Epithelial Cells and Nasal Polyps from Cystic Fibrosis Patients	IND 74633 5/19/2006
C166	I	Effects of VX-770 on Activity of the R117H Mutant of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Expressed in FRT Cells	IND 74633 6/5/2007
C172	I	VX-770 Effectively Potentiates ΔF508-CFTR Activity When Added to Either Apical or Basolateral Side of Human Bronchial Epithelia In Vitro	IND 74633 6/5/2007
C181	M1	In Vitro Activity of VRT-837018, a Putative Metabolite of VX-770, on Chloride Secretion in Cystic Fibrosis Bronchial Epithelia	IND 74633 6/5/2007
C187	I	Effects of VX-770 on CFTR-Mediated Chloride Transport in Human Bronchial Epithelia Heterozygous for the 2789+5G->A Splice Site Mutation and ΔF508-CFTR	IND 74633 6/5/2007
D058	I	Effects of VX-770 on G551D-CFTR in Recombinant Cells and in Human Bronchial Epithelia Isolated from a G551D/ΔF508 Heterozygous Cystic Fibrosis Subject	IND 74633 1/13/2012
D143	L	Effects of VRT-826809 on CFTR-Mediated Chloride Secretion in Human Bronchial Epithelia Isolated From Cystic Fibrosis Subjects (Version 1)	IND 79521 1/3/2008
D144	L	Determination of the Efficacy Criteria for CFTR Modulators Based on Genotype-to-Clinical Phenotype Correlations in Cystic Fibrosis Subjects	IND 79521 1/3/2008
D146	L	Effects of VRT-826809 on Protein Conformation, Trafficking and Channel Gating of ΔF508-CFTR (Version 1)	IND 79521 1/3/2008
E111	M6	In vitro Activity of VRT-842917, a Metabolite (M6) of VX-770, on Chloride Secretion in Cystic Fibrosis Bronchial Epithelia	IND 74633 1/13/2012
E156	M1	In vitro Activity of VRT-837018, a Metabolite (M1) of VX-770, on Chloride Secretion in Cystic Fibrosis Bronchial Epithelia	IND 74633 1/13/2012
F081	L, I	Effects of VX-770 and VX-809 combinations on F508del-CFTR function in cultured F508del/F508del-HBE	IND 79521 4/17/2009
G010	M28	Effects of VRT-0995096 on CFTR activity in cultured human bronchial epithelial isolated from the bronchi of a F508del homozygous cystic fibrosis patient	IND 79521 3/10/2010
G205	I	Effect of VX-770 on multiple mutant CFTR forms in vitro	IND 74633 1/13/2012
H189	I	Time Course of VX-770 Potentiation of F508del CFTR In Vitro	IND 74633 1/13/2012
4.2.1.2 Secondary Pharmacodynamics			
1054946-1016520	I	Lead Profiling Screen Data Report: VRTXSD135 (VES-40)	IND 74633 5/19/2006
1054946-1016920	I	Spectrum Screen Data Report: VRTXSD135 (VES-40)	IND 74633 5/19/2006
1083320-1025114	L	Lead Profiling Screen Data Report: VRTXSD294 (VES-108)	IND 79521 1/3/2008
1083320-1025509	L	Spectrum Screen Data Report: VRTXSD294 (VES-108)	IND 79521 1/3/2008
D145	L	Effects of VRT-826809 in a Functional Assay for TP Prostanoid Receptor Activity in Rat Aorta Rings In Vitro	IND 79521 1/3/2008

Study #	Test Article	Study Title	Review
D147	L	Effects of VRT-826809 on Misfolded Proteins Other Than CFTR	IND 79521 1/3/2008
D148	L	The Effects of VRT-826809 on Wild-Type Non-CFTR Cellular Proteins	IND 79521 1/3/2008
4.2.1.3 Safety Pharmacology			
B231	I	Effects on Motor Coordination in the Rotarod Test in Sprague Dawley Rats Following a Single Oral Administration of VRT-813077	IND 74633 5/19/2006
B241	I	An In Vivo Telemetry Study of the Effects of VRT-813077 Following a Single Oral Dose in Sprague Dawley Rats	IND 74633 5/19/2006
VRT-813077 -TX-011	I	VRT-813077: Cardiovascular Effects Following Oral (Gavage) Administration in Conscious, Telemetered Beagle Dogs	IND 74633 5/19/2006
VRT-813077 -TX-012	I	VRT-813077: Effects on HERG Tail Current Recorded from Stably Transfected HEK293 Cells	IND 74633 5/19/2006
VRT-813077 -TX-013	I	VRT-813077: Effects in the Irwin Test in Sprague Dawley Rats	IND 74633 5/19/2006
VRT-813077 -TX-014	I	VRT-813077: Effects on Respiration Rate and Tidal Volume in Sprague Dawley Rats	IND 74633 5/19/2006
VRT-813077 -TX-017	I	VRT-813077: Effects on Gastrointestinal Transit of a Charcoal Meal in Sprague Dawley Rats	IND 74633 5/19/2006
VRT-826809 -TX-012	L	VRT-826809: Effects on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	IND 79521 1/3/2008
VRT-826809 -TX-013	L	VRT-826809: Cardiovascular Effects Following Oral (Gavage) Administration in Conscious, Telemetered Beagle Dogs (GLP)	IND 79521 1/3/2008
VRT-826809 -TX-014	L	VRT-826809: Effects of Oral (Gavage) Administration on the Functional Observational Battery in Rats (GLP)	IND 79521 1/3/2008
VRT-826809 -TX-015	L	VRT-826809: Effects of Oral (Gavage) Administration on Respiration Rate and Tidal Volume in Rats (GLP)	IND 79521 1/3/2008
VRT-826809 -TX-016	L	VRT-826809: Effects of Oral (Gavage) Administration on Gastrointestinal Transit of a Charcoal Meal in Rats (GLP)	IND 79521 1/3/2008
4.2.2.2 Absorption			
B177	I	Pharmacokinetics of VRT-813077 in Male Cynomolgus Monkeys following Single Intravenous Administration	IND 74633 5/19/2006
B178	I	Pharmacokinetics of VRT-813077 in Male CD-1 Mice Following Single Intravenous or Oral Administration	IND 74633 5/19/2006
B204	I	Pharmacokinetics of VRT-813077 Following Intravenous and Oral Administration in Male Beagle Dogs	IND 74633 5/19/2006
B239	I	Pharmacokinetics of VRT-813077 in Male Sprague Dawley Rats following Single Intravenous Administration	IND 74633 5/19/2006
B240	I	Pharmacokinetics of VRT-813077 in Male Sprague Dawley Rats following Oral Administration at Seven Dose Levels in Three Formulations	IND 74633 5/19/2006
B256	I	Pharmacokinetics of VRT-813077 in Male Sprague Dawley Rats Following Oral Administration in Suspension and PEG400-based Solutions	IND 74633 5/19/2006

Study #	Test Article	Study Title	Review
C001	I	Pharmacokinetics of VRT-813077 in Male Beagle Dogs Following Oral Administration in Suspension and PEG400-based Solutions	IND 74633 5/19/2006
C021	I	Absorption, Metabolism, Distribution, and Excretion of 14C-VRT-813077 Following Oral and Intravenous Administration to Intact or BDC Rats	IND 74633 5/19/2006
D047	L	Pharmacokinetics of VRT-826809 Following Intravenous and Oral Administration of the HCl Salt in Male Beagle Dogs	IND 79521 1/3/2008
D048	L	Pharmacokinetics of VRT-826809 in Male Cynomolgus Monkeys following Single Intravenous Administration	IND 79521 1/3/2008
D049	L	Pharmacokinetics of VRT-826809 in Male CD-1 Mice Following Single Intravenous Administration	IND 79521 1/3/2008
D081	L	Pharmacokinetics of VRT-826809 in Male Sprague Dawley Rats following Single Intravenous Administration	IND 79521 1/3/2008
D127	L	Pharmacokinetics of VRT-826809 Following Oral Administration in Male Sprague Dawley Rats	IND 79521 1/3/2008
D133	L	Oral Pharmacokinetics of the HCl Salt and Free Form of VRT-826809 and Comparison of the Free Form under Fed and Fasted Conditions in Male Beagle Dogs	IND 79521 1/3/2008
H130	I	In Vitro Assessment of VX-770 as a Substrate of P-glycoprotein	NDA 203188 1/17/2012
VX-770-DMPK -DM-041	I, M1, M6	P-gp Substrate and Inhibitor Assessment of the Customer's Test Compound (VX-770) and Two Metabolites (VRT-837018 and VRT-842917)	NDA 203188 1/17/2012
4.2.2.3 Distribution			
B237	I	In Vitro Binding of VRT-813077 to Plasma Proteins in Mouse, Rat, Dog, Monkey, and Human Plasma	IND 74633 5/19/2006
D051	L	Tissue Distribution of VRT-826809 in Male Sprague Dawley Rats Following Single Oral Administration	IND 79521 1/3/2008
D152	L	In Vitro Binding of VRT-826809 to Plasma Proteins in Mouse, Rat, Dog, Monkey, and Human Plasma	IND 79521 1/3/2008
VX-770-DMPK -DM-046	I	Placental Transfer of 14C-VX-770 Following Administration of a Single Oral Dose to Pregnant Rabbits	NDA 203188 1/17/2012
VX-809-DMPK -DM-021	L	In Vitro Protein Binding of [14C]VX-809 to Mouse, Rat, Rabbit, Dog, and Human Plasma, HSA, AGG, and HGG, and Protein Binding Displacement Interactions between VX-809 and Warfarin	IND 79521 9/24/2010
4.2.2.4 Metabolism			
B202	I	Evaluation of Induction of Liver Cytochrome P450 Isozymes in Male and Female Sprague-Dawley Rats Following Repeated Oral Administration of VRT-813077	IND 74633 5/19/2006
B224	I	Assessment of VRT-813077 Inhibition Potential of Human Cytochrome P450 Isozymes	IND 74633 5/19/2006
B230	I	In Vitro Assessment of Metabolic Stability of VRT-813077: Evaluation of Stability in Liver Subfractions (Microsomes and S9) from Mouse, Rat, Dog, Monkey, and Human and Stability in Hepatocytes from Rat, Dog, Monkey, and Human	IND 74633 5/19/2006

Study #	Test Article	Study Title	Review
B236	I	Identification of VRT-813077 Metabolites from In Vitro and In Vivo Matrices	IND 74633 5/19/2006
B242	I	The In Vitro Stability of VRT-813077 in Human Recombinant CYP450 Isozymes	IND 74633 5/19/2006
D071	L	The In Vitro Stability of VRT-826809 in Human Recombinant CYP450 Isozymes	IND 79521 1/3/2008
D072	L	In Vitro Assessment of Metabolic Stability of VRT-826809 in Hepatocytes from Rat, Dog, Monkey, and Human	IND 79521 1/3/2008
D079	L	Assessment of VRT-826809 Inhibition Potential of Human Cytochrome P450 Isozymes	IND 79521 1/3/2008
D083	L	Characterization of VRT-826809 Putative Metabolites from In Vitro and In Vivo Matrices	IND 79521 1/3/2008
D128	L	Evaluation of Induction of Liver Cytochrome P450 Isozymes in Human Hepatocytes Following In Vitro Exposure of Hepatocytes to VRT-826809	IND 79521 1/3/2008
DMPK-DM-028	L,I	Evaluation of the Induction Potential of VX-809 and VX-661 on Cytochromes P450 in Human Hepatocyte Cultures and Associated Effects on the Metabolism of VX-770	IND 79521 9/1/2011
H191	I, M1, M6	In vitro Metabolic Human Cytochrome P450s Enzyme Mapping for VX-770, VRT-837018, and VRT-842917	NDA 203188 1/17/2012
VX-770-DMPK -DM-038	I, M1, M6	Induction Potential of VX-770 and Metabolites VRT-842917 and VRT-837018 on Cytochromes P450 in Human Hepatocyte Cultures and Cytotoxicity in Cultured Human Hepatocytes	NDA 203188 1/17/2012
VX-770-DMPK -DM-039	I, M1, M6	Inhibitory Potential of VX-770 and Metabolites VRT-842917 and VRT-837018 on Human Hepatic Microsomal Cytochromes P450	NDA 203188 1/17/2012
VX-809-DMPK -DM-018	L	Inhibitory Potential of VX-809 on Human Hepatic Microsomal Cytochromes P450	IND 79521 9/24/2010
VX-809-DMPK -DM-028	L, I	Evaluation of the Induction Potential of VX-809 and VX-661 on Cytochromes P450 in Human Hepatocyte Cultures and Associated Effects on the Metabolism of VX-770	IND 79521 9/1/2011
4.2.2.5 Excretion			
VX-770 - DMPK-DM-043	I	Placental Transfer and Lacteal Excretion of 14C-VX-770 Following Administration of a Single Oral Dose to Pregnant and Lactating Rats	NDA 203188 1/17/2012
4.2.3.1 Single-dose Toxicity			
VRT-826809 -TX-008	L	VRT-826809: Single-dose Oral Toxicity Study in Mice with a 14-day Observation Period (GLP)	IND 79521 1/3/2008
VRT-813077 -TX-007	I	VRT-813077: A Single-dose Oral (Gavage) Toxicity and Toxicokinetics Study in Mice with 2- and 14-day Observation Periods	IND 74633 5/19/2006
VRT-826809 -TX-009	L	VRT-826809: Single-dose Oral Toxicity Study in Rats with a 14-day Observation Period (GLP)	IND 79521 1/3/2008
VRT-813077 -TX-008	I	VRT-813077: A Single-dose Oral (Gavage) Toxicity and Toxicokinetics Study in Rats with 2- and 14-day Observation Periods	IND 74633 5/19/2006

Study #	Test Article	Study Title	Review
		4.2.3.2 Repeat-dose Toxicity	
VX-809-TX-023	L	VX-809: A 28-Day Oral Toxicity and Toxicokinetic Study in Transgenic Mice with a 5-Day Preliminary Range- Finding Toxicity Study (GLP)	IND 79521 11/2/2012 # 1, IND 79521 11/30/2012
VX-809-TX-015	L	VX-809: 3-month Oral (Gavage) Carcinogenicity Range-finding Study in CD-1 Mice	IND 79521 11/2/2012 #1
VX-770-TX-012	I	VX-770: 3-month Oral (Gavage) Carcinogenicity Range-finding Study in Mice	IND 74633 1/16/2009
VRT-826809 -TX-002	L	VRT-826809: A 7-day Oral (BID) Dose Range-finding Toxicity and Toxicokinetic Study in Rats (Non-GLP)	IND 79521 1/3/2008
VRT-826809 -TX-010	L	VRT-826809: A 14-day Oral (Gavage) Toxicity and Toxicokinetic Study in Rats (GLP)	IND 79521 1/3/2008
VX-809-TX-001	L	VX-809: A 28-day Oral (Gavage) Toxicity and Toxicokinetic Study in Rats with a 14-day Recovery Period (GLP)	IND 79521 2/2/2010 #1
VX-809-TX-007	L	VX-809: A 3-month Oral (Gavage) Toxicity and Toxicokinetic Study in Rats with a 1-month Recovery Period	IND 79521 11/2/2012 #2
VRT-813077 -TX-001	I	VRT-813077: A 7-day Oral Toxicity Study in Rats (Non-GLP)	IND 74633 5/19/2006
VRT-813077 -TX-009	I	VRT-813077: 14-day Oral (Gavage) Toxicity and Toxicokinetic Study in Rats (GLP)	IND 74633 5/19/2006
VX-809-TX-009 VX-770-TX-015	L, I	VX-809 and VX-770: A 28-day Oral (Gavage) Combination Toxicity and Toxicokinetic Study in Rats with 14-day Recovery Period	IND 79521 12/10/2013
VX-809-TX-012 VRT-0995096 - TX-006	L, M28	VX-809 and VRT-0095096: A 6-month Oral (Gavage) Toxicity and Toxicokinetic Study in Rats with a 1-month Recovery Period	IND 79521 11/2/2012 #2
VX-770-TX-010	I	VX-770: 6-month Oral (Gavage) Toxicity and Toxicokinetic Study in Rats with a 1-month Recovery Period	IND 74633 1/16/2009
VRT-826809 -TX-004	L	VRT-826809: A 7-Day Oral Dose Range-finding Toxicity Study in Dogs (Non-GLP)	IND 79521 1/3/2008
VRT-826809 -TX-011	L	VRT-826809: 14-day Oral (Gavage) Toxicity and Toxicokinetic Study in Dogs (GLP)	IND 79521 1/3/2008
VX-809-TX-002	L	VX-809: 28-day Oral (Gavage) Toxicity and Toxicokinetic Study in Dogs with a 28-day Recovery Period (GLP)	IND 79521 2/2/2010 #1
VRT-813077 -TX-004	I	VRT-813077: Preliminary 7-day Oral (QD) Toxicity Study in Dogs (Non-GLP)	IND 74633 5/19/2006
VRT-813077 -TX-010	I	VRT-813077: 14-day Oral (Gavage) Toxicity and Toxicokinetic Study in Dogs	IND 74633 5/19/2006
VX-809-TX-010	L, I	VX-809 and VX-770: A 28-day Oral (Gavage) Combination Toxicity and Toxicokinetic Study in Dogs with 14-day Recovery Period	IND 79521 12/10/2013
VX-809-TX-008	L	VX-809: A 3-month Oral (Gavage) Toxicity and Toxicokinetic Study in Dogs with a 1-month Recovery Period	IND 79521 12/10/2013
VX-770-TX-002	I	VX-770: 3-month Oral Toxicity and Toxicokinetic Study in Dogs with a 28-day Recovery Period	IND 74633 6/5/2007

Study #	Test Article	Study Title	Review
VX-809-TX-014	L	VX-809: 12-month Oral Toxicity and Toxicokinetic Study in Dogs with a 6-month Interim Sacrifice and a 1-month Recovery Period	IND 79521 12/10/2013
VX-770-TX-011	I	VX-770: 12-month Oral Toxicity and Toxicokinetic Study in Dogs with a 6-month Interim Sacrifice and a 1-month Recovery Period	IND 74633 2/26/2010
VX-770-TX-001	I	VX-770: 3-month Oral Toxicity and Toxicokinetic Study in Rats with a 28-day Recovery Period (GLP)	IND 74633 6/5/2007
VX-809-TX-013 VX-770-TX-026 VRT-0995096 - TX-005	L, I, M28	VX-809, VX-770, and VRT-0995096: A 3-month Oral (Gavage) Combination Toxicity Study in Rats with a 28-day Recovery Period	IND 79521 12/10/2013
4.2.3.3 Genotoxicity			
VRT-826809-TX-005	L	VRT-826809: Bacterial Reverse Mutation Assay	IND 79521 1/3/2008
VRT-826809-TX-006	L	VRT-826809: In Vitro Mammalian Chromosome Aberration Test	IND 79521 1/3/2008
VRT-813077-TX-003	I	VRT-813077: Bacterial Reverse Mutation Assay	IND 74633 5/19/2006
VRT-813077-TX-005	I	VRT-813077: In vitro Mammalian Chromosome Aberration Test	IND 74633 5/19/2006
VRT-826809-TX-007	L	Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of VRT-826809	IND 79521 1/3/2008
VRT-813077-TX-006	I	VRT-813077: Mammalian Erythrocyte Micronucleus Test	IND 74633 5/19/2006
4.2.3.4 Carcinogenicity			
VX-809-TX-019	L	VX-809: 26-week Repeated Dose Oral Carcinogenicity Study in Tg.RasH2 Mice	IND 79521 11/20/2014
VX-770-TX-013	I	VX-770: A 24-month Oral Carcinogenicity Study in Mice	NDA 203188 1/3/2012
VX-770-TX-014	I	VX-770: A 24-month Oral Carcinogenicity Study in Rats	NDA 203188 1/3/2012
4.2.3.5 Reproductive and Developmental Toxicity			
VX-809-TX-016	L, M28	VX-809 and VRT-0995096: An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation in Rats	IND 79521 10/16/2014
VX-770-TX-008	I	VX-770: Oral (Gavage) Fertility Study in Rats (GLP)	NDA 203188 1/6/2012
VX-809-TX-005	L	Oral (Gavage) Developmental Toxicity Study of VX-809 in Rats	IND 79521 10/16/2014
VX-809-TX-006	L	Oral (Stomach Tube) Developmental Toxicity Study of VX-809 in Rabbits	IND 79521 10/16/2014
VX-770-TX-006	I	VX-770: Oral (Gavage) Developmental Toxicity Study in Rats (GLP)	NDA 203188 1/6/2012
VX-770-TX-007	I	VX-770: Oral (Stomach Tube) Developmental Toxicity Study in Rabbits (GLP)	NDA 203188 1/6/2012
VX-809-TX-017	L, M28	VX-809 / VRT-0995096: Oral (Gavage) Developmental and Perinatal / Postnatal Reproduction Study in Rats	IND 79521 10/16/2014

Study #	Test Article	Study Title	Review
VX-770-TX-009	I	VX-770: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral/Functional Evaluation (GLP)	NDA 203188 1/6/2012
VX-770-TX-024	I	VX-770: An Oral (Gavage) Dose Range-Finding Study in Juvenile Rats	NDA 203188 8/27/2012
VX-770-TX-025	I	VX-770: An Oral (Gavage) Toxicity Study in Juvenile Rats, with Recovery	NDA 203188 8/27/2012
VX-809-TX-003	L	Oral (Gavage) Dosage-Range Developmental Toxicity Study of VX-809 in Rats	IND 79521 10/16/2014
VX-809-TX-004	L	Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of VX-809 in Rabbits	IND 79521 10/16/2014
VX-770-TX-003	I	VX-770: Oral (Gavage) Dosage-Range Finding Developmental Toxicity and Toxicokinetics Study in Pregnant Rats (non-GLP)	NDA 203188 1/6/2012
VX-770-TX-005	I	VX-770: Oral (Stomach Tube) Dosage-Range Developmental Toxicity and Toxicokinetics Study in Rabbits (non-GLP)	NDA 203188 1/6/2012
4.2.3.7 Other Toxicity Studies			
VRT-0995096-TX-003	M28	VRT-0995096: A 28-day Oral (Gavage) Toxicity and Toxicokinetic Study in Rats	IND 79521 11/2/2012 #2
VRT-0995096-TX-001	M28	VRT-0995096: Bacterial Reverse Mutation Assay	IND 79521 11/2/2012 #2
VRT-0995096-TX-002	M28	VRT-0995096: In Vitro Mammalian Chromosome Aberration Test	IND 79521 11/2/2012 #2
VRT-0995096-TX-008	M28	VRT-0995096: An Oral (Gavage) Study of the Effects on Embryo/Fetal Development in Rats	IND 79521 10/16/2014
VX-809-TX-031	L IMP	In Silico Analysis of Process Intermediates and Impurities in the Synthesis of VX-809 for Mutagenic Potential	NDA 206038 2/2/2015
[redacted]			(b) (4)
VRT-(b) (4)-TX-001	L IMP	(b) (4) Bacterial Reverse Mutation Assay	NDA 203188 1/13/2012
VRT-(b) (4)-TX-001	L IMP	(b) (4) Bacterial Reverse Mutation Assay	IND 74633 2/22/2010
VRT-(b) (4)-TX-001	L IMP	(b) (4) Bacterial Reverse Mutation Assay	IND 74633 2/22/2010
VRT-(b) (4)-TX-001	L IMP	(b) (4): Bacterial Reverse Mutation Assay	NDA 203188 1/13/2012

I = ivacaftor / VX-770 / VRT-813077
L = lumacaftor / VX-809 / VRT-826809
M28 = lumacaftor metabolite VRT-0995096
M1 = ivacaftor metabolite VRT-837018
M6 = ivacaftor metabolite VRT-842917
L IMP = other lumacaftor impurities

4 Pharmacology

4.1 Primary Pharmacology

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor (alone or in combination with ivacaftor)
Certain pharmacology studies have been previously reviewed under IND 79521 by Dr. Timothy Robison on January 3, 2008. Updated and newly submitted study reports are review in this section. An integrated summary, including a review of the scientific literature, is provided in **Section 11**.

Effects of VRT-826809 on Protein Conformation, Trafficking and Channel Closing of ΔF508-CFTR
Report #D146 (Version 1: August 8, 2007; Version 2: August 10, 2014)
Study conducted January 2007 – May 2014
Biology Department, Vertex Pharmaceuticals, San Diego, CA
Good Laboratory Practices (GLP) compliance: No

Study D146 was previously reviewed by Dr. Timothy Robison (January 3, 2008), as summarized in Section 11. However, modifications to the study report made by the sponsor and submitted as Version 2 with the NDA are briefly noted below.

In Version 2, the data in the section entitled "Effects of VRT-826809 on the Intrinsic Channel Gating Activity of ΔF508-CFTR" has been completely replaced when compared to Version 1. The experiments presented in each version of the study report involved treatment of cell lines recombinantly expressing F508del CFTR with lumacaftor (with or without ivacaftor) in order to assess effects on channel gating.

In Version 1, recombinant NIH 3T3 cells were used to assess CFTR channel open probability under the following conditions: 1) F508del CFTR, untreated; 2) F508del CFTR, corrected by incubation at 27°C for 24-72 hours; 3) F508del CFTR treated with 3 mcM lumacaftor for 48-72 hours; and 4) WT CFTR. As shown in the sponsor's figure below, treatment with lumacaftor restored F508del CFTR open probability to a level approximately equivalent to that in cells expressing WT CFTR. Note that the temperature-corrected cells were used as a comparator because none of the patches from untreated cells expressing F508del CFTR exhibited functional channel activity.

Figure 3. Effect of Lumacaftor on F508del CFTR Open Probability in 3T3 Cells

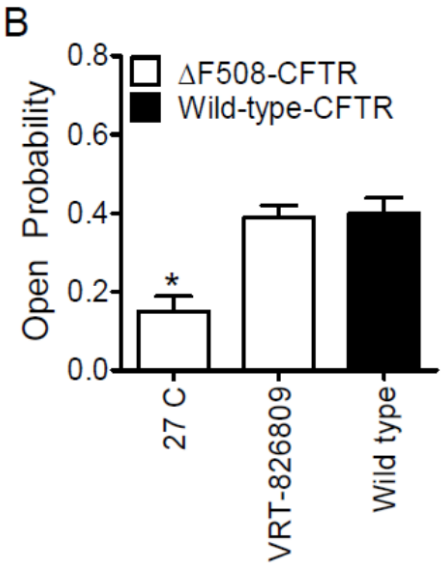
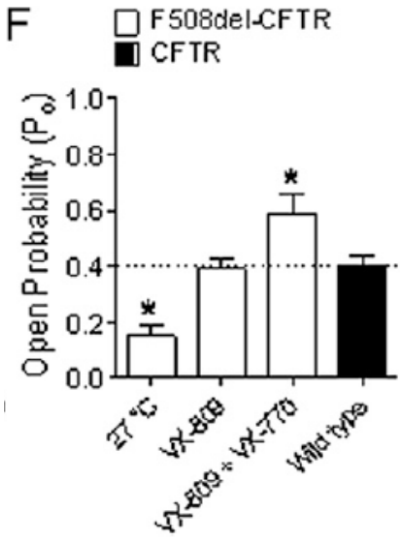


Figure 8-5B from Vertex study D146 (Version 1)

In the sponsor's publication regarding the pharmacology of lumacaftor, the 3T3 data presented (confirmed by the sponsor to be from the same experiment) also included a lumacaftor-ivacaftor combination data point. As shown below, this data suggests that lumacaftor and ivacaftor in combination are superior to lumacaftor alone in terms of increasing F508del CFTR channel open probability in 3T3 cells.[1]

Figure 4. Effect of Lumacaftor With or Without Ivacaftor on F508del CFTR Channel Open Probability in 3T3 Cells



Van Goor et al. Figure 2-F.

[1] Van Goor et al. (2011) Correction of the F508del CFTR protein processing defect in vitro by the investigational drug VX-809. *Proceedings of the National Academy of Sciences*. 108 (46): 18843-18848.

However, in Version 2, the sponsor presents data from separate experiments in Fischer Rat Thyroid (FRT) cells instead of NIH 3T3 cells. The new data presented channel open probability data in recombinant FRT cells under the following conditions: 1) F508del CFTR, untreated; 2) F508del CFTR, pre-treated for undisclosed duration with 3 mcM lumacaftor; 3) F508del CFTR pre-treated with 3 mcM lumacaftor and acutely treated with an unspecified ivacaftor concentration; and 4) WT CFTR. In this study, very low channel open probability was observed in FRT cells expressing F508del CFTR, as expected. Treatment with lumacaftor alone partially restored, and treatment with the lumacaftor-ivacaftor combination fully restored, channel open probabilities to levels equivalent to that observed in cells expressing WT CFTR.

Figure 5. Effect of Lumacaftor on F508del CFTR Open Probability in FRT Cells

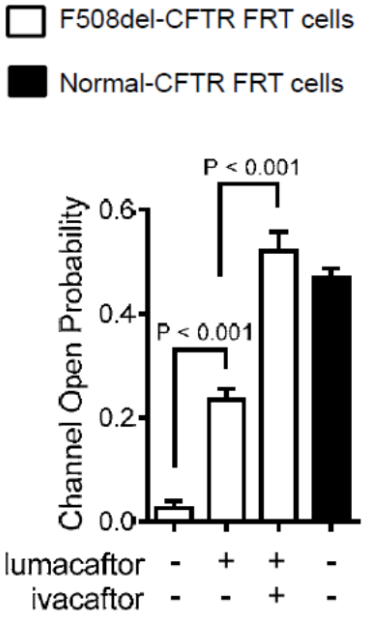


Figure 8-5B from Vertex study D146 (Version 2)

In a response to Information Request received May 1, 2015, the sponsor indicated that the FRT data represented improvements in the experimental methodology compared to the prior 3T3 studies, allowing for more accurate estimation of the total number of channel in the isolated membrane patch. Altogether, the data in both 3T3 and FRT cells support the conclusion that the lumacaftor-ivacaftor combination results in a greater effect on F508del CFTR channel open probability than either agent alone. The sponsor provided the summary table reproduced below.

Table 5. Summary of Lumacaftor-Ivacaftor Single-Channel Patch-Clamp Data

Treatment	CFTR Channel Open Probability mean ± SEM (replicates)			
	NIH-3T3 Cells		FRT Cells	
	CFTR	F508del-CFTR	CFTR	F508del-CFTR
None	0.40 ± 0.04 (6)	No Channels	0.47 ± 0.02 (5)	0.03 ± 0.01 (3)
3 μM LUM (24 – 48 hrs)	Not tested	0.39 ± 0.04 (9)	Not tested	0.23 ± 0.02 (7)
3 μM LUM (24 – 48 hrs) + Acute IVA	0.80 ± 0.04 (6)	0.59 ± 0.07 (3)	0.80 ± 0.06 (5)	0.52 ± 0.04 (7)
27 °C for 24- 48 hrs	Not tested	0.15 ± 0.04 (9)	Not tested	Not tested

Single-channel recordings of channel gating activity in excised inside-out membrane patches isolated from NIH-3T3 or FRT cells expressing normal-CFTR or F508del-CFTR. Prior to recording channel activity, NIH-3T3 or FRT-cells expressing normal CFTR or F508del CFTR were incubated for 24-to-48 hours at 37 °C with vehicle (no pre-treatment) or 3 μM lumacaftor. NIH-3T3 cells expressing F508del-CFTR were also incubated at 27 °C to temperature correct F508del-CFTR. Following activation of CFTR by application of 1 mM adenosine triphosphate and 75 nM protein kinase A to the cytoplasmic surface of the channel (bath solution), channel activity was recorded to quantify the baseline channel open probability, followed by application of 1 or 3 μM ivacaftor to record the response to acute ivacaftor treatment.

Effects of VRT-826809 on CFTR-Mediated Chloride Secretion in Human Bronchial Epithelia Isolated From Cystic Fibrosis Subjects

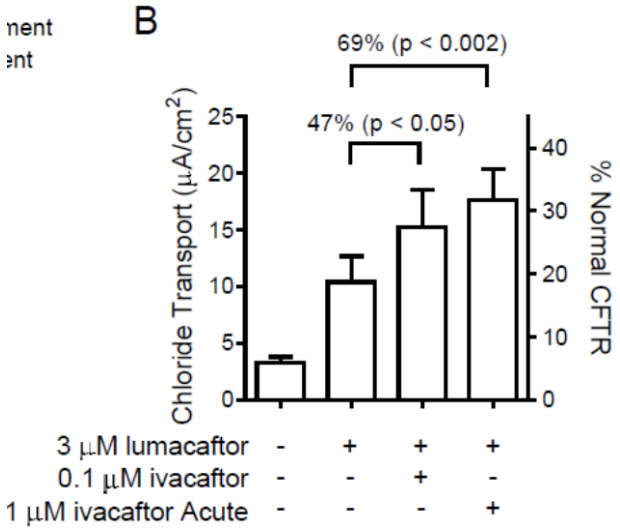
Report #D143 (Version 1: August 8, 2007; Version 2: August 10, 2014; Version 3: February 27, 2015)
Study conducted August 2006 – January 2015
Biology Department, Vertex Pharmaceuticals, San Diego, CA
GLP compliance: No

Study D143 was previously reviewed by Dr. Timothy Robison (January 3, 2008), as summarized in Section 11. However, modifications to the study report were made by the sponsor and submitted to the NDA as Version 2 (November 2014) and Version 3 (March 2015).

In Version 2, the sponsor provided additional data from experiments conducted using HBE from CF donors homozygous for the *F508del CFTR* mutation. Ussing chamber recordings of forskolin-stimulated chloride transport were captured under the following conditions: untreated, lumacaftor (3 mcM for 24 hours), lumacaftor plus "chronic" ivacaftor (100 nM for 24 hours), and lumacaftor plus "acute" ivacaftor (1 mcM). As shown in the sponsor's figure below, treatment with lumacaftor increased chloride transport from 5.8% to 18.6% of normal (WT CFTR). Addition of chronic or acute ivacaftor combination resulted in a further, statistically significant, increase in chloride transport to 27.2 and 31.4% of normal, respectively. In Version 3, the sponsor provided additional data to supplement these results. When the F508del CFTR HBE cells were treated with 100 nM ivacaftor for 24 hours, average forskolin-stimulated chronic transport increased from 3.4 mcA/cm^2 (6.0% of normal) to 4.5 mcA/cm^2 (8.1% of normal). Taken together, these data provide support for the hypothesis that the

complementary mechanisms of action of lumacaftor and ivacaftor combine to increase chloride transport in F508del CFTR HBE cells to a greater degree than either agent alone.

Figure 6. Effect of Lumacaftor and Ivacaftor on Chloride Transport in F508del/F508del HBE cells



Vertex study D143, Version 2, Figure 8-8B

The sponsor conducted additional studies of the effect of ivacaftor on chloride transport in F508del CFTE HBE cells in the monotherapy development program under IND 74633 and NDA 203188.[2] These experiments involved the more typical use of acute ivacaftor treatment at 1 mcM to potentiate CFTR activity, compared to the continuous low-dose ivacaftor treatment described above. In an Information Request dated April 24, 2015, the sponsor was requested to provide a summary of all relevant in vitro studies of the effect of ivacaftor, lumacaftor and the combination on chloride transport in F508del CFTR HBE cells. The sponsor summary table, provided in a response dated May 1, 2015, is reproduced below.

[2] Vertex study B229 and Van Goor et al. (2009) *Proceedings of the National Academy of Sciences*. 106 (44): 18825-18830.

Table 6. Summary of Experiments Evaluating the Effects of Lumacaftor and Ivacaftor on Chloride Transport in F508del CFTR HBE Cells

	Study	Chloride Transport	Normal HBE	F508del/F508del-HBE					
				No Treatment	LUM [b]	IVA [c] (continuous)	IVA [d] (acute)	LUM [b] + IVA [c] (continuous)	LUM [b]+ IVA [d] (acute)
1	Vertex Study D143, Table 8-3 "Study 1" [a]	Raw Data ($\mu A/cm^2$)	56 ± 6	1.9 ± 0.4	7.8 ± 1.3	-	4.8 ± 1.1	-	13.9 ± 3.9
		% WT	100 ± 11	3.4 ± 0.7	13.9 ± 2.3	-	8.5 ± 2.0	-	25.1 ± 6.9
		# Donors	4	7	7	-	7	-	7
2	Vertex Study D143, Table 8-3 "Study 2"	Raw Data ($\mu A/cm^2$)	56 ± 6	3.3 ± 0.5	10.4 ± 2.3	4.5 ± 0.5	-	15.3 ± 3.3	17.6 ± 2.8
		% WT	100 ± 11	6.0 ± 0.7	18.6 ± 4.1	8.1 ± 0.4	-	27.2 ± 5.8	31.4 ± 5.0
		# Donors	4	4	4	6	-	4	4
3	Vertex Study B229	Raw Data ($\mu A/cm^2$)	56 ± 6	-	-	-	-		
		% WT	100 ± 11	-	-	-	7 – 12		
		# Donors	4	-	-	-	4		
4	Vertex Study Van Goor et al., PNAS 2009 [e]	Raw Data ($\mu A/cm^2$)	56 ± 6	2.2 ± 0.9	-	-	9.0 ± 1.0	-	-
		% WT	100 ± 11	4.0 ± 0.6	-	-	16.0 ± 4.0	-	-
		# Donors	4	6	-	-	6	-	-

[a] Data presented in Table 8-3 "study 1" was published in Van Goor et al., 2011 PNAS
[b] Pretreatment with 3 µM lumacaftor (LUM)
[c] Continuous ivacaftor treatment; 100 nM ivacaftor was added during the 24 hour pretreatment and throughout the Ussing chamber recording. In the presence of 100 nM ivacaftor, amiloride was added to block the epithelial sodium channel, followed by sequential addition of 10 µM forskolin and a CFTR inhibitor. To quantify the total CFTR-mediated chloride current, the difference between the peak response to forskolin and the steady-state current following addition of the CFTR inhibitor was quantified and expressed as mA/cm2 or as a percentage of the 10 µM forskolin-stimulated response in normal HBE (% normal).
[d] Acute ivacaftor treatment; 1 µM ivacaftor was added following activation of the channel with 10 µM forskolin. The experimental protocol and the quantification of chloride transport were similar to that described in c, with the exception that ivacaftor was not added during the pre-treatment period.

Notwithstanding the limitations of cross-study comparisons, the aggregate data provided by the sponsor support the following conclusions:

- Both ivacaftor and lumacaftor alone increase chloride transport in HBE cells derived from CF patients homozygous for the *F508del CFTR* mutation.
- The combination of ivacaftor and lumacaftor results in a greater effect on F508del CFTR chloride transport than either monoproduct alone.
- The HBE cell data have limited translation to the clinical outcomes in the lumacaftor-ivacaftor clinical development program (e.g., lumacaftor has a robust in vitro effect but decreased pulmonary function in CF patients).

Effect of VX-809 and VX-770 on airway surface liquid height and cilia beat frequency in HBE cells isolated from people who are F508 del homozygous
Report #K248 (February 13, 2014)
Study conducted April – December 2013
Biology Department, Vertex Pharmaceuticals, San Diego, CA
GLP compliance: No

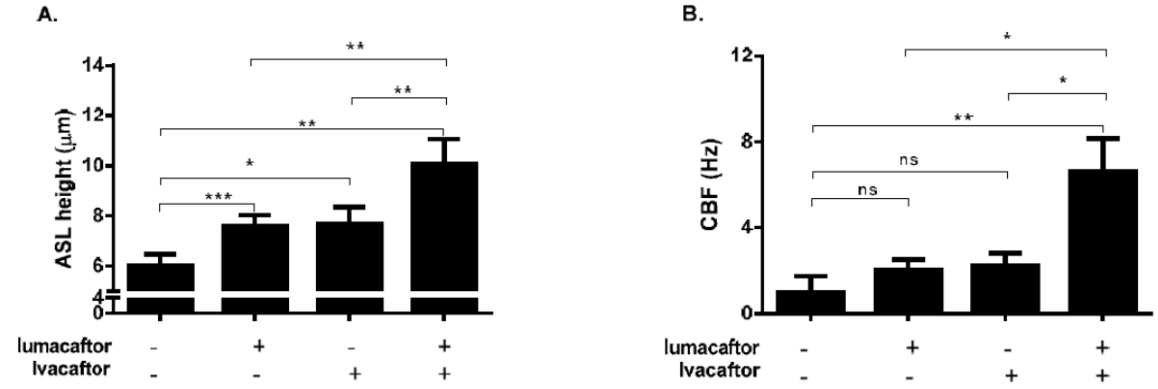
Methods

This study evaluated the effects of lumacaftor and ivacaftor on airway surface liquid (ASL) height and cilia beat frequency (CBF) in HBE cells isolated from five CF donors homozygous for the *F508del CFTR* mutation. Experiments were performed in the presence of 30 nM vasoactive intestinal peptide.

Results

After treatment with 3 mcM lumacaftor or 3 mcM ivacaftor for 72 hours, there was a statistically significant increase in ASL height, but no significant effect on CBF. Combined treatment with lumacaftor plus ivacaftor resulted in statistically significant increases in ASL height and CBF as compared to vehicle controls and each individual test article.

Figure 7. Effect of Lumacaftor and Ivacaftor on ASL and CBF in F508del/F508del HBE cells



Vertex study K248, Figure 9-1.

VX-809 directly and specifically interacts with MSD1 domain of CFTR
Report #K124 (August 30, 2014)
Study conducted: July 2014
Biology Department, Vertex Pharmaceuticals, San Diego, CA
GLP compliance: No

Methods

Plasmids expressing a 6xHis-tagged MSD1 domain of CFTR (aa 1-437), 6xHis MSD2 (aa 837-1172) or full-length F508del CFTR were stably expressed in HEK293 or Sf9 cells. The interaction of lumacaftor with CFTR expressed by these cells lines was investigated in photoaffinity labeling studies utilizing a radioactive benzophenone derivative of lumacaftor (VRT-1121619) and cold competitor analogs. In addition, the functional effect in terms of correcting the F508del CFTR trafficking defect was evaluated via immunoblotting (detection of CFTR B and C or His-tagged MSD1) and Ussing chamber experiments (measurement of chloride transport).

Results

The sponsor first demonstrated that VRT-1121619 functioned as a CFTR "(b) (4)" by demonstrating that the photoactive analog could induce increases in CFTR maturation, 6xHis-MSD1 protein levels and chloride transport commensurate with lumacaftor. Molecular weight profiling of labeled protein species after incubation of cell lysate from Sf9 cells expressing MSD1 with VRT-1121619 with or without 20-fold excess of cold or inactive analog indicated that the interaction with MSD1 was specific and selective. Experiments with other plasmid constructs confirmed that the regulatory insertion (aa 401-436) was not required for interaction and that VRT-1121619 did not bind to the MSD2 region. Finally, the sponsor demonstrated that lumacaftor competes with VRT-1121619 for interaction with MSD1 in a dose-dependent manner.

4.2 Secondary Pharmacology

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
Refer to nonclinical review filed to IND 79521 by Dr. Timothy Robison on January 3, 2008.

Lumacaftor-ivacaftor combination
No secondary pharmacology studies were conducted with the combination of lumacaftor plus ivacaftor.

4.3 Safety Pharmacology

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
Lumacaftor was evaluated in an in vitro human ether-à-go-go related gene (hERG) assay, central nervous system, respiratory and gastrointestinal safety pharmacology studies in rats, and a cardiovascular pharmacology study in dogs. These studies have been previously reviewed under IND 79521 by Dr. Timothy Robison on January 3, 2008.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
An extensive review of pharmacokinetics, including absorption, distribution, metabolism and elimination (ADME) studies with lumacaftor has been conducted previously by Dr. Timothy Robison and filed to IND 79521 on January 3, 2008. Certain additional studies are reviewed below.

Absorption, Distribution, Metabolism, and Excretion of ^{14}C-VX-809 Following Oral and Intravenous Administration to Rats
Report #6536-451 (April 29, 2008)
Study conducted
(b) (4)
GLP compliance and QA statement: Yes

Methods

Sprague-Dawley and pigmented Long Evans rats received ^{14}C-lumacaftor by oral or intravenous (IV) administration as shown in the table below. The animals in Group 1 were bile duct-cannulated by the supplier. All animals were observed for mortality and clinical condition.

Table 7. Rat ADME Study Design

Group	Number of Animals		Strain	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)[b]	Samples Collected
	Male	Female					
1	4[a]	4[a]	SD	Oral	30	5	Urine, Feces, Bile, Expired Air, and Carcass
2	27	27	SD	Oral	30	5	Blood
3	27	27	SD	IV	3	1	Blood
4	18	NA	SD	Oral	30	5	Blood and Tissues
5	18	NA	LE	Oral	30	5	Blood and Tissues

For excretion analysis, the following samples were collected from Group 1 animals:

- Urine at intervals: 0-4 hours, 4-8 hours, 8-24 hours and then every 24 hours through 168 hours post-dose.
- Feces at 24-hour intervals through 168 hours post-dose.
- Bile at intervals: 0-4 hours, 4-8 hours, 8-24 hours and then every 24 hours through 168 hours post-dose.
- Expired air at 24-hour intervals through 168 hours post-dose

Blood samples for measurement of plasma TK parameters were collected from Group 2 and 3 animals at 5 minutes (IV only), 0.25, 0.5 (oral only), 1, 2, 4, 8, 24, 48 and 72 hours post-dose. Group 4 and 5 animals were sacrificed for tissue distribution analysis at 1, 4, 24, 48, 72 and 168 hours post-dose (3 animals per time point). The following tissues were collected from Group 4 animals (bold font denotes tissues also collected from Group 5 animals):

- Adrenal glands
- Bladder (urinary)
- **Blood**
- Bone (both femurs)
- Bone marrow (from both femurs)
- Brain
- **Eyes (both)**
- Fat (reproductive)
- Heart
- **Kidneys**
- Large intestine including cecum
- (without contents)
- **Liver**
- Lungs
- Lymph nodes (mesenteric)
- Muscle (thigh)
- Pancreas
- **Plasma**
- Prostate
- Salivary glands
- **Skin (dorsal, shaved)**
- Small intestine (without contents)
- **Spleen**
- Stomach (without contents)
- **Testes**
- Thymus
- Thyroid

Results

After a single oral dose in bile duct-cannulated rats, essentially all radioactivity was recovered with <1% in urine, 62% in feces, and 38% in bile. The majority of radioactivity (79-87%) in urine, feces and bile was unchanged ^{14}C-lumacaftor, with the glucuronide metabolite being the next most abundant species (6-10%). In plasma, unchanged

parent represented ≥93% of radioactivity at early time points after oral or IV administration, decreasing to 57% and 74% at 24 hours post-dose in males and females, respectively.

After a single oral dose in Sprague-Dawley or Long Evans rats, radioactivity was distributed throughout the body with T_{max} generally occurring at 4 hours post-dose. Only in the liver was C_{max} greater than in plasma, and there was no evidence that ^{14}C-lumacaftor bound to melanin.

Pharmacokinetic parameters were determined for lumacaftor after single oral and IV doses. These results are consistent with previously reviewed studies and are summarized in the sponsor's table below.

Table 8. Rat ADME Study PK Results

Sex	T_{max} (Hour)	C_{max} (ng/g)	$t_{1/2}$ (Hour)	AUC $_{0-t}$ (ng·h/g)	$AUC_{0-\infty}$ (ng·h/g)	%F
			Group 2 (Oral)			
Male	2.00	66200	6.46	410000	410000	69.7
Female	2.00	66100	7.88	743000	743000	92.2
			Group 3 (IV)			
Male	0.083	24600	8.62	58900	58900	NA
Female	0.083	26500	8.05	80500	80600	NA

Bronchoalveolar Lavage Fluid and Epithelial Lining Fluid Concentrations of VX-809 Following Oral Administration to Male Sprague Dawley Rats

Report #I353 (November 12, 2012)
Study conducted July – August 2007
Biology Department, Vertex Pharmaceuticals, San Diego, CA
GLP compliance: No

Methods

Male Sprague-Dawley rats received single oral doses of lumacaftor at 30, 100 or 300 mg/kg (N=3 per group). Samples were collected after 4 hours to assess the concentration of lumacaftor in plasma, bronchoalveolar lavage fluid (BALF) and epithelial lining fluid (ELF) by LC/MS/MS.

Results

Lumacaftor exposure in the lungs was ~18% of plasma levels at all doses evaluated. Exposure in the ELF was about 1-2% of that in lung tissue, with even lower concentrations noted in the BALF.

Table 9. Distribution of Lumacaftor to the Lungs in Male Rats

Dose (mg/kg)	Plasma (ng/mL)	Lung (ng/mL)	BALF (ng/mL)	ELF (ng/mL)	Lung/ Plasma	ELF/Lung (%)	ELF Penetation*
30	30800 (12300)	5430 (1670)	25.2 (5.08)	617 (101)	0.18 (0.04)	1.40 (0.28)	150 (76.0)
100	95100 (48400)	15730 (5990)	220 (141)	860 (792)	0.17 (0.03)	2.38 (2.21)	370 (458)
300	130000 (20400)	23400 (289)	91.4 (46.4)	2210 (597)	0.18 (.03)	1.19 (0.389)	117 (45.1)

n=3, vehicle: 0.5% MC/0.5% SLS/water, 5 mL/kg
*Elf penetration = concentration of drug in ELF/free fraction of drug in plasma[1]
Table excerpted from sponsor report

Placental Transfer and Lacteal Excretion of ^{14}C-VX-809 Following Administration of a Single Oral Dose to Pregnant and Lactating Rats

Report #K045 (May 7, 2014)
Study conducted September – December 2013
(b) (4)
GLP compliance and QA statement: Yes

Methods

A total of 25 pregnant female Sprague-Dawley rats received a single oral 100 mg/kg (100 mcCi/kg) dose of ^{14}C-lumacaftor on Gestation Day (GD) 13 (N=5), GD 18 (N=5), or postpartum day 9-11 (N=15). Animals were observed daily for mortality and clinical condition. The animals dosed on GD 13 and GD 18 were prepared for quantitative whole-body autoradiography (QWBA) with one animal per group at each time point (2, 6, 12, 24 and 48 hours post-dose). For the postpartum dosing group, milk was collected from 3 animals per time point at 1, 2, 4, 8 and 24 hours post-dose. In addition, blood samples were collected from all animals to determine plasma lumacaftor concentrations.

Results

After dosing on GD 13 or GD 18, radioactivity was detected in most maternal tissues by 2 hours post-dose and reached maximal concentrations by 12 hours, with the highest levels (~2 times plasma concentrations) seen in the liver. In general, other tissue: plasma ratios were <1 and radioactivity was cleared from tissues by 48 hours post-dose. Quantifiable radioactivity was also present in fetuses, indicative of placental transfer. Radiolabeled lumacaftor was rapidly excreted into milk and was detectable at each time point from 1-24 hours post-dose at 22-43% of plasma concentrations.

Placental Transfer of VX-809 Following Administration of a Single Oral Dose to Pregnant Rabbits
Report #K046 (April 10, 2014)
Study conducted October – November 2013
(b) (4)
GLP compliance and QA statement: Yes

Methods

15 timed-pregnant New Zealand White rabbits received a single oral dose of 50 mg/kg lumacaftor on GD 25. Animals were observed daily for mortality and clinical condition. Three does were sacrificed per time point at 1, 4, 8, 12 and 36 hours post-dose. Maternal and fetal blood samples were collected for determination of plasma concentrations of lumacaftor.

Results

Lumacaftor exposure was detected in maternal and fetal plasma with T_{max} of 1 and 8 hours, respectively. The elimination half-life of lumacaftor was calculated as ~10 and 15 hours in maternal and fetal plasma, respectively. The study indicated that lumacaftor transfer across the placental barrier occurs in pregnant rabbits, leading to fetal plasma exposure that was ~20% of that observed in maternal plasma.

5.2 Toxicokinetics

Toxicokinetic parameters were evaluated in conjunction with the review of toxicology studies, as referenced elsewhere in this review.

6 General Toxicology

6.1 Single-Dose Toxicity

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
Single-dose toxicity studies with lumacaftor were conducted in mice and rats. Refer to nonclinical review filed by Dr. Timothy Robison to IND 79521 on January 3, 2008.

Lumacaftor-ivacaftor combination
No single-dose toxicity studies were conducted with the combination of lumacaftor plus ivacaftor.

6.2 Repeat-Dose Toxicity

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
Repeat-dose toxicity studies with lumacaftor were conducted in mice, rats and dogs. These studies have been reviewed previously under IND 79521 as follows:

- 28-day Tg.RasH2 mouse study (Dr. Jane Sohn, November 2, 2012 and November 30, 2012)
- 14-day rat and 14-day dog studies (Dr. Timothy Robison, January 3, 2008)
- 28-day rat and 28-day dog studies (Dr. Timothy W. Robison, February 2, 2010; not included in appendices)
- 3- and 6-month rat studies (Dr. Timothy Robison, November 2, 2012)
- 3-, 6- and 12-month dog studies (Dr. Timothy Robison, December 10, 2013)

Lumacaftor-ivacaftor combination
Repeat-dose studies evaluating the combination of lumacaftor plus ivacaftor were conducted in rats (28-day and 3-month) and dogs (28-day). These studies have been reviewed previously under IND 79521 by Dr. Timothy Robison on February 2, 2010 (not included in appendices) and December 10, 2013.

7 Genetic Toxicology

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
Lumacaftor was evaluated in a bacterial reverse mutation assay, an in vitro mammalian chromosomal aberration assay, and a mouse bone marrow erythrocyte micronucleus assay. These studies have been previously reviewed under IND 79521 by Dr. Timothy Robison on January 3, 2008. Also, refer to nonclinical review filed by Dr. Andrew Goodwin to NDA 206038 on February 2, 2015 in response to a consultation request from reviewing Chemist Dr. Edwin Jao.

Lumacaftor-ivacaftor combination
No genetic toxicology studies were conducted with the combination of lumacaftor plus ivacaftor.

8 Carcinogenicity

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
Refer to nonclinical review filed by Dr. Andrew Goodwin to IND 79521 on November 20, 2014 evaluating the results of the 6-month carcinogenicity study in Tg.RasH2 transgenic mice. A two-year carcinogenicity study in Sprague-Dawley rats is ongoing and will be submitted as a PMR (see **Section 2.7**).

Lumacaftor-ivacaftor combination
No carcinogenicity studies were conducted with the combination of lumacaftor plus ivacaftor.

9 Reproductive and Developmental Toxicology

Ivacaftor
A complete pharmacology and toxicology program with ivacaftor was reviewed previously under NDA 203188. Refer to nonclinical review filed by Dr. Marcie Wood on January 17, 2012.

Lumacaftor
Lumacaftor was evaluated in a fertility and early embryonic development study in rats, embryo-fetal development studies in rats and rabbits, and a peri-/post-natal development study in rats. These studies have previously been reviewed under IND 79521 by Dr. Andrew Goodwin on October 16, 2014.

Lumacaftor-ivacaftor combination
No reproductive and developmental toxicology studies were conducted with the combination of lumacaftor plus ivacaftor.

10 Special Toxicology Studies

Not applicable. No *Special Toxicology* studies were considered pivotal to the evaluation of the nonclinical safety evaluation of NDA 206038.

11 Integrated Summary and Safety Evaluation

Vertex Pharmaceuticals has submitted NDA 206038 for ORKAMBI, a fixed-dose combination product containing lumacaftor and ivacaftor co-formulated in tablets for oral administration. The product's proposed indication is the treatment of CF in patients who are homozygous for the *F508del CFTR* mutation. ORKAMBI is administered as two tablets twice daily separated by 12 hours, for a total daily dose of 800 mg lumacaftor and 500 mg ivacaftor.

Ivacaftor is FDA-approved as a monoproduct therapy for CF patients with certain *CFTR* mutations (*G551D*, *G1244E*, *G1349D*, *G178R*, *G551S*, *S1251N*, *S1255P*, *S549N*, *S549R*, and *R117H*) under the trade name KALYDECO. The sponsor completed a full nonclinical program evaluating ivacaftor, including pharmacology, safety pharmacology, ADME, general toxicology, genetic toxicology, carcinogenicity, and reproductive and developmental toxicology. These studies were reviewed under NDA 203188 and considered to be adequate to characterize the pharmacological and toxicological profile of ivacaftor in support of the proposed clinical use at a daily dose of 300 mg.

To support NDA 206038, the sponsor has completed a full nonclinical program evaluating lumacaftor, including pharmacology, safety pharmacology, ADME, general toxicology, genetic toxicology, and reproductive and developmental toxicology. The sponsor has completed a 6-month carcinogenicity study in transgenic mice, but the two-year carcinogenicity study in rats remaining outstanding. As discussed in **Section 2.7**, DPARP agreed that the rat study could be submitted as a PMR. In addition, the sponsor has conducted in vitro pharmacology assays and general toxicology studies in rats and dogs evaluating the lumacaftor-ivacaftor combination.

The remainder of the **Integrated Summary and Safety Evaluation** is organized as follows:

11.1. Lumacaftor pharmacology and toxicology
11.2. Review of studies with the lumacaftor-ivacaftor combination
11.3. Brief summary of ivacaftor pharmacology and toxicology
11.4 Nonclinical Recommendation
11.5. Labeling review and recommended edits

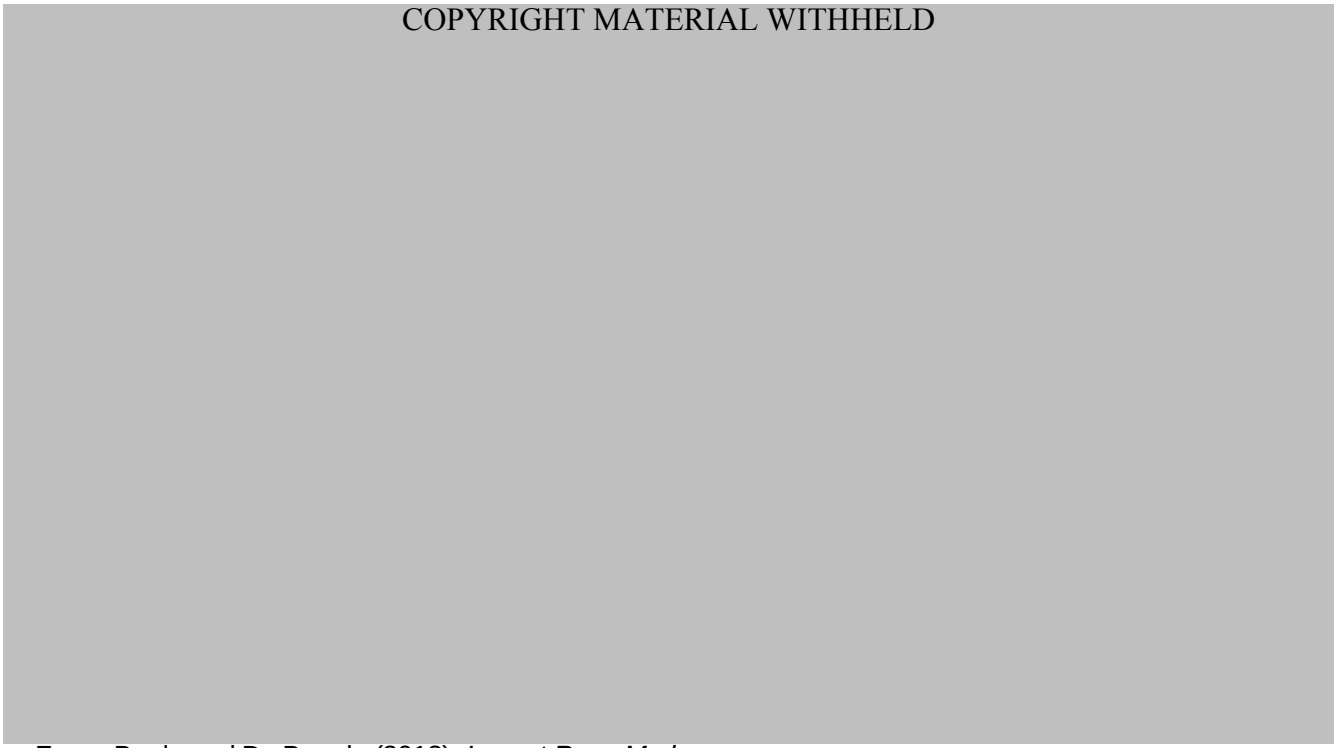
11.1 Lumacaftor

Primary Pharmacology

Introduction: CFTR and F508del mutation
The cystic fibrosis transmembrane conductance regulator (CFTR) plays a crucial homeostatic role in the maintenance of the airway surface liquid layer of the lungs. A spectrum of >1900 mutations in the *CFTR* gene have been described which result in reduced quantity of cell-surface CFTR protein and/or impaired functional chloride channel activity, leading to CF. The single most common mutation is the deletion of a phenylalanine residue at position 508 (referred to as *F508del*). 90% of CF patients

harbor at least one *F508del CFTR* mutation and approximately half are *F508del* homozygotes.[3]

Figure 8. Summary of CFTR Mutation Classes and Associated Defects

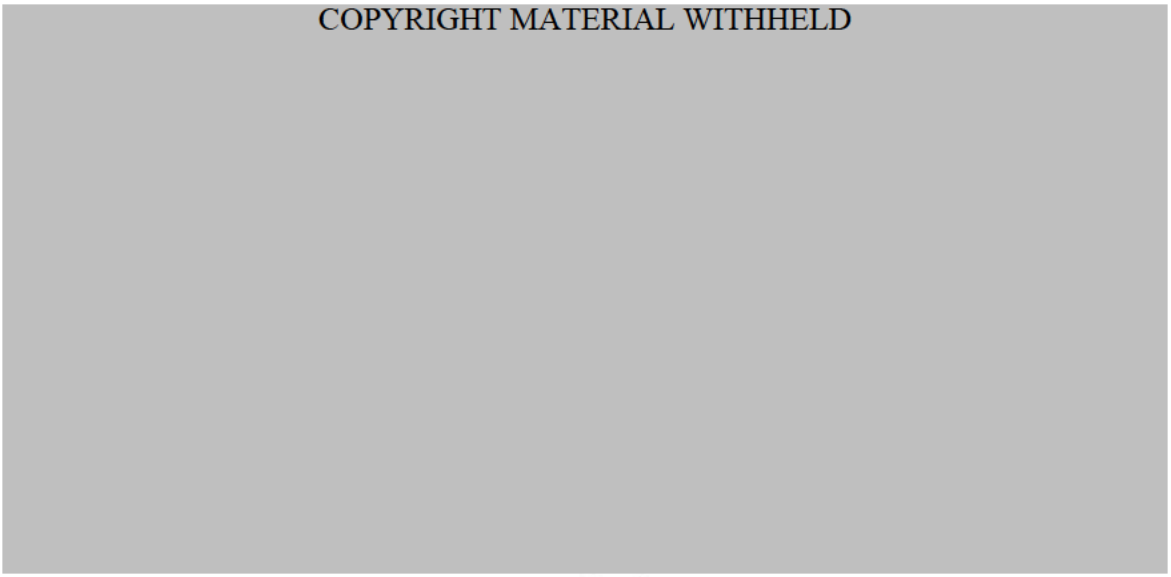


From: Boyle and De Boeck. (2013). *Lancet Resp Med*.

While the full CFTR structure has not been solved, there is some information available regarding the organization and conformation of the protein. CFTR is a member of the ATP-binding cassette (ABC) superfamily, with nucleotide-binding domains (NBDs), membrane-spanning domains (MSDs, or transmembrane domains, TMDs), and a regulatory (R) domain. Noncovalent interactions between the MSDs and NBDs via the intracellular loops (ICLs) are critical for CFTR structure and function. However, CFTR is unique in that, unlike typical ABC family members, it functions as a channel for chloride diffusion down its concentration gradient and not as an active transporter. The F508 residue is localized in NBD1, and deletion of F508del is believed affect the folding and stability of this domain, interaction with the ICLs, conformation of the MSDs, and domain-domain assembly of the CFTR protein.[4,5,6,7]

[3] Boyle and De Boeck. (2013) A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect. *Lancet Resp Med*. 1 (2): 158-163.
[4] Chiaw et al. (2011). Insights into the mechanisms underlying the CFTR channel activity, the molecular basis for cystic fibrosis and strategies for therapy. *Essays Biochem*. 50: 233-248.
[5] Lukacs and Verkman. (2012). CFTR: folding, misfolding and correcting the ΔF508 conformational defect. *Trends in Molecular Medicine*. 18 (2): 81-91.
[6] Du et al. (2005). The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. *Nature Struct Mol Biol*. 12 (1): 17-25.

Figure 9. Structural Organization of the CFTR Protein



From: Chiaw et al. (2011). *Essays Biochem.*

Wild-type and *F508del CFTR* genes are transcribed, translated into CFTR polypeptides, inserted in the ER and subject to core glycosylation. At this stage, the folding status of the ~135-140 kDa immature form of CFTR (termed "Band B" in immunoblotting studies) is subject to ER quality control assessment governed by calnexin, heat shock proteins and other mediators. As an integral membrane protein, wild-type CFTR is trafficked through the Golgi complex, further glycosylated to form the mature ~170-180 kDa "Band C", and subsequently inserted into the plasma membrane. In contrast, F508del CFTR exhibits incorrect folding, which results in retention in the ER and targeting for degradation via the ubiquitin-proteasome pathway.[8] Consequently, little F508del CFTR reaches the cell surface and that which does has impaired stability and gating activity compared to wild-type CFTR (Study D146). A study employed proteomic analysis of microsomal WT and F508del CFTR via small-angle x-ray scattering demonstrated that the protein complex associated with the mutant protein was altered in both composition and conformation.[9] In addition, F508del CFTR exhibits impaired phosphorylation of the R domain by protein kinase A, which is required for channel function.[10]

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[7] Okiyoneda et al. (2014). Mechanism-based corrector combination restores ΔF508-CFTR folding and function. *Nature Chemical Biology*. 9: 444-454.
[8] Farinha et al. (2013). Control of cystic fibrosis transmembrane conductance regulator membrane trafficking: not just from the endoplasmic reticulum to the Golgi. *FEBS Journal*. 280 (18): 4396-4406.
[9] Baroni et al. (2014). Functional and pharmacological induced structural changes of the cystic fibrosis transmembrane conductance regulator in the membrane solved using SAXS. *Cell Mol Life Sci*. 72 (7): 1363-1375.
[10] Pasyk et al. (2015). The major cystic fibrosis causing mutation exhibits defective propensity for phosphorylation. *Proteomics*. 15: 447-461.

Mechanism of action of lumacaftor on F508del CFTR
The sponsor refers to lumacaftor as a [redacted] and proposes that the drugs affects the cellular processing and trafficking of F508del CFTR, resulting in an increased amount of protein being delivered to the cell surface. The widespread and longstanding use of the term [redacted] in the pharmaceutical, academic and CF communities to describe pharmacological agents such as lumacaftor is noted. However, the assignment of potential EPC for lumacaftor is still under discussion by the review team. [redacted]. Any reference to the term "[redacted]" in this review should be not be interpreted to represent a decision to assign this term as the EPC for lumacaftor.

In recombinant HEK293 cells, 3 mcM lumacaftor decreased the proteolytic sensitivity of F508del CFTR, indicating that drug treatment allowed a fraction of the mutant protein population to attain a WT-like conformation. Further, exposure of F508del HBE cells to increasing concentrations of lumacaftor resulted in generation of the mature Band C CFTR (EC_{50} = 100 nM) and increased chloride secretion. Likewise, in HEK293 cells expressing F508del CFTR, lumacaftor treatment induced a six-fold increase in the formation of Band C, representing approximately 34% maturation efficiency compared to WT CFTR. The effect of lumacaftor on CFTR gating activity was assessed via single-channel recordings in NIH-3T3 and FRT cells expressing F508del CFTR. Results indicated that treatment with lumacaftor increased the density of F508del CFTR at the cell surface and partially (FRT) or fully (3T3) restored channel opening probability of lumacaftor-treated F508del CFTR to WT levels. Finally, Ussing chamber recordings of chloride transport in HBE cells over time demonstrated that lumacaftor increased the half-life of F508del CFTR to levels comparable to WT, indicative of cell surface stability and resistance to internalization and degradation.[11] Additional experiments published by the sponsor showed that lumacaftor did not affect the stability of immature F508del CFTR or inhibit the activity of the 16s proteasome.[12]

The effect of lumacaftor on CFTR-mediated chloride secretion was further evaluated in Ussing chamber studies of HBE isolated from CF donor lungs. After 48-hour exposure to lumacaftor, chloride secretion in homozygous F508del CFTR isolates increased six-fold to ~14% of WT levels with EC_{50} and EC_{90} values of 94 and 631 nM, respectively (though potency was five-fold lower in the presence of 20% human serum which may be more representative of in vivo effects). Based on a study of the correlation between clinical disease severity and in vitro chloride section across a spectrum of CFTR mutations, the sponsor asserts that achieving at least 10% of WT chloride secretion may be clinically meaningful.[13]

In a study investigating the interaction of a photoreactive derivative of lumacaftor with a variety of recombinant CFTR fragments, the sponsor demonstrated that lumacaftor

[11] Vertex study D146
[12] Van Goor et al. (2011) Correction of the F508del CFTR protein processing defect in vitro by the investigational drug VX-809. *Proceedings of the National Academy of Sciences*. 108 (46): 18843-18848.
[13] Vertex studies D143 and D144

interacts specifically and directly with and stabilizes the MSD1 region of CFTR.[14] An expanded description of these and related studies was subsequently published by the sponsor and collaborators. This study showed that lumacaftor altered the protein conformation of MSD1 to suppress folding defects and be more resistance to ER-associated degradation. The paper identified key residues required for MSD1 to acquire a conformation which could be stabilized by lumacaftor, but did not identify the specific binding site. By evaluating the effects of numerous other mutations, the authors demonstrated that various changes to CFTR conformation and maturation had substantial impact on the efficacy of lumacaftor.[15] A separate study confirmed that lumacaftor interacted with and stabilized MSD1, but not other CFTR domains, to facilitate rescue of CFTR maturation.[16]

Farinha and colleagues utilized lumacaftor, chemical chaperones and genetic CFTR revertants to characterize the molecular mechanism of action of lumacaftor. Lumacaftor was additive to chemical chaperones (VRT-325, Corr-4a), temperature correction, and genetic revertants in terms of rescue of CFTR maturation, indicating complementary mechanisms of action. He and colleagues presented data (inconsistent with the sponsor's) suggesting that lumacaftor-rescued F508del CFTR has a shorter half-life and decreased thermal stability compared to WT-CFTR. Nonetheless, the paper showed that while lumacaftor leads to the maturation of only a small fraction of CFTR, the assembled protein's conformation was similar to WT in terms of resistance to proteolysis and restoration of domain-domain cysteine cross-linking. A separate proteomic study of microsomal CFTR complexes reported that lumacaftor modified the F508 del CFTR-associated protein complex, but did not restore the WT conformation. Interestingly, ligand binding modeling studies by both Farinha and He identified the NBD1:ICL4 interface of F508del CFTR as a putative lumacaftor binding site.[17,18,19]

Okiyoneda and colleagues investigated lumacaftor in studies involving the characterization of the molecular mechanisms of action of pharmacological "(b) (4) and genetic F508del CFTR rescue mutations. Lumacaftor was categorized as a (b) (4) based on its ability to stabilize the NBD1-MSD2 interface rather than the NBD1 domain. Based on in silico docking studies combined with mutational experiments, the authors propose that lumacaftor targets the interdomain interface between NBD1 and MSD1 (via ICL1) and MSD2 (via ICL4). This target site overlaps

[14] Vertex study K124
[15] Ren et al. (2013) VX-809 corrects folding defects in cystic fibrosis transmembrane conductance regulator protein through action on membrane-spanning domain 1. *Molecular Biology of the Cell*. 24: 3016-3024.
[16] Loo et al. (2013) Corrector VX-809 stabilizes the first transmembrane domain of CFTR. *Biochemical Pharmacology*. 86: 612-619.
[17] Farinha et al. (2013). Revertants, Low Temperature, and Correctors Reveal the Mechanism of F508del CFTR Rescue by VX-809 and Suggest Multiple Agents for Full Correction. *Chemistry & Biology*. 20: 943-955.
[18] He et al. (2013). Correctors of ΔF508 CFTR restore global conformational maturation without thermally stabilizing the mutant protein. *FASEB Journal*. 27: 536-545.
[19] Baroni et al. (2014). Functional and pharmacological induced structural changes of the cystic fibrosis transmembrane conductance regulator in the membrane solved using SAXS. *Cell Mol Life Sci*. 72 (7): 1363-1375.

with that identified by the Farinha and He papers, and as shown by the sponsor, Ren and Loo, the presence of the MSD1 domain was critical for the activity of lumacaftor. Overall, the authors proposed a model in which lumacaftor "binds MSD1 and stabilizes the [I]CL1-[I]CL4 coupling helix, a critical step to form the proper interactions of NBD1, first with MSD1 then with MSD2, and ultimately facilitating cooperative domain assembly upon completion of NBD2 translation."[20]

The studies described above employed lumacaftor, other pharmacological chaperones, chemical or temperature correction, and additional *CFTR* mutations known to affect CFTR conformation, stability and trafficking. Likewise, synergistic effects of lumacaftor and other correctors have been described by other groups.[21,22] The results highlight that individual corrector molecules can act to restore different F508del CFTR defects, supporting the notion that improved rescue of CFTR function may be achieved with a combination approach.

As detailed earlier, the sponsor's data suggests that lumacaftor-rescued F508del CFTR at the cell surface exhibits increased stability. A publication by Eckford and colleagues proposes that this phenomenon results from a direct interaction of lumacaftor with cell surface CFTR, in addition to the co-translational effects on folding and trafficking of F508del CFTR. The authors demonstrated that lumacaftor or a structurally related compound (C18, VRT-534) directly binds membrane-associated F508del CFTR, increases channel activity, and decreases susceptibility to protein unfolding and aggregation. When lumacaftor was added to BHK cells in which maturation of F508del CFTR had been induced by temperature correction, the rate of degradation of "Band C" protein was diminished. Further, acute exposure of temperature-corrected F508del CFTR to lumacaftor resulted in increased channel activity in BHK cells and patient-derived organoids, suggesting "potentiator" type activity. [23] This result is in contrast to the sponsor's data in study D143 with acute lumacaftor treatment of F508del CFTR HBE cells. The experiments were notably different based on 1) the low temperature treatment used by Eckford and colleagues to increase cell surface F508del CFTR prior to the acute lumacaftor exposure and 2) the corresponding fact that the studies showing potentiator-like activity of lumacaftor were also conducted at the lower 27˚C temperature.

Na^+/H^+ exchanger regulatory factor 1 (NHERF1) is a scaffolding protein that has been shown to link CFTR to the actin cytoskeleton and affects its phosphorylation status. The interaction of NHERF1 with F508del CFTR is approximately five-fold weaker than with

[20] Okiyoneda et al. (2014). Mechanism-based corrector combination restores ΔF508-CFTR folding and function. *Nature Chemical Biology*. 9: 444-454.
[21] Boinot et al. (2014). Searching for Combinations of Small-Molecule Correctors to Restore F508del–Cystic Fibrosis Transmembrane Conductance Regulator Function and Processing. *The Journal of Pharmacology and Experimental Therapeutics*. 350: 624-634.
[22] Phuan et al. (2014). Synergy-Based Small-Molecule Screen Using a Human Lung Epithelial Cell Line Yields DF508-CFTR Correctors That Augment VX-809 Maximal Efficacy. *Molecular Pharmacology*. 86: 42-51.
[23] Eckford et al. (2014). VX-809 and Related Corrector Compounds Exhibit Secondary Activity Stabilizing Active F508del CFTR after Its Partial Rescue to the Cell Surface. *Chemistry & Biology*. 21: 666-678.

WT-CFTR, and overexpression of this protein was shown to decrease turnover of cell surface F508del CFTR. Treatment with lumacaftor was shown to specifically mediate increased cell-surface F508del CFTR stability (via preservation of functional activity and decreased diffusiveness) and binding to NHERF1 in an HEK293 cell system. The authors also demonstrated lumacaftor-medicated protein-protein interactions between F508del CFTR and NHERF1 in organoids derived from mouse crypt intestinal stem cells via a proximal ligation assay.[24]

The sponsor also conducted studies aimed at evaluating the specificity of the activity of lumacaftor. Since some chemical chaperones have been demonstrated to correct other proteins with processing defects in addition to F508del CFTR, the activity of lumacaftor on other WT or misfolded proteins was assessed. While the panel was small, there was no evidence that lumacaftor increased the cell surface expression of proteins that utilize similar trafficking pathways, were members of the same superfamily, or have other defects causing ER arrest.[25]

The reviewer identified one paper in the scientific literature which purported to show that lumacaftor acted to restore trafficking of a protein other than CFTR, the kidney anion exchanger 1 (kAE1). The G701D kAE1 mutant is retained in the Golgi and can cause distal renal tubular acidosis. Lumacaftor treatment (3 mcM in 0.4% DMSO) increased cell surface G701D kAE1 levels by immunostaining. However, several concerns were noted including 1) lumacaftor actually decreased functional activity of C701D kAE1 and 2) the results appear confounded by the activity of the DMSO vehicle as a chemical chaperone and an inducer of kAE1 protein synthesis.[26] While the limitations of the "off-target" studies described above are noted, the reviewer considered the data in this manuscript inadequate to raise concern about the specificity of the activity of lumacaftor for the CFTR protein.

In summary, the mechanism of action of lumacaftor has been extensively studied, but key details such as the specific binding site on F508del-CFTR remain to be elucidated. Unraveling the molecular details is complicated by technical challenges such as limited protein structure information, as well as by the fact that interaction of corrector molecules at one site on F508del CFTR may exert allosteric effects on folding or interactions elsewhere in the protein assembly. Nonetheless, the collective data indicate that the activity of lumacaftor is dependent on the specific interaction of lumacaftor with the CFTR protein. Lumacaftor stabilizes the interface between the MSD1 and NBD1 domains to alleviate folding defects and shifting the balance from ER retention and degradation towards increased maturation and delivery to the plasma membrane. Intriguingly, the recent studies by Eckford and Arora also suggest activity of lumacaftor on cell surface F508del CFTR to further improve stability and potentiate chloride transport activity.

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[24] Arora et al. (2014). Stabilizing Rescued Surface-Localized ΔF508 CFTR by Potentiation of Its Interaction with Na^+/H^+ Exchanger Regulatory Factor 1. *Biochemistry*. 53: 4169-4179.
[25] Vertex studies D147 and D148
[26] Chu et al. (2013) Functional Rescue of a Kidney Anion Exchanger 1 Trafficking Mutant in Renal Epithelial Cells. *PLOS One*. 8 (2): e57062.

Secondary Pharmacology

Lumacaftor was evaluated in two secondary pharmacology studies in which radioligand assays were employed to evaluate binding to a collection of enzyme, receptor and channel targets. The only significant finding at a concentration of 10 mcM was a 69-75% inhibition of the thromboxane A_2 receptor. A follow-up study conducted in rat aorta rings indicated that lumacaftor acts as a thromboxane A_2 receptor antagonist.[27]

Safety Pharmacology

There were no notable effects of lumacaftor compared to vehicle (0.5% Tween-80 and 0.5% methylcellulose in water) controls in a battery of GLP-compliant neurological, cardiovascular, respiratory and gastrointestinal safety pharmacology studies.[28]

Potential central nervous system effects were assessed in male Sprague-Dawley rats after single oral doses of 250, 500 or 1000 mg/kg lumacaftor. No effects were observed in any Functional Observation Battery parameter at 4 or 10 hours post-dose. Increased motor activity was noted 4 hours post-dose at ≥500 mg/kg/day and 10 hours post-dose at all doses, but the effects were not statistically significant vs. the vehicle control.

Lumacaftor had no effects in a pair of cardiovascular safety pharmacology studies. There were no test article-related effects on hERG channel current in an in vitro assay in HEK293 cells at concentrations up to 4.6 mcM (higher doses could not be achieved due to solubility issues). Likewise, lumacaftor had no effect on heart rate, blood pressure or any ECG parameter (PR, QRS, RR, QT, and QTc intervals) in conscious male Beagle dogs after single oral doses of up to 200 mg/kg.

Respiratory effects were assessed in male Sprague-Dawley rats after single oral doses of 250, 500 or 1000 mg/kg lumacaftor. There were no effects on respiratory rate, tidal volume or minute volume at time points ranging from 1 to 24 hours post-dose compared to the vehicle control.

Gastrointestinal effects were assessed in male Sprague-Dawley rats after single oral doses of 250, 500 or 1000 mg/kg lumacaftor. There was no test article-related effect on the intestinal transit of a charcoal meal.

ADME

The absorption, distribution, metabolism and elimination (ADME) of lumacaftor was evaluated in mice, rats, dogs, monkeys and in vitro studies.

The half-life of lumacaftor was ~2 to 17 hours in mice, rats, dogs and monkeys after IV administration and ~2 to 8 hours in rats and dogs after oral administration. When

[27] Vertex studies 1083320-1025114, 1083320-1025509 and D145
[28] Vertex studies VRT-826809-TX-012, -TX-013, -TX-014, -TX-015 and –TX-016

lumacaftor was administered orally, there were no food effects and bioavailability was 47-131% in rats (generally lower with higher doses) and 24-49% in dogs. Lumacaftor was highly protein bound (>99%) in mouse, rat, dog, monkey and human plasma. Following a single oral dose of 10 or 75 mg/kg to male rats, the highest lumacaftor concentrations were noted in the plasma and liver (1.2 tissue: plasma ratio), followed by the lung (0.37), pancreas (0.17) and brain (0.03). In a separate study in male rats at 30, 100 or 300 mg/kg, lung: plasma exposure ratios were 0.17-0.18 and lumacaftor concentrations in bronchoalveolar lavage fluid and epithelial lung fluid were <2% of that in lung tissue.[29]

In pregnant rats, radiolabeled lumacaftor was detected in most maternal tissues by 2 hours post-dose and was cleared from tissues within 48 hours. Radioactivity was also detected in fetuses, indicating that placental transfer occurred. In rats, lumacaftor was detected in milk 1 to 24 hours post-dose at concentrations ranging from 22-43% of those observed in plasma at the same time point. Placental transfer of lumacaftor was also observed in pregnant rabbits, leading to fetal plasma exposure that was ~20% of maternal levels.[30]

Lumacaftor was slowly metabolized in vitro in dog, monkey and human hepatocytes and was stable in rat hepatocytes. Predicted clearance was low (≤10%) compared to hepatic blood flow. Studies indicated that lumacaftor was metabolized by human CYP 3A4 and did not inhibit a panel of CYP enzymes (IC_{50} >30 mcM) in vitro. In human hepatocytes, lumacaftor induced the activity of CYPs 1A2 and 3A4. The role of lumacaftor as a CYP 3A4 inducer was shown to be important clinically, as exposure to ivacaftor (a CYP 3A4 substrate) is significantly reduced when administered in combination with lumacaftor.[31]

The metabolism of lumacaftor was characterized using rat, dog, monkey and human in vitro systems. Metabolites M1 (methyl alcohol) and M2 (glucuronide conjugate) were the primary metabolites identified after incubation with liver microsomes and cryopreserved hepatocytes, respectively. M2 was the most abundant metabolite in vivo after oral administration of lumacaftor to rats and dogs.[32]

In a phase 1 clinical study (VX08-809-004), the sponsor identified M28 (hydroxyl-pyrrolidone) as a human-specific metabolite which comprised 13% of total exposure after a single 200 mg lumacaftor dose in healthy male subjects. M28 was initially designated as a disproportionate human metabolite and was therefore assessed in certain genetic toxicology, general toxicology, reproductive toxicology and carcinogenicity studies. M28 was administered in the 6-month rat general toxicology study and the NOAEL was considered as 25 mg/kg/day. The exposure at the NOAEL provided a safety margin of at least 25-fold compared to the proposed clinical dose on an AUC basis, which is sufficient to qualify the safety of the metabolite. However, as development proceeded and the clinical dose of lumacaftor increase to 800 mg daily, the contribution of M28 to total human exposure decreased to ~7%. Therefore, the

[29] Vertex studies D152 and D051
[30] Vertex studies K045 and K046

nonclinical studies with M28 were not considered pivotal to the safety evaluation of lumacaftor and are not discussed further in this review.

A small fraction (≤1%) of the administered lumacaftor dose was excreted unchanged in the bile and urine of rats (non-bile duct-cannulated) or the urine of dogs. The M2 glucuronide was the predominant form in rat and dog urine, and was present at ~72 times higher levels than the parent in rat bile. After a single oral dose in bile duct-cannulated rats, essentially all radioactivity was recovered with <1% in urine, 62% in feces, and 38% in bile. The majority of radioactivity (79-87%) in urine, feces and bile was unchanged ^{14}C-lumacaftor, with the glucuronide metabolite being the next most abundant species (6-10%).[33]

The table below summarizes comparative pharmacokinetic properties of lumacaftor in mice, rats, dogs and monkeys.

Table 10. Cross-Species Comparison of Pharmacokinetic Parameters

Species	Route	Dose (mg/kg)	C_{max} (mcg/mL)	AUC (mcg*hr/mL)	Clearance (mL/min/kg)	$T_{1/2}$ (hr)	V_{ss} (L/kg)	F (%)
Mouse	IV	2.5	13000	27723	1.4	4.3	0.4	
Rat	IV	1-5	7.1-24.6	19.2-62.3	0.8-1.6	5.9-7.9	0.4-0.7	
	Oral	1-600	1.1-203	13.7-3085		5.9-7.7		47-131
Dog	IV	0.2-1.0	1.6-8.3	1.08-6.37	2.4-2.5	1.6-3.5	0.2-0.3	
	Oral	1.0-200	0.37-101	2.19-523		4.9-8.8		24-49
Monkey	IV	1.0	7.7	21.0	0.7	16.7	0.9	

Adapted from review by Dr. Timothy Robison, January 3, 2008

General Toxicology

Pivotal general toxicology studies with lumacaftor were conducted in rats (up to 6 months) and dogs (up to 12 months).

In the 26-week toxicology study, Sprague-Dawley rats received lumacaftor by oral gavage at doses of 250, 500, or 1000 mg/kg/day in combination with the M28 metabolite at a dose of 25 mg/kg/day. There were no test article-related deaths or effects on body weights, hematology, coagulation or clinical pathology parameters. Histopathological examination did not identify any target organs of toxicity. Lumacaftor C_{max} and AUC increased in a less than dose-proportional manner. The NOAEL was considered as the high-dose of 1000 mg/kg/day lumacaftor plus 25 mg/kg/day M28, corresponding to lumacaftor exposure of 1300 and 3160 ug*hr/mL in males and females, respectively.[34]

[31] Vertex studies D072, D071, D079 and D128
[32] Vertex study D083
[33] Vertex studies D083 and 6536-451
[34] Vertex study VX-809-TX-012

The results of a 3-month rat study are briefly noted. In animals receiving lumacaftor at a dose of 2000 mg/kg/day (2-fold higher than in the chronic study), there was no effect on survival but body weight gains were reduced ~20% vs. controls. Target organs were identified as the liver (hepatocellular hypertrophy, a rodent-specific adaptive response[35]) and spleen (extramedullary hematopoiesis, considered a compensatory response to red blood cell depletion) but these findings were not considered dose-limiting.[36]

In the 12-month Beagle dog study with an interim 6-month sacrifice, animals received lumacaftor by oral gavage at doses of 125, 250 or 500 mg/kg/day. There were no unscheduled deaths in the study. Clinical signs included abnormal stool at all doses and vomiting at the high-dose. Test article-related effects on hematological parameters at ≥250 mg/kg/day, consisting of decreased red blood cells counts, reticulocytes, hemoglobin and hematocrit as well as increased platelet counts, were considered to be clinically monitorable. Serum biochemistry parameters including cholesterol, triglycerides, total protein, albumin and globulin were decreased in all treatment groups but the effects were not considered adverse. Histopathological examination did not reveal any target organs of toxicity and therefore the NOAEL was considered as the high-dose level of 500 mg/kg/day. Lumacaftor exposure increased in a dose-proportional manner from 125 to 500 mg/kg/day, and 2- to 4-fold accumulation was noted over the course of the 12-month study. At the NOAEL of 500 mg/kg/day, lumacaftor AUC values were 750 and 860 ug*hr/mL in males and females, respectively.[37]

The results of a previous 3-month dog study are briefly noted. In animals receiving lumacaftor at a dose of 1000 mg/kg/day (2-fold higher than in the chronic study), 3 of 12 animals were sacrificed prematurely in moribund condition. Clinical signs in the premature decedents and other animals in the 1000 mg/kg/day dose group included irregular gait, jerky movements and muscle rigidity. Histopathological findings were noted at 1000 mg/kg/day in the thymus (exacerbation of lymphoid depletion), male reproductive organs (delayed maturation of testes and prostate, sloughed germ cells in epididymides) and liver (extramedullary hematopoiesis). The NOAEL in the 3-month study was considered as 500 mg/kg/day, consistent with the 6-/12-month study described above.[38]

Genetic Toxicology

Lumacaftor exhibited no potential for genotoxicity in a battery of scientifically valid and GLP-compliant genotoxicity assessments including a bacterial reverse mutation assay, an in vitro mammalian chromosome aberration assay, and an in vivo micronucleus assay.[39]

[35] Thoolen et.al. (2010) Proliferative and Nonproliferative Lesions of the Rat and Mouse Hepatobiliary System. *Toxicological Pathology*. 38: 5S-81S.
[36] Vertex study VX-809-TX-007
[37] Vertex study VX-809-TX-014
[38] Vertex study VX-809-TX-008
[39] Vertex studies VRT-826809-TX-005, -TX-006, and -TX-007

A bacterial reverse mutation assay was conducted in *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A at lumacaftor doses of up to 5000 mcg per plate, with or without metabolic activation via addition of S9 mix. There were no test article-related increases in revertant colonies and the study was considered to be negative.

A mammalian chromosomal aberration assay was conducted in Chinese hamster ovary (CHO) cells. The effects of lumacaftor concentrations of up to 250 mcg/mL were evaluated after 4 hours (with or without metabolic activation by S9 mix) and concentrations up to 125 mcg/mL were evaluated after 20 hours (without metabolic activation). There were no toxicologically significant increases in the number of cells with structural aberrations under any of the conditions tested.

A bone marrow erythrocyte micronucleus assay was conducted in which male ICR mice received a single oral dose of 500, 1000 or 2000 mg/kg lumacaftor and were sacrificed 24 hours (all doses) or 48 hours (2000 mg/kg only) later. Clinical signs at the 2000 mg/kg dose included lethargy and piloerection. There was no evidence of bone marrow toxicity or increased incidence of micronucleated polychromatic erythrocytes compared to animals receiving the vehicle control.

Carcinogenicity

Lumacaftor was evaluated in a 6-month oral carcinogenicity study in Tg.RasH2 transgenic mice. Dose levels were 200, 700 and 2000 mg/kg/day in males and 200, 500 and 1500 mg/kg/day in females. The CDER Executive Carcinogenicity Assessment Committee (ECAC) provided its concurrence with the design of the study via a Special Protocol Assessment (SPA). There were no test article-related effects on survival or neoplastic findings in the study.[40]

The sponsor is also conducting a two-year oral carcinogenicity study in Sprague-Dawley rats. Dose levels in males and females were set at 75, 150 and 1000 mg/kg/day lumacaftor and 25 mg/kg/day VRT-0995096 (M28 metabolite). The ECAC provided its concurrence with the study design via an SPA. As discussed above, the division agreed that the results of the two-year rat study were not required to be included in the NDA submission. A PMR will be implemented regarding the completion of the study and submission of the results for review and incorporation into the product labeling.

Reproductive Toxicology

Lumacaftor was not associated with any adverse effects in developmental and reproductive toxicology studies, including male / female fertility, embryofetal survival, teratogenicity, and peri-/post-natal development and sexual maturation.[41]

[40] Vertex study VX-809-TX-019
[41] Vertex studies VX-809-TX-005, -TX-006, -TX-016 and –TX-017

Male fertility, female fertility and early embryonic development were evaluated in a Sprague-Dawley rat study in which lumacaftor was administered in combination with a 20 mg/kg/day dose of the M28 metabolite. There was no effect on fertility in males receiving up to 1000 mg/kg/day lumacaftor for 28 days prior to mating. There was no effect on fertility or early embryonic development in females receiving up to 1000 mg/kg/day lumacaftor from 14 days prior to mating through GD 7.

There was no evidence of maternal or embryofetal toxicity in a Sprague-Dawley rat study in which females received up to 2000 mg/kg/day lumacaftor from GD 7 to GD 17.

An embryo-fetal development study rabbits was conducted in which females received lumacaftor at doses of 50, 100 or 200 mg/kg/day from GD 7 to GD 19. Excessive maternal toxicity was observed at ≥100 mg/kg/day including premature deaths, body weight loss, and abortions. There was no effect on embryofetal survival and no evidence of teratogenicity in the study at any dose level.

Peri- and post-natal development was evaluated in a Sprague-Dawley rat study in which lumacaftor was administered in combination with a 20 mg/kg/day dose of the M28 metabolite. F_0 females received lumacaftor at doses of up to 1000 mg/kg/day from GD 6 through parturition and continuing for 21 days thereafter. The development and reproductive performance of F_1 offspring was assessed and the status of F_2 litters was evaluated by Cesarean section of F_1 dams at GD 14. There was no evidence of any adverse effects on F_0 maternal health and reproduction, F_1 survival and development, or F_2 litter viability in the study.

11.2 Lumacaftor-Ivacaftor Combination

Primary Pharmacology

The sponsor investigated the effect of the lumacaftor-ivacaftor combination on the function of F508del CFTR in several in vitro systems. Lumacaftor monotherapy treatment led to partial improvement in FRT cells and fully restored channel open probability to WT levels in 3T3 cells. In each cell line, combination of lumacaftor with ivacaftor resulted in a greater increase in channel open probability compared to results with lumacaftor alone. In HBE isolated from CF donors homozygous for the F508del mutation, the increases in chloride transport, airway surface liquid height and cilia beat frequency were all greater with combined lumacaftor-ivacaftor treatment than with either individual test article alone.[42,43]

Two recent publications also evaluated the pharmacological interaction of lumacaftor and ivacaftor. In F508del CFTR HBE cells, “chronic” (48 hour) treatment with 5 mcM each lumacaftor and ivacaftor abrogated the lumacaftor-mediated increase F508del CFTR chloride transport. This effect was attributed to increased turnover and decreased

[42] Vertex studies D143 (Version 3), D146 (Version 2) and K248
[43] Van Goor et al. (2011) Correction of the F508del CFTR protein processing defect in vitro by the investigational drug VX-809. *Proceedings of the National Academy of Sciences*. 108 (46): 18843-18848.

protein half-life in the presence of ivacaftor. In addition to counteracting the effects of lumacaftor, prolonged treatment with ivacaftor decreased basal intracellular maturation, cell surface levels, stability and functional activity of F508del CFTR.[44,45] The relevance of these findings in the context of chronic clinical treatment with ORKAMBI is uncertain.

General Toxicology

As described above, the sponsor conducted complete nonclinical development programs for ivacaftor and lumacaftor, the two active ingredients in ORKAMBI. In addition, the sponsor evaluated the toxicity of the lumacaftor-ivacaftor combination in rats (28-day and 3-month studies) and dogs (28-day study). Novel findings in the combination studies were noted in both rats (stomach necrosis / erosion) and dogs (ECG changes and histopathological findings in male reproductive organs).

In the 28-day rat study, animals received the combination by oral gavage at doses of 100/25, 300/50, 1000/50, and 1000/100 mg/kg/day (shown as lumacaftor / ivacaftor). The major target organs were identified as the kidneys (increased incidence of chronic progressive nephropathy) and stomach (glandular mucosal erosion). The kidney effects were judged to represent the exacerbation of a rat-specific finding[46] which has little or no relevance to humans. The stomach findings (minimal to moderate, focal or multifocal erosions without significant inflammatory response) were noted in a fraction mid-high dose females and high-dose males and females. The sponsor did not perform histopathological assessment of animals sacrificed after a 14-day recovery period, so no assessment of reversibility is possible. Based on the proposed indication of cystic fibrosis and the judgment of the Medical Officer that these findings were clinically monitorable, the NOAEL was considered as the high-dose of 1000 mg/kg lumacaftor and 100 mg/kg ivacaftor per day. Lumacaftor exposure increased in a less than dose-proportional manner. As noted above, lumacaftor enhances the metabolism of ivacaftor and as a result, ivacaftor exposure in the 300/50 dose group was equal to or greater than that in the 1000/50 and 1000/100 dose groups. At the NOAEL, lumacaftor exposure was 2830 (males) and 3410 (females) mcg*hr/mL and ivacaftor exposure was 285 (males) and 479 (females) mcg*hr/mL.[47]

In the 3-month rat study, animals received lumacaftor / ivacaftor / M28 by oral gavage at doses of 500/10/10, 500/25/10, 1000/25/20, and 1000/100/20 mg/kg/day. Consistent with the 28-day study, test article-related findings were noted in the stomach and kidneys. An increased incidence of basophilic tubules was observed in the kidneys of males and females at the 1000/100/20 mg/kg/day dose level, but was not considered dose-limiting and is of uncertain relevance to humans. In the glandular stomach, findings of focal necrosis and/or erosion with edema and inflammatory cell infiltrate were

[44] Cholon et al. (2014) Potentiator ivacaftor abrogates pharmacological correction of ΔF508 CFTR in cystic fibrosis. *Science Translational Medicine*. 6 (246): 246ra96.
[45] Veit et la. (2014) Some gating potentiators, including VX-770, diminish ΔF508-CFTR functional expression. *Science Translational Medicine*. 6 (246): 246ra97.
[46] Hard and Khan. (2004) A contemporary review of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. *Toxicol Pathol*. 32 (2): 171-180.
[47] Vertex study VX-809-TX-009

noted without a consistent relationship to dose. The findings of gastric mucosal necrosis / erosion were reversible following a 28-day recovery period. As noted above, the stomach toxicity was considered to be clinically monitorable in cystic fibrosis patients. The observation of bilateral posterior subcapsular cataracts of the lens in one 1000/100/20 mg/kg/day female was attributed to treatment with ivacaftor. Based on the prior observation of cataracts in the ivacaftor juvenile rat study, there is ongoing clinical concern regarding this risk in children with CF who are treated with KALYDECO. However, the isolated cataract finding in the combination study was judged to represent an acceptable risk for the proposed ORKAMBI patient population of CF patients age 12 years and older. The potential ocular toxicity is considered clinically monitorable, but not reversible. Exposure at the 1000/100/20 mg/kg/day dose level for lumacaftor was 2430 (males) and 3010 (females) mcg*hr/mL and for ivacaftor was 372 (males) and 653 (females) mcg*hr/mL.[48]

In the 28-day dog study, animals received the lumacaftor-ivacaftor combination by oral gavage at doses of 300/5, 300/15, 600/15 and 600/60 mg/kg/day. ECG changes attributed to the lumacaftor-ivacaftor combination included prolonged PR intervals (first degree AV block) in 3/12 and 10/12 animals at the 600/15 mg/kg and 600/60 mg/kg dose levels, respectively. In addition, early depolarization (SVPC) was noted in the majority of animals at the highest dose level. At the 600/60 mg/kg/day dose level, a spectrum of male reproductive findings were identified in the testes (increased multinucleated degenerate germ cells), epididymides (sloughed testicular germ cells) and prostate (acinar contraction, decreased secretion, lower organ weights). These findings were reversible following a 14-day recovery period and were judged to represent a slight retardation of sexual maturation, which may have been secondary to decreased body weight gains. At the NOAEL of 600/15 mg/kg/day, lumacaftor exposure was 503 (males) and 976 (females) mcg*hr/mL and ivacaftor exposure was 124 (males) and 102 (females) mcg*hr/mL.[49]

The nonclinical studies conducted in rats and dogs with ivacaftor, lumacaftor and the combination provide adequate nonclinical support for the proposed clinical dose level of 800 mg lumacaftor and 500 mg ivacaftor per day. Note that while the nominal daily ivacaftor dose is higher in ORKAMBI (500 mg) than in KALYDECO (300 mg), the exposure is substantially lower due to the drug-drug interaction described above, leading to increased safety margins on an AUC basis.

[48] Vertex study VX-809-TX-013
[49] Vertex study VX-809-TX-010

Table 11. Nonclinical Safety Margins (AUC Basis) for ORKAMBI

Species (Study)	Dose (mg/kg/day)		Exposure (mcg*hr/mL)		Safety Margin (AUC basis)	
	LUMA	IVA	LUMA	IVA	LUMA	IVA
Human (Phase 3 studies in CF patients*)	13.3[a]	8.3[a]	429[b]	3.4[b]	-	-
Rat (6-month lumacaftor study)	1000	-	1300 (M) 3160 (F)	-	3.0 (M) 7.4 (F)	-
Rat (6-month ivacaftor study)	-	50	-	445 (M) 561 (F)	-	131 (M) 165 (F)
Rat (3-month combination study)	1000	100	2430 (M) 3010 (F)	372 (M) 653 (F)	5.7 (M) 7.0 (F)	109 (M) 192 (F)
Dog (12-month lumacaftor study)	500	-	750 (M) 860 (F)	-	1.7 (M) 2.0 (F)	-
Dog (12-month ivacaftor study)	-	60	-	351 (M) 254 (F)	-	103 (M) 75 (F)
Dog (28-day combination study)	600	15	503 (M) 976 (F)	124 (M) 102 (F)	1.2 (M) 2.3 (F)	36 (M) 30 (F)

LUMA: Lumacaftor; IVA: ivacaftor
[a] Converted from 800 mg LUMA, 500 mg IVA daily dose based on 60 kg human weight
[b] Human exposure values represent median $AUC_{0\text{-}24hr}$ for phase 3 clinical trials -103 and -104 from population PK study K050, as provided by Clinical Pharmacology reviewers Dr. Jianmeng Chen and Anshu Marathe.

11.3 Ivacaftor

The pharmacological and toxicological profile of ivacaftor was characterized under NDA 203188, and the reader is referred to Dr. Marcie Wood's nonclinical review for a more detailed summary. Ivacaftor is a first-in-class drug with a novel mechanism of action as a CFTR potentiator. The small molecular drug acts upon the CFTR protein to increase the channel open probability (also known as gating activity) and enhance chloride transport. Ivacaftor is most active (in vitro and in clinical trials) against the subset of CFTR mutations which affect channel gating (e.g., *G551D*). However, ivacaftor monotherapy also potentiates chloride transport in F508del CFTR HBE cells.[50]
Pivotal general toxicology studies for ivacaftor were conducted in rats for up to 6 months and dogs for up to 12 months. In the 6-month rat study, test article-related deaths were noted at ≥100 mg/kg/day and target organs of toxicity were identified as the heart (cardiomyopathy and coronary medial degeneration), kidneys (chronic progressive nephropathy) and liver (increased ALT). The heart and kidney toxicities were judged to represent exacerbations of rat-specific findings that were likely not relevant to humans. The NOAEL was identified as 50 mg/kg/day, corresponding to AUC values of 445 and

[50] Vertex study B229 and Van Goor et al. (2009) *Proceedings of the National Academy of Sciences*. 106 (44): 18825-18830.

561mcg*hr/mL in males and females, respectively. In the 12-month dog study, gastrointestinal (emesis and abnormal stool) and cardiac (SVPCs) effects were observed but there were no correlating histopathological findings. The NOAEL was considered as 30 mg/kg/day; however, the cardiac toxicity was judged to be clinically monitorable. Therefore, safety margin calculations were based on 60 mg/kg/day dose level, corresponding to an AUC of 351 and 254 mcg*hr/mL in males and females, respectively.

Two disproportionate human metabolites (M1 and M6) of ivacaftor were identified and were evaluated in the chronic toxicology studies. The 0.5-1.1x AUC safety margins achieved in male rats in the 6-month study were considered adequate to qualify the nonclinical safety of M1 and M6 for the KALYDECO product. The M1 and M6 exposure levels in CF patients receiving the proposed dose level 400 mg lumacaftor plus 250 mg ivacaftor twice daily in study VX09-809-102 was substantially lower than those in the KALYDECO product. Therefore there are no nonclinical safety concerns regarding the qualification of M1 and M6 for the ORKAMBI product.[51]

Ivacaftor was negative in a standard battery of genetic toxicology assays. Two-year carcinogenicity studies were conducted in mice and rats and there was no evidence of test article-related tumorigenicity. Ivacaftor impaired male and female fertility in rats in the presence of severe toxicity at 200 mg/kg/day, but fertility was unaffected at lower doses. It was not teratogenic in rats or rabbits and had no effects on peri- or post-natal development in rats.

11.4 Nonclinical Recommendation

The sponsor has adequately characterized the pharmacology and toxicology of ORKAMBI and there are no outstanding nonclinical issues at this time. Therefore, NDA 206038 is recommended for approval from the nonclinical perspective.

The nonclinical evaluation of lumacaftor will be completed via a Post-Marketing Requirement (PMR) for the submission of the results of a two-year carcinogenicity study in rats. Labeling recommendations are provided below.

11.5 Labeling

The reviewer's recommended nonclinical labeling is provided below, along with rationale for changes from the sponsor's proposed language. The reviewer's proposed insertions and deletions are indicated in blue font and ~~red strikethrough~~ text, respectively.

Comparisons between human and animal doses for lumacaftor and ivacaftor were calculated based on exposure ($AUC_{0\text{-}24hr}$). The following table describes the derivation

[51] See VX09-809-102 clinical study report (Table 11-11) and NDA 203188 nonclinical review by Dr. Marcie Wood (January 17, 2012, Table 12)

and calculation of the exposures and dose ratios that will be used in the labeling text. Note that values >10 are rounded to the nearest 5 and values >100 are rounded to the nearest 10 in the labeling text.

Table 12. Summary of Dose Ratios for ORKAMBI Labeling

Species (Study)	Dose (mg/kg/day)		AUC_{0-24hr} (mcg*hr/mL)		Dose Ratio (exposure basis)	
	LUMA	IVA	LUMA	IVA[a]	LUMA	IVA
Human (Phase 3 studies in CF patients)	13.3[b]	8.3[b]	429[c]	30.2[d]	-	-
Teratogenicity (Rat)	2000	200	3320	1134[e]	7.7	38
Teratogenicity (Rabbit)	200	100	1950	338	4.5	132[f]
Carcinogenicity (Mouse)	2000 (M) 1500 (F)	200	1390 (M) 2090 (F)	284 (M) 141 (F)	3.2 (M) 4.9 (F)	9.4 (M) 4.7 (F)
Carcinogenicity (Rat)	NA	50	NA	1041 (M) 718 (F)	NA	34 (M) 24 (F)
Fertility and reproductive performance (Rat)[e]	1000	100	1300 (M) 3160 (F)[e]	681 (M) 663 (F)[e]	3.0 (M) 7.4 (F)	23 (M) 22 (F)
		200		1419 (M) 1134 (F)[e]		47 (M) 38 (F)
Juvenile (Rat)	NA	10	NA	20.1 (M) 31.9 (F)	NA	0.7 (M) 1.1 (F)

LUMA: Lumacaftor; IVA: ivacaftor

[a] To maintain consistency with KALYDECO labeling, sum of IVA+M1+M6 exposure was used for calculation of dose ratios for studies evaluating IVA (with the exception of the rabbit study, for which no metabolite exposure data is available)

[b] Converted from 800 mg LUMA, 500 mg IVA daily dose based on 60 kg human weight

[c] Human LUMA exposure represents median AUC_{0-24hr} for phase 3 clinical trials -103 and -104 from population PK study K050, as provided by Clinical Pharmacology reviewers Dr. Jianmeng Chen and Anshu Marathe.

[d] Represents sum of IVA (2.56 mcg*hr/mL) + M1 (8.85 mcg*hr/mL) + M6 (18.8 mcg*hr/mL) exposures in study VX09-809-102 cohort receiving 400 mg LUMA plus 250 IVA BID.

[e] Exposure values extrapolated from 6-month rat toxicology studies (VX-809-T012 and VX-770-TX-010)

[f] As noted in footnote (a), this exposure multiple is in terms of IVA alone (without metabolites).

INDICATIONS AND USAGE

To be determined.

Reviewer's Comment:

As noted above, the determination of an EPC for lumacaftor is still under discussion by the review team. A supplemental labeling review will provide recommended edits.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Teratogenic effects: Pregnancy Category B. There are ~~N~~no adequate and well-controlled (b) (4) trials of ORKAMBI or its individual components, lumacaftor or ivacaftor, in pregnant women (b) (4). Embryofetal development studies in rats and rabbits were conducted with the individual components of ORKAMBI, lumacaftor and ivacaftor. Because animal reproduction studies are not always predictive of human response, ORKAMBI should be used during pregnancy only if clearly needed.

Lumacaftor was not teratogenic in rats at approximately 8 times the maximum recommended human dose (MRHD) (b) (4) (on a lumacaftor AUC basis at a maternal oral dose of 2000 mg/kg/day). Lumacaftor was not teratogenic in rabbits at approximately 5 times the MRHD (b) (4) (on a lumacaftor AUC basis at a maternal oral dose of 200 mg/kg/day). Placental transfer of lumacaftor was observed in pregnant rats and rabbits.

Ivacaftor was not teratogenic in rats at approximately (b) (4) times the MRHD (b) (4) (based on summed AUCs for ivacaftor and its metabolites at a maternal oral dose of 200 mg/kg/day (b) (4). Ivacaftor was not teratogenic in rabbits at approximately 1 (b) (4) times the MRHD (b) (4) (on an ivacaftor AUC basis at a maternal oral dose of 100 mg/kg/day). Placental transfer of ivacaftor was observed in pregnant rats and rabbits.

(b) (4)

8.3 Nursing Mothers

Both lumacaftor and ivacaftor are excreted into the milk of lactating female rats. Excretion of ORKAMBI into human milk is probable. (b) (4) There are no human studies that have investigated the effects of lumacaftor and ivacaftor on breast-fed infants. Caution should be exercised when ORKAMBI is administered to a nursing woman.

Reviewer's Comment:
Exposure margins have been adjusted to reflect the method of calculation described above. To maintain consistency with the KALYDECO label, the dose multiple for the rabbit EFD study is expressed on the basis of ivacaftor exposure alone. This is in contrast to the other ivacaftor exposure comparisons which are based on the sum of ivacaftor, M1 and M6 AUC values for both animal and human studies. For added context, it is noted that the rabbit ivacaftor exposure (metabolites were not quantified) was 10 times higher than the human combined exposure of ivacaftor and its metabolites. Section 8.3 has been revised to reflect the wording used in the current KALYDECO labeling (e.g., "probable" excretion into human breast milk). Other minor

labeling edits were made to harmonize with the approach used for other combination product labels.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

To be determined.

Reviewer's Comment:
As noted above, the determination of a potential EPC for lumacaftor is still under discussion by the review team. As part of this process, the totality of the data regarding the molecular mechanism of action of lumacaftor remains under review. A supplemental labeling review will provide recommended Section 12.1 labeling once a final determination is made.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with ORKAMBI; however, studies are available for individual components, lumacaftor and ivacaftor, as described below.

Lumacaftor:
A 26-week study was conducted in transgenic Tg.rasH2 mice to assess carcinogenic potential of lumacaftor. No evidence of tumorigenicity was observed in Tg.rasH2 mice at lumacaftor oral doses up to 1500 and 2000 mg/kg/day in female and male mice, respectively (b) (4)

Lumacaftor was negative for genotoxicity in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, and *in vivo* mouse micronucleus test.

Lumacaftor had no effects on fertility and reproductive performance indices in male and female rats at 1000 mg/kg/day (approximately 3 and (b) (4) times, respectively, the MRHD (b) (4) on a lumacaftor AUC basis (b) (4)

Ivacaftor:
Two-year studies were conducted in mice and rats to assess carcinogenic potential of ivacaftor. No evidence of tumorigenicity was observed in mice and rats at ivacaftor oral doses up to 200 mg/kg/day and 50 mg/kg/day, respectively (approximately equivalent to (b) (4) and (b) (4) times the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites).

Ivacaftor was negative for genotoxicity in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, and *in vivo* mouse micronucleus test.

Ivacaftor impaired fertility and reproductive performance indices in male and female rats at 200 mg/kg/day (approximately (b) (4) the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites (b) (4) Increases in prolonged diestrus were observed in females at 200 mg/kg/day. Ivacaftor also increased the number of females with all nonviable embryos and decreased corpora lutea, implantations, and viable embryos in rats at 200 mg/kg/day (approximately (b) (4) times the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites (b) (4) when dams were dosed prior to and during early pregnancy. These impairments of fertility and reproductive performance in male and female rats at 200 mg/kg/day were attributed to severe toxicity. No effects on male or female fertility and reproductive performance indices were observed at ≤100 mg/kg/day (approximately (b) (4) times the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites (b) (4) .

13.2 Animal Toxicology and/or Pharmacology

Cataracts were seen in juvenile rats dosed with ivacaftor from postnatal day 7-35 at dose levels of 10 mg/kg/day and higher (approximately (b) (4) times the MRHD for the ivacaftor component of ORKAMBI based on summed AUCs of ivacaftor and metabolites). This finding has not been observed in older animals.

Reviewer's Comment:

The introductory sentence for Section 13.1 was added to harmonize with other combination product labels. As transgenic mouse carcinogenicity studies are considered to represent hazard identification only, (b) (4) *have been removed.* (b) (4) *have been edited to reflect the calculations described above. The sponsor's additional details regarding* (b) (4) (b) (4) *was not considered necessary for product labeling and has been deleted. The sponsor's inserted sentence regarding effects on estrous cycling was considered acceptable. With regard to Section 13.2 the reviewer determined that insertion of discussion related to* (b) (4) *was not warranted.* (b) (4)

12 Appendix/Attachments

Reviews of pivotal nonclinical studies are attached.

1. Nonclinical review, IND 79521, Dr. Timothy Robison, January 3, 2008
2. Nonclinical review, IND (b) (4) Dr. Timothy Robison, November 2, 2012
3. Nonclinical review, IND 79521, Dr. Timothy Robison, December 10, 2013
4. Nonclinical review, IND 79521, Dr. Andrew Goodwin, October 16, 2014
5. Nonclinical review, IND 79521, Dr. Andrew Goodwin, November 20, 2014

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

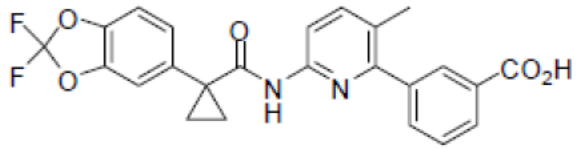
IND number: 79,521
Review number: #01
Sequence number/date/type of submission:
#001/October 18, 2007/Initial Submission
#002/November 13, 2007/Amendment
#003/November 14, 2007/Amendment
#004/November 28, 2007/Amendment
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Vertex Pharmaceuticals Incorporated
130 Waverly Street
Cambridge, MA 02139-4242
Manufacturer for drug substance: (b) (4) [redacted]

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.
Division name: Pulmonary and Allergy Products
HFD #: 570
Review completion date: January 3, 2008

Drug:

Code name: VRT-826809 or VX-809
Chemical name: 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid
Molecular formula/molecular weight: $C_{24}H_{18}F_2N_2O_5$ / 452 g/mole
Structure:

Figure 1 Structure of VX-809 Drug Substance



Chemical Formula: $C_{24}H_{18}F_2N_2O_5$
Molecular Weight: 452.41

Relevant INDs/NDAs/DMFs: None

Drug class: Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) (b) (4) [redacted]

Intended clinical population: Cystic Fibrosis

Clinical formulation: VX-809 drug product will be prepared at the clinical site as a suspension for oral administration. Each 30 g of suspension will contain the specified amount of VX-809 drug substance for the dose regimens as defined in the clinical protocol VX07-809-001. Lower doses will be prepared by substituting the VX-809 drug substance with additional vehicle, which contains sterile water for irrigation, methyl cellulose, polysorbate 80 and simethicone.

Table 1 Composition of VX-809 Suspension for Oral Administration

Ingredient	Quantity (g/30 g of suspension)	Content (%)	Function
VX-809	0.010-1.00	0.033-3.33[a]	Active Ingredient
Sterile Water for Irrigation, USP	(b) (4)		
Methyl Cellulose USP			
Polysorbate 80 NF			
Simethicone USP			
Total	30.0	100.0	-

[a] The actual percentage depends on the dosed administered as detailed in the clinical study protocol.

Table 1 Composition of Placebo Suspension for Oral Administration

Ingredient	Quantity (g/30 g of suspension)	Content (%)	Function
Microcrystalline Cellulose, USP	(b) (4)		
Sterile Water for Irrigation, USP			
Methyl Cellulose USP			
Polysorbate 80 NF			
Simethicone USP			
Total	30.0	100.0	-

Route of administration: Oral

Original proposed clinical protocol:
A Phase I, Randomized, Double-Blinded, Single-Dose Escalation Study in Healthy Subjects Followed by a Multiple-Dose Escalation Study of VRT-826809 (VX-809) in Healthy Subjects (Vertex Study Number VX07-809-001):

The sponsor has proposed a 4-part, 5-panel, double-blind, placebo-controlled, randomized study with healthy male and female subjects (18 to 55 years old) as shown in the table below. A total of 65 patients will enrolled in the study as follows: Part A, 16 healthy male subjects; Part B, 9 female healthy subjects; Part C, 10 healthy male subjects; and Part D, 30 healthy male and female subjects. A minimum of 2 females are required for each dose group in Part D. Subjects in Parts A, B, and C must agree to use 2 highly effective methods of contraception, including at least one barrier method. Female subjects in Part D must be of documented non-childbearing potential.

Dosing Groups

PART	PANEL	DOSES
A	1	A single suspension dose of 75, 150, 300, 600, or 750 mg of VX-809 or placebo administered to male subjects in the morning of the first day in the dosing period, following an overnight (10-hour) fast.
	2	A single suspension dose of 750, 1000, 1350, or 1500 mg of VX-809 or placebo administered to male subjects in the morning of the first day in the dosing period, following an overnight (10-hour) fast.
B	3	A single suspension dose of 600 or 1350 mg VX-809 or placebo, administered to female subjects in the morning of the first day in the dosing period, following an overnight (10-hour) fast.
C	4	A single suspension dose of 750 mg VX-809 or placebo administered to male subjects in the morning within 30 minutes from the start of a high-fat breakfast or following an overnight (10-hour) fast.
D	5	Multiple suspension doses of 175, 350, or 700 mg VX-809 or placebo administered to male and female subjects q12h over 14 days. The food status of subjects at dosing will be specified based on PK data observed in Panel 4, Part C (Food Effect).

Part A (Panels 1 and 2): To evaluate the safety, tolerability, and pharmacokinetics of single ascending doses of VRT-826809 suspension administered to healthy male subjects in the fasted state. Part A contains 2 single-dose escalation panels (Panels 1 and 2), which extend over 4 dosing occasions each, and consists of 2 groups of 8 healthy male subjects (Panel 1 is composed of Subjects 1 to 8 and Panel 2 is composed of Subjects 9 to 16). Subjects in each panel will receive a single dose of study drug (VRT-826809 or placebo) in the fasted state at the beginning of each of the 4 periods within a panel (3 doses of VRT-826809 and one dose of placebo). Subjects will be randomly assigned to 1 of the dose escalation sequences specified for Panels 1 and 2 and the safety of the drug at preceding lower doses will be evaluated by the Medical

Monitor and study team before administering the next higher dose in the dose escalation sequence. A minimum 7-day washout period is mandatory between each dosing occasion for Part A); however, the range between dosing occasions can extend to a period of 21 days for Part A only.

In the single-dose escalation part of the study in male subjects (Part A), the first subjects who will be exposed to VRT-826809, 2 approaches are being used: (1) a leading-dose approach during Panel 1 whereby, after the first dosing occasion, only 1 subject is exposed to the next dose level before more subjects are exposed to the same dose on the next dosing occasion, and (2) a mixture of dose doubling and Fibonacci dose escalation schemes. Dose doubling is used for escalation at lower doses (75 to 600 mg) and Fibonacci at higher doses (750 to 1500 mg) producing an intentionally gradual increase in dose levels. Within-subject dose escalation was chosen to permit the characterization of linearity or non-linearity of PK, and a 7-day washout is included between study drug doses to eliminate any possible carryover effects that may confound safety assessments. In addition, subjects serve as their own control by receiving placebo on one of the dosing occasions. This has the advantage of minimizing bias in the assessment of safety and tolerability.

Part B (Panel 3): To evaluate the safety, tolerability, and pharmacokinetics of single ascending doses of VRT-826809 suspension administered to healthy female subjects in the fasted state. Part B contains a single-dose escalation panel (Panel 3), which consists of 9 female subjects (Subjects 17 to 25), and extends over 3 dosing occasions. Subjects in this panel will receive a single dose of study drug (VRT-826809 or placebo) in the fasted state at the beginning of each of the 3 occasions within the panel. Subjects will be randomly assigned to 1 of the dose escalation sequences specified for Panel 3 and the safety of the drug at preceding lower doses will be evaluated by the Medical Monitor and study team before administering the next higher dose in the dose escalation sequence. A minimum 7-day washout period is mandatory between each dosing occasion for Part B.

Part C (Panel 4): To evaluate the safety, tolerability, and pharmacokinetics of single doses of VRT-826809 administered to healthy male subjects. Part C contains a single-dose, food-effect crossover panel (Panel 4), which consists of 10 healthy male subjects (Subjects 26 to 35) and extends over 2 dosing occasions. Subjects in this panel will receive a single dose of study drug on 2 separate occasions, with the suspension formulation in fed (high-fat) and fasted states. Subjects will be randomly assigned to 1 of the treatment sequences. A minimum 7-day washout period is mandatory between each dosing occasion for Part C.

Part D (Panel 5): To evaluate the safety, tolerability, and pharmacokinetics of multiple ascending doses of VRT-826809 suspension administered for 14 days to healthy male and female subjects. The food status of subjects (fed/fasted) will be based upon the bioavailability observed in Part C. Part D is a multiple-dose panel (Panel 5) consisting of 30 healthy male and female (of non-child bearing potential) subjects (Subjects 36 to 65). Dosing will extend over a single 14-day dosing period. Subjects will be randomized into

3 dose groups, with 10 subjects in each group (a minimum of 2 female subjects is required for each dose group), to receive multiple doses of VRT-826809 or placebo each day over a 14-day period. Total daily doses of VRT-826809 will be 350, 700, or 1400 mg/day (175, 350, or 700 mg q12hr, respectively).

Revised clinical protocol:

In accordance with the Division's request dated November 13, 2007, the sponsor amended the protocol as follows (Amendment #002 dated November 14, 2007) :

Part A: Doses of 1000, 1350 and 1500 mg were excluded, leaving the following single doses to be studied in 4 dosing occasions: 75 mg, 200 mg, 375 mg, and 750 mg. The sponsor modified the dose escalation to accommodate the 4 dose levels in one panel, as shown below in the revised protocol scheme.

Part B: The 600 mg and 1350 mg doses have been reduced to 375 mg and 750 mg, respectively.

Part C: Doses will remain unchanged (750 mg fed and 750 mg fasted).

Part D: Doses have been reduced to 100 mg q12hr, 200 mg q12hr, and 375 mg q12hr, for a maximum total daily dose of 750 mg.

A total of 57 subjects will now be enrolled in the study. The revised protocol scheme is shown below.

Single Dose																		
Part A	Panel 1 (8 males) Subjects 1–8		P	200	375	750												
			75	P	375	750												
			75	200	P	750												
			75	200	375	P												
			75	P	375	750												
			75	200	375	P												
			75	375	P	750												
			P	200	750	375												
Part B	Panel 2 (9 females) Subjects 9–17					P	375	750										
						375	P	750										
						375	750	P										
Part C	Panel 3 Food Effect (10 males) Subjects 18–27							P fast	P fed									
								750 fast	750 fed									
								P fed	P fast									
								750 fed	750 fast									
Multiple Dose																		
Part D	Panel 4 (30 males and females) Subjects 28–57										3 week delay		P 100 q12h					
															P 200 q12h			
																	P 375 q12h	

Previous clinical experience: None.

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

Studies reviewed within this submission:

Title:	**Study/Report Number**:
PHARMACOLOGY:	
SAFETY PHARMACOLOGY:	
Neurological Effects:	
Effects of oral (gavage) administration on the functional observational battery in rats	VRT-826809-TX-014
Cardiovascular Effects:	
Effects of VRT-826809 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	VRT-826809-TX-012
Cardiovascular Effects following Oral (Gavage) Administration in Conscious Telemetered Beagle Dogs	VRT-826809-TX-013
Pulmonary Effects:	
Effects of Oral (Gavage) Administration on Respiration Rate and Tidal Volume in Rats	VRT-826809-TX-015
Gastrointestinal Effects:	
Effects of Oral (Gavage) Administration on Gastrointestinal Transit of a Charcoal Meal in Rats	VRT-826809-TX-016
PHARMACOKINETICS/TOXICOINETICS:	
Absorption:	
Mice	
Pharmacokinetics of VRT-826809 in Male CD-1 Mice Following Single Intravenous Administration	D049
Rats	
Pharmacokinetics of VRT-826809 in Male Sprague-Dawley Rats following Single Intravenous Administration	D081
Pharmacokinetics of VRT-826809 following Oral Administration in Male Sprague Dawley Rats	D127
Dogs	
Pharmacokinetics of VRT-826809 Following Intravenous and Oral Administration of the HCl Salt in Male Beagle Dogs	D047
Oral Pharmacokinetics of the HCl Salt and Free Form of VRT-826809 and Comparison of the Free Form under Fed and Fasted Conditions in Male Beagle Dogs	D133
Monkeys	
Pharmacokinetics of VRT-826809 in Male Cynomolgus Monkeys following Single Intravenous Administration	D048
Distribution:	
In Vitro Binding of VRT-826809 to Plasma Proteins in Mouse, Rat, Dog, Monkey, and Human Plasma	D152
Tissue Distribution of VRT-826809 in Male Sprague Dawley Rats Following Single Oral Administration	D051
Metabolism:	
In Vitro	
In Vitro Assessment of Metabolic Stability of VRT-826809 in Hepatocytes from Rat, Dog, Monkey, and Human	D072
The In Vitro Stability of VRT-826809 in Human Recombinant CYP450	D071

Isozymes	
Assessment of VRT-826809 Inhibition Potential of Human Cytochrome P450 Isozymes	D079
Evaluation of Induction of Liver Cytochrome P450 Isozymes in Human Hepatocytes Following In Vitro Exposure of Hepatocytes to VRT-826809	D128
In Vivo	
Characterization of VRT-826809 Putative Metabolites from In Vitro and In Vivo Matrices	D083
TOXICOLOGY:	
Single Dose:	
Single-Dose Oral Toxicity Study in Mice with a 14-Day Observation Period	VRT-826809-TX-008
Single Dose Oral Toxicity Study in Rats with a 14-Day Observation Period	VRT-826809-TX-009
Repeat Dose:	
A 7-day range finding study in rats (Summary only)	VRT-826809-TX-002
A 14-Day Oral (Gavage) Toxicity and Toxicokinetic Study in Rats	VRT-826809-TX-010
A 7-day range finding study in dogs (Summary only)	VRT-826809-TX-004
A 14-Day Oral (Gavage) Toxicity and Toxicokinetic Study in Dogs	VRT-826809-TX-011
Genetic Toxicology:	
Bacterial Reverse Mutation Assay	VRT-826809-TX-005
In Vitro Mammalian Chromosome Aberration Test	VRT-826809-TX-006
Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of VRT-826809	VRT-826809-TX-007

Studies not reviewed within this submission: None

TABLE OF CONTENTS

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

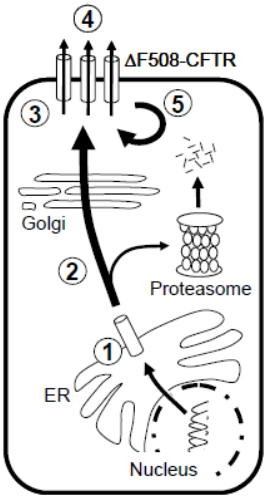
CF is caused by a defect in the CFTR gene, which encodes for the cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation is deletion of phenylalanine 508 (ΔF508), which is found on at least one chromosome in 90% of patients with cystic fibrosis. The ΔF508 CFTR protein is defective in folding, so it is retained in the endoplasmic reticulum and then rapidly degraded. This leads to reduced trafficking of ΔF508 CFTR to the apical membrane, reduced cell surface stability, and impaired channel gating. The reduced cell surface density and function of ΔF508-CFTR severely impair chloride secretion in airway epithelia, causing a chain of deleterious events that ultimately leads to bronchiectasis.

Studies on processing mutants of the sister protein of CFTR, the multi-drug resistance P-glycoprotein, showed that specific pharmacological chaperones could be used to rescue mutants defective in folding. Therefore, high-throughput screening of chemical libraries was performed to identify pharmacological chaperones that could promote folding of CFTR processing mutants such as ΔF508. These molecules have been referred to as CFTR (b) (4) VRT-826809 (VX-809) is such a (b) (4)

Pharmacology studies suggest that VRT-826809 corrects the processing, trafficking, and function of ΔF508-CFTR by promoting the proper protein conformation in a fraction of the available pool of ΔF508-CFTR. Proteolytic digestion experiments showed that VRT-826809 treatment resulted in a wild-type-like digestion pattern in a fraction of the ΔF508-CFTR population. VRT-826809 increased the intrinsic channel gating activity of ΔF508-CFTR to wild-type levels. VRT-826809 significantly increased the cell surface stability of ΔF508-CFTR. The increased maturation efficiency of ΔF508-CFTR led to improved trafficking to the cell surface, improved surface stability, and increased channel gating. The magnitude of the changes in ΔF508-CFTR trafficking and improved channel function are consistent with the increased ΔF508-CFTR-mediated Cl^- secretion observed in ΔF508-HBE.

Figure 5 Summary of VX-809 Mechanism of Action in ΔF508-HBE

1. Acts at the ER to promote proper protein folding in 10 – 15 % of the ΔF508-CFTR pool
2. Increases ΔF508-CFTR trafficking to the cell surface
3. Increases cell surface density of ΔF508-CFTR
4. Restores gating behavior of ΔF508-CFTR to normal
5. Restores cell surface stability of ΔF508-CFTR to normal



In safety pharmacology study, single oral doses up to 1000 mg/kg in rats had no effect on CNS, respiratory, or gastrointestinal functions. Single oral doses up to 200 mg/kg in dogs had no effects on cardiovascular functions.

2.6.2.2 Primary pharmacodynamics

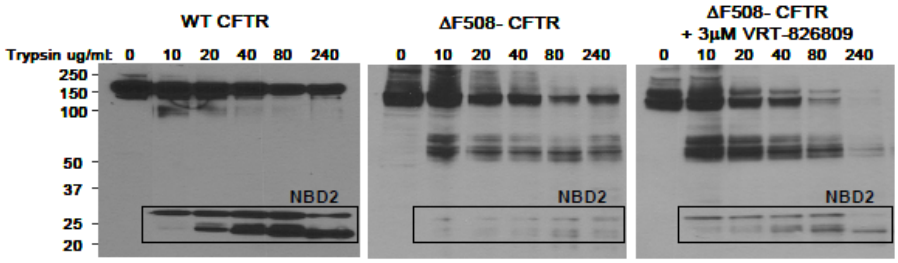
Mechanism of action:

Effects of VRT-826809 on Protein Conformation, Trafficking, and Channel Gating of ΔF508-CFTR (Report number D146): The ΔF508 mutation disrupts the folding and domain assembly of the cystic fibrosis transmembrane conductance regulator protein (CFTR), leading to reduced trafficking to the cell surface, reduced cell surface stability, and impaired channel gating. In this study, biochemical and electrophysiological techniques were used to investigate the effects of VRT-826809 on the protein conformation of ΔF508-CFTR and its ability to restore the trafficking, cell surface stability, and channel gating activity.

The ΔF508 mutation impairs the co-translational folding of NBD1 (nucleotide binding domain 1) and post-translational folding of NBD2 and domain-domain assembly of CFTR. The proteolytic sensitivity of NBD2 was significantly different between wild-type-CFTR and ΔF508-CFTR.To determine whether VRT-826809 enabled ΔF508-CFTR to attain a more wild-type-like conformation, microsome membranes were isolated from HEK-293 cells, expressing wild-type-CFTR or ΔF508-CFTR, that were treated for 24 hr with DMSO or 3 μM VRT-826809. The CFTR-containing microsomes were digested with varying concentrations of trypsin and the proteolytic digestion pattern was analyzed by SDS-PAGE and immunoblotting with M3A7 antibody which recognizes NBD2 in CFTR. Similar to published reports, a clear difference between wild-type-CFTR and ΔF508-CFTR was observed in the proteolytic sensitivity of the NBD2 domain as wild-type-CFTR generated two stable 20-25 kDa NBD2 tryptic fragments while NBD2 in ΔF508-CFTR was extremely sensitive to trypsin digestion. Digestion of ΔF508-CFTR

from cells treated with 3 µM VRT-826809 showed the presence of the stable NBD2 fragments seen in the wild-type-CFTR sample suggesting that VRT-826809 treatment allowed a fraction of the ΔF508-CFTR population to attain a wild-type conformation.

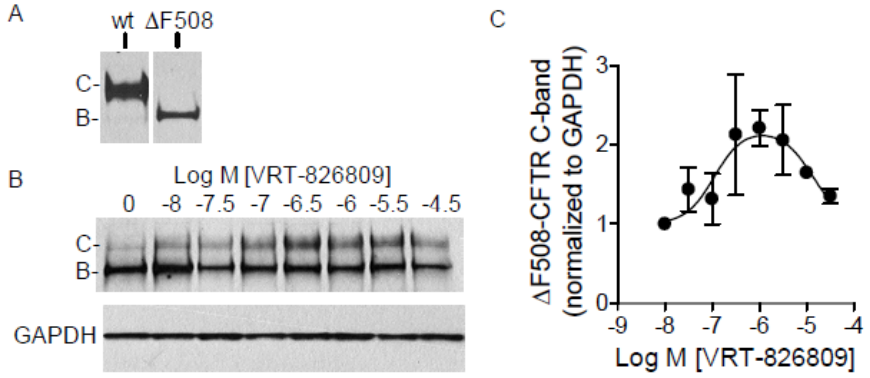
Figure 8-1 Effect of VRT-826809 on the Proteolytic Sensitivity of ΔF508-CFTR



Immunoblot of trypsin-digested microsomes from cells expressing wild-type-CFTR or ΔF508-CFTR treated with DMSO or 3 µM VRT-826809 for 24 hrs. The characteristic 20-25 kDa NBD2 fragments are indicated. A representative example from 4 separate experiments is shown.

Western Blot techniques were used to determine if the increase in ΔF508-CFTR mediated Cl^- secretion observed in HBE treated with VRT-826809 was due to an increase in the trafficking of ΔF508-CFTR to the cell surface. A hallmark of normal CFTR trafficking is the appearance of a high molecular weight extensively glycosylated, mature CFTR (band C), which was indicative of exit from the endoplasmic reticulum (ER) and passage through the Golgi complex, serving as a measure of efficient processing and trafficking. Because ΔF508-CFTR does not exit the ER, only the core-glycosylated, immature form (band B) is present. Incubation of ΔF508-HBE cells for 48 hr in the presence of VRT-826809 resulted in a dose-dependent increase in the steady-state levels of mature, fully glycosylated ΔF508-CFTR with an EC_{50} of 100 nM. At high concentrations of VRT-826809, the magnitude ΔF508-CFTR maturation was reduced compared to the peak response resulting in a "bell-shaped" curve with an IC_{50} of 15 µM. Due to the 16-fold window between the EC_{90} (900 nM) and IC_{50}, these effects observed in vitro at high compound concentrations were likely of minimal concern in a therapeutic setting. A similar "bell-shaped" curve was observed for the effects of VRT-826809 on ΔF508-CFTR-dependent Cl^- secretion in ΔF508-HBE. These results indicate that VRT-826809 restores the trafficking of a fraction of ΔF508-CFTR to increase its cell surface density and this likely underlies the increase in CFTR-mediated Cl^- secretion.

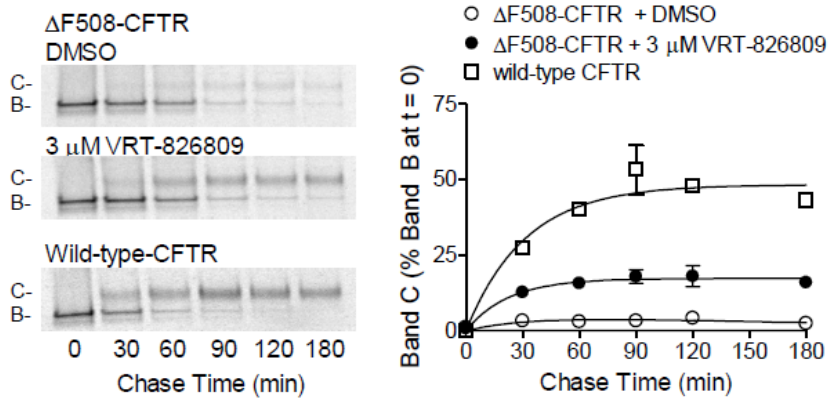
Figure 8-3 Effects of VRT-826809 on the Maturation of ΔF508-CFTR in HBE



Panel A. Representative immunoblot of the glycosylation pattern of CFTR in cell lysates prepared from wild-type- and ΔF508-HBE.
Panel B. Representative immunoblot of ΔF508-HBE treated with 0 to -4.5 M VRT-826809 for 48 hours prior to preparing the cell lysates and monitoring the glycosylation pattern of ΔF508-CFTR.
Panel C. Dose dependent increase in ΔF508-CFTR maturation normalized to the GAPDH load control in each lane (mean ± SEM, n = 3).

The ability of ΔF508-CFTR to form a more wild-type like conformation in the presence of VRT-826809 is expected to facilitate its exit out of the ER and traffic to the Golgi complex where it undergoes maturation. The maturation efficiency of VRT-826809-corrected ΔF508-CFTR was monitored by measuring the fraction of newly synthesized, core-glycosylated (band B) CFTR that is converted into the extensively glycosylated (band C) form using pulse-chase analysis. In HEK-293 cells expressing ΔF508-CFTR, 24-hr incubation with 3 μM VRT-826809 increased the amount of mature ΔF508-CFTR, with a maximum conversion of core-glycosylated ΔF508-CFTR to the mature form of 18 ± 2 % in VRT-826809-treated cells vs. 3 ± 1 % for the DMSO treatment. The conversion of core-glycosylated ΔF508-CFTR to the mature form in VRT-826809-treated cells represented 34 ± 4 % of the maturation efficiency observed for wild-type CFTR. The mature form of ΔF508-CFTR in VRT-826809-treated cells showed no evidence of decline during chases up to 180 min, consistent with the formation of a more wild-type like conformation that is resistant to degradation. These results are consistent with the formation of an ER export competent, wild-type like conformation for ΔF508-CFTR in the presence of VRT-826809.

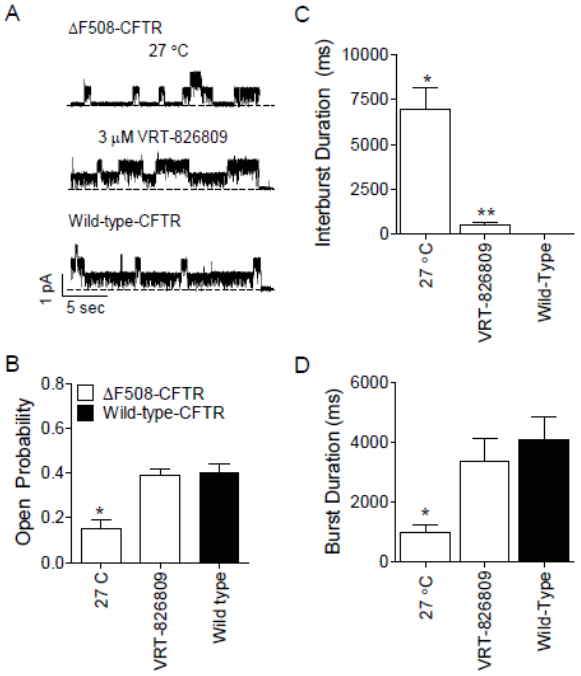
Figure 8-2 Effects of DMSO and VRT-826809 on the Maturation Efficiency of ΔF508-CFTR Compared to Wild-Type CFTR Expressed in HEK-293 Cells



Results are mean ± SEM, n = 3

The non-native protein conformation of ΔF508-CFTR impairs its intrinsic gating activity compared to wild-type CFTR. Based upon observations of the formation of a more "wild-type-like" protein conformation in the limited proteolysis experiments, it was expected that VRT-826809 would restore the intrinsic gating activity of ΔF508-CFTR to wild-type levels. To monitor the effects of VRT-826809 on the intrinsic gating activity of ΔF508-CFTR, inside-out single-channel recording techniques were used. In previous studies, the gating activity of ΔF508-CFTR has been monitored in cells incubated at 27 ºC for 24 to 72 hr to increase ΔF508-CFTR trafficking to the membrane. Although this temperature-correction increases the cell surface density of ΔF508-CFTR, its gating activity remains defective compared to wild-type-CFTR. NIH-3T3 cells expressing ΔF508-CFTR were incubated at 37 ºC for 48 to 72 hr with 3 μM VRT-826809 The gating activity of ΔF508-CFTR in VRT-826809-treated cells was compared to wild-type CFTR and temperature-corrected ΔF508-CFTR. In 3 μM VRT-826809-treated cells, ΔF508-CFTR activity was observed in 44% (23/52) of the patches tested. The greater number of patches containing functional ΔF508-CFTR in VRT-826809-treated cells compared to untreated cells was consistent with an increase in the cell surface density of ΔF508-CFTR. The open probability (Po) of VRT-826809-corrected ΔF508-CFTR was similar to untreated wild-type CFTR and significantly higher than temperature-corrected ΔF508-CFTR. The higher Po in VRT-826809-corrected ΔF508-CFTR and wild-type-CFTR compared to temperature-corrected ΔF508-CFTR was due to shorter channel closures (interburst interval) and longer channel openings (burst duration). There was no significant difference in the single-channel amplitude between VRT-826809- or 27 ºC-corrected ΔF508-CFTR and wild-type CFTR. These results indicated that VRT-826809 increased the cell surface density of ΔF508-CFTR and restored its channel gating activity to that observed for wild-type CFTR.

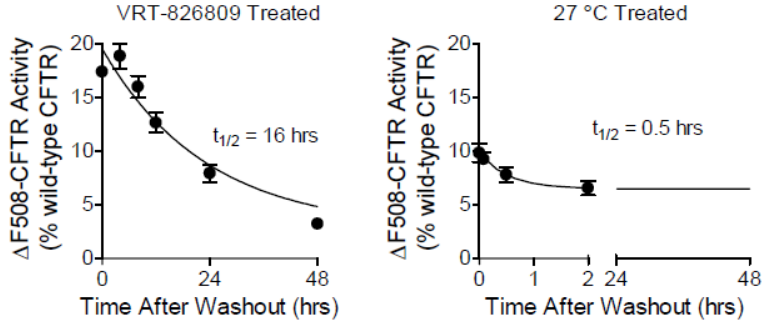
Figure 8-5 Effects of VRT-826809 and Temperature Correction (27 °C) on the Intrinsic Gating Activity of ΔF508-CFTR Compared to Wild-type-CFTR Expressed in NIH-3T3 Cells



Panel A. Single-channel tracings of wild-type-CFTR activity and ΔF508-CFTR activity following incubation at 27 °C or with 3 μM VRT-826809. Dotted line indicates closed state.
Panel B. Open probability (P_o) of wild-type-CFTR (filled bars) and ΔF508-CFTR (open bars) following 48 to 72 hr incubation of the cells at 27 °C or with 3 μM VRT-826809 (mean ± SEM, n = 9).
Panel C. The interburst duration for wild-type-CFTR (filled bars) and ΔF508-CFTR (open bars) under the different treatment conditions (mean ± SEM, n = 9).
Panel D. The burst duration for wild-type-CFTR (filled bars) and ΔF508-CFTR (open bars) under the different treatment conditions (mean ± SEM, n = 9).
* significantly different from wild-type CFTR ($P < 0.05$)
**significant different both wild-type-CFTR and 27 °C -treated ΔF508-CFTR ($P < 0.05$)

Ussing chamber recording techniques were used to monitor the effects of VRT-826809 on the cell surface stability of ΔF508-CFTR in the apical membrane of ΔF508-HBE. VRT-826809 was added to the basolateral surface for 24 hr to correct ΔF508-CFTR and subsequently removed prior to recording CFTR function at regular intervals up to 48 hr. ΔF508-CFTR dependent Cl^- secretion in HBE returned to levels observed in untreated HBE within 48 hr after removal of VRT-826809 with a half-life (t½) of 16 hr. These results indicate that, like wild-type-CFTR, VRT-826809-corrected ΔF508-CFTR is less susceptible to rapid internalization and degradation. This, along with the increase in trafficking to the membrane, will increase the steady-state cell surface density of ΔF508-CFTR.

Figure 8-6 VRT-826809 Increases the Cell Surface Stability of ΔF508-CFTR in HBE



Left Panel: ΔF508-HBE were pre-incubated with the EC_{99} of VRT-826809 for 24 hours to correct ΔF508-CFTR. The cultures were subsequently washed 3X and ΔF508-CFTR activity was monitored 0 – 48 hours after compound removal (n = 4 for each time point).
Right Panel: ΔF508-HBE were pre-incubated at 27 °C for 24 hours to correct ΔF508-CFTR. The cultures were subsequently returned to 37 °C and ΔF508-CFTR activity was monitored 0 – 48 hours after compound removal (n = 4 for each time point).

Effects of VRT-826809 on Misfolded Proteins Other Than CFTR (Report number D147): VRT-826809 corrects the processing, trafficking, and function of the misfolded cystic fibrosis transmembrane conductance regulator (CFTR) protein, ΔF508-CFTR. Because some pharmacological correctors of ΔF508-CFTR also correct a number of other misfolded proteins, the present study assessed the effects of VRT-826809 on a panel of misfolded proteins that included the G601S-human ether-a-go-go (G601S-hERG) cardiac K+ channel, G268V-P-glycoprotein (G268V-P-gp), α1-antitrypsin Z (α1-ATZ), and N370S-β-glucosidase. These proteins either utilized similar trafficking pathways as CFTR (i.e., G601S-hERG), were from the same superfamily as CFTR (i.e., G268V-Pgp), or were other endoplasmic reticulum (ER)-arrested misfolded proteins that likely utilize chaperone pathways distinct from CFTR (i.e., α1-ATZ and N370S-β-glucosidase). VRT-826809 had no effects on the cell surface expression of a trafficking deficient mutant hERG channel (G601S-hERG) when incubated with HEK-293 cells expressing G601S-hERG at concentrations up to 10^{-4} M for 16 hr, trafficking of G268V-P-gp in Fischer rat thyroid (FRT) cells, transfected with a pcDNA3.1-based vector containing cDNA for the G268V form of P-glycoprotein, when incubated at a concentration of 3 μM for 16 hr, intracellular and extracellular levels of α1-antitrypsin in Hela cells, transfected with a pcDNA3.1-based vector containing cDNA for the E342K form of the α1-antitrypsin (AAT) Z mutant, when incubated with a concentration of 3 μM for 16 hr, or β-glucosidase activity in primary skin fibroblasts isolated from a 29 year-old male Type I Gaucher patient (GM00372) when incubated at concentrations up to 10^{-4} M for 3 days. Less efficacious correctors of ΔF508-CFTR were shown to correct misfolded proteins in addition to CFTR. These results suggest that the pharmacological action of VRT-826809 is selective for the correction of CFTR relative to other misfolded proteins.

The Effects of VRT-826809 on Wild-Type Non-CFTR Cellular Proteins (Report number D148): VRT-826809 corrects the processing, trafficking, and function of the misfolded cystic fibrosis transmembrane conductance regulator (CFTR) protein, ΔF508-CFTR. Because some pharmacological chaperones demonstrate broad activity against both misfolded and wild-type proteins, the present study assessed the effects of VRT-826809 on the activities of wild-type versions of G601S-hERG, N370S-β-glucosidase,

and G268V-P-glycoprotein. In addition, effects on the expression of 36 intracellular and cell surface proteins from human peripheral blood cells were assessed. VRT-826809 had no effects on the cell surface levels or density of human ether-a-go-go (hERG) cardiac K^+ channels in HEK cells when incubated at concentrations up 30 μM for 16 hr, β-glucosidase activity in HEK cells when incubated at concentrations up to 10 μM for 4 days, or trafficking of P-glycoprotein in BHK cells when incubated at a concentration of 3 μM for 16 hr. VRT-826809 had no effects on 5 ng/mL PMA + 300 nM ionomycin stimulated expression of 36 intracellular and cell surface proteins from peripheral blood cells when incubated at a concentration of 4 μM VRT-826809 for a total of 8 hr. VRT-826809 had no notable effects on the biosynthesis and trafficking of wild-type versions of several mutant misfolded proteins or on any of the proteins studied in human peripheral blood cells (granulocytes and lymphocytes), suggesting that it is a relatively selective corrector of ΔF508-CFTR trafficking. For mechanistic reasons, the range of proteins tested was somewhat biased toward proteins that, like CFTR, utilize the secretory pathway for their biogenesis. However, the panel of proteins also included a number of cytoplasmic proteins which also showed no significant effects of VRT-826809 on expression.

Drug activity related to proposed indication:

Effects of VRT-826809 on CFTR-Mediated Chloride Secretion in Human Bronchial Epithelia Isolated From Cystic Fibrosis Subjects (Report number D143): Effects of the (b) (4), VRT-826809, on CFTR-mediated Cl^- secretion from monolayers of human bronchial epithelia (HBE) isolated from lungs of ΔF508 heterozygous and -homozygous CF patients were assessed. To assess the level of correction, ΔF508-CFTR-mediated Cl^- secretion was compared to wild-type CFTR-mediated Cl^- secretion in HBE isolated from the lung of non-CF subjects. Genomic DNA was isolated from the HBE and assayed for CFTR mutations.

To monitor the effect of VRT-826809 on ΔF508-CFTR-mediated Cl- secretion, the compound was added to the basolateral surface of ΔF508-HBE for 48 hr to allow for de novo protein synthesis and trafficking to the membrane. VRT-826809 was then removed prior to recording ΔF508-CFTR-mediated Cl- secretion using the Ussing chamber technique. Based on Ussing chamber recordings using HBE from 7 different ΔF508-homozygous subjects, VRT-826809 caused a 6-fold increase in CFTR-mediated Cl^- secretion from 3 ± 1 % to 14 ± 2 % wild-type CFTR with an EC_{50} and EC_{90} of 94 ± 13 nM and 631 ± 153 nM, respectively. In the presence of 20% human serum, the EC_{50} shifted 5-fold, indicating that the potency in vivo may be lower.

Correlations between disease severity and in vivo measurements of Cl^- secretion in CF subjects suggest that increasing mutant CFTR-mediated Cl^- secretion to 10% of wild-type CFTR-mediated Cl- secretion should be sufficient to ameliorate severe lung disease in CF subjects. The mean VRT-826809 concentration needed to reach 10% wild-type CFTR in ΔF508-HBE isolated from 7 CF subjects was 153 ± 66 nM with an EC_{90} of 631 ± 153 nM. Based on these in vitro data, the estimated therapeutic dose should achieve a target tissue concentration between 153 and 631 nM.

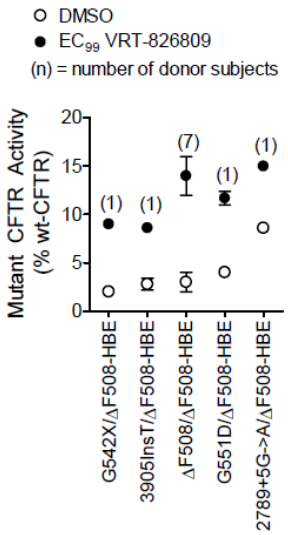
Table 8-1 Potency and Efficacy of VRT-826809 on CFTR-Mediated Cl^- Secretion From ΔF508-HBE Isolated From CF Subjects

HBE (CF-Subject)	n	EC_{90} (nM)	Efficacy @ EC_{90} (nM) (% wt-CFTR Activity)	Concentration (nM) @ 10% wt-CFTR activity
ACD#13749	12	690	13	158
ACD#13777	3	192	8	∞
ACD#13865	27	749	15	126
ACD#13825	4	1398	7	∞
ACD#13763	6	493	18	32
ACD#13838	12	221	16	50
ACD#13778	8	672	11	398
All Patients	7	631 ± 153	13 ± 2	153 ± 66

∞ Indicates activity did not reach 10% wt-CFTR at the concentrations tested; n = number of replicate dose responses in ΔF508-HBE isolated from each of the 7 individual CF subjects

VRT-826809 also increased CFTR-mediated Cl^- secretion in HBE from donors heterozygous for the ΔF508 mutation and either a Class I (G542X/ΔF508-HBE, 3905InsT/ΔF508-HBE), Class III (G551D/ΔF508-HBE), or Class V (2789+5G->A/ΔF508-HBE) mutation. In HBE containing a Class I mutation, which prevents the synthesis of CFTR, VRT-826809 did not increase Cl- secretion by more than 10% wild-type-CFTR. In contrast, mutant CFTR-mediated Cl- secretion exceeded 10% of that observed for wild-type-CFTR in VRT-826809-treated G551D/ΔF508- and 2789+5G->A/ΔF508-HBE. These data suggest that CF subjects with a Class I mutation in at least one allele may not benefit from a corrector monotherapy. It is not known if the effects of VRT-826809 on G551D/ΔF508- and 2789+5G->A/ΔF508-HBE are due to correction of G551D- or 2789+5G->A-CFTR in addition to ΔF508-CFTR.

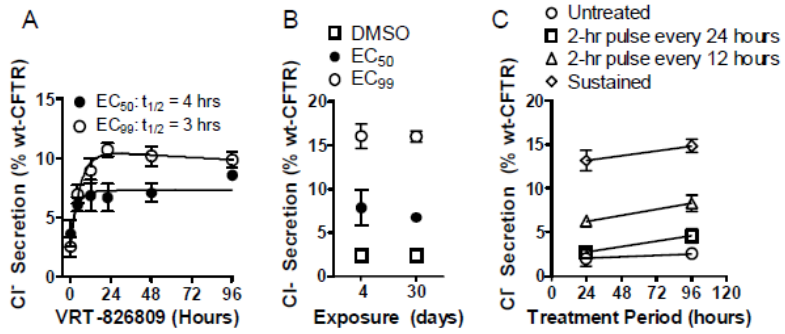
Figure 8-3 Effects of VRT-826809 on Mutant CFTR-Mediated Cl^- Secretion Isolated From ΔF508-Heterozygous CF Subjects



Increase in mutant CFTR-dependent Cl^- secretion in response to 48-hour pre-incubation with DMSO (○) or the EC_{99} of VRT-826809 (●). Results are mean (SEM) from n = 3 to 6 replicate wells in mutant HBE isolated from CF subjects. The number of CF subjects for each genotype listed is indicated by (n).

Correctors of CFTR require sustained treatment to allow for de novo synthesis, processing, and trafficking of ΔF508-CFTR to the cell surface. In contrast, CFTR potentiators act acutely (< 5 minutes) to increase the gating activity of CFTR. The maximum effect of VRT-826809 at the EC_{50} or EC_{99} was reached after 24 hr of treatment. No decrease in efficacy was observed following 30 days of sustained treatment with VRT-826809, suggesting that the molecular target or pathway that VRT-826809 acts on is not desensitized or down-regulated. Acute addition of VRT-826809 did not increase ΔF508-CFTR-mediated Cl- secretion, indicating that it is not a CFTR potentiator.

Figure 8-5 Kinetics of VRT-826809 Correction of ΔF508-CFTR in HBE

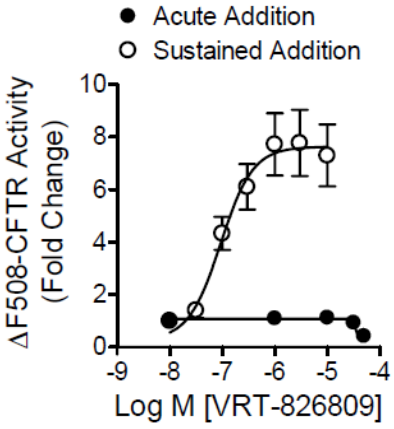


Panel A: The duration of exposure required to reach peak ΔF508-CFTR-mediated Cl⁻ secretion was monitored following 0 to 96 hours of sustained basolateral treatment of ΔF508-HBE with the EC_{50} or EC_{99} of VRT-826809.

Panel B: ΔF508-CFTR activity was monitored after 4 or 30 days of continuous treatment with DMSO- (open squares) or the EC_{50} (filled circles) and EC_{99} (open circles) of the compound.

Panel C: Effects of pulsatile and sustained VRT-826809 treatment on ΔF508-CFTR-mediated Cl⁻ secretion in ΔF508-HBE. ΔF508-HBE cultures were treated for 2 hours with the EC_{99} once (squares) or twice daily (triangles) for 96 hours (pulsatile treatment) or throughout the 96 hour treatment period (diamonds; sustained treatment). After 96 hours, ΔF508-CFTR-mediated Cl⁻ secretion was measured using Ussing chamber recording techniques.

Figure 8-6 Effects of Acute and Sustained Addition of VRT-826809 on ΔF508-CFTR-Mediated Cl⁻ Secretion

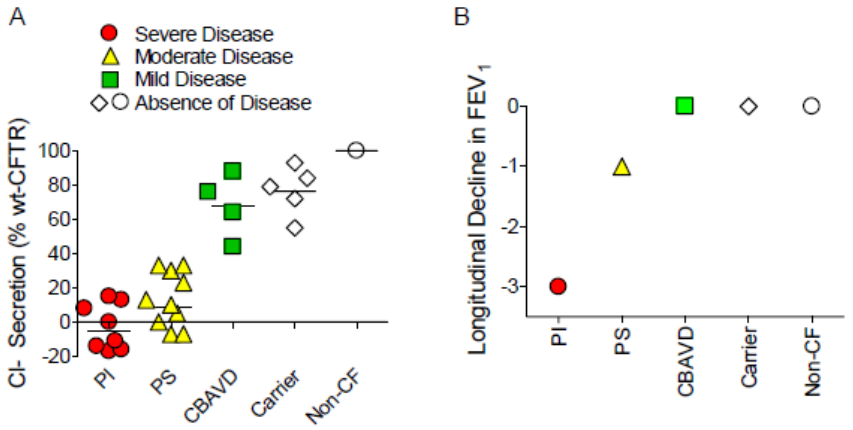


Increase in ΔF508-CFTR-mediated Cl⁻ secretion in response to acute (●; 5 min) or sustained (○; 48 hours) addition of VRT VRT-826809. The data were expressed as the fold change in the forskolin response compared to that in the absence of VRT-826809. Results are mean (SEM) from n = 5 replicate wells in ΔF508-HBE isolated from CF subject, ACD#13865.

Determination of the Efficacy Criteria for CFTR Modulators Based on Genotype-to-Clinical Phenotype Correlations in Cystic Fibrosis Subjects (Report number D144): The heterogeneity of disease severity among CF patients with different genotypes was used to estimate the minimum efficacy criteria for CFTR modulators, because there are no animal models of CF lung disease or therapies that rescue CFTR function.

Several published studies have used nasal potential difference recording techniques to measure in vivo CFTR mediated Cl^- secretion in CF and non-CF control subjects, including obligate heterozygotes that carry one copy of the mutant gene and were clinically unaffected. For each study, CF subjects were grouped into three categories based on their pancreatic and reproductive status. The three CF disease groups were referred to as pancreatic insufficient (PI), pancreatic sufficient (PS), and congenital bilateral absence of the vas deferens (CBAVD). The amount of CFTR-mediated Cl^- secretion reported in each CF disease group and the obligate heterozygotes (carriers) was then normalized to that in the non-CF control from each study and expressed as percent wild-type CFTR. The combined data from each CF disease group was then plotted against percent wild-type-CFTR-mediated Cl- secretion. Lung function in each of the three CF disease groups was then categorized as severe, moderate, or mild based on published reports of the longitudinal decline in FEV1 in CF patients with PI, PS, and CBAVD. Based on the pooled data from multiple published studies, the calculated the amount of mutant CFTR-mediated Cl^- secretion in patients with severe, moderate, and mild lung disease to be < 1%, 9 ± 6 % (0 to 33 %), and 68 ± 9 % (44 to 88 %) wild-type-CFTR, respectively. This data suggested that a CFTR modulator must increase mutant CFTR-mediated Cl^- secretion in CF patients with severe lung disease to a minimum of 10% wild-type-CFTR-mediated Cl^- secretion to produce a moderate decline in lung function.

Figure 8-1 Correlation Between Mutant CFTR-Mediated Cl Secretion and Disease Severity Using Combined NPD Data From Published Studies



In vivo data were pooled from multiple published studies using NPD techniques to measure CFTR-mediated Cl secretion in CF subjects with pancreatic insufficiency (PI), pancreatic sufficiency (PS), CBAVD and non CF-subjects, including obligate heterozygotes (carriers). CFTR-mediated Cl secretion in each study was normalized to the non-CF control subject and expressed as percent wild-type CFTR (Panel A). The severity of lung disease in each group was determined from the longitudinal decline in FEV1 and classified as severe (●), moderate (▲), or mild (■) disease severity (Panel B). No lung disease was observed in the carrier and non-CF groups.

Another approach to determine the amount of CFTR–mediated Cl- secretion that could be beneficial was to measure the amount of wild-type CFTR mRNA in subjects with missense mutations and mutations in introns that regulate mRNA splicing. In these subjects, a small amount of wild type-CFTR mRNA is produced and leads to the synthesis of normal CFTR protein. The percent wild-type CFTR mRNA produced was used to estimate the cell surface density of CFTR and to calculate total CFTR-dependent Cl- secretion (ICFTR) based on equation 1; Equation 1: ICFTR = cell surface density * open probability * channel amplitude. Because the small amount of protein made by the wild-type-CFTR mRNA was expected to be the native form, it was assumed that trafficking to the membrane, open probability, and single channel conductance were normal. Subjects with 4 - 10 % wild type-CFTR mRNA typically have more moderate lung disease, whereas patients with >30 % wild type-CFTR mRNA have mild to no lung disease. Carriers of CF, who do not exhibit CF-related lung disease, had only 50% of the expected CFTR activity. These results were consistent with in vivo functional studies, indicating that 10% wild-type CFTR is sufficient to produce moderate lung disease, whereas as much as 30% is needed to produce mild to no lung disease.

Table 8-1 Severity of Lung Disease and Predicted CFTR Activity Based on Wild-type mRNA Levels in CF Subjects with Splice Mutations

Genotype[a]	Channel Density (wt-mRNA)	Channel Gating (P_o)	Channel Conductance	Total CFTR Activity	Severity of Lung Disease
wt-CFTR	1	1	1	1	None
ΔF508/wt-CFTR	0.5	1	1	0.5	
5T	0.3	1	1	0.3	Mild
5T-TG12-M470V	0.11	1	1	0.11	Moderate
A455E	0.1	1	1	0.10	
3849+10kbC->T	0.08	1	1	0.08	
3272-26A->G	0.05	1	1	0.05	
2789+5G->A	0.04	1	1	0.04	
1811+1.6A->G	0.01	1	1	0.01	Severe
G551D	1	0.025	1	0.025	
ΔF508/ΔF508	0.01	0.25	1	0.0025	

[a] The wt-, ΔF508/wt-, G551D- and ΔF508-genotypes were added for comparison. All other genotypes are missense or splice mutations. P_o = open probability determined from single channel recordings normalized to the wild-type P_o of 0.4. Because the small amount of protein produced by the splice mutations is expected to be normal, the P_o and conductance were normalized to wild-type.

Key indicators of lung disease severity include the patient genotype, age of diagnosis, age of death or transplant, and incidence of pancreatic, sweat gland, intestinal, liver, and reproductive dysfunction. Indicators associated with mild, moderate, or severe lung disease, along with the percent wild-type CFTR activity or mRNA from the analysis of published reports are shown in the table below.

Table 8-2 Clinical Indicators of Disease Severity in CF Subjects

Severity of Lung Disease	Mild	Moderate	Severe
Genotypes (ΔF508/X)	R117H-7T	A455E 3849+10kbC->T 3272-26A->G 2789+5G->A	ΔF508 G551D 1811+1.6kbA->G G542X N1303K
Age at diagnosis	Adulthood	Adolescence	Infant
Age at death/transplant; mean (range)	>50	38 (28 – 48)	24 (8 – 40)
Pancreatic insufficient	0%[a]	50%	100%
Sweat [Cl^-] mmol/L; mean (range)	54 (22 - 100)	81 (28 – 116)	103 (65 - 158)
Other clinical presentations	CBAVD[b] Pancreatitis Sinusitis	CBAVD	CBAVD Meconium Ileus Diabetes,Cirrhosis
Lung function (FEV) decline per year; mean (range)	ND[c]	-0.9% (0.8 – 1.1)	-3% (2.3 – 3.6)
Percent wild type-CFTR activity Surface Density (% wild type-CFTR mRNA)	68 (44 – 90) 33	9 (0 – 33) 7 (4 – 11)	<1 (0 – 15) 4
% Patient Population	1 -2[d]	10 – 20[e]	80 - 90

[a] Mild mutations are associated with chronic pancreatitis
[b] Congenital bilateral absence of the vas deferens
[c] Although a longitudinal assessment of FEV_1 in CBAVD patients has not been done, these patients typically have normal FEV_1 scores. However, occasional mild respiratory disorders have been reported in some patients. These patients have one or two CFTR mutations and typically have normal sweat Cl^- levels and FEV_1 scores.
[d] Accounts for 1 – 2% of male infertility.
[e] Based on the patient population with mild mutations, pancreatic sufficiency, and residual CFTR activity.

In vivo measurements of mutant CFTR-mediated Cl- secretion in airway epithelia of CF patients with moderate or mild disease suggest that ≥10% wild type-CFTR-mediated Cl secretion was associated with a moderate decline in lung function. Estimates of CFTR mediated Cl- secretion based on the amount of wild-type CFTR mRNA in patients with splice mutations also indicate that 10% wild-type CFTR activity was associated with moderate lung disease. Based on these studies, it was concluded that increasing mutant CFTR-mediated Cl secretion to ≥10% wild-type CFTR would decrease the rate of lung function decline in patients with severe lung disease to levels observed in patients with moderate lung disease. Further improvement in lung function would require increasing CFTR activity to levels >30% to 40% wild-type CFTR. The relationship between mutant CFTR activity and disease severity was based on a set amount of CFTR activity since birth, as CFTR function was believed to be unchanged during the lifespan of CF patients. It was not known if increasing mutant CFTR activity to these levels would impact established CF disease. In addition, symptomatic treatment strategies, genetic modifiers, and environment factors can also influence disease severity in CF patients and may impact the amount of CFTR function needed to ameliorate severe lung disease.

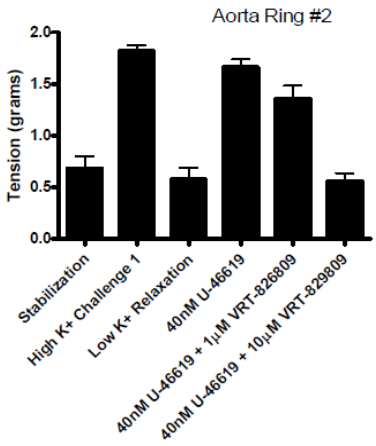
2.6.2.3 Secondary pharmacodynamics

Lead Profiling Screen Data Report of VRTXSD294 for Vertex Pharmaceuticals LLC-San Diego: The activity of VRT-826809 was evaluated in series of radioligand binding assays at a concentration of 10 μM. No significant results were observed.

(b) (4) **Pharmacology Data Report On Compound VES-108, VRTXSD294 for Vertex Pharmaceuticals LLC-San Diego** : The activity of VRT-826809 was evaluated in series of radioligand binding assays at a concentration of 10 µM. VRT-826809 at 10 µM produced a 69 to 75% inhibition of thromboxane A_2 receptor. The IC_{50} and K_i were estimated to be 4.56 and 2.97 µM, respectively.

Effects of VRT-826809 in a Functional Assay for TP Prostanoid Receptor Activity in Rat Aorta Rings In Vitro (Report number D145): Agonist or antagonist activity of VRT-826809 was assessed in an in vitro functional assay of TP prostanoid (thromboxane) receptor activity in rat aorta rings. U-44619, a stable prostaglandin H_2 analog and TP receptor agonist, produced contractile activity in all 4 aorta rings tested with EC_{50} values of 9 to 16 nM. VRT-826809 did not produce contractile responses in 2 aorta rings at concentrations ranging from 1 to 30 µM, indicating that it did not possess TP receptor agonist activity. VRT-826809 at 1 µM partially inhibited and at 10 µM completely blocked the contractile response to U-44619 in a rat aorta ring. These results are consistent with antagonist but not agonist activity of VRT-826809 at TP prostanoid receptors.

Figure 8-3 Inhibition by VRT-826809 at 1 and 10 µM of the Contractile Response to U-46619 in a Rat Aorta Ring



2.6.2.4 Safety pharmacology

Neurological effects:

Effects of Oral (Gavage) Administration on the Functional Observational Battery in Rats (Vertex Study Number VRT-826809-TX-014):

Methods: Potential effects of VRT-826809 on the central nervous system were assessed in male Sprague-Dawley rats. Male rats (8/group) were gavaged once with 0 (0.5% Tween-80 (w/w) + 0.5% methylcellulose (w/w) in water), 250, 500, or 1000 mg/kg/day VRT-826809 or 4 mg/kg Chlorprozamine (positive control). Parameters evaluated during the study were viability, clinical observations, body weights, functional observational battery and motor activity.

Results: VRT-826809 administered as single oral doses up to 1000 mg/kg to rats had no effects on parameters measured by the functional observation battery at 4 and 10 hr postdose. Motor activity values (number of beam breaks) appeared to be elevated for the 500 and 1000 mg/kg groups at 4-hr postdose and for the 250, 500, and 1000 mg/kg groups at 10-hr postdose although differences were not statistically significant.

Cardiovascular effects:

Effects of VRT-826809 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Vertex Study VRT-826809-TX-012)

Methods: The in vitro effects of VRT-826809 on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for IKr, the rapidly activating, delayed rectifier cardiac potassium current) using HEK293 cells were stably transfected with hERG cDNA.

Results: VRT-826809 inhibited hERG current by 0.2 ± 0.2% at the target concentration of 4.6 µM (the highest achievable concentration based on its solubility) versus 0.7 ± 0.2% in control. HERG inhibition at the target concentration of 4.6 µM was not statistically significant when compared to vehicle control values.

Cardiovascular Effects following Oral (Gavage) Administration in Conscious Telemetered Beagle Dogs (Sponsor Study number. VRT-826809-TX-013):

Methods: Potential cardiovascular effects of VRT-826809 were assessed in male beagle dogs. Four telemetered, male beagle dogs received VRT-826809 as single oral doses of 0, 50, 100, and 200 mg/kg using a Latin square design. There was a 3-4 day washout period between doses. Animals were monitored for cardiovascular parameters up to 24 hr after dosing.

Results: VRT-826809 at oral doses up to 200 mg/kg had no effects on heart rate, ECG parameters (PR, QRS, RR, QT, and QTc intervals) and systolic, diastolic, and mean blood pressures.

Pulmonary effects:

Effects of Oral (Gavage) Administration on Respiration Rate and Tidal Volume in Rats (Sponsor Study Number VRT-826809-TX-015):

Methods: Potential respiratory effects of VRT-826809 were assessed in male Sprague-Dawley rats. Male rats (8 /group) were orally administered a single dose of vehicle (0.5% Tween-80 (w/v) + 0.5% methylcellulose (w/v) in water), 250, 500, or 1000 mg/kg VRT-826809 or 30 mg/kg Baclofen (positive control). Respiratory parameters, including respiratory rate, tidal volume and minute volume, were collected pretest, predose, and again 1, 3, 6, 9 and 24 hours after dose administration. At least 40 minutes of

acclimation to the whole body plethysmograph chamber was allowed at each time point, which immediately preceded a 20-minute data collection interval.

Results: VRT-826809 administered as single oral doses up to 1000 mg/kg to rats had no effects on respiratory rate, tidal volume, and minute volume at time points ranging from 1- to 24-hr postdose.

Gastrointestinal effects:

Effects of Oral (Gavage) Administration on Gastrointestinal Transit of a Charcoal Meal in Rats (Sponsor Study Number VRT-826809-TX-016).

Methods: Potential gastrointestinal effects of VRT-826809 were assessed in male Sprague-Dawley rats. Male rats (10/group) were administered a single oral dose of 0 (0.5% Tween-80 (w/v) + 0.5% methylcellulose (w/v) in water), 250, 500 and 1000 mg/kg VRT-826809. Group 5 animals (10 animals) received morphine at a dose level of 30 mg/kg and dose volume of 10 mL/kg. Parameters evaluated during the study were: viability, clinical observations, intestine length, and the distance traversed by charcoal down the gastrointestinal tract.

Results: VRT-826809 administered as single oral doses up to 1000 mg/kg to rats had no effects on intestinal transit of a charcoal meal.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Half-lives of VRT-826809 following intravenous administration to mice, rats, dogs, and monkeys were 4.28, 5.9 to 7.9, 1.62-3.45, and 16.7 hr, respectively. Half-lives following oral administration to rats and dogs were 5.86-7.65 and 1.62-3.45 hr, respectively. Oral bioavailability in rats and dogs was 47-131% and 24-49%, respectively. Volume of distribution values in mice, rats, dogs, and monkeys indicated distribution into tissues. Clearance values in mice, rats, dogs, and monkeys were low as compared to hepatic blood volume.

2.6.4.2 Methods of Analysis

Plasma concentrations of VRT-826809 were measured using a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method, which had a lowest limit of quantitation (LLOQ) of 1 ng/mL and a linearity range between 1 and 10000 ng/mL.

2.6.4.3 Absorption

Mice

Pharmacokinetics of VRT-826809 in Male CD-1 Mice Following Single Intravenous Administration (Report number D049)

Methods: For intravenous administration, male CD-1 mice were administered a single dose of VRT-826809 (HCl salt, Lot 6) formulated in dimethylisosorbide/ethanol/ PEG400/5% dextrose in water (D5W) (10%/15%/35%/40%) at a nominal dose of 2.5 mg/kg free base weight. Plasma concentrations of VRT-826809 were determined using a LC/MS/MS method.

Results: Following intravenous administration in male CD-1 mice at a nominal dose of 2.5 mg/kg free base, VRT-826809 had a very low systemic clearance value of 1.4 mL/min/kg, representing approximately 1% of the hepatic blood flow rate in mice. The volume of distribution at steady state (V_{SS}) was 0.38 L/kg. The terminal half-life of VRT-826809 was 4.3 hours in male mice following intravenous administration.

Rats

Pharmacokinetics of VRT-826809 in Male Sprague-Dawley Rats following Single Intravenous Administration (Report number D081)

Methods: Male Sprague Dawley rats were administered a single IV dose of VRT-826809 (lots 2 and 6) at doses of 1.0, 2.5, and 5.0 mg/kg formulated in dimethylisosorbide/ethanol/propylene glycol/5% dextrose in water (D5W) (10%/15%/35%/40%), with polyethylene glycol 400 (PEG400) replacing propylene glycol for the 2.5 mg/kg dose group. Blood samples were collected at pre-dose, 5, 15, and 30 min and at 1, 2, 4, 6, 8, 12, and 24 hr. Plasma concentrations of VRT-826809 were measured with a LC/MS/MS method.

Results: Following IV administration in male Sprague-Dawley rats, C_{max} and AUC values with doses of 1.0 and 2.5 mg/kg were relatively comparable, while C_{max} and AUC values at 5.0 mg/kg were approximately 3-times higher. VRT-826809 at doses of 1.0, 2.5, and 5.0 mg/kg had relatively low systemic clearance values of 0.8, 1.6, and 1.1 mL/min/kg, respectively, representing approximately 1% to 3% of the hepatic blood flow rate in rats. The volume of distribution at steady state (VSS) was 0.4, 0.7, and 0.5 L/kg for the 1.0, 2.5, and 5.0 mg/kg dose groups, respectively, consistent with low to moderate distribution into tissues. The terminal half-life of VRT-826809 was long at 7.0 hr for the 1.0 mg/kg group, 5.9 hr for the 2.5 mg/kg group, and 7.9 hr for the 5.0 mg/kg group.

Pharmacokinetics of VRT-826809 following Oral Administration in Male Sprague Dawley Rats (Report number D127):

Methods: Male Sprague-Dawley rats with free access to food were administered single oral doses of 1, 30, 150, 300, or 600 mg/kg VRT-826809 (free form, lot 12) in a vehicle consisting of 0.5% Tween80/0.5% methylcellulose/water. In a second study, male Sprague-Dawley rats were either allowed free access to food or fasted for 14 hr prior to

dosing and were administered a dose of 30 mg/kg (free form equivalent) of VRT-826809 (HCl salt, lot 6) formulated in 0.5% methyl-cellulose in water. Plasma concentrations of VRT-826809 were measured with a LC/MS/MS method.

Results: For oral doses of 1, 30, 150, 300, and 600 mg/kg, $AUC_{0\text{-}INF}$ and C_{max} values increased with increasing dose, but increases were generally less than dose proportional. Half-life values ranged from 5.86 to 8.07 hr. Bioavailability with doses of 1, 30, 150, 300, and 600 mg/kg were 131, 115, 47, 81, and 50%, respectively.

In a study evaluating the oral pharmacokinetics of VRT-826809 (HCl salt) under fed and fasted conditions in male Sprague-Dawley rats at a dose of 30 mg/kg (free form equivalent), pharmacokinetic parameters were generally comparable (see table below). The C_{max} value for VRT-826809 (HCl salt) obtained under fed conditions (36.1 µg/mL) was similar to that obtained for the free form (28.2 µg/mL) but was lower than that under fasted conditions (52.3 µg/mL), possibly due to decreased gastric emptying time in the presence of food.

Table 1-2 Summary of Mean (SD) Pharmacokinetic Parameters in Male Rats after Oral Administration of VRT-826809 (HCl Salt) in 0.5% MC/Water under Fed and Fasted Conditions

Nominal Dose (mg/kg)	Vehicle	$AUC_{0\text{-}INF}$ (hr*µg/mL)	C_{max} (µg/mL)	t_{max} (hr)	$t_{1/2}$ (hr)	F (%)	C_{last} (ng/mL)	t_{last} (hr)
30, Fasted	MC	383 (41)	52.3 (3.9)	3.33 (1.15)	5.36 (0.98)	123	60 (18)	48
30, Fed	MC	340 (137)	36.1 (10.2)	3.67 (2.52)	6.07 (0.67)	110	240 (202)	48

Mean (SD); n = 3; MC: 0.5% methylcellulose/water

Dogs

Pharmacokinetics of VRT-826809 Following Intravenous and Oral Administration of the HCl Salt in Male Beagle Dogs (Report Number D047).

Methods: Pharmacokinetic properties of VRT-826809 were assessed in male Beagle dogs after single intravenous and oral doses. For IV administration, male beagle dogs were administered a single dose of VRT-826809 (lot 15) formulated in 10% dimethylisosorbide, 15% ethanol, 35% propylene glycol, and 40% D5W (5% dextrose in water) at nominal doses of 0.2 and 1.0 mg/kg. For oral administration, male beagle dogs were administered single doses of 1, 3, or 10 mg/kg VRT-826809 (lot 5) in a vehicle consisting of 0.5% methylcellulose/water; or single doses of 5, 25, 50, or 100 mg/kg VRT-826809 (lot 6) or 200 mg/kg dose of VRT-826809 (lot 9) formulated in 0.5% Tween80 /0.5% methylcellulose in water. Plasma concentrations of VRT-826809 were determined with a LC/MS/MS method.

Results:

Following a single IV administration of VRT-826809 at nominal doses of 0.2 and 1.0 mg/kg, C_{max} and AUC values were generally dose proportional. Systemic clearance values with doses of 0.2 and 1.0 mg/kg (2.48 and 2.40 mL/min/kg) were low representing approximately 7% of hepatic blood flow in dogs. The volume of distribution at steady state was also low at 0.22 and 0.35 L/kg for the 0.2 and 1.0 mg/kg doses, respectively. Terminal half-lives of VRT-826809 after IV administration of doses of 0.2 and 1.0 mg/kg were 1.62 and 3.45 hr, respectively.

Following oral administration of VRT-826809 at doses of 1, 3, and 10 mg/kg in 0.5% methylcellulose/water, C_{max} and AUC values were generally dose proportional. Bioavailability values at doses of 1, 3, and 10 mg/kg were 34.8, 49.2, and 40.2%, respectively. Half-lives at 1, 3, and 10 mg/kg were 5.99, 5.25, and 8.84 hr, respectively.

Following oral administration of VRT-826809 at doses of 5, 25, 50, 100, and 200 mg/kg in 0.5% Tween80 /0.5% methylcellulose in water, C_{max} and AUC values were generally dose proportional. Bioavailability values at doses of 5, 25, 50, 100, and 200 mg/kg were 24.3, 41.3, 26.7, and 45.7%, respectively. Half-lives at 5, 25, 50, 100, and 200 mg/kg were 6.99, 5.87, 4.88, and 5.36 hr, respectively.

Longer half-lives following oral administration as compared to IV administration indicated that the rate of absorption was longer than the rate of elimination. The average Tmax ranged from 1 to 2.3 hours at all doses studied, indicating that the absorption of VRT-826809 in dogs was not dissolution rate-limited.

Oral Pharmacokinetics of the HCl Salt and Free Form of VRT-826809 and Comparison of the Free Form under Fed and Fasted Conditions in Male Beagle Dogs (Report number D133)

Methods: For evaluation of the effects of food on the pharmacokinetic parameters of VRT-826809, male dogs were administered a single dose of 10 mg/kg VRT-826809 (free form, lot 12) in a vehicle consisting of 0.5% Tween80/0.5% methylcellulose/water under fed or fasted conditions. In a another study, the free form (lot 12) or HCl salt (lot 15) of VRT-826809 was administered to male dogs at an oral dose of 200 mg/kg (free form) in the same vehicle and dose volume. Plasma concentrations of VRT-826809 were determined with a LC/MS/MS method.

Results: In a study evaluating the oral pharmacokinetic parameters of VRT-826809 at a dose of 10 mg/kg under fed and fasted conditions in male beagle dogs, $AUC_{0\text{-}INF}$ values of 35.8 and 35.7 µg·hr/mL achieved under fasted and fed conditions, respectively, were comparable. The C_{max} value obtained under fasted conditions (7.87 µg/mL) was higher than that obtained under fed conditions (4.07 µg/mL). T_{max} values were shorter in the fasted state (1.5 hours) relative to the fed state (2.7 hours). The terminal half-life of VRT-826809 was similar under fasted or fed conditions, 6.3 and 6.6 hr, respectively. The variability of the systemic exposure of VRT-826809 was higher under fed condition than under fasted conditions, possibly due to changes in stomach

emptying time under fed conditions. Bioavailability of the free form at a dose of 10 mg/kg was similar under fasted (F = 52.9%) and fed (F = 52.8%) conditions.

In a study comparing oral administration of the free form or HCl salt of VRT-826809 at 200 mg/kg, higher systemic $AUC_{0\text{-}INF}$ and C_{max} values were observed for VRT-826809 after administration of the HCl salt compared to the free form; 755 and 288 µg·hr/mL ($AUC_{0\text{-}INF}$) and 133 and 52 µg/mL (C_{max}), respectively. Bioavailability was also higher for the HCl salt (F = 53.4%) than for the free form (F = 20.3%). T_{max} values of 1.7 and 2.5 hr, and t1/2 values 6.1 and 5.5 hours, were similar after administration of the free form and HCl salt of VRT-826809, respectively.

Monkeys

Pharmacokinetics of VRT-826809 in Male Cynomolgus Monkeys following Single Intravenous Administration (Report number D048)

Methods: Male Cynomolgus monkeys were administered a single IV dose of VRT-826809 (Lot 6) formulated in dimethylisosorbide/ethanol/PEG400/5% dextrose in water (D5W) (10%/15%/35%/40%) at a nominal dose of 1.0 mg/kg. Blood samples were collected at pre-dose, 5, 15, 30, and 45 min and at 1, 2, 4, 6, 8, 12, 24, 36, and 48 hr following IV administration. Plasma concentrations of VRT-826809 were measured with a LC/MS/MS method.

Results: Following IV administration in male cynomolgus monkeys at a dose of 1.0 mg/kg, VRT-808409 had a low systemic clearance value of 0.72 mL/min/kg, representing approximately 2% of the hepatic blood flow rate in monkeys. The volume of distribution at steady state (VSS) was 0.86 L/kg. The terminal half-life of VRT-826809 in male monkeys following IV administration was 16.7 hours.

2.6.4.4 Distribution

In Vitro Binding of VRT-826809 to Plasma Proteins in Mouse, Rat, Dog, Monkey, and Human Plasma (Report number D152)

Methods: The objective of this study was to determine the extent of binding of VRT-826809 to plasma proteins in male CD-1 mouse, male Sprague-Dawley rat, male Beagle dog, male Cynomolgus monkey, and male human plasma in vitro. In the plasma protein-binding experiments, VRT-826809 was incubated with mouse, rat, dog, monkey, or human plasma at initial concentrations of 0.5, 1, 5 and 10 µM. VRT-826809 bound to plasma proteins was separated from unbound VRT-826809 using equilibrium dialysis against buffer.

Results: For mouse, rat, dog, monkey and human plasma, the percentage of VRT-826809 bound to plasma varied from 99.7 to 99.8%, 99.6 to 99.9%, 98.5 to 99.4%, 99.3 to 99.5% and 99.2 to 99.4%, respectively. At an initial concentration of 0.5 µM, the

plasma protein binding followed this trend across species with mouse = rat = human = monkey > dog.

Table 8-1 Mean Percent (%) Binding of VRT-826809 to Plasma Proteins Following Equilibrium Dialysis of Plasma Against Buffer.

VRT-826809 (µM)	Percent Protein Bound (Mean ± SD)				
	Mouse	Rat	Dog	Monkey	Human
0.5	99.7[a]	99.6 ± 0.5	98.5[a]	99.3[a]	99.4 ± 0.1
1	99.8 ± 0.1	99.7[a]	98.9 ± 0.3	99.5 ± 0.1	99.2 ± 0.2
5	99.8 ± 0.3	99.0 ± 0.5	99.0 ± 0.5	99.4 ± 0.3	99.3 ± 0.1
10	99.8 ± 0.2	99.9 ± 0.0	99.4[a]	99.3[a]	99.4 ± 0.1

Positive controls: glipizide (89.6 ± 4.0)
[a]n=2

Tissue Distribution of VRT-826809 in Male Sprague Dawley Rats Following Single Oral Administration (Report number D051)

Methods: The distribution of VRT-826809 in liver, lung, pancreas, and brain of male Sprague-Dawley rats was assessed following single oral doses of 10 and 75 mg/kg. Male rats were administered a dose of 10 mg/kg (freebase weight) VRT-826809 HCl salt (lot #6) in 0.5% methylcellulose/water by oral gavage. At 1 hr postdose, plasma and perfused brain, liver, lung, and pancreas were obtained from one group, and plasma and non-perfused brain, liver, lung, and pancreas were obtained from another group. Tissues were homogenized. To evaluate tissue distribution and elimination rates, male Sprague-Dawley rats were administered a single 75 mg/kg dose of VRT-826809 HCl salt (lot 6) in 0.5% methylcellulose/water by oral gavage. Blood samples were collected at 1, 4, 12, and 48 hr postdose. At each time point, a systemic perfusion was performed and the brain, liver, lung and pancreas were removed, weighed, and homogenized. Concentrations of VRT-826809 in plasma and tissue homogenates were determined with a LC/MS/MS method.

Results: Following single oral doses of 10 or 75 mg/kg, the highest concentrations were observed in plasma and liver, which were relatively comparable, followed by lung, pancreas, and brain. The terminal half-lives for elimination of VRT-826809 from all tissues and plasma were comparable at 6.5 to 8.1 hr.

Table 1-1 Mean (SD) Concentrations of VRT-826809 in Perfused Versus Nonperfused Tissues and Plasma of Male Sprague Dawley Rats at 1 Hour After Oral Administration of 10 mg/kg

Perfusion status	VRT-826809 (ng/mL or ng/g)					Tissue-to-Plasma Concentration Ratio			
	Plasma	Liver	Lung	Pancreas	Brain	Liver/ plasma	Lung/ plasma	Pancreas/ plasma	Brain/ plasma
Nonperfused	13683 (7874)	14587 (4910)	4487 (1945)	2133 (758)	402 (159)	1.19 (0.43)	0.37 (0.19)	0.17 (0.05)	0.03 (0.01)
Perfused	9177 (5063)	9397 (4859)	2717 (1595)	1857 (1028)	238 (228)	1.03 (0.17)	0.29 (0.02)	0.20 (0.02)	0.02 (0.01)

Table 1-2 Summary of Tissue Pharmacokinetic Parameters of VRT-826809 in Male Sprague Dawley Rats After Oral Administration of 75 mg/kg

Tissue	$AUC_{0-\infty}$ (ng·hr/mL)	C_{max} (ng/mL)	t_{max} (hr)	C_{last} (ng/mL)	t_{last} (hr)	$t_{1/2}$ (hr)	C_{max} Ratio (Tissue/Plasma)
Plasma	980266	59067	4	542	48	6.51	--
Liver	885161	38067	4	965	48	8.06	0.64
Lung	187039	9140	1	189	48	7.79	0.15
Pancreas	132270	6833	4	86	48	6.87	0.12
Brain	9304[a]	957	4	546	12	--	0.02

[a] AUC_{all}

2.6.4.5 Metabolism

In Vitro

In Vitro Assessment of Metabolic Stability of VRT-826809 in Hepatocytes from Rat, Dog, Monkey, and Human (Report number D072)

Methods: The objective of this study was to evaluate the extent of in vitro metabolism of VRT-826809 in cryopreserved hepatocytes from various species. VRT-826809 at 1 µM or testosterone (positive control) at 30 µM were incubated with male rat, male dog, male Cynomolgus monkey, or male human hepatocytes at a concentration of 1×10^6 cells/mL. The samples were analyzed using LC/MS/MS and the percentage of VRT-826809 or testosterone remaining based on the zero time point was calculated.

Results: The rate of metabolism of VRT-826809 at 1 µM was slow in hepatocytes from dog, monkey, and human with 84% to 89% remaining after incubation for 240 min. VRT-826809 was stable in rat hepatocytes. The corresponding predicted hepatic clearances were 3.2, 3.3, and 1.6 mL/min/kg in dog, monkey, and human, respectively. The corresponding percentages of hepatic blood flow were 10.3%, 7.5%, and 7.6% in dog, monkey and human, respectively.

Table 1-1 Metabolism of VRT-826809 at 1 µM Initial Concentration in Cryopreserved Hepatocytes Isolated from Rat, Dog, Monkey and Human

Species	Mean (SD) Percent Remaining at 240 min	$t_{1/2}$ (min)	Intrinsic Clearance (mL/min/kg)	Predicted Hepatic Clearance (mL/min/kg)
Rat	115[a] (6)	Stable	NC[b]	NC
Dog	89[a] (4)	754	3.5	3.2
Monkey	85 (8)	766	3.5	3.3
Human	84 (5)	1240	1.7	1.6

[a]Incubation time = 225 minutes

[b]NC: not calculated

The In Vitro Stability of VRT-826809 in Human Recombinant CYP450 Isozymes (Report number D071):

Methods: The objective of this study was to investigate the potential involvement of various human CYP450 isozymes in the metabolism of VRT-826809 using recombinant human CYP450 isozymes. VRT-826809 was incubated at initial concentrations of 2 or 20 µM with recombinant human CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 for 1 hr. The extent of metabolism was determined by measuring the VRT-826809 remaining at the end of the incubation using a LC/MS/MS method.

Results: The only CYP450 isozyme tested that detectably metabolized VRT-826809 was CYP3A4, with 82% remaining at 0.2 µM and 87% remaining at 2 µM after 1 hour incubation at 37°C. There was no detectable reduction in VRT-826809 after incubation at 2 and 20 µM with CYP1A2, CYP2C9, CYP2C19, and CYP2D6.

Table 1-1 Summary of Stability Data (Percent Remaining) for VRT-826809 and Control Compounds after 1 Hour Incubation at 37°C with Human CYP450 Isozymes

Initial VRT-826809 concentration	Mean (SD) Percent of VRT-826809 Remaining (%)				
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
2 µM	99 (7)	103[a]	99 (3)	106 (1)	87 (3)
20 µM	102 (2)	107 (16)	128 (22)	124 (6)	92 (4)

Assessment of VRT-826809 Inhibition Potential of Human Cytochrome P450 Isozymes (Report number D079)

Methods: The purpose of this study was to determine the potential of VRT-826809 to inhibit human cytochrome P450 (CYP450) isozymes. The CYP450 reversible inhibition potential of VRT-826809 was evaluated using pooled, mixed-gender human liver microsomes. VRT-826809 was incubated at 8 different concentrations with liver microsomes supplemented with NADPH and 30 µM phenacetin as CYP1A2 substrate, 5 µM diclofenac as CYP2C9 substrate, 30 µM mephenytoin as CYP2C19 substrate, 10

μM dextramethorphan as CYP2D6 substrate, and 50μM testosterone or 2.5 μM midazolam as CYP3A4 substrates. The appearance of metabolites acetaminophen, 4-hydroxydiclofenac, 4-hydroxymephenytoin, dextorphan, 6β-hydroxytestosterone, and 1-hydroxymidazolam, respectively, was monitored by LC/MS/MS.

For the CYP2C9 and CYP3A4 time-dependent inhibition assays, reaction mixtures were prepared with one set of vials (time-dependent inhibition samples) containing 8 different concentrations of VRT-826809 while the other set (reversible samples) did not contain VRT-826809. Human liver microsomes (0.2 mg/mL) supplemented with 1 mM NADPH were pre-incubated with the reaction mixtures for 30 min in a 37°C water bath. After the preincubation, the microsomes were diluted and VRT-826809 was added at 8 different concentrations to the reversible samples. A CYP3A4 or CYP2C9 specific substrate, 250 μM testosterone or 25 μM diclofenac, respectively, was added and vials were incubated for the substrate appropriate incubation time. The appearance of each metabolite was monitored and the enzyme activities in the pre-incubated samples were compared to the reversible samples.

Results: In a human liver microsome-based reversible inhibition assay, VRT-826809 inhibited the CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 isozymes with IC_{50} values of >30 μM, >30 μM, >90 μM, >30 μM, and >30 μM (testosterone and midazolam). In the time-dependent inhibition assays, diclofenac and testosterone hydroxylation activities did not decrease when human liver microsomes were pre-incubated with VRT-826809. IC_{50} values >30 μM were obtained for both isozymes.

Evaluation of Induction of Liver Cytochrome P450 Isozymes in Human Hepatocytes Following In Vitro Exposure of Hepatocytes to VRT-826809 (Report number D128)

Methods: Effects of VRT-826809 on expression and activities of selected Cytochrome P450 enzymes (CYP3A4 and CYP1A2) were evaluated in human hepatocytes following in vitro exposure to VRT-826809 at 0.1, 1, and 10 μM for 48 hr. The conversion of the CYP1A2 and CYP3A4 probe substrates to their metabolites by hepatocytes treated with VRT-826809 was compared to hepatocytes treated with the prototypical CYP1A2 and CYP3A4 inducers, β-naphthoflavone and rifampicin, respectively. Following incubation, hepatocyte supernatants were removed and assayed by HPLC-UV for CYP3A4 activity, or fluorescence detection for CYP1A2 activity.

Results: Induction of CYP1A2 activity in the presence of the VRT-826809 was approximately 1 to 2-fold higher than in the control hepatocytes. Induction with the protypical CYP1A2 inducer, β-naphthoflavone, was approximately 2 to 4-fold higher than in the control hepatocytes. Induction of CYP3A4 activity in the presence of the VRT-826809 was approximately 2 to 4-fold higher than in the control hepatocytes. Induction in the presence of the protypical CYP3A4 inducer, rifampicin, was approximately 4-fold to 23-fold higher than in the control hepatocytes.

In Vivo

Characterization of VRT-826809 Putative Metabolites from In Vitro and In Vivo Matrices (Report number D083)

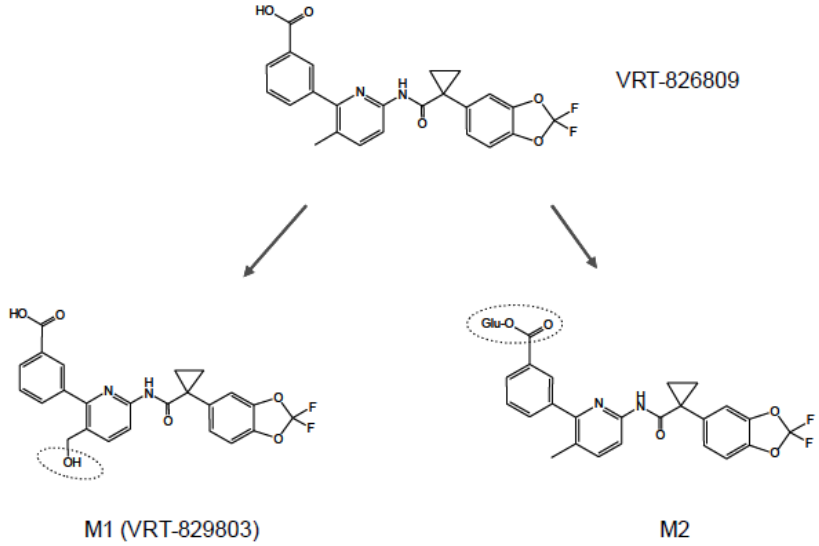
Methods: Putative in vitro metabolites of VRT-826809 were characterized with in vitro rat, dog, monkey, and human liver microsomes and cryopreserved hepatocytes. The in vivo metabolic profile of VRT-826809 was assessed in rats and dogs following oral dosing. VRT-826809 at a concentration of 10 µM was incubated for 60 min with liver microsomes from rat, dog, monkey and human. VRT-826809 at a concentration of 30 µM was incubated for 120 min with cryopreserved hepatocytes from rat, dog, monkey, and human. Plasma, urine and bile samples were collected from 4 bile duct-cannulated male Sprague-Dawley rats after oral administration of 100 mg/kg VRT-826809 (HCl salt, lot 6). Plasma and urine samples were collected from 3 male beagle dogs after oral administration of 10 mg/kg VRT-826809 (HCl salt, lot 5). Samples were collected from 0 to 72 hr postdose and were pooled for analysis. The in vitro and in vivo sample extracts were analyzed for VRT-826809 and its metabolites using LC/MS/MS methods.

Results:

In vitro: The metabolites detected in rat, dog, monkey and human in vitro samples were designated M1 and M2. Metabolite M1 (*m/z* 469) is the methyl alcohol of VRT-826809, with oxidation at the aromatic methyl group. Metabolite M2 (*m/z* 629) is a direct glucuronide conjugate of VRT-826809. M1 was the primary metabolite observed following incubation of VRT-826809 with liver microsomal preparations, while M2 was the primary metabolite detected following incubation of VRT-826809 with hepatocytes.

In vivo: The direct glucuronide conjugate (M2) was the most abundant metabolite in the plasma of rats and dogs after oral administration of VRT-826809. Only a small percentage of the administered dose of VRT-826809 was excreted unchanged in the bile of rat (1.1%) or in the urine of rat (0.3%) or dog (0.8%). The glucuronide (M2) was the primary metabolite in urine of rat and dog, and was excreted in rat bile at much higher levels than the parent.

Figure 1-1 Structures of VRT-826809 and Proposed Metabolites



2.6.4.9 Discussion and Conclusions

Half-lives of VRT-826809 following intravenous administration to mice, rats, dogs, and monkeys were 4.28, 5.9 to 7.9, 1.62-3.45, and 16.7 hr, respectively. Half-lives following oral administration to rats and dogs were 5.86-7.65 and 1.62-3.45 hr, respectively. Oral bioavailability in rats and dogs was 47-131% and 24-49%, respectively. For mouse, rat, dog, monkey and human plasma, the percentage of VRT-826809 bound to plasma varied from 99.7 to 99.8%, 99.6 to 99.9%, 98.5 to 99.4%, 99.3 to 99.5% and 99.2 to 99.4%, respectively. Volume of distribution values in mice, rats, dogs, and monkeys indicated distribution into tissues. Following single oral doses of 10 or 75 mg/kg administered to rats, the highest concentrations were observed in plasma and liver, which were relatively comparable, followed by lung, pancreas, and brain. Clearance values in mice, rats, dogs, and monkeys were low as compared to hepatic blood volume. The metabolites of VRT-826809 detected in rat, dog, monkey and human in vitro samples were designated M1 (methyl alcohol of VRT-826809) and M2 (glucuronide conjugate of VRT-826809). M1 was the primary metabolite observed following incubation of VRT-826809 with liver microsomal preparations, while M2 was the primary metabolite detected following incubation of VRT-826809 with hepatocytes. The direct glucuronide conjugate (M2) was the most abundant metabolite in the plasma of rats and dogs after oral administration of VRT-826809. Only a small percentage of the administered dose of VRT-826809 was excreted unchanged in the bile of rat (1.1%) or in the urine of rat (0.3%) or dog (0.8%). The glucuronide (M2) was the primary metabolite in urine of rat and dog, and was excreted in rat bile at much higher levels than the parent.

2.6.4.10 Tables and figures to include comparative TK summary

Species comparison of pharmacokinetic parameters

Species	Route	Doses mg/kg	C_{max} µg/mL	AUC µg·hr/mL	CL mL/min/kg	T½ hr	V_{ss} L/kg	F %
CD-1 mice	IV	2.5	13000	27723	1.40	4.28	0.38	
Rats	IV	1.0 2.5 5.0	7.1 to 24.6	19.2 to 62.3	0.80 to 1.6	5.9 to 7.9	0.4 to 0.7	
Rats	Oral	1 to 600	1.10 to 203	13.7 to 3085		5.86 to 7.65		47 to 131
Dog	IV	0.2 1.0	1.6 8.3.	1.08 6.37	2.40 2.48	1.62 3.45	0.22 0.34	
Dog	Oral	1 to 200	0.37 to 101	2.19 to 523		4.88 to 8.84		24 to 49
Monkey	IV	1.0	7.7	21.0	0.72	16.7	0.86	

There were no significant differences in drug exposures for rats and dogs in the fasted and fed states.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:
In a single oral dose toxicity study, CD-1 mice received VRT-826809 at 0, 500, 1000, or 2000 mg/kg and were observed for 14 days postdose. There were no deaths or treatment-related clinical signs. Body weight gains and food consumption were reduced for male treatment groups; however, these parameters were unaffected for female treatment groups.

In a single oral dose toxicity study, Sprague-Dawley rats received VRT-826809 at 0, 500, 1000, or 2000 mg/kg and were observed for 14 days postdose. There were no deaths. Clinical signs were confined to male and female rats that received a single dose of 2000 mg/kg. Clinical signs for both males and females included ano-genital stains, decreased activity, hunched appearance, red/brown stains on the snout, and unformed stool. Except for ano-genital staining, which was observed in males until day 6, all clinical signs subsided after day 4. Potential treatment-related macroscopic findings were observed in the epididymides, jejunum, liver, lungs, stomach, and uterus w/cervix.

In a 14-day toxicology study with rats, VRT-826809 was administered at oral doses of 0, 150, 300, or 600 mg/kg/day. There were minor effects on hematology parameters in treatment groups, primarily the high dose group, consisting of slight decreases of RBC counts, hemoglobin levels, and hematocrit and slight increases of WBC counts, which appeared to have no toxicological significance. Total bilirubin levels were slightly increased for females in the 600 mg/kg/day group and ALT values were slightly increased for males and females in the 600 mg/kg/day group; however, these increases

did not appear to achieve toxicological significance. No target organs of toxicity were observed with oral doses up to 600 mg/kg/day for 14 days. The NOAEL was identified as the high dose of 600 mg/kg/day based upon no treatment-related histopathological findings.

In a 14-day toxicology study with dogs, VRT-826809 was administered at oral doses of 0, 50, 100, or 200 mg/kg/day. Dogs were 5 to 6 months old at the start of treatment, which was considered to be unusually young for a standard toxicology study. There were increased incidences of unformed stool in treatment groups although there was no dose-response. There were minor effects on hematology parameters for females in the high dose group, consisting of slight decreases of RBC counts, hemoglobin levels, and hematocrit and slight increases of WBC counts, which appeared to have no biological significance. Prostate weights were decreased for male treatment groups, which was attributed to the immaturity of the dogs. Histopathological findings in the kidneys were judged to be potentially related to treatment. Findings in other tissues were judged to be spontaneous background findings and unrelated to treatment. Histopathological findings of concern in the kidneys were observed for one male dog from the 200 mg/kg/day group and consisted of medullary findings of bilateral interstitial stromal hyperplasia and bilateral inflammatory cell infiltrate in the stroma. The sponsor judged that these findings reflected the presence of a previous low grade infection; however, there was no historical control data from the testing laboratory to support that these particular findings were incidental and not test article-related. The bilateral nature of these findings increased the concern of a potential relationship to treatment. Further, these findings were judged to be unmonitorable in a clinical setting. Based upon the histopathological findings in the kidneys, the NOAEL was identified as the mid dose of 100 mg/kg/day.

Genetic toxicology:

VRT-826809 was negative in the in vitro bacterial/microsomal activation assay, in vitro Chinese hamster ovary cell chromosomal aberration assay, and in vivo mouse micronucleus assay.

2.6.6.2 Single-dose toxicity

Study title: **Single-Dose Oral Toxicity Study in Mice with a 14-Day Observation Period.**

Key study findings:

● In a single oral dose toxicity study, CD-1 mice received VRT-826809 at 0, 500, 1000, or 2000 mg/kg and were observed for 14 days postdose.

● There were no deaths or treatment-related clinical signs.

● Body weight gains and food consumption were reduced for male treatment groups; however, these parameters were unaffected for female treatment groups.

Study no.: Sponsor Study number VRT-826809-TX-008
Volume #, and page #: Electronic IND, Pages 1-241
Conducting laboratory and location: (b) (4)

Date of study initiation: May 31, 2007
GLP compliance: No, Draft Final Report
QA report: yes ( ) no (X)
Drug, lot #, and % purity: VRT-826809, Lot number 12

Methods
Doses: VRT-826809 was administered as single oral doses of 0, 500, 1000, and 2000 mg/kg.

	Daily Dose[a]			Number of Animals		
				Total	Toxicokinetics Day 1	Necropsy Day 15
Group	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M/F	M/F	M/F
1	0	20	0	16/16	6/6	10/10
2	500	20	25	28/28	18/18	10/10
3	1000	20	50	28/28	18/18	10/10
4	2000	20	100	28/28	18/18	10/10

[a] Doses represent active ingredient.
Numbers shown under M/F represent the total numbers of males and females in each dose group.
The first day of dosing was defined as Day 1 of the study.

Concentrations of dosing solutions ranged from 106.2 to 114.0% of nominal concentrations.

Species/strain: Crl:CD-1®(ICR)BR mice were obtained from (b) (4)
Number/sex/group or time point (main study): 10 mice/sex/group
Route, formulation, volume, and infusion rate: Vehicle (0.5% Tween 80 (w/v) + 0.5% methylcellulose (w/v) in water) and dosing suspensions were administered by oral gavage using a dose volume of 20 mL/kg.
Satellite groups used for toxicokinetics or recovery: For toxicokinetic analysis, there were 6 mice/sex/group for the control group and 18 mice/sex/group for treatment groups.
Age: Animals were 7 weeks old at dosing.
Weight: At dosing, body weight ranges for main study animals were 23.5-34.3 g for males and 20.2-29.2 g for females.

Observation and Times:
Clinical signs: Animals were observed in their cages for mortality and general condition twice daily up to 14 days after dosing. Observations for signs of toxic or pharmacologic effects were made twice daily for each toxicity study animal. Physical examinations were conducted twice per week.
Body weights: Body weights were measured twice weekly.

Food consumption: Food consumption was measured twice weekly.
Ophthalmoscopy: Not performed.
EKG: Not performed
Hematology: Not performed
Clinical chemistry: Not performed
Urinalysis: Not performed
Gross pathology: Animals were sacrificed 14 days after dosing and submitted to macroscopic examinations. Organs and tissues were collected and preserved.
Organ weights: Absolute and relative organ weights were measured for the adrenal glands, brain, heart, liver, ovaries, spleen, testes, and thymus,
Histopathology: Not performed
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on day 1 at 0.5, 1, 2, 4, 10, and 24 hr postdose. There were 3 mice/sex/group/time point. The concentration of VRT-826809 in the individual plasma samples was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a lower limit of quantitation (LLOQ) of 2 ng/mL.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical signs.

Body weights: Mean body weight gains for male treatment groups up to day 11 postdose ranged from 56.6 to 67.7% of the control group; however, mean body weight gains were unaffected for female treatment groups.

Food consumption: Food consumption for male treatment groups over the 14-day observation period after dosing was reduced by 78.9 to 82.4%; however, food consumption was unaffected for female treatment groups.

Gross pathology: One male in the 2000 mg/kg group was observed with enlarged kidneys.

Organ weights: Absolute and relative thymus weights for male treatment groups were increased by 112.4 to 135.8%; however, there was no dose-response. Absolute and relative liver weights for males in the 2000 mg/kg group were decreased by 82.3 to 87.3% of the control.

Toxicokinetics: C_{max} and AUC values increased with increasing dose, but in a less than dose proportional manner. There were no apparent sex-related differences in C_{max} and AUC values. Elimination half-life values ranged from 4.1 to 8.6 hr.

Table 1.3-1 Summary of Toxicokinetic Parameters for VRT-826809 in Female and Male Mice

Dose (mg/kg)	Gender							
	Female				Male			
	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	$DN\_AUC_{0\text{-}24hr}$[a] (µg*hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	$DN\_AUC_{0\text{-}24hr}$ (µg*hr/mL)
500	173.00	0.50	1836.67	3.67	142.00	0.50	1966.16	3.93
1000	196.00	2.00	1848.50	1.85	185.00	2.00	2104.45	2.10
2000	325.00	1.00	2899.05	1.45	264.00	1.00	2677.90	1.34

[a]Dose-normalized to 1 mg/kg. Source: Table 10.1-9.

Study title: **Single Dose Oral Toxicity Study in Rats with a 14-Day Observation Period.**

Key study findings:

● In a single oral dose toxicity study, Sprague-Dawley rats received VRT-826809 at 0, 500, 1000, or 2000 mg/kg and were observed for 14 days postdose.

● There were no deaths. Clinical signs were confined to male and female rats that received a single dose of 2000 mg/kg. Clinical signs for both males and females included ano-genital stains, decreased activity, hunched appearance, red/brown stains on the snout, and unformed stool. Except for ano-genital staining, which was observed in males until day 6, all clinical signs subsided after day 4.

● Potential treatment-related macroscopic findings were observed in the epididymides, jejunum, liver, lungs, stomach, and uterus w/cervix.

Study no.: Sponsor Study number VRT-826809-TX-009
Volume #, and page #: Electronic IND, Pages 1 to 245
Conducting laboratory and location: (b) (4)

Date of study initiation: June 1, 2007
GLP compliance: No, Audited Draft Final Report
QA report: yes ( ) no (X)
Drug, lot #, and % purity: VRT-826809, Lot number 12 (Purity, 98.86%)

Methods
Doses: VRT-826809 was administered at single oral doses of 0, 500, 1000, and 2000 mg/kg.

Group	Daily Dose[a]			Number of Animals		
				Total	Toxicokinetics	Necropsy
					Day 1	Day 15
	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M/F	M/F	M/F
1	0	20	0	13/13	3/3	10/10
2	500	20	25	19/19	9/9	10/10
3	1000	20	50	19/19	9/9	10/10
4	2000	20	100	19/19	9/9	10/10

[a] Doses represent active ingredient.

Actual concentrations of dosing solutions were 102.8-109.2% of nominal concentrations.

Species/strain: Sprague-Dawley (Crl: CD® (SD) IGS BR) rats were obtained from (b) (4)

Number/sex/group or time point (main study): 10 rats/sex/group
Route, formulation, volume, and infusion rate: Vehicle (0.5% Tween 80 (w/v) + 0.5% methylcellulose (w/v) in water) and dosing suspensions were administered by oral gavage using a dose volume of 20 mL/kg.
Satellite groups used for toxicokinetics or recovery: For toxicokinetic analysis, there 3 rats/sex/group for the control group and 9 rats/sex/group for treatment groups.
Age: Rats were approximately 9 weeks old at dosing.
Weight: Body weight ranges for toxicity animals were 262-300 g for males and 172-222 g for females.

Observation and Times:
Clinical signs: Animals were observed in their cages for mortality and general condition twice daily up to 14 days postdose. Observations for signs of toxic or pharmacologic effects were made twice daily for each toxicity study animal. Physical examinations were conducted twice weekly.
Body weights: Body weights were measured twice weekly.
Food consumption: Food consumption was measured twice weekly.
Ophthalmoscopy: Not performed.
EKG: Not performed.
Hematology: Not performed.
Clinical chemistry: Not performed.
Urinalysis: Not performed.
Gross pathology: Necropsy was performed on 10 toxicity animals/sex/group 14 days after receiving a single dose. Macroscopic examinations were conducted on each animal. Organs and tissues were collected and preserved.
Organ weights: Absolute and relative organ weights were measured for the adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, testes, and thymus.
Histopathology: Not performed.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on day 1 at 0.5, 1, 2, 4, 10, and 24-hr postdose. The concentration of VRT-

826809 in the individual plasma samples was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), with a lower limit of quantitation (LLOQ) of 2 ng/mL.

Results:

Mortality: None.

Clinical signs: Clinical signs were confined to male and female rats that received a single dose of 2000 mg/kg. Clinical signs for both males and females included ano-genital stains, decreased activity, hunched appearance, red/brown stains on the snout, and unformed stool. Additional signs observed for males (generally only 1 animal) included dark discoloration in eyes, no stool, red exudate from snout, scabs on snout, slow breathing, and snout swollen. Additional signs observed for females (generally only 1 animal) included decreased fecal volume and lethargic. Except for ano-genital staining, which was observed in males until day 6, all clinical signs subsided after day 4.

Body weights: Body weights gains for male and female rats in the 2000 mg/kg group up to day 11 postdose were decreased to 77.4 and 65.2% of the control, respectively.

Food consumption: Food consumption for male and female rats in the 2000 mg/kg group from days 1 to 14 postdose were decreased to92.9 and 91.5% of the control, respectively.

Gross pathology: Potential treatment-related macroscopic findings were observed in the epididymides, jejunum, liver, lungs, stomach, and uterus w/cervix. For male #4002 at 2000 mg/kg, there were findings of epididymides abscess, jejunum abscess, liver discolored, and stomach adhesion.

Gross pathological findings on day 14, n = 10 rats/sex/group

Organ/Tissue	Males				Females			
	0	500	1000	2000	0	500	1000	2000
Epididymides -abscess	0	0	0	2				
Jejunum -abscess, serosa	0	0	0	1	0	0	0	0
Liver -discolored, all lobes	0	0	0	1	0	0	0	0
Lungs -discolored	0	0	1	1	0	0	0	1
Stomach -adhesions, serosa	0	0	0	1	0	0	0	0
Uterus w/Cervix -distended					0	1	1	1

Organ weights: There were no treatment-related effects on organ weights.

Toxicokinetics: C_{max} and AUC values increased with increasing doses. C_{max} values for males and females as well as AUC values for females increased in a less than dose proportional manner. AUC values for males increased in a dose-related manner. C_{max} and AUC values for females were greater than values for males at 500 and 1000 mg/kg; however, values were relatively comparable at 2000 mg/kg. Observed elimination half-life (T½) values of VRT-826809 ranged from 9.4 to 10.8 hr in the 500 mg/kg group. The elimination half-life could not be calculated with doses of 1000 and 2000 mg/kg.

Table 1.3-1 Summary of Toxicokinetic Parameters for VRT-826809 in Female and Male Sprague-Dawley Rats Following a Single Oral Administration of 500, 1000 or 2000 mg/kg of VRT-826809

Dose (mg/kg)	Gender							
	Female				Male			
	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	DN_AUC_{0-24hr}[a] (µg*hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	DN_AUC_{0-24hr}[a] (µg*hr/mL)
500	243.0	4.00	3345.65	6.69	135.00	4.00	1388.62	2.78
1000	263.00	4.00	5044.75	5.04	177.00	10.00	3534.28	3.53
2000	305.00	10.00	6750.25	3.38	306.00	24.00	6106.50	3.05

[a]Dose-normalized to 1 mg/kg. Source: Table 10.1-9.

2.6.6.3 Repeat-dose toxicity

Rats

Study title: **VRT-826809: A 14-Day Oral (Gavage) Toxicity and Toxicokinetic Study in Rats.**

Key study findings:

● In a 14-day toxicology study with rats, VRT-826809 was administered at oral doses of 0, 150, 300, or 600 mg/kg/day.

● There were minor effects on hematology parameters in treatment groups, primarily the high dose group, consisting of slight decreases of RBC counts, hemoglobin levels, and hematocrit and slight increases of WBC counts, which appeared to have no toxicological significance.

● Total bilirubin levels were slightly increased for females in the 600 mg/kg/day group and ALT values were slightly increased for males and females in the 600 mg/kg/day group; however, these increases did not appear to achieve toxicological significance.

● No target organs of toxicity were observed with oral doses up to 600 mg/kg/day for 14 days.

● The NOAEL was identified as the high dose of 600 mg/kg/day based upon no treatment-related histopathological findings.

Study no.: Study No. 07-2016 (Sponsor Study No. VRT-826809-TX-010)
Volume #, and page #: Electronic Submission, Pages 1-478
Conducting laboratory and location: (b) (4)

Date of study initiation: May 30, 2007 (June 4, 2007 - Dosing Initiation)
GLP compliance: No. Draft Final Report
QA report: yes ( ) no (X)
Drug, lot #, and % purity: VRT-826809, Lot number 12 (Purity, (b) (4) % AUC by HPLC)

CHROMATOGRAPHIC PURITY (HPLC)[3]	Report Results	(b) (4) % AUC
RELATED SUBSTANCES (HPLC)[3]	Report Results	
(b) (4)		(b) (4) % AUC
		% AUC
		% AUC
		% AUC
TOTAL RELATED SUBSTANCES		% AUC

Methods
Doses: VRT-826809 was administered at oral doses of 0, 150, 300, or 600 mg/kg/day for 14 consecutive days.

	Daily Dose*			Number of Animals [b]									
				Toxicity Animals		Toxicokinetic		Clinical Pathology		Necropsy		Microscopic Pathology	
						Days 1 and 14		Termination		Day 15			
Group	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M	F	M	F	M	F	M	F	M	F
1	0	5	0	10	10	3	3	10	10	10	10	10	10
2	150	5	30	10	10	9	9	10	10	10	10	0	0
3	300	5	60	10	10	9	9	10	10	10	10	0	0
4	600	5	120	10	10	9	9	10	10	10	10	10	10

*Doses represent active ingredient.
[b] Numbers shown under M/F represent the total numbers of males and females in each dose group. The first day of dosing was defined as Day 1 of the study.

Dose confirmation analysis on days 1 and 14 of the study found that actual concentrations of dosing solutions were 102.4 to 114.6% of nominal concentrations.

Species/strain: Sprague-Dawley Crl: CD® (SD) IGS BR rats were obtained from (b) (4)

Number/sex/group or time point (main study): 10 rats/sex/group
Route, formulation, volume, and infusion rate: The vehicle (0.5% Tween-80 (w/v) + 0.5% methylcellulose (w/v) in purified water) or drug suspensions were administered by oral gavage using a dose volume of 5 mL/kg.
Satellite groups used for toxicokinetics or recovery: For toxicokinetic analysis, there were 3 rats/sex/group in the control group and 9 rats/sex/group in each treatment group.
Age: Rats were approximately 9 weeks old at the initiation of dosing.
Weight: Body weight ranges at the initiation of dosing were 231.7-277.9 g for male rats and 173.1-200.6 g for female rats.
Unique study design or methodology (if any): Dose selection was based upon a 7-day range finding study in which 5 rats/sex/group received doses of 0, 15, 75, or 150 mg/kg twice per day (total doses of 0, 30, 150, or 300 mg/kg/day, respectively) for 7 days (Study number 06-2991/Sponsor study number VRT-826809-TX-002). There were no deaths or treatment-related clinical signs. Body weight gain for males in the 300 mg/kg/day group was reduced to 54.9% of the control although body weight gains were unaffected in the 14-day study with doses up to 600 mg/kg/day Food consumption was unaffected for male and female treatment groups. White blood cell counts were slightly increased for males in the 100 and 300 mg/kg/day groups, which were attributed to increases of lymphocytes, monocytes, and large unstained cells. Potassium levels were decreased for females in the 300 mg/kg/day group. Adrenal gland weights were increased for male and female treatment groups although there were no corresponding histopathological findings. Histopathological findings were observed in the heart (minimal myocardial inflammatory focus), kidneys (minimal inflammatory foci and minimal to slight mineral deposition in the cortex/corticomedullary junction), liver (minimal inflammatory foci), and uterus with cervix (slight dilated lumen); however, these findings were not confirmed in the present 14-day toxicology study with doses up to 600 mg/kg/day. Lung to plasma ratios of VRT-826809 for male and female treatment groups ranged from 0.14 to 0.53. Liver to plasma ratios of VRT-826809 for male and female treatment groups ranged from 0.95 to 3.20. AUC and C_{max} values for VRT-826809 increased in a generally dose proportional manner.

Observation and Times:
Clinical signs: Animals were observed for mortality and general condition at least twice daily. Physical examinations were conducted once per week.
Body weights: Body weights were measured weekly.
Food consumption: Food consumption was measured weekly.
Ophthalmoscopy: Ophthalmic examinations were conducted at pretest and day 12.
EKG: Not performed.
Hematology: Blood samples for measurement of hematology and coagulation parameters were collected at study termination.
Clinical chemistry: Blood samples for measurement of clinical chemistry parameters were collected at study termination.
Urinalysis: At study termination, urine was obtained by a 16-hr overnight collection period.

Gross pathology: Necropsy examinations were conducted on day 15 after animals had been dosed for 14 consecutive days. Tissues were collected and preserved for all toxicity animals.
Organ weights: Absolute and relative organ weights were obtained for the adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid (w/parathyroid), uterus (horns/body/cervix).
Histopathology: Organs and tissues were examined by light microscopy for the control and high dose groups. Femoral marrow was also examined for the low and mid dose groups.
Toxicokinetics: Blood samples for measurement of plasma concentrations of VRT-826809 were collected on days 1 and 14 at 0.5, 1, 2, 4, 10, and 24 hr postdose using 3 animals/sex/group for each time point. Plasma concentrations of VRT-826809 were measured using a LC-MS/MS method. The lower limit of quantitation was 2 ng/mL.
Tissue collection: Liver, heart, lung, colon, pancreas, salivary gland, and blood samples were collected from 24-hr toxicokinetic animals for biomarker analysis. Samples of liver, lung, colon, pancreas, and salivary gland were collected for biomarker analysis.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical signs.

Body weights: Body weight gains for rats that received doses up to 600 mg/kg/day for 14 days were unaffected.

Food consumption: Food consumption was unaffected.

Ophthalmoscopy: There were no treatment-related ophthalmic findings.

Hematology: Slight decreases of hemoglobin, hematocrit, and red blood cell counts, accompanied by elevations of reticulocytes, were observed for male and female treatment groups, which might be indicative of slight anemia. There were additional findings of increased MCHC and RDW for female treatment groups. These findings were judged to be monitorable in a clinical setting.

White blood cell counts were slightly increased for male and female treatment groups. These increases were generally attributed to elevations of lymphocytes, neutrophils, eosinophils, and/or monocytes.

Hematology parameters at termination (day 15)

Parameters	Males				Female			
	0	150	300	600	0	150	300	600
Hemoglobin g/dL	14.8	14.3 (97%)	14.2* (96%)	14.1* (95%)	14.7	14.3 (97%)	14.2 (97%)	13.7* (93%)
Hematocrit %	42.7	41.5 (97%)	41.2* (97%)	40.9* (96%)	41.9	40.4 (96%)	39.5* (94%)	39.0* (93%)

RBC x 10^6/µL					7.86	7.57 (96%)	7.48* (95%)	7.20* (92%)
Reticulocytes x 10^9/L	224.2	256.0 (114%)	277.0* (124%)	295.2* (132%)	170.6	169.5	197.3 (116%)	210.2 (123%)
MCHC g/dL					35.1	35.5	35.9* (102%)	35.3* (101%)
RDW %					11.1	11.2	11.6* (105%)	11.9* (108%)
WBC x 10^3/µL	9.43	11.38 (121%)	11.41 (121%)	12.01 (128%)	9.36	10.6 (114%)	11.16 (119%)	14.49* (155%)
Neutrophils x 10^3/µL					0.89	0.85	0.89	1.26 (142%)
Lymphocytes x 10^3/µL	7.76	9.27 (120%)	9.35 (121%)	10.01 (129%)	7.98	9.26 (116%)	9.78 (123%)	12.56* (157%)
Eosinophils x 10^3/µL					0.10	0.10	0.10	0.15* (150%)
Monocytes X10^3/µL	0.28	0.32 (114%)	0.32 (114%)	0.35 (125%)				

Clinical chemistry: Total bilirubin levels were increased for females in the 600 mg/kg/day group although the highest value (0.26 mg/dL) was not 2.5 times greater than either the mean or upper end of the control range (0.06-0.15 mg/dL). Four females in the 600 mg/kg/day group had total bilirubin levels (0.23, 0.21, 0.20, and 0.26 mg/dL) outside the control range. Two males in the 600 mg/kg/day group had total bilirubin levels (0.17 and 0.17 mg/dL) outside the control range (0.05-0.12 mg/dL) although these increases appeared to have no toxicological significance. ALT values were increased for males and females in the 600 mg/kg/day group. Increased ALT values for females in the 600 mg/kg/day group were primarily attributed to 1 female (#4508, 63 U/L) although the value was 2.5 times greater than either the mean or upper end of the control range (17-36 U/L). Three males in the 600 mg/kg/day group had ALT values (47, 49, and 47 U/L) outside the control range (21-40 U/L) although these increases appeared to have no toxicological significance. There were no corresponding histopathological findings in the liver. These clinical chemistry changes were judged to be monitorable in a clinical setting.

Cholesterol levels were increased for males and females in the 600 mg/kg/day group. Triglyceride levels were slightly decreased for males in the 300 and 600 mg/kg/day groups. Total protein and albumin levels were slightly decreased for females in the 300 and 600 mg/kg/day groups.

Clinical chemistry parameters at termination (day 15)

Parameters	Males				Female			
	0	150	300	600	0	150	300	600
Total bilirubin mg/dL	0.09	0.09	0.10	0.10	0.11	0.13	0.11	0.17* (155%)
ALT U/L	30	30	31	37* (123%)	26	28	25	38* (146%)
Cholesterol mg/dL	63	70	68	76* (121%)	77	89	83	112* (146%)
Triglyceride	54	52	42	43				

mg/dL			(78%)	(80%)				
ALKP U/L					74	74	79	83 (112%)
Albumin g/dL					4.0	3.8	3.6* (90%)	3.7* (93%)

*p<0.05

Urinalysis: Urine volumes were increased for males and females in the 600 mg/kg/day group. Specific gravity was decreased for males in the 600 mg/kg/day group, which appeared to correlate with increased urinary volume. Urinary pH was slightly increased for males in the high dose group. There were no corresponding histopathological findings in the kidneys.

Urinalysis parameters at termination (day 15)

Parameters	Males				Female			
	0	150	300	600	0	150	300	600
Volume mL	15	17	15	41* (273%)	11	12	9	23 (209%)
Specific gravity	1.021	1.024	1.022	1.010* (99%)				
pH	6.7	6.8	6.9	7.1				

Gross pathology: There were no treatment-related gross pathological findings. Gross pathological findings for the stomach, testes, and epididymides appear to correspond to histopathological findings.

Gross pathological findings in rats after treatment for 14 days (10 rats/sex/group)

Organ/Tissue	Sex	0	150	300	600
Stomach					
-discolored	M	0/10	0/10	0/10	1/10
	F	0/10	0/10	0/10	0/10
Testes					
-small	M	0/10	0/10	0/10	1/10
-adhesion	M	0/10	0/10	0/10	1/10
-undescended	M	0/10	0/10	0/10	1/10
Epididymides					
-small	M	0/10	0/10	0/10	1/10
Skin					
-scab	M	0/10	0/10	1/10	1/10
	F	0/10	0/10	0/10	0/10
Skin protocol					
-hair thin/absent	M	0/10	0/10	1/10	1/10
	F	0/10	0/10	0/10	1/10

Organ weights: Uterus weights were decreased for females in the 300 and 600 mg/kg/day groups; however, these findings were attributed to variations in sexual maturity. There were no corresponding histopathological findings.

Histopathology: There were no treatment-related histopathological findings. Observed histopathological findings were judged to be spontaneous in nature and unrelated to treatment with VRT-826809.

For one male in the high dose group, there were findings of severe aspermia in the right epididymis and tubular degeneration/atrophy in the right testes. These findings were attributed to the failure of the right testes to descend as it was located in the abdominal cavity. These findings were attributed to a congenital abnormality and not to the drug treatment.

Minimal erosion was observed in the glandular stomach for 1 of 20 rats (males and females combined) in the high dose group. The incidence in the present study was comparable to the published background incidence of this finding at 5 to 6.5% (based upon examinations of 2000 males and 2000 females).

Minimal subacute/chronic inflammation of the Harderian gland was observed for 3 high dose females. It is a common finding for rats and might be attributed to the sialodacryoadenitis virus.

Slight sinus histiocytosis in the mesenteric LN was observed for 1 high dose female. Sinus histiocytosis in the mesenteric LN is a common finding in rats and might be a response to inflammation or systemic infection.

Tension lipidosis in the liver was observed for 1 high dose male. Lipidosis in the liver occurs spontaneously in random patterns and increases with age. The published background incidence ranges from 16.9 to 23.5% (based upon examinations of 2000 males and 2000 females).

Minimal dilated tubules in the kidneys were observed for 1 high dose female. These findings may be associated with spontaneous chronic progressive nephropathy.

Secretory activity of the mammary protocol was observed for 1 high dose male. The toxicological significance of this finding was unclear. It appears to be monitorable in a clinical setting.

Cysts in the pars distalis of the pituitary gland were observed for 2 high dose males. These cysts were judged to be remnants of the hypophyseal cleft (Rathke's pouch).

Inflammatory cells or cell debris on the surface of the skin was observed for 1 high dose male. The toxicological significance of this finding was unclear and judged to be monitorable in a clinical setting.

Histopathological findings in rats after treatment for 14 days

Organ/Tissue	Sex	0	150	300	600
Epididymides -aspermia, unilateral, severe	M	0/10	-	-	1/10
Testes					

-tubular degeneration/atrophy, unilateral minimal marked	M	 1/10	 -	 -	 1/10
Stomach -glandular stomach erosion, minimal	 M F	 0/10 0/10	 - -	 - -	 1/10 0/10
Harderian gland -subacute (chronic active)/chronic inflammation, minimal	 M F	 0/10 0/10	 - -	 - -	 0/10 3/10
Mesenteric LN -sinus histiocytosis, slight	 M F	 0/10 0/10	 - -	 - -	 0/10 1/10
Liver -tension lipidosis (P)	 M F	 0/10 0/10	 - -	 - -	 1/10 0/10
Kidneys -dilated tubules(s), minimal	 M F	 0/10 0/10	 - -	 - -	 0/10 1/10
Mammary protocol -secretory activity	 M F	 0/9 0/10	 - -	 - -	 1/7 0/10
Pituitary gland -pars distalis: cysts	 M F	 0/10 0/10	 - -	 - -	 2/10 0/10
Skin -surface inflammatory cells/cell debris	 M F	 - -	 - -	 - -	 1/1 -

Toxicokinetics: C_{max} and AUC values for VRT-826809 on days 1 and 14 generally increased with elevating doses although increases were less than dose proportional. AUC values on day 14 were slightly higher than values on day 1. C_{max} and AUC values for females on days 1 and 14 were generally greater than values observed for males.

Table 1.3-1 Summary of Toxicokinetic Parameters for VRT-826809 in Female and Male Sprague-Dawley Rats on Day 1 and Day 14

Study Day	Dose (mg/kg)	Gender							
		Female				Male			
		C_{max} (μg/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (μg*hr/mL)	$DN\_AUC_{0\text{-}24hr}$ (μg*hr/mL)	C_{max} (μg/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (μg*hr/mL)	$DN\_AUC_{0\text{-}24hr}$ (μg*hr/mL)
Day 1	150	145.00	4.00	2001.85	13.35	145.00	4.00	1105.03	7.37
Day 1	300	199.00	4.00	2996.15	9.99	132.00	4.00	1995.70	6.65
Day 1	600	273.00	4.00	4426.90	7.38	164.00	10.00	2656.98	4.43
Day 14	150	231.00	4.00	2404.85	16.03	73.60	2.00	993.48	6.62
Day 14	300	206.00	4.00	3463.75	11.55	135.00	10.00	2142.62	7.14
Day 14	600	272.00	10.00	5501.25	9.17	181.00	10.00	3218.60	5.36

Source: Table 10.1-17.

Dogs

Study title: **VRT-826809: 14-Day Oral (Gavage) Toxicity and Toxicokinetic Study in Dogs.**

Key study findings:

● In a 14-day toxicology study with dogs, VRT-826809 was administered at oral doses of 0, 50, 100, or 200 mg/kg/day. Dogs were 5 to 6 months old at the start of treatment, which was considered to be unusually young for a standard toxicology study.

● There were increased incidences of unformed stool in treatment groups although there was no dose-response.

● There were minor effects on hematology parameters for females in the high dose group, consisting of slight decreases of RBC counts, hemoglobin levels, and hematocrit and slight increases of WBC counts, which appeared to have no biological significance.

● Prostate weights were decreased for male treatment groups, which was attributed to the immaturity of the dogs.

● Histopathological findings in the kidneys were judged to be potentially related to treatment. Findings in other tissues were judged to be spontaneous background findings and unrelated to treatment.

● Histopathological findings of concern in the kidneys were observed for one male dog from the 200 mg/kg/day group and consisted of medullary findings of bilateral interstitial stromal hyperplasia and bilateral inflammatory cell infiltrate in the stroma. The sponsor judged that these findings reflected the presence of a previous low grade infection; however, there was no historical control data from the testing laboratory to support that these particular findings were incidental and not test article-related. The bilateral nature of these findings increased the concern of a potential relationship to treatment. Further, these findings were judged to be unmonitorable in a clinical setting.

● Based upon the histopathological findings in the kidneys, the NOAEL was identified as the mid dose of 100 mg/kg/day.

Study no.: Study number 07-3261 or Sponsor study number VRT-826809-TX-011
Volume #, and page #: Electronic IND, Pages 1 to 462
Conducting laboratory and location: (b) (4)

Date of study initiation: May 29, 2007 (June 7, 2007 Dosing Initiation)
GLP compliance: No. Draft Final Report
QA report: yes ( ) no (X)
Drug, lot #, and % purity: VRT-826809, Lot number 12 (Purity, (b) (4) by HPLC)

CHROMATOGRAPHIC PURITY (HPLC)[3]	Report Results	(b) (4) % AUC
RELATED SUBSTANCES (HPLC)[3]	Report Results	
(b) (4)		(b) (4) % AUC
		% AUC
		% AUC
TOTAL RELATED SUBSTANCES		% AUC

Methods

Doses: VRT-826809 was administered at oral doses of 0, 50, 100, or 200 mg/kg/day for 14 consecutive days.

	Daily Dose[a]			Number of Animals			
				Total	Toxicokinetics[b]	Clinical Pathology[c]	Necropsy Histopathology
					Days 1 & 14	Pretest & Termination	Day 15
Group	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M/F	M/F	M/F	M/F
1	0	5	0	3/3	3/3	3/3	3/3
2	50	5	10	3/3	3/3	3/3	3/3
3	100	5	20	3/3	3/3	3/3	3/3
4	200	5	40	3/3	3/3	3/3	3/3

[a] Doses represent active ingredient.
[b] Toxicokinetic samples were collected on Days 1 and 14 as follows: 0.5, 1, 2, 4, 8, 12, and 24 hours postdose
[c] Hematology, coagulation, clinical chemistry and urinalysis samples were collected from animals pretest and at termination.
Numbers shown under M/F represent the total numbers of males and females in each dose group.
The first day of dosing was defined as Day 1 of the study.

Concentrations of dosing formulations on days 1 and 15 were 107.5 to 114.5% of nominal concentrations. Homogeneity of the 10 and 40 mg/mL dosing formulations were 110.7 and 107.5% of nominal concentrations, respectively. Stability of 10 mg/mL dosing formulation after 24 hr at room temperature was 108.9% of the nominal concentration.

Species/strain: Beagle dogs were obtained from (b) (4)

Number/sex/group or time point (main study): 3 dogs/sex/group
Route, formulation, volume, and infusion rate: The vehicle (0.5% Tween-80 (w/v) + 0.5% methylcellulose (w/v) in purified water) or drug suspensions were administered by oral gavage using a dose volume of 5 mL/kg.
Satellite groups used for toxicokinetics or recovery: None
Age: Dogs were 5 to 6 months old at the initiation of dosing. These dogs appeared to be unusually low for a standard toxicology study where the standard age generally runs from 9 to 12 months.
Weight: Body weight ranges at the initiation of dosing were 7.8 to 10.1 kg for males and 6.2 to 8.1 kg for females.

Unique study design or methodology (if any): Dose selection was based upon a 7-day range finding study in which 1 beagle dog/sex/group received VRT-826809 at oral doses of 0, 25, 50, or 100 mg/kg/day (Sponsor Study Number VRT-826809-TX-004). Dogs were 6 months old at the initiation of dosing. Incidences of unformed stools were increased for males in the 50 and 100 mg/kg/day groups and females in the 100 mg/kg/day group. Cholesterol levels were decreased for males in the 100 mg/kg/day group and females in the 50 and 100 mg/kg/day groups. Triglyceride levels were decreased for male and female treatment groups although dose-response relationships were not present. Body weight gains and food consumption were unaffected. ECG, hematology, coagulation, and urinalysis parameters were unaffected. There were no treatment-related microscopic findings. On day 1, C_{max} values for males and females and AUC values for females increased with elevating dose although increases were less than dose proportional. The highest AUC for male treatment groups was achieved at the mid dose of 50 mg/kg/day. On day 7, the highest AUC for male treatment groups was achieved at the low dose of 25 mg/kg/day. The highest C_{max} value for male treatment groups was achieved at the high dose although the value at the mid dose was smaller than the value at the low dose. The highest C_{max} and AUC values for female treatment groups were achieved at the high dose although values at the mid dose were smaller than those for the low dose. Half-life values ranged from 4.8 to 7.9 hr in males and 3.8 to 18.3 hr in females. Ratios of liver to plasma drug concentrations ranged from 1.47 to 2.99 for males and 0.78 to 1.63 for females. Ratios of lung to plasma concentrations ranged from 0.30 to 0.62 for males and 0.11 to 0.37 for females.

Observation and Times:
Clinical signs: Animals were observed for mortality and general condition twice daily. During the dosing period, observations were made once prior to once after dosing. Physical examinations were conducted once per week.
Body weights: Dogs were weighted weekly during the treatment period.
Food consumption: Food consumption was estimated by visual inspection on a daily basis.
Ophthalmoscopy: Ophthalmic examinations were conducted pretest and at the end of the dosing period.
EKG: ECG recordings were conducted on unanesthetized dogs during pretest and termination at approximately 1 to 4 hr postdose. The following leads were used at each interval: I, II, III, aVR, aVL, aVF, rV2, V2, and V10. Heart rate and durations of the P wave, and PR, QRS, and QT intervals were calculated.
Hematology: Blood samples were collected at pretest and study termination for measurement of hematology parameters.
Clinical chemistry: Blood samples were collected at pretest and study termination for measurement of clinical chemistry parameters.
Urinalysis: Urine, obtained via a minimum 16-hour overnight collection period, was analyzed for all animals pretest and from 3 animal/sex/group at study termination. Animals were fasted and water-deprived during the collection period.
Gross pathology: Necropsy was performed on 3 animals/sex/group after animals had been dosed for 14 consecutive days.

Organ weights: Absolute and relative organ weights were obtained for adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, thyroid/parathyroid, and uterus (horns/body/cervix).
Histopathology: After fixation, the tissues and organs from all animals were routinely processed, embedded in paraffin, cut at a microtome setting of 4-7 microns, mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy.
Toxicokinetics: Blood samples were collected on days 1 and 14 at 0.5, 1, 2, 4, 8, 12, and 24-hr postdose.
Collection of tissues for biomarker analysis: Samples of heart, liver, and blood were collected and frozen.

Results:

Mortality: None

Clinical signs: Incidences of unformed stool, watery stool, or mucus in the stool were generally increased for male and female treatment groups although dose-response relationships were not present.

Clinical observations, days 1-15

Observation	Males				Females			
	0	50	100	200	0	50	100	200
Unformed stool	12	44	33	41	12	30	5	37
Watery stool	0	3	0	5	0	1	0	1
Mucus in stool	0	4	0	3	3	16	1	5

Body weights: Body weight gains were slightly decreased for male and female dogs in the high dose group.

Body weight change (%) over the 14-day treatment period

Dose, mg/kg/day	Males	Females
0	1.1	2.7
15	2.2	2.8
100	1.2	2.9
200	-1.1	-1.3

Food consumption: Qualitative food consumption appeared to be lower for male and female treatment group although the toxicological significance appeared to be relatively minimal.

Ophthalmoscopy: There were no treatment related ophthalmic findings.

EKG: There were no treatment-related effects on heart rate or ECG parameters (P wave and PR, QRS, and QT intervals).

Hematology: Hemoglobin levels and hematocrit were decreased for female treatment groups. Reticulocyte counts were decreased for male treatment groups and females in

the 200 mg/kg/day group although relationships to treatment were questionable. These changes might be indicative of a very slight anemia. Eosinophil and basophil counts were decreased for female treatment groups. Large unstained cell counts were decreased for male treatment groups. The APTT was slightly decreased for males in the 200 mg/kg/day although a treatment relationship was questionable.

Hematology parameters at pretreatment and termination (Day 15)

Parameter	Time	Males				Females			
		0	50	100	200	0	50	100	200
Hemoglobin g/dL	Pre-					14.2	13.3	13.3	13.4
	Term					14.2	12.6 (89%)	13.0 (92%)	12.7* (89%)
Hematocrit %	Pre-					43.1	40.6	40.2	40.9
	Term					42.5	37.9 (89%)	39.5 (93%)	38.3* (90%)
Reticulocytes x 10^9/L	Pre-	55.1	42.7	54.7	48.6	43.4	55.7	46.3	33.0
	Term	72.6	37.7 (52%)	37.1 (51%)	41.7 (57%)	67.4	65.3	42.8	29.1 (43%)
Eosinophils x 10^3/µL	Pre-					0.30	0.23	0.26	0.22
	Term					0.38	0.16 (42%)	0.20 (53%)	0.11* (29%)
Basophils x 10^3/µL	Pre-					0.10	0.07	0.08	0.07
	Term					0.10	0.05* (50%)	0.05* (50%)	0.04* (40%)
Large unstained cells x 10^3/µL	Pre-	0.07	0.08	0.10	0.08				
	Term	0.12	0.07 (58%)	0.07 (58%)	0.06 (50%)				
APTT sec	Pre-	18.1	16.4	16.7	15.9	17.3	20.7	18.2	17.0
	Term	17.8	16.4	15.7	14.7* (83%)	22.3	20.7	18.2	17.0

Clinical chemistry: Cholesterol and triglyceride levels were decreased for male and female treatment groups. BUN levels were increased for female treatment groups although a dose-response relationship was not present. Phosphate levels were slightly decreased for female treatment groups.

Hematology parameters at termination (Day 15)

Parameter	Time	Males				Females			
		0	50	100	200	0	50	100	200
Cholesterol mg/dL	Term	192	109 (57%)	106 (55%)	102* (53%)	135	115 (85%)	105 (78%)	106 (78%)
Triglyceride mg/dL	Term	37	17* (46%)	23* (62%)	20* (54%)	36	28 (78%)	22 (61%)	16* (44%)
BUN mg/dL	Term					12	14 (117%)	18* (150%)	15* (125%)

Phosphate mg/dL	Term					6.1	5.9	5.7 (93%)	5.5* (90%)

Urinalysis: There were no treatment-related changes of urinalysis parameters.

Gross pathology: Gross pathological findings with potential relationships to treatment were observed in the prostate, uterus, and ileoceccal junction. Small prostates were observed with an increased incidence in all male treatment groups. Histopathological findings of prostate immaturity were reported for all control and treated dogs as dogs were only 5 to 6 months old at the start of treatment. The severity of immaturity was increased for treatment groups. These gross pathological findings for the prostate did correlate with decreased absolute and relative prostate weights. Small uterus was reported for 1 female in each of the 100 and 200 mg/kg/day groups. Discoloration of the ileoceccal junction correlated with histopathological findings of congestion.

Gross pathological findings at termination (Day 15), n = 3/sex/group

Organ/Tissue	Males				Females			
	0	50	100	200	0	50	100	200
Prostate -small	0	2	3	3				
Uterus w/Cervix -small					0	0	1	1
Ileoceccal junction -discolored	0	0	0	1	0	0	0	0

Organ weights: Differences in organ weights between control and treatment groups were observed for the prostate, spleen, thymus, and uterus with cervix. Prostate weights were decreased for all male treatment groups although the dose-response relationship was flat. These findings correlated with gross pathological findings of small prostates for all male treatment groups. Histopathological examinations found immature prostates for all male control and treatment groups. Spleen weights were decreased for all male and female treatment groups although dose-response relationships were not present and there were no corresponding histopathological findings. Thymus weights were decreased for females in the 200 mg/kg/day group although there were no corresponding histopathological findings. Uterus weights were decreased for all female treatment groups although anestrus was reported for all female control and treatment groups.

Organ weights at termination (Day 15)

Parameter	Males				Females			
	0	50	100	200	0	50	100	200
Prostate g	2.952	1.332* (45%)	1.103* (37%)	1.133* (38%)				
Prostate %BW	0.035	0.015* (43%)	0.014* (40%)	0.013* (37%)				
Prostate %BrW	3.739	1.783* (48%)	1.463* (39%)	1.715* (46%)				
Spleen g	119.738	93.403 (78%)	93.836 (78%)	90.063 (75%)	108.108	75.912 (70%)	89.985 (83%)	80.850 (75%)

Spleen %BW	1.404	1.071 (76%)	1.139 (81%)	1.043 (74%)	1.536	1.101 (72%)	1.319 (86%)	1.115 (73%)
Spleen %BrW	153.615	125.711 (82%)	125.743 (82%)	136.257 (89%)	149.912	105.804 (71%)	137.651 (92%)	105.628 (71%)
Thymus g					11.216	10.232	10.337	8.540 (76%)
Thymus %BW					0.161	0.149	0.152	0.121 (75%)
Thymus %BrW					15.662	14.402	15.857	11.160 (71%)
Uterus w/Cervix g					2.674	2.167 (81%)	2.103 (79%)	1.564 (59%)
Uterus w/Cervix %BW					0.038	0.031 (82%)	0.031 (82%)	0.022 (58%)
Uterus w/Cervix %BrW					3.716	2.977 (80%)	3.234 (87%)	2.015 (54%)

Histopathology: Histopathological findings in the kidneys were judged to be potentially related to treatment. Findings in other tissues were judged to be spontaneous background findings and unrelated to treatment.

For the kidneys, most dogs in control and treatment groups had findings of dilated medullary tubules and medullary mineral deposits. Histopathological findings of concern in the kidneys were observed for one male dog (#4076) from the 200 mg/kg/day group and consisted of medullary findings of bilateral interstitial stromal hyperplasia and bilateral inflammatory cell infiltrate in the stroma. The sponsor judged that these findings reflected the presence of a previous low grade infection; however, there was no historical control data from the testing laboratory to support that these particular findings were incidental and not test article-related (Amendment #001 dated November 13, 2007). The bilateral nature of these findings increased the concern of a potential relationship to treatment. Further, these findings were judged to be unmonitorable in a clinical setting. This dog had an additional finding of unilateral tubular epithelial hyperplasia although there was less concern for this particular finding given that it was unilateral in nature.

Findings in the prostate, consisting of immaturity, dilated/cystic acini, and fibrosis, were judged to be unrelated to treatment. Given that dogs were 5 to 6 months old at the start of treatment, immature prostate was observed for all control and treated males. The severity of immaturity was increased for treatment groups; however, there was not a dose-response relationship (i.e., severity was greater for the low dose as compared to the mid dose). Prostate weights also reflected that immaturity was greater in treatment group although the dose-response relationship was flat. The incidence of dilated/cystic acini was increased for treatment groups. From eleven studies conducted at the testing laboratory, the mean and range of this finding were 28.75% and 0 to 100%, respectively (Amendment #001 dated November 13, 2007). Fibrosis was observed for 1 male dog in the high dose group. From eleven studies conducted at the testing laboratory, one control male was observed with fibrosis. The mean and range of this finding were 1.53%

and 0 to 16.67% (1 of 6 control males), respectively (Amendment #001 dated November 13, 2007).

Findings in the pituitary gland consisting of cyst(s) in the pars distalis were judged to be common findings for beagle dogs. Most of these cysts are remnants of the craniopharyngeal duct and are located at the periphery of the pars tuberalis and distalis. Cysts were reported in 24-36% of young beagles.

The finding in the parathyroid gland consisting of a thyroglossal cyst for 1 male dog in the 200 mg/kg/day group was judged to be unrelated to treatment. Although uncommon (1% of young beagles), small cysts may be found within or near the parathyroid apparently arising from the duct connecting the thymus and parathyroid primordia.

The finding in the heart consisting of a cyst (clear with hard material inside) on the atrioventricular valve for 1 male dog in the 200 mg/kg/day group was judged to be unrelated to treatment. Valvular angiectasis (telangiectasis, hematocyst, congenital hematoma) is occasionally observed (4/1000 = 0.4%) involving atrioventricular valves, usually the right.

Congestion of ileocecal junction was observed for 1 male in the 200 mg/kg/day group was judged to be unrelated to treatment. Mucosal blood vessels are commonly congested as a result of digestive processes.

Histopathological findings in dogs after treatment for 14 days

Organ/Tissue	Sex	0	50	100	200
Kidneys					
- medulla: tubular epithelium - hyperplasia, unilateral, minimal	M	0/3	0/3	0/3	1/3
	F	0/3	0/3	0/3	0/3
- medulla: papilla – interstitial stromal hyperplasia, bilateral, slight	M	0/3	0/3	0/3	1/3
	F	0/3	0/3	0/3	0/3
- medulla: papillary stroma – mixed inflammatory cell infiltrate, bilateral, slight	M	0/3	0/3	0/3	1/3
	F	0/3	0/3	0/3	0/3
Prostate					
- immature (total)	M	3/3	3/3	3/3	3/3
minimal		1/3	0/3	0/3	0/3
slight		1/3	0/3	0/3	0/3
moderate		1/3	1/3	2/3	0/3
marked		0/3	1/3	0/3	2/3
severe		0/3	1/3	1/3	1/3
- acini: dilated/cystic (total)	M	1/3	2/3	2/3	3/3
slight		1/3	2/3	0/3	2/3
moderate		0/3	0/3	2/3	1/3
- fibrosis (total)	M	0/3	0/3	0/3	1/3
slight		0/3	0/3	0/3	1/3
Pituitary gland					

-pars distalis: cyst(s) (total)	M F	0/3 0/3	1/3 1/3	1/3 1/3	1/3 1/3
minimal	M F	0/3 0/3	1/3 1/3	0/3 0/3	0/3 0/3
slight	M F	0/3 0/3	0/3 0/3	1/3 1/3	1/3 1/3
Parathyroid -soft tissue: thyroglossal cyst	M F	0/3 0/3	0/3 0/3	0/3 0/3	1/3 0/3
Heart - atrio-ventricular valve(s): cyst(s), slight	M F	0/3 0/3	0/3 0/3	0/3 0/3	1/3 0/3
Ileocecal Junction - congestion, slight	M F	- -	- -	- -	1/1 -

Toxicokinetics: C_{max} and AUC values for VRT-826809 on days 1 and 14 increased with elevating dose. Increases of C_{max} values were approximately dose proportional. Increases of AUC values for male treatment groups on day 1 were approximately dose proportional. Increases of AUC values for female treatment groups on day 1 and male and female treatment groups on day 14 were less than dose proportional between 50 and 100 mg/kg/day and approximately dose proportional between 100 and 200 mg/kg/day. There were no consistent differences of C_{max} and AUC values between males and females or between days 1 and 14.

Low concentrations of VRT-826809 were observed in some of the samples collected from the control group male dogs on Day 14 indicating possible contamination during blood sampling or handling of plasma samples. These concentrations were approximately 1000 times lower than the C_{max} for the low dose group.

Table 11.2-1 – Summary of Toxicokinetic Parameters for VRT-826809 in Female and Male Beagle Dogs on Days 1 and 14

Dose (mg/kg/day)	Gender		Study Days	
			Day 1	Day 14
50	Female	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	221.88 ± 4.16	199.60 ± 28.12
		C_{max} (µg/mL)	19.37 ± 3.07	22.23 ± 5.90
		T_{max} (hr)	3.33 ± 1.15	1.67 ± 0.58
	Male	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	60.50 ± 19.07	226.83 ± 112.86
		C_{max} (µg/mL)	8.35 ± 1.08	22.63 ± 8.66
		T_{max} (hr)	0.67 ± 0.29	2.33 ± 1.53
100	Female	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	315.45 ± 179.16	337.45 ± 115.13
		C_{max} (µg/mL)	35.10 ± 22.04	49.60 ± 13.17
		T_{max} (hr)	3.33 ± 1.15	2.00 ± 0.00
	Male	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	169.95 ± 147.09	252.24 ± 35.36
		C_{max} (µg/mL)	26.30 ± 10.83	23.47 ± 2.62
		T_{max} (hr)	1.33 ± 0.58	3.33 ± 4.04
200	Female	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	573.51 ± 176.50	719.83 ± 163.92
		C_{max} (µg/mL)	55.80 ± 7.16	96.37 ± 23.17
		T_{max} (hr)	4.00 ± 0.00	2.67 ± 1.15
	Male	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	690.82 ± 89.72	598.17 ± 86.22
		C_{max} (µg/mL)	77.23 ± 22.25	71.77 ± 25.35
		T_{max} (hr)	2.67 ± 1.15	2.67 ± 1.15

[a]Mean ± SD (N=3 dogs/group).

Histopathology inventory (optional)

Study	14-day study	14-day study
Species	Rats	Dogs
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	NE	NE
Bone (femur)	X (w/marrow)	X (w/marrow)
Brain	X*	X*
Cecum	X	X
Cervix	X (w/uterus)	X (w/uterus)
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions	X	X
Harderian gland	X	
Heart	X*	X*
Ileum	X	X

Injection site		
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	X
Larynx		
Liver	X*	X*
Lungs	X	X
Lymph nodes, cervical		
Lymph nodes mandibular		
Lymph nodes, mediastinal	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X* (w/thyroid)	X* (w/thyroid)
Peripheral nerve		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X (w/sternum)	X (w/marrow)
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X* (w/parathyroid)	X* (w/parathyroid)
Tongue		
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: **VRT-826809: Bacterial Reverse Mutation Assay**

Key findings:

● VRT-826809 was tested in the bacterial reverse mutation assay with *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A at doses up to 5000 µg/plate ± S9.

● Under the conditions of this study, VRT-826809 was judged to be negative in the bacterial reverse mutation assay.

Study no.: Sponsor study number VRT-826809-TX-005

Volume #, and page #: Electronic IND, Pages 1 to 70
Conducting laboratory and location: (b) (4)

Date of study initiation: June 1, 2007
GLP compliance: No, Draft Report
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: VRT-826809, Lot number 12

CHROMATOGRAPHIC PURITY (HPLC)[3]	Report Results	(b) (4) % AUC
RELATED SUBSTANCES (HPLC)[3]	Report Results	(b) (4)
(b) (4)		% AUC
		% AUC
		% AUC
TOTAL RELATED SUBSTANCES		% AUC

Methods

Strains: *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A

Doses used in definitive study: 50, 150, 500, 1500 and 5000 µg per plate ± S9

Basis of dose selection: In an initial toxicity-mutation assay, VRT-826809 was tested at dose levels of 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate ± S9 (two plates per dose ± S9). Precipitate was observed at 5000 µg per plate in most test conditions. Toxicity was observed at 5000 µg per plate with tester strain WP2 *uvr*A ± S9. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg per plate.

Negative controls: DMSO

Positive controls:

Table 3.0-1			
Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene (b) (4) Lot No. 12317CE Exp. Date 01-Feb-2009 CAS No. 613-13-8 Purity 99.9%	1.0
WP2 *uvr*A			10
TA98	None	2-nitrofluorene (b) (4) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		Sodium azide (b) (4) Lot No. 073K0119 Exp. Date 31-Jul-2008 CAS No. 26628-22-8 Purity 99.9%	1.0
TA1537		9-aminoacridine (b) (4) Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. 90-45-9 Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (b) (4) Lot No. 05713JD Exp. Date 15-Jan-2009 CAS No. 66-27-3 Purity 99.9%	1,000

Metabolic activation: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was lot prepared and purchased from (b) (4) The S9 mix was prepared immediately before its use and contained 10% S9.

Incubation and sampling times: The plate incorporation method was used. There were 3 plates per dose ± S9. Plates were inverted and incubated for approximately 48 to 72 hours at 37± 2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted.

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

Criteria for a valid test included all cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98,

10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60. The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.

Results

Study validity: VRT-826809 was tested to the limit dose of 5000 µg/plate. Precipitate was observed at 5000 µg per plate in most test conditions. Toxicity was observed beginning at 1500 or at 5000 µg per plate in the absence of S9 activation with tester strains TA1537 and TA98, respectively. Positive controls produced expected increases of revertant colony counts.

Study outcome: Slight increases of revertant colony frequencies (1.4 to 1.5 times the vehicle-control) were observed with TA100 at 5000 µg/plate ± S9 although these results were not judged to be indicative of a positive response. VRT-826809 tested up to the limit dose of 5000 µg per plate ± S9 with strains TA98, TA100, TA1535, TA1537, and WP2 uvrA did not produce increased revertant colony frequencies indicative of a positive response.

Study title: ***In Vitro* Mammalian Chromosome Aberration Test**

Key findings:

● VRT-826809 was tested in the in vitro CHO cell chromosomal aberration assay as follows: for the 4-hr treatment in the absence of S9, doses up to 250 µg/mL were examined; for the 4-hr treatment in the presence of S9, doses up to 250 µg/mL were examined; and for the 20-hr treatment in the absence of S9, doses up to 125 µg/mL were examined.

● Under these testing conditions, VRT-826809 was negative in the in vitro CHO cell chromosomal aberration assay.

Study no.: Sponsor study number VRT-826809-TX-006

Volume #, and page #: Electronic IND, Pages 1 to 53
Conducting laboratory and location: (b) (4)

Date of study initiation: June 5, 2007
GLP compliance: No, Draft Report.
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: VRT-826809, Lot number 12

CHROMATOGRAPHIC PURITY (HPLC)[3]	Report Results		(b) (4) % AUC
RELATED SUBSTANCES (HPLC)[3]	Report Results		(b) (4)
(b) (4)			% AUC
			% AUC
			% AUC
TOTAL RELATED SUBSTANCES			% AUC

Methods

Cell line: Chinese hamster ovary (CHO-K_1) cells

Doses used in definitive study: Doses for the definitive studies are shown in the table below. Dose selection was based a preliminary toxicity assay.

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	12.5, 25, 100, 150, 200, 250, 300, and 350
	20 hr	0 hr	6.25, 12.5, 25, 50, 75, 100, 125, and 150
S9-activated	4 hr	16 hr	12.5, 25, 100, 150, 200, 250, 300, and 350

Basis of dose selection: Doses used in the preliminary toxicity assay are shown in the table below. Significant cytotoxicity (greater than 50% cell growth inhibition, relative to the solvent control) was observed at dose levels ≥452 µg/mL in both the non-activated and S9-activated 4-hour exposure groups, and at dose levels ≥135.6 µg/mL in the non-activated 20-hour continuous exposure group.

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	12.5, 25, 100, 150, 200, 250, 300, 350
	20 hr	0 hr	6.25, 12.5, 25, 50, 75, 100, 125, 150
S9-activated	4 hr	16 hr	12.5, 25, 100, 150, 200, 250, 300, 350

Negative controls: DMSO

Positive controls: Mitomycin C (0.1 and 0.2 µg/mL) and cyclophosphamide (10 and 20 µg/mL) were used as positive controls in the absence and presence of metabolic activation, respectively.

Metabolic activation: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 (Lot No. 2088) was obtained from (b) (4). The concentration of S9 was 20 µL/mL medium (2%).

Incubation and sampling times: In the non-activated study, cells were exposed to the test article for 4 hours or continuously for 20 hours. In the 4-hour exposure group, after the exposure period, the treatment medium was removed, the cells washed, refed, and returned to the incubator. Two hours prior to the scheduled harvest, Colcemid® (0.1 µg/mL) was added to duplicate flasks for each treatment condition. In the S9-activated study, cells were exposed for 4 hours. After the exposure period, the treatment medium was removed, the cells washed, refed with complete medium, and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® (0.1 µg/mL) was added to duplicate flasks for each treatment condition. Metaphase cells were harvested, processed, placed on slides, and stained with 5% Giemsa.

Selection of doses for analysis of chromosome aberrations was based upon toxicity of the test article. The highest dose selected for evaluation was the dose which induced at least 50% toxicity, as measured by cell growth inhibition with a sufficient number of scorable metaphase cells. In treatment groups with excessive mitotic inhibition at dose levels with ≥ 50% reduction in cell growth, selection of doses for microscopic analysis was based on mitotic index (the lowest dose with at least 50% reduction in mitotic index). Two additional lower dose levels were included in the evaluation.

To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Slides were coded using random numbers by an individual not involved with the scoring process. Metaphase cells with 20±2 centromeres were examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations. The number of metaphase spreads that were examined and scored per duplicate flask was reduced when the percentage of aberrant cells reached a significant level (at least 10%) before 100 cells were scored.

Statistical analysis of the percent aberrant cells was performed using the Fisher's Exact test. Fisher's Exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's Exact test at any test article dose level, the Cochran-Armitage test was used to measure dose-responsiveness. However, values that are statistically significant but do not exceed the range of historical solvent controls may be judged as not biologically significant.

Results

Study validity: For the 4-hr treatment in the absence of S9, doses of 150, 200, and 250 µg/mL were examined for chromosomal aberrations. At 250 µg/mL - S9, there was a

32% inhibition of cell growth and a 50% inhibition of mitotic index. For the 4-hr treatment in the presence of S9, doses of 25, 150, and 250 µg/mL were examined for chromosomal aberrations. At 250 µg/mL + S9, there was a 45% inhibition of cell growth and a 66% inhibition of mitotic index. For the 20-hr treatment in the absence of S9, doses of 25, 50, and 125 µg/mL were examined for chromosomal aberrations. At 125 µg/mL - S9, there was a 54% inhibition of cell growth and a 53% inhibition of mitotic index. Levels of cytotoxicity at the highest dose examined for each treatment condition appeared to be adequate. Positive controls produced expected increases of chromosomal aberrations.

Study outcome: For the 4-hr and 20-hr treatments in the absence of S9, VRT-826809 had no effects on the incidence of cells with numerical or structural aberrations. For the 4-hr treatment in the presence of S9, the incidence of structural aberrations was significantly increased to 4% at 250 µg/mL as compared to the vehicle-control; however, the testing laboratory historical control range was 0 to 5% indicating that the observed increase was not toxicologically significant. At the next highest dose of 300 µg/mL + S9, there was excessive cytotoxicity (i.e., 79% inhibition of cell growth and 85% inhibition of mitotic index). Numerical aberrations were unaffected by the 4-hr treatment in the presence of S9. Under these testing conditions, VRT-826809 was negative in the CHO cell chromosomal aberration assay.

TABLE 6
CONCURRENT TOXICITY TEST USING VRT-826809
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment µg/mL	Flask	Cell Count Averages ($x10^6$)	Cell Viability (%)	Mean Cells per Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)	Mitotic Index (%)	Mitotic Inhibition (%)
DMSO	A	1.14	99%					
	B	1.28	100%	1.20	100%		10.2%	
VRT-826809								
12.5	A	1.01	100%					
	B	1.04	100%	1.02	85%	15%	9.9%	3%
25	A	1.24	100%					
	B	1.11	100%	1.18	98%	2%	10.2%	0%
100	A	1.17	99%					
	B	1.14	97%	1.13	94%	6%	9.7%	5%
150	A	1.03	97%					
	B	0.96	100%	0.98	81%	19%	7.7%	25%
200	A	0.97	96%					
	B	0.92	97%	0.91	76%	24%	7.5%	26%
250	A	0.75	95%					
	B	0.64	96%	0.67	55%	45%	3.5%	66%
300	A	0.34	75%					
	B	0.32	77%	0.25	21%	79%	1.5%	85%
350	A	0.14	66%					
	B	0.09	63%	0.08	6%	94%	0.8%	92%
CP	A	0.98	96%					
10	B	1.00	98%	0.96	80%	20%	3.0%	71%
CP	A	0.89	98%					
20	B	1.05	97%	0.94	78%	22%	1.0%	90%

Treatment: CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.
Cell Viability: determined by trypan blue dye exclusion.
Mean Viable Cells/Flask = cell count x % viable cells, reported as mean of Flasks A and B.
Cell Growth Index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.
Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

TABLE 10
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	10.7	200	200	0.000	±0.000	2.5	0.0
VRT-826809									
150	-S9	4	9.6	200	200	0.005	±0.071	4.5	0.5
200	-S9	4	9.3	200	200	0.010	±0.100	4.0	1.0
250	-S9	4	5.3	200	200	0.015	±0.122	3.5	1.5
MMC, 0.2	-S9	4	6.8	200	50	0.240	±0.476	2.0	22.0**
DMSO	+S9	4	10.2	200	200	0.000	±0.000	3.5	0.0
VRT-826809									
25	+S9	4	10.2	200	200	0.000	±0.000	4.5	0.0
150	+S9	4	7.7	200	200	0.015	±0.122	4.0	1.5
250	+S9	4	3.5	200	200	0.050	±0.260	3.0	4.0**
CP, 10	+S9	4	3.0	200	100	0.250	±0.557	2.5	19.0**
DMSO	-S9	20	11.9	200	200	0.000	±0.000	2.5	0.0
VRT-826809									
25	-S9	20	12.3	200	200	0.005	±0.071	3.5	0.5
50	-S9	20	11.7	200	200	0.005	±0.071	4.0	0.5
125	-S9	20	5.6	200	200	0.000	±0.000	3.5	0.0
MMC, 0.1	-S9	20	6.3	200	50	0.220	±0.465	2.5	20.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	0.7	20.9
±SD[1]	1.0	9.3
Range	0.0-5.0	8.0-84.3

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.1-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-20 µg/mL).

Study title: **Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of VRT-826809.**

Key findings:

● A single oral administration of VRT-826809 at doses up to 2000 mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow of male ICR mice. Therefore, VRT-826809 was concluded to be negative in the mouse micronucleus assay.

Study no.: Sponsor Study number VRT-826809-TX-007

Volume #, and page #: Electronic IND, Pages 1 to 53
Conducting laboratory and location: (b) (4)

Date of study initiation: June 4, 2007
GLP compliance: No, Draft Report.
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: VRT-826809, Lot number 12 (Purity 98.86%)

Methods

Species: ICR mice were obtained from (b) (4) At the time of dose administration for each phase of the study, the mice were approximately 6 to 8 weeks old. Body weight ranges for the pilot toxicity study were 26.2-29.9 g for males and 25.2-28.8 g for females. The body weight range of males for the definitive micronucleus study was 25.8-31.5 g.

Doses used in definitive study: For the definitive micronucleus study, VRT-826809 was administered as single doses at 0, 500, 1000, and 2000 mg/kg/day. Dosing solutions were 25, 50, and 100 mg/mL. The dosing volume was 20 mL/kg. The dose formulation analysis was not provided in this draft report.

Basis of dose selection: In a pilot toxicity study, 5 mice/sex/group received a single oral dose of VRT-826809 at 1000 or 2000 mg/kg. Mice were observed after dose administration and daily thereafter for 3 days for clinical signs of toxicity. Body weights were recorded before dose administration on day 0 and on days 1 and 3. No mortality was observed. Lethargy and piloerection were seen in all males and females. In the absence of mortality, the high dose for the definitive micronucleus study was set at 2000 mg/kg. Since there was no apparent difference in toxicity between males and females, the definitive study was conducted using males only.

Negative controls: 0.5% Tween-80 (w/v) + 0.5% methylcellulose (w/v) in water was used as the test article vehicle

Positive controls: Cyclophosphamide (50 mg/kg)

Incubation and sampling times: The definitive micronucleus study consisted of seven groups, each containing 5 male mice. Mice in five of these groups were dosed either with the controls (vehicle or positive) or with VRT-826809 at 500, 1000, or 2000 mg/kg and were euthanized 24 hr postdose. Mice in the other two groups were dosed either with the vehicle control or VRT-826809 at a dose of 2000 mg/kg and were euthanized 48 hr postdose.

Dose Group (20 mL/kg)	Number of Mice Dosed	Number of Mice Used for Bone Marrow Collection at	
		24 hrs post-dose	48 hrs post-dose
Vehicle Control: 0.5% Tween-80 (w/v) + 0.5% methylcellulose (w/v) in water	10	5	5
Test Article: VRT-826809			
Low dose (500 mg/kg)	5	5	0
Mid dose (1000 mg/kg)	5	5	0
High dose (2000 mg/kg)	15*	5	5
Positive Control: CP (50 mg/kg)	5	5	0

*Including 5 replacement mice/sex to ensure the availability of five mice for micronucleus analysis..

Bone marrow cells were collected, placed on slides, and stained with May-Gruenwald-Giemsa. Two thousand polychromatic erythrocytes (PCEs) per mouse were screened (scored) for the presence of micronuclei resulting in evaluation of a total of 10000 PCEs per each dose group. The number of NCEs and micronucleated NCEs (MNCEs) in the field of 1000 total erythrocytes (ECs) was determined for each animal in order to determine the proportion of polychromatic erythrocytes to total erythrocytes (PCEs/ECs). The proportion of polychromatic erythrocytes to total erythrocytes was also recorded per 1000 erythrocytes per each animal (PCEs/ECs ratio).

The test article would have been considered to induce a positive response if a dose responsive increase in the incidence of micronucleated polychromatic erythrocytes was observed and one or more doses were statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time.

Results

Study validity: The limit dose of 2000 mg/kg was used for the definitive micronucleus study. The positive control produced the expected increase in the incidence of micronucleated PCEs.

Study outcome: In the definitive micronucleus study, male mice were exposed to VRT-826809 at single doses of 500, 1000 or 2000 mg/kg. No mortality was observed. Lethargy was seen in 8/15 males at 2000 mg/kg and piloerection in 2/5 males at 500

mg/kg and in all males at 1000 and 2000 mg/kg. There were no appreciable reductions in the ratio of polychromatic erythrocytes to total erythrocytes in treatment groups as compared to the vehicle-control. There were no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes in treatment groups as compared to the vehicle-control at 24 or 48 hours after dose administration.

2.6.6.9 Discussion and Conclusions

In 14-day toxicology studies, rats received oral doses up to 600 mg/kg/day and dogs received oral doses up to 200 mg/kg/day. No clear target organs of toxicity or dose limiting toxicity were identified in these studies although a potential treatment relationship for kidneys findings in one dog from the 200 mg/kg/day group could not be excluded. The sponsor should consider using higher doses in future toxicology studies with rats and dogs in order to identify target organs of toxicity and/or dose limiting toxicity.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

VRT-826809 is under development (b) (4) for the treatment of cystic fibrosis. In the present submission, the sponsor proposed to assess the safety and tolerability of VRT-826809 in healthy male and female volunteers. Proposed studies consist of single dose clinical trials and a 14-day repeat dose clinical trial.

Pivotal nonclinical studies to evaluate the safety of the proposed clinical trial were 14-day toxicology studies in rats and dogs and a standard battery of genotoxicity tests.

In a 14-day toxicology study with rats, VRT-826809 was administered at oral doses of 0, 150, 300, or 600 mg/kg/day. There were minor effects on hematology parameters in treatment groups, primarily the high dose group, consisting of slight decreases of RBC counts, hemoglobin levels, and hematocrit and slight increases of WBC counts, which appeared to have no toxicological significance. Total bilirubin levels were slightly increased for females in the 600 mg/kg/day group and ALT values were slightly increased for males and females in the 600 mg/kg/day group; however, these increases did not appear to achieve toxicological significance. No target organs of toxicity were observed with oral doses up to 600 mg/kg/day for 14 days. The NOAEL was identified as the high dose of 600 mg/kg/day based upon no treatment-related histopathological findings.

In a 14-day toxicology study with dogs, VRT-826809 was administered at oral doses of 0, 50, 100, or 200 mg/kg/day. Dogs were 5 to 6 months old at the start of treatment, which was considered to be unusually young for a standard toxicology study. There were increased incidences of unformed stool in treatment groups although there was no dose-response. There were minor effects on hematology parameters for females in the high dose group, consisting of slight decreases of RBC counts, hemoglobin levels, and hematocrit and slight increases of WBC counts, which appeared to have no biological significance. Prostate weights were decreased for male treatment groups, which was

attributed to the immaturity of the dogs. Histopathological findings in the kidneys were judged to be potentially related to treatment. Findings in other tissues were judged to be spontaneous background findings and unrelated to treatment. Histopathological findings of concern in the kidneys were observed for one male dog from the 200 mg/kg/day group and consisted of medullary findings of bilateral interstitial stromal hyperplasia and bilateral inflammatory cell infiltrate in the stroma. The sponsor judged that these findings reflected the presence of a previous low grade infection; however, there was no historical control data from the testing laboratory to support that these particular findings were incidental and not test article-related. The bilateral nature of these findings increased the concern of a potential relationship to treatment. Further, these findings were judged to be unmonitorable in a clinical setting. Based upon the histopathological findings in the kidneys, the NOAEL was identified as the mid dose of 100 mg/kg/day.

VRT-826809 was negative in the in vitro bacterial/microsomal activation assay, in vitro Chinese hamster ovary cell chromosomal aberration assay, and in vivo mouse micronucleus assay.

Safety margins for revised clinical doses are shown in the tables below. Safety margins for clinical doses were judged to be adequate given they were greater than 10 based upon the NOAEL of the 14-day toxicology study with rats and greater than 6 based upon the NOAEL of the 14-day toxicology study with dogs.

Safety margin for the proposed clinical start dose of 75 mg (single dose)

Study	NOAEL mg/kg (mg/m^2)	Safety margin for proposed clinical start dose
		75 mg 55.5 mg/m^2
14-day rat	600 (3600)	64.9
14-day dog	100 (2000)	36.0

Safety margins for revised clinical doses in the proposed single dose trials

Study	NOAEL mg/kg	Safety margins for revised doses in single dose clinical trials			
		75 mg (1.5 mg/kg)	200 mg (4 mg/kg)	375 mg (7.5 mg/kg)	750 mg (15 mg/kg)
14-day rat	600	400	150	80	40
14-day dog	100	66.7	25	13.3	6.7

Safety margins for revised clinical doses in the proposed 14-day repeat dose trial

Study	NOAEL mg/kg	Safety margins for revised doses in the 14-day repeat dose clinical trial		
		200 mg (100 mg q12hr) 4 mg/kg	400 mg (200 mg q12hr) 8 mg/kg	750 mg (375 mg q12hr) 15 mg/kg
14-day rat	600	150	75	40
14-day dog	100	25	12.5	6.7

Doses greater than 750 mg/day might be supported for trials with duration less than or equal to 14 days if clinical AUC values do not exceed 252 µg*hr/mL (AUC for male dogs

at the NOAEL of 100 mg/kg/day) or if longer duration toxicology studies with dogs using appropriate doses do not reproduce the kidney findings (These comments were conveyed to the sponsor in a FAX dated November 13, 2007).

No clear target organs of toxicity or dose limiting toxicity were identified in 14-day toxicology studies with rats and dogs although a potential treatment relationship for kidneys findings in one dog from the 200 mg/kg/day group could not be excluded. Toxicology studies of longer duration may provide more information with regard to target organs of toxicity and/or dose limiting toxicity.

Recommendation: From a nonclinical perspective, the proposed clinical trials appear reasonably safe to proceed.

Signatures (optional):

Reviewer Signature _____
Timothy W. Robison, Ph.D.

Supervisor Signature _____
C. Joseph Sun, Ph.D.
Concurrence Yes ___ No ___

cc:
IND 79,521, HFD-570 Division Files
RaggioM, HFD-570
DurmowiczA, HFD-570
SunC, HFD-570
RobisonT, HFD-570

Linked Applications	Sponsor Name	Drug Name
IND 79521	VERTEX PHARMS	VX809

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

/s/

TIMOTHY W ROBISON
01/03/2008
Non-Clinical Reviewer

Ching-Long J J SUN
01/03/2008
Non-Clinical Reviewer, Quality Microbiology Reviewer
I concur.

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	79,521
Supporting document/s:	Numbers 44, 46, 73, 76, 77, 78, 80, 81 (SPA), 82 (SPA), 83, 84
Sponsor's letter date:	44: January 14, 2011 46: March 11, 2011 73: July 19, 2012 76: August 16, 2012 77: August 22, 2012 78: September 14, 2012 80: September 24, 2012 81 and 82: September 24, 2012 83: September 27, 2012 84: September 27, 2012
CDER stamp date:	44: January 14, 2011 46: March 11, 2011 73: July 19, 2012 76: August 16, 2012 77: August 22, 2012 78: September 14, 2012 80: September 24, 2012 81 and 82: September 24, 2012 83: September 28, 2012 84: September 28, 2012
Product:	VX-809 (b) (4)
Indication:	Cystic Fibrosis
Sponsor:	Vertex Pharmaceuticals Incorporated 130 Waverly Street Cambridge, MA 02139-4242
Review Division:	Pulmonary, Allergy, and Rheumatology Products
Reviewer/Team Leader:	Timothy W. Robison, Ph.D., D.A.B.T.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Angela Ramsey

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

This review evaluates nonclinical data to address a Special Protocol Assessment for a proposed 2-year rat carcinogenicity bioassay protocol submitted by Vertex Pharmaceuticals Incorporated (Vertex). The drug product Lumacaftor (VX-809) is a cystic fibrosis transmembrane conductance regulator (CFTR) [redacted] (b) (4) proposed for the treatment of cystic fibrosis (CF). VX-809 is proposed to act as a chaperone that can rescue the processing, trafficking, and function of cystic fibrosis transmembrane conductance regulator (CFTR) carrying a deletion at phenylalanine 508 (ΔF508). Ninety percent of patients with CF carry a mutation that leads to misfolded ΔF508 CFTR.

In Phase I studies, the sponsor identified a unique metabolite (VRT-0995096 or M28), which represented 13% of the total parent and metabolites (based on radio-labeling) and was not formed in nonclinical test species.

1.2 Brief Discussion of Nonclinical Findings

To support a 2-year repeat dose oral carcinogenicity study in Sprague-Dawley rats, Vertex submitted ADME studies, a 3-month toxicology study with VX-809 in rats, a 6-month toxicology study with VX-809 + VRT-0995096 in rats, a 1-month toxicology study with VRT-0995096 in rats, and *in vitro* genetic toxicology studies with VRT-0995096.

In a 3-month oral toxicology study, rats received VX-809 at doses of 0, 250, 500, 1000, or 2000 mg/kg/day. Body weight gains for male rats at 500, 1000, and 2000 mg/kg/day were reduced to 92.7, 90.3, and 80.7% of the control and for female rats at 500, 1000, and 2000 mg/kg/day were reduced to 88.7, 82.1, and 81.4% of the control, respectively. Treatment-related histopathological findings were evident in the liver (hepatocellular hypertrophy) and spleen (extramedullary hematopoiesis and increased erythrocytes in the red pulp), although these findings were not considered dose-limiting and were not evident in the 6-month study. The NOAEL was judged to be 2000 mg/kg/day. However, the MTDs were identified as 1000 and 500 mg/kg/day for males and female rats, respectively, based decreases of body weight gain relative to the controls.

In a 26-week toxicology study, Sprague-Dawley rats received VX-809 by oral gavage at doses of 250, 500, or 1000 mg/kg/day in combination with VRT-0995096 at a dose of 25 mg/kg/day. Absolute body weights were unaffected by treatment for 26 weeks. There were no biologically significant changes of hematology, coagulation, or clinical chemistry parameters. No target organs of toxicity were identified. Plasma C_{max} and AUC values for VX-809 increased with elevating doses; however, these increases were significantly less than dose proportional. Plasma C_{max} and AUC values for VRT-0995096 were comparable for Group 2, 3, and 4 at each time point suggesting that VX-809 had no effects on exposure to VRT-0995096. The MTD was judged to be greater than 1000 mg/kg/day VX-809 + 25 mg/kg/day VRT-0995096.

In a 28-day toxicology study, rats received VRT-0995096 (M28) at doses of 0, 25, 50, and 100 mg/kg/day. No dose-limiting toxicity or target organs of toxicity were identified in rats that received doses up to 100 mg/kg/day for 28 days.

VRT-0995096 (M28) was negative in the *in vitro* bacterial reverse mutation assay and *in vitro* Chinese hamster ovary cell chromosomal aberration assay.

Exposure margins for clinical exposures to VX-809 with doses of 200, 800, and 1200 mg/day relative to exposures in the 3- and 6-month rat studies were greater than 1.7-fold.

In the clinical setting, the metabolite to parent drug ratio (M28/VX-809) based on AUC on Days 1 and 28 decreased from 33% at 25 mg/day to 15% at 200 mg/day after 28 days of VX-809 treatment. Exposure margins were greater than 1 for clinical exposures to M28 obtained with clinical doses of VX-809 at 200, 800, and 1200 mg/day, assuming a M28/VX-809 ratio of 15%, relative to the 1-month toxicology study with M28 in rats and 6-month toxicology study with VX-809 + M28 in rats.

1.3 Recommendations

(b) (4)

(b) (4)

2 Drug Information

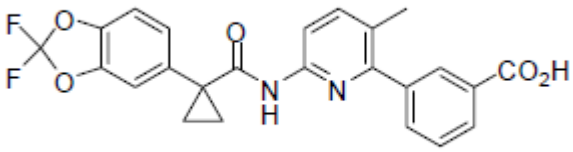
2.1 Drug
Code name: VRT-826809 or VX-809

Chemical name: 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid

Molecular formula/molecular weight: $C_{24}H_{18}F_2N_2O_5$ / 452 g/mole

Structure:

Figure 1 Structure of VX-809 Drug Substance



Chemical Formula: $C_{24}H_{18}F_2N_2O_5$
Molecular Weight: 452.41

Pharmacologic class: CFTR (b) (4)

2.2 Relevant INDs, NDAs, and DMFs
IND 74,633 (Vertex Pharmaceuticals Inc., VX-770)

2.3 Drug Formulation

VX-809 and matching placebo will be supplied as pink film-coated tablets of similar size and appearance containing 200 mg and 0 mg of VX-809, respectively. VX-770 and matching placebo will be supplied by Vertex as blue film-coated tablets containing 100 mg, 150 mg, and 0 mg of VX-770, respectively. The active drug and placebo tablets will be similar in appearance.

Table 11-1 Identity of Study Drugs, Dosage, and Storage

Drug Name	Strength/Formulation/ Route	Dosage	Storage Condition
VX-809	200 mg Tablet/Oral	200 mg (1 tablet) qd 400 mg (2 tablets) qd 600 mg (3 tablets) qd (AM) Days 1 to 21 (Cohort 1) (AM) Days 1 to 56 (Cohort 2)	15°C to 30°C (59°F to 86°F)
VX-809 matching placebo[a]	0 mg Tablet/Oral	0 mg (1 tablet) qd 0 mg (2 tablets) qd 0 mg (3 tablets) qd (AM) Days 1 to 21 (Cohort 1) (AM) Days 1 to 56 (Cohort 2)	15°C to 30°C (59°F to 86°F)
VX-770	150 mg Tablet/Oral	150 mg (1 tablet) q12h (AM and PM) Days 15 to 21 (Cohort 1) (AM and PM) Days 29 to 56 (Cohort 2)	15°C to 30°C (59°F to 86°F)
VX-770	100 mg Tablet/Oral	100 mg (1 tablet) q12h (AM and PM) Days 15 to 21 (Cohort 1) (AM and PM) Days 29 to 56 (Cohort 2)	15°C to 30°C (59°F to 86°F)
VX-770 (150 mg) matching placebo	0 mg tablet/Oral	0 mg (1 tablet) q12h (AM and PM) Days 15 to 21 (Cohort 1) (AM and PM) Days 29 to 56 (Cohort 2)	15°C to 30°C (59°F to 86°F)
VX-770 (100 mg) matching placebo	0 mg tablet/Oral	0 mg (1 tablet) q12h (AM and PM) Days 15 to 21 (Cohort 1) (AM and PM) Days 29 to 56 (Cohort 2)	15°C to 30°C (59°F to 86°F)

AM: morning; PM: evening; qd :once daily; q12h: every 12 hours

Note: For Cohort 1 on Day 21, the morning doses of VX-809, VX-770, and placebo will be the final doses in the study. For Cohort 2 on Day 56, the morning doses of VX-809, VX-770, and placebo will be the final doses in the study.

[a] In Cohort 1, placebo group will be dosed with 1 VX-809 matched placebo tablet corresponding to the VX-809 200 mg dose. In Cohort 2, placebo group will be dosed with 3 VX-809 matched placebo tablets in order to maintain the blind with regards to VX-809 dose level.

2.4 Proposed Clinical Population and Dosing Regimen

Male or Female subjects with CF (18 years or older) who are homozygous or heterozygous for the F508del-CFTR mutation will be administered VX-809 alone or in combination with VX-770.

2.5 Comments on Impurities/Degradants of Concern

(b) (4) is present as (b) (4) in VX809 and VX-770 (Kalydeco™, Ivacaftor).

The following information request was sent to the Sponsor on August 13, 2012:

"The proposed specification of (b) (4) *at* (b) (4) *ppm is acceptable for Phase 1 and 2 clinical trials with VX-809. We note for a daily dose of VX-809 at 1200 mg, the daily exposure to* (b) (4) *would be approximately* (b) (4) *mg. If the daily exposure to* (b) (4) *exceeds* (b) (4) *mg/day (level in Kalydeco at 300 mg/day) for*

the Phase 3 clinical trials and an NDA, it is our position that the exposure should be supported by a GLP 3-month toxicology study in one species that includes clinical signs, body weight, hematology, clinical chemistry, organ weights, and gross pathologic and histopathologic examinations of a full panel of tissues. This study should characterize the toxicity of (b) (4) and identify a NOAEL that provides adequate safety margins for the proposed specification of (b) (4).”

The Sponsor requested a teleconference in their submission dated August 21, 2012. In addition, they made reference to

(b) (4)

A teleconference was held with the Sponsor on September 12, 2012. A synopsis of the teleconference is provided below.

“Dr. Timothy Robison referenced (b) (4) Pending review of the study and verification of the Sponsor’s NOAEL at 1000 mg/kg/day, this might be an adequate approach for qualifying the proposed clinical dose of (b) (4). The dose of (b) (4) at the NOAEL was approximately (b) (4) mg/kg/day or (b) (4) mg/m2/day. Clinical doses of (b) (4) from VX-809 at 1200 mg/day and VX-770 at 1050 mg/day were estimated to be (b) (4) and (b) (4) mg/day, respectively, for a total of (b) (4) mg/day (equivalent to (b) (4) mg/m^2/day for a 60 kg patient). On a mg/m^2 basis, the dog dose provides a safety factor of approximately 2-fold for the estimated human dose. Dr. Robison noted that the Division prefers at least a 2-fold safety margin on a mg/m^2 basis. Dr. Robison stated that the safety margin is acceptable for a 60 kg adult; however, the safety margin may be inadequate for children. Vertex will submit a revised Certificate of Analysis for Lot PT-C07062202-G10001 that documents the concentration of (b) (4) and a revised estimate for the (b) (4) dose in humans.”

2.8 Regulatory Background

The sponsor is developing VX-809, a CFTR (b) (4) for the treatment of CF as a monoproduct, and in combination with the approved CFTR potentiator VX-770. The

opening IND submission was submitted on October 18, 2007 to support a Phase 1 SAD and MAD oral clinical trial in healthy volunteers. The sponsor is currently studying the combination of VX-809 and VX-770 in a Phase II multiple dose study with VX-809 alone and co-administered with VX-770 (protocol version 1 submitted July 27, 2010).

On February 19, 2010, the sponsor submitted plans for nonclinical studies to support the safety of the major metabolite (VRT-0995096 or M28), which was identified in Phase I studies and was not formed in nonclinical test species. M28 represented 13% of the total parent and metabolites (based on radio-labeling). The sponsor stated that preliminary data showed that oral administration of M28 in rats resulted in adequate exposure to M28, and therefore they proposed to conduct oral toxicology studies in rat to ensure adequate safety coverage for M28 exposure in clinical studies. The sponsor also proposed to conduct genetic toxicology, and *in vitro* drug metabolism studies with M28.

3 Studies Submitted

3.1 Studies Reviewed

Repeat Dose Toxicology

1. VX-809: A 3-MONTH ORAL (GAVAGE) TOXICITY AND TOXICOKINETIC STUDY IN RATS WITH A 1-MONTH RECOVERY PERIOD
2. VX-809 AND VRT-0095096: A 6-MONTH ORAL (GAVAGE) TOXICITY AND TOXICOKINETIC STUDY IN RATS WITH A 1-MONTH RECOVERY PERIOD
3. VRT-0995096: A 28-DAY ORAL (GAVAGE) TOXICITY AND TOXICOKINETIC STUDY IN RATS

Genetic Toxicology

1. VRT-0995096: Bacterial Reverse Mutation Assay
2. VRT-0995096: In Vitro Mammalian Chromosome Aberration Test

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

1. Pharmacology and Toxicology Review of IND 79,521 dated February 2, 2010
2. Pharmacology and Toxicology Review of IND 79,521 dated March 10, 2010
3. Pharmacology and Toxicology Review of IND 79,521 dated September 30, 2010
4. Pharmacology and Toxicology Review of IND 79,521 dated September 28, 2011
5. Pharmacology and Toxicology Review of IND 79,521 dated July 15, 2012

6 General Toxicology

Repeat-Dose Toxicity

Study title: A 3-MONTH ORAL (GAVAGE) TOXICITY AND TOXICOKINETIC STUDY IN RATS WITH A 1-MONTH RECOVERY PERIOD

Study no.: VX-809-TX-007
Study report location: EDR
Conducting laboratory and location: (b) (4)

Date of study initiation: December 23, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity:

Lot Numbers	Purity	Description	Dates Received	Retest Dates
PT-C07091701-M08001	Assume 100%	White to off white solid	31 Dec 2008	22 Aug 2009
PT-C07091701-M08002	Assume 99.9%	White to off-white solid	16 Feb 2009	20 Sep 2009

Key Study Findings

• In a 3-month oral toxicology study, rats received VX-809 at doses of 0, 250, 500, 1000, or 2000 mg/kg/day.

• Body weight gains for male rats at 500, 1000, and 2000 mg/kg/day were reduced to 92.7, 90.3, and 80.7% of the control and for female rats at 500, 1000, and 2000 mg/kg/day were reduced to 88.7, 82.1, and 81.4% of the control, respectively.

• There were changes of several hematology parameters at the end of the dosing period (increases of platelet counts, mean platelet volume, reticulocytes, RBC distribution width, and white blood cell and lymphocyte counts and decreases of RBC counts, hemoglobin, hematocrit, and eosinophil counts).

• Treatment-related histopathological findings were evident in the liver (hepatocellular hypertrophy) and spleen (extramedullary hematopoiesis and increased erythrocytes in the red pulp), Extramedullary hematopoiesis in the spleen appeared to be a compensatory response to decreased RBC counts. These findings were not considered dose-limiting and were not evident in the 6-month study with doses of VX-809 up to 1000 mg/kg/day.

• Administration of VX-809 to male and female rats at doses of 1000 and 2000 mg/kg/day resulted in small increases of microsomal protein yield, total cytochrome P450 content, and CYP4A activity. In addition, there were increases of CYP3A activity in female animals. There were larger increases of CYP3A activity in male animals at a dose of 2000 mg/kg/day and CYP2B and CYP1A activities in male and female animals at doses of 1000 and 2000 mg/kg/day.

• The NOAEL was 2000 mg/kg/day. However, the MTDs were identified as 1000 and 500 mg/kg/day for males and female rats, respectively, based decreases of body weight gain.

Methods

Doses:	0, 250, 500, 1000, and 2000 mg/kg/day
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% Methylcellulose/0.5% Tween 80/0.05% Simethicone in water
Species/Strain:	Albino Rats (Outbred) VAF/Plus® Sprague-Dawley derived (CD®) Crl:CD®(SD)IGS BR Obtained from (b) (4)
Number/Sex/Group:	10 rats/sex/group
Age:	Approximately 8 weeks
Weight:	Males: Mean 251, Range 220 to 295; Females: Mean 188, Range 156 to 212
Satellite groups:	An additional 5 rats/sex/group were included in the 0, 1000, and 2000 mg/kg/week groups for a 4-week recovery period. For toxicokinetic assessments, an additional 3 rats/sex were included in the control group and 6 rats/sex were included in each drug-treated group.
Unique study design:	None
Deviation from study protocol:	Deviations were minor and did not affect the integrity of the study

Table 1 Design of the 13-week toxicology study with VX-809 in rats

9.3.1 Study Design

The test and control articles were administered by daily oral intubation (gavage) to rats for 3-months followed by a 1-month recovery period.

	Daily Dosage[a]			Number of Animals									
				Toxicity Animals		TK Animals	Clinical Pathology		Necropsy		Microscopic Pathology	
				Main	Recovery	Days 1, 45, and 90	Main (Term)	Recovery	Main (Day 92)	Recovery	Main	Recovery
Group	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F
1	0	10	0	10/10	5/5	3/3	10/10	5/5	10/10	5/5	10/10	5/5
2	250	10	25	10/10	0/0	9/9	10/10	0/0	10/10	0/0	10/10[b]	0/0
3	500	10	50	10/10	0/0	9/9	10/10	0/0	10/10	0/0	10/10[b]	0/0
4	1000	10	100	10/10	5/5	9/9	10/10	5/5	10/10	5/5	10/10	5/5
5	2000	10	200	10/10	5/5	9/9	10/10	5/5	10/10	5/5	10/10	5/5

[a] Doses represent active ingredient.
[b] The liver was identified as a target organ and examined in Groups 2 and 3.
The first day of dosing was designated at Day 1 of the study.

Observations and Results

Mortality: Animals were observed in their cages for mortality and general condition twice daily (once in the morning and once in the afternoon).

There were no treatment-related deaths.

Two control animals (1011M and 1509F) were sacrificed due to accidental snout trauma.

Clinical Signs: Observations for signs of toxic or pharmacologic effects were made at least once daily for each toxicity study animal throughout the pre-dosing, dosing and recovery periods. All toxicity animals were removed from their cages and given a physical examination once pretest and once weekly throughout the dosing and recovery periods.

One high-dose male (5012M) had clinical signs beginning month 3 of the study consisting of irregular gait, trembling/shaking, decreased activity, and thin appearance. These findings were still evident during the recovery period. There were no histopathological findings that appeared to correlate with these clinical observations.

Body Weights: Non-fasted body weights were recorded twice pretest, twice weekly during the dosing and recovery periods and terminally after fasting. Terminal fasted body weights were obtained prior to necropsy.

Body weight gains were decreased >10% for males at 2000 mg/kg/day and females at ≥500 mg/kg/day as compared to controls.

Table 2 Body weight gains for male and female rats during the 3-month dosing period

Body weight gain	Males					Females				
	0	250	500	1000	2000	0	250	500	1000	2000
BW (g), Rand	253.6	250.4	248.0	244.1	245.7	190.5	189.6	192.2	184.4	185.3
BW (g), Day 91	574.7	563.2	540.8	525.0	498.5	306.0	301.6	295.6	276.2	276.7
Δ (g)	323.1	312.8	292.8	280.9	252.7	115.5	112.0	103.4	91.8	91.4
% Initial BW	127.4	124.9	118.1	115.1	102.9	60.6	59.1	53.8	49.8	49.3
% Control BW	100	98.0	92.7	90.3	80.7	100	97.4	88.7	82.1	81.4

Figure 1 Body weights for male rats during the 3-month dosing period and 4-week recovery period

Males	Mean Body Weights (grams)	Figure 1

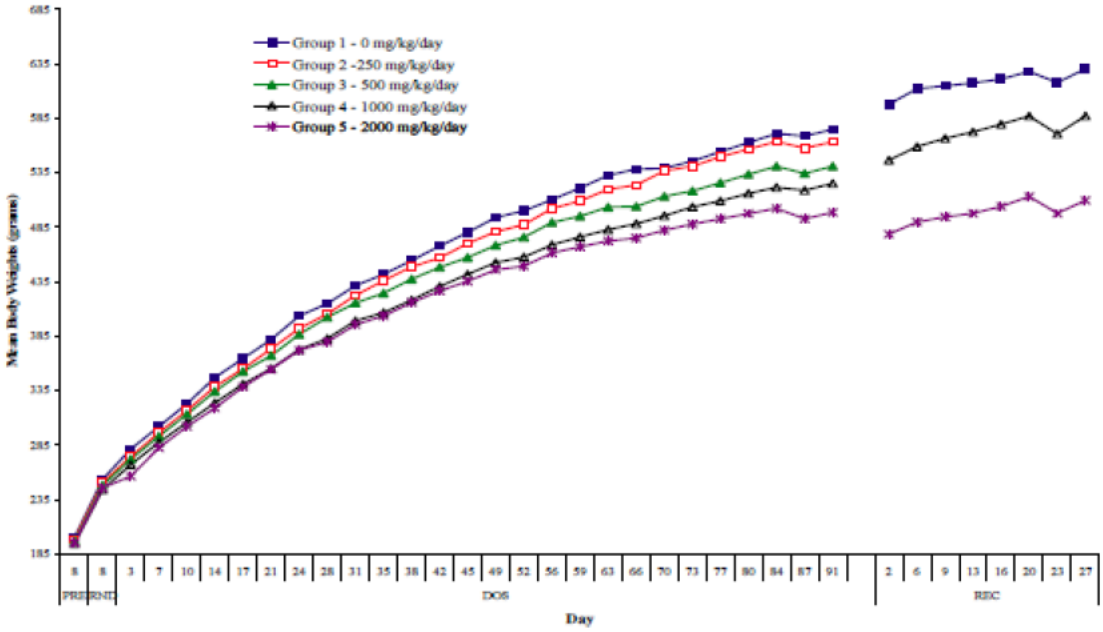


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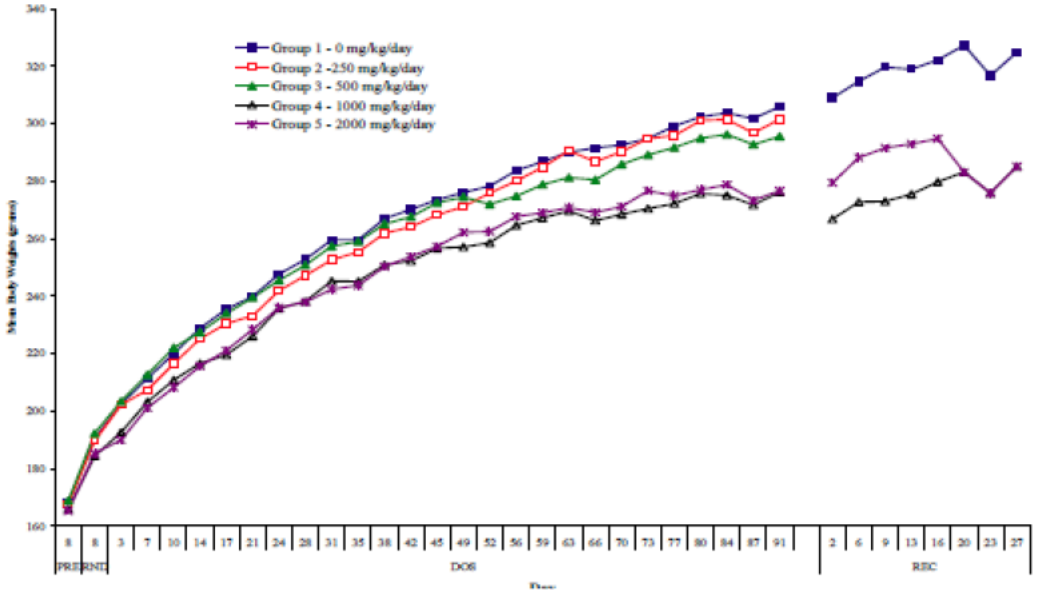
Figure 2 Body weights for female rats during the 3-month dosing period and 4-week recovery period

Females	Mean Body Weights (grams)	Figure 2



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Feed Consumption: Feed was available without restriction 7 days per week. Animals were presented with full feeders of known weight. After 5, 6 or 7 days, feeders were reweighed and the resulting weight was subtracted from the full feeder weight to obtain the grams consumed per animal over the 5-, 6- or 7-day period. Feed consumption was measured (weighed) weekly for all toxicity animals commencing the last week of pretest and extending throughout the dosing and recovery periods (for toxicity animals only).

There were no treatment-related effects on food consumption.

Figure 3 Food consumption for male rats during the 3-month dosing period and 4-week recovery period

Males	Mean Feed Consumption (grams/day)	Figure 3

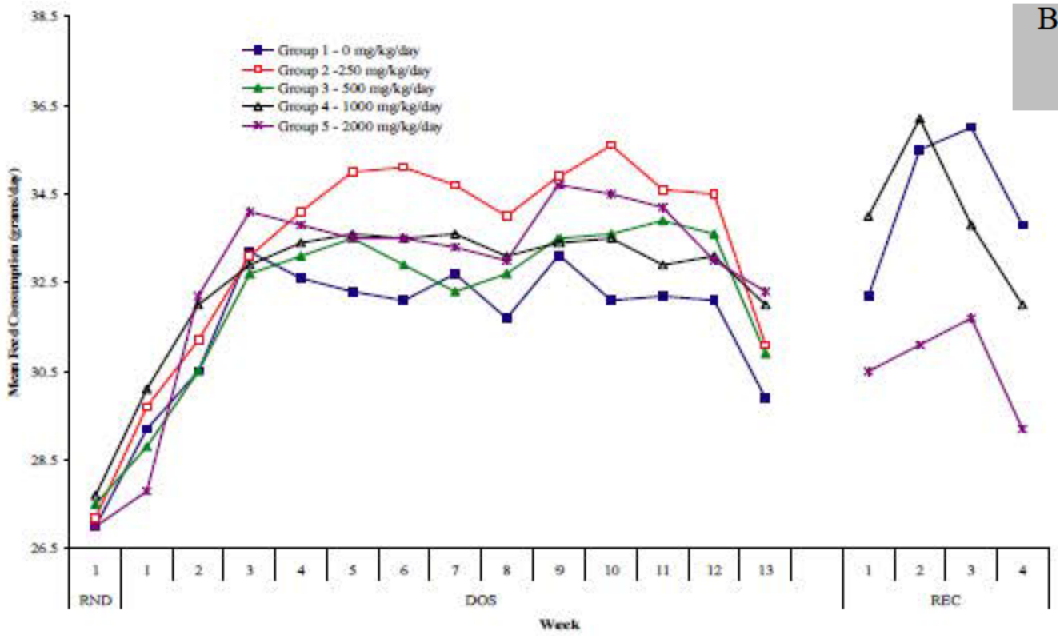
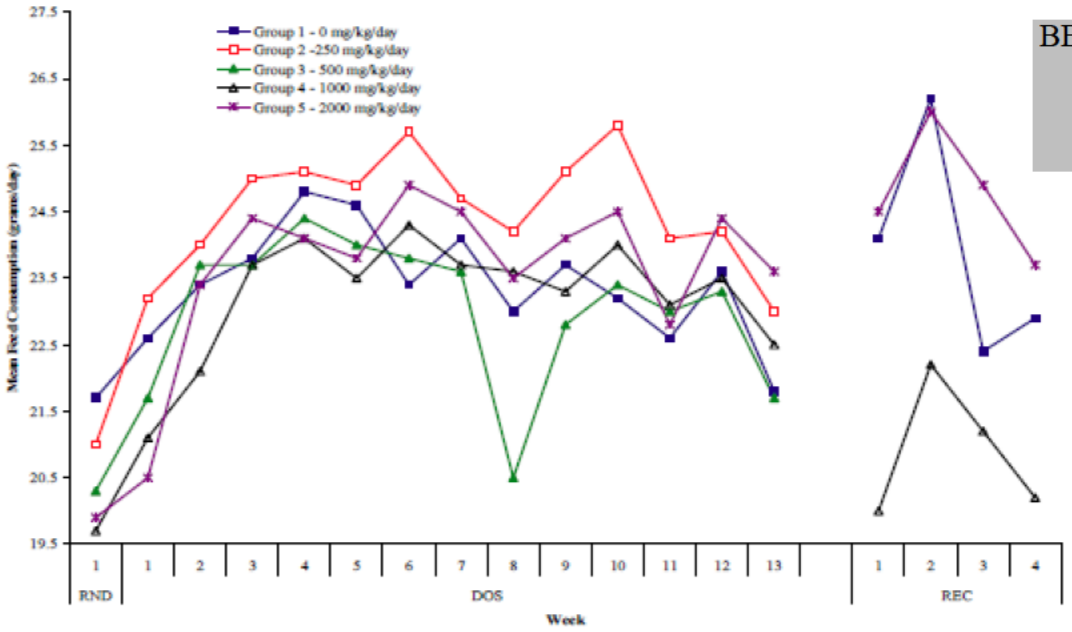


Figure 4 Food consumption for female rats during the 3-month dosing period and 4-week recovery period

Females	Mean Feed Consumption (grams/day)	Figure 4



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Ophthalmoscopy: Ophthalmic examinations were performed at pretest, week 2, study termination at week 13, and at Recovery week 4.

Male #4012 in the 1000 mg/kg/day group was observed with a myelinated retinal nerve fiber at the end of the 13-week dosing period; however, this finding was not evident at the end of the 4-week recovery period. Male #4012 was also observed with focal posterior polar cataract at the end of the recovery period.

There were no findings for the 2000 mg/kg/day group at the end of the treatment or recovery periods.

Hematology: Blood obtained at necropsy via puncture of the vena cava under deep anesthesia (isoflurane) was used to analyze a complete panel of clinical chemistry parameters for up to 10 animals/sex/group at study termination and up to 5 animals/sex/Groups 1, 4 and 5 at recovery. Animals were fasted overnight prior to each blood collection interval.

There were changes of several hematology parameters at the end of the dosing period (increases of platelet counts, mean platelet volume, reticulocytes, RBC distribution width, and white blood cell and lymphocyte counts and decreases of RBC counts, hemoglobin, hematocrit, and eosinophil counts). Increases of white blood cell and lymphocyte counts were relatively small and not dose-related.

Table 3 Changes in hematology parameters after drug treatment for 3 months

Table 10.8.1-1: Changes in Hematology Parameters[1] in Rats Dosed Orally with VX-809 for 3 Months

	Males				Females			
	Dose Level (mg/kg/day)				Dose Level (mg/kg/day)			
	250	500	1000	2000	250	500	1000	2000
PLT	+15%*	+11%*	+10%*	+9%*	-	-	+17%*	+26%***
MPV	-	+8%**	+13%***	-	+8%***	+11%***	+13%***	+20%***
HGB	-	-	-	-7%**	-5%*	-7%**	-9%***	-15%***
HCT	-	-	-	-6%**	-	-6%*	-8%***	-13%***
RBC	-	-	-	-7%**	-	-	-7%**	-14%***
RETIC	-	-	+29%*	+74%***	-	-	+44%***	+63%***

*Absolute values were statistically significantly different from controls.
[1] Percentage change compared to corresponding control values.
PLT = Platelet; MPV=Mean Platelet Volume; HBG= Hemoglobin; HCT=Hematocrit
RBC=Red Blood Cell; RETIC=Reticulocytes

Table 4 Additional changes in hematology parameters after drug treatment for 3 months

Parameter	Males					Females				
	0	250	500	1000	2000	0	250	500	1000	2000
WBC $x10^3/\mu L$	9.57	12.56	11.04	11.51	10.91	5.09	7.21	6.94	6.38	6.34
Lymphocytes $x10^3/\mu L$	7.79	10.53	8.89	9.58	8.95	04.28	5.87	5.96	5.56	5.38
RDW %	14.0	14.5	15.4	15.3	16.2*	11.8	12.3*	12.4*	13.2*	14.5*

*Significantly different from control

Clinical Chemistry: Blood obtained at necropsy via puncture of the vena cava under deep anesthesia (isoflurane) was used to analyze a complete panel of clinical chemistry parameters for up to 10 animals/sex/group at study termination and up to 5 animals/sex/Groups 1, 4 and 5 at recovery. Animals were fasted overnight prior to each blood collection interval.

Decreases of triglyceride and glucose and increases of cholesterol and phosphate were evident as shown in the table below. Small changes of total bilirubin, AST, total protein, albumin, the A/G ratio, BUN, and creatinine were also evident.

Table 5 Changes in clinical chemistry parameters after drug treatment for 3 months

Table 10.8.3-1 Main Changes in Clinical Chemistry Parameters in Rats Dosed Orally with VX-809 for 3 Months

	Males				Females			
	Dose Level (mg/kg/day)				Dose Level (mg/kg/day)			
	250	500	1000	2000	250	500	1000	2000
TRIG	-51%***	-46%***	-51%***	-68%***	-	-	-	-
CHOL	-	-	-	+26%*	-	-	+26%*	+50%***
GLU	-	-	-	-20%*	-	-	-	-24%**
PHOS	-	-	-	+10%*	-	-	-	-

*Absolute values were statistically significantly different from controls.
TRIG = Triglycerides; CHOL = Cholesterol; GLU = Glucose; PHOS = Phosphorous

Table 6 Additional changes in clinical chemistry parameters after drug treatment for 3 months

Parameter	Males					Females				
	0	250	500	1000	2000	0	250	500	1000	2000
Total bilirubin mg/dL	0.15	0.15	0.14	0.13	0.17	0.16	0.17	0.18	0.21	0.26*
AST U/L	81	82	98	84	101*	NC	NC	NC	NC	NC
Total protein g/dL	NC	NC	NC	NC	NC	7.0	6.8	6.6*	6.6*	6.7*
Albumin g/dL	NC	NC	NC	NC	NC	4.2	4.0	4.0*	3.9*	4.0*
A/G	1.3	1.3	1.3	1.3	1.4*	NC	NC	NC	NC	NC
BUN mg/dL	13	14	15	16	15*	NC	NC	NC	NC	NC
Creatinine mg/dL	0.2	0.3*	0.3*	0.3*	0.3*	NC	NC	NC	NC	NC
Phosphate mg/dL	6.9	6.8	7.0	7.1	7.6*	6.0	6.1	6.5	6.4	6.9

*Significantly different from control
NC = No change

At the end of the 4-week recovery period for males at 2000 mg/kg/day, triglyceride levels were still decreased and phosphate levels were still increased.

Urinalysis: Urine obtained via a 16-hour overnight collection period was analyzed for 10 animals/sex/group at study termination and up to 5 animals/sex/Groups 1, 4 and 5 at recovery.

There were several changes of urinalysis parameters although there was no significant evidence of histopathological changes in the kidneys.

Table 7 Changes of urinalysis parameters after drug treatment for 3 months

Table 10.8.4-1: VX-809 Effects[1] on Urinalysis Parameters in Rats Dosed At 3 Months

	Males				Females			
	Dose Level (mg/kg/day)				Dose level (mg/kg/day)			
	250	500	1000	2000	250	500	1000	2000
↑U.Vol	2.8x**	2.4x**	5.6x***	4.0x***	-	-	2.2x*	2.8x**
↑pH	-	-	1.1x*	1.1x*	-	-	-	-
↓Creat	.64x*	.53x**	.28x***	.24x***	-	-	.40x*	.23x***
↓Ca++	.44x**	.32x**	.20x**	.20x**	-	-	.42x*	.30x**

*Absolute values were statistically significantly different from controls
[1] fold difference from control values ↑: increase ↓: decrease

Gross Pathology: Necropsy was performed on up to 10 animals/sex/group after animals had been treated for 3 months, and on up to 5 animals/sex/Group 1, 4 and 5 after a 28-day recovery period. Animals were fasted overnight prior to necropsy.

Gross pathological changes were observed in the brain, cecum, ovaries, stomach, and thymus; however, there were no correlates to treatment-related histopathological findings.

Table 8 Gross pathological findings in rats at the end of 3-month dosing period

Parameter	Males					Females				
	0	250	500	1000	2000	0	250	500	1000	2000
N =	10	10	10	10	10	9	10	10	10	10
Brain -discolored	0	0	0	0	0	0	0	0	0	1
Cecum -abnormal contents	0	0	0	0	1	0	0	0	0	1
Ovaries -cyst						0	0	0	0	1
Stomach -discolored	0	0	0	1	4	0	3	1	4	5
Thymus -small	0	0	0	1	1	0	0	0	0	0

Organ Weights: Absolute and relative organ weights were measured for the adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroids/parathyroids, and uterus with cervix.

Relative liver weights for males at ≥1000 mg/kg/day and absolute/relative liver weights of females at 2000 mg/kg/day were increased compared to controls and most likely represented an adaptive change. In males, this correlated with minimal centrilobular hypertrophy at ≥1000 mg/kg/day.

Minimally increased spleen weights were noted in both sexes at 2000 mg/kg/day, and were considered secondary to hematological changes at this dose level.

Decreased thymus weights were noted in males at ≥ 500 mg/kg/day. There was no histopathological correlation. At the end of the recovery period, thymus weights of males at 2000 mg/kg/day were still decreased compared to control values after a 4-week recovery period.

Table 9 Organ weight changes in the 13-week toxicology study with rats

Table 10.9.1-1: Changes in Absolute Organ Weight Values[1] in Rats Dosed Orally with VX-809

	Males			Females		
	Dose Level (mg/kg/day)			Dose Level (mg/kg/day)		
	500	1000	2000	500	1000	2000
Spleen	-	-	+16%	-	-	+13%
Liver	-	-	+3.6%	-	-	+14%*
Thymus	-23%*	-22%*	-35%**	-	-	-

*Absolute values were statistically significantly different from controls
[1]Percentage change compared to corresponding control values

Histopathology:

Adequate Battery: A complete panel of tissues and organs was submitted to histopathological examination. After fixation, the tissues and organs from all Toxicity animals in Groups 1, 4 and 5 were routinely processed, embedded in paraffin, cut at a microtome setting of 4-7 microns, mounted on glass slides, stained with hematoxylin and eosin and examined by light microscopy. The bones were decalcified in Decalcifier II.

Table 10 Tissue collected for the 13-week toxicology study with rats

Table 9.3.15.4-1 – Tissues weighed, preserved and examined microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY Group 1, 4 and 5
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X	
bone (sternum,distal femur)		X	X
bone marrow (sternum, femur)		X	X[a]

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY Group 1, 4 and 5
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
epididymides		X	X
esophagus		X	X
eyes		X	X
Harderian gland		X	X
heart	X	X	X
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
liver	X	X	X[d]
lungs (with mainstem bronchi)		X	X
lymph nodes (mesenteric and mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
optic nerve		X	X
ovaries	X	X	X
pancreas		X	X
pituitary gland	X	X	X
prostate gland	X	X	X
salivary glands (submandibular)		X	X
seminal vesicles		X	X
skeletal muscle (*Biceps femoris*)		X	X
skin		X	X
small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumbar)		X	X
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X[b]	X	X
trachea		X	X
urinary bladder		X	X
uterus (horns/body with cervix)	X	X	X
vagina		X	X
gross lesions[c]		X	X

[a]Qualitative examination (no differential count).
[b]Weighed post-fixation
[c]Up to 360 total gross lesions from all toxicity animals in Groups 1, 4 and 5 were preserved and examined.
[d]The liver was identified as a target organ and was examined in all animals in Groups 2 and 3.

Peer Review: None

Histological Findings: Treatment-related histopathological findings were evident in the liver (hepatocellular hypertrophy) and spleen (extramedullary hematopoiesis and increased erythrocytes in the red pulp) as shown in the table below. Findings of hepatocellular hypertrophy were considered adaptive and not regarded as adverse. Extramedullary hematopoiesis was considered a compensatory responses to observed changes of red blood cell parameters. Findings in the stomach were of unclear relationship to treatment. Other findings were considered background in nature, but were included for comparisons to findings in the sponsor's toxicology studies with the combination of VX-809 + VX-770 (Ivacaftor).

Table 11 Histopathological findings in rats at the end of 3-month dosing and 4-week recovery periods

Organ/Tissue	Sex	End of Dosing Period					End of Recovery Period		
		0	250	500	1000	2000	0	1000	2000
Liver									
-hepatocellular	M	0/10	-	-	3/10	6/10	0/4	0/5	0/5
hypertrophy, minimal	F	0/9	-	-	0/10	0/10	0/5	0/5	0/5
Spleen									
-increased extramedullary	M	0/10	-	-	0/10	0/10	0/4	0/5	0/5
hematopoiesis, slight	F	0/9	-	-	0/10	3/10	0/5	0/5	0/5
-red pulp, increased	M	0/10	-	-	0/10	2/10	0/4	0/5	0/5
erythrocytes, slight	F	0/9	-	-	0/10	0/10	0/5	0/5	0/5
Mediastinal LN									
-intrasinusoidal	M	1/10	-	-	0/10	1/9	1/4	0/5	0/5
erythrocytes with or w/o	F	1/8	-	-	1/9	3/10	0/5	0/5	0/5
brown pigment									
Mesenteric LN									
-intrasinusoidal	M	0/10	-	-	1/10	0/10	0/4	0/5	0/5
erythrocytes	F	0/9	-	-	0/10	1/10	0/5	0/5	0/5
-increased mast cells	M	0/10	-	-	0/10	1/10	0/4	0/5	0/5
	F	0/9	-	-	0/10	0/10	0/5	0/5	0/5
-increased sinus	M	0/10	-	-	0/10	0/10	0/4	0/5	0/5
histiocytes	F	0/9	-	-	0/10	1/10	0/5	0/5	0/5
Kidneys									
-mineral deposits, minimal-	M	0/10	-	-	0/10	0/10	0/4	0/5	1/5
slight	F	1/9	-	-	2/10	2/10	1/5	1/5	2/5
-chronic progressive	M	3/10	-	-	2/10	2/10	0/4	5/5	3/5
nephropathy, minimal	F	2/9	-	-	3/10	0/10	0/5	0/5	0/5
Stomach									
-congestion, minimal	M	0/10	-	-	1/10	0/10	0/4	0/5	0/5
	F	0/10	-	-	0/10	1/10	0/5	0/5	0/5
-glandular stomach:	M	0/10	-	-	0/10	0/10	0/4	0/5	0/5
necrosis, minimal	F	0/10	-	-	1/10	0/10	0/5	0/5	0/5
-hemorrhage, slight	M	0/10	-	-	0/10	0/10	0/4	0/5	0/5
	F	0/10	-	-	0/10	1/10	0/5	0/5	0/5

Special Evaluation: Liver samples collected at terminal necropsy from animals in Groups 1, 4 and 5 were shipped on dry ice to for analysis of cytochrome P450 activities to (b) (4).

The microsomal fractions were analyzed for the following parameters:

Parameter	Assay Method
Microsomal protein	Lowry protein assay
Total Cytochrome P450	Difference spectroscopy
CYP1A Activity	7-Ethoxyresorufin O-deethylase
CYP2B Activity	7-Pentoxyresorufin O-dealkylase
CYP3A Acivity	Testosterone 6β-hydroxylase
CYP4A Activity	Lauric acid 12-hydroxylase

Administration of VX-809 to male rats for 3 months at dose levels of 1000 and 2000 mg/kg/day resulted in slight increases of microsomal protein yield (1.2- and 1.3-fold, respectively) compared to vehicle controls. In female rats, no overall change in protein yield was observed at 1000 mg/kg/day; however, a slight increase (1.3-fold) in protein yield was observed in females at 2000 mg/kg/day.

Total cytochrome P450 content was increased in male and female rats at doses of 1000 and 2000 mg/kg/day ≥1.4-fold compared to vehicle controls.

Ethoxyresorufin O-deethylase activity, a marker for CYP1A activity, was increased in male and female rats at doses of 1000 and 2000 mg/kg/day ≥4-fold compared to vehicle controls.

Pentoxyresorufin O-dealkylase activity, a marker for CYP2B activity, was increased in male and female rats at doses of 1000 and 2000 mg/kg/day ≥2-fold as compared to vehicle controls.

Testosterone 6β-hydroxylase activity, a marker for CYP3A activity, was increased in male rats at doses of 1000 and 2000 mg/kg/day ≥1.6-fold as compared to vehicle controls. In female rats, smaller increases (1.3- and 1.5-fold) in CYP3A activity were observed at both dose levels.

Lauric acid 12-hydroxylase activity, a marker for CYP4A activity, was increased in male rats at 2000 mg/kg/day to 1.6-fold as compared to the vehicle control. In female rats, 1.4- and 1.8-fold increases of CYP4A activity were observed at 1000 and 2000 mg/kg/day, respectively.

Table 1
Mean protein yield, total cytochrome P450 content, and CYP450 isoenzyme activities in hepatic microsomes from male rats administered VX-809 for 3 months

		Mean[a] ± Standard Deviation (Percent of Control)		
	Group No.	1	4	5
Parameter	Dose Level[b]	0 (Control)	1000	2000
Protein yield (mg/g liver)		31.1 ± 4.0 (NA)	35.8 ± 3.2 (115)	39.0 ± 2.5 (125)
Total CYP450 content (nmol/mg protein)		0.550 ± 0.050 (NA)	0.744 ± 0.060 (135)	0.824 ± 0.101 (150)
CYP1A (EROD) (pmol/min/mg protein)		48.1 ± 4.7 (NA)	409 ± 235 (851)	1200 ± 90 (2490)
CYP2B (PROD) (pmol/min/mg protein)		10.9 ± 3.3 (NA)	23.1 ± 9.9 (212)	39.6 ± 5.1 (363)
CYP3A (6β-OH) (pmol/min/mg protein)		501 ± 84 (NA)	855 ± 550 (171)	1050 ± 150 (210)
CYP4A (12-LAH) (pmol/min/mg protein)		691 ± 142 (NA)	752 ± 157 (109)	1120 ± 80 (162)

NA Not applicable.
a Mean of three animals.
b Dose level units are mg/kg/day.

Table 2
Mean protein yield, total cytochrome P450 content, and CYP450 isoenzyme activities in hepatic microsomes from female rats administered VX-809 for 3 months

		Mean[a] ± Standard Deviation (Percent of Control)		
	Group No.	1	4	5
Parameter	Dose Level[b]	0 (Control)	1000	2000
Protein yield (mg/g liver)		33.9 ± 11.5 (NA)	36.4 ± 0.9 (108)	43.8[c] (129)
Total CYP450 content (nmol/mg protein)		0.400 ± 0.037 (NA)	0.544 ± 0.018 (136)	0.677 ± 0.022 (169)
CYP1A (EROD) (pmol/min/mg protein)		88.3 ± 23.2 (NA)	865 ± 240 (979)	1570 ± 340 (1780)
CYP2B (PROD) (pmol/min/mg protein)		6.54 ± 1.09 (NA)	22.5 ± 3.8 (345)	37.8 ± 3.1 (578)
CYP3A (6β-OH) (pmol/min/mg protein)		97.3 ± 25.2 (NA)	121 ± 27 (125)	148 ± 4 (152)
CYP4A (12-LAH) (pmol/min/mg protein)		666 ± 193 (NA)	916 ± 138 (138)	1160 ± 50 (175)

NA Not applicable.
a Mean of three animals unless otherwise noted.
b Dose level units are mg/kg/day.
c Value is the average of two values.

Toxicokinetics: On Days 1, 45 and 90, blood samples for measurement of plasma drug concentrations were obtained from 3 animals/sex/time point from toxicokinetic animals in Groups 2 to 5 as follows: 0.5, 1, 2, 4, 10, and 24 hours post dose. Blood samples were collected from the control article TK rats (Group 1) at a single time point: 10 hours post dose. Plasma samples were shipped to the sponsor for analysis. The concentration of VX-809 in the individual plasma samples was determined using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, where the lowest limit of quantitation (LLOQ) was 2 ng/mL. The calibration standard concentrations over the range of 2.00 to 2000 ng/mL were analyzed using a linear regression method.

All the 24-hour TK animals (up to 3/sex/Groups 2 to 5) and the 10-hour control TK animals (up to 3/sex/Group 1) were transferred to necropsy and sacrificed on Day 91 (immediately after the last scheduled 24-hour TK blood collection for Groups 2 to 5), blood was collected and the targeted organs (lung, heart, and liver) harvested as soon as possible for possible biomarker analyses or determination of tissue concentrations of VX-809 and its major metabolites.

Toxicokinetic parameters are shown in the table. In most cases, C_{max} and AUC values increased with elevating dose although increases were less than dose proportional. In some cases C_{max} and AUC values did not increase with elevating dose (nonlinear

toxicokinetics). C_{max} and AUC values were generally higher in females as compared to males. There was no evidence of accumulation when comparing values on days 45 and 90 to day 1.

Table 12 Toxicokinetic parameters for VX-809 in male and female rats on days 1, 45, and 90

Table 2 Summary of Toxicokinetic Parameters for VX-809 in Female and Male Rats on Days 1, 45 and 90

Study Day	Dose (mg/kg)	Gender									
		Female					Male				
		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/mL)	DN_AUC_{0-24hr}[a] (ng*hr/mL)	$T_{1/2}$ (hr)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/mL)	DN_AUC_{0-24hr}[a] (ng*hr/mL)	$T_{1/2}$ (hr)
Day 1	250	173000	4.00	1920000	7680	5.61	132000	10.00	1950000	7800	N/A
Day 1	500	223000	4.00	3530000	7070	7.91	161000	10.00	2670000	5350	N/A
Day 1	1000	253000	4.00	3590000	3590	7.01	170000	10.00	3250000	3250	16.12
Day 1	2000	266000	4.00	5180000	2590	N/A	243000	10.00	4690000	2350	N/A
Day 45	250	167000	2.00	2100000	8390	5.05	93000	4.00	1140000	4550	4.06
Day 45	500	192000	4.00	2690000	5370	5.04	101000	2.00	1610000	3230	10.00
Day 45	1000	229000	4.00	3190000	3190	5.65	147000	4.00	1760000	1760	6.23
Day 45	2000	249000	4.00	2680000	1340	7.95	133000	10.00	2160000	1080	N/A
Day 90	250	220000	4.00	2530000	10100	7.16	137000	4.00	1630000	6510	5.49
Day 90	500	243000	4.00	3860000	7720	6.76	185000	4.00	3050000	6110	16.03
Day 90	1000	296000	2.00	4850000	4850	11.22	171000	4.00	2340000	2340	6.12
Day 90	2000	356000	10.00	5880000	2940	N/A	182000	2.00	3010000	1500	9.34

Source: PKS Study: CF-VX-809-TX-007-RAT-13 WK-TK
[a] Dose normalized to 1 mg/kg
N/A: not applicable as the elimination half-life for VX-809 could not be estimated based on the concentrations observed in the terminal elimination portion of the plasma concentration versus time curve.

Dosing Formulation Analysis: Homogeneity and dose concentration verification analyses were performed by the Testing Facility.

Homogeneity Analysis: Duplicate samples (1.0 mL each) were taken from the top, middle and bottom of the dose formulations for Groups 2 to 5 for use on Days 1 and 56. Dose Confirmation Analysis: Triplicate samples (1.0 mL each) taken from the Days 45 and 91 formulations on the day prepared were used for concentration analysis. Results from the middle homogeneity samples on Day 1 were used for Day 1 concentration verification. On Days 1, 45 and 90, duplicate control samples (1.0 mL each) were obtained and analyzed to verify that no cross contamination had occurred.

Actual concentrations were within ±10% of nominal concentrations.

Table 10.1-1: Analytical Results: Nominal vs Actual Concentration

GROUP	NOMINAL CONCENTRATION (mg/mL)	ACTUAL CONCENTRATION (% of Nominal)
Day 1 Dose Prep		
2	25	104.9
3	50	101.2
4	100	100.0
5	200	102.9
Day 45 Dose Prep		
2	25	110.0
3	50	102.5[a]
4	100	106.7[a]
5	200	110.2[a]
Day 90 Dose Prep		
2	25	103.0
3	50	104.7
4	100	94.8
5	200	104.9

[a]Re-analysis results. Original results were outside of the protocol acceptance criteria (the mean concentration was >10% of the nominal concentration).

Study title: <u>VX-809 AND VRT-0095096: A 6-MONTH ORAL (GAVAGE) TOXICITY AND TOXICOKINETIC STUDY IN RATS WITH A 1-MONTH RECOVERY PERIOD</u>

Study no.: VX-809-TX-012 and VRT-0995096-TX-006
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: May 17, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: VX-809:

Lot Number	Purity	Description	Date Received	Expiration Date
PT-C07091701-M09002	99.6%	White to off-white (b) (4) powder	25 May 2011 9 August 2011	(b) (4)
PT-C07091701-M09003	99.9%		29 Sep 2011 25 Oct 2011	

VRT-0995096 (Also known as M28): M28 is a major human metabolite of VX-809 (hydroxypyrrolidone-VX-809), which is not formed in animals. The VRT-0995096 test article is a 50%:49%:1% (by weight) (b) (4) mixture of VRT-0995096, hydroxypropylmethylcellulose acetate succinate (HPMC-AS), and sodium lauryl sulfate (SLS) and is a (b) (4) **Lot Number** BJL-E16681-20-A (Purity: 99.9%)

Key Study Findings

▪ In a 26-week toxicology study, Sprague-Dawley rats received VX-809 by oral gavage at doses of 250, 500, or 1000 mg/kg/day in combination with VRT-0995096 at a dose of

25 mg/kg/day. A control group received the vehicle consisting of 0.5% Tween 80 (w/v) + 0.5% methylcellulose (w/v) + 0.05% simethicone in water. At the end of the 26-week dosing period, 15 rats/sex/group were sacrificed. At the end of an additional 4-week recovery period, 5 rats/sex/group were sacrificed.

▪ There were no treatment-related deaths. Absolute body weights were unaffected by treatment for 26 weeks. There were no biologically significant changes of hematology, coagulation, or clinical chemistry parameters.

▪ No target organs of toxicity were identified. A number of low incidence findings, primarily confined to the high dose, were observed although relationships to treatment were unclear.

▪ Plasma C_{max} and AUC values for VX-809 increased with elevating doses; however, these increases were significantly less than dose proportional. Plasma C_{max} and AUC values for VRT-0995096 were comparable for Group 2, 3, and 4 at each time point suggesting that VX-809 had no effects on exposure to VRT-0995096.

▪ The MTD was judged to be greater than 1000 mg/kg/day VX-809 + 25 mg/kg/day VRT-0995096.

Methods

Doses:

Group	Test Article	Dose (mg/kg)
1	Control	0
2	VX-809	250
	VRT-0995096*	25
3	VX-809	500
	VRT-0995096*	25
4	VX-809	1000
	VRT-0995096*	25

* VRT-0995096 was administered at a dose of 25 mg/kg/day in 5 mL/kg of the vehicle.

Frequency of dosing: Daily

Route of administration: Oral gavage

Dose volume: Control, 10 mL/kg
VX-809, 5 mL/kg
VRT-0995096, 5 mL/kg

Formulation/Vehicle: Vehicle was 0.5% Tween 80 (w/v) + 0.5% methylcellulose (w/v) + 0.05% simethicone in water

Species/Strain: Albino Rats (Outbred) VAF/Plus
Sprague-Dawley derived (CD®)
Crl: CD®(SD)IGS BR
obtained from (b) (4)

Number/Sex/Group: 15 rats/sex/group – sacrificed after the 26-week treatment period

Age: Approximately 8 weeks

Weight:

	Mean	Range
Males	262.0	211.6 to 314.4
Females	211.1	181.4 to 247.5

Satellite groups: 5 rats/sex/group were included for a 4-week recovery period following the 26-week treatment period
For toxicokinetic assessments, 3 rats/sex/group were included in the control group and 9 rats/sex/group were included in each treatment group

Unique study design: Groups 2, 3, and 4 received VX-809 + VRT-0995096

Deviation from study protocol: Deviations were generally minor and did not affect the integrity of the study.

The sponsor rationalized that because VX-809 will be administered orally to humans on a daily basis, the same route and frequency of administration was used in this study. In addition, since humans will be exposed systemically to VRT-0995096 (the major metabolite of VX-809 also known as hydroxypyrrolidone-VX-809 or M28) after oral administration of VX-809, and because high systemic exposures to M28 have been

demonstrated in rats after oral administration, the oral route was considered the most appropriate route of administration for toxicology studies of this human metabolite.

Figure 5 Design of the 26-week toxicology study with VX-809 in combination with VRT-0995096 in rats

9.3.1 Study Design

The test and control articles were administered by daily oral intubation (gavage) to rats for 6-months followed by a 1-month recovery period.

Group	Test Article[a]	Daily Doses[a]			Number of Animals									
					Total on Study		Toxicity Study						TK Study[c]	
							Total		Terminal Necropsy		Recovery Necropsy		Day1 & Months 3 & 6	
		Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M	F	M	F	M	F	M	F	M	F
1	Control[b]	0	10	0	23	23	20	20	15	15	5	5	3	3
2	VX-809	250	5	50	29	29	20	20	15	15	5	5	9	9
	VRT-0995096	25	5	10										
3	VX-809	500	5	100	29	29	20	20	15	15	5	5	9	9
	VRT-0995096	25	5	10										
4	VX-809	1000	5	200	29	29	20	20	15	15	5	5	9	9
	VRT-0995096	25	5	10										

[a]Doses of VX-809 represent active ingredient. Dose Groups 2 through 4 received the VRT-0995096 test article: a (b) (4)% VRT-0995096, 49% hydroxypropylmethylcellulose acetate succinate (HPMC-AS) and 1% sodium lauryl sulfate (SLS) by weight, at a dose level of 25 mg/kg in 5 mL/kg of the vehicle (concentration = 10 mg/mL).

[b]Vehicle alone, consisting of 0.5% w/v methylcellulose (MC) + 0.5% Tween 80 and 0.05% simethecone in water.

[c]TK samples were collected on Day 1 and during Months 3 and 6.

The first day of dosing was defined as Day 1 of the study.

Observations and Results

Mortality: Animals were observed twice daily for mortality and general condition.

No deaths were attributed to the two test articles.

Three main study rats and 1 toxicokinetic rat died were found dead or sacrificed in a moribund condition. Deaths of two animals, Female #4501 (Day 42) in the high dose group and Female #3520 (Day 150) in the mid dose group, were attributed oral gavage accidents. Both animals had findings of pleural adhesions to the chest wall and diaphragm with inflammation and/or granulation tissue. Female #4501 also had findings of pleural abscess or granuloma with plant material.

The cause of death for Male #2013 in the low dose could not be determined. Histopathological findings were generally unremarkable and there were no treatment-related deaths at higher doses.

Male #2029 in the low dose toxicokinetic group was euthanized on Day 105 due to an apparent head injury. These deaths due to oral gavage errors, lack of a dose-response, or trauma were judged to be unrelated to the two test articles.

Clinical Signs: Observations for any signs of poor health or toxic or pharmacologic effects were made once daily for each toxicity animal throughout the predosing, dosing,

and recovery periods. Physical examinations were conducted on main study animals weekly during the study period.

No treatment-related related clinical signs were identified from daily examinations. During weekly physical examinations, incidences of alopecia on the limbs were slightly increased in drug-treated groups, although a dose-response was not evident and these observations probably had little or no toxicological significance.

Table 13 Findings from weekly physical examinations during the 26-week treatment and 4-week recovery periods

Clinical signs	Time	Males				Females			
	Treatment	G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4
Alopecia (limbs)	Dosing	144/7	53/4	185/13	172/11	42/2	89/6	46/3	79/6
	Recovery	2/1	4/1	11/3	8/2	0/0	4/1	4/1	2/1

Body Weights: Toxicity animals were removed from their cages and weighed once pretest, prior to dosing on Day 1, weekly during the treatment and the recovery periods, and terminally (after fasting).

Absolute body weights at week 26 relative to the control group were unaffected by treatment with VX-809.

Table 14 Body weight changes from Day 1 to Week 26

Males	**0**	**250/25**	**500/25**	**1000/25**
BW (g), Day 1	260.6	257.8	260.7	268.4
BW (g), Wk 26	716.9	667.4	704.7	711.8
BW, % of Control	**100.0**	**93.1**	**98.3**	**99.3**
Δ (g), Wk 26 - Day 1	456.3	409.6	444.0	443.4
Δ, % of Initial BW	175.1	158.9	170.3	165.2
BW Gain, % of Control	100.0	90.7	97.3	94.3
Females	**0**	**250/25**	**500/25**	**1000/25**
BW (g), Day 1	210.3	211.9	209.0	210.0
BW (g), Wk 26	401.5	388.7	398.7	383.6
BW, % of Control	**100.0**	**96.8**	**99.3**	**95.5**
Δ (g), Wk 26 - Day 1	191.2	176.8	189.7	173.6
Δ, % of Initial BW	90.9	83.4	90.8	82.7
BW Gain, % of Control	100.0	91.8	99.8	90.9

Figure 6 Body weights for male control and treatment groups

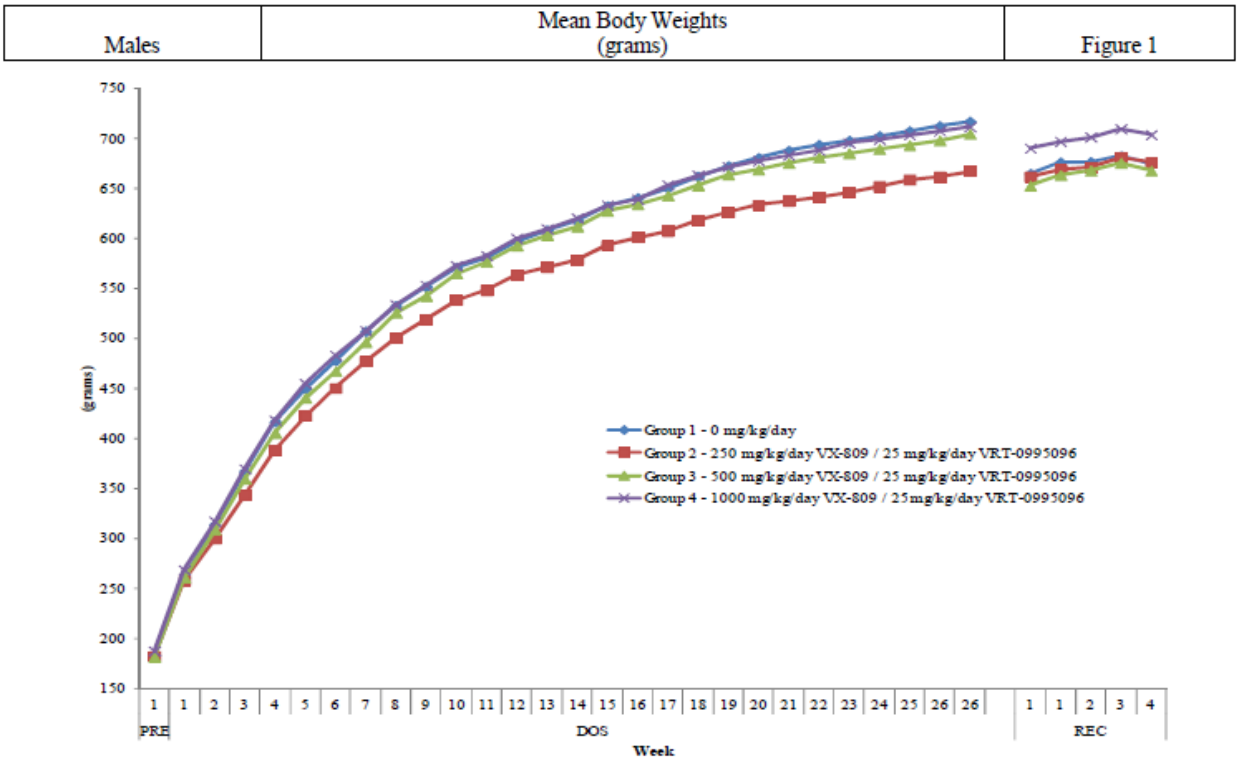
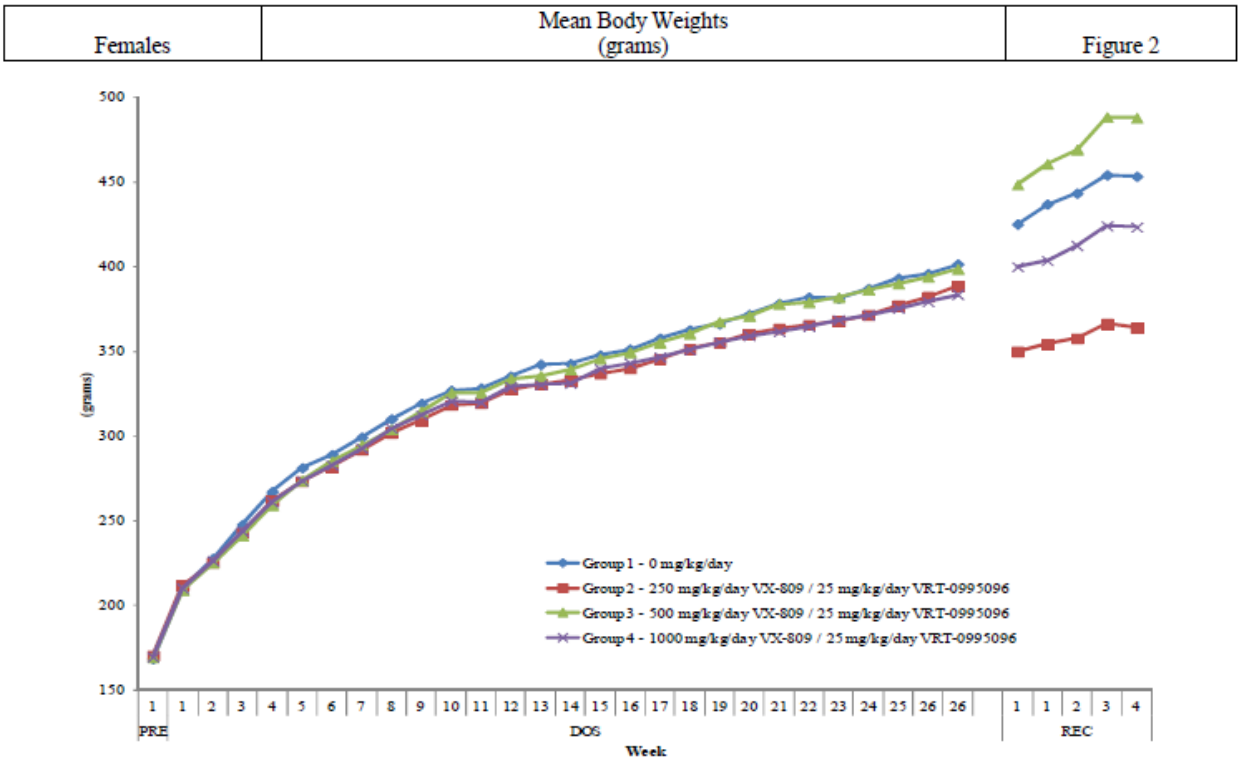


Figure 7 Body weights for female control and treatment groups



Feed Consumption: Feed was available without restriction 7 days/week. Toxicity animals were presented with full feeders of known weight. After 6 or 7 days, feeders were reweighed and the resulting weight was subtracted from the full feeder weight to obtain the grams consumed per animal over the 6 or 7-day period.

There were no treatment-related effects on food consumption.

Figure 8 Food consumption for male control and treatment groups

Males	Mean Food Consumption (grams/animal/day)	Figure 3

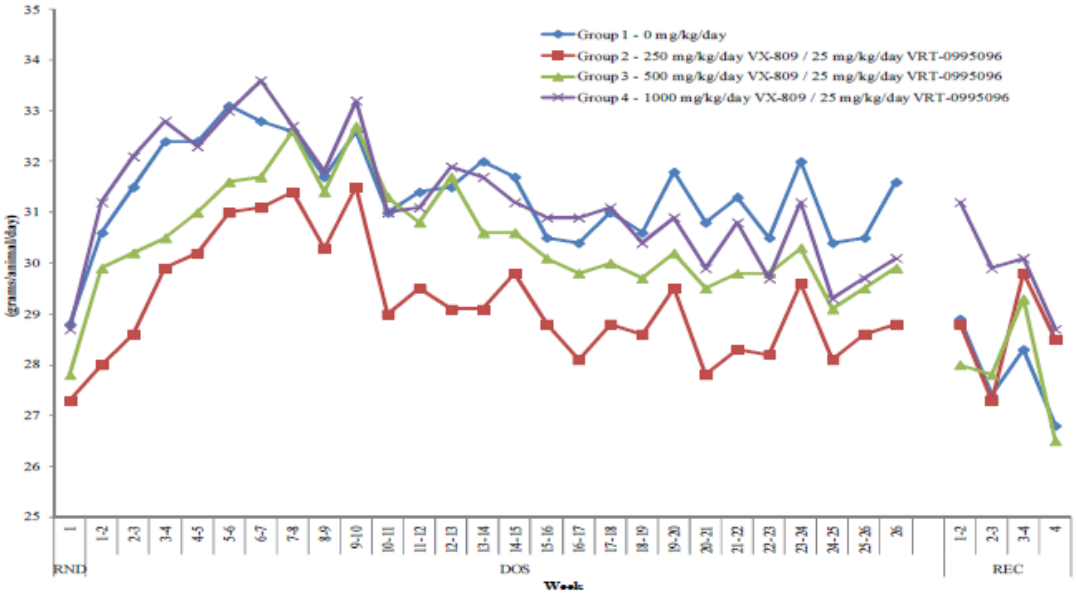
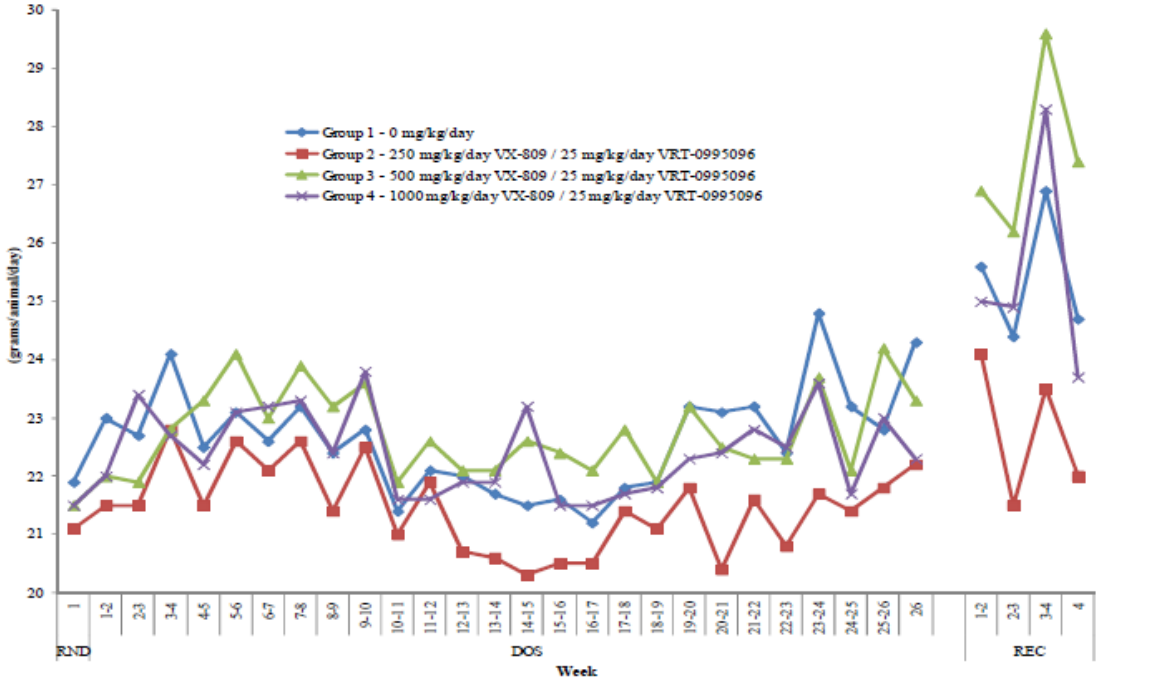


Figure 9 Food consumption for female control and treatment groups

Females	Mean Food Consumption (grams/animal/day)	Figure 4



Ophthalmoscopy: Ophthalmic examinations were conducted pretest and at the end of dosing and recovery periods.

There were no treatment-related ophthalmic effects.

Hematology: Blood samples were collected to analyze hematology and coagulation parameters for up to 15 animals/sex/group at study termination and up to 5 animals/sex/group following the 1-month recovery period.

There were no biologically significant changes of hematology and coagulation parameters.

Clinical Chemistry: Blood samples were collected to analyze clinical chemistry parameters for up to 15 animals/sex/group at study termination and up to 5 animals/sex/group following the 1-month recovery period.

At the end of the dosing period, glucose levels were slightly decreased in male and female drug-treated groups. Cholesterol levels were slightly decreased in male drug-treated groups; however, levels were slightly increased in female drug-treated groups. These changes of clinical chemistry parameters appear to have no biological significance.

Figure 10 Clinical chemistry parameters at the end of the dosing and 1-month recovery periods

Parameter	Time	Males				Females			
		G1	G2	G3	G4	G1	G2	G3	G4
Glucose mg/dL	Dosing	136	123	114*	114*	129	118*	115*	115*
Triglyceride mg/dL	Dosing	116	84*	78*	96*	102	112	128*	127*

Urinalysis: Urine samples for measurement of urinalysis parameters were collected during a 17-hour overnight collection period for up to 15 animals/sex/group at study termination and up to 5 animals/sex/group following the 1-month recovery period. Animals were fasted and but had access to water during the collection period.

There were no biologically significant changes of urinalysis parameters.

Gross Pathology: Necropsy examinations were performed on up to 15 toxicity animals/sex/group after animals had been treated for up to 26 weeks and up to 5 toxicity animals/sex/group following a 28-day recovery period.

Incidences of discolored stomach were increased for male and female drug-treated group, although there was no evidence of a dose-response relationship. These gross findings may have correlated with histopathological findings of nonglandular stomach limiting ridge: cystic epithelial – degeneration, glandular dilatation, mixed inflammatory cell infiltrate, and congestion.

Incidences of thin absent hair were increased for female drug-treated group, although this observation probably had little or no toxicological significance.

Table 15 Gross necropsy observations

Organ/Tissue	Time	Males				Females			
		G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4
N =		15	14	15	15	15	15	15	14
Stomach -discolored	Dosing	0	2	2	2	1	4	2	4
Skin (other) -hair thin absent	Dosing	1	0	0	0	0	1	1	2

Organ Weights: Absolute and relative organ weights were measured for the adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, thyroid/parathyroid glands, and uterus.

Changes of absolute and relative heart, prostate, pituitary gland, and ovary weights were observed for drug-treated groups; however, there were no corresponding histopathological changes.

Absolute and relative heart weights for males in the high dose group were increased by 24 to 29% above the control; however, there were no corresponding histopathological changes. Absolute and relative prostate weights for males in the high dose group were increased by 14 to 17.7% above the control; however, there were no corresponding histopathological changes. Absolute and relative thyroid/parathyroid weights for males in the high dose group were increased by 14 to 17.7% above the control; however, there were no corresponding histopathological changes.

Absolute and relative pituitary gland weights for females in the high dose group were decreased by 28-30% below the control; however, there were no corresponding histopathological changes. Absolute and relative ovaries weights for females in high dose group were increased by 29 to 38% above the control; however, there were no corresponding histopathological changes.

Histopathology:

Adequate Battery: An adequate panel of tissues and organs was submitted to histopathological evaluation. After fixation, the tissues and organs from all toxicity animals in Group 1 and 4 and toxicity animals euthanized in moribund condition, sacrificed for humane reasons or found dead in Groups 2 and 3 were routinely processed, embedded in paraffin, cut at a microtome setting of 4-7 microns, mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy.

Table 16 Tissues collected in the 6-month toxicology study with rats

Table 9.3.15.4-1 – Tissues Weighed, Preserved and Examined Microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY (Groups 1 and 4)[a]
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (femur)		X	
bone (sternum, distal femur)		X	X
bone marrow (sternum, distal femur)		X	X[b]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
cecum		X	X
colon		X	X
epididymides		X	X
esophagus		X	X
eyes		X	X
heart	X	X	X
kidneys	X	X	X
lacrimal glands/Harderian gland		X	X
liver	X	X	X
lungs (with mainstem bronchi)		X	X
lymph nodes (mesenteric, mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
optic nerve		X	
ovaries	X	X	X
pancreas		X	X
pituitary gland	X[c]	X	X
prostate gland	X	X	X
rectum		X	
salivary glands (submandibular)		X	X
seminal vesicles		X	X
skeletal muscle (*rectus femoris*)		X	X
skin (dorsal –base of tail)		X	X
small intestine (duodenum, ileum, jejunum and Peyer's patches/GALT)		X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY (Groups 1 and 4)[a]
spinal cord (cervical)		X	X
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X[c]	X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix	X	X	X
vagina		X	X
tissues with macroscopic findings including tissue masses		X	X

[a]Including toxicity animals euthanized in moribund condition, sacrificed for humane reasons or found dead in Groups 2 and 3.
[b]Qualitative examination (no differential count).
[c]Weighed post-fixation.

Peer Review: None.

Histological Findings: No target organs of toxicity were identified with doses up to 1000/25 mg/kg/day. A number of low incidence findings, primarily confined to the high dose, were observed although relationships to treatment were unclear.

One of 14 males in the high dose group was observed with findings in the lung of minimal necrosis with lymphohistiocytic inflammation. There were no similar findings for females in the high dose group.

Other findings would not be expected to affect animal survival.

Table 17 Microscopic observations

Organ/Tissue	Time	Males				Females			
		G-1	G-2	G-3	G-4	G-1	G-2	G-3	G-4
Lungs -necrosis with lympho-histiocytic inflammation, G1	Dosing	15 0	0 -	0 -	14 1	15 0	0 -	0 -	14 0
Adrenal glands -cortical vacuolation, G2	Dosing	15 0	0 -	0 -	14 1	15 0	0 -	0 -	14 0
Pancreas -perivascular mixed inflammatory cell infiltrate, G1	Dosing	15 0	0 -	0 -	15 0	15 0	0 -	0 -	14 1
Pancreas -acinar atrophy, G1	Dosing	15 0	0 -	0 -	15 2	15 0	0 -	0 -	14 0
Pancreas -acinar atrophy, G1	Rec	5 1	0 -	0 -	5 0	5 0	0 -	0 -	5 0
Skin -hypotricosis	Dosing	1 0	0 -	0 -	0 -	0 -	0 -	0 -	2 2
Stomach	Dosing	15	0	0	15	15	0	0	14

-nonglandular stomach, limiting ridge: cystic epithelial – degeneration, G1-2		0	-	-	2	2	-	-	3
Stomach -nonglandular stomach, limiting ridge: cystic epithelial – degeneration, G1-2	Rec	5 1	0 -	0 -	5 1	5 2	0 -	0 -	5 1
Stomach -mixed inflammatory cell infiltrate, G1	Dosing	15 0	0 -	0 -	15 1	15 2	0 -	0 -	14 1
Stomach -congestion	Dosing	15 0	0 -	0 -	15 0	15 0	0 -	0 -	14 2
Ovaries -reduced/absent corpora lutea	Dosing					15 4	0 -	0 -	14 7
Kidneys -tubular dilatation, G1	Dosing	15 0	0 -	0 -	15 0	15 0	0 -	0 -	14 1
Kidneys -tubular dilatation, G1	Rec	5 0	0 -	0 -	5 1	5 1	0 -	0 -	5 1
Heart -brown pigment, G1	Dosing	15 0	0 -	0 -	14 1	15 0	0 -	0 -	14 0
Mediastinal LN -increased plasma cells, G1-2	Dosing	15 2	0 -	0 -	15 2	14 0	0 -	0 -	14 3
Mesenteric LN -intrasinusoidal erythrocytes	Dosing	15 0	0 -	0 -	14 1	15 0	0 -	0 -	14 0

Special Evaluation: None

Toxicokinetics: On Day 1 and during Month 3, blood samples for measurement of plasma drug concentrations were collected from 3 animals/sex/time point from each drug-treated group at 0.5, 1, 2, 4, 10, and 24 hours after dosing and during Month 6 from 3 animals/sex/time point from each drug-treated group at group 0.5, 1, 2, 4, 10, 24, 48, and 72 hours after dosing. Each TK animal in Groups 2 through 4 was bled on 6-7 occasions during the entire 6-month dosing period. At the 6-month collection, TK animals used for collection of the 72-hour TK samples from Groups 2 to 4, as well as TK animals from Group 1, were transferred to necropsy, where blood was collected for biomarker analysis (under deep anesthesia, using isoflurane). Following blood collection, these TK animals were euthanized (by exsanguination), followed by final tissue (heart, liver, and lung) collection.

Plasma C_{max} and AUC values for VX-809 increased with elevating doses; however, these increases were significantly less than dose proportional. C_{max} and AUC values for VX-809 on days 90 and 180 were generally greater than values on day 1. C_{max} and AUC values for VX-809 on day 180 were generally greater than values on day 90. There was drug accumulation in the process to achieve steady state levels. C_{max} and AUC values for VX-809 were generally greater in female rats than male rats.

Plasma C_{max} and AUC values for VRT-0995096 were comparable for Group 2, 3, and 4 at each time point suggesting that VX-809 had no effects on exposure to VRT-0995096. C_{max} and AUC values for VRT-0995096 on days 90 and 180 were generally greater than

values on day 1; however, values on days 90 and 180 were relatively comparable. C_{max} and AUC values for VRT-0995096 were generally greater in female rats than male rats.

Table 18 Toxicokinetic parameters for VX-809 in male and female rats on days 1, 90, and 180

Table 10.2-1 Summary of Selected Plasma Toxicokinetic Parameters of VX-809 in Female and Male Rats on Day 1, Day 90 and Day 180

Study Day	Group	VX-809 (mg/kg/day)[a]	Sex	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	$DN\_AUC_{0\text{-}24hr}$[b] (µg*hr/mL)	C_{last} (µg/mL)	t_{last} (hr)
Day 1	2	250	Female	86.8	10.0	1350	5.41	6.00	24.00
	2	250	Male	74.2	10.0	1080	4.33	4.33	24.00
	3	500	Female	133	10.0	2010	4.03	8.08	24.00
	3	500	Male	60.3	4.00	1000	2.01	9.21	24.00
	4	1000	Female	130	10.0	2170	2.17	29.3	24.00
	4	1000	Male	92.1	10.0	1510	1.51	14.5	24.00
Day 90	2	250	Female	143	4.00	1520	6.09	16.4	24.00
	2	250	Male	73.9	4.00	853	3.41	10.4	24.00
	3	500	Female	138	2.00	1660	3.31	24.1	24.00
	3	500	Male	66.8	4.00	881	1.76	10.6	24.00
	4	1000	Female	164	4.00	2010	2.01	44.1	24.00
	4	1000	Male	93.2	4.00	1140	1.14	11.6	24.00
Day 180	2	250	Female	154	2.00	2150	8.59	0.711	72.00
	2	250	Male	80.9	4.00	943	3.77	0.152	72.00
	3	500	Female	200	4.00	2500	4.99	0.605	72.00
	3	500	Male	80.3	4.00	973	1.95	0.375	72.00
	4	1000	Female	189	2.00	3160	3.16	0.787	72.00
	4	1000	Male	117	4.00	1300	1.30	0.217	72.00

Source: Vertex TK Report I019.

[a]All doses of VX-809 were co-administered 25 mg/kg/day of VRT-0995096

[b]Please note that $DN\_AUC_{0\text{-}24hr}$ is the $AUC_{0\text{-}24hr}$ (µg*hr/mL) normalized by dose (mg/kg/day).

Table 19 Toxicokinetic parameters for VRT-0995096 (M28) in male and female rats on days 1, 90, and 180

Table 10.2-2 Summary of Selected Plasma Toxicokinetic Parameters of VRT-0995096 in Female and Male Rats on Day 1, Day 90 and Day 180

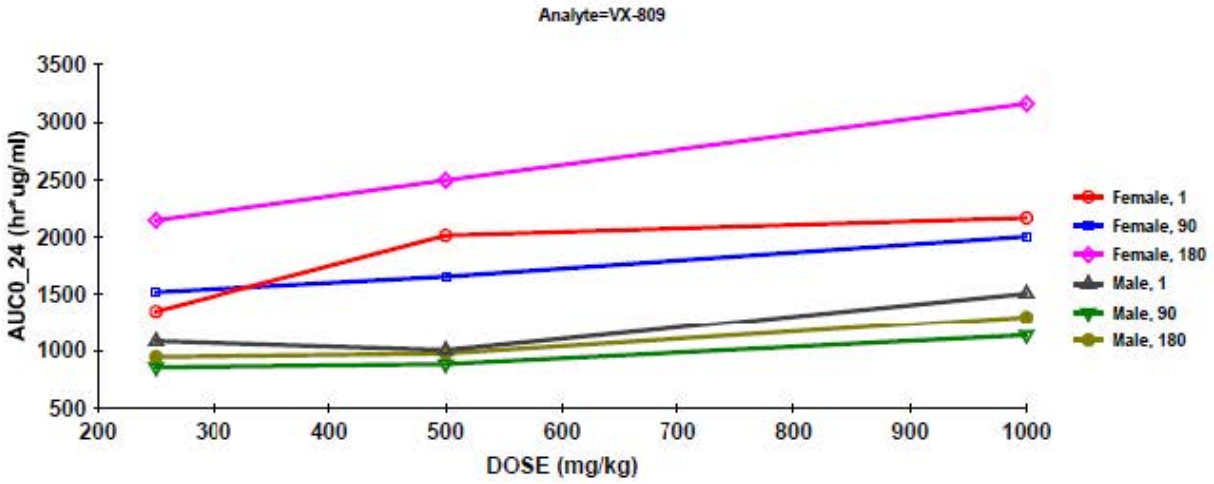
Study Day	Group	VRT-0995096 (mg/kg/day)[a]	Sex	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	$DN\_AUC_{0\text{-}24hr}$[b] (µg*hr/mL)	C_{last} (µg/mL)	t_{last} (hr)
Day 1	2	25	Female	40.7	1.00	586	NA	4.41	24.0
	2	25	Male	21.4	2.00	304	NA	2.32	24.0
	3	25	Female	34.2	0.50	504	NA	5.57	24.0
	3	25	Male	24.3	1.00	334	NA	2.64	24.0
	4	25	Female	31.2	1.00	546	NA	8.12	24.0
	4	25	Male	28.0	1.00	323	NA	3.35	24.0
Day 90	2	25	Female	129	1.00	1220	NA	12.6	24.0
	2	25	Male	74.6	0.50	727	NA	8.95	24.0
	3	25	Female	139	0.50	1370	NA	23.3	24.0
	3	25	Male	84.3	1.00	733	NA	8.44	24.0
	4	25	Female	113	0.50	1100	NA	17.9	24.0
	4	25	Male	70.6	0.50	712	NA	10.2	24.0
Day 180	2	25	Female	125	0.50	1110	NA	3.21	72.0
	2	25	Male	92.0	0.50	765	NA	0.308	72.0
	3	25	Female	139	0.50	1200	NA	2.29	72.0
	3	25	Male	83.3	1.00	621	NA	0.650	72.0
	4	25	Female	116	0.50	1180	NA	0.999	72.0
	4	25	Male	104	0.50	751	NA	0.461	72.0

Source: Vertex TK Report I019.
[a]All doses of VRT-0995096 were co-administered with 250, 500, or 1000 mg/kg/day VX-809.
[b]Please note that $DN\_AUC_{0\text{-}24hr}$ is the $AUC_{0\text{-}24hr}$ (µg*hr/mL) normalized by dose (mg/kg/day).
NA: not applicable.

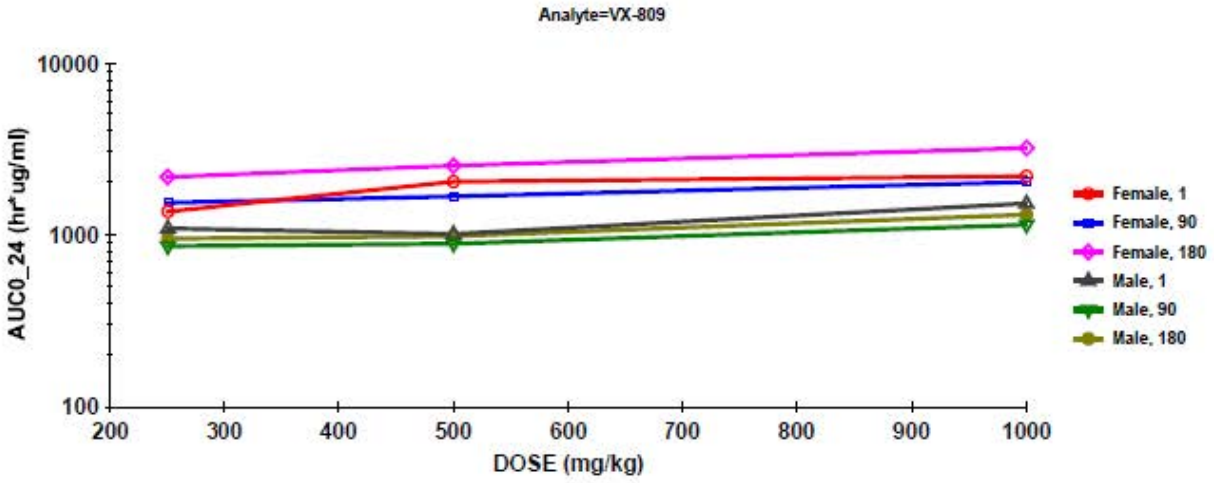
Figure 11 Relationship of VX-809 AUC to the Administered Dose of VX-809 in Male and Female Rats on Days 1, 90, and 180

Figure 13 Relationship of $AUC_{0\text{-}24hr}$ of VX-809 versus Administered Doses of VX-809 in Male and Female Rats on Day 1, 90, and 180

A) Rectilinear Plot



B) Semi-log plot

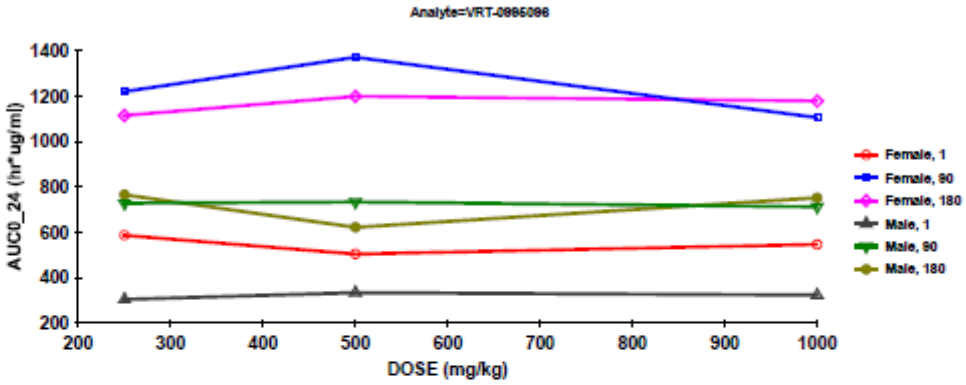


Source: Table 1

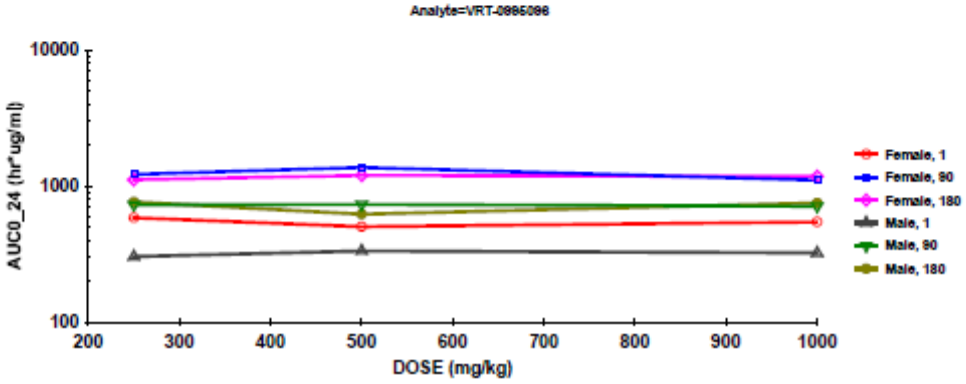
Figure 12 Relationship of VRT-0995096 AUC to the Administered Dose of VX-809 in Male and Female Rats on Days 1, 90, and 180

Figure 14 Relationship of $AUC_{0\text{-}24hr}$ of VRT-0995096 versus Administered Doses of VX-809 in Male and Female Rats on Day 1, 90, and 180

A) Rectilinear Plot



B) Semi-log plot



Dosing Formulation Analysis:

Homogeneity Analysis: On Day 1 of dosing, batches of the dose formulations for Groups 2 to 4 were prepared. Duplicate (4.0 mL each) samples each from the top, middle and bottom portion of each mixture were taken for analysis.

Dose Confirmation Analysis: Duplicate (4.0 mL each) samples from the middle portion the formulations prepared for Groups 2 to 4 were taken from Months 3 and 6 toxicokinetic sampling day preparations. Results from the middle homogeneity samples on Day 1 were used for Day 1 concentration verification. On Day 1 and Months 3 and 6, duplicate vehicle control samples (2.5 mL each) were analyzed to verify that no cross contamination occurred.

Homogeneity analyses on Day 1 of the study demonstrated that the mixing procedure produced homogenous dose formulations for both VX-809 and VRT-0995096. The concentrations of the 2 samples at each of the 3 levels were within ± 15% of each other and the mean concentration of each level were within ± 15% of each other. Dose

concentration analyses performed on Day 1, Months 3 and 6 confirmed that dosing formulations of the appropriate concentration were administered throughout the study. The concentrations of duplicate samples were within ± 15% of each other and the mean concentrations were within ± 15% of the nominal concentrations.

Study title: <u>VRT-0995096: A 28-DAY ORAL (GAVAGE) TOXICITY AND TOXICOKINETIC STUDY IN RATS</u>

Study no.:	VRT-0995096-TX-003
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 25, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	The test article is a 50%:49%:1% (by weight) (b) (4) mixture of VRT-0995096 and hydroxypropyl-methylcellulose acid succinate (HPMC-AS) and sodium lauryl sulfate (SLS). Lot numbers A2851-288 and A3379-041 (Purity, 100%) The (b) (4) (b) (4) powder was formulated as a suspension in the vehicle.

Key Study Findings

• In a 28-day toxicology study, rats received VRT-0995096 (M28) at doses of 0, 25, 50, and 100 mg/kg/day.

• No dose-limiting toxicity or target organs of toxicity were identified in rats that received doses up to 100 mg/kg/day for 28 days.

• AUC values for males and females at 100 mg/kg/day on day 28 were 1800 and 2910 µg*hr/mL, respectively.

Methods

Doses:	0, 25, 50, and 100 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose, 0.5% (w/v) Tween-80, and 0.05% (w/v) simethicone in water
Species/Strain:	Albino Rats (Outbred) VAF/Plus® Sprague-Dawley derived (CD®) Crl: CD®(SD)IGS BR
	Obtained from (b) (4)
Number/Sex/Group:	10 rats/sex/group
Age:	Approximately 8 weeks

Weight:

	Mean	**Range**
Males	316.5	259.7 to 357.2
Females	232.7	203.9 to 262.1

Satellite groups:	For toxicokinetic assessments, an additional 6 rats/sex/group were in the control group and 10 rats/sex/group were included in each drug-treated group
Unique study design:	None
Deviation from study protocol:	Deviations were generally minor and did not affect the integrity of the study.

Table 20 Design of 1-month toxicology study with VRT-0995096 (M28) in rats

The test and control article was administered orally, by gavage, to rats for 28 consecutive days.

	Daily Doses[a]			Number of Animals							
				Total on Study		Toxicity Study				TK Study[d]	
						Total Main		Terminal Necropsy		Days 1, 8 and 28	
Group	Dose (mg/kg)	Volume (mL/kg)	Conc.[b] (mg/mL)	M	F	M	F	M	F	M	F
1	0	5	20[c]	16	16	10	10	10	10	6	6
2	25	5	10	21	21	10	10	10	10	11	11
3	50	5	20	21	21	10	10	10	10	11	11
4	100	5	40	21	21	10	10	10	10	11	11

[a]Test article (TA) was a 50%/49%/1% (by weight) solid dispersion mixture of VRT-0995096 and hydroxypropylmethylcellulose acid succinate (HPMC-AS) and sodium lauryl sulfate (SLS). The [(b) (4)] powder was formulated as a suspension in a vehicle consisting of 0.5% w/v methylcellulose (MC) + 0.5% Tween-80 and 0.05% simethicone in water.

[b]VRT-0995096 concentrations were ½ of the TA concentration listed here.

[c]Control article (HPMC-AS-HF) was added to the vehicle at the highest concentration level used in the TA.

[d]Toxicokinetic samples were collected on Days 1, 8 (24 hours post-Day 7) and 28.

The first day of dosing was designated as Day 1 of the study.

Observations and Results

Mortality: All animals were observed in their cages for mortality and general condition twice daily.

There were no deaths in the study.

Clinical Signs: During the dosing period, observations for signs of toxic or pharmacologic effects were made twice daily. Toxicity animals were removed from their cages and submitted to physical examinations twice pretest and weekly during the study period.

There were no treatment-related clinical signs.

Body Weights: Non-fasted body weights for toxicity animals were recorded twice pretest, once weekly during the dosing period and terminally after fasting. Terminal fasted body weights were obtained just prior to necropsy.

Body weight gain was slightly decreased for females in the 100 mg/kg/day group.

Table 21 Body weight gains during the 28-day dosing period

BW Parameters	Males			
	0	25	50	100
BW (g), Rnd1	316.5	317.6	314.8	309.1
BW (g), Week 4	445.6	457.4	445.9	437.3
Δ (g)	129.1	139.8	131.1	128.2
%Initial BW	40.79	44.02	41.65	41.48
%Control	100.00	107.91	102.10	101.68
BW Parameters	Females			
	0	25	50	100
BW (g), Rnd1	235.3	232.2	230	236.3
BW (g), Week 4	284.7	282.5	284.1	280.1
Δ (g)	49.4	50.3	54.1	43.8
%Initial BW	20.99	21.66	23.52	18.54
%Control	100.00	103.18	112.04	88.29

Figure 13 Body weights for male rats during the 28-day dosing period

Males	Mean Body Weights (grams)	Figure 1

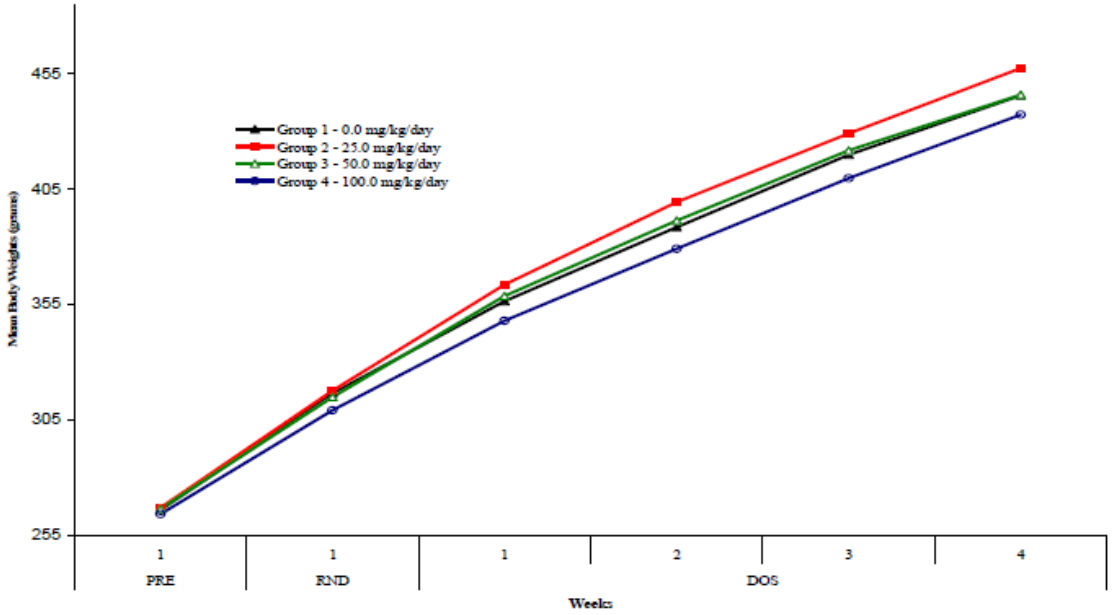
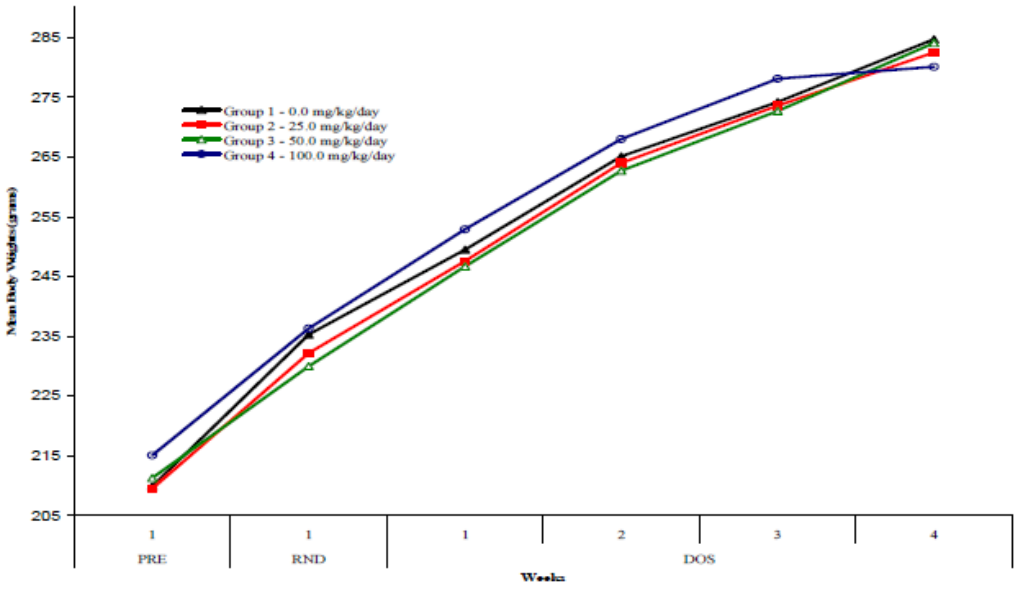


Figure 14 Body weights for female rats during the 28-day dosing period

Females	Mean Body Weights (grams)	Figure 2



Feed Consumption: Feed was available without restriction 7 days/week. Animals were presented with full feeders of known weight. After 5 or 7 days, feeders were reweighed and the resulting weight was subtracted from the full feeder weight to obtain the grams

consumed per animal over the 5 or 7-day period. Food consumption was measured (weighed) for all toxicity study animals once pretest and weekly during the dosing phase.

There were no treatment-related effects on food consumption.

Figure 15 Food consumption for male rats during the 28-day dosing period

Males	Mean Food Consumption (grams/day)	Figure 3

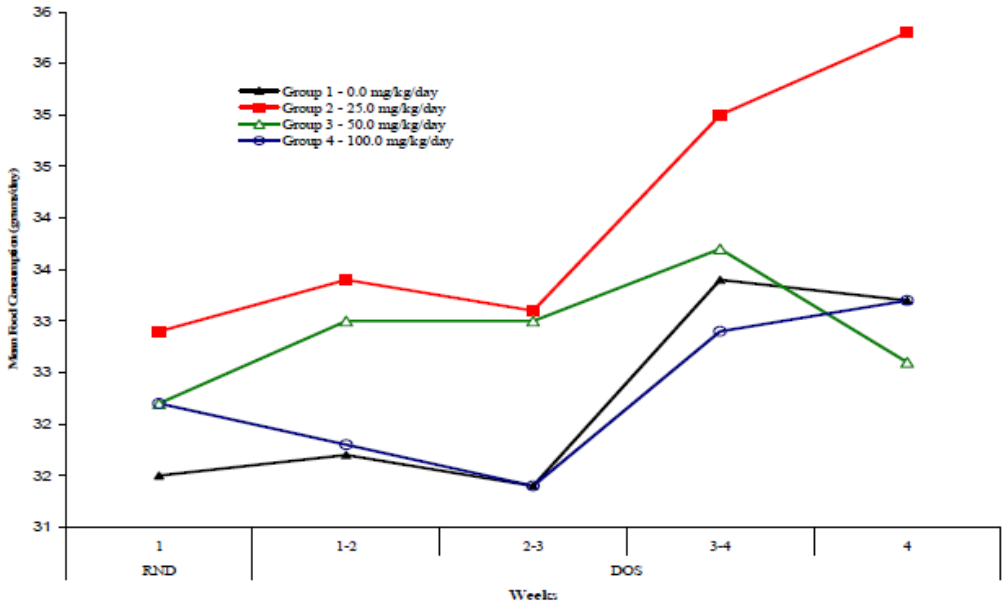
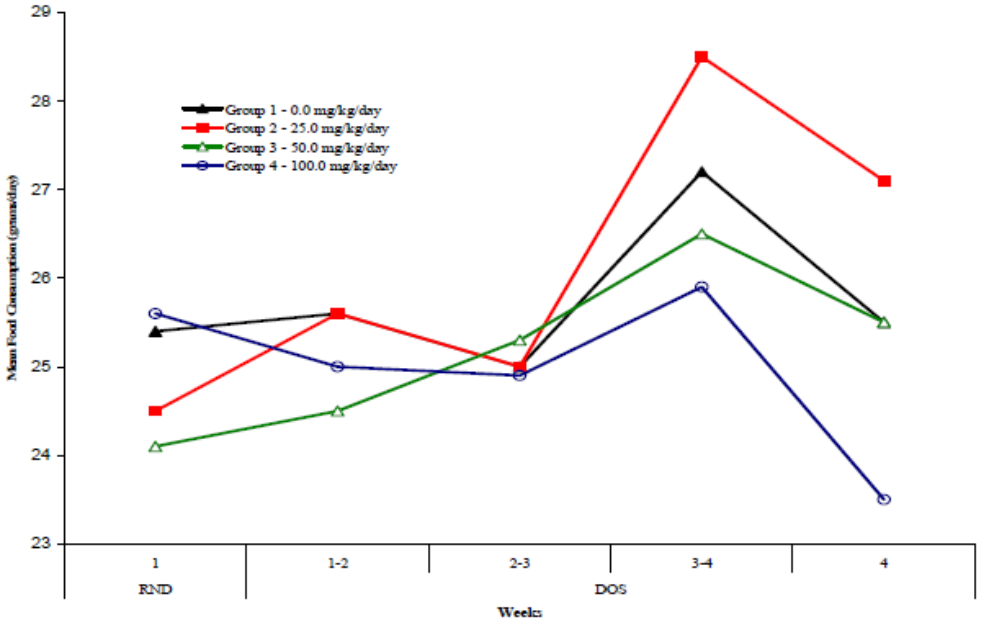


Figure 16 Food consumption for female rats during the 28-day dosing period

Females	Mean Food Consumption (grams/day)	Figure 4



Ophthalmoscopy: All animals were submitted to ophthalmic examinations pretest and toxicity animals at study termination.

There were no treatment-related ophthalmic findings.

Hematology: Blood obtained at terminal necropsy via the aorta/vena cava under isoflurane anesthesia was used to analyze a complete panel of hematology and coagulation parameters for 10 toxicity animals/sex/group. Animals were fasted overnight prior to the blood collection.

A number of small, statistically significant changes of hematology parameters were observed for males and females in the high dose group; however, there biologically significance was questionable.

Table 22 Changes of hematology parameters at termination

Parameter	Males				Females			
	0	25	50	100	0	25	50	100
Reticulocytes x 10^9/L	199.8	226.1	207.0	**253.9***	159.1	167.4	158.6	**177.2**
Platelets x 10^3/L	1014	1029	1056	**1139***	1035	1042	1061	1066
MPV fL	7.3	6.8	7.0	**7.8***	7.5	7.0	7.3	**8.1***
RDW %	12.4	12.9	13.0	**13.9***	11.5	11.7	**12.1***	**12.2***
WBC x 10^3/L	11.28	12.95	11.15	**9.96**	NC	NC	NC	NC
Neutrophils x 10^3/L	1.71	1.30	1.87	**1.30**	NC	NC	NC	NC
Lymphocytes x 10^3/L	9.02	11.09	8.69	8.19	NC	NC	NC	NC

*Achieved statistical significance
NC = No change

Clinical Chemistry: Blood obtained at terminal necropsy via the aorta/vena cava under isoflurane anesthesia was used to analyze a complete panel of chemistry parameters for 10 toxicity animals/sex/group. Animals were fasted overnight prior to the blood collection.

AST activity was increased for males in the high dose group (males #4002, 4004, and 4006 had values of 177, 211, and 161 U/L, respectively). Potassium levels were increased for male drug treated groups. The biologically significance of these changes was unclear. There were no corresponding histopathological findings to these changes of clinical chemistry parameters.

Table 23 Changes of clinical chemistry parameters at termination

Parameter	Males				Females			
	0	25	50	100	0	25	50	100
AST U/L	92	113	112	127*	NC	NC	NC	NC
K+ mEq/L	4.8	5.0	5.1*	5.0*	NC	NC	NC	NC

*Achieved statistical significance

NC = No change

Urinalysis: Urine obtained via a 16-hour overnight collection period, was analyzed for 10 toxicity animals/sex/group at study termination. Animals were fasted but had access to water during the collection period.

Finding of white blood cells in the urinary sediment were increased for males and females at 50 and 100 mg/kg/day. The significance of this finding was unclear as there were no treatment-related histopathological findings in the kidneys.

Changes of urinalysis parameters at termination

Parameter	Males				Females			
	0	25	50	100	0	25	50	100
WBC	7 at 0 3 at 1+	5 at 0 4 at 1+ 1 at 2+	2 at 0 8 at 1+	1 at 0 9 at 1+	8 at 0 2 at 1+	9 at 0 1 at 1+	2 at 0 7 at 1+ 1 at 2+	4 at 0 6 at 1+

Gross Pathology: Necropsy was performed on 10 toxicity animals/sex/group after animals had been dosed for 28 consecutive days. Animals were fasted overnight prior to necropsy.

Gross pathological changes were evident for the liver, lungs, and spleen; however, there were no corresponding treatment-related histopathological changes.

Table 24 Gross necropsy changes

Organs/Tissues	Males				Females			
	0	25	50	100	0	25	50	100
Liver	10	10	10	10	10	10	10	10
-discolored	0	0	0	1	0	0	0	0
Lungs	10	10	10	10	10	10	10	10
-discolored	0	0	0	1	0	0	0	0
-adhesion	0	0	0	1	0	0	0	0
-small	0	0	0	1	0	0	0	0
Spleen	10	10	10	10	10	10	10	10
-irregular surface	0	0	0	0	0	0	0	1

Organ Weights:
Absolute and relative organ weights were measured for the adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, seminal vesicles, spleen, testes, thymus, thyroids/parathyroids, and uterus.

Changes of absolute and relative pituitary gland, kidney, and liver weights were evident; however, there were no corresponding treatment-related histopathological changes.

Absolute and relative pituitary gland weights were increased by 10.6 to 13.1% for males in the 100 mg/kg/day group. Absolute and relative kidney weights were increased by 9.2

to 11.3% for females in the 100 mg/kg/day group. Absolute and relative liver weights were increased by 3 to 5% for females in the 100 mg/kg/day group.

Histopathology:

Adequate Battery: An adequate panel of tissues and organs was submitted to histopathological examination. After fixation, the tissues and organs from all animals were routinely processed, embedded in paraffin, cut at a microtome setting of 4-7 microns, mounted on glass slides, stained with hematoxylin and eosin and examined by light microscopy. The bones were decalcified in Decalcifier II.

Table 9.3.15.4-1 – Tissues weighed, preserved and examined microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY Groups 1 and 4
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (femur)		X	
bone (sternum, distal femur)		X	X
bone marrow (sternum, distal femur)		X	X[a]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
Harderian gland		X	X
heart	X	X	X
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum[b])		X	X
liver	X	X	X
lungs (with mainstem bronchi)		X	X
lymph nodes (mesenteric and mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
optic nerve		X	
ovaries	X	X	X
pancreas		X	X
pituitary gland	X[c]	X	X
prostate gland	X	X	X
salivary glands (submandibular)		X	X
seminal vesicles	X	X	X
skeletal muscle (*Rectus femoris*)		X	X
skin (dorsal – base of tail)		X	X
small intestine (duodenum, ileum, jejunum and Peyer's patches/GALT)		X	X
spinal cord (cervical)		X	X
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X[c]	X	X
trachea		X	X
urinary bladder		X	X
uterus (horns/body/cervix)	X	X	X
vagina		X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY Groups 1 and 4
tissues with macroscopic findings including tissue masses		X	X

[a]Qualitative examination (no differential count).
[b]Not examined.
[c]Weighed post-fixation

Peer Review: None

Histological Findings: There were no target organs of toxicity. A number of minimal findings were observed at low incidences for males and females in the high dose group.

Table 25 Histopathological changes at the end of the 28-day dosing period

Organ/Tissue	Males				Females			
	0	25	50	100	0	25	50	100
Liver	10	0	0	10	10	0	0	10
-centrilobular hepatocellular hypertrophy, G1	0	-	-	1	0	-	-	0
-fatty vacuolation, G1	0	-	-	1	0	-	-	0
-extramedullary hematopoiesis, G1	0	-	-	1	0	-	-	0
-tension lipidosis, G1	0	-	-	1	0	-	-	0
Kidneys	10	0	0	10	10	0	0	10
-subacute/chronic inflammatory cell infiltrate, G1	0	-	-	0	0	-	-	1
-tubule: eosinophilic material, G1	0	-	-	1	0	-	-	0
Lungs	10	0	0	10	10	0	0	10
-perivascular mixed inflammatory cell infiltrate, G1	1	-	-	1	1	-	-	3
Spleen	10	0	0	10	10	0	0	10
-increased extramedullary hematopoiesis, G1	0	-	-	1	0	-	-	0

Special Evaluation: On Day 8, an additional 5 toxicokinetic animals/sex used for the 24-hour time point (post-Day 7 dose) in Groups 2, 3 and 4 and 3 animals/sex in Group 1 were transferred to necropsy at approximately 24 hours postdose and liver samples were collected for cytochrome P450 analyses.

All the 72-hour TK animals (up to 3/sex/group, Groups 2-4) and the control TK animals (up to 3/sex, Group 1) were transferred to necropsy and sacrificed on Day 30 (after the last scheduled 72-hour TK blood collection for Groups 2 to 4); blood was collected and the targeted organs (heart, liver, lung harvested as soon as possible for possible biomarker analyses or determination of tissue concentrations of VRT-0995096.

No results were provided in the study report.

Toxicokinetics: Blood was collected for measurement of plasma drug concentrations at the time points listed in the table below. Plasma samples were shipped to the sponsor for analysis of drug concentrations. Bioanalytical samples were analyzed with a validated liquid chromatographic-triple quadrupole mass spectrometric (LC-MS/MS) assay at the Sponsor-designated site.

Table 26 Blood collection time points for measurement of plasma drug concentrations

Table 9.3.14-1 – Collection Times and Number of Animals

No. of Animals	Interval/Time points
Day 1	
3 animals/ sex/group/timepoint	0.5, 1, 2, 4, 8 and 24 hrs post-dose
Day 8	
3 animals/sex/Group1 5 animals/ sex/Groups 2-4	24 hours post Day 7 dose
Day 28	
3 animals/ sex/group/timepoint	0, 1, 2, 4, 8, 24, 48 and 72 hrs post-dose

Blood samples were collected from Group 1 animals at 1 time point (4 hours) on Days 1 and 28, which is near the t_{max} for VRT-0995096 in rats.

AUC values for VRT-099509 increased in a dose proportional manner for females on days 1 and 28 and males on day 1; however, AUC values for males on day 28 increased in a less than dose proportional manner. C_{max} values increased in a less than dose proportional manner for females on days 1 and 28 and males on day 28. C_{max} values increased in a dose proportional manner for males on day 1. Cmax and AUC values were relatively comparable for males and females at the low dose of 25 mg/kg/day; however, C_{max} and AUC values were generally greater for females at the mid and high doses of 50 and 100 mg/kg/day, respectively.

Table 10.2-1. Summary of Toxicokinetic Parameters for VRT-0995096 in Female and Male Sprague-Dawley Rats Following Once Daily Oral Administration of 25, 50 and 100 mg/kg/day of VRT-0995096 on Day 1 and Day 28

Study Day	Dose of VRT-099509 6 (mg/kg)	Sex							
		Female				Male			
		C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)
Day 1	25	57.7	1.00	571	6.97	32.5	8.00	535	5.26
Day 1	50	167	1.00	1480	5.61	70.5	2.00	1030	5.29
Day 1	100	174	8.00	2830	5.61	139	2.00	2080	4.07
Day 28	25	78.7	1.00	738	11.1	58.4	1.00	868	8.75
Day 28	50	109	1.00	1440	10.6	112	1.00	1200	9.14
Day 28	100	202	1.00	2910	10.6	121	1.00	1800	8.96

Source: PKS Study: CF-VRT-0995096-TX-003

Dosing Formulation Analysis: Dosing solutions were analyzed for homogeneity, stability, and concentration. On Day 1 of the study, batches of low- and high-concentration dose formulations were prepared and three samples (1.5 mL each) were

collected from the top, middle and bottom portion of each mixture were taken for analysis. Duplicate samples of the low- and high-concentration dose solutions were assayed after preparation at 5 hr (room temperature) and 24 hr (refrigerated). All 4 dose levels were assayed on Days 2, 8, and 29 (to coincide with the day of toxicokinetic sampling). Duplicate samples (1.5 mL each) were taken from the middle region of each formulation on the day of dose preparation.

The concentrations of all 3 samples at each of the 3 levels (top, middle, bottom) were within ±10% of each other, and the mean concentration of each level were within ±15% of the nominal concentration. Refrigerated (24 hr) and 5-hr room temperature stability at the concentrations used in this study were also confirmed.

7 Genetic Toxicology

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

Study title: VRT-0995096: Bacterial Reverse Mutation Assay

Study no.:	VRT-0995096-TX-001
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 23, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test Article Lot No.: E17512-020 Test Article Purity: 98.54% (per Pre-study Certificate of Analysis) and 98.20% (per Post-study Certificate of Analysis)

Key Study Findings

▪ VRT-0995096 (M28) at doses up to 5000 µg/plate in the presence and absence of S9 metabolic activation showed no evidence of mutagenicity activity with *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A.

Methods

Strains: *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A

Concentrations in definitive study: The doses tested were 15, 50, 150, 500, 1500 and 5000 µg/plate with all *Salmonella* tester strains and 50, 150, 500, 1500 and 5000 µg/plate with tester strain WP2 *uvr*A. Due to unacceptable vehicle control values, tester strain TA1537 was not evaluated for mutagenicity, but was retested. In the retest of the confirmatory mutagenicity assay, the doses tested were 15, 50, 150, 500, 1500 and 5000 µg/plate.

Basis of concentration selection: An initial toxicity-mutation assay was conducted with doses of 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate. Toxicity was generally evident for all tester strains at 1500 and 5000 µg/plate based upon thinning of the bacterial lawn and/or reductions of the revertant colony counts.

Negative control: DMSO

Positive control:

Table 3.0-1

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535, TA1537	Rat	2-aminoanthracene (Aldrich Chemical Co., Inc.) Lot No. 03403ED Exp. Date 22-Jun-2012 CAS No. 613-13-8 Purity 99.8%	1.0
TA100			2.0
WP2 *uvr*A			10
TA98	None	2-nitrofluorene (Aldrich Chemical Co., Inc.) Lot No.03319JD Exp. Date 28-Feb-2011 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide (Sigma Aldrich Chemical Co.) Lot No. 71980 Exp. Date 28-Dec-2010 CAS No. 26628-22-8 Purity 99.8%	1.0
TA1537		9-aminoacridine (Sigma Chemical Co.) Lot No. 106F06682 Exp. Date 30-Oct-2010 CAS No. 90-45-9 Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (Sigma Aldrich Chemical Co.) Lot No. 76296KJ Exp. Date 02-Jun-2012 CAS No. 66-27-3 Purity 99.8%	1,000

Formulation/Vehicle: DMSO

Incubation & sampling time: The plate incorporation method was used for an initial toxicity-mutation assay and a subsequent confirmatory mutagenicity assay. Assays were conducted in the presence and absence of S9 metabolic activation. The S9 was prepared by and purchased from (b) (4) The S9 mix was prepared immediately before its use and contained 10% S9, 5 mM glucose-6-phosphate, 4 mM β-nicotinamide-adenine dinucleotide phosphate, 8 mM MgCl2 and 33 mM KCl in a 100 mM phosphate buffer

at pH 7.4. All dose levels of test article, vehicle control and positive controls were plated in triplicate. The plates were incubated for approximately 48 to 72 hr at 37°C. Plates were counted manually or with an automated colony counter.

Criteria for a positive response: For a test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article as specified. For strains TA1535 and TA1537, data sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-times the mean vehicle control value. For strains TA98, TA100 and *WP2 uvrA, d*ata sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

Study Validity: The sponsor's bacterial tester strain selection was adequate per the ICH S2A(R1) Guidance. Positive controls were observed with expected increases of revertant colony counts. The limit dose of 5000 µg/plate was used in both the initial and confirmatory assays. Toxicity was evident with the test article at 1500 and/or 5000 µg/plate based upon findings of bacterial lawn thinning or reductions of revertant colony counts.

Results: VRT-0995096 (M28) at doses up to 5000 µg/plate in the presence and absence of S9 metabolic activation showed no evidence of mutagenic activity with *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: VRT-0995096: In Vitro Mammalian Chromosome Aberration Test

Study no.:	VRT-0995096-TX-002
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 15, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test Article Lot Number: E17512-020 Test Article Purity: 98.54% (per Pre-study Certificate of Analysis) and 98.20% (per Post-study Certificate of Analysis)

Key Study Findings

▪ CHO cells treated with the test article for 4 or 20 hr in the absence of S9 metabolic activation or 4 hr in the presence of S9 metabolic activation did not produce any significant changes of the frequency of structural aberrations. Thus, VRT-0995096 was negative in the *in vitro* CHO cell chromosomal aberration assay.

Methods

Cell line: Chinese hamster ovary (CHO-K1) cells (repository number CCL 61) were obtained from (b) (4) In order to assure the karyotypic stability of the cell line, working cell stocks were not used beyond passage 20. The frozen lot of cells was tested using the Hoechst staining procedure and found to be free of mycoplasma contamination. This cell line has an average cell cycle time of 10-14 hours with a modal chromosome number of 20.

Concentrations in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	50, 100, 150, 200, 250, 350, 500
	20 hr	0 hr	25, 50, 75, 100, 150, 175
S9-activated	4 hr	16 hr	10, 25, 50, 100, 150, 200

Basis of concentration selection: Dose levels for the chromosome aberration assay were selected following a preliminary toxicity test and were based upon a reduction of cell growth (cell growth inhibition) relative to the solvent control. CHO cells were exposed to solvent alone and to nine concentrations of test article ranging from 0.5 to 5000 µg/mL in the absence and presence of an S9 reaction mixture. Visible precipitate was observed in the treatment medium at doses ≥1500

µg/mL and dose levels ≤500 µg/mL were soluble in the treatment medium at the beginning of the treatment period. At the conclusion of the treatment period, visible precipitate was observed in the treatment medium at 5000 µg/mL and dose levels ≤1500 µg/mL were soluble in the treatment medium in all three treatment group.

For the 4-hr incubation in the absence of S9, 37% inhibition of cell growth was observed at 150 µg/mL and ≥75% inhibition was observed at concentrations ≥500 µg/mL. For the 4-hr incubation in the presence of S9, 51% inhibition of cell growth was observed at 50 µg/mL and 59% inhibition at 150 µg/mL. For the 20-hr incubation in the absence of S9, 30% inhibition of cell growth was observed at 50 µg/mL and ≥67% inhibition was observed at concentrations ≥150 µg/mL.

Negative control: DMSO

Positive control: MMC was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2 µg/mL. CP was used as the positive control in the S9-activated study at final concentrations of 10 and 15 µg/mL. For both positive controls, one dose level exhibiting a sufficient number of scorable metaphase cells was selected for analysis.

Formulation/Vehicle: DMSO

Incubation & sampling time: In the non-activated study, the cells were exposed to the test article for 4 hr or continuously for 20 hr up to the cell harvest at 37°C. In the 4-hr exposure group, after the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, re-fed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks returned to the incubator until cell collection.

In the S9-activated study, the cells were exposed for 4 hours at 37°C. After the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, re-fed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks were returned to the incubator until cell collection.

A concurrent toxicity test was conducted in both the non-

activated and the S9-activated test systems. After cell harvest an aliquot of the cell suspension was removed from each culture and counted using a Coulter counter. Cell viability was determined by trypan blue dye exclusion.

Two hr after the addition of Colcemid®, metaphase cells were harvested. The cell pellet was resuspended in 2-4 mL 0.075 M potassium chloride (KCl) for 4-8 min, centrifuged, and washed. The cells were treated with fixative (methanol:glacial acetic acid, 3:1, v/v). Cell were placed on slides and the slides were stained with 5% Giemsa, air dried, and permanently mounted.

The selection of dose levels for analysis of chromosome aberrations in CHO cells was based upon toxicity of the test article. The highest dose level selected for analysis was the dose which induced at least 50% toxicity, as measured by cell growth inhibition, relative to the solvent control, with sufficient number of scorable metaphase cells. Two additional lower dose levels were included in the analysis. To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Metaphase cells with 20 ± 2 centromeres were examined. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored.

Statistical analysis of the percentage of aberrant cells will be performed using the Fisher's exact test. The Fisher's test will be used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's exact test at any test article dose level, the Cochran-Armitage test will be used to measure dose-responsiveness. All conclusions will be based on sound scientific basis; however, as a guide to interpretation of the data, the test article will be considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). However, values that are statistically significant and fall within or just outside the range of historical control values for the solvent may be judged as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

Study Validity: CHO cells were treated with the test article at sufficient concentrations, in the presence and absence of S9, to produce acceptable levels of cytotoxicity (see below). Positive control produced expected increases in the frequency of structural chromosomal damage.

Toxicity of VRT-0995096 (cell growth inhibition relative to the solvent control) in CHO cells when treated for 4 hr in the absence of S9 activation was 55% at 200 µg/mL, the highest test concentration evaluated for chromosome aberrations. The dose levels selected for microscopic analysis were 50, 100 and 200 µg/mL.

Toxicity of VRT-0995096 (cell growth inhibition relative to the solvent control) in CHO cells when treated for 4 hr in the presence of S9 activation was 50% at 150 µg/mL, the highest test concentration evaluated for chromosome aberrations. The dose levels selected for microscopic analysis were 25, 50 and 150 µg/mL.

Toxicity of VRT-0995096 (cell growth inhibition relative to the solvent control) in CHO cells when treated for 20 hours in the absence of S9 activation was 51% at 75 µg/mL, the highest test concentration evaluated for chromosome aberrations. The dose levels selected for microscopic analysis were 25, 50 and 75 µg/mL.

Results: CHO cells treated with the test article for 4 or 20 hr in the absence of S9 metabolic activation or 4 hr in the presence of S9 metabolic activation did not produce any significant changes of the frequency of structural aberrations. The incidence of numerical aberrations was increased following treatment with the test article for 4 hr in the absence of S9 metabolic activation although the change was not statistically significant and has not been correlated with any adverse health outcome within the context of this *in vitro* assay. Thus, VRT-0995096 was negative in the *in vitro* CHO cell chromosomal aberration assay.

Table 27 Summary of the CHO cell chromosomal aberration assay

SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	10.7	200	200	0.015	±0.122	0.5	1.5
VRT-0995096									
50	-S9	4	10.6	200	200	0.015	±0.122	2.0	1.5
100	-S9	4	10.3	200	200	0.020	±0.140	1.5	2.0
200	-S9	4	9.7	200	200	0.025	±0.157	8.0	2.5
MMC, 0.2	-S9	4	6.9	200	50	0.340	±0.519	2.0	32.0**
DMSO	+S9	4	10.9	200	200	0.005	±0.071	2.5	0.5
VRT-0995096									
25	+S9	4	10.4	200	200	0.010	±0.100	1.0	1.0
50	+S9	4	10.1	200	200	0.015	±0.122	1.0	1.5
150	+S9	4	10.6	200	200	0.020	±0.140	3.0	2.0
CP, 10	+S9	4	3.9	200	50	0.720	±1.526	2.0	42.0**
DMSO	-S9	20	11.0	200	200	0.005	±0.071	2.0	0.5
VRT-0995096									
25	-S9	20	9.9	200	200	0.000	±0.000	1.0	0.0
50	-S9	20	9.0	200	200	0.015	±0.122	1.5	1.5
75	-S9	20	5.2	200	200	0.015	±0.158	1.5	1.0
MMC, 0.1	-S9	20	6.2	200	100	0.280	±0.552	2.0	23.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

8 Carcinogenicity

CARCINOGENICITY STUDY DRAFT PROTOCOL: 2-YEAR RAT STUDY

(b) (4)

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(b) (4)

11 Integrated Summary and Safety Evaluation

Vertex submitted a Special Protocol Assessment on September 24, 2012, in which they propose to evaluate the carcinogenic potential of VX-809, a CFTR (b) (4) for the treatment of cystic fibrosis, in Sprague-Dawley rats. A Phase 2, multiple dose study is ongoing with VX-809 (1200 mg/day) alone and co-administered with VX-770 (CFTR potentiator) in adult CF patients who carry the F508del-CFTR mutation (study VX10-809-102).

In Phase I studies, the sponsor identified a unique metabolite (VRT-0995096 or M28), which represented 13% of the total parent and metabolites (based on radio-labeling) and was not formed in nonclinical test species.

To support a 2-year repeat dose oral carcinogenicity study in Sprague-Dawley rats, Vertex submitted ADME studies, a 3-month toxicology study with VX-809 in rats, a 6-month toxicology study with VX-809 + VRT-0995096 in rats, a 1-month toxicology study with VRT-0995096 in rats, and *in vitro* genetic toxicology studies with VRT-0995096.

ADME Studies: VX-809 and M28 metabolite exhibited high protein binding in plasma from mouse, rat, rabbit, dog, and human. The percentage of bound in human plasma was 99.97 to 100% over the plasma concentration range of 0.5 to 100 µM. The mean percentage of bound in plasma from nonclinical species ranged from 97.02 to 100%. The binding data from individual human plasma proteins suggested that VX-809 was highly bound to serum albumin and binding to alpha acid glycoprotein and gamma globulin proteins was negligible.

The metabolic profile of VX-809 was qualitatively similar across various species following in vitro incubations with liver fractions and plasma from nonclinical species, including rats and dogs. VX-809 was mainly metabolized via oxidation and glucuronidation. Hydroxy-VX-809 (M1; hydroxylation of methyl pyridine) and VX-809 acyl glucuronide (M2; acyl glucuronidation) were observed to be the circulating metabolites of VX-809 in rats and dogs; however, levels of these metabolites were less than 10%.

In human plasma, most of the circulating radioactivity was associated with parent drug and metabolite hydroxy-pyrrolidone-VX-809 (VRT-0995096 or M28). Comparison of AUC values in plasma for the parent drug versus total radioactivity indicated that approximately 52% of the radioactivity was associated with unchanged VX-809. M28 was the major metabolite observed in plasma and represented 13% of the total radioactivity and a metabolite:parent AUC ratio of 25%. In metabolic profiling studies, the M28 metabolite was not observed in either *in vitro* or *in vivo* nonclinical studies with rats and dogs.

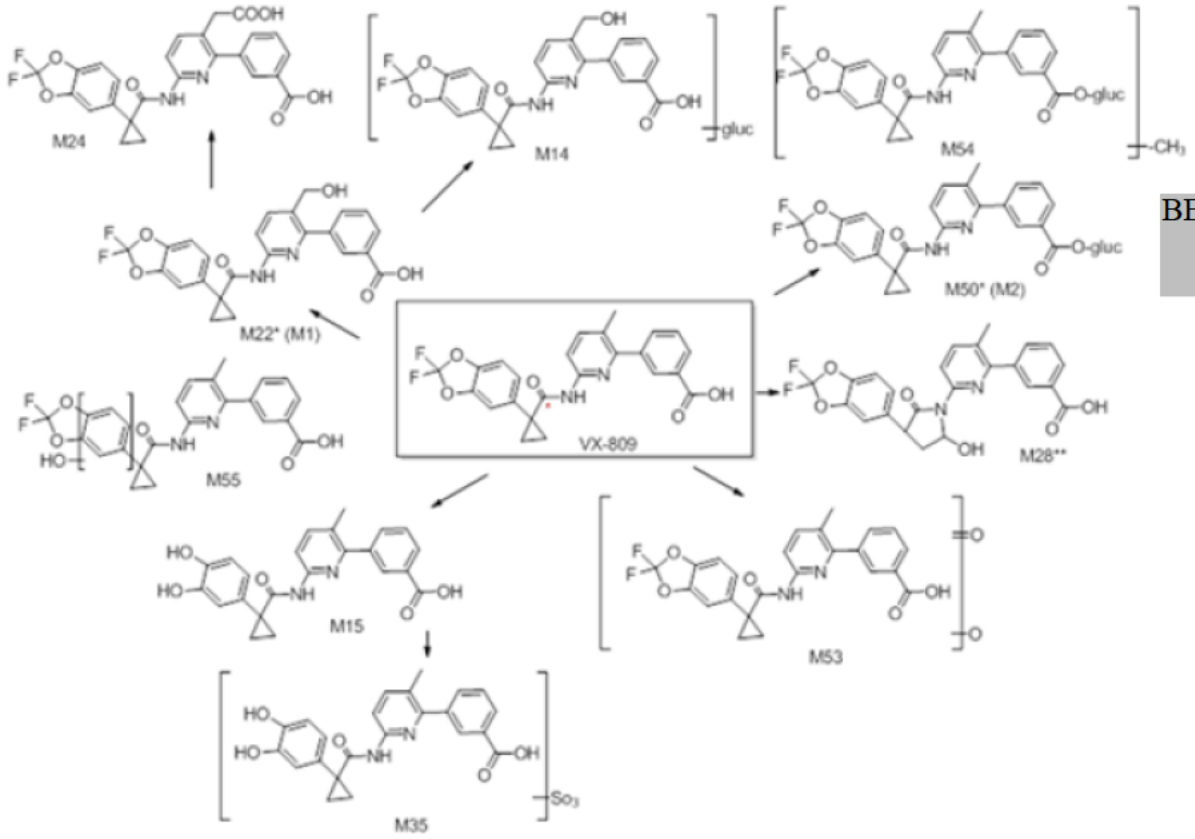
Table 30 Metabolites of VX-809

Table 3 Summary of Metabolites Detected In Vitro and In Vivo

ID	*Protonated m/z*	Metabolic Transformation
VX-809	453	NA (Parent)
M1 (M22)	469	Oxidation on methyl pyridine group
M2 (M50)	629	Direct glucuronidation
M14	645	Hydroxyl-glucuronidation
M15	405	desdifluoromethylene-VX-809
M24	483	Carboxylic acid derivative of VX-809
M28	469	hydroxy-pyrrolidone-VX-809
M35	485	desdifluoromethylene-VX-809-sulfate
M53	483	Dioxo-VX-809
M54	643	VX-809 glucuronide-methyl ester
M55	469	Oxidation of difluoromethylene-VX-809

Figure 17 VX-809 and its metabolites in healthy male human subjects

Figure 3 Structures of VX-809 and its Proposed Metabolites from a ^{14}C-VX-809 Study in Healthy Male Subjects



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Nonclinical toxicology studies with VX-809 and VRT-0995096: In a 3-month oral toxicology study, rats received VX-809 at doses of 0, 250, 500, 1000, or 2000 mg/kg/day. Body weight gains for male rats at 500, 1000, and 2000 mg/kg/day were reduced to 92.7, 90.3, and 80.7% of the control and for female rats at 500, 1000, and 2000 mg/kg/day were reduced to 88.7, 82.1, and 81.4% of the control, respectively. There were changes of several hematology parameters at the end of the dosing period (increases of platelet counts, mean platelet volume, reticulocytes, RBC distribution width, and white blood cell and lymphocyte counts and decreases of RBC counts, hemoglobin, hematocrit, and eosinophil counts). Treatment-related histopathological findings were evident in the liver (hepatocellular hypertrophy) and spleen (extramedullary hematopoiesis and increased erythrocytes in the red pulp), although these findings were not considered dose-limiting and were not evident in the 6-month toxicology study. Extramedullary hematopoiesis in the spleen appeared to be a compensatory response to decreased RBC counts. Administration of VX-809 to male and female rats at doses of 1000 and 2000 mg/kg/day resulted in small increases of microsomal protein yield, total cytochrome P450 content, and CYP4A activity. In

addition, there were increases of CYP3A activity in female animals. There were larger increases of CYP3A activity in male animals at a dose of 2000 mg/kg/day and CYP2B and CYP1A activities in male and female animals at doses of 1000 and 2000 mg/kg/day. The NOAEL was 2000 mg/kg/day. However, the MTDs were identified as 1000 and 500 mg/kg/day for males and female rats, respectively, based decreases of body weight gain.

In a 26-week toxicology study, Sprague-Dawley rats received VX-809 by oral gavage at doses of 250, 500, or 1000 mg/kg/day in combination with VRT-0995096 at a dose of 25 mg/kg/day. A control group received the vehicle consisting of 0.5% Tween 80 (w/v) + 0.5% methylcellulose (w/v) + 0.05% simethicone in water. At the end of the 26-week dosing period, 15 rats/sex/group were sacrificed. At the end of an additional 4-week recovery period, 5 rats/sex/group were sacrificed. There were no treatment-related deaths. Absolute body weights were unaffected by treatment for 26 weeks. There were no biologically significant changes of hematology, coagulation, or clinical chemistry parameters. No target organs of toxicity were identified. A number of low incidence findings, primarily confined to the high dose, were observed although relationships to treatment were unclear. Plasma C_{max} and AUC values for VX-809 increased with elevating doses; however, these increases were significantly less than dose proportional. Plasma C_{max} and AUC values for VRT-0995096 were comparable for Group 2, 3, and 4 at each time point suggesting that VX-809 had no effects on exposure to VRT-0995096. The MTD was judged to be greater than 1000 mg/kg/day VX-809 + 25 mg/kg/day VRT-0995096.

In a 28-day toxicology study, rats received VRT-0995096 (M28) at doses of 0, 25, 50, and 100 mg/kg/day. No dose-limiting toxicity or target organs of toxicity were identified in rats that received doses up to 100 mg/kg/day for 28 days. AUC values for males and females at 100 mg/kg/day on day 28 were 1800 and 2910 µg*hr/mL, respectively.

Genetic Toxicology: VRT-0995096 (M28) was negative in the *in vitro* bacterial reverse mutation assay and *in vitro* Chinese hamster ovary cell chromosomal aberration assay.

Exposure Margins for VX-809: Exposure margins for clinical exposures to VX-809 with doses of 200, 800, and 1200 mg/day relative to exposures in the 3- and 6-month rat studies are shown in the table below.

Exposure margins were ≥1.7-fold, which was considered sufficient.

Table 31 Safety margins for clinical doses of VX-809 at 200, 800, and 1200 mg/day

Study	NOAEL, mg/kg/day	Sex	AUC µg*hr/mL	Safety margins for proposed clinical doses of VX-809 (Lumacaftor)		
				200 mg QD (126 µg*hr/mL)	400 mg q12hr (504 µg*hr/mL)	400 mg q8hr (756 µg*hr/mL)
3-month rat	2000	M	3010	23.9	6.0	4.0
		F	5880	46.7	11.7	7.8
	1000	M	2340	18.6	4.6	3.1
		F	4850	38.5	9.6	6.4
6-month rat	1000	M	1300	10.3	2.6	1.7
		F	3160	25.1	6.3	4.2

Exposure Margins for VRT-0995096: The PK of metabolite M28 has been assessed in studies with healthy subjects and cystic fibrosis patients. The mean AUC and C_{max} of metabolite M28 on Day 1 and Day 28 appeared to increase less than dose proportionally from 25 to 200 mg, which may be due to the saturation of the metabolism pathway producing M28 or to the increase in other VX-809 metabolism/elimination processes. The terminal half life of metabolite M28 in plasma was estimated to be approximately 100 hr across dose levels. From predose plasma concentration of metabolite M28 measured on Days 7, 14, 21 and 28, steady state was reached by Day 21 at VX-809 doses of 25, 50 and 100 mg/day whereas a longer treatment duration appeared to be necessary for 200 mg/day. Based on AUC, the average accumulation ratio of metabolite M28 in plasma ranged from 8.2 to 10 over the VX-809 dose range tested. The metabolite to parent drug ratio (M28/VX-809) based on AUC on Days 1 and 28 decreased from 33% at 25 mg/day to 15% at 200 mg/day after 28 days of VX-809 treatment. This decrease in metabolic ratio observed with increasing VX-809 dose was related to the dose proportional increase in VX-809 concentrations versus the less than dose proportional increase in metabolite M28 concentrations over the tested dose range. Exposure margins for clinical exposures to M28 obtained with clinical doses of VX-809 at 200, 800, and 1200 mg/day, assuming a M28/VX-809 ratio of 15%, and expressed relative to the 1-month toxicology study with M28 in rats and 6-month toxicology study with VX-809 + M28 in rats are shown in the table below. Exposure margins were greater than 1, which was considered sufficient.

Table 32 Safety margins for estimated exposures to VRT-0995096 achieved with clinical doses of VX-809 at 200, 800, and 1200 mg/day (Assuming M28/VX-809 = 0.15)

Study	NOAEL for VRT-0995096, mg/kg/day	Sex	AUC µg*hr/mL	Safety margins for estimated exposures to VRT-0995096 (M28) achieved with clinical doses of VX-809 (Lumacaftor)		
				200 mg QD VX-809 (M28: 18.9 µg*hr/mL)	400 mg q12hr VX-809 (M28: 75.6 µg*hr/mL)	400 mg q8hr VX-809 (M28: 113.4 µg*hr/mL)
1-month rat with VRT-0995096	100	M	2910	154	38.5	25.7
		F	1800	95.2	23.8	15.9
6-month Rat with VX-809 + VRT-0995096	25	M	1163	61.5	15.4	10.3
		F	712	37.7	9.4	6.3

Sponsor's Proposed Doses for the 2-Year Carcinogenicity Study with Rats: (b) (4)

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/s/

TIMOTHY W ROBISON
11/02/2012

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	79,521
Supporting document/s:	#033, 090, 096, 107, 113, 117, 119, 120, and 125
Sponsor's letter date:	January 8, 2010, October 23, 2012, December 20, 2012, March 7, 2013, April 26, 2013, May 16, 2013, June 24, 2013, June 25, 2013, and July 19, 2013
CDER stamp date:	January 11, 2010, October 23, 2012, December 20, 2012, March 7, 2013, April 26, 2013, May 16, 2013, June 24, 2013, June 25, 2013, and July 19, 2013
Product:	VX-809
Indication:	CF subjects who are homozygous or heterozygous for the F508del-CFTR mutation
Sponsor:	Vertex Pharmaceuticals Incorporated 130 Waverly Street Cambridge, MA 02139-4242
Review Division:	Pulmonary, Allergy, and Rheumatology Products
Reviewer/Team Leader:	Timothy W. Robison, Ph.D., D.A.B.T.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Angela Ramsey

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Lumacaftor (VX-809) is a cystic fibrosis transmembrane conductance regulator (CFTR) (b) (4) proposed for the treatment of cystic fibrosis (CF). VX-809 is proposed to act as a chaperone that can rescue the processing, trafficking, and function of cystic fibrosis transmembrane conductance regulator (CFTR) carrying a deletion at phenylalanine 508 (ΔF508). Ninety percent of patients with CF carry a mutation that leads to misfolded ΔF508 CFTR.

Monotherapy with lumacaftor or ivacaftor alone has been shown to have no clinical benefit in subjects homozygous for the *F508del-CFTR* mutation; however, the combination of lumacaftor and ivacaftor was reported to have clinically significant benefit.

The Sponsor has proposed Phase 3 clinical studies designed to demonstrate clinical efficacy and safety of lumacaftor in combination with ivacaftor in subjects with CF homozygous for the *F508del-CFTR* mutation. These studies will evaluate the efficacy and safety of 2 dose levels of lumacaftor (600 mg qd and 400 mg q12hr) each in combination with ivacaftor (250 mg q12hr). The 24-week primary endpoint was selected because a significant response in FEV_1 is anticipated to be observed after 14 to 28 days of treatment with lumacaftor in combination with ivacaftor. This permits FEV_1 to be measured for an additional 5 months to obtain a more robust assessment that is less affected by short-term variability in FEV_1 and to allow for an assessment of the durability of response. The 24-week duration is also anticipated to facilitate demonstration of significant changes in the secondary efficacy endpoints such as body mass index.

Patients that complete the 24-week clinical trial are eligible to enroll in a 95-week clinical trial. In addition, CF subjects who are heterozygous for the *F508del-CFTR* mutation that complete a 56-day clinical trial (Cohort 4 of Clinical Trial VX09-809-102) are also eligible to enroll in this 95-week clinical trial.

1.2 Brief Discussion of Nonclinical Findings

In support of the proposed Phase 3 clinical trials with the combination of lumacaftor and ivacaftor, the sponsor submitted supporting nonclinical toxicology studies with lumacaftor alone, ivacaftor alone (see IND 74,633 and NDA 203-188), and the combination of lumacaftor and ivacaftor. For lumacaftor alone, toxicology studies up to 6 months in rats and 12 months in dogs were conducted. For the combination of lumacaftor and ivacaftor, 28-day toxicology studies in rats and dogs and a 3-month toxicology study in rats were conducted.

In Phase I clinical studies with VX-809, the sponsor identified a unique metabolite (VRT-0995096 or M28), which represented 13% of the total parent and metabolites (based on radio-labeling) and was not formed in nonclinical test species. The Sponsor included VRT-0995096 in the 6-month toxicology study in rats with VX-809 and 3-month

toxicology study in rats with the combination of VX-809 and VX-770 to assess its potential toxicity.

In chronic toxicology studies with VX-809, rats received doses up to 1000 mg/kg/day and dogs received doses up to 500 mg/kg/day. No dose-limiting toxicity or target organs of toxicity were identified in these studies.

Adverse findings observed in the 3-month toxicology study in dogs with VX-809 at 1000 mg/kg/day, which included death, trembling, jerky movements, and/or muscular rigidity, were not evident in the 12-month dog study with doses up to 500 mg/kg/day.

No dose-limiting toxicity or target organs of toxicity were identified with VRT-0995096 (or M28), a metabolite of VX-809.

Toxicology studies with the combination of VX-809 and VX-770 in rats up to 3 months and dogs up to 28 days identified the following findings that were not generally observed with the individual monoproducts:

1. In rats treated with the combination of VX-809 and VX-770, focal necrosis and/or erosions (minimal to slight) were present in the glandular mucosa of the stomach for all male and female drug-treated groups. The findings were dose-responsive for male drug-treated groups, but not for female drug-treated groups. Mucosal necrosis and/or erosions were reversible following the recovery period. The squamous epithelium along the limiting ridge of the forestomach showed cystic degeneration (minimal to moderate) in males and females dosed at ≥500/10/10 mg/kg/day VX-809/VX-770/VRT-0995096. Variable numbers of the superficial keratinized cells along the limiting ridge contained small cysts filled with pink material. There was no clear dose relationship in males. Females had an inverse relationship to dose. Epithelial cystic degeneration along the limiting ridge was not reversible. Focal inflammation and edema were variably present. In one male (No. 2038) in Group 2 at 1000/100/20 mg/kg/day, the edema was moderate and diffuse. It is noted that humans have no analogous structure to the rat forestomach; however, findings in the rat glandular stomach would be considered potentially relevant to humans. Based upon discussions with the Medical Officer, findings in the stomach were considered to be monitorable in a clinical setting based upon the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation.

2. Following co−administration of VX-770/VX-809 to dogs, prolonged PR intervals (first degree AV block) were observed in 3/12 and 10/12 dogs in the 600/15, and 600/60 mg/kg/day, respectively. Co−administration of VX-770 and VX-809 also produced early depolarization of supraventricular origin (supraventricular premature complex: SVPC) for 8/12 dogs in the 600/60 mg/kg/day group. Some SVPCs occurred during the inspiratory phase of the respiratory cycle; therefore, early supraventricular depolarization with the P wave in contact with the previous T wave, not buried in the previous T wave, could be resulting from exaggerated

respiratory sinus arrhythmia. These ECG changes were not observed with VX-809 alone. SVPCs were increased as compared to VX-770 alone. These findings were considered monitorable in a clinical setting.

3. In the 600/60 mg/kg/day group, 2 of 4 male dogs had increased numbers of multinucleate degenerate germ cells in the testes (moderate vs. minimal) and one of these dogs also had increased sloughed testicular germ cells in the epididymides. The incidence and severity of prostatic acinar contraction and reduced secretion was also greater in this group than in the other groups and this correlated with a decrease in prostatic weight in this group. Given the peripubertal status of the animals, it was likely that these changes represented a slight retardation of sexual maturation in the high dose group animals, which was probably associated with the decreased body weight gain that occurred in these animals during the study. These changes might be an indirect effect of the test article. The NOAEL was identified as 600/15 mg/kg/day based upon histopathological findings in the male reproductive organs (testes, epididymides, and prostate) at the high dose of 600/60 mg/kg/day. Findings were reversible at the end of a 14-day recovery period.

In the 90-day oral (gavage) toxicology study, rats were dosed once daily by oral gavage with various combinations of VX-809, VX-770, and VRT-0995096 co-formulated as a suspension in the vehicle. Female #2559 in Group 2 (1000/100/20 mg/kg/day) was observed with bilateral posterior subcapsular cataracts of the lens at the end of the 90-day dosing period. The NOAEL was identified at 1000/25/20 mg/kg/day based upon a finding of bilateral, subcapsular cataracts for one animal at 1000/100/20 mg/kg/day. However, based upon the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation, the finding of cataracts for one rat at 1000/100/20 mg/kg/day might be considered an acceptable risk that is monitorable in a clinical setting, but not reversible.

NOAELs for VX-809, VX-770 and its metabolites, and VRT-0995096 obtained from 6-month rat and 12-month dog toxicology studies with VX-809 alone and VX-770 alone as well as the 3-month toxicology study with the combination of VX-809, VX-770, and VRT-0995096 in rats provide adequate safety margins for the proposed clinical doses of the combination of VX-809 (lumacaftor) and VX-770 (ivacaftor).

1.3 Recommendations

1.3.1 Clinical Studies Safe to Proceed: Yes

From the nonclinical perspective, the proposed Phase 3 clinical trials (103, 104, and 105) appear reasonably safe and should be allowed to proceed.

2 Drug Information

2.1 Drug

Generic Name: Lumacaftor

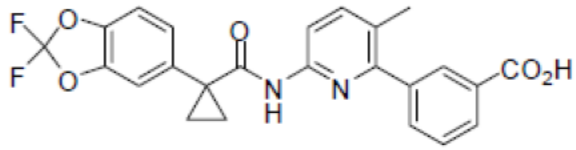
Code name: VX-809 (or VRT-826809)

Chemical name: 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido) -3-methylpyridin-2-yl)benzoic acid

Molecular formula/molecular weight: $C_{24}H_{18}F_2N_2O_5$ / 452 g/mole

Structure:

Figure 1 Structure of VX-809 Drug Substance



Chemical Formula: $C_{24}H_{18}F_2N_2O_5$
Molecular Weight: 452.41

Pharmacologic class: CFTR [(b) (4)]

2.2 Relevant INDs, NDAs, and DMFs

NDA 203-188 and IND 74,633 for KALYDECO® (Vertex Pharmaceuticals Inc., Ivacaftor (VX-770))

2.3 Drug Formulation

Lumacaftor/ivacaftor tablets are a co-formulation of the active ingredients, lumacaftor (VX-809) and ivacaftor (VX-770), in a single oral dosage form. Each tablet contains 200 mg of lumacaftor and 125 mg of ivacaftor. The target composition of 200 mg lumacaftor/125 mg ivacaftor tablets along with the quality reference and function of each component is provided in the table below. Ivacaftor drug substance is provided as a [(b) (4)] Tablets are manufactured from [(b) (4)] ivacaftor [(b) (4)] and lumacaftor drug substance with excipients. The tablets are film coated.

Table 1 Composition of 200 mg lumacaftor/125 mg ivacaftor CG tablets

Table 1 Composition of 200 mg Lumacaftor/125 mg Ivacaftor CG Tablets

Component	Quality Standard	Component Function	Amount per Tablet (mg)	Content (% w/w)
(b) (4)				
(b) (4)				
Lumacaftor drug substance	In house standard	Active Ingredient	200.0	(b) (4)
Ivacaftor (b) (4) (b) (4)	NDA 203188	(b) (4)		(b) (4)
Microcrystalline cellulose	USP/NF			
Croscamellose sodium	USP/NF			
Sodium lauryl sulfate	USP/NF			
Povidone	USP/NF			
(b) (4)	USP/NF			
	USP/NF			
Magnesium stearate	USP/NF			
(b) (4)				
Film Coat				
(b) (4)	(b) (4) standard USP/NF			
Total			**582.5**	
(b) (4)				

For clinical trials VX12-809-103 and VX12-809-104, lumacaftor/ivacaftor (200/125) and matching placebo will be supplied as pink film-coated tablets of similar size and appearance containing 200 mg lumacaftor/125 mg ivacaftor and 0 mg lumacaftor/0 mg ivacaftor, respectively. Lumacaftor/ivacaftor (200/83) and matching placebo will be supplied as pink film-coated tablets of similar size and appearance containing 200 mg lumacaftor/83 mg ivacaftor and 0 mg lumacaftor/0 mg ivacaftor, respectively. Ivacaftor and matching placebo will be supplied as blue film-coated tablets of similar size and appearance containing 125 and 0 mg of ivacaftor, respectively.

Table 2 Study drugs for clinical trials VX12-809-103 and VX12-809-104

Table 11-2 Identity of Study Drugs, Dosage, and Storage

Drug Name	Strength/Formulation/Route	Dosage	Storage Condition
Lumacaftor/ivacaftor (200/125)	200 mg/125 mg tablet; oral	400 mg q12h/ 250 mg q12h	≤30°C (≤86°F)
Lumacaftor/ivacaftor (200/83)	200 mg/83 mg tablet; oral	400 mg q12h/ 250 mg q12h	≤30°C (≤86°F)
Lumacaftor/ivacaftor (200/125) placebo	0 mg/0 mg tablet; oral	0 mg q12h/ 0 mg q12h	≤30°C (≤86°F)
Lumacaftor/ivacaftor (200/83) placebo	0 mg/0 mg tablet; oral	0 mg q12h/ 0 mg q12h	≤30°C (≤86°F)
Ivacaftor	125 mg tablet; oral	250 mg q12h	≤30°C (≤86°F)
Ivacaftor placebo	0 mg tablet; oral	0 mg q12h	≤30°C (≤86°F)

qd: once daily; q12h: every 12 hours.

For clinical trial VX13-809-011, Vertex will supply the 200-mg lumacaftor/125-mg ivacaftor tablets and 125-mg ivacaftor tablets in (b) (4) weekly blister cards. Study drug labeling will be in compliance with applicable local and national regulations. Additional details regarding packaging, labeling, and dispensing for lumacaftor and ivacaftor will be included in the Pharmacy Manual.

Table 3 Study drugs for clinical trial VX13-809-011

Table 11-1 Study Drug

Drug Name	Formulation/ Route	Packaging (Formulation Strength)	Storage Condition
Lumacaftor/ivacaftor (fixed-dose)	fixed-dose tablet/ Oral	Supplied as 200-mg lumacaftor /125-mg ivacaftor tablets	15°C to 30°C (59°F to 86°F), protected from light
Ivacaftor (film-coated)	Tablet/ Oral	Supplied as 125-mg tablets	15°C to 30°C (59°F to 86°F), protected from light

2.4 Proposed Clinical Population and Dosing Regimen

Male or Female subjects with CF who are homozygous or heterozygous for the F508del-CFTR mutation will be administered VX-809 in combination with VX-770.

2.5 Comments on Impurities/Degradants of Concern

(b) (4) found in the drug product. Tentative qualification of this (b) (4) has been provided, although the exact level in the drug product needs to be provided with an NDA.

2.6 Proposed Clinical Protocol

A Phase 3, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Evaluate the Efficacy and Safety of Lumacaftor in Combination With Ivacaftor in Subjects Aged 12 Years and Older With Cystic Fibrosis, Homozygous for the F508del-CFTR Mutation (Vertex Study Number: VX12-809-103)

The Sponsor has proposed a Phase 3, randomized, double-blind, placebo-controlled, parallel-group, multicenter study to evaluate the efficacy, safety, and pharmacokinetics of lumacaftor in combination with ivacaftor through week 24 in subjects with CF who are homozygous for the *F508del-CFTR* mutation. Clinical subjects will be male and female subjects aged 12 years or older with CF who are homozygous for the *F508del-CFTR* mutation. This study will evaluate 2 dose levels of lumacaftor in combination with ivacaftor. This study includes a Screening Period, a Treatment Period, and a Safety Follow-up Visit.

Approximately 501 subjects will be randomized, stratified by age (<18 versus ≥18 years of age), sex (male versus female), and FEV1 severity determined at the Screening Visit (<70% versus ≥70% predicted), and then randomized (1:1:1) to 1 of the 3 treatment arms.

The Treatment Period will last approximately 24 weeks. Subjects will be randomized to 1 of 3 treatment arms: 2 combination treatment arms and 1 placebo arm. The dosing regimen for each treatment arm is as follows:

- Treatment Arm A: 600 mg lumacaftor daily (qd) + 250 mg ivacaftor every 12 hours (q12hr)
- Treatment Arm B: 400 mg lumacaftor q12hr + 250 mg ivacaftor q12hr
- Treatment Arm C: lumacaftor placebo q12hr + ivacaftor placebo q12hr

Active substance: lumacaftor and ivacaftor (fixed-dose combinations with lumacaftor and ivacaftor); 200-mg lumacaftor/125-mg ivacaftor and 200-mg lumacaftor/83-mg ivacaftor tablets for oral administration

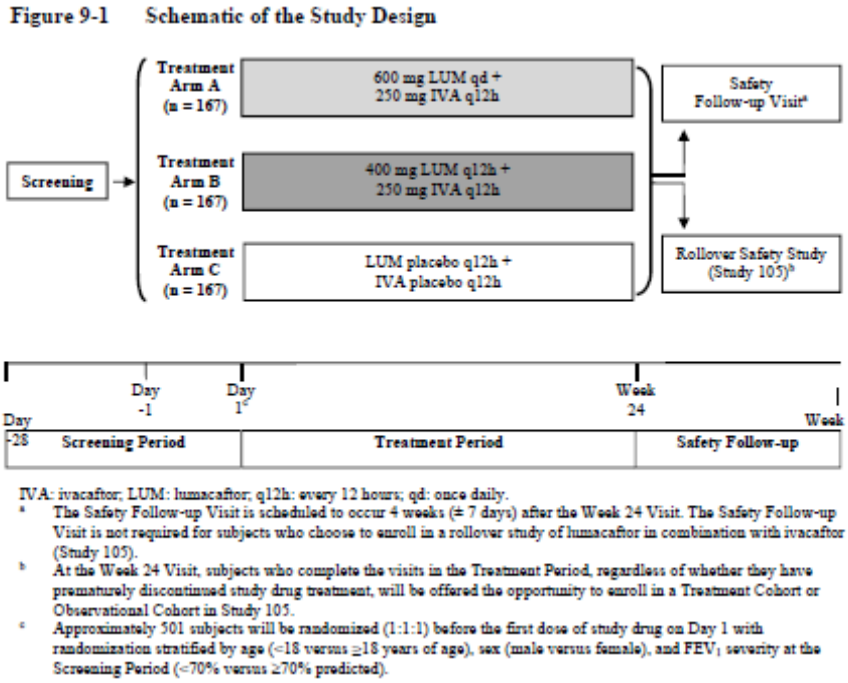
Placebo: Lumacaftor and ivacaftor ([200/125] and [200/83]) matching placebos; 0-mg lumacaftor/0-mg ivacaftor tablets for oral administration

Active substance: ivacaftor (film-coated); 125-mg tablets for oral administration

Placebo: Ivacaftor matching placebo; 0-mg tablets for oral administration

At the Week 24 Visit, subjects who complete the visits in the Treatment Period, regardless of whether they have prematurely discontinued study drug treatment, will be offered the opportunity to enroll in a Treatment Cohort or Observational Cohort in a rollover study of lumacaftor in combination with ivacaftor (VX12-809-105 [Study 105]).

Figure 1 Study Designs of Clinical Trials VX12-809-103 and VX12-809-104



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At this stage in the development of lumacaftor in combination with ivacaftor, participation in this study requires a commitment from the research subject and his/her partner to use 2 methods of birth control. All contraceptive methods must be used throughout the study and for 90 days following the last dose of study drug.

Contraception for the couple is waived for the following:
- True abstinence for the subject, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, and post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- If the male is infertile (e.g. bilateral orchiectomy). Infertility may be documented through examination of a semen specimen or by demonstration of the absence of the vas deferens by ultrasound before the first dose of the study drug.
- If the female is of non-childbearing potential, per the following:
    Postmenopausal: spontaneous amenorrhea for at least 12 consecutive months and have a serum FSH level ≥40 mIU/mL at Screening or Documented hysterectomy or a bilateral oophorectomy/salpingo-oophorectomy.
- Has not achieved menarche (has not had her first menstrual period). Girls who fall into this category are considered not to be of childbearing potential only as long as they have not had their first menstrual period.

All other female subjects who have had their first menstrual period (including subjects with tubal ligations and subjects who do not have a documented hysterectomy) will be considered to be of childbearing potential.

Acceptable contraceptive methods:
Acceptable contraceptive methods for the **male** include the following:
- Vasectomy 6 months or more previously, with a negative post-vasectomy semen analysis for sperm.
- Condom with spermicide (either as a single product if commercially available and/or allowed according to local regulations; otherwise condom and spermicide as separate products), and the female must use an additional acceptable method of contraception.

These methods should be in successful use at least 14 days before the first dose of study drug because pregnancy tests are not considered reliable if the pregnancy is of less than 14 days' duration.

Acceptable contraceptive methods for the **female** include the following:
- Bilateral tubal ligation performed at least 6 months previously.
- An intrauterine device (non-hormone-releasing) in place for at least 90 days.
- Barrier contraception (such as diaphragm, cervical cap, or female condom) with spermicide and the male must use an additional acceptable method of contraception.

These methods should be in successful use at least 14 days before the first dose of study drug because pregnancy tests are not considered reliable if the pregnancy is of less than 14 days' duration.

Additional notes:
- Female condom cannot be used with male condom (as a double method of contraception) due to risk of tearing.
- Male and female subjects who are not sexually active at the time of screening must agree to follow the contraceptive requirements of this study if they become sexually active with a partner of the opposite sex.
- If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.
- Female partners of male subjects may use hormonal contraceptives as a method of highly effective contraception.
- For female subjects, hormonal contraceptives are not considered highly effective, because lumacaftor may alter efficacy of hormonal contraceptives
- Male subjects must not donate sperm after the first dose of study drug, throughout the study, and for 90 days following the last dose of study drug.
- Female subjects and female partners of male subjects should not plan to become pregnant during the study through 90 days following the last dose of study drug.
- Female subjects should not nurse a child from the start of study drug dosing through 90 days following the last dose of study drug.

A Phase 3, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Evaluate the Efficacy and Safety of Lumacaftor in Combination With Ivacaftor in Subjects Aged 12 Years and Older With Cystic Fibrosis, Homozygous for the F508del-CFTR Mutation (Vertex Study Number: VX12-809-104)

Study VX12-809-104 is identical to Study VX-809-103 (see description above).

A Phase 1, Open-Label Study to Evaluate the Pharmacokinetics and Safety of Lumacaftor in Combination With Ivacaftor in Subjects 6 Through 11 Years of Age With Cystic Fibrosis, Homozygous for the F508del-CFTR Mutation (Vertex Study Number: VX13-809-011)

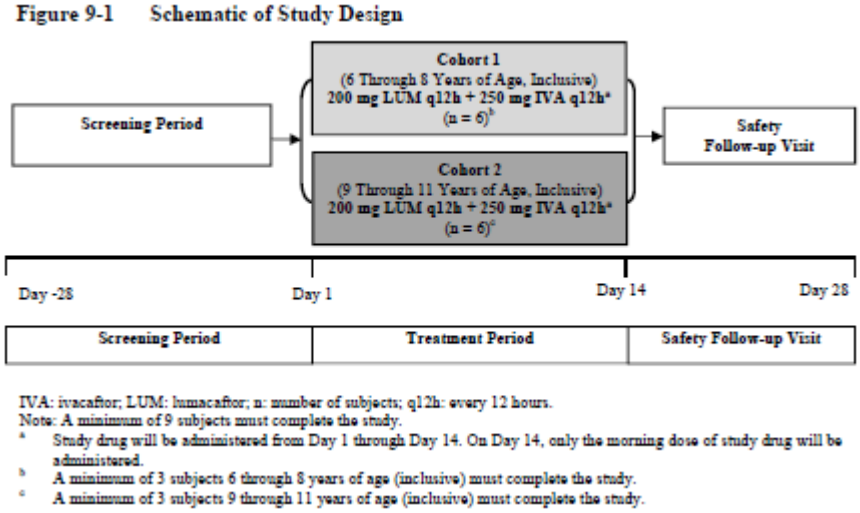
The Sponsored proposed a Phase 1, open-label, multiple-cohort, multiple-dose, multicenter study evaluating the pharmacokinetics and safety of lumacaftor in combination with ivacaftor in subjects 6 through 11 years of age (inclusive) with CF who are homozygous for the *F508del-CFTR* mutation. Approximately 12 subjects are planned for enrollment. A minimum of 9 subjects must complete the study. Of these 9 subjects, 3 subjects must be 6 through 8 years of age (inclusive) and 3 subjects must be 9 through 11 years of age (inclusive).

After obtaining written informed consent and assent (if applicable), screening visit assessments will be completed at any time during the period of approximately 4 weeks (Day -28 through Day -1) before the first dose of the study drug (Day 1). During the treatment period, subjects will be administered 200 mg lumacaftor q12hr in combination with 250 mg ivacaftor q12hr (1 × 200-mg lumacaftor/125-mg ivacaftor tablet q12hr + 1 × 125-mg tablet of ivacaftor q12hr) for approximately 14 days. A safety follow-up visit is scheduled to occur 10 (±4) days after the last dose of study drug. Subjects who prematurely discontinue from this study for any reason (except withdrawal of consent) will be asked to return to the clinical site 10 (±4) days after their last dose of study drug.

Based on AUC simulations for subjects 6 through 11 years of age, the lumacaftor dose of 200 mg q12hr is expected to yield a distribution of exposures that are comparable to those of the adult population (subjects 18 years and older) who were administered 400 mg of lumacaftor q12hr in Study 102. The ivacaftor dose of 250 mg q12hr is chosen for the pediatric population in this study (6 through 11 years of age). The anticipated clinical dose of ivacaftor in subjects 12 years of age and older with CF who are homozygous for the *F508del-CFTR* mutation is 250 mg q12hr, which was shown to be safe and efficacious in combination with both the 600 mg once daily (qd) and 400 mg q12hr lumacaftor treatment regimens in Study 102. The approved clinical dose for ivacaftor monotherapy in both pediatric patients (6 through 17 years) and in adult patients (18 years and older) with CF who have the *G551D-CFTR* mutation on at least 1 allele is 150 mg q12hr. Based on the achievement of the comparable PK target observed for the adult subjects who received 150 mg of ivacaftor q12hr in Study VX08-770-102, no dose adjustment from the adult clinical dose was needed for children aged 6 through 17 years (Study VX08-770-102 and Study VX08-770-103). When given in combination, lumacaftor is expected to markedly reduce (by approximately 80%) the exposures of ivacaftor due to the induction of cytochrome (CYP)3A enzyme (Study VX09-809-005 [Study 005] and VX10-809-006 [Study 006]), which is primarily responsible for the metabolism of ivacaftor. As the CYP3A enzyme system is expected to be fully matured

in subjects 6 through 11 years of age; a similar induction effect of lumacaftor on ivacaftor is assumed in this population. The duration of 14 days was selected to evaluate PK, safety, and tolerability when the induction effect of lumacaftor on the metabolism of ivacaftor is anticipated to have reached steady state.

Figure 2 Study Design of Clinical Trial VX13-809-011



A Phase 2, Multicenter, Double-Blind, Placebo-Controlled, Multiple-Dose Study to Evaluate the Safety, Tolerability, Efficacy, Pharmacokinetics, and Pharmacodynamics of Lumacaftor Monotherapy, and Lumacaftor and Ivacaftor Combination Therapy in Subjects With Cystic Fibrosis, Homozygous or Heterozygous for the F508del-CFTR Mutation (Vertex Study Number: VX09-809-102)

Versions 1, 2, and 3 of the Protocol were submitted on July 27, 2010, August 11, 2011, and June 1, 2012, respectively. This protocol was amended to include an additional cohort (Cohort 4) to investigate one dose regimen of lumacaftor in combination with ivacaftor using a fixed-dose formulation in subjects who are heterozygous for the *F508del-CFTR* mutation. Subjects will receive 400mg of lumacaftor q12hr in combination with 250mg of ivacaftor for 56 days.

This is a Phase 2, multicenter, double-blind, placebo-controlled, multiple-dose study of lumacaftor monotherapy and lumacaftor and ivacaftor combination therapy in subjects with CF who are homozygous or heterozygous for the *F508del-CFTR* mutation. The primary aims of the study are to evaluate the safety, tolerability, and efficacy of lumacaftor in combination with ivacaftor. In Cohort 4, approximately 120 male and female subjects (≥18 years old) who are heterozygous for the *F508del-CFTR* mutation will be randomized to 1 of 2 treatment groups (active drug or placebo) and study drug will be administered for 56 days. The active drug will be administered as a film-coated, fixed-dose combination tablet containing 200 mg lumacaftor and 125 mg ivacaftor. The

placebo will be a matching tablet (0-mg lumacaftor/0-mg ivacaftor tablet) for oral administration

Subjects will be stratified by sex (male versus female) and FEV1 severity collected at the Screening Visit (<70% versus ≥70% predicted), and then randomized (1:1) to 1 of the following treatment groups in Cohort 4:

Group 1 (N = 60): Subjects heterozygous for the *F508del-CFTR* mutation will receive 400 mg of lumacaftor q12hr in combination with 250 mg of ivacaftor q12hr (Day 1 through Day 56).

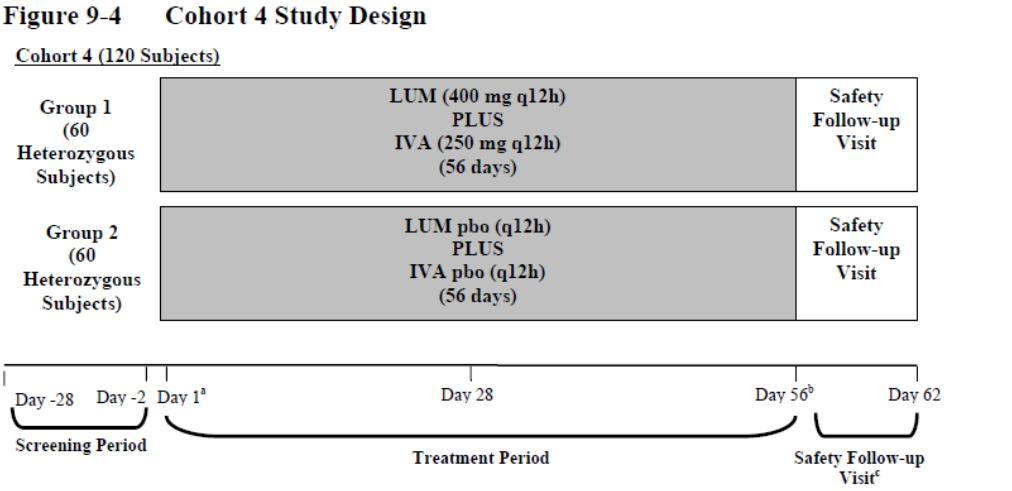
Group 2 (N = 60): Subjects heterozygous for the *F508del-CFTR* mutation will receive lumacaftor in combination with ivacaftor matched placebo q12hr (Day 1 through Day 56).

Treatment groups in Cohort 4 will be studied in a parallel manner. One interim analysis (IA) assessing safety, efficacy, PK, and PD will be performed when approximately 40 subjects in Cohort 4 have completed 8 weeks of treatment with study drug. Additional IAs may take place at any other time during the study if warranted by the ongoing data, and/or if deemed necessary by an internal Vertex team (separate from the blinded study team). An independent DMC will review the safety data from Cohort 4 when approximately 40 subjects have completed 14 days of treatment with study drug.

At the Day 56 Visit, only subjects in Cohort 4 who remained on study drug and completed the visits in the Treatment Period may be offered the opportunity to enroll in a Treatment Cohort in Study 105.

Contraception requirements are similar to those described above for Clinical Trial VX12-809-103.

Figure 3 Study Design for Cohort 4 of Clinical Trial VX09-809-102



A Phase 1, Randomized, Single-Dose, Open-Label Crossover Study to Investigate the Effect of Food on the Relative Bioavailability of 2 Fixed-Dose Combinations of Lumacaftor and Ivacaftor Tablet Formulations in Healthy Adult Subjects (Vertex Study Number: VX13-809-012)

The sponsor proposed a single-center, Phase 1, randomized, open-label, 2-part (2-sequence, 2-period per part), crossover study in healthy adult subjects. This study is designed to investigate the effect of food on the relative bioavailability of 2 different strengths of fixed-dose combinations of lumacaftor and ivacaftor tablet formulations. This study will include a screening period, 2 treatment periods, and a safety follow-up visit. The screening period will occur between 2 and 21 days before administration of study drug to confirm that subjects meet the eligibility criteria. Each of 28 subjects will be randomized to 1 of 4 dosing sequences before administration of study drug on Day 1 of Treatment Period 1. There will be a washout of at least 14 days between each dosing occasion. Subjects will have a safety follow-up visit 10 (± 2) days after the last dose of study drug in Treatment Period 2.

The doses of lumacaftor (400 and 600 mg) and ivacaftor (250 mg) reflect those selected for Phase 3 studies investigating the lumacaftor and ivacaftor combination treatment. Washout periods are generally considered adequate if they encompass 5 half-lives of the administered compound. The duration of the washout period (14 days) is related to the apparent long terminal half-life of lumacaftor (mean = 26 hours, range = 17 to 40 hours). The terminal half-life of ivacaftor is shorter than that of lumacaftor. Patient will receive single doses consisting of 2 or 3 tablets containing the combination of lumacaftor and ivacaftor at doses of 200 mg/125 mg or 200 mg/83 mg, respectively

Male and female subjects will be between the ages of 18 and 55 years, inclusive, and healthy, as defined by no clinically relevant abnormalities identified by a detailed medical history, full physical examination (PE), including blood pressure and pulse rate measurement, standard 12-lead ECG, and clinical laboratory tests. No more than 4 subjects 45 through 55 years of age will be enrolled in each part of the study

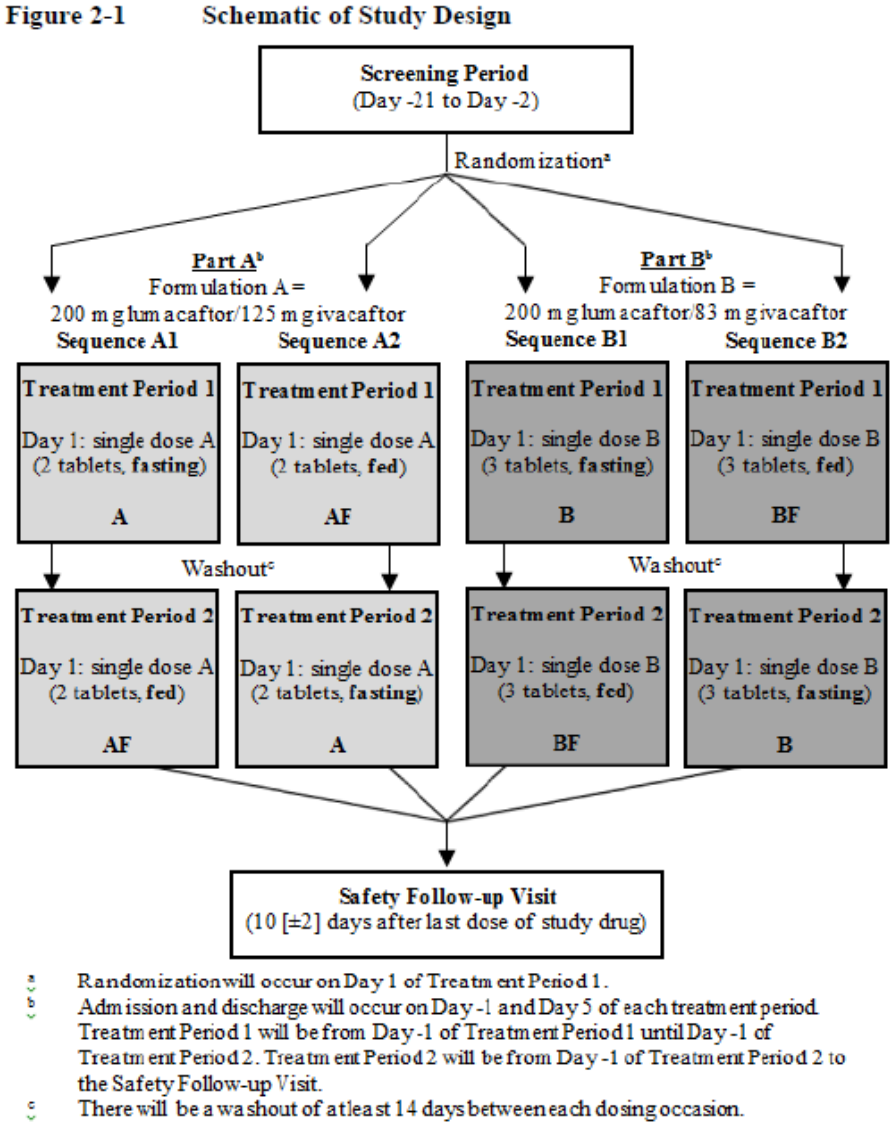
Table 4 Study Drug for Clinical Trial VX13-809-012

Table 11-2 Study Drug

Study	Drug Name	Formulation/ Route	Dosage	Packaging	Storage Condition
Part A	Lumacaftor/ivacaftor formulation	Tablet/oral	400/250 mg	Supplied as 200-mg lumacaftor/125-mg ivacaftor tablets	15°C to 30°C (59°F to 86°F)
Part B	Lumacaftor/ivacaftor formulation	Tablet/oral	600/250 mg	Supplied as 200-mg lumacaftor/83-mg[a] ivacaftor tablets	15°C to 30°C (59°F to 86°F)

[a] Each tablet in Part B contains approximately 83.3 mg ivacaftor.

Figure 4 Study Design of Clinical Trial VX13-809-012



Contraception requirements are similar to those described above for Clinical Trial VX12-809-103.

A Phase 3, Rollover Study to Evaluate the Safety and Efficacy of Long-term Treatment With Lumacaftor in Combination With Ivacaftor in Subjects Aged 12 Years and Older With Cystic Fibrosis, Homozygous or Heterozygous for the F508del-CFTR Mutation (Vertex Study Number: VX12-809-105)

This is a Phase 3, parallel-group, multicenter, rollover study in subjects with CF who are homozygous or heterozygous for the *F508del-CFTR* mutation and who participated in Study 103, Study 104, or Cohort 4 of Study 102. Study 105 is designed to evaluate the safety and efficacy of long-term treatment of lumacaftor in combination with ivacaftor. Study 105 consists of 2 parts (Part A and Part B), which will be enrolled in parallel. Part

A will enroll subjects from Study 103 and Study 104. Part B will enroll subjects from Cohort 4 of Study 102.

Part A (Subjects From Study 103 and Study 104)
Part A consists of a Part A Treatment Cohort and a Part A Observational Cohort, which will enroll subjects from Study 103 and Study 104. The Part A Treatment Cohort and the Part A Observational Cohort will be enrolled in parallel.

Part A Treatment Cohort
The following subjects from Study 103 or Study 104 who meet the study criteria and elect to enroll in Study 105 are eligible for enrollment in the Part A Treatment Cohort:

- Subjects who are receiving study drug treatment (i.e., lumacaftor in combination with ivacaftor or placebo) at the end of treatment in Study 103 or Study 104
- Subjects who are not receiving study drug treatment at the end of treatment in Study 103 or Study 104 AND who have received Vertex approval for entry. Subjects who prematurely discontinued study drug treatment are not eligible for enrollment in the Part A Treatment Cohort.

The Part A Treatment Cohort will be double-blind and will consist of 2 treatment arms:

- Treatment Arm 1: 600 mg lumacaftor once daily (qd) + 250 mg ivacaftor every 12 hours (q12hr)
- Treatment Arm 2: 400 mg lumacaftor q12hr + 250 mg ivacaftor q12hr

Subjects who received lumacaftor in combination with ivacaftor in Study 103 or Study 104 will continue to receive the same dose and regimen of study drug in a double-blind fashion in Study 105 for 96 weeks as follows:

- Subjects who were randomized to Treatment Arm A in Study 103 or Study 104 are eligible for enrollment in Treatment Arm 1
- Subjects who were randomized to Treatment Arm B in Study 103 or Study 104 are eligible for enrollment in Treatment Arm 2
- Subjects who received placebo in Study 103 or Study 104 (Treatment Arm C in Study 103 or Study 104) will be randomized (1:1) to 1 of the 2 double-blind treatment arms (Treatment Arm 1 or Treatment Arm 2)

Part A Observational Cohort
The following subjects from Study 103 or Study 104 who meet the study criteria and elect to enroll in Study 105 are eligible for enrollment in the Part A Observational Cohort:

- Subjects who received at least 4 weeks of study drug in Study 103 or 104 and who are not eligible for the Part A Treatment Cohort with lumacaftor in combination with ivacaftor
- Subjects who received at least 4 weeks of study drug in Study 103 or 104 and who elect not to continue treatment with lumacaftor in combination with ivacaftor

Subjects in the Part A Observational Cohort will not receive study drug and will have regularly scheduled telephone calls for approximately 2 years after their last dose of

study drug in Study 103 or Study 104 to assess post-treatment safety of lumacaftor and ivacaftor combination therapy.

Part B (Subjects From Cohort 4 of Study 102)
Part B will consist of a Part B Treatment Cohort that will enroll subjects from Cohort 4 of Study 102. The following subjects from Cohort 4 of Study 102 who meet the study criteria and elect to enroll in Study 105 are eligible for enrollment in the Part B Treatment Cohort:

- Subjects who are receiving study drug treatment at the end of treatment in Cohort 4 of Study 102
- Subjects who are not receiving study drug treatment at the end of treatment in Cohort 4 of Study 102 AND who have received Vertex approval for entry. Subjects who prematurely discontinued study drug treatment are not eligible for enrollment in the Part B Treatment Cohort.

The Part B Treatment Cohort will be open-label and will consist of 1 treatment arm:

- Treatment Arm 3: 400 mg lumacaftor q12hr + 250 mg ivacaftor q12hr

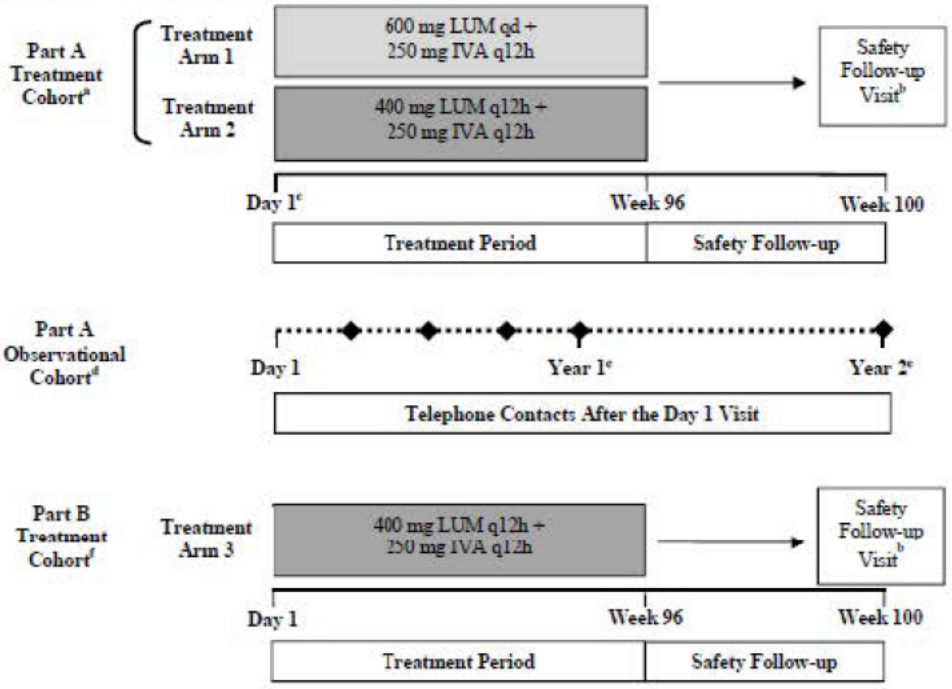
Subjects who received lumacaftor in combination with ivacaftor in Cohort 4 of Study 102 will be enrolled in Treatment Arm 3 and will continue to receive the same dose and regimen of study drug in Study 105 for 96 weeks as follows:

- Subjects who received active study drug in Cohort 4 of Study 102 are eligible for enrollment in Treatment Arm 3
- Subjects who received placebo in Cohort 4 of Study 102 are eligible for enrollment in Treatment Arm 3

Contraception requirements are similar to those described above for Clinical Trial VX12-809-103.

Figure 5 Study Design of Clinical Trial VX12-809-105

Figure 9-1 Schematic of the Study Design



AE: adverse event; IVA: ivacaftor; LUM: lumacaftor; q12h: every 12 hours; qd: once daily.

[a] The following subjects from Study 103 or Study 104 may be eligible for enrollment in the Part A Treatment Cohort: (1) subjects who are receiving study drug treatment (i.e., lumacaftor in combination with ivacaftor or placebo) at the end of treatment in Study 103 or Study 104 and (2) subjects who are not receiving study drug treatment at the end of treatment in Study 103 or Study 104 AND who have received Vertex approval for entry. Subjects who prematurely discontinued study drug treatment are not eligible for enrollment in the Part A Treatment Cohort.

[b] The Safety Follow-up Visit is scheduled to occur 4 weeks (± 7 days) after the last dose of study drug.

[c] Subjects who are eligible for the Part A Treatment Cohort and were randomized to Treatment Arm A in Study 103 or Study 104 are eligible for enrollment in Treatment Arm 1. Subjects who are eligible for the Part A Treatment Cohort and were randomized to Treatment Arm B in Study 103 or Study 104 are eligible for enrollment in Treatment Arm 2. Subjects who are eligible for the Part A Treatment Cohort and received 24 weeks of placebo in Study 103 or Study 104 (Treatment Arm C) will be randomized (1:1) to 1 of the 2 treatment arms in the Part A Treatment Cohort.

[d] The following subjects from Study 103 or Study 104 may be eligible for enrollment in the Part A Observational Cohort: (1) subjects who received at least 4 weeks of study drug in Study 103 or 104 and who are not eligible for the Part A Treatment Cohort with lumacaftor in combination with ivacaftor and (2) subjects who received at least 4 weeks of study drug in Study 103 or 104 and who elect not to continue treatment with lumacaftor in combination with ivacaftor.

[e] A telephone contact will be made every 3 to 4 months during the first year and at approximately 2 years (± 4 weeks).

[f] The following subjects from Cohort 4 of Study 102 may be eligible for enrollment in the Part B Treatment Cohort: (1) who are receiving study drug treatment at the end of treatment in Cohort 4 of Study 102 and (2) who are not receiving study drug treatment at the end of treatment in Cohort 4 of Study 102 AND who have received Vertex approval for entry. Subjects who prematurely discontinued study drug treatment are not eligible for enrollment in the Part B Treatment Cohort.

Table 5 Study Drug Administration in Clinical Trial VX12-809-105

Table 11-1 Study Drug Administration

Treatment Arm	Time	LUM/IVA (200/125 mg per tablet)	LUM/IVA 200/125 matching placebo	LUM/IVA (200/83 mg per tablet)	LUM/IVA 200/83 matching placebo	IVA (125 mg/ tablet)	IVA matching placebo
Part A: Treatment Cohort							
Arm 1 600 mg LUM qd + 250 mg IVA q12h	AM	None	2 tablets	3 tablets	None	None	None
	PM	None	2 tablets	None	None	2 tablets	None
Arm 2 400 mg LUM q12h + 250 mg IVA q12h	AM	2 tablets	None	None	3 tablets	None	None
	PM	2 tablets	None	None	None	None	2 tablets
Part B: Treatment Cohort							
Arm 3 400 mg LUM q12h + 250 mg IVA q12h	AM	2 tablets	None	None	3 tablets	None	None
	PM	2 tablets	None	None	None	None	2 tablets

IVA: ivacaftor; LUM: lumacaftor; q12h: every 12 hours; qd: daily.

2.7 Previous Clinical Experience

Lumacaftor monotherapy has been investigated in 2 clinical studies in subjects with CF (Study VX08-809-101 [Study 101] and VX09-809-102 [Study 102]).

Study 101 was a 28-day double-blind, placebo-controlled, multiple-dose, dose finding study investigating lumacaftor monotherapy in subjects with CF who are homozygous for the *F508del-CFTR* mutation. Study 101 demonstrated that lumacaftor monotherapy at doses up to 200 mg was well tolerated, but did not show a clinically or statistically significant change in FEV_1 despite a dose-dependent decrease observed in sweat chloride levels in subjects who received lumacaftor compared to those who received placebo.

Study 102 is a Phase 2 double-blind, placebo-controlled, multiple-dose, dose finding study evaluating the safety, tolerability, and efficacy of lumacaftor monotherapy and lumacaftor and ivacaftor combination therapy in subjects with CF. Study 102 consists of a period of lumacaftor monotherapy followed by a period of lumacaftor and ivacaftor combination therapy in subjects with CF who are homozygous or heterozygous for the *F508del-CFTR* mutation. During the 28-day lumacaftor monotherapy period in Cohort 2 of Study 102, there was no evidence of improvement in lung function; however, there was a 9.65% relative increase (6.05% absolute increase) in percent predicted FEV_1 during the 28-day period of administration of 600 mg lumacaftor qd in combination with 250 mg ivacaftor q12hr in subjects homozygous for the *F508del-CFTR* mutation that was both statistically and clinically significant. A similar increase in FEV_1 was observed in Cohort 3 of Study 102 during the period of treatment with 400 mg lumacaftor q12hr in combination with 250 mg ivacaftor q12hr. The Sponsor contended that the data from Study 102 demonstrated proof of concept that pharmacologic modulation of CFTR

function through treatment with lumacaftor in combination with ivacaftor can result in clinical benefit in subjects who are homozygous for the *F508del-CFTR* mutation. The results of Study 102 are also consistent with *in vitro* nonclinical studies of airway epithelial cells from patients homozygous for the *F508del-CFTR* mutation in which the response to lumacaftor and ivacaftor combination therapy was greater than that observed when either compound was administered alone.

Given the lack of efficacy of lumacaftor monotherapy in clinical studies, coupled with a low response in vitro to lumacaftor alone in airway epithelial cells from patients homozygous for the *F508del-CFTR* mutation, further clinical evaluation of lumacaftor monotherapy is unlikely to reveal significant benefit.

- **Rationale for dose of 600 mg Lumacaftor QD**

In Study 102, all treatment groups either remained stable or demonstrated a modest reduction in FEV_1 during the 28-day period of lumacaftor monotherapy. In contrast, during the 28-day period of combination therapy, an increase in FEV_1 was observed in the active treatment cohorts, while a decrease in FEV_1 was observed in the placebo group. The 600 mg lumacaftor qd dosage in combination with 250 mg ivacaftor q12hr demonstrated a significant improvement in FEV_1 in subjects with CF who are homozygous for the *F508del-CFTR* mutation. In subjects who received 200 and 400 mg lumacaftor qd in combination with 250 mg ivacaftor q12hr, a smaller increase in FEV_1 was observed during the period of combination therapy; however, the within-group analysis revealed that the increase in FEV_1 was not statistically or clinically significant. Previously in the ivacaftor development program, ivacaftor monotherapy had shown no statistically significant difference in FEV_1 over 16 weeks of treatment compared with placebo in subjects with CF who are homozygous for the *F508del-CFTR* mutation (see below). The Sponsor contended that these results suggested that the minimum clinically effective dose combination in subjects with CF who are homozygous for the *F508del-CFTR* mutation is 600 mg lumacaftor qd in combination with 250 mg ivacaftor q12hr.

- **Rationale for dose of 400 mg Lumacaftor q12hr**

Nonclinical studies of lumacaftor in airway epithelial cells derived from patients with CF and other model cell systems demonstrated a sigmoidal exposure-response relationship and suggest that a sufficiently high level of lumacaftor must be maintained throughout the dosing interval to maintain CFTR correction. To explore the potential for an advantageous PK profile and additional efficacy beyond the 600 mg lumacaftor qd regimen, a 400 mg lumacaftor q12hr dosage in combination with 250 mg ivacaftor q12hr was added to the Phase 2 study (Cohort 3 of Study 102). The 400 mg lumacaftor q12hr regimen allows for an approximately 2-fold increase in the expected trough concentration relative to the 600 mg lumacaftor qd regimen and reduced peak-to-trough ratio while incurring only a modest increase in the total daily dose and exposure of lumacaftor. The regimen of 400 mg lumacaftor q12hr in combination with 250 mg ivacaftor q12hr was shown to be safe and efficacious in Cohort 3 of Study 102. Although the 400 mg lumacaftor q12hr regimen could not be differentiated from the 600 mg lumacaftor qd regimen in the Phase 2 study, both doses will be studied in Phase 3 given the potentially advantageous PK profile of the former.

- **Rationale for dose of 250 mg Ivacaftor q12hr**

In the initial drug interaction study (Study VX09-809-005 [Study 005]) between 200 mg lumacaftor qd and the approved dosage of ivacaftor (150 mg, q12hr), a significant reduction in the plasma concentrations of ivacaftor was observed when lumacaftor was administered in combination with ivacaftor. Based on the observed reduction in ivacaftor exposure, the dosage of ivacaftor was increased to 250 mg q12hr from the approved ivacaftor dosage of 150 mg q12hr when administered alone. In the ivacaftor monotherapy program, the concentration at which effect is at 90% of the maximum (EC_{90}) was estimated using a population PK/pharmacodynamic (PD) model for the G551D-CFTR population. Nonclinical studies of ivacaftor in airway epithelial cells derived from patients with CF have shown that ivacaftor has increased potency in F508del-CFTR-expressing cells relative to G551D-CFTR-expressing cells. With the dose of 250 mg ivacaftor q12hr given in combination with lumacaftor, the ivacaftor trough concentration is projected to be above the EC_{90} for the F508del-CFTR population when adjusted for the shift in potency between F508del-CFTR and G551D-CFTR populations. The dose of 250 mg ivacaftor q12hr dosage was shown to be safe and efficacious in combination with both the 600 mg qd and 400 mg q12hr lumacaftor regimens in Study 102.

Study VX08-770-104 (Study 770-104) was a Phase 2, randomized, double-blind, placebo-controlled, parallel-group, multiple-dose study that evaluated the effects of ivacaftor monotherapy for 16 weeks in subjects with CF homozygous for the *F508del-CFTR* mutation. No significant benefit was observed from ivacaftor monotherapy treatment in this population. The Sponsor contends that the data from Study 102 and Study 770-104 are consistent with the mechanistic hypothesis that the combination of lumacaftor and ivacaftor leads to increased epithelial cell chloride transport, exceeding the additive benefits of each agent alone.

2.8 Regulatory Background

The IND was opened on October 19, 2007.

An EOP2 Meeting was held between the Division and Sponsor on February 12, 2013 (See Meeting Minutes dated March 20, 2013).

3 Studies Submitted

3.1 Studies Reviewed

1. VX-809: A 3-Month Oral (Gavage) Toxicity and Toxicokinetic Study in Dogs with a 1-Month Recovery Period (VX-809-TX-008)
2. VX-809: 12-Month Oral Toxicity and Toxicokinetic Study in Dogs with a 6-Month Interim Sacrifice and a 1-Month Recovery Period (VX-809-TX-014)
3. VX-809 AND VX-770: A 28-Day Oral (Gavage) Combination Toxicity and Toxicokinetic Study in Rats with a 14-Day Recovery Period (VX-809-TX-009 and VX-770-TX-015).
4. VX-809, VX-770, and VRT-0995096: A 3-Month Oral (Gavage) Combination Toxicity and Toxicokinetic Study in Rats with a 28-Day Recovery Period (VX-809-TX-013, VX-770-TX-026, and VRT-0995096-TX-005)
5. VX-809 AND VX-770: A 28-Day Oral (Gavage) Combination Toxicity and Toxicokinetic Study in Dogs with a 14-Day Recovery Period (VX-809-TX-010 and VX-770-TX-016)

3.2 Studies Not Reviewed

Reviews of reproductive toxicology studies will follow shortly

3.3 Previous Reviews Referenced

6 General Toxicology

6.2 Repeat-Dose Toxicity

DOGS

Study title: VX-809: A 3-MONTH ORAL (GAVAGE) TOXICITY AND TOXICOKINETIC STUDY IN DOGS WITH A 1-MONTH RECOVERY PERIOD

Study no.: VX-809-TX-008
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: December 30, 2008 (Signed protocol)
January 13, 2009 (Dosing initiation)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity:

Lot Numbers	Purity	Description	Dates Received	Retest Dates
PT–C07091701–M08001	Assume 100%	White to off–white (b) (4)	31 Dec 2008	(b) (4)
PT–C07091701–M08002	Assume 100%	White to off–white (b) (4)	16 Feb 2009	

Key Study Findings

● In a 3-month oral (gavage) toxicology study, dogs received VX-809 at doses of 0, 125, 250, 500, or 1000 mg/kg/day. At the end of the dosing period, 4 dogs/sex/group were sacrificed. After a 1-month recovery period, an additional 2 dogs/sex/group in the 0, 500, and 1000 mg/kg/day groups were sacrificed.

● Treatment-related moribund sacrifices occurred for 2 males and 1 female in the 1000 mg/kg/day group. Two dogs developed irregular gait, jerky movements and/or muscle rigidity prior to sacrifice.

● Irregular gait, trembling, jerky movements, and/or muscle rigidity were observed sporadically in individual animals at 1000 mg/kg/day after approximately 2 months of VX−809 administration (a total of 5 males and 3 females at 1000 mg/kg/day were observed with these findings). There were no histopathological findings that correlated to clinical signs or moribund sacrifices.

● Histopathological findings were evident for dogs at 1000 mg/kg/day and included the thymus (increased severity of lymphocyte depletion), male reproductive organs (delays of maturation in the testes and prostate and sloughed germ cells in the epididymides), and liver (extramedullary hematopoiesis). Findings were generally reversible by the end of the recovery period with the exception of the epididymides. Findings in the thymus and liver were not considered dose-limiting.

● The NOAEL was identified 500 mg/kg/day based upon deaths, neurological clinical signs, and histopathological findings in the male reproductive organs at 1000

mg/kg/day. $AUC_{0\text{-}24\ hr}$ values for females and males at 500 mg/kg/day on day 91 were 932 and 897 µg·hr/mL, respectively.

Methods

Doses:	0, 125, 250, 500, or 1000 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% (w/v) Tween–80 + 0.5% (w/v) methylcellulose + 0.05% (w/v) simethicone in water
Species/Strain:	Beagle dogs were obtained from (b) (4)
Number/Sex/Group:	4 dogs/sex/group
Age:	Approximately 5.5-6.5 months old at the start of dosing
Weight:	

	Mean	Range
Males	9.1	7.6 – 11.1
Females	6.7	6.1 – 7.6

Satellite groups:	After a 1-month recovery period, an additional 2 dogs/sex/group in the 0, 500, and 1000 mg/kg/day groups were sacrificed.
Unique study design:	None
Deviation from study protocol:	Deviations were generally minor and did not affect the overall integrity of the study. Animal #5367 was placed on recovery on Day 85 (due to clinical signs and decreased food consumption), therefore receiving 83 days of dosing rather than the protocol required 91 days of dosing. In addition, the end of Month 3 urinalysis and electrocardiogram procedures were not performed (due to the clinical condition of the animal). This animal was subsequently sacrificed in a moribund condition.

Table 6 Design of the 3-month toxicology study with VX-809 in dogs

9.3.1 Study Design

The test and control articles were administered by daily oral intubation (gavage) to Beagle dogs, once daily for 3 months followed by a 1–month recovery period.

	Daily Dose[a]			Number of Animals							
				Toxicity Animals		Clinical Pathology		Necropsy		Microscopic Pathology	
				Main[b]	Recovery	Main (Term)	Recovery	Main (Day 92)	Recovery	Main	Recovery
Group	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F
1	0	5	0	4/4	2/2	4/4	2/2	4/4	2/2	4/4	2/2
2	125	5	25	4/4	0/0	4/4	0/0	4/4	0/0	4/4	0/0
3	250	5	50	4/4	0/0	4/4	0/0	4/4	0/0	4/4	0/0
4	500	5	100	4/4	2/2	4/4	2/2	4/4	2/2	4/4	2/2
5	1000	5	200	4/4	2/2	4/4	2/2	4/4	2/2	4/4	2/2

[a] Doses represent active ingredient.
[b] Toxicokinetic samples were collected on Days 1, 45 and 91 at 0.5, 1, 2, 4, 8, 12 and 24 hours post–dose.

Numbers shown under M/F represent the total numbers of males and females in each dose group.
The first day of dosing was defined as Day 1 of the study.

Observations and Results

Mortality: Observations for mortality and general condition were made at least twice daily.

Treatment-related moribund sacrifices occurred for 2 males and 1 female in the 1000 mg/kg/day group.

Two 1000 mg/kg/day dogs were sacrificed after exhibiting irregular gait, jerky movements and/or muscle rigidity (1 male (5370M) on day 74 and 1 female (5868F) on day 71). In addition, a male (5367M) from the 1000 mg/kg/day group that showed progressive weight loss and little or no food consumption during the dosing phase was taken off dosing. In the absence of any improvement after an 8-day dose-free period, it was sacrificed on day 93. There were no histopathological findings in these 3 dogs that explained causes of death (moribund sacrifice).

Clinical Signs: Observations for general condition were made at least twice daily. Physical examinations were conducted once pretest and once weekly during the dosing and recovery periods.

The administration of VX−809 was associated with an increase in the incidence/occurrence of abnormal stool in both genders at ≥125 mg/kg/day (unformed, watery, and red exudate in stool) with the highest incidence occurring at ≥ 500 mg/kg/day. Apparent test article in stool at ≥250 mg/kg/day and yellow stool at ≥ 125 mg/kg/day were also observed. An increase in the incidence/occurrence of vomiting (with food/test article) was observed in both genders, most frequently at ≥ 500 mg/kg/day. Irregular gait, trembling, jerky movements, and/or muscle rigidity were observed sporadically in individual animals at 1000 mg/kg/day after approximately 2

months of VX−809 administration (a total of 5 males and 3 females at 1000 mg/kg/day were observed with these findings).

Body Weights: Body weights were recorded for all animals three times during the pretest period (including the day prior to dose initiation), weekly throughout the dosing and recovery periods, and terminally after fasting. Terminal fasted body weights were obtained just prior to necropsy.

Decreased body weight gain or body weight loss were observed for males and females at ≥250 mg/kg/day.

Feed Consumption: A visual estimate of the amount of feed consumed per day was made for each dog on a daily basis beginning one week prior to study initiation and continuing throughout the dosing and recovery periods.

Decreased feed consumption (under 50 % consumed) was noted in individual animals of both sexes at 1000 mg/kg/day. Some animals with little or no feed consumption had to be supplemented with canned food. Decreased feed consumption was also noted in individual females at ≥ 250 mg/kg/day. During the recovery period, feed consumption was considered comparable between control and test article groups.

Ophthalmoscopy: Ophthalmic examinations were conducted during the pretest period, at the end of the dosing period (Week 12), and recovery period (Week 4).

There were no treatment-related ophthalmic effects.

ECG: Tracings were taken on unanesthetized dogs positioned in right lateral recumbancy, pretest, at study termination at approximately 3-4 hours after completion of dosing, and at the end of recovery. A 9-lead electrocardiogram recording was made using standard limb leads I, II and III; augmented leads aVR, aVL, and aVF as well as chest leads V2, rV2 and V10. Quantitative assessments included heart rate and duration of the P wave, and PR, RR, QRS and QT and QTc intervals.

ECG examinations at the end of the dosing period found small increases of the PR, QT, and QTc intervals for males at 1000 mg/kg/day and small increases of the QT and QTc intervals for all female treatment groups. There was some suggestion of QTc prolongation (>250 msec) for males at 1000 mg/kg/day. Observed changes for females were small.

Table 7 ECG data at the end of the 3-month dosing period

Parameter	Males					Females				
	0	125	250	500	1000	0	125	250	500	1000
PR interval msec	115	109	115	107	**120**	105	111	109	105	107
QT interval msec	224	214	230	226	**251**	213	215	**228**	**223**	**234**
QTcV internal msec	248	244	245	250	**268**	244	**252**	**250**	**252**	**254**

Hematology: Blood obtained from unanesthetized animals by jugular or femoral venipuncture was used to analyze a complete panel of hematology and coagulation parameters for all animals pretest, up to 6 animals/gender/group at Week 9 and study termination, and up to 2 animals/gender/Groups 1, 4 and 5 at the end of recovery. Animals were fasted overnight prior to the blood collection interval.

There were changes of several hematology parameters at 9 weeks and 3 months.

Increased platelet counts and decreased RBC parameters were evident at 9 weeks. Increased platelet counts were observed for males at 500 and 1000 mg/kg/day and females at 1000 mg/kg/day. Mean platelet volumes were decreased for females in all drug-treated groups, although there was no dose-response relationship. Red blood cell counts, hemoglobin levels, and hematocrit were decreased for males and females at 500 and 1000 mg/kg/day.

Increased platelet counts and decreased RBC parameters were evident at 3 months. Increased platelet counts were observed for males at 500 and 1000 mg/kg/day and females in all drug-treated groups. Mean platelet volumes were decreased for females in all drug-treated groups, although there was no dose-response relationship. Red blood cell counts, hemoglobin levels, and hematocrit were decreased for males and females at 250, 500, and 1000 mg/kg/day.

Table 8 Hematology changes in dogs treated with VX-809 for 3 months

Table 10.9.1–1: Changes in Hematology Values[1] in Dogs Dosed Orally with VX–809 for 3 Months

Dose Level (mg/kg/day)	125	250	500	1000	125	250	500	1000
	Males				Females			
Week 9								
PLT	–	–	+31%	+49%*	–	–	–	+83%**
MPV	–	–	–	–	–19%*	–20%*	–10%**	–22%***
HGB	–		–8%	– 20%***	–	–	–6%	–17%***
HCT	–	–	–7%	– 22%***	–	–	–5%	–18%***
RBC	–	–	–9.5%	– 24%***	–	–	–10%*	–21%***
Month 3								
PLT	–	–	+28%	+21%	+54%*	+65%*	+64%*	+71%**
MPV	–	–	–	–	–14%*	–17%*	–15%*	–16%*
HGB	–	–8%	–9%	–24%**	–	–15%*	–12%*	–21%***
HCT	–	–9%	–9%	– 25%***	–	–15%*	–13%**	–21%***
RBC	–	–8%	–11%	– 28%***	–	–16%**	–17%**	–25%***

*Absolute values were statistically significantly different from controls.
[1] Percentage change or fold increase compared to corresponding control values.
PLT = Platelet; MPV=Mean Platelet Volume; HBG= Hemoglobin; HCT=Hematocrit
RBC=Red Blood Cell; * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$

Statistically significant shorter APTT intervals were observed for males at ≥250 mg/kg/day and females at ≥125 mg/kg/day. This may parallel increased platelet counts.

Table 9 Changes of APTT in dogs treated with VX-809 for 3 months

Table 10.9.2–1: Shorter APTT (seconds) in Dogs Dosed Orally with VX–809 for 3 Months

Dose Level (mg/kg/day)	125	250	500	1000	125	250	500	1000
	Males				Females			
Week 9								
APTT	–	3.7**	2.9**	3.6***	3.8***	3.0***	2.0***	3.9***
Month 3								
APTT	–	4.0*	3.6*	4.0*	4.3***	3.4***	3.2***	4.5***

*Absolute values were statistically significantly different from controls.
APTT= Activated Partial Thromboplastin Time
* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$

Clinical Chemistry: Blood obtained from unanesthetized animals by jugular or femoral venipuncture was used to analyze a complete panel of clinical chemistry parameters for all animals pretest, up to 6 animals/gender/group at Week 9 and study termination, and up to 2 animals/gender/Groups 1, 4 and 5 at the end of recovery. Animals were fasted overnight prior to the blood collection interval.

Treatment-related decreases of triglyceride, cholesterol, total protein, and globulin were observed at 9 weeks and 3 months.

At 9 weeks, BUN levels were increased for males at 1000 mg/kg/day and females at doses ≥250 mg/kg/day, although no treatment-related histopathological findings were evident in the kidneys. Triglyceride and cholesterol levels were decreased for all male and female drug-treated groups. Total protein and globulin levels were decreased for males and females at 1000 mg/kg/day. Total protein levels were also slightly decreased for females at 500 mg/kg/day.

At 3 months, BUN levels were increased for males and females at 1000 mg/kg/day, although no treatment-related histopathological findings were evident in the kidneys. Triglyceride and cholesterol levels were decreased for all male and female drug-treated groups. Total protein levels were decreased for males at 1000 mg/kg/day and females at 500 and 1000 mg/kg/day. Globulin levels were decreased for all male drug-treated groups and females at 500 and 1000 mg/kg/day. Small changes of electrolytes were evident for females at 1000 mg/kg/day (i.e., sodium, potassium, calcium, and magnesium) that appeared to have little or no biological significance.

Table 10 Changes of clinical chemistry parameters in dogs treated with VX-809 for 3 months

Table 10.9.3–1: Main Changes in Clinical Chemistry in Dogs Dosed Orally with VX–809 for 3 Months

Dose Level (mg/kg/day)	125	250	500	1000	125	250	500	1000
	Males				Females			
Week 9								
TRIG	–63%***	–42%***	–49%***	–74%***	–46%**	–46%**	–62%***	–65%***
CHOL	–35%**	–30%**	–29%**	–40%***	–32%**	–29%**	–37%***	–43%***
TP	–	–	–	–9%**	–	–	–8%	–19%***
GLOB	–	–	–	–17%***	–	–	–	–23%***
BUN	–	–	–	+53%***	–	+60%***	+33%***	+53%***
Month 3								
TRIG	–43%*	–37%*	–26%*	–57%**	–46%**	–67%***	–64%***	–54%***
CHOL	–29%*	–26%*	–23%*	–48%***	–32%**	–37%***	–40%***	–45%***
TP	–	–	–	–15%***	–	–	–9%*	–20%***
GLOB	–12%*	–12%*	–8%*	–25%***	–	–	–9%*	–23%***
BUN	–	–	–	+59%**	–	–	–	+44%***

*Absolute values were statistically significantly different from controls.
TRIG = Triglycerides; CHOL = Cholesterol; TP=Total Protein; GLOB=Globulin;
BUN=Blood Urea Nitrogen; * – p<0.05; ** – p<0.01; *** – p<0.001

Urinalysis: Urine obtained by an approximate 16-hr overnight collection period was analyzed for a complete panel of urinalysis parameters from all animals pretest and up to 6 animals/sex/group at study termination and up to 2 animals/sex/ group for Groups 1, 4, and 5 at the end of recovery. Animals did not have access to food or water during urine collection.

There were no treatment-related changes of urinalysis parameters.

Gross Pathology: Necropsy was performed on up to 4 animals/sex/group after animals had been dosed for at least 3 months and 2 animals/sex/group for Groups 1, 4 and 5 after the 1-month recovery period. Samples of heart, liver, and blood were collected, frozen to -80°C, and shipped to the sponsor for possible biomarker analysis.

At the end of 3-month dosing period, treatment-related gross pathological findings of small thymus were observed for 1 male each at 500 and 1000 mg/kg/day, 1 female at 250 mg/kg/day, and 3 females at 1000 mg/kg/day.

In the 1000 mg/kg/day group, a small thymus (severe) was present in male #5366M that survived to the end of the dosing period and a small thymus (moderate) was present in male #5367M that was euthanized after an 8 day drug free period. For females at 1000 mg/kg/day, slight to moderate small thymus was observed in all 3 females (Numbers 5865F, 5866F, and 5867F). Small thymus size correlated with decreased organ weight for females at 1000 mg/kg/day, but not males. Small thymuses were also observed in a

500 mg/kg male (No. 4367M, moderate) and a 250 mg/kg female (No. 3868F, slight). Decreased size correlated with microscopic findings of lymphoid depletion.

Organ Weights: Organs weights were measured for the adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, testes, thyroid/parathyroid, and uterus from all animals at the scheduled sacrifice intervals.

Decreased thymus weights were observed in males at 1000 mg/kg/day and females at ≥125 mg/kg/day that correlated with histopathological findings of lymphoid depletion. Spleen weights were decreased for males at 500 and 1000 mg/kg/day and females at 1000 mg/kg/day, although there were no corresponding histopathological findings. Liver weights were increased for females at 1000 mg/kg/day, although histopathological findings were limited to increased hematopoiesis.

Table 11 Changes of thymus weight in dogs treated with VX-809 for 3 months

Table 10.10–1: Changes in Absolute Organ Weight[1] Values in Dogs Dosed Orally with VX–809

Dose Level (mg/kg/day)	125	250	500	1000	125	250	500	1000
	Males				Females			
Thymus	–	–	–	–59%	–42%*	–56%**	–55%**	–75%**

*Absolute values were statistically significantly different from controls
[1]Percentage change compared to corresponding control values
* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$

Histopathology: Tissues and organs from all animals were routinely processed, embedded in paraffin, cut at a microtome setting of 4–7 microns, mounted on glass slides, stained with hematoxylin and eosin and, examined by light microscopy.

Adequate Battery: A complete panel of tissues was submitted to histopathological examination.

Table 9.3.14.4–1 – Tissues preserved and examined microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X	
bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X[a]
brain (medulla, pons, cerebrum, and cerebellum)	X	X	X
epididymides		X	X
esophagus		X	X
eyes		X	X
gall bladder		X	X
heart	X	X	X
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
liver	X	X	X
lungs (with mainstem bronchi)	X	X	X
lymph nodes (mesenteric and mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
optic nerve		X	X
ovaries	X	X	X
pancreas		X	X
pituitary gland	X	X	X
prostate gland	X	X	X
salivary glands (submandibular)		X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
skeletal muscle (*Biceps femoris*)–longitudinal and transverse		X	
skeletal muscle (*m. gastrocnemius*)– longitudinal and transverse		X	X
skeletal muscle (*m. psoas)*– longitudinal and transverse		X	
skin		X	X
small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumbar)		X	X
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid with parathyroid	X	X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns/cervix)	X	X	X
vagina		X	X
gross lesions		X	X

[a]Qualitative examination (no differential count).

Peer Review: None.

Histological Findings: Histopathological findings were evident at the high dose of 1000 mg/kg/day that included the thymus (increased severity of lymphocyte depletion), male reproductive organs (delays of maturation in the testes and prostate and sloughed germ cells in the epididymides), and liver (extramedullary hematopoiesis) as shown in the table below. Findings were generally reversible by the end of the recovery period with

the exception of the epididymides. Findings in the thymus were not considered dose-limiting based upon the high background incidence in the control group. Findings in the liver (extramedullary hematopoiesis) may have been compensatory responses to decreased RBC counts and were not considered dose-limiting.

Table 12 Histopathological findings in dogs at the end of the 3-month dosing and 1-month recovery periods

Organ/Tissue	Sex	End of dosing period					End of recovery period		
		0	125	250	500	1000	0	500	1000
Thymus									
-decreased lymphocytes	M	4/4	4/4	4/4	3/3	2/2	2/2	1/2	0/2
	F	3/4	4/4	4/4	3/4	2/3	2/2	2/2	1/2
-minimal	M	0	1	0	1	0	1	0	0
	F	2	2	1	2	0	0	0	0
-slight	M	2	0	0	2	0	0	1	0
	F	1	2	1	1	0	1	2	1
-moderate	M	2	3	4	0	1	1	0	0
	F	0	0	1	1	2	1	0	0
-marked	M	0	0	0	0	1	0	0	0
	F	0	0	1	0	1	0	0	0
Testes									
-immaturity, minimal	M	0/4	0/4	0/4	0/4	1/2	0/2	0/2	0/5
Epididymides									
-sloughed germ cells and germ cell debris, slight	M	0/4	0/4	0/4	0/4	1/2	0/2	0/2	1/2
Prostate									
-immaturity	M	0/4	0/4	0/4	0/4	1/2	0/2	0/2	0/2
Liver									
-extramedullary hematopoiesis, slight	M	0/4	1/4	0/4	0/4	1/2	0/2	0/2	0/2
	F	1/4	0/4	0/4	0/4	2/3	0/2	0/2	0/1
Thyroid									
-C cell hyperplasia, minimal	M	0/4	0/4	0/4	0/4	0/2	0/2	0/2	0/2
	F	0/4	0/4	0/4	0/4	1/2	0/2	0/2	0/2

Special Evaluation: None

Toxicokinetics: On Days 1, 45 and 91, blood samples for toxicokinetic determinations were obtained from 6 animals/gender/dose Groups 1, 4 and 5, and 4 animals/sex/dose Groups 2 and 3 per time point at 0.5, 1, 2, 4, 8, 12, and 24 hr postdose. Blood samples were collected from Group 1 animals at all time points; however, a single time point, 4 hr postdose, was analyzed. The DMPK-NCD group at Vertex Pharmaceuticals Inc. (Cambridge, MA) conducted the plasma sample analyses using a fully validated bioanalytical method with liquid chromatography/tandem mass spectrometry (LC-MS/MS), which had a lower limit of quantitation (LLOQ) of 2.00 ng/mL. The calibration standard concentrations over the range of 2.00 and 2000 ng/mL were analyzed using a

linear regression method. Reversed-phase high performance liquid chromatography was utilized as the separation method and mass spectrometry was used as the detection method in quantitative analysis. Samples were extracted by methyl tertiary-butyl ether (MTBE) using liquid–liquid extraction.

C_{max} and AUC values increased with elevating dose; however, increases were generally less than dose proportional. There was no evidence of drug accumulation over the 3-month dosing period. There were no sex-related differences in exposure.

Table 13 Toxicokinetic parameters for dogs

Table 10.2–1– Summary of Toxicokinetic Parameters for VX–809 in Female and Male Dogs Following Once Daily Oral Administration of 125, 250, 500 and 1000 mg/kg/day of VX–809 on Day 1, Day 45 and Day 91

Study Day	Dose of VX–809 (mg/kg)	Gender							
		Female				Male			
		C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)
Day 1	125	33.6±2.53	1.33±0.58	185±86.6	5.07±1.70	31.2±13.6	4.25±5.19	315±123	13.86±8.
	250	63.1±8.76	2.00±0.00	467±236	11.03±5.28	45.4±14.8	1.75±0.50	215±76.5	5.52±0.7
	500	73.9±29.8	3.50±1.22	501±222	5.31±0.59	57.6±20.4	2.67±1.51	431±136	7.42±4.9
	1000	123±35.0	3.67±0.82	1410±596	7.35±7.07	108±23.8	4.67±1.63	1180±598	5.04±2.7
Day 45	125	41.6±13.4	6.00±4.90	358±272	5.45±2.11	32.9±4.27	7.00±5.77	376±121	4.16±0.7
	250	74.1±28.9	7.00±5.77	641±408	5.19±0.81	49.8±13.2	4.50±5.00	266±134	5.42±2.5
	500	99.6±11.3	5.33±3.01	868±250	3.99±1.05	62.6±15.5	2.83±1.33	645±346	7.19±9.7
	1000	170±71.9	4.33±1.97	1570±791	3.62±1.46	129±31.3	5.00±2.45	1170±408	4.44±1.6
Day 91	125	38.7±6.67	1.50±0.58	206±110	15.48±18.51	40.6±11.1	4.25±5.19	326±232	6.80±1.7
	250	91.6±13.2	4.25±5.19	688±308	5.05±1.32	66.7±17.1	5.00±4.76	473±147	4.98±0.8
	500	108±41.7	2.50±1.22	932±369	5.67±3.80	107±23.2	7.00±5.59	897±630	5.02±2.4
	1000	200±78.5	3.60±0.89	1460±553	3.52±0.76	167±25.3	2.00±0.00	1350±715	4.06±1.1

Dosing Formulation Analysis: Triplicate samples (1.0 mL each) were taken from the Days 45 and 91 formulations on the day prepared and used for concentration analysis. Results from the Day 1 middle homogeneity samples were used for Day 1 concentration verification. On Days 1, 45 and 91, duplicate control samples (1.0 mL each) were analyzed to verify that no cross contamination had occurred.

Dosing solutions were homogeneous. Actual concentrations of the dosing solutions on days 1, 45, and 91 ranged from 99.5 to 110.8% of nominal concentrations.

Study title: VX-809: 12-MONTH ORAL TOXICITY AND TOXICOKINETIC STUDY IN DOGS WITH A 6-MONTH INTERIM SACRIFICE AND A 1-MONTH RECOVERY PERIOD

Study no.: VX-809-TX-014
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: August 23, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity:

Supplier	Lot Number	Date Received	Expiration/ Retest Date
(b) (4)	L0300634 (Batch No. PT-C07091701-M09003)	29 Sep 2011 25 Oct 2011 13 Dec 2011 20 Dec 2011	(b) (4)
	L0300631 (Batch No. PT-C07091701-M09002)	25 May 2011 9 Aug 2011	
	11-401	13 Dec 2011	
	11-401	09 Mar 2012 30 May 2012	
	4281-P54-12-401	10 Aug 2012	
	121056	17 Aug 2012	

NA – not available

Key Study Findings

● In a 12-month oral (gavage) toxicology study with a 6-month interim sacrifice, dogs received VX-809 at doses of 0, 125, 250, or 500 mg/kg/day. At the end of the 6- and 12-month dosing periods, 4 dogs/sex/group were sacrificed. After 1-month recovery periods following treatment for 6 or 12 months, an additional 2 dogs/sex/group in the 500 mg/kg/day group were sacrificed. Concurrent control dogs were not included in the recovery periods.

● No deaths were attributed to treatment with VX-809.

● The administration of VX-809 was associated with increases in the incidence/occurrence of abnormal stool in both sexes at ≥125 mg/kg/day (unformed, watery, pale, white and/or yellow) with the highest incidence occurring at 500 mg/kg/day. A slight increase in the incidence/occurrence of vomiting (food) was observed in both sexes at 500 mg/kg/day.

● Red blood parameters (RBC counts, hemoglobin, and hematocrit) were slightly decreased for males and females at 250 and 500 mg/kg/day during months 3, 6, 9, and 12. Platelet counts were slightly increased for male drug-treated groups and females at 250 and 500 mg/kg/day during months 3, 6, 9, and 12. Slight decreases of absolute reticulocyte counts were observed for males and females at 500 mg/kg/day during months 9 and 12 and also for males and females at 250 mg/kg/day during month 12. These changes would be expected to be monitorable in a clinical setting.

● Cholesterol, triglyceride, total protein, albumin, and globulin levels were decreased for male and female drug-treated groups at months 3, 6, 9, and 12.

● Liver weights were increased for male and female drug-treated groups at 6 and 12 months; however, there were no corresponding histopathological findings.

● Judging the totality of the histopathological findings from the 6-month interim sacrifice and 12-month terminal sacrifice as well as the respective 1-month recovery periods, no treatment-related target organs of toxicity were identified with doses up to 500 mg/kg/day.

● The NOAEL was identified as the high dose of 500 mg/kg/day. $AUC_{0\text{-}24hr}$ values for males and females at 500 mg/kg/day on day 363 were 750 and 860 µg*hr/mL, respectively. Average steady-state $AUC_{0\text{-}24hr}$ values for males and females at 500 mg/kg/day from days 90 to 363 were 429 and 515 µg*hr/mL, respectively.

Methods

Doses:	125, 250, or 500 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral intubation using a funnel and catheter, a syringe of appropriate size and a flush system of approximately 5 mL pre-dose and 10 mL postdose of tap water.
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (w/v) + 0.5% Tween-80 (w/v) + 0.05% simethicone (w/v) in water
Species/Strain:	Beagle dogs
Number/Sex/Group:	4 dogs/sex/group were sacrificed at 6 and 12 months.
Age:	Approximately 17 to 19 weeks
Weight:	**Mean** **Range** Males 6.2 kg 4.8-7.5 kg Females 4.4 kg 3.2-5.3 kg
Satellite groups:	2 dogs/sex in the 500 mg/kg/day were sacrificed after a 28-day recovery period following drug treatment for 6 or 12 months. No concurrent control dogs were included in recovery groups.
Unique study design:	12-month dosing period with a 6-month interim sacrifice
Deviation from study protocol:	Deviations were generally minor and did not impact the integrity of the study.

Table 14 Design of 12-month toxicology study with dogs

The test and control articles were administered by oral intubation using a dosing syringe system to beagle dogs, once daily, for 12 months.

				Number of Animals							
						Toxicity Study				TK Study[c]	
						Necropsy				Day 1 and end of Months 3, 6, 9 and 12	
	Daily Doses[a]			Total on Study		Month 6		Month 12			
Group	VX-809 Dose (mg/kg)	Volume (mL/kg)	TA Conc (mg/mL)	M	F	Int M/F	Rec M/F	Term M/F	Rec M/F	M	F
1[b]	0	5	0	8	8	4/4	0/0	4/4	0/0	8	8
2	125	5	25	8	8	4/4	0/0	4/4	0/0	8	8
3	250	5	50	8	8	4/4	0/0	4/4	0/0	8	8
4	500	5	100	12	12	3/4	2/2	4/4	2/2	12	12

[a]Doses of VX-809 represent active ingredient.
[b]Vehicle alone, consisting of 0.5%w/v methylcellulose (MC) + 0.5% Tween 80 and 0.05% simethicone in water.
[c]Toxicokinetic samples were collected on Day 1, and at the end of Months 3, 6, 9 and 12.
The first day of dosing was defined as Day 1 of the study. Dose initiation was staggered for males and females

Observations and Results

Mortality: Animals were observed in their cages for mortality and general condition twice daily.

One dog (4180M) receiving the high dose at 500 mg/kg/day was euthanized on Day 176 prior to the interim sacrifice due to lameness in the left rear leg and radiographic evidence that the Sponsor diagnosed as “Legg-Perthes Disease”.

On Day 87, the animal was reported to vocalize when the left hind paw was touched, although movement in the cage was normal. A radiographic examination of the left hind paw was conducted on Day 90 and there was a diagnosis of potential femoral head necrosis. A radiographic examination was conducted on Day 164 and findings were indicative of osteonecrosis of the femoral head and the left femoral head and neck showed progressive irregularities of the margin. The animal walked and ran with no signs of acute pain, but the gait was slightly stilted (still fully weight bearing) with atrophy of left hind musculature evident. The animal resisted manual extension of left hind limb. On Day 175, the radiographic examination indicated femoral head necrosis.

Macroscopic findings included irregular shape and opaque white discoloration of the femoral head and neck and thickening of the coxofemoral joint capsule. Microscopically, there was marked necrosis of the femoral head and neck and marked fibrosis of the coxofemoral joint capsule. The Sponsor considered these findings to be consistent with “Legg-Perthes Disease”.

No other dogs (23 of 24 male and female dogs combined) in the 500 mg/kg/day group were noted with these findings for the joint capsule or femoral head at the end of the 6- or 12-month treatment periods or respective recovery periods. The findings for dog 4180M would appear to be congenital and unrelated to treatment with VX-809.

Table 15 Veterinary findings from Days 87 to 175 for Dog #4180M that received the high dose at 500 mg/kg/day

Animal Number	Test Day	Technician Findings	Veterinary Findings	Treatment	Follow up
Group 4 – 500 mg/kg/day					
4180	87	Favoring left hind limb	BAR; animal moving about normally on cage floor; vocalizes when left hind paw is touched; no Injuries visible, using limb gingerly when in home cage		
	90			Anesthetized with Propofol IV and radiographed	BAR; active; animal moves about cage normally; when left hind limb manipulated animal has sling pain response and then begins to favor left hind limb. Appears to be femoral head necrosis.
	104				Animal radiographed for favoring left hind limb; BAR; active; moves about normally outside of cage; no favoring seen at this time; no pain response when both hind limbs extended; left hind limb seems slightly stiff when trying to extend.

Animal Number	Test Day	Technician Findings	Veterinary Findings	Treatment	Follow up
Group 4 – 500 mg/kg/day					
4180 (cont.)	139				Animal previously diagnosed with possible femoral head necrosis of left hind limb; BAR; active; animal shows no signs or pain response when hind limbs manipulated ;hind limb appears very slightly stiff when animal moves about outside of cage; no favoring seen.
	163	Favoring left hind leg	Animal previously radiographed on Day 90; appears to have femoral head necrosis (left hind limb); BAR; very active; intermittently favoring left hind limb; no apparent swelling; limb appears WNL otherwise; slight pain response when left hind limb extended (vocalizing)	Anesthetized with Propofol IV and radiographed	
	164				Radiographed findings indicative of osteonecrosis of femoral head. Left femoral head and neck show progressive irregularity of margins. Animal walks and runs with no signs of acute pain but gait is slightly stilted (still fully weight bearing) with atrophy of left hind musculature evident. Resists manual extension of left hind limb.

Animal Number	Test Day	Technician Findings	Veterinary Findings	Treatment	Follow up
Group 4 – 500 mg/kg/day					
4180 (cont.)	175	Favoring left hind leg	Animal intermittently favors limb; radiographs show femoral head necrosis; BAR; very active; animal intermittently non-weight bearing in home cage; will run outside of cage without favoring; animal vocalizes when left hind leg manipulated.		Left hind limb atrophy.

Table 16 Gross pathological and histopathological findings at the hip joint and femoral head for Dog #4180M that received the high dose at 500 mg/kg/day

Prob Cause Death . . .	No gross observations on tissue.	
		FEMORAL HEAD NECROSIS (LEGG-PERTHES DISEASE), Present.
Joint		
	Irregular Shape, Hip, White, Diffuse, Severe/ Left hind leg,cartilage of femoral head is opaque. The head is moderately small and is ventrally flattened. The femoral neck is enlarged and irregular.	Examined; 1 correlation found: FEMORAL HEAD NECROSIS, Marked.
	Thickened joint capsule, Hip, White, Diffuse, Severe/ Left hindleg	Examined; 1 correlation found: JOINT CAPSULE FIBROSIS, Marked.

Clinical Signs: On dosing days, clinical observations were performed prior to and after dosing. For physical examinations, all study animals were removed from their cages and examined once pretest and once weekly during the study period.

Dosing Phase
The administration of VX-809 was associated with increases in the incidence/occurrence of abnormal stool in both sexes at ≥125 mg/kg/day (unformed, watery, pale, white and/or yellow) with the highest incidence occurring at 500 mg/kg/day. A slight increase in the incidence/occurrence of vomiting (food) was observed in both sexes at 500 mg/kg/day.

Recovery Phases
After a 28 recovery period, following 6 months of dosing, unformed stool and/or mucus in stool was observed in males at 500 mg/kg/day (6 observations in 2 males). No test article-related findings were noted after in the recovery period following 12 months of dosing.

Table 17 Clinical signs during the 12-month drug-treatment period (#observations/#dogs)

Clinical signs	Males				Female			
	0	125	250	500	0	125	250	500
Behavior, favoring limbs	3/1	8/1	0/0	31/1	32/1	0/0	0/0	2/1
Gastrointestinal, pale stool	0	6/5	49/8	114/12	0/0	0/0	11/7	93/12
Gastrointestinal, unformed stool	151/8	484/8	476/8	2063/12	68/6	359/8	539/8	1135/12

Gastrointestinal, watery stool	10/4	36/7	35/8	368/12	2/2	27/8	31/7	130/12
Gastrointestinal, white stool	0/0	51/8	156/8	728/12	0/0	13/6	99/8	642/12
Gastrointestinal, yellow stool	0/0	251/8	657/8	1381/12	0/0	199/8	583/8	1000/12
Oral, Vomit - Food	8/6	9/5	15/7	27/7	16/2	11/7	23/6	30/12
General appearance-thin	138/2	320/2	59/2	194/2	0/0	22/1	181/2	45/2
Abdominal distension	-	-	-	-	0/0	1/1	6/1	20/3

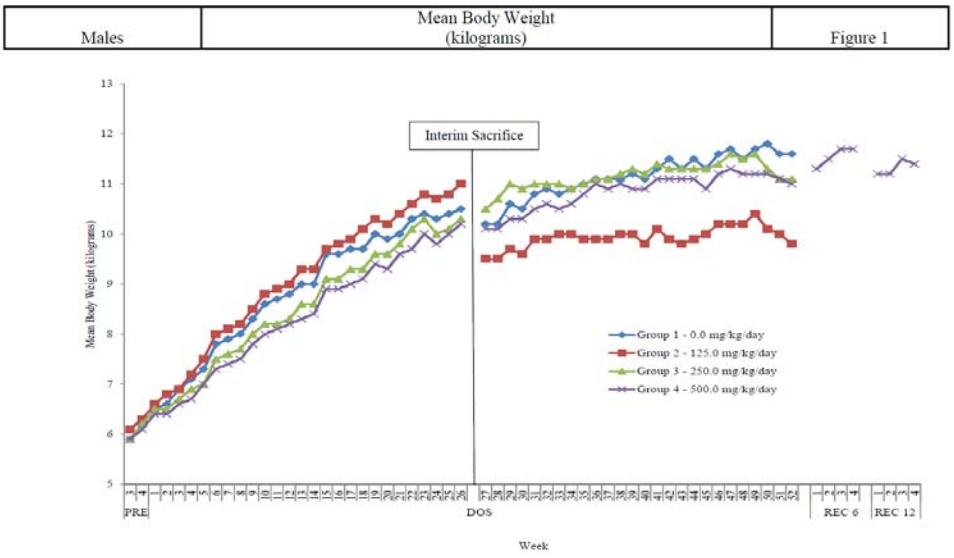
Body Weights: Non-fasted body weights for toxicity study animals were recorded twice pretest and weekly during the study period, and terminally on the day of the scheduled necropsy. Fasted body weights were obtained on the days of the scheduled necropsy intervals.

During the first 6-month of dosing, body weight gains for comparable for male control and drug-treated groups; however, body weight gains for females at 250 and 500 mg/kg/day were lower than the concurrent control. From weeks 27 to 52, body weight gains for male drug-treated group were lower than the concurrent control; however, body weight gains for female drug-treated groups were unaffected.

Table 18 Body weight gains for drug-treated male and female rats during the 6- and 12-month dosing periods

Body weight, kg	Male Dogs				Female Dogs			
	0	125	250	500	0	125	250	500
BW, Wk 1	6.5	6.6	6.5	6.4	5.1	5.0	5.0	5.0
BW, Wk 26	10.5	11.0	10.3	10.2	8.9	8.5	8.3	8.2
▲, Wk 1 to Wk 26	4.0	4.4	3.8	3.8	3.8	3.5	3.3	3.2
% of Wk 1	61.5	66.7	58.4	59.3	74.5	70.0	66.0	64.0
BW, Wk27	10.2	9.5	10.5	10.1	8.2	8.3	7.7	8.3
BW, Wk52	11.6	9.8	11.1	11.0	8.7	9.1	8.3	8.9
▲, Wk 27 to Wk 52	1.4	0.3	0.6	0.9	0.5	0.8	0.6	0.6
% of Wk 27	13.7	3.2	5.7	8.9	6.1	9.6	7.8	7.2

Figure 6 Body weights for male and female control and drug-treated dogs during the 6- and 12-month dosing periods



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Feed Consumption: A visual estimate of the amount of food consumed per day was made for each dog 7 times per week beginning one week prior to initiation of dosing (fewer observations were made when animals were fasted prior to blood collection or when urinalysis was performed).

Food consumption for male and female drug-treated groups appeared to qualitatively parallel body weight gains as described above.

Ophthalmoscopy: Ophthalmic examinations were conducted on all animals at pretest and the end of the Months 6 and 12.

No treatment-related ophthalmic changes were identified after treatment for either 6 or 12 months.

ECG: Nine-lead electrocardiogram tracings were collected from unanesthetized dogs positioned in right lateral recumbancy, pretest and during months 3, 6 9 and 12 (at T_{max} [approximately 3 hours post dose]) using standard limb leads I, II and III; augmented leads aVR, aVL, and aVF as well as chest leads V_2, rV_2 and V_{10}. Quantitative assessments including heart rates and PR, QRS, RR, QT and QTc intervals were calculated using EMKA Technologies ECG-Auto software. The corrected QT interval (QTc) was evaluated using the following formulas: QTcM = (Log $_{10}$ 600 x QT interval)/Log $_{10}$ RR interval [Matsunaga, 1997] and QTcV = QT – 0.087 x (RR-1000) [Van de Water, 1989].

There were no treatment-related changes of heart rate or PR, QRS, QT, RR, QTcM, and QTcV intervals during months 3, 6, 9, or 12.

Hematology: Blood samples for measurement of a complete panel of hematology and coagulation parameters were collected at pretest and the end of Months 3, 6, 9, and 12. Blood was also obtained from 2 animals/sex in Group 4 at the end of the Month 6 and Month 12 recovery periods. Animals were fasted overnight prior to blood collections except at the pretest collection.

Red blood parameters (RBC counts, hemoglobin, and hematocrit) were slightly decreased for males and females at 250 and 500 mg/kg/day during months 3, 6, 9, and 12. Platelet counts were slightly increased for male drug-treated groups and females at 250 and 500 mg/kg/day during months 3, 6, 9, and 12. Slight decreases of absolute reticulocyte counts were observed for males and females at 500 mg/kg/day during months 9 and 12 and also for males and females at 250 mg/kg/day during month 12. These changes would be expected to be monitorable in a clinical setting.

Table 19 Hematology changes in dogs at 3, 6, 9, and 12 months

Parameter	Time	Males				Females			
		0	125	250	500	0	125	250	500
Hemoglobin g/dL	3	13.7	12.9	12.6	11.6*	14.0	13.2	12.6*	11.4*
	6	14.5	14.7	13.3	12.9*	14.7	14.9	14.6	13.4*
	9	14.9	14.0	13.0	13.3*	14.9	14.1	13.0*	13.0*
	12	15.7	15.0	13.5*	13.4*	16.3	15.5	14.2*	12.9*
Hematocrit %	3	42.5	39.4	38.7*	35.9*	43.2	40.5	38.4*	35.0*
	6	43.1	43.7	39.6	38.7*	44.1	44.4	43.4	39.8*
	9	44.6	41.5	37.6*	39.1*	44.2	41.4	37.7*	37.9*
	12	48.3	45.4	40.9*	41.0*	49.8	47.0	43.4*	39.2*
Red blood cells $x10^6/\mu L$	3	6.19	5.78	5.72	5.09*	6.36	5.96	5.51*	5.00*

	6	6.38	6.43	5.93	5.62*	6.56	6.62	6.42	5.85*
	9	6.58	6.17	5.83	5.75*	6.59	6.23	5.65*	5.68*
	12	6.99	6.68	6.22	5.91*	7.33	6.93	6.41*	5.80*
Reticulocytes $x10^9$/L	3	44.5	29.0	45.5	39.8	41.3	63.7	40.1	40.6
	6	39.8	37.7	39.7	32.3	30.4	58.0	53.4	42.0
	9	43.2	46.0	37.2	25.8	33.2	34.8	36.6	28.8
	12	33.2	34.2	25.7	23.9	46.5	36.9	29.3	28.5
RDW %	12	12.3	13.1*	12.7*	12.8*				
Platelets $x10^3$/µL	3	310	363	377	407*	292	319	373	389*
	6	291	353	348	382*	308	316	359	395*
	9	302	354	367	386	326	336	423	389
	12	219	334	371*	402*	260	260	387*	424*
APTT Seconds	3	NC	NC	NC	NC	19.7	19.0	18.1	16.4*
	6	NC	NC	NC	NC	17.6	18.5	16.9	16.4
	9	NC	NC	NC	NC	16.3	17.1	16.1	16.0
	12	NC	NC	NC	NC	15.4	15.3	14.6	15.0
Eosinophils $x10^3$/µL	3	NC	NC	NC	NC	0.24	0.14	0.13*	0.11*
	6	0.34	0.33	0.27	0.20*	0.38	0.27	0.26	0.16*
	9	NC	NC	NC	NC	0.48	0.24*	0.30*	0.22*
	12	NC	NC	NC	NC	0.28	0.24	0.22	0.20

*$p < 0.05$

Clinical Chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected at pretest and the end of Months 3, 6, 9, and 12. Blood was also obtained from 2 animals/sex in Group 4 at the end of the Month 6 and Month 12 recovery periods. Animals were fasted overnight prior to blood collections except at the pretest collection.

Cholesterol, triglyceride, total protein, albumin, and globulin levels were decreased for male and female drug-treated groups at months 3, 6, 9, and 12. ALT activities were slightly increased for males and females at 250 and 500 mg/kg/day, although these increases did not achieve toxicological significance. AST and ALKP activities and total bilirubin levels were slightly decreased for male and female drug-treated groups, although the toxicological significance of these changes was unclear. Small changes of electrolyte levels (e.g., Na^+, Cl^-, and Ca^{2+}) were observed in drug-treated groups at 3 and/or 6 months; however, these changes were not evident at later time points and the toxicological significance of these changes was unclear based upon the small

magnitude of observed differences between control and drug-treated groups. The Sponsor correlated these changes to clinical observations of increases in the incidence/occurrence of abnormal stool in both sexes at ≥125 mg/kg/day.

Table 20 Clinical chemistry changes in dogs at 3, 6, 9, and 12 months

Parameter	Time	Males				Females			
		0	125	250	500	0	125	250	500
Cholesterol mg/dL	3	171	153	137*	118*	160	141*	131*	94*
	6	171	169	154	142*	192	170	157*	135*
	9	189	160	143*	138*	166	166	143	121*
	12	171	151	132	116*	167	154	120*	111*
Triglyceride mg/dL	3	37	25*	22*	11*	35	30	19*	12*
	6	36	35	26*	22*	47	39	33*	23*
	9	39	21*	16*	20*	44	21*	21*	16*
	12	53	32*	21*	18*	55	35*	17*	14*
Total protein g/dL	3	5.3	5.0*	5.0*	4.7*	5.2	4.9*	4.9*	4.5*
	6	5.5	5.4	5.3	5.0*	5.3	5.3	5.3	5.0*
	9	5.6	5.4	5.2*	5.2*	5.3	5.2	5.1	5.1
	12	5.7	5.4	5.0*	4.9*	5.4	5.1	4.9*	4.9*
Albumin g/dL	3	3.2	3.1	3.1	2.9*	3.2	3.1	3.1	2.9*
	6	3.2	3.3	3.2	3.1*	3.3	3.3	3.3	3.2
	9	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
	12	3.2	3.1	3.0	3.0*	3.3	3.1	3.0	3.0
Globulin g/dL	3	2.1	1.9*	1.9*	1.8*	2.0	1.8*	1.8*	1.6*
	6	2.3	2.1	2.1	1.9*	2.1	1.9	2.0	1.9*
	9	2.4	2.2	2.0*	2.0*	2.1	2.0	1.9	1.9
	12	2.6	2.3	2.0*	1.9*	2.1	2.0	1.8	1.8
ALT U/L	3	34	29	32	27*	32	36	40*	49*
	6	39	40	46	52*	33	40	46*	46*
	9	40	41	57	56	35	37	55	42
	12	39	37	51	46	28	29	39	47*
AST U/L	3	34	29	32	27*	35	32	29	32*
	6	40	33*	32*	31*	31	31	30	31
	9	40	29*	31*	29*	33	27	32	29

	12	37	29	32	31	27	24	23	26
ALKP U/L	3	110	100	95	92	118	89	92	80*
	6	82	66	59	61	85	67	68	51*
	9	91	63	40	58	76	51	45*	35*
	12	89	61	41	59	89	42*	46*	37*
Total bilirubin mg/dL	3	0.05	0.02	0.05	0.03*	0.07	0.05	0.07	0.05
	6	0.09	0.06*	0.05*	0.04*	0.11	0.05*	0.09*	0.04*
	9	0.13	0.08*	0.08*	0.06*	0.09	0.11	0.10	0.07
	12	0.10	0.05*	0.03*	0.02*	0.09	0.06	0.06	0.05*
Na^+ mEq/L	3	146	146	145*	145*	149	148	147*	146*
	6	147	147	146	146*	147	148	147	148
	9	145	146	145	145	146	145	142	145
	12	147	146	144	144	146	146	145	145
Cl^- mEq/L	3	111	112	112	114*	113	114	114	114*
	6	113	113	113	113	113	114	114	115*
	9	113	113	113	114	114	114	112	115
	12	113	113	111	113	113	113	112	112
Ca^{2+} mg/dL	3	11.0	10.8	10.7	10.4*	11.0	10.6*	10.3*	10.3*
	6	10.3	10.4	10.2	10.2	10.3	10.3	10.0	10.1
	9	10.6	10.5	10.6	10.7	10.5	10.8	10.1	10.6
	12	10.1	9.8	9.6	9.6	10.1	10.1	9.5	9.8

*$p < 0.05$

Urinalysis: Urine obtained by an approximate 16-hour overnight collection period, was analyzed for a complete panel of urinalysis parameters at pretest and the end of Months 3, 6, 9, and 12. Urine was also obtained from 2 animals/sex in Group 4 at the end of the Month 6 and Month 12 recovery periods. Animals were fasted and water-deprived (except for Animal Nos. 4178, 4179 and 4679 at the interim sacrifice) during the collection period.

There were no treatment-related changes of urinalysis parameters at months 3, 6, 9, or 12 months.

Gross Pathology: A 6-month interim necropsy and a 12-month terminal sacrifice were performed.

6 Month Interim Necropsies
A 6 month interim necropsy was performed on up to 4 animals/sex/group after animals were dosed for at least 6 months. A Month 6 recovery necropsy was performed on 2 animals/sex in Group 4 after animals had been dosed for at least 6 months and then allowed to recover for at least 28 days. A concurrent current group was not included for 28-day recovery period. Animals were fasted overnight prior to necropsy.

Gross pathological findings observed at the 6-month interim sacrifice or the sacrifice after the 28-day recovery period did not correlate with potential treatment-related histopathological findings.

12 Month Terminal Necropsies
A 12 month terminal necropsy was performed on 4 animals/sex/group after animals were dosed for at least 12 months. A Month 12 recovery necropsy was performed on 2 animals/sex in Group 4 after animals had been dosed for at least 12 months and then allowed to recover for at least 28 days. Animals were fasted overnight prior to necropsy. A concurrent current group was not included for 28-day recovery period. Animals were fasted overnight prior to necropsy.

Gross pathological findings observed at the 12-month terminal sacrifice or the sacrifice after the 28-day recovery period did not correlate with potential treatment-related histopathological findings.

Organ Weights: Organ weights (absolute and relative) were measured for the adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, thyroid gland/parathyroid gland, and uterus with cervix.

Liver weights were increased for male and female drug-treated groups at 6 and 12 months; however, there were no corresponding histopathological findings.

Differences of absolute and relative organ weights between control and drug-treated groups were also observed for the pituitary gland, thyroid/parathyroid glands, thymus, prostate, and spleen; however, the findings were considered unrelated to treatment for one or more the following reasons: no correlating histopathological findings, changes were evident at 6 months and not at 12 months, there were lack of dose-response relationships, changes were not consistent between male and female drug-treated groups, or differences were small and appeared to lack biological significance.

Table 21 Organ weights in dogs at 6 and 12 months

Parameter	Time	Males				Females			
		0	125	250	500	0	125	250	500
Liver g	6	290.453	347.273	332.788	321.127	259.456	249.513	297.443	278.809
	12	314.106	307.322	350.766	351.805	234.266	272.937	291.402	315.641*
Liver %BW	6	2.805	3.068	3.582*	3.415*	2.876	3.139	3.630*	3.773*
	12	2.883	3.336	3.393	3.419	2.910	3.199	3.816*	4.111*

Liver %BrW	6	349.706	456.179	426.320	406.201	340.774	362.924	397.533	387.018
	12	376.628	378.719	472.474	439.808	331.699	368.201	414.976	421.339

*p < 0.05

Histopathology: Tissues and organs from all animals were routinely processed, embedded in paraffin, cut at a microtome setting of 4-7 microns, mounted on glass slides, stained with hematoxylin and eosin and examined by light microscopy. The bones were decalcified.

Adequate Battery: A complete panel of tissues was submitted to histopathological examination.

Table 9.3.16.4-1 Tissues weighed, preserved and examined microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X	
bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X[a]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
epididymides		X	X
esophagus		X	X
eyes		X	X
gall bladder		X	X
heart	X	X	X
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum[b])		X	X
liver	X	X	X
lungs (with mainstem bronchi)	X	X	X
lymph nodes (mesenteric, mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
optic nerve		X	X
ovaries	X	X	X
pancreas		X	X
pituitary gland	X	X	X
prostate gland	X	X	X
salivary glands (submandibular)		X	X
skeletal muscle (*biceps femoris*) longitudinal and transverse		X	
skeletal muscle (*m.gastrocnemious*) longitudinal and transverse		X	X
skeletal muscle (*m.psosas*) longitudinal and transverse		X	
skin (base of tail)		X	X
small intestine (duodenum, ileum, jejunum and Peyer's patches/GALT)		X	X
spinal cord (cervical)		X	X
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X	X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix	X	X	X
vagina		X	X
tissues with macroscopic findings including tissue masses		X	X

[a]Qualitative examination
[b]Not examined microscopically

Peer Review: None.

Histological Findings: Potential target organs of toxicity identified from the 6-month interim and 12-month terminal sacrifices were identified as the heart, mesenteric LN, thymus, pituitary gland, brain, kidneys, and liver.

At the 6-month interim sacrifice, potential target organs of toxicity were identified as heart, mesenteric LN, thymus, and pituitary gland.

In the heart, mesothelial proliferation was observed for 1 male dog at 500 mg/kg/day. At the 12-month terminal sacrifice, this finding was observed 1 male dog at 250 mg/kg/day, but was not evident at the high dose of 500 mg/kg/day.

In the mesenteric LN, decreased lymphocytes were observed for 1 male dog at 500 mg/kg/day. This finding was not evident at the 12-month terminal sacrifice.

In the thymus, the severity of decreased lymphocytes was increased for males at 500 mg/kg/day. At the 12-month terminal sacrifice, the severity of decreased lymphocytes was generally comparable between control and drug-treated groups.

There were findings of pituitary cysts in drug-treated male and female groups at the 6-month interim sacrifice, although there was no evidence of dose-response relationships. At the 12-month terminal sacrifice, pituitary cysts were observed with increased incidences for males and females in the 500 mg/kg/day, although there was one control male observed with this finding. Based upon the observed incidences in drug-treated groups, the general lack of dose-response relationships, and the finding in the one control male, it was judged that these findings were more than likely background in nature and unrelated to treatment.

Table 22 Histopathological findings at 6 months (Interim Sacrifice)

Organ/Tissue	Time	Males				Females			
		0	125	250	500	0	125	250	500
Heart									
-mesothelial proliferation,	IS	0/4	0/4	0/4	1/3	0/4	0/4	0/4	0/4
Grade 2	Rec	-	-	-	0/2	-	-	-	0/2
Mesenteric LN									
-decreased lymphocytes,	IS	0/4	0/4	0/4	1/3	0/4	0/4	0/4	0/4
Grade 2	Rec	-	-	-	0/2	-	-	-	0/2
Thymus									
-decreased lymphocytes	IS	4/4	4/4	4/4	3/3	4/4	4/4	4/4	4/4
Grade 1		0	0	0	0	2	3	1	2
Grade 2		4	4	4	1	2	1	3	2
Grade 3		0	0	0	2	0	0	0	0
Thymus									
-decreased lymphocytes	Rec	-	-	-	2/2	-	-	-	2/2
Grade 1		-	-	-	0	-	-	-	1
Grade 2		-	-	-	2	-	-	-	1
Pituitary									
-cysts	IS	0/4	2/4	2/4	1/4	0/4	2/4	0/4	1/4
	Rec	-	-	-	2/2	-	-	-	1/2

IS = Interim Sacrifice
Rec = Recovery
Dashes (-): Group not examined

At the 12-month terminal sacrifice, the potential target organs of toxicity were identified as the heart, pituitary, thymus, brain, kidneys, and liver.

In the heart, mesothelial proliferation was observed for 1 male dog in the mid dose group of 250 mg/kg/day at the 12-month termination sacrifice; however, there were no findings in the high dose group. At the 6-month interim sacrifice. 1 male at the high dose of 500 mg/kg/day was observed with mesothelial proliferation. The lack of this finding in the high dose group at the 12-month terminal sacrifice suggests that the finding was unrelated to treatment.

A grade 1 hemocyst was observed in the heart of one high dose male at the 12-month terminal sacrifice. Based upon the isolated occurrence for one animal, the relationship to treatment was unclear.

In the brain, Grade 1 fibrosis of choroid plexus was observed for 1 male and 1 female in the high dose group at the end of the 1-month recovery period. There was no similar finding at the end of the 12-month treatment period. The relationship of the finding to treatment was unclear.

In the kidneys, tubular degeneration/regeneration was observed for 1 high dose male at the end of the 1-month recovery period. There was no similar finding at the end of the 12-month treatment period. The relationship of the finding to treatment was unclear.

In the liver, increased glycogen was observed for 2 females in the high dose group at the end of the 1-month recovery period. There was no similar finding at the end of the 12-month treatment period. The relationship of the finding to treatment was unclear.

Table 23 Histopathological findings at 12 months (Terminal Sacrifice)

Organ/Tissue	Time	Males				Females			
		0	125	250	500	0	125	250	500
Heart -mesothelial proliferation, Grade 2	Term	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
	Rec	-	-	-	0/2	-	-	-	0/2
Heart -hematocyst, Grade 1	Term	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Rec	-	-	-	0/2	-	-	-	0/2
Pituitary -cysts	Term	1/4	0/4	1/4	2/4	0/4	1/4	1/4	2/4
	Rec	-	-	-	1/2	-	-	-	2/2
Thymus -decreased lymphocytes Grade 2 Grade 3	Term	4/4 4 0	4/4 3 1	4/4 4 0	4/4 3 1	4/4 2 2	4/4 3 1	4/4 2 2	4/4 3 1
Thymus -decreased lymphocytes Grade 2	Rec	-	-	-	2/2 2	-	-	-	2/2 2
Brain -choroid plexus: fibrosis, Grade 1	Rec	-	-	-	1/2	-	-	-	1/2
Kidney -tubular degeneration/ regeneration, Grade 1	Rec	-	-	-	1/2	-	-	-	0/2
Liver -increased glycogen Grade 1 Grade 3	Rec	-	-	-	0/2	-	-	-	2/2 1 1

Term = Terminal Sacrifice
Rec = Recovery
Dashes (-): Group not examined

Conclusion: Judging the totality of the histopathological findings from the 6-month interim sacrifice and 12-month terminal sacrifice as well as the respective 1-month recovery periods, no treatment-related target organs of toxicity were identified with doses up to 500 mg/kg/day.

Special Evaluation: To further investigate target organ toxicity of VX-809 (if any), blood, serum, liver and heart samples were collected for possible biomarker analysis at the 6-month interim and 12-month terminal sacrifices (from up to 4 animals/sex/group), and at the respective recovery sacrifices (from up to 2 animals/sex/Group 4 only). No data was submitted.

Toxicokinetics: Blood samples were obtained for the determination of plasma concentrations of VX-809 on Day 1 and predose and postdose at the end of Months 3, 6, 9, and beginning and end of Month 12. Plasma samples were shipped to the Sponsor for analysis.

Table 9.3.15-1 Collection Times and Number of Animals

Number of animals	Interval/Timepoints
Day 1	
8/sex/Groups 1, 2 & 3/timepoint 12/sex/Group 4/timepoint	0.5, 1, 2, 4, 8 & 24 hours post dose
End of Months 3 and 6	
8/sex/Groups 1, 2 & 3/timepoint 12/sex/Group 4/timepoint	Pre-dose and 1, 2, 4, 8, & 24 hours post dose
End of Month 9	
4/sex/Groups 1, 2 & 3/timepoint 6/sex/Group 4/timepoint	Pre-dose and 1, 2, 4, 8, & 24 hours post dose
Beginning of Month 12	
4/sex/Groups 2 & 3/timepoint 6/sex/Group 4/timepoint	Pre-dose and 1, 2, 4, 8, & 24 hours post dose
End of Month 12	
4/sex/Groups 1, 2 & 3/timepoint 6/sex/Group 4/timepoint	Pre-dose and 1, 2, 4, 8, 12, 18 & 24 hours post dose

Plasma C_{max} and AUC values for VX-809 increased with elevating dose on days 1, 90, 180, 270, 330, and 363. On day 363, AUC values for VX-809 increased in a dose proportional manner and there were no differences in exposures between males and females. C_{max} values increased in a less than dose proportional manner. Values on day 363 were 2- to 4-fold higher than values on day 1 suggesting significant accumulation in the process to achieve steady-state drug levels. The Sponsor indicated that steady-state levels had been achieved by day 90.

Table 24 Toxicokinetic parameters in dogs treated with VX-809 for up to 12 months

Table 10.2-1 Summary of Toxicokinetic Parameters for VX-809 in Female and Male Beagle Dogs Following Once Daily Oral Administration of 125, 250 and 500 mg/kg/day of VX-809 on Days 1, 90, 180, 270, 330 and 363

Study Day	Group	Dose (mg/kg)	Sex					
			Female			Male		
			C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)
Day 1	2	125	21.0±6.44	1.31±0.59	83.2±20.0	26.1±4.40	1.75±0.46	99.0±20.8
	3	250	27.1±8.28	1.88±0.35	100±18.9	41.9±14.9	1.50±0.54	221±91.6
	4	500	28.7±12.0	1.58±0.63	194±82.0	41.8±10.0	1.67±0.49	261±185
Day 90	2	125	30.8±8.47	1.00±0.00	111±33.2	26.6±8.02	1.88±0.99	132±62.0
	3	250	42.6±19.6	1.13±0.35	172±119	58.2±21.5	3.25±2.96	511±542
	4	500	42.4±22.1	3.25±6.88	294±265	57.4±26.2	4.67±6.61	619±316
Day 180	2	125	23.8±5.26	1.25±0.46	88.6±20.2	21.3±5.44	1.38±0.52	89.0±16.0
	3	250	32.7±12.8	1.38±0.52	148±73.7	40.3±19.4	2.13±2.42	261±279
	4	500	31.3±9.07	2.17±2.79	220±131	38.2±27.7	2.50±2.61	296±377
Day 270	2	125	28.5±6.72	1.00±0.00	122±18.1	29.8±13.2	1.00±0.00	79.1±24.7
	3	250	50.7±13.4	1.25±0.50	365±150	31.8±10.3	2.75±3.50	245±236
	4	500	56.2±19.2	3.83±3.43	644±330	62.2±26.4	1.17±0.41	450±299
Day 330	2	125	46.3±9.96	1.25±0.50	152±8.98	40.6±16.5	1.25±0.50	142±76.0
	3	250	75.1±17.0	1.75±0.50	237±39.1	70.3±23.8	1.00±0.82	449±398
	4	500	87.6±18.5	1.83±0.41	470±102	87.0±37.2	2.67±2.66	576±379
Day 363	2	125	52.4±15.1	1.50±0.58	208±69.1	46.8±10.3	1.00±0.00	145±79.6
	3	250	79.5±10.8	1.75±0.50	431±230	77.1±36.2	3.75±5.50	468±499
	4	500	101±28.7	3.00±2.68	860±330	88.6±30.0	2.67±2.66	750±497
Steady State Day 90 to 363	2	125	33.8±13.3	1.18±0.39	126±50.5	30.4±13.0	1.39±0.69	115±56.5
	3	250	50.8±23.1	1.39±0.50	239±159	53.7±26.3	2.61±3.06	386±402
	4	500	56.0±31.8	2.79±4.19	429±322	61.3±33.5	2.98±4.09	515±387

Dosing Formulation Analysis: Dose formulations were evaluated for homogeneity and concentration during the study.

Homogeneity of the dose formulations using the proposed preparation procedure was demonstrated by taking 3 top, 3 middle and 3 bottom samples (9 samples per batch of 2.5 mL) from formulations of the low-, mid-, and high concentrations from the Day 1 preparation. Homogeneity was also performed on Day 184/185. Similarly, homogeneity of the dose formulations from the high concentration only was performed at the beginning of Month 12.

Dose confirmation analyses were performed in duplicate on Day 2 and during Months 3, 6, 9, and beginning of Month 12 (to coincide with the days of toxicokinetic sampling for both males and females). Two samples (2.5 mL each) were taken from the middle region of each formulation (including control) on the day of dose preparation. An

average of the Day 1 middle sample for homogeneity was used for the Day 1 dose confirmation.

Analysis of Day 1, Month 6 and Month 12 dose formulations confirmed that the preparation procedure used for this study produced homogeneous mixtures. Analyses conducted during the dosing period confirmed that dose suspensions of appropriate concentration were administered (actual concentrations ranged from 97.8 to 111.9% of nominal concentrations).

STUDIES WITH THE COMBINATION OF VX-809 AND VX-770

RATS

Study title: VX-809 AND VX-770: A 28-DAY ORAL (GAVAGE) COMBINATION TOXICITY AND TOXICOKINETIC STUDY IN RATS WITH 14-DAY RECOVERY PERIOD

Study no.: VX-809-TX-009 and VX-770-TX-015
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: May 26, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity:

Test Articles	Lot Numbers	Purity	Description	Dates Received	Retest Dates
VX-770	17QB02.HQ00016	99.2%	White powder	24 Mar 2009	(b) (4)
VX-809	PT-C07091701-M09001	99%	White (b) (4)	05 Jun 2009	

VX-770: The test article was a 50:49.5:0.5 (b) (4) mixture of VX-770 (API) and hydroxypropylmethylcellulose acid succinate (HPMC-AS) and sodium lauryl sulfate (SLS). The (b) (4) (b) (4) powder was formulated as a suspension in the vehicle.

Key Study Findings

● In a 28-day oral (gavage) toxicology study, rats received the combination of VX-809 and VX-770 at doses of 0/0, 100/25, 300/50, 1000/50, and 1000/100 mg/kg/day. VX-809 and VX-770 were formulated as separate suspensions for co-administration by oral gavage. At the end of the 28-day dosing period, 10 rats/sex/group were sacrificed. At the end of a 14-day recovery period, 5 rats/sex/group were sacrificed.

● Histopathological findings at the end of the dosing period were evident in the kidneys, duodenum, stomach, mediastinal LN, mesenteric LN, and Harderian gland. With the

exception of the findings in the kidneys and mesenteric LN, incidences of findings were low.

● Increased incidences of chronic progressive nephropathy were observed for males in the 1000/50 and 1000/100 mg/kg/day groups. This is a common background finding in rats, particularly males; however, treatment with doses of 1000/50 and 1000/100 mg/kg/day exacerbated the incidence of this finding. Chronic progressive nephropathy has generally been judged to be a rat-specific finding with little or no relevance to humans (Toxicologic Pathology 32:171-80, 2004). Test article-related exacerbation of this finding has been observed with many diverse agents and has little or no relevance to humans.

● Erosions of the gastric glandular mucosa were present in 2 of 10 females at 1000/50 mg/kg/day, 2 of 10 males at 1000/100 mg/kg/day, and 1 of 10 females at 1000/100 mg/kg/day. This finding was also identified in 1 female at 100/25 mg/kg/day (No. 2503) examined for gross lesions. Minimal to moderate, focal or multifocal erosions of the glandular mucosa occurred without significant inflammatory response. These findings were observed with low incidences and not observed with a dose-response relationship. In discussions with the Medical Officer, based upon the indication for cystic fibrosis, these findings were considered monitorable in a clinical setting.

● Slight erosion of the duodenal mucosa was identified for one female in the 1000/100 mg/kg/day group.

● The incidence of free erythrocytes and erythrophagocytosis in the mesenteric LN was increased for males in the 1000/100 mg/kg/day group. This finding was also evident in the mediastinal LN where incidences displayed no relationship to treatment.

● Treatment with doses of 1000/50 and 1000/100 mg/kg/day increased the incidences of subacute and chronic inflammation in Harderian gland. These findings were rats-specific and have no relevance to humans.

● The sponsor did not perform histopathological examinations of recovery animals.

● AUC and C_{max} values for VX-809 increased with elevating dose although they were generally less than dose proportional. AUC and C_{max} values for VX-770 and its metabolites increased in an approximate dose proportional manner from 100/25 to 300/50 mg/kg/day. AUC and C_{max} values for VX-770 and its metabolites at 1000/50 mg/kg/day were lower than values observed at 300/50 mg/kg/day. AUC and C_{max} values for VX-770 and its metabolites at 1000/100 mg/kg/day were approximately equal to values observed at 300/50 mg/kg/day. VX-809 was found to enhance the metabolism of VX-770.

● The NOAEL was identified as the high dose of 1000/100 mg/kg/day given that observed histopathological findings were either considered irrelevant to humans or of low incidences with little evidence of dose-response relationships and considered

monitorable in a clinical setting. $AUC_{0\text{-}24hr}$ values for VX-809 in females and males were 3410 and 2830 µg.hr/mL, respectively. Respective $AUC_{0\text{-}24hr}$ values for VX-770 and its M1 and M6 metabolites in females were 479, 145, and 6.17 µg·hr/mL and in males were 285, 216, and 18.6 µg·hr/mL.

Methods

Doses:	The combination of VX-809 and VX-770 at doses of 0/0, 100/25, 300/50, 1000/50, and 1000/100 mg/kg/day. VX-809 and VX-770 were formulated as separate suspensions for co-administration by oral gavage.
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	5 mL/kg/day for each vehicle and test article in all groups (20 mg/g)
Formulation/Vehicle:	The vehicle for VX-809 consisted of 0.5% methylcellulose (MC), 0.5 % Tween80, and 0.05% simethicone in distilled water. The vehicle for VX-770 consisted of 0.5 % MC, 0.5% sodium lauryl sulfate, and 0.01% simethicone in distilled water. The placebo for VX-770, hydroxypropylmethylcellulose acetate succinate (HPMC-AS), was suspended in the VX-770 vehicle at the highest concentration dosed in the VX-770 TA groups (20 mg/g).
Species/Strain:	Sprague-Dawley Crl: CD® (SD)IGS BR rats were obtained from (b) (4) (b) (4)
Number/Sex/Group:	10 rats/sex/group were sacrificed at the end of the 28-day dose period
Age:	Approximately 12 weeks at the start of dosing
Weight:	**Mean** **Range** Males 404 357 to 457 Females 241 211 to 289
Satellite groups:	5 rats/sex/group were sacrificed at the end of a 14-day recovery period
Unique study design:	None
Deviation from study protocol:	Deviations were minor and did not impact the integrity of the study

Table 25 Design of 28-day toxicology study with the combination of VX-809 and VX-770 in rats

The test and control articles were administered orally, by gavage, to rats for 28 consecutive days followed by a 14-day recovery period as shown below.

Group	Test Article	Daily Doses[a]			Number of Animals									
					Total on Study		Toxicity Study						TK Study[d]	
							Total		Terminal Necropsy		Recovery Necropsy		Days 1 and 28	
		Dose (mg/kg)	Volume (mL/kg)	Conc.[b]	M	F	M	F	M	F	M	F	M	F
1	Control	0	5	20[c]	18	18	15	15	10	10	5	5	3	3
	Control	0	5	0										
2	VX-770	25	5	10	24	24	15	15	10	10	5	5	9	9
	VX-809	100	5	20										
3	VX-770	50	5	20	24	24	15	15	10	10	5	5	9	9
	VX-809	300	5	60										
4	VX-770	50	5	20	24	24	15	15	10	10	5	5	9	9
	VX-809	1000	5	200										
5	VX-770	100	5	40	24	24	15	15	10	10	5	5	9	9
	VX-809	1000	5	200										

[a]VX-770 test article (TA) was a 50:49.5:0.5 solid dispersion mixture of VX-770 (active pharmaceutical ingredient, or API) and hydroxypropylmethylcellulose acid succinate (HPMC-AS) and sodium lauryl sulfate (SLS).. The (b) (4) (b) (4) powder was formulated as a suspension in a vehicle consisting of 0.5% w/v methylcellulose (MC) + 0.5% w/v sodium lauryl sulfate (SLS) + 0.01% w/v simethicone in water. Group 1 animals received the vehicle control for VX-770 plus its control article (see footnote c, below) as well as the vehicle control for VX-809, which consisted of 0.5% Tween-80 + 0.5% MC + 0.05% simethicone in water.

[b]API concentrations for VX-770 were ½ of the TA concentrations listed here. API concentrations for VX-809 were equal to the TA concentrations. VX-770 was prepared on a weight/weight basis (mg/g) whereas VX-809 was prepared on a weight to volume basis (mg/mL).

[c]Control article (HPMC-AS-HF) for VX-770 was added to the vehicle control for VX-770 at the highest concentration level used in the VX-770 TA animals.

[d]Toxicokinetic (TK) samples were collected from Groups 2-5 on Day 1 at 6 timepoints as follows: 0.5, 1, 2, 4, 8 and 24 hours post dose and on Day 28 predose and 1, 2, 4, 8, and 24 hours postdose. TK samples were collected from 3 animals/gender/group/time point. TK samples were collected from Group 1 only at the 8 hour timepoint.

The first day of dosing was defined as Day 1 of the study. The dosing initiation was staggered.

Observations and Results

Mortality: All animals were observed in their cages for mortality and general condition twice daily.

There were no treatment-related deaths.

Clinical Signs: All animals were observed in their cages for mortality and general condition twice daily. Physical examinations were conducted twice pretest and once per week during the treatment and recovery periods.

Low incidences of clinical signs including thin, ano-genital staining, piloerection, dry rales, and labored breathing were observed for rats in the 1000/100 mg/kg/day group.

Table 26 Clinical signs for rats treated with the combination of VX-809 and VX-770 for 28 days

Clinical signs	Males					Females				
	0/0	100/25	300/50	1000/50	1000/100	0/0	100/25	300/50	1000/50	1000/100
Thin	0/0	0/0	0/0	0/0	15/3	0/0	0/0	0/0	0/0	1/1
Ano-genital staining	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0
Piloerection	0/0	0/0	0/0	0/0	5/1	0/0	0/0	0/0	0/0	0/1
Dry rales	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1
Labored breathing	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1

Body Weights: Non-fasted body weights were recorded twice pretest, once weekly during the dosing and recovery periods and terminally after fasting. Terminal fasted body weights were obtained prior to necropsy.

Body weight gains were decreased >17.1% for males in the 300/50, 1000/50, and 1000/100 mg/kg/day groups and >20.7% for females in the 300/50, 1000/50, and 1000/100 mg/kg/day groups.

Table 27 Body weights for male rats treated with the combination of VX-809 and VX-770 for 28 days

Males	Mean Body Weights (grams)	Figure 1

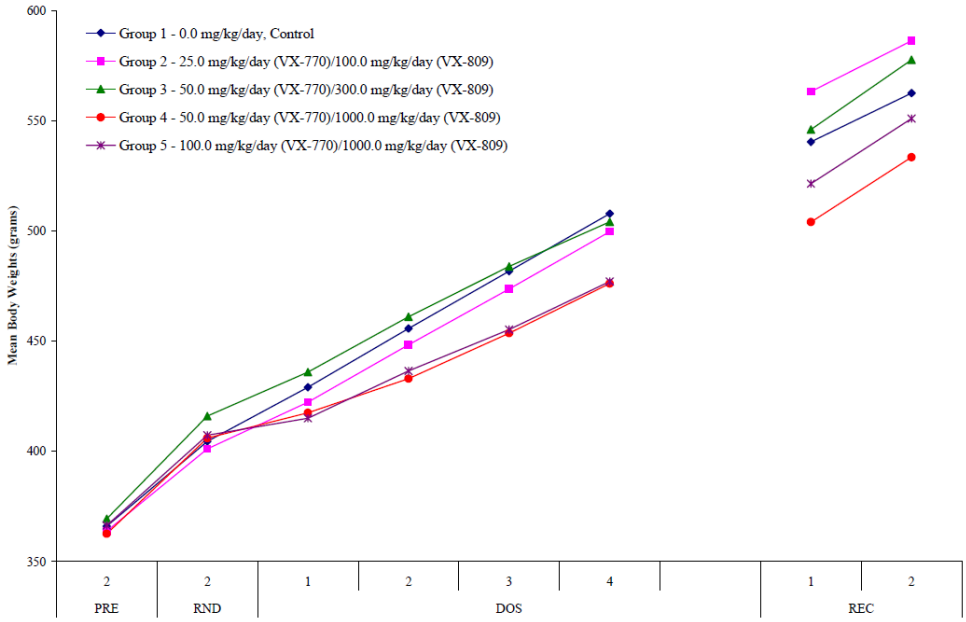
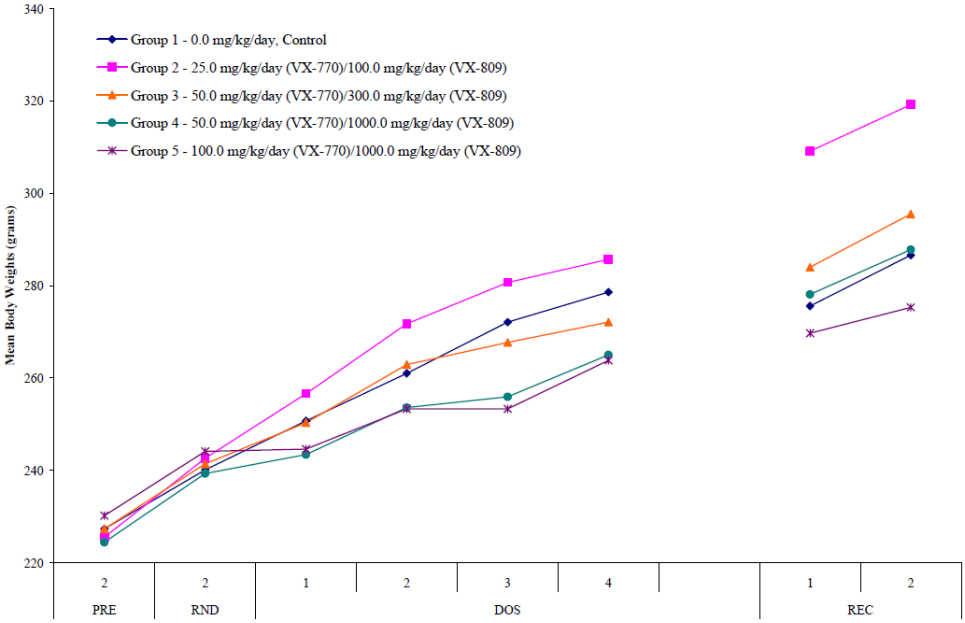


Table 28 Body weights for female rats treated with the combination of VX-809 and VX-770 for 28 days

Females	Mean Body Weights (grams)	Figure 2



Feed Consumption: Food consumption was measured (weighed) weekly for all toxicity animals commencing the last week of pretest and extending throughout the dosing and recovery periods (for toxicity animals only).

Food consumption of males in Groups 4 and 5 was lower than concurrent control values primarily during the first week of dose administration.

Food consumption of females in Groups 3, 4 and 5 was lower than concurrent control values throughout the study.

Figure 7 Food consumption for male rats treated with the combination of VX-809 and VX-770 for 28 days

Males	Mean Feed Consumption Values (grams/day)	Figure 3

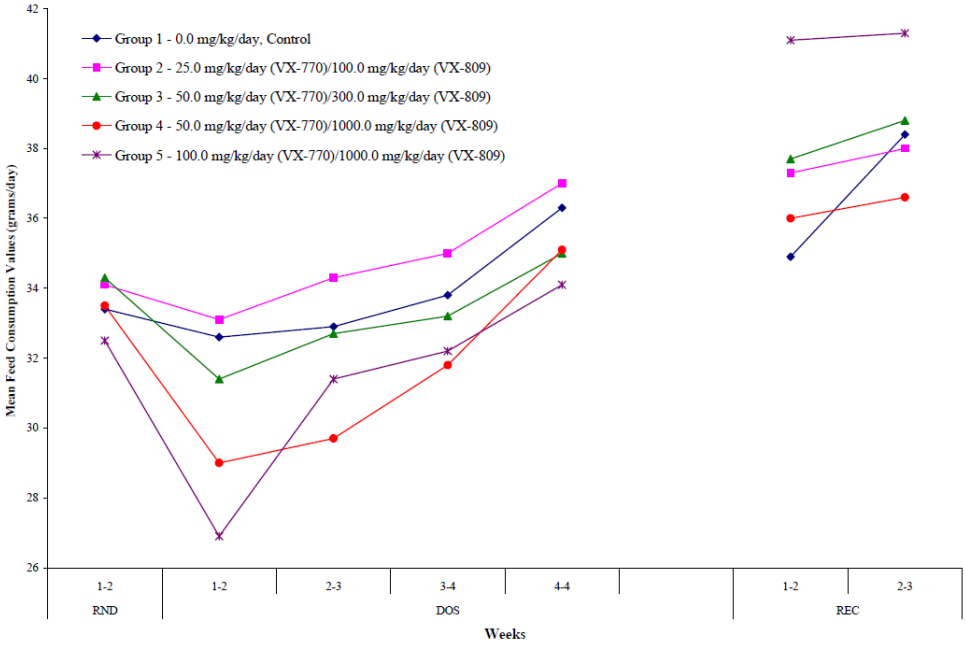
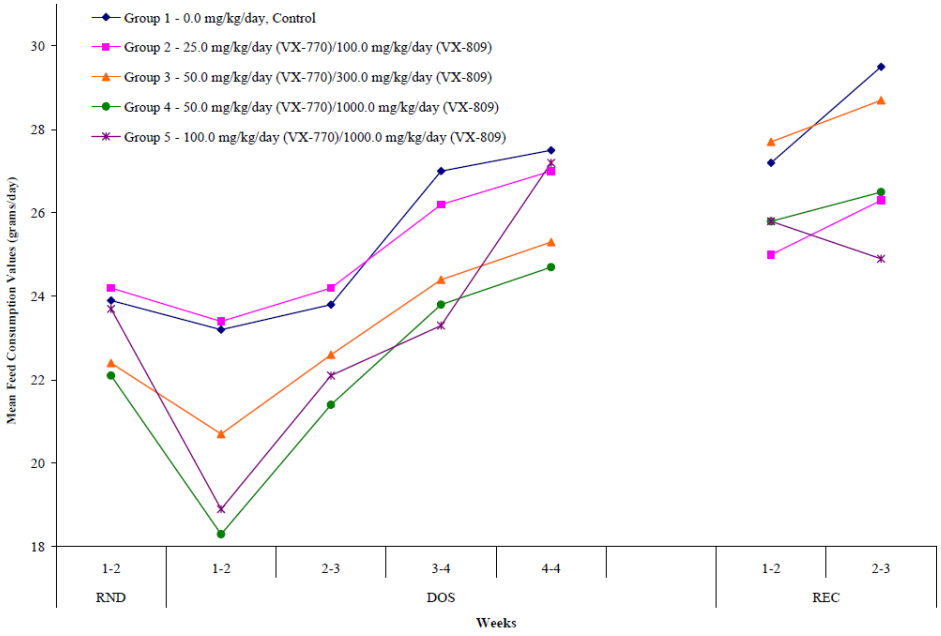


Figure 8 Food consumption for female rats treated with the combination of VX-809 and VX-770 for 28 days

Females	Mean Feed Consumption Values (grams/day)	Figure 4



Ophthalmoscopy: Ophthalmic examinations were conducted on all animals at pretest and toxicity animals at the end of the dosing period.

There were no treatment-related ophthalmic findings for rats treated with the combination of VX-809 and VX-770 for 28 days.

Hematology: Blood samples for measurement of a complete panel of hematology and coagulation parameters were collected from 10 animals/gender/group at study termination and 5 animals/gender/group at recovery. Animals were fasted overnight prior to each blood collection interval.

Changes of several hematology parameters were observed at the end of the dosing period. Decreased RBC counts, hemoglobin, and hematocrit were observed for males and females in the 1000/50 and 1000/100 mg/kg/day groups. Increased reticulocytes, were observed for females at doses ≥300/50 mg/kg/day. Increased platelet counts were observed for males at doses ≥100/50 mg/kg/day and females at dose ≥300/50 mg/kg/day. Mean platelet volume was increased for males at doses ≥300/50 mg/kg/day and females at dose ≥1000/50 mg/kg/day. The significance of hematological changes was unclear, although all changes appeared to be reversible.

Table 29 Changes of hematology parameters in rats treated with the combination of VX-809 and VX-770 for 28 days

Table 10.8.1-1: Changes in Hematology Values[1] in Rats Dosed Orally with VX-770 and VX-809 for 28 Days

	Males				Females			
Group	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
VX-770/VX-809 (mg/kg/day)	**25/ 100**	**50/ 300**	**50/ 1000**	**100/ 1000**	**25/ 100**	**50/ 300**	**50/ 1000**	**100/ 1000**
RBC	-	-	-5%*	-11%***	-	-	-8%**	-7%**
RETIC	-	-	-	-	-	+21%	+72%***	+45%***
HGB	-	-	-6%*	-9%***	-	-	-6%**	-7%***
HCT	-	-	-5%*	-9%***	-	-	-6%**	-6%**
PLT	-	-	+21%**	+18%**	-	+14%**	+27%***	+21%***
MPV	-	+4%*	+8%***	+15%***	-	-	+8%**	+9%***

*Absolute values were statistically significantly different from controls.
* - p<0.05; ** - p<0.01; *** - p<0.001
[1] Percentage change compared to corresponding control values.
PLT = Platelet; MPV=Mean Platelet Volume; HBG= Hemoglobin; HCT=Hematocrit
RBC=Red Blood Cell; RETIC=Reticulocytes

Clinical Chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected from 10 animals/gender/group at study termination and 5 animals/gender/group at recovery. Animals were fasted overnight prior to each blood collection interval.

There were changes of several clinical chemistry parameters at the end of the treatment period. BUN was increased for males at doses ≥1000/50 mg/kg/day and females at 1000/100 mg/kg/day. Phosphate levels were increased for males at doses ≥1000/50 mg/kg/day and females in all drug-treated groups. Sodium and chloride levels were slightly decreased for males at 1000/100 mg/kg/day. ALT activities were increased for males at doses ≥1000/50 mg/kg/day, although toxicological significance was not achieved. Cholesterol levels were increased for males at doses ≥1000/50 mg/kg/day and females at doses ≥300/50 mg/kg/day. These changes may correlate with histopathological findings of chronic progressive nephropathy for males in the 1000/50 and 1000/100 mg/kg/day groups.

Table 30 Changes of clinical chemistry parameters in rats treated with the combination of VX-809 and VX-770 for 28 days

Table 10.8.3-1: Changes in Clinical Chemistry Values[1] in Rats Dosed Orally with VX-770 and VX-809 for 28 Days

	Males				Females			
Group	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
VX-770/VX-809 (mg/kg/day)	**25/ 100**	**50/ 300**	**50/ 1000**	**100/ 1000**	**25/ 100**	**50/ 300**	**50/ 1000**	**100/ 1000**
BUN	-	-	+23%*	+54%***	-	-	-	+47%***
PHOS	–	–	+16%***	+11%***	+11%*	+13%*	+24%***	+18%***
Na	-	-	-	-1.4%*	-	-	-	-
Cl	-	-	-	-2%*	-	-	-	-
ALT	-	-	+50%***	+53%***	-	-	-	-
CHOL	-	-	+32%**	+37%**		+36%**	+48%***	+36%***

*Absolute values were statistically significantly different from controls.
* - p<0.05; ** - p<0.01; *** - p<0.001
[1] Percentage change compared to corresponding control values.
ALT = Alanine aminotransferase; BUN=Blood Urea Nitrogen; CHOL= Cholesterol;
PHOS=Phosphorus Na= Sodium Cl=Chloride

Urinalysis: Urine samples (obtained by a 16-hr overnight collection period) were analyzed for a complete panel of urinalysis parameters from 10 animals/sex/group at study termination and for 5 animals/sex/group at recovery. Animals were fasted but had access water.

Several changes of urinalysis parameters were observed at the end of the treatment period. These changes may correlate with findings of chronic progressive nephropathy for male rats in the 1000/50 and 1000/100 mg/kg/day groups.

Increased urine volumes were observed for males and females at doses ≥300/50 mg/kg/day. Decreased urinary creatinine levels were observed for males at doses ≥1000/50 mg/kg/day and all female drug-treated groups. Decreased urinary sodium levels were observed for females at doses ≥300/50 mg/kg/day. An increased urinary sodium: creatinine ratio was observed for females at 1000/100 mg/kg/day. Decreased urinary potassium levels were observed for males at doses ≥1000/50 mg/kg/day and females at doses ≥300/50 mg/kg/day. Increased urinary potassium: creatinine ratios were observed for females at doses ≥1000/50 mg/kg/day. Decreased urinary calcium levels were observed for females at doses ≥300/50 mg/kg/day. Decreased urinary phosphate levels were observed for males at doses ≥300/50 mg/kg/day and all female drug-treated groups. Decreased urinary potassium: creatinine ratios were observed for males at doses ≥1000/50 mg/kg/day.

Table 31 Changes of urinalysis parameters in rats treated with the combination of VX-809 and VX-770 for 28 days

Table 10.8.4-1: Changes in Urinalysis Parameters[1] in Rats Dosed Orally with VX-770 and VX-809 for 28 Days

	Males				Females			
Group	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
VX-770/VX-809 (mg/kg/day)	**25/ 100**	**50/ 300**	**50/ 1000**	**100/ 1000**	**25/ 100**	**50/ 300**	**50/ 1000**	**100/ 1000**
↑U.Vol	-	2.0x	3.0x*	3.6x***	-	2.0x*	2.8x***	4.8x***
↓CREAT	-	-	0.59x*	0.37x**	0.64x*	0.56x**	0.33x***	0.26x***
↓Na+	-	-	-	-	-	0.45x*	0.38x*	0.34x*
↑Na/Cre	-	-	-	-	-	-	-	1.5x*
↓K+	-	-	0.62x*	0.39x***	-	0.53x*	0.46x**	0.36x*
↑K/Cre	-	-	-	-	-	-	1.5x**	1.4x*
↓Ca++	-	-	-	-	-	0.27x***	0.30x***	0.24x***
↓PHOS	-	0.56x**	0.45x**	0.30x***	0.64x*	0.61x*	0.39x***	0.33x***
↓PHOS/CREAT	-	-	0.91x*	0.79x**	-	-	-	-

*Absolute values were statistically significantly different from controls.
* - p<0.05; ** - p<0.01; *** - p<0.001
[1] fold difference from control values ↑: increase ↓: decrease

Gross Pathology: Necropsy was performed on 10 animals/sex/group after animals had been dosed for 28 days and on 5 animals/sex/group after a 14-day recovery period. Animals were fasted overnight prior to necropsy (and terminal blood collections).

There was a dose related incidence of slight to moderate, red or black discoloration of the glandular mucosa in the stomach of test article-treated females in the 100/25, 300/50, 1000/50, and 1000/100 mg/kg/day groups (Numbers 2503, 3503, 3506, 3510, 4501, 4506, 4509, 5503, 5506, 5510) and in 2 of 10 males from each of the 1000/50 mg/kg/day group (Numbers 4001, 4007) and 1000/100 mg/kg/day group (5003, 5008).

The duodenal mucosa of one female (Number 5503) in the 1000/100 mg/kg/day group was slightly red. Discoloration of the gastric and duodenal mucosa correlated microscopically with erosions in the majority of the test article-treated rats that had microscopic evaluation performed (1000/50 and 1000/100 mg/kg/day groups only). Moderate amounts of abnormal black contents were present in the ileal lumen of one female (No. 4509) in the 1000/50 mg/kg/day group, but there were no microscopic abnormalities in the mucosa of this tissue. This animal also had gastric erosion.

Table 32 Gross pathological findings in rats treated with the combination of VX-809 and VX-770 for 28 days

Organ/Tissue	Males					Females				
	0/0	100/ 25	300/ 50	1000/ 50	1000/ 100	0/0	100/ 25	300/ 50	1000/ 50	1000/ 100
N =	10	10	10	10	10	10	10	10	10	10
Stomach -discolored	0	0	0	2	2	0	1	3	3	4
Duodenum -discolored	0	0	0	0	0	0	0	0	0	1
Ileum -abnormal contents	0	0	0	0	0	0	0	0	1	0

Organ Weights: Organs (adrenals, brain, epididymides, heart, kidneys, liver, lung, ovaries, pituitary gland, prostate, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid, and uterus) were weighed for all toxicity animals at the scheduled sacrifice intervals.

Dose-related increases in liver weight were observed for males and females in the 1000/50 and 1000/100 mg/kg/day groups. Increases in absolute liver weights compared to controls were 6-8% in the 1000/50 mg/kg/day group and 13% in the 1000/100 mg/kg/day group.

Histopathology:

Adequate Battery: Tissues were obtained at the scheduled sacrifice intervals and preserved for all toxicity animals. After fixation, tissues and organs from all toxicity animals in Groups 1, 4 and 5 at termination were routinely processed, embedded in paraffin, cut at a microtome setting of 4-7 microns, mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy. Slides were prepared by (b) (4). Additionally, the stomach was processed at (b) (4) and examined as a target organ for all animals in Groups 2 and 3 where the stomach exhibited gross lesions at the terminal sacrifice. Any abnormalities not noted during macroscopic examinations which were seen during histology processing were recorded. Recovery animals were not examined.

Table 9.3.15.4-1 - Tissues weighed, preserved and examined microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY Group 1, 4 and 5
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (femur)		X	
bone (sternum,distal femur)		X	X
bone marrow (sternum,distal femur)		X	X[a]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
Harderian gland		X	X
heart	X	X	X
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
liver	X	X	X
lungs (with mainstem bronchi)	X	X	X
lymph nodes (mesenteric and mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
optic nerve		X	X
ovaries	X	X	X
pancreas		X	X
pituitary gland	X[b]	X	X
prostate gland	X	X	X
salivary glands (submandibular)		X	X
seminal vesicles	X	X	X
skeletal muscle (*rectus femoris*) longitudinal and transverse		X	X
skeletal muscle (*m.gastrocnemious*) longitudinal and transverse		X	
skeletal muscle (*m.psosas*) longitudinal and transverse		X	
skin (inguinal)		X	X
small intestine (duodenum, ileum, jejunum and Peyer's patches)		X	X
spinal cord (cervical)		X	X
spleen	X	X	X
stomach[c]		X	X
testes	X	X	X
thymus	X	X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY Group 1, 4 and 5
thyroid/parathyroid glands	X[b]	X	X
trachea		X	X
urinary bladder		X	X
uterus (horns/body with cervix)	X	X	X
vagina		X	X
gross lesions		X	X

[a]Qualitative examination (no differential count).
[b]Weighed post-fixation
[c] The stomach was examined as a target organ for all animals in Groups 2 and 3 where the stomach exhibited gross lesions at the terminal sacrifice.

Peer Review: None

Histological Findings:
Histopathological findings at the end of the dosing period were evident in the kidneys, stomach, duodenum, mediastinal LN, mesenteric LN, Harderian gland, ovaries, mammary gland, and urinary bladder. With the exception of the kidneys and mesenteric LN, incidences of findings were low and dose-response relationships were not generally present.

Increased incidences of chronic progressive nephropathy were observed for males in the 1000/50 and 1000/100 mg/kg/day groups. This is a common background finding in rats, particularly males; however, treatment with doses of 1000/50 and 1000/100 mg/kg/day exacerbated the incidence of this finding. Chronic progressive nephropathy has generally been judged to be a rat-specific finding with little or no relevance to humans (Toxicologic Pathology 32:171-80, 2004). Test article-related exacerbation of this finding has been observed with many diverse agents and has little or no relevance to humans.

Erosions of the gastric glandular mucosa were present in 2 of 10 females at 1000/50 mg/kg/day (Numbers 4501, 4509), 2 of 10 males at 1000/100 mg/kg/day (Numbers 5003 and 5008), and 1 of 10 females at 1000/100 mg/kg/day (Number 5510). This findings was also identified in 1 female at 100/25 mg/kg/day (No. 2503) examined for gross lesions after 28 days of drug treatment. Minimal to moderate, focal or multifocal erosions of the glandular mucosa occurred without significant inflammatory response. These microscopic findings correlated with some of the red or black discoloration observed grossly in the glandular gastric mucosa. These findings had low incidences and generally lacked a dose-response relationship. In discussions with the Medical Officer, based upon the indication for cystic fibrosis, these findings were considered monitorable in a clinical setting.

Slight erosion of the duodenal mucosa was identified for one female (No. 5503) in the 1000/100 mg/kg/day group. These findings correlated with the red discoloration observed during the necropsy. Discoloration (gross finding) of the gastric glandular mucosa also occurred in this female.

The incidence of free erythrocytes and erythrophagocytosis in the mesenteric LN was increased for males in the 1000/100 mg/kg/day group. This finding was also evident in the mediastinal LN where incidences displayed no relationship to treatment.

Treatment with doses of 1000/50 and 1000/100 mg/kg/day increased the incidences of subacute and chronic inflammation in Harderian gland. These findings were considered rat-specific and have no relevance to humans.

Findings in the ovaries, mammary gland, and urinary bladder appeared to be background in nature.

The sponsor did not perform histopathological examinations of recovery animals.

Table 33 Histopathological findings in rats at the end of the 28-day dosing period

Organ/Tissue	Sex	0/0	100/25	300/50	1000/50	1000/100
Kidneys						
-chronic progressive nephropathy (characterized by predominantly basophilic tubules), minimal-slight	M	2/10	-	-	7/10	7/10
	F	2/10	-	-	1/10	2/10
-cortex: tubules - eosinophilic material	M	0/10	-	-	0/10	1/10
	F	0/10	-	-	0/10	0/10
Duodenum						
-mucosa: erosion, slight	M	0/10	-	-	0/10	0/10
	F	0/10	-	-	0/10	1/10
Stomach						
-glandular mucosa: erosion, minimal to moderate	M	0/10	-	-	0/10	2/10
	F	0/10	1/1	0/3	2/10	1/10
Mesenteric LN						
sinuses: free erythrocytes/ erythrophagocytosis, minimal-slight	M	0/10	-	-	2/10	6/10
	F	0/10	-	-	2/10	1/10
Mediastinal LN						
-sinuses: free erythrocytes/ erythrophagocytosis, minimal-moderate	M	2/7	-	-	3/8	1/10
	F	0/8	-	-	3/10	1/8
Harderian gland						
-subacute (chronic active)/chronic inflammation	M	1/10	-	-	2/10	3/10
	F	0/10	-	-	1/10	2/10

Dashes (-) indicate the tissue was not examined for the group

Special Evaluation: All the 24-hour TK animals (up to 3/sex/Groups 2 to 5) and the 8-hour control TK animals (up to 3/sex/Group 1) were transferred to necropsy and sacrificed on Day 29 (immediately after the last scheduled 24-hour TK blood collection for Groups 2 to 5). Blood was collected and the targeted organs (heart, liver, and lung) harvested as soon as possible for possible biomarker analyses or determination of tissue concentrations of VX-770 and VX-809 and any known major metabolites. Results were not provided in the study report.

Toxicokinetics: Blood samples were obtained for the determination of plasma concentrations of VX-770, VX-809, and the major metabolites of VX-770, VRT−837018 (M1, hydroxymethyl−VX−770) and VRT−842917 (M6, carboxy−VX−770). On Days 1 and 28 blood samples for toxicokinetic determinations were obtained from 3 animals/sex/time point from toxicokinetic animals in Groups 2 to 5 as follows: predose (Day 28 only), and at 0.5 (Day 1 only), 1, 2, 4, 8, and 24 hr postdose. Blood samples were collected from the control article TK rats (Group 1) at a single time point: 8 hours postdose.

Toxicokinetic parameters for VX-809 and VX-770 and its metabolites are shown in the table below. AUC and C_{max} values for VX-809 increased with elevating dose although they were generally less than dose proportional.

AUC and C_{max} values for VX-770 and its metabolites increased in an approximate dose proportional manner from 100/25 to 300/50 mg/kg/day. AUC and C_{max} values for VX-770 and its metabolites at 1000/50 mg/kg/day were lower than values observed at 300/50 mg/kg/day. AUC and C_{max} values for VX-770 and its metabolites at 1000/100 mg/kg/day were approximately equal to values observed at 300/50 mg/kg/day.

Table 34 Toxicokinetic parameters for VX-809, VX-770, VRT-842917 (M6), and VRT-837018 (M1) in female and male dogs following co-administration of VX-809 and VX-770 on day 1

Table 2 Summary of Toxicokinetic Parameters for VX-809, VX-770, VRT-842917 (M6) and VRT-837018 (M1) in Female and Male Rats Following Co-administration of VX-770 and VX-809 on Day 1

Analyte	Study Day	Dose (mg/kg)	Gender							
			Female				Male			
			C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (ng*hr/mL)	$DN\_AUC_{0\text{-}24hr}$[a] (ng*hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (ng*hr/mL)	$DN\_AUC_{0\text{-}24hr}$[a] (ng*hr/mL)
VX-809	Day 1	100	170000	4.00	1830000	18300	136000	8.00	2130000	21300
		300	235000	8.00	4520000	15100	186000	8.00	3430000	11400
		1000	329000	8.00	6940000	6940	289000	24.00	5960000	5960
		1000	316000	24.00	6300000	6300	289000	8.00	5990000	5990
VX-770	Day 1	25	4340	8.00	83000	3320	3620	4.00	73200	2930
		50	8300	4.00	134000	2680	6270	8.00	119000	2380
		50	7500	4.00	123000	2460	4340	8.00	89200	1780
		100	9000	4.00	140000	1400	6040	24.00	116000	1160
VRT-842917	Day 1	25	51.2	4.00	597	N/A	176	8.00	2470	N/A
		50	76.5	4.00	1620	N/A	182	8.00	3370	N/A
		50	126	24.00	2230	N/A	179	24.00	2820	N/A
		100	139	24.00	2240	N/A	193	24.00	3590	N/A
VRT-837018	Day 1	25	1700	4.00	26600	N/A	2950	8.00	47200	N/A
		50	3010	4.00	42700	N/A	3330	8.00	62900	N/A
		50	2880	4.00	44500	N/A	2480	8.00	48400	N/A
		100	2910	4.00	49100	N/A	3050	24.00	59800	N/A

Source: PKS Study: CF-VX-809-TX-009-VX-770-TX-015 RAT 28-DAY TK

[a] Dose normalized to 1.00 mg/kg

N/A: not applicable as the elimination half-life for VX-809 could not be estimated based on the concentrations observed in the terminal elimination portion of the plasma concentration versus time curve.

Table 35 Toxicokinetic parameters for VX-809, VX-770, VRT-842917 (M6), and VRT-837018 (M1) in female and male dogs following co-administration of VX-809 and VX-770 on day 28

Table 3 Summary of Toxicokinetic Parameters for VX-809, VX-770, VRT-842917 (M6) and VRT-837018 (M1) in Female and Male Rats Following Co-administration of VX-770 and VX-809 on Day 28

Analyte	Study Day	Dose (mg/kg)	Gender							
			Female				Male			
			C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (ng*hr/mL)	DN_$AUC_{0\text{-}24hr}$[a] (ng*hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-}24hr}$ (ng*hr/mL)	DN_$AUC_{0\text{-}24hr}$[a] (ng*hr/mL)
VX-809	Day 28	100	145000	2.00	1180000	11800	103000	2.00	971000	9710
		300	190000	2.00	2900000	9670	129000	8.00	1950000	6500
		1000	278000	2.00	3650000	3650	176000	2.00	2320000	2320
		1000	230000	2.00	3410000	3410	167000	2.00	2830000	2830
VX-770	Day 28	25	7990	2.00	131000	5240	6050	4.00	110000	4400
		50	12700	2.00	252000	5040	10200	8.00	201000	4020
		50	14000	8.00	259000	5180	10700	8.00	191000	3820
		100	23900	8.00	479000	4790	16200	4.00	285000	2850
VRT-842917	Day 28	25	103	4.00	968	N/A	236	2.00	4220	N/A
		50	275	2.00	3140	N/A	577	4.00	8940	N/A
		50	235	4.00	3140	N/A	725	8.00	12300	N/A
		100	518	4.00	6170	N/A	1190	8.00	18600	N/A
VRT-837018	Day 28	25	3300	4.00	43600	N/A	4340	8.00	77600	N/A
		50	5740	2.00	94100	N/A	8580	8.00	150000	N/A
		50	5480	4.00	90300	N/A	9060	8.00	149000	N/A
		100	9240	4.00	145000	N/A	12200	8.00	216000	N/A

Source: PKS Study: CF-VX-809-TX-009-VX-770-TX-015 RAT 28-DAY TK
[a] Dose normalized to 1.00 mg/kg
N/A: not applicable as the elimination half-life for VX-809 could not be estimated based on the concentrations observed in the terminal elimination portion of the plasma concentration versus time curve.

Dosing Formulation Analysis: The dose formulations of the test article, VX-770, a 50:49.5:0.5 (b) (4) mixture of VX-770 (API) and Hydroxypropylmethylcellulose Acid Succinate (HPMC-AS) and Sodium Lauryl Sulfate (SLS), in control vehicle [0.5% (w/v) Methylcellulose (MC) with 0.5% (w/v) Sodium Lauryl Sulfate (SLS) and 0.01% (w/v) Simethicone in Water] were analyzed to confirm that the prepared dose formulations were homogeneous and the administered VX- 770 concentrations were appropriate under the study conditions.

The dose formulations of the test article, VX-809 (assumed 100% pure) in control vehicle [0.5% Tween 80 (w/v) + 0.5% Methylcellulose (w/v) + 0.05% (w/v) Simethicone in Water] were also analyzed to confirm that the prepared dose formulations were homogeneous and the administered VX-809 concentrations were appropriate under the study conditions.

Actual concentrations of VX-770 dose formulations (10, 20, 20, and 40 mg/mL for Groups 2, 3, 4, and 5, respectively) and VX-809 dose formulations (20, 60, 200, and 200 mg/mL for Groups 2, 3, 4, and 5, respectively) on Days 1 and 28 ranged from 95.0 to 112.0% of target concentrations.

Study title: VX-809, VX-770, and VRT-0995096: A 3-MONTH ORAL (GAVAGE) COMBINATION TOXICITY AND TOXICOKINETIC STUDY IN RATS WITH A 28-DAY RECOVERY PERIOD

Study no.:	VX-809-TX-013, VX-770-TX-026,VRT-0995096-TX-005
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 4, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Rats were dosed once daily by oral gavage with various combinations of VX-809, VX-770, and VRT-0995096 co-formulated as a suspension in the vehicle. **VX-809**: Test article alone (b) (4) **VX-770**: The test article was a 50:49.5:0.5% (by weight) (b) (4) mixture of VX-770 (API) and hydroxypropylmethylcellulose acid succinate (HPMC-AS) and sodium lauryl sulfate (SLS).The (b) (4) (b) (4) powder was formulated as a suspension in the vehicle. **VRT-0995096**: The test article is a 50%:49%:1% (by weight) (b) (4) mixture of VRT-0995096, HPMC-AS, and SLS. The (b) (4) (b) (4) powder was formulated as a suspension in the vehicle.

9.2.1.2. Test Article, Supplier, Lot Numbers, Dates Received and Description

Test Article	Supplier	Lot Numbers	Dates Received	Retest Date	Description
VX-770	(b) (4)	17QB02.HQ00016	12 Jan 2011	(b) (4)	White (b) (4)
VX-809		L0300634 (Batch No. PT-C07091701-M09003)	29 Sept 2011 25 Oct 2011 13 Dec 2011		White to off-white (b) (4) powder
VRT-0995096		A3379-274 Manufacturer Lot: BJL-E16681-20-A	26 May 2011		White powder

9.2.1.3. Purity

100 %

Key Study Findings

● In a 90-day oral (gavage) toxicology study, rats were dosed once daily by oral gavage with various combinations of VX-809, VX-770, and VRT-0995096 co-formulated as a suspension in the vehicle. Doses of VX-809, VX-770, and VRT-0995096 in Groups 2, 3, 4, and 5 were 1000/100/20, 1000/25/20, 500/25/10, and 500/10/10 mg/kg/day, respectively. Group 2 was the high dose group and Group 5 was the low dose group.

● Female #2559 in Group 2 (1000/100/20 mg/kg/day) was observed with bilateral posterior subcapsular cataracts of the lens at the end of the 90-day dosing period. This finding was attributed to treatment with VX-770 and judged to be monitorable in a clinical setting, but not reversible.

● Target organs of toxicity were identified as the stomach, kidneys, thymus, mesenteric LN, and heart.

● Focal necrosis and/or erosions (minimal to slight) were present in the glandular mucosa of the stomach for all male drug-treated groups and females in Groups 2, 3, and 5. These foci correlated with macroscopic findings of red, brown and black discoloration. The findings were dose-responsive for male drug-treated groups, but not for female drug-treated groups. Mucosal necrosis and/or erosions was reversible following the recovery period. The squamous epithelium along the limiting ridge of the forestomach showed cystic degeneration (minimal to moderate) in males and females dosed at ≥500/10/10 mg/kg/day VX-809/VX-770/VRT-0995096. Variable numbers of the superficial keratinized cells along the limiting ridge contained small cysts filled with pink material. There was no clear dose relationship in males. Females had an inverse relationship to dose. Epithelial cystic degeneration along the limiting ridge was not reversible. Focal inflammation and edema were variably present. In one male (No. 2038) in Group 2 at 1000/100/20 mg/kg/day, the edema was moderate and diffuse. Based upon discussions with the Medical Officer, findings in the stomach were considered to be monitorable in a clinical setting given the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation.

● Basophilic tubules (minimal) were observed in 6 of 10 males dosed at 1000/100/20 mg/kg/day VX-809/VX-770/VRT-0995096. This finding was also evident for 2 of 10 females in Group 2 and 1 of 10 females in Group 3. The kidneys from recovery animals were not examined microscopically. The sponsor contended that basophilic and dilated tubules are common background findings in rats and the slightly increased incidence observed in terminal sacrifice animals was a known class effect of the test article as this finding was observed at increased incidences in the 28-day toxicology study with rats that received the combination of VX-809 and VX-770.

● An increased severity of decreased lymphocytes was observed in the thymus for males in Group 2 at 1000/100/20 mg/kg/day.

● Decreased lymphocytes were observed in the mesenteric LN for one male in Group 2 at 1000/100/20 mg/kg/day.

● Urothelial hyperplasia in the urinary bladder was observed for one male in Group 2 at 1000/100/20 mg/kg/day.

● Minimal cardiomyopathy was observed with an increased incidence for 4 of 10 females in Group 2 at 1000/100/20 mg/kg/day. The significance of this finding was unclear as the finding was observed for 1 of 9 control females and 2 of 10 control males. The incidence in male drug-treated groups ranged from 2 of 10 to 5 of 10 with no dose-response relationship.

● There were no findings that suggested any toxicokinetic interactions between VX-809, VX-770 and it metabolites, or VRT-0995096.

● Histopathological findings in the stomach, kidneys, thymus, mesenteric LN, and heart were not considered dose-limiting. The NOAEL was identified at 1000/25/20 mg/kg/day based upon a finding of bilateral, subcapsular cataracts for one animal at 1000/100/20 mg/kg/day. However, based upon the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation, the findings at 1000/100/20 mg/kg/day might be considered an acceptable risk that is monitorable in a clinical setting, but not reversible.

Methods

Doses:	Rats were dosed once daily by oral gavage for 90 days with various combinations of VX-809, VX-770, and VRT-0995096 co-formulated as a suspension in the vehicle
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	The dose volume was 5 mL/kg/day for all dose groups
Formulation/Vehicle:	The vehicle for VX-809 was 0.5% methylcellulose (w/v) + 0.5% Tween-80 (w/v) + 0.05% simethicone (w/v) in water The vehicle for VX-770 and VRT-0995096, hydroxypropylmethylcellulose acetate succinate (HPMC-AS), was added to the vehicle control suspension for Group 1 at the highest concentration level used in the VX-770 or VRT-0995096 treated animals (20 mg/mL).
Species/Strain:	CD® IGS Rats Crl: CD(SD) obtained from (b) (4)
Number/Sex/Group:	10 rats/sex/group for terminal sacrifice after treatment for 90 days
Age:	Approximately 12 weeks at the start of dosing
Weight:	Mean Range Males 426.7 347.3 to 536.9 Females 261.4 210.8 to 313.6
Satellite groups:	Recovery: 5 rats/sex/group for a recovery sacrifice after a 90-day treatment period and 28-day recovery period Toxicokinetics: For toxicokinetic determinations on days 1 and 90, 3 rats/sex/group were included in the control group and 9 rats/sex/group were included in test article-treated groups.
Unique study design:	Rats were dosed once daily by oral gavage with various combinations of VX-809, VX-770, and VRT-0995096 co-formulated as a suspension in the vehicle
Deviation from study protocol:	Deviations were generally minor and did not impact the integrity of the study.

The test and control articles were administered orally, by gavage, to rats for 3-months followed by a 28-day recovery period as shown in the following table.

Table 36 Design of 90-day combination toxicology study with rats that were dosed once daily by oral gavage with various combinations of VX-809, VX-770, and VRT-0995096 co-formulated as a suspension in the vehicle

					Number of Animals									
							Toxicity Study						TK Study[d]	
		Daily Doses[a]			Total on Study		Total		Terminal Necropsy		Recovery Necropsy		Days 1 and 90	
Group	Test Article	Dose (mg/kg)	Volume (mL/kg)	Conc.[b] (mg/mL)	M	F	M	F	M	F	M	F	M	F
1	HPMC-AS-NF	0	5	[c]	18	18	15	15	10	9	4	5	3	3
	Vehicle	0		0										
2	VX-770	100	5	40	24	24	15	15	9	10	5	5	9	9
	VX-809	1000		200										
	VRT-0995096	20		8										
3	VX-770	25	5	10	24	24	15	15	10	10	5	5	9	9
	VX-809	1000		200										
	VRT-0995096	20		8										
4	VX-770	25	5	10	24	24	15	15	10	9	5	5	9	9
	VX-809	500		100										
	VRT-0995096	10		4										
5	VX-770	10	5	4	24	24	15	15	10	10	5	5	9	9
	VX-809	500		100										
	VRT-0995096	10		4										

[a]VX-770 test article (TA) was a 50:49.5:0.5 solid dispersion mixture of VX-770 (API) and hydroxypropylmethylcellulose acid succinate (HPMC-AS), and sodium lauryl sulfate (SLS). VRT-0995096 TA was also a 50%/49%/1% (by weight) (b) (4) mixture of VRT-0995096, HPMC-AS, and SLS. Group 1 animals received the vehicle control plus the control article for VX-770 and VRT-0995096 TA's (see footnote c below).

[b]API concentrations for VX-770 and VRT-0995096 were ½ of the TA concentrations listed here. API concentrations for VX-809 were equal to the TA concentrations listed here.

[c]Control article (HPMC-AS-HF) for VX-770 and VRT-0995096 TA's was added to the vehicle control at the highest concentration level used in the VX-770 or VRT-0995096 TA animals (20 mg/mL).

[d]Toxicokinetic (TK) samples were collected from Groups 2-5 on Day 1 at 6 timepoints as follows: 0.5, 1, 2, 4, 8 and 24 hours post dose and on Day 90 predose and 1, 2, 4, 8, and 24 hours postdose. Group 1 animals were bled at the 8 hour timepoint. TK sample collections were from 3 animals/sex/group/time point.

The first day of dosing was defined as Day 1 of the study.

Observations and Results

Mortality: Animals were observed in their cages for mortality and general condition twice daily (once in the morning and once in the afternoon).

There were 4 deaths in the study that were attributed to oral gavage errors. There were no deaths attributed to drug treatment with the combination.

During the dosing phase, 1 male (Animal #2036) in Group 2 was found dead on Day 31, 1 female (Animal #4608) in Group 4 was euthanized in a moribund condition on Day 40 and a control female (Animal #1524) was found dead on Day 57. These deaths were the results of oral gavage errors. Macroscopically, the esophagus was perforated in 2 rats (#2036 and #1524) and a third rat (#4608) had a large amount of white material in the thoracic cavity. Other macroscopic findings in these animals, including fluid in the thoracic cavity, discoloration of the heart and/or lungs and adhesions, correlated microscopically with necrosis and inflammation and were consistent with gavage errors.

During the recovery phase, a control male (Animal #1013) was found dead on recovery Day 8. The control recovery male unscheduled decedent had no macroscopic findings, but a slight amount of pale eosinophilic material in the lungs was observed with microscopic examination. The cause of death for Animal #1013 was undetermined.

Clinical Signs: Observations for signs of toxic or pharmacologic effects were made once daily for each study animal. Unusual signs were recorded. These observations were made concurrently with one of the viability checks. Toxicity study animals were removed from their cages and examined twice pretest and once weekly during the study period. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration.

Incidences of alopecia of the limbs and torso were increased in an approximate dose-related manner for female drug-treated groups.

Table 37 Clinical signs in male and female rats over the 13-week treatment period

Clinical signs	Males					Females				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10
Alopecia (Limbs)	26/3	34/7	30/3	30/3	33/3	37/4	89/9	47/6	51/5	75/8
Alopecia (Torso)	0/0	1/1	0/0	13/1	1/1	0/0	47/6	33/4	8/1	8/2

Body Weights: Non-fasted body weights for toxicity study animals were recorded three times pretest and weekly during the study and recovery periods. Fasted body weights were obtained on the day of the scheduled necropsy.

Body weight gains were reduced ≥10% in male and female rats treated with the drug combinations for 13 weeks relative to concurrent control groups. A dose-response for reduced body weight gain was generally evident for male drug-treated groups. However, a dose-response was not evident for female drug-treated groups.

Table 38 Body weight (BW) gains in male and female rats over the 13-week treatment period

	Males				
	Control	Group 2	Group 3	Group 4	Group 5
BW, Week 1	465.3	443.4	461.2	451.8	445.5
BW, Week 13	663.2	568.3	627.4	604	616.4
Δ	197.9	124.9	166.2	152.2	170.9
%Initial BW	42.5	28.2	36.0	33.7	38.4
% Control	100.0	66.2	84.7	79.2	90.2
	Females				
	Control	Group 2	Group 3	Group 4	Group 5
BW, Week 1	285.6	267.8	268.6	266.1	275.5
BW, Week 13	366.2	323.6	316.3	329.5	332.7
Δ	80.6	55.8	47.7	63.4	57.2
%Initial BW	28.2	20.8	17.8	23.8	20.8
% Control	100.0	73.8	62.9	84.4	73.6

Figure 9 Body weights in Groups 1 to 5 of male rats

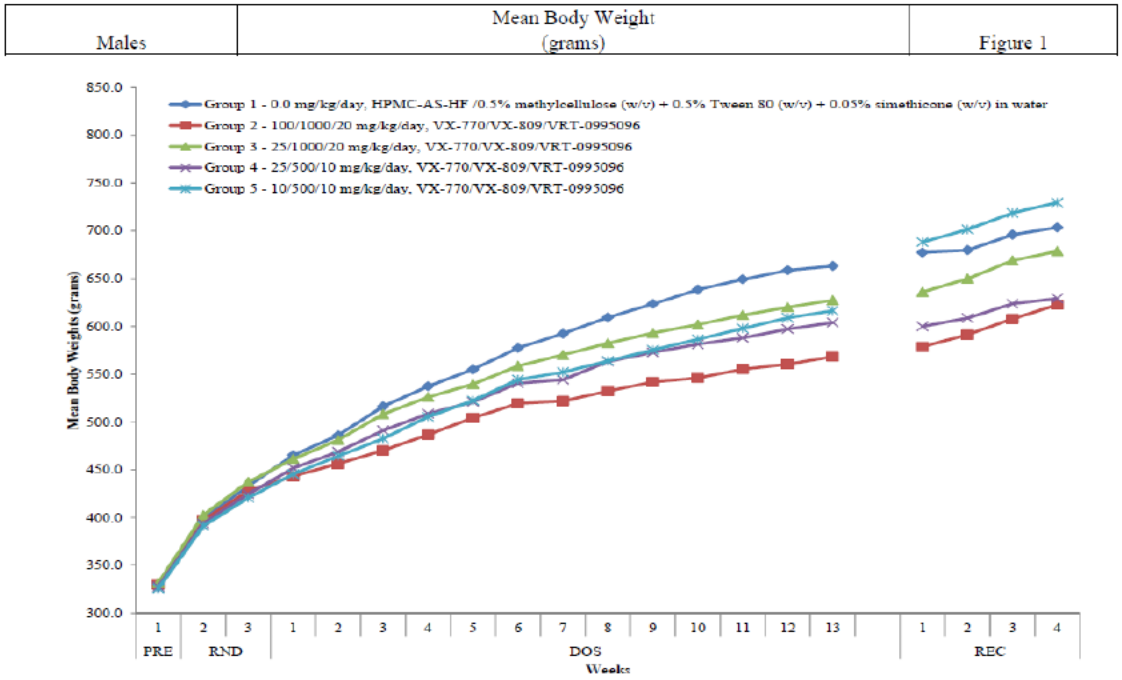
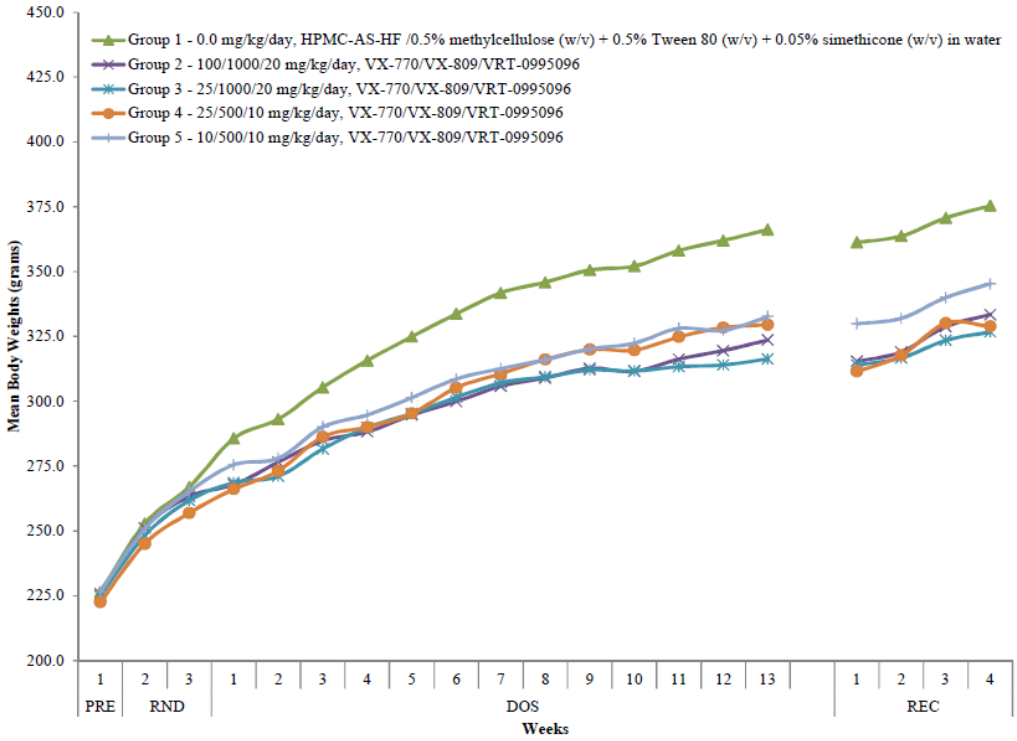


Figure 10 Body weights in Groups 1 to 5 of female rats

Females	Mean Body Weight (grams)	Figure 2



Feed Consumption: Feed was available without restriction 7 days/week. Food consumption was measured (weighed) for all toxicity study animals 2 weeks prior to start of dosing and weekly during treatment and the recovery period.

Food consumption was slightly reduced for males in Group 2 (1000/100/20 mg/kg/day).

Figure 11 Food consumption for male rats over the 90-day dosing period and 28-day recovery period

Males	Mean Food Consumption (grams/day)	Figure 3

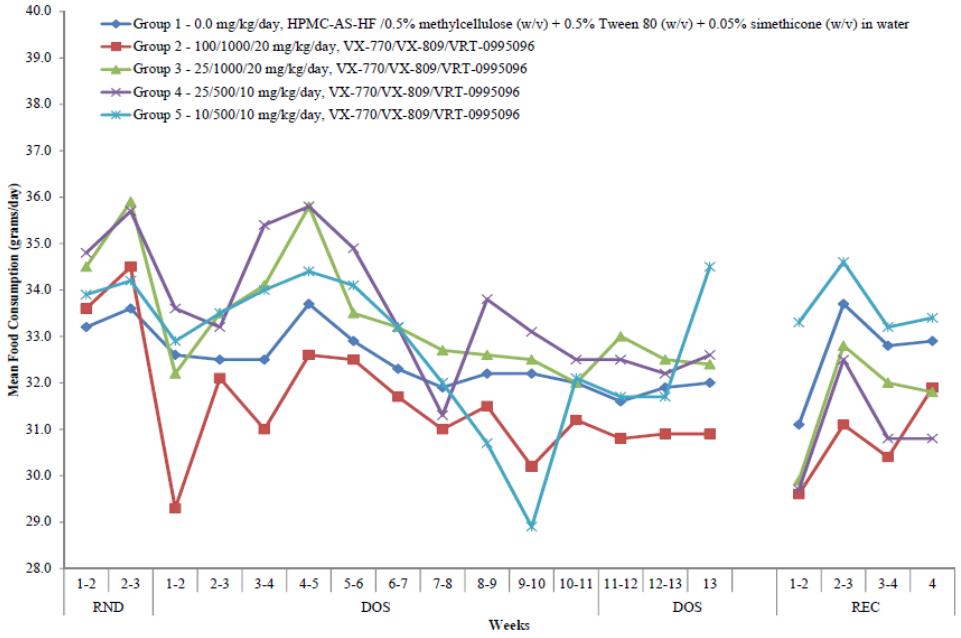
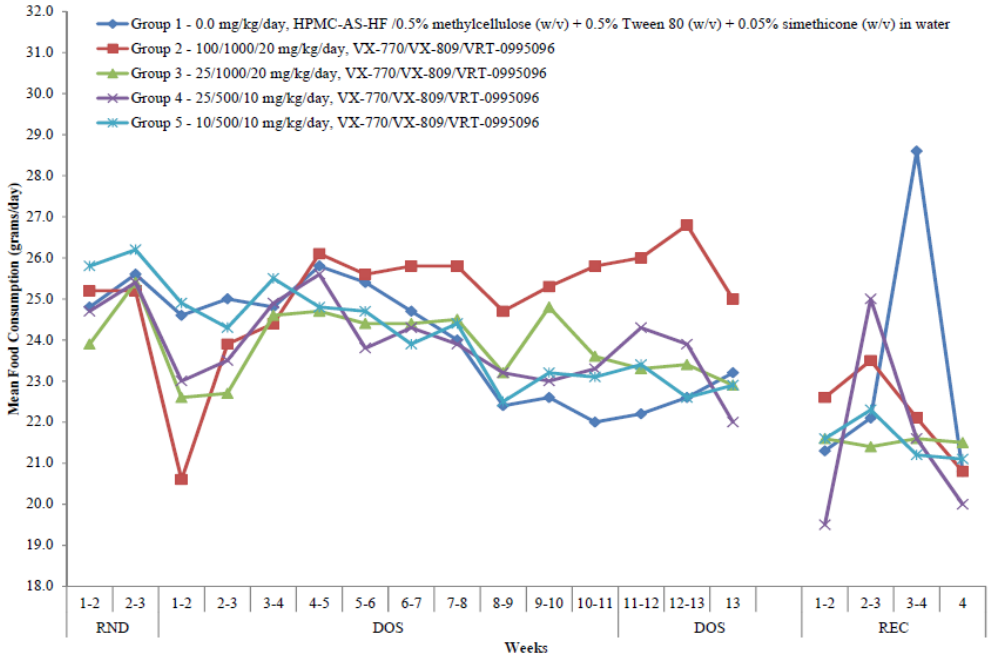


Figure 12 Food consumption for female rats over the 90-day dosing period and 28-day recovery period

Females	Mean Food Consumption (grams/day)	Figure 4



Ophthalmoscopy: Ophthalmic examinations were performed on all animals at pretest and at the end of the dosing period. Lids, lacrimal apparatus and conjunctiva were examined visually. The cornea, anterior chamber, lens, iris, vitreous humor, retina and optic disc were examined by indirect ophthalmoscopy.

Female #2559 in Group 2 (1000/100/20 mg/kg/day) was observed with bilateral posterior subcapsular cataracts of the lens at the end of the 90-day dosing period. The findings of bilateral posterior subcapsular cataracts were attributed to VX-770 (ivacaftor) based upon the results of a 28-day toxicology study with juvenile rats that received VX-770 alone (see Pharmacology and Toxicology Review of IND 74,633 dated June 28, 2012; Reference ID: 3152535).

Hematology: Blood was collected from up to 10 toxicity animals/sex/group at study termination and up to 5 toxicity animals/sex/group at the end of the recovery period for measurement of a complete panel of hematology and coagulation parameters.

Slight decreases of hemoglobin levels, hematocrit, and RBC counts were observed for males in Groups 2, 3, and 4 and females in Group 2. Platelet counts were increased for male and female drug-treated groups. Neutrophil, monocyte, and eosinophil counts were decreased for males in Group 2. Prothrombin times (PT) were decreased for male drug-treated groups. Activated partial thromboplastin times (APTT) were decreased for male and female drug-treated groups, although statistical significance was not achieved. Decreases of PT and APTT may have correlated with increased with platelet counts. Most of these changes were reversible by the end of the recovery period with the possible exception of APTT.

Table 39 Hematology and Coagulation parameters in rats at the end of the 90-day dosing period and 28-day recovery period

Parameter	Time	Males					Females				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
		0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10
Hemoglobin g/dL	Term	14.3	13.5*	13.6*	13.8	13.8	13.7	13.2	13.6	13.8	14.0
Hematocrit %	Term	44.0	40.8*	41.5*	42.0*	42.5	41.3	39.0*	40.7	41.7	41.8
RBC $x10^6/\mu L$	Term	8.36	7.88*	8.16	8.08	8.34	7.42	7.14	7.40	7.63	7.70
Platelets $x10^3/\mu L$	Term	1007	1150*	1162*	1136*	1191*	808	1050*	1010*	951	983
Neutrophils $x10^3/\mu L$	Term	1.98	1.31*	1.66	1.76	1.78	0.69	0.89	0.72	0.63	0.70
Monocytes $x10^3/\mu L$	Term	0.40	0.28	0.32	0.36	0.30	0.16	0.16	0.13	0.17	0.15
Eosinophils $x10^3/\mu L$	Term	0.17	0.09*	0.15	0.14	0.17	0.09	0.07	0.07	0.09	0.07
PT sec	Term	17.0	15.8*	15.9*	15.5*	15.9*	15.4	14.9	14.9	14.8	15.1
APTT sec	Term	17.0	16.2	15.0	15.9	16.6	17.0	16.2	16.4	15.8	16.6
	Rec	18.8	15.7	17.1	16.5	17.6	17.2	14.9	16.4	15.8	17.2

* p≤0.05
Term = termination after 90 days of dosing

Clinical Chemistry: Blood was collected from up to 10 toxicity animals/sex/group at study termination and up to 5 toxicity animals/sex/group at the end of the recovery period for measurement of a complete panel of clinical chemistry parameters.

Cholesterol levels were increased for male drug-treated groups. Triglyceride and glucose levels were decreased for male and female drug-treated groups. BUN levels were increased for males and females in the high dose Group 2. Albumin levels and A/G ratios were decreased for female drug-treated groups. Sodium and chloride levels were slightly decreased for male drug-treated groups. Phosphate levels were slightly increased for females in Groups 2 and 3. AST activities were decreased for males and females in Group 2. Total bilirubin levels were slightly decreased for male drug-treated groups and females in Group 2. The significance of decreased AST activities and total bilirubin were unclear. Most changes were reversible with the exception of triglycerides and AST activities.

Table 40 Clinical chemistry parameters in rats at the end of the 90-day dosing period and 28-day recovery period

Parameter	Time	Males					Females				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
		0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10
Cholesterol mg/dL	Term	72	98*	109*	96*	94*	101	117	114	112	104
Triglyceride mg/dL	Term	76	40*	63	61	60	93	39*	47*	56*	49*
	Rec	128	91	86	90	123	85	52	55	83	75
Glucose mg/dL	Term	142	98*	119*	119*	114*	155	114*	105*	121*	112*
BUN mg/dL	Term	13	17*	14	13	15	15	21*	13	14	14
Albumin g/dL	Term	3.5	3.5	3.5	3.5	3.6	4.4	4.1*	3.9*	4.0*	4.1
A/G ratio	Term	1.3	1.3	1.3	1.3	1.3	1.6	1.4*	1.5	1.5	1.5
Na^+ mEq/L	Term	145	143*	143*	144	143	142	141	141	142	142
Cl^- mEq/L	Term	103	102*	102*	102*	101*	100	100	100	101	100
Phosphate mg/dL	Term	6.7	7.0	6.8	7.3	6.9	5.6	6.7*	6.4*	5.8	6.2
AST U/L	Term	154	95*	128	128	138	135	81*	131	129	120
	Rec	133	97	114	111	123					
Total bilirubin mg/dL	Term	0.17	0.12*	0.12*	0.13	0.15	0.17	0.13*	0.18	0.17	0.17

* p≤0.05
Term = termination after 90 days of dosing

Urinalysis: Urine, obtained by a 16-hour overnight collection period, was obtained from up to 10 toxicity animals/sex/group at study termination and up to 5 toxicity animals/sex/group at the end of the recovery period. Animals were fasted and water-deprived during the collection period.
Potential treatment-related changes of urinalysis parameters were observed for urinay volume, specific gravity, and pH. Increased urinary volume and decreased urinary specific gravity was observed for males and females in Groups 2 and 3. Urinary pH was increased for males in Groups 2 and 3. These findings may correlate with the histopathological finding of basophilic tubules. The relevance of these findings to humans was unclear.

Table 41 Urinalysis parameters in rats treated with combinations of VX-809, VX-770, and VRT-0995096 for 3 months

Parameter	Time	Males					Females				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
		0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10
Volume mL	Term	6 5	10* 8*	12* 2	7 6	5 6	5 3	12* 4	9 3	6 2	5 3
pH	Term	6.4	6.7*	6.8*	6.6	6.4	6.2	6.4	6.3	6.1	6.3
Specific Gravity	Term	1.037 1.054	1.026* 1.035	1.025* 1.075	1.035 1.044	1.038 1.047	1.028 1.038	1.013* 1.035	1.021 1.036	1.030 1.056	1.030 1.035

*p≤0.05

Gross Pathology: Necropsy examinations were performed on up to 10 toxicity animals/sex/group after treatment for at least 3 months and on up to 5 animals/sex/group 28 days after termination of dosing. Animals were not fasted overnight prior to necropsy. A necropsy schedule was established to ensure that examination of animals of both sexes was performed at similar times of the day throughout the necropsy period.

Test article-related macroscopic findings were observed in the stomach in drug-treated male rats at doses ≥25/500/25/10 mg/kg/day and in female rats at doses ≥500/10/10 mg/kg/day. Eleven rats had foci of red, brown, or black discoloration or discolored streaks in the gastric mucosa, 5 rats had depressed areas in the gastric mucosa, and 1 rat had severe edema of the fundic mucosa and red material in the stomach lumen. These findings correlated microscopically with foci of mucosal necrosis and erosion with variable amounts of submucosal inflammation and edema.

There were no gross pathological findings in the stomach at the end of the recovery period.

Table 42 Gross pathological finding in rats treated with combinations of VX-809, VX-770, and VRT-0995096 for 3 months

Parameter	Time	Males					Females				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
		0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10
N =	Term	10	9	10	10	10	9	10	10	9	10
Stomach	Term										
-discolored		0	2	1	1	0	0	2	2	0	3
-abnormal contents		0	1	0	0	0	0	0	0	0	0
-depressed area		0	1	0	0	0	0	1	0	0	0
-edematous		0	1	0	0	0	0	0	0	0	0

Organ Weights: Absolute and relative organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid glands, and uterus with cervix.

Organ weight changes were observed for the thymus, liver, prostate, thyroid/parathyroid, and adrenals as detailed in the table below. Decreased thymus weights observed for males in Group 2 appeared to correlate with a histopathological finding of an increased severity of decreased lymphocytes in the thymus. Other organ weight changes had no correlations to histopathological findings.

Table 43 Organ weights in rats treated with combinations of VX-809, VX-770, and VRT-0995096 for 3 months

Parameter	Time	Males					Females				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
		0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10
Thymus g	Term	0.3328	**0.2228***	0.3063	0.3199	0.2969	0.2585	0.2377	0.2293	0.2529	0.2477
Thymus %BW	Term	0.0523	**0.0413**	0.0513	0.0537	0.0524	0.0730	0.0779	0.0767	0.0772	0.0772
Thymus %BrW	Term	14.6698	**10.2020***	13.8208	14.5617	13.5579	13.1703	12.0397	11.6778	12.7296	12.4590
Liver g	Term	14 0802	**15.0495**	14.0509	14.0525	13.0190	8.4789	**9.4594**	7.7514	8.2212	7.8986
Liver %BW	Term	2.2118	**2.7730***	2.3346	2.3705	2.3105	2.4215	**3.0997***	2.5952	2.522	2.4912
Liver %BrW	Term	617.505	**691.391**	634.703	640.124	595.329	430.554	**478.624**	394.843	413.847	397.799
Prostate g	Term	3.7740	**3.1854**	**3.0226**	3.5575	3.5109					
Prostate %BW	Term	0.5951	**05875**	**0.5007**	0.6129	0.6254					
Prostate %BrW	Term	165.303	**146.117**	**136.340**	162.494	160.559					
Thyroid/Parathyroid, g	Term	0.0315	**0.0384***	**0.0394***	**0.0375***	**0.0373***	0.0256	0.0275	0.0259	0.0286	0.0245
Thyroid/Parathyroid, %BW	Term	0.0050	**0.0070***	**0.0067***	**0.0064***	**0.0066***	0.0076	0.0090	0.0087	0.0089	0.0077
Thyroid/Parathyroid, %BrW	Term	1.3848	**1.7651***	**1.7901***	**1.7129***	**1.7045***	1.3039	1.3878	1.3282	1.4372	1.2318
Adrenal g	Term	0.0632	0.0618	0.0592	0.0583	0.0629	0.0696	**0.0817**	**0.0764**	0.0714	0.0679
Adrenal %BW	Term	0.0100	0.0114	0.0101	0.0099	0.0112	0.0201	**0.0268***	**0.0256***	0.0222	0.0215
Adrenal %BrW	Term	2.7764	2.8367	2.6901	2.6682	2.8759	3.5483	**4.1359**	**3.8824**	3.5923	3.4176

*p≤0.05
Bold text denotes potential differences between control and drug-treated groups

Histopathology:

Adequate Battery: The tissues listed in the table below were obtained at the scheduled sacrifice intervals and preserved for all toxicity animals. In addition, slides of the indicated tissues were prepared and examined microscopically for all toxicity animals Groups 1, 4 and 5 sacrificed at termination. Tissues were also examined for any toxicity animals in the intermediate dose groups which died prior to study termination. Any abnormalities not noted during macroscopic examinations which were seen during histology processing were recorded.

Table 9.3.15.4-1: Tissues weighed, preserved and examined microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY (Groups 1 - 5)[a]
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (femur)		X	
bone (sternum, distal femur)		X	X
bone marrow (sternum, distal femur)		X	X[b]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
Harderian gland		X	X
heart	X	X	X
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
liver	X	X	X
lungs (with mainstem bronchi)	X	X	X
lymph nodes (mesenteric, mediastinal)		X	X
mammary gland (inguinal)		X	X
nerve (sciatic)		X	X
optic nerve		X	X
ovaries	X	X	X
pancreas		X	X
pituitary gland	X[c]	X	X
prostate gland	X[d]	X	X
salivary glands (submandibular)		X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY (Groups 1 - 5)[a]
seminal vesicles	X[d]	X	X
skeletal muscle (*rectus femoris*) longitudinal and transverse		X	X
skeletal muscle (*m.gastrocnemious*) longitudinal and transverse		X	
skeletal muscle (*m.psosas*) longitudinal and transverse		X	
skin (base of tail)		X	X
small intestine (duodenum, ileum, jejunum and Peyer's patches/GALT)		X	X
spinal cord (cervical)		X	X
spleen	X	X	X
stomach		X	X[e]
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X[c]	X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix	X	X	X
vagina		X	X
tissues with macroscopic findings including tissue masses		X	X

[a]These tissues were also examined for toxicity study animals in Groups 2-3 which died prior to study termination.
[b]Qualitative examination
[c]Weight taken post-fixation
[d]Prostate and seminal vesicles were weighed together.
[e]Examined in the Groups 1 to 5 male and female recovery animals.

Peer Review: None

Histological Findings: Target organs of toxicity were identified as the stomach, kidneys, thymus, mesenteric LN, and heart.

Stomach: Focal necrosis and/or erosions (minimal to slight) were present in the glandular mucosa of the stomach for all male drug-treated groups and females in Groups 2, 3, and 5. These foci correlated with macroscopic findings of red, brown and

black discoloration. The findings were dose-responsive for male drug-treated groups, but not for female drug-treated groups. Mucosal necrosis and/or erosions was reversible following the recovery period.

The squamous epithelium along the limiting ridge of the forestomach showed cystic degeneration (minimal to moderate) in males and females dosed at ≥500/10/10 mg/kg/day VX-809/VX-770/VRT-0995096. Variable numbers of the superficial keratinized cells along the limiting ridge contained small cysts filled with pink material. There was no clear dose relationship in males. Females had an inverse relationship to dose. Epithelial cystic degeneration along the limiting ridge was not reversible.

Focal inflammation and edema were variably present. In one male (No. 2038) in Group 2 at 1000/100/20 mg/kg/day, the edema was moderate and diffuse.

Kidney: Basophilic tubules (minimal) were observed in 6 of 10 males dosed at 1000/100/20 mg/kg/day VX-809/VX-770/VRT-0995096. This finding was also evident for 2 of 10 females in Group 2 and 1 of 10 females in Group 3. The kidneys from recovery animals were not examined microscopically. The sponsor contended that basophilic and dilated tubules are common background findings in rats and the slightly increased incidence observed in terminal sacrifice animals was a known class effect of the test article as this finding was observed at increased incidences in the 28-day toxicology study with rats that received the combination of VX-809 and VX-770.

Thymus: An increased severity of decreased lymphocytes was observed in the thymus for males in Group 2 at 1000/100/20 mg/kg/day. It is noted that the spontaneous incidence of this finding in the control group was high.

Mesenteric LN: Decreased lymphocytes were observed in the mesenteric LN for one male in Group 2 at 1000/100/20 mg/kg/day.

Urinary bladder: Urothelial hyperplasia in the urinary bladder was observed for one male in Group 2 at 1000/100/20 mg/kg/day.

Heart: Minimal cardiomyopathy was observed with an increased incidence for 4 of 10 females in Group 2 at 1000/100/20 mg/kg/day. The significance of this finding was unclear as the finding was observed for 1 of 9 control females and 2 of 10 control males. The incidence in male drug-treated groups ranged from 2 of 10 to 5 of 10 with no dose-response relationship.

Table 44 Histopathological finding in rats treated with combinations of VX-809, VX-770, and VRT-0995096 for 3 months

Organ/Tissue	Time	Male					Female				
		G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
		0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10	0 0 0	1000 100 20	1000 25 20	500 25 10	500 10 10
Stomach -necrosis and/or erosions with edema and inflammatory cell infiltrate	Term	0/10	3/10	3/10	2/10	1/10	0/9	1/10	3/10	0/9	2/10
Grade 1		0	1	1	1	1	0	0	2	0	0
Grade 2		0	1	2	1	0	0	1	1	0	2
Grade 3		0	1	0	0	0	0	0	0	0	0
Stomach -necrosis and/or erosions with edema and inflammatory cell infiltrate	Rec	0/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Stomach -limiting ridge: epithelial cystic degeneration	Term	0/10	2/9	4/10	3/10	4/10	0/9	1/10	4/10	3/9	6/10
Grade 1		0	2	2	1	4	0	1	4	1	5
Grade 2		0	0	2	1	0	0	0	0	2	0
Grade 3		0	0	0	1	0	0	0	0	0	1
Stomach -limiting ridge: epithelial cystic degeneration, Grade 1	Rec	0/4	2/5	1/5	0/5	2/5	0/5	0/5	0/5	3/5	3/5
Stomach -diffuse submucosal edema, Grade 3	Term	0/10	1/9	0/10	0/10	0/10	0/9	0/10	0/10	0/9	0/10
Stomach -diffuse submucosal edema	Rec	0/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Stomach -congestion	Term	0/10	1/9	0/10	0/10	0/10	0/9	0/10	0/10	0/9	0/10
Stomach -congestion	Rec	0/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Kidneys -basophilic tubules, Grade 1	Term	1/9	6/9	1/10	3/10	1/10	0/9	2/10	1/10	0/9	0/10
Thymus -decreased lymphocytes	Term	6/10	6/9	5/10	7/10	8/10	8/9	7/10	5/10	1/9	9/10
Grade 1		6	3	5	7	8	8	7	5	1	9
Grade 2		0	3	0	0	0	0	0	0	0	0
Mesenteric LN -decreased lymphocytes	Term	0/10	1/9	0/10	0/10	0/10	0/9	0/10	0/10	0/9	0/10
Urinary bladder -urothelial hyperplasia, Grade 1	Term	0/10	1/9	0/10	0/10	0/10	0/9	0/10	0/10	0/9	0/10
Heart -cardiomyopathy, G1-2	Term	2/10	3/10	2/10	5/10	0/10	1/9	4/10	0/10	1/9	1/10

Note that Group 2 is the high dose group and Group 5 is the low dose group. Doses progressively decreased from Group 2 to Group 5.

Special Evaluation: The 24-hour toxicokinetic animals (up to 3/sex/group, Groups 2-5) and the control toxicokinetic animals (up to 3/sex, Group 1) were transferred to necropsy and sacrificed on Day 91 (after the last scheduled 24-hour toxicokinetic blood collection for Groups 2 to 5); blood was collected and the targeted organs (heart, liver, lung) harvested as soon as possible for eventual biomarker analyses or determination of tissue concentrations of VX-770 and VX-809 and their characterized major

metabolites. The decision to analyze these frozen tissue samples will be by protocol amendment, which will outline the purpose for any such analysis and how the data will be reported. No data was provided in the report.

Toxicokinetics: Blood samples were obtained from test article-treated rats on days 1 and 91 for the determination of plasma concentrations of VX-770, VX-809 and VRT-0995096.

Table 9.3.14-1: Collection Times and Number of Animals

No. of Animals	Interval/Time points
Day 1	
3/sex/Groups 2 to 5/timepoint[a]	0.5, 1, 2, 4, 8 and 24 hrs post-dose
Day 90	
3/sex/Groups 2 to 5/timepoint[a]	Predose, 1, 2, 4, 8 and 24 hrs post-dose

[a]Blood samples were collected from Group 1 animals on Day 1 and Day 90 at only one timepoint (8 hours) which was near the t_{max} for all three test articles in rats.

Concentrations of VX-770, VRT-837018, VRT-842917, VX-809 and VRT-0995096 in rat plasma samples were determined by the Vertex DMPK-Dev BA Laboratory (Cambridge, MA) using LC-MS/MS detection methods. Lower and upper limits of quantitation (LLOQ and ULOQ) for VX-770, VRT-837018, VRT-842917, VX-809 and VRT-0995096 are reported in the table below.

Table 45 Lower and upper limits of quantitation (LLOQ and ULOQ, ng/mL) for VX-770, VRT-837018, VRT-842917, VX-809 and VRT-0995096

Analyte	LLOQ	ULOQ
VX-770	2.00	2000
VRT-837018	2.00	2000
VRT-842917	2.00	2000
VX-809	2.00	2000
VRT-0995096	2.00	2000

On day 90, C_{max} and AUC values for VX-809 were relatively comparable at doses of 500 and 1000 mg/kg/day in males and females showed no evidence of any relationship to dose and suggested a saturation of exposure. Exposures were generally higher in females as compared to males. Exposures were generally higher on day 1 as compared to day 90 that might be suggestive of enhanced metabolism of VX-770.

Table 46 Toxicokinetic parameters for VX-809 in rats following once daily oral administration of VX-770, VX-809, and VRT-0995096 for 90 days

Table 2 Summary of Toxicokinetic Parameters for VX-809 in Female and Male Rats Following Once Daily Oral Co-administration of VX-770, VX-809 and VRT-0995096 on Day 1 and Day 90

Study Day	Group	Dose of VX-809 (mg/kg)	Matrix	Gender							
				Female				Male			
				C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	DN_$AUC_{0\text{-}24hr}$* (µg*hr/mL)	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	DN_$AUC_{0\text{-}24hr}$* (µg*hr/mL)
Day 1	2	1000	Plasma	245	8.00	5070	5.07	178	8.00	3570	3.57
	3	1000	Plasma	205	8.00	3390	3.39	179	8.00	3610	3.61
	4	500	Plasma	168	4.00	2150	4.31	213	8.00	2990	5.98
	5	500	Plasma	225	4.00	2870	5.74	134	8.00	2030	4.06
Day 90	2	1000	Plasma	190	8.00	3010	3.01	140	8.00	2430	2.43
	3	1000	Plasma	222	4.00	3400	3.40	145	4.00	2150	2.15
	4	500	Plasma	216	4.00	3040	6.07	135	8.00	2250	4.51
	5	500	Plasma	225	4.00	3120	6.24	145	8.00	2300	4.60

Source: PKS Study: CF-VX-809-TX-013-Rat-3-month-Combo

* Dose normalized to 1 mg/kg

On day 90, C_{max} and AUC values for VRT-0995096 increased in an approximate dose proportional manner with doses of 10 and 20 mg/kg/day. Exposures were slightly higher in females as compared to males. Exposures on days 1 and 90 were relatively comparable.

Table 47 Toxicokinetic parameters for VRT-0995096 in rats following once daily oral administration of VX-770, VX-809, and VRT-0995096 for 90 days

Table 3 Summary of Toxicokinetic Parameters for VRT-0995096 in Female and Male Rats Following Once Daily Oral Co-administration of VX-770, VX-809 and VRT-0995096 on Day 1 and Day 90

Study Day	Group	Dose of VRT-0995096 (mg/kg)	Matrix	Gender							
				Female				Male			
				C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	DN_$AUC_{0\text{-}24hr}$* (µg*hr/mL)	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	DN_$AUC_{0\text{-}24hr}$* (µg*hr/mL)
Day 1	2	20	Plasma	70.8	1.00	828	41.4	40.0	1.00	535	26.7
Day 1	3	20	Plasma	77.7	0.50	707	35.3	52.2	0.50	685	34.2
Day 1	4	10	Plasma	41.1	0.50	387	38.7	25.6	0.50	344	34.4
Day 1	5	10	Plasma	51.2	0.50	476	47.6	27.8	2.00	340	34.0
Day 90	2	20	Plasma	78.1	1.00	938	46.9	51.9	1.00	725	36.3
Day 90	3	20	Plasma	79.6	1.00	875	43.7	70.1	1.00	788	39.4
Day 90	4	10	Plasma	39.0	1.00	473	47.3	34.3	1.00	430	43.0
Day 90	5	10	Plasma	44.5	1.00	507	50.7	37.1	1.00	443	44.3

Source: PKS Study: CF-VX-809-TX-013-Rat-3-month-Combo

* Dose normalized to 1 mg/kg

On day 90, C_{max} and AUC values for VX-770 increased in an approximate dose proportional manner with doses of 10, 25, and 100 mg/kg/day. With a dose of 100 mg/kg/day, exposures were generally higher in females than males; however, at lower doses, there were no sex-related differences in exposures. Exposures were higher on day 90 as compared to day 1 suggesting accumulation in the process to achieve steady-state levels.

Table 48 Toxicokinetic parameters for VX-770 in rats following once daily oral administration of VX-770, VX-809, and VRT-0995096 for 90 days

Table 4 Summary of Toxicokinetic Parameters for VX-770 in Female and Male Rats Following Once Daily Oral Co-administration of VX-770, VX-809 and VRT-0995096 on Day 1 and Day 90

Study Day	Group	Dose of VX-770 (mg/kg)	Matrix	Gender							
				Female				Male			
				C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	DN_$AUC_{0\text{-}24hr}$* (µg*hr/mL)	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	DN_$AUC_{0\text{-}24hr}$* (µg*hr/mL)
Day 1	2	100	Plasma	10.4	8.00	221	2.21	7.65	8.00	157	1.57
Day 1	3	25	Plasma	4.24	8.00	77.9	3.12	3.19	4.00	68.5	2.74
Day 1	4	25	Plasma	4.75	4.00	71.5	2.86	5.36	8.00	95.5	3.82
Day 1	5	10	Plasma	2.11	4.00	27.0	2.70	1.54	4.00	28.8	2.88
Day 90	2	100	Plasma	35.9	4.00	653	6.53	19.1	4.00	372	3.72
Day 90	3	25	Plasma	8.74	4.00	138	5.51	7.56	4.00	143	5.70
Day 90	4	25	Plasma	8.34	4.00	120	4.79	7.73	8.00	151	6.06
Day 90	5	10	Plasma	3.14	2.00	39.4	3.94	2.91	4.00	47.3	4.73

Source: PKS Study: CF-VX-809-TX-013-Rat-3-month-Combo

* Dose normalized to 1 mg/kg

On day 90, C_{max} and AUC values for VRT-837018 increased in an approximate dose proportional manner. On day 1, there were no sex-related differences in exposures. However, on day 90, exposures were slightly in males as compared females. Exposures were higher on day 90 as compared to day 1 suggesting accumulation in the process to achieve steady-state levels.

Table 49 Toxicokinetic parameters for VRT-837018 (M1) in rats following once daily oral administration of VX-770, VX-809, and VRT-0995096 for 90 days

Table 5 Summary of Toxicokinetic Parameters for VRT-837018 in Female and Male Rats Following Once Daily Oral Co-administration of VX-770, VX-809 and VRT-0995096 on Day 1 and Day 90

Study Day	Group	Dose of VX-770 (mg/kg)	Matrix	Gender					
				Female			Male		
				C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)
Day 1	2	100	Plasma	1.51	24.00	30.0	1.55	8.00	31.6
Day 1	3	25	Plasma	0.654	2.00	11.4	0.737	8.00	15.0
Day 1	4	25	Plasma	0.804	4.00	12.3	1.31	8.00	22.0
Day 1	5	10	Plasma	0.327	2.00	5.00	0.470	4.00	7.89
Day 90	2	100	Plasma	3.98	4.00	65.9	4.93	8.00	97.4
Day 90	3	25	Plasma	1.32	2.00	22.9	1.76	8.00	35.9
Day 90	4	25	Plasma	1.32	2.00	22.0	1.79	8.00	35.4
Day 90	5	10	Plasma	0.594	2.00	8.34	0.773	4.00	13.1

Source: PKS Study: CF-VX-809-TX-013-Rat-3-month-Combo

On day 90, C_{max} and AUC values for VRT-842917 increased in an approximate dose proportional manner. On day 1, there were no sex-related differences in exposures. However, on day 90, exposures were slightly in males as compared females. Exposures were higher on day 90 as compared to day 1 suggesting accumulation in the process to achieve steady-state levels.

Table 50 Toxicokinetic parameters for VRT-842917 (M6) in rats following once daily oral administration of VX-770, VX-809, and VRT-0995096 for 90 days

Table 6 Summary of Toxicokinetic Parameters for VRT-842917 in Female and Male Rats Following Once Daily Oral Co-administration of VX-770, VX-809 and VRT-0995096 on Day 1 and Day 90

Study Day	Group	Dose of VX-770 (mg/kg)	Matrix	Gender					
				Female			Male		
				C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)	C_{max} (µg/mL)	t_{max} (hr)	$AUC_{0\text{-}24hr}$ (µg*hr/mL)
Day 1	2	100	Plasma	0.145	24.00	2.22	0.159	24.00	2.76
Day 1	3	25	Plasma	0.0442	8.00	0.822	0.0666	4.00	1.40
Day 1	4	25	Plasma	0.0507	4.00	0.711	0.263	4.00	1.77
Day 1	5	10	Plasma	0.0361	8.00	0.491	0.0601	8.00	0.865
Day 90	2	100	Plasma	0.375	4.00	6.46	0.861	8.00	17.1
Day 90	3	25	Plasma	0.155	8.00	2.62	0.309	8.00	5.97
Day 90	4	25	Plasma	0.132	2.00	1.98	0.301	4.00	4.93
Day 90	5	10	Plasma	0.0691	4.00	0.996	0.145	8.00	2.32

Source: PKS Study: CF-VX-809-TX-013-Rat-3-month-Combo

There were no findings that suggested any toxicokinetic interactions between VX-809, VX-770 and it metabolites, or VRT-0995096.

Dosing Formulation Analysis: Prior to dosing of the study, homogeneity of VX-809 and VRT-0995096 in dose formulations prepared using the proposed preparation procedure were determined by taking 3 top, 3 middle, and 3 bottom samples (9 samples/4 mL each per batch) of from formulations of the low and high concentrations for the study. Homogeneity of VX-770 was determined from dose formulations prepared on Day 1 of the study by taking 3 top, 3 middle and 3 bottom samples (9 samples/2 mL each per batch) from formulations of the low and high concentrations for the study. Stability under storage conditions to be used in this study was determined from the homogeneity preparations for 24 hours at room temperature and refrigerated. Two 4 (predose) or 2 mL samples (Day 1) were taken from the middle of the low- and high concentrations (Groups 2 and 5) prepared for homogeneity analyses. Dose confirmation analyses were performed in duplicate on Day 1 and Day 90 (to coincide with the day of toxicokinetic sampling). Two samples (4 mL each) were taken from the middle region of each formulation (including the control group) on the day of dose preparation.

Homogeneity results for VX-809, VX-770, and VRT-0995096 confirmed that the preparation procedure used for this study produced homogeneous mixtures and that the test article was stable in the vehicle, under storage conditions used in this study (at room temperature and refrigerated for 24 hours). Analyses conducted during the treatment period confirmed that dose suspensions of appropriate concentration were administered with the exception of VX-809 on Day 90 which was slightly above the + 15% of nominal concentration.

DOGS

Study title: VX-809 AND VX-770: A 28-DAY ORAL (GAVAGE) COMBINATION TOXICITY AND TOXICOKINETIC STUDY IN DOGS WITH 14-DAY RECOVERY PERIOD

Study no.: VX-809-TX-010 and VX-770-TX-016
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: June 2, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity:

Test Articles	Lot Numbers	Purity	Description	Dates Received	Retest Dates
VX-770	17QB02.HQ00016	99.2%	White powder	24 Mar 2009	(b) (4)
VX-809	PT-C07091701-M08001	100%	White (b) (4)	31 Dec 2008	
	PT- C07091701-M09001	99%	White	5 Jun 2009	
	PT-C07091701-M08002	99.9%	White to off-white (b) (4)	16 Feb 2009	

VX-770 was supplied as a 50:49.5:0.5 (b) (4) (b) (4) mixture of VX-770 (API) and hydroxypropylmethylcellulose acid succinate (HPMC-AS) and sodium lauryl sulfate (SLS). The (b) (4) (b) (4) powder was formulated as a suspension in the vehicle.

Key Study Findings

● In a 28-day oral (gavage) toxicology study, dogs received the combination of VX-809 and VX-770 at doses of 0/0, 300/5, 300/15, 600/15, and 600/60 mg/kg/day. Combinations of VX-809 and VX-770 were formulated as separate suspensions for co-administration by oral gavage. At the end of the dosing period, 4 dogs/sex/group were sacrificed. At the end of a 14-day recovery period, an additional 2 dogs/sex/group in the control and 600/15 and 600/60 mg/kg/day groups were sacrificed.

● Following co−administration of VX-770/VX-809, prolonged PR intervals (first degree AV block) were observed in 3/12 and 10/12 dogs in the 600/15, and 600/60 mg/kg/day, respectively.

● Co−administration of VX-770 and VX-809 also produced early depolarization of supraventricular origin (supraventricular premature complex: SVPC) for 8/12 dogs in the 600/60 mg/kg/day group. Some SVPCs occurred during the inspiratory phase of the respiratory cycle; therefore, early supraventricular depolarization with the P wave in contact with the previous T wave, not buried in the previous T wave, could be resulting from exaggerated respiratory sinus arrhythmia. These ECG changes were not observed with VX-809 alone. SVPCs were increased as compared to VX-770 alone.

● Histopathological findings were evident in the thymus, testes, epididymides, prostate, and gallbladder.

● There was a dose-related depletion of lymphocytes in the thymus. The severity of lymphoid depletion exceeded control levels in males dosed at ≥300 mg/kg/day VX−809 + ≥ 5 mg/kg/day VX−770 (Groups 3 through 5) and in females at all dose levels. Thymic weights were decreased for all male and female treatment groups. These findings were not considered dose-limiting based upon the high spontaneous incidence in the control group.

● In the 600/60 mg/kg/day group, 2 of 4 males had increased numbers of multinucleate degenerate germ cells in the testes (moderate vs. minimal) and one of these dogs also had increased sloughed testicular germ cells in the epididymides. The incidence and severity of prostatic acinar contraction and reduced secretion was also greater in this group than in the other groups and this correlated with a decrease in prostatic weight in this group. Given the peripubertal status of the animals, it was likely that these changes represented a slight retardation of sexual maturation in the high dose group animals, which was probably associated with the decreased body weight gain that occurred in these animals during the study. These changes might be an indirect effect of the test article.

● The epithelial cells lining the gall bladder contained slightly increased amounts of mucinous secretory product in males and females at 600/60 mg/kg/day and in one male at 600/15 mg/kg/day compared to control animals. This finding was not considered adverse as there was no evidence of structural damage in the gallbladder.

● The NOAEL was identified as 600/15 mg/kg/day based upon histopathological findings in the male reproductive organs (testes, epididymides, and prostate) at the high dose of 600/60 mg/kg/day. Findings were reversible at the end of a 14-day recovery period. ECG findings were considered monitorable in a clinical setting. $AUC_{0\text{-}24hr}$ values for VX-809 in females and males were 976 and 503 µg.hr/mL, respectively. Respective $AUC_{0\text{-}24hr}$ values for VX-770 and its M1 and M6 metabolites in females were 102, 21.2, and 2.32 µg·hr/mL and in males were 124, 16.7, and 1.31 µg·hr/mL.

Methods

Doses: Combinations of VX-809 and VX-770 were administered at doses of 0/0, 300/5, 300/15, 600/15, and 600/60 mg/kg/day. Combinations of VX-809 and VX-770 were formulated as separate suspensions for co-administration by oral gavage.

Frequency of dosing: Once per day

Route of administration: Oral gavage

Dose volume: The dose volume was 5 mL/kg/day for each vehicle and test article in all groups.

Formulation/Vehicle: The vehicle for VX-809 consisted of 0.5% methylcellulose (MC), 0.5% Tween 80, and 0.05% simethicone in distilled water.
The vehicle for VX-770 consisted of 0.5 % MC, 0.5% sodium lauryl sulfate, and 0.01% simethicone in distilled water.

The placebo for VX-770, hydroxypropyl-methylcellulose acid succinate (HPMC-AS) was suspended in the VX-770 vehicle [0.5% (w/v) Methylcellulose (MC) with 0.5% (w/v) Sodium Lauryl Sulfate (SLS) and 0.01% (w/v) Simethicone in Water] at the highest concentration dosed in the VX-770 test article groups (12 mg/g). Vehicle-control animals in Group 1 received both vehicle control suspensions, which were co-administered daily for 28 days.

Species/Strain: Beagle dogs (male and female) were obtained from (b) (4)

Number/Sex/Group: 4 dogs/sex/group were sacrificed at the end of the dosing period

Age: Animals were 7.5 to 8.5 months at the start of dosing

Weight:

Kg	Mean	Range
Males	8.2	7.0 - 9.5
Females	7.2	6.0 - 8.5

Satellite groups: 2 dogs/sex/group in the control and 600/15 and 600/60 mg/kg/day groups were sacrificed at the end of the 14-day recovery period

Unique study design: For potential biomarker analysis, sections of heart, liver, kidneys, and serum were collected and frozen at -70°C.

Deviation from study protocol: Deviations were minor and did not impact the integrity of the study.

Table 51 Design of the 28-day oral toxicology study with the combination of VX-809 and VX-770 in dogs

Group	Test Article	Daily Doses[a]			Number of Animals							
					Total on Study		Toxicity Study				TK Study[d]	
							Terminal Necropsy		Recovery Necropsy		Days 1 and 28	
		Dose (mg/kg)	Volume (mL/kg)	Conc.[b]	M	F	M	F	M	F	M	F
1	Control	0	5	12[c] mg/g	6	6	4	4	2	2	6	6
	Control	0	5	0								
2	VX-770	5	5	2 mg/g	4	4	4	4	0	0	4	4
	VX-809	300	5	60 mg/mL								
3	VX-770	15	5	6 mg/g	4	4	4	4	0	0	4	4
	VX-809	300	5	60 mg/mL								
4	VX-770	15	5	6 mg/g	6	6	4	4	2	2	6	6
	VX-809	600	5	120 mg/mL								
5	VX-770	60	5	24 mg/g	6	6	4	4	2	2	6	6
	VX-809	600	5	120 mg/mL								

[a] VX-770 test article (TA) was a 50:49.5:0.5 solid dispersion mixture of VX-770 (API) and hydroxypropylmethylcellulose acid succinate (HPMC-AS) and sodium lauryl sulfate (SLS),. The (b) (4) (b) (4) powder was formulated as a suspension in a vehicle consisting of 0.5% w/v methylcellulose (MC) + 0.5% w/v sodium lauryl sulfate (SLS) + 0.01% w/v simethicone in water. Group 1 animals received the vehicle control for VX-770 plus its placebo control article, HPMC-AS-HF (see footnote c, below), as well as the vehicle control for VX-809, which consisted of 0.5% w/v MC + 0.5% w/v Tween-80 + 0.05% w/v simethicone in distilled water.

[b] API concentrations for VX-770 were ½ of the TA concentrations listed here. API concentrations for VX-809 were equal to the TA concentrations. VX-770 was prepared on a weight/weight basis (mg/g) whereas VX-809 was prepared on a weight to volume basis (mg/mL).

[c] Control article (HPMC-AS-HF) for VX-770 was added to the vehicle control for VX-770 at the highest concentration level used in the VX-770 TA animals.

[d] Toxicokinetic (TK) samples were collected from Groups 1-5 on Day 1 at 6 timepoints as follows: 0.5, 1, 2, 4, 8 and 24 hours post dose and on Day 28 predose and 1, 2, 4, 8, and 24 hours postdose. TK samples were collected from all animals.

The first day of dosing was defined as Day 1 of the study. Animals were dosed in the order of TA presented in this summary.

Observations and Results

Mortality: Animals were observed in their cages for mortality at least twice daily.

There were no treatment-related deaths.

One male (#3416) dosed with the combination at 300/15 mg/kg/day was euthanized on day 9 following aspiration of the test article following an episode of emesis during dose administration. Microscopic findings of acute inflammation in the lungs, trachea and mediastinal lymph node were consistent with aspiration of the test article.

No deaths were observed in the 600/15 or 600/60 mg/kg/day groups.

Clinical Signs: Animals were observed in their cages for general condition at least twice daily. On dosing days, all animals were observed once prior to and once after test

article administration. Animals were removed from their cages for physical examinations once pretest and once weekly during the dosing and recovery periods.

The administration of VX−770 in combination with VX−809 was associated with an increase in the incidence/occurrence of abnormal stool (unformed, watery, and/or red exudate in stool) in both genders in Groups 3, 4 and 5 (≥300 mg/kg/day VX−809 + ≥15 mg/kg/day VX−770) with the highest incidence occurring in Groups 4 and 5 (≥600 mg/kg/day VX−809 + ≥15 mg/kg/day VX−770). White substance/stool and yellow substance/stool was noted in all test article groups with the highest frequency in Groups 4 and 5. An increase in the incidence/occurrence of vomiting (with food/test article) was observed in both genders, most frequently in males in Groups 4 and 5 and females in Group 4.

Table 52 Clinical signs in dogs treated with the combination of VX-809 and VX-770 for 28 days

Clinical signs	Males					Females				
	0/0	300/5	300/15	600/15	600/60	0/0	300/5	300/15	600/15	600/60
Oral, vomit - food	1/1	1/1	3/2	7/4	12/6	1/1	0/0	1/1	2/2	2/2
Oral, vomit white substance	1/1	6/3	4/2	28/6	48/6	0/0	8/4	14/3	30/6	23/6
Gastrointestinal, unformed stool	72/6	84/4	88/4	164/6	117/6	37/6	67/4	53/4	185/6	154/6
Gastrointestinal, watery stool	0/0	18/4	54/4	71/6	102/6	2/2	12/4	34/4	45/6	139/6
Gastrointestinal, red exudate in stool	0/0	0/0	0/0	0/0	8/6	0/0	0/0	2/1	0/0	1/1
Gastrointestinal, white substance in stool	0/0	16/4	36/4	56/6	123/6	0/0	12/4	25/4	76/6	117/6
Gastrointestinal, yellow substance in stool	0/0	11/4	3/3	27/6	18/6	0/0	10/4	9/4	36/6	22/6
Gastrointestinal, yellow stool	0/0	13/4	12/4	39/6	26/6	0/0	14/4	19/4	47/6	26/6
Gastrointestinal, white stool	0/0	4/3	4/2	46/6	49/6	0/0	2/1	2/2	32/6	45/6

Body Weights: Non-fasted body weights were recorded for all animals three times during the pretest period, weekly during the remainder of the study and terminally after fasting. Terminal fasted body weights were obtained just prior to necropsy.

Decreased body weight gain or body weight loss was evident for all male and female treatment groups.

Table 53 Body weight changes in dogs treated with the combination of VX-770 and VX-809 for 28 days

BW (kg)/Time point	Males					Females				
	0/0	300/5	300/15	600/15	600/60	0/0	300/5	300/15	600/15	600/60
BW, Pre2	8.3	8.0	8.4	8.1	8.3	7.3	7.1	7.4	7.2	7.1
BW, Week 4	8.8	8.3	7.9	7.8*	7.9*	7.7	7.1	7.3	6.7*	6.8*
BW, Rec2	8.7			8.9	9.0	8.2			7.8	7.9
BW Gain Week 4 - Pre2	0.5	0.3	-0.5	-0.3	-0.4	0.4	0	-0.1	-0.5	-0.3
BW Gain % of Pre2	6%	-3.8%	-6%	-3.7%	-5.1%	5.5%	0	-1.4	-7%	-4.2%
BW Gain Rec2 - Week 4	-0.1	-	-	-	1.1	0.5	-	-	-	1.1
BW Gain % of Week 4	-1.1%	-	-	-	13.9%	6.5%	-	-	-	1.6%

Feed Consumption: A visual estimate of the amount of feed consumed per day was made for each dog on a daily basis beginning one week prior to study initiation and continuing throughout the dosing and recovery periods.

Qualitative food consumption was relatively comparable for control and drug-treated groups.

Ophthalmoscopy: All animals were examined by a veterinary ophthalmologist during the pretest period, at the end of the dosing period (Week 4), and the end of the recovery period (Week 2).

There were no treatment-related ophthalmic findings in dogs treated with the combination of VX-809 and VX-770 at oral doses up to 600/60 mg/kg/day for 28 days.

ECG: ECG tracings were taken on conscious dogs positioned in right lateral recumbancy, pretest, at study termination at approximately 3 hours after completion of dosing, and at the end of recovery. A 9-lead electrocardiogram recording was made using standard limb leads I, II and III; augmented leads aVR, aVL and aVF as well as chest leads V2, rV2 and V10. Quantitative assessments including heart rates and PR, QRS, RR, QT and QTc intervals were calculated using EMKA Technologies ECG-Auto software.

Following co−administration of VX-770/VX-809, prolonged PR intervals (first degree AV block) were observed in 3/12 and 10/12 dogs in the 600/15 and 600/60 mg/kg/day groups, respectively.

These ECG changes were not observed with VX-809 alone. SVPCs were increased as compared to VX-770 alone.

Table 54 Prolongation of PR intervals in dogs after treatment with VX-809 and VX-770 at doses of 600/15 and 600/60 mg/kg/day for 28 days

Table 10.8-1: Test article-related changes in PR interval

Animal No.	Pretest	Term	Recovery
VX-770/VX-809 (15/600 mg/kg/day)			
4419m	104	130	107
4420m	116	143	112
4918f	114	135	na
VX-770/VX-809 (60/600 mg/kg/day)			
5417m	97	147	na
5418m	114	168	na
5419m	119	177	109
5420m	117	183	114
5915f	104	143	na
5916f	105	132	na
5917f	115	151	na
5918f	105	147	na
5919f	112	158	100
5920f	110	170	109

na: not applicable

Table 55 Mean PR interval at pretreatment, termination of the treatment period, and end of the recovery period

ECG Parameter	Males					Females				
	0/0	300/5	300/15	600/15	600/60	0/0	300/5	300/15	600/15	600/60
PR Interval, msec Pretest	112	106	103	110	111	101	100	113	107	108
PR Interval, msec Termination	111	103	111	**119**	**148**	104	106	**123**	**120**	**150**
PR Interval, msec Recovery	104	-	-	109	111	100	-	-	97	105

Co−administration of VX-770 and VX-809 also produced early depolarization of supraventricular origin (supraventricular premature complex: SVPC) for 8/12 dogs in the 600/60 mg/kg/day group. Some SVPCs occurred during the inspiratory phase of the respiratory cycle; therefore, early supraventricular depolarization with the P wave in contact with the previous T wave, not buried in the previous T wave, could be resulting from exaggerated respiratory sinus arrhythmia.

Two dogs (5419m, 5920f) in the 600/60 mg/kg/day group with these findings were in the recovery groups and had no abnormalities two weeks after the last dose was administered.

Table 56 Incidences of supraventricular premature complexes in dogs treated with the combination of VX-809 and VX-770 at doses of 600/15 and 600/60 mg/kg/day

Table 10.8-2: Summary Table: Incidence of Arrhythmias at Termination

Dose Level VX-770/VX-809 mg/kg/day	Animal No.	Finding	Frequency	T-P configuration of the SVPC	
				A	B
15/600	4419m	SVPC	1	0	1
60/600	5416m		1	0	1
	5417m		3	1	2
	5419m		2	2	0
	5915f		2	2	0
	5916f		7	6	1
	5917f		2	1	1
	5918f		6	3	3
	5920f		9	1	8
		2AVB	4	Na	

SVPC: supraventricular premature complex, 2AVB: second degree AV block
na: not applicable
A: P-wave is in contact with the previous T-wave
B: P-wave is buried in the previous T wave (embedded)

Hematology: Blood samples were obtained by jugular venipuncture from conscious dogs for measurement of a complete panel of hematology and coagulation parameters for all animals pretest, up to 6 dogs/sex/group in Groups 1, 4 and 5 and up to 4 dogs/sex/group in Groups 2 and 3 at study termination, and 2 dogs/sex/group in Groups 1, 4 and 5 at the end of recovery. Animals were fasted overnight prior to the blood collection interval.

There were changes of several hematology parameters observed at the end of the dosing period in males at doses ≥300/5 mg/kg/day and females at doses ≥300/15 mg/kg/day that included decreases of RBC counts, hemoglobin, hematocrit, reticulocytes, lymphocyte, basophils, and LUC counts, and APTT and increases of platelet counts and MCHC. Decreased APTT and eosinophil counts and increased platelet counts were also observed in females at a dose of 300/5 mg/kg/day. These changes were generally reversible by the end of the recovery period. Eosinophil counts were decreased in males at doses ≥300/15 mg/kg/day.

Table 57 Changes of hematology parameters in dogs that received the combination of VX-809 and VX-770 for 28 days

Table 10.9.1-1: Changes in Hematology Values[1] in Dogs Dosed Orally with VX-770 and VX-809 for 28 Days

	Males				Females			
Group	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
VX-770/ VX-809 (mg/kg/day)	**5/ 300**	**15/ 300**	**15/ 600**	**60/ 600**	**5/ 300**	**15/ 300**	**15/ 600**	**60/ 600**
RBC	-10%	-9%	-9%	-17%**	-	−15%**	-15%**	-13%**
RETIC	-31%	-33%	-41%**	-50%**	-	-26%	-52%*	-19%*
HGB	-8%	-10%	-9%*	-16%**	-	-12%*	-14%**	-12%**
HCT	-10%%	-12%	-10%*	-18%***	-	-15%**	-16%***	-15%***
PLT	+33%*	+64%*	+34%**	+32%**	+60%	+39%	+24%	+32%

Absolute values were statistically significantly different from controls.=p <0.05 **=p <0.01***=p<0.001 [1] Percentage change compared to corresponding control values.
PLT = Platelet; HBG= Hemoglobin; HCT=Hematocrit, RBC=Red Blood Cell; RETIC=Reticulocytes

Table 58 Hematology parameters in dogs at the end of the 28-day dosing period (continued)

Parameter	Time	Males					Females				
		0/0	300/ 5	300/ 15	600/ 15	600/ 60	0/0	300/ 5	300/ 15	600/ 15	600/ 60
Lymphocytes $x10^3/\mu L$	Term	3.99	3.47	2.81*	2.93*	2.87*	3.49	3.12	2.80	2.81	3.32
	Rec	3.61			3.16	2.84					
Eosinophils $x10^3/\mu L$	Term	0.27	0.26	0.13*	0.13*	0.11*	0.23	0.09*	0.10*	0.06*	0.11*
Basophils $x10^3/\mu L$	Term	0.07	0.08	0.05	0.04	0.04*	0.07	0.06	0.05	0.05	0.05
LUC $x10^3/\mu L$	Term	0.08	0.06	0.05	0.04*	0.05*	0.05	0.05	0.04	0.03	0.04

Table 59 Changes of coagulation parameters in dogs that received the combination of VX-809 and VX-770 for 28 days

Table 10.9.2-1: Shorter APTT (seconds) in Dogs Dosed Orally with VX-809 for 28 Days

	Males				Females			
Group	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
VX-770/VX-809 (mg/kg/day)	**5/300**	**15/300**	**15/600**	**60/600**	**5/300**	**15/300**	**15/600**	**60/600**
APTT	4.3***	4.7***	4.9***	3.6***	3.7***	3.9***	2.9***	3.1***

*Absolute values were statistically significantly different from controls. . *=p <0.05 **=p <0.01***=p <0.001
APTT= Activated Partial Thromboplastin Time

Clinical Chemistry: Blood samples were obtained by jugular venipuncture from conscious dogs for measurement of a complete panel of clinical chemistry parameters for all animals pretest, up to 6 dogs/sex/group in Groups 1, 4 and 5 and up to 4 dogs/sex/group in Groups 2 and 3 at study termination, and 2 dogs/sex/group in Groups 1, 4 and 5 at the end of recovery. Animals were fasted overnight prior to the blood collection interval.

Changes were observed for several clinical chemistry parameters. Cholesterol levels were decreased for males and females at doses ≥300/5 mg/kg/day. Triglyceride levels were decreased for males at doses ≥300/5 mg/kg/day and females at doses ≥600/15 mg/kg/day. Total protein and globulin levels were decreased for males at doses ≥300/5 mg/kg/day. Chloride levels were decreased for males at 600/60 mg/kg/day and females at doses ≥600/15 mg/kg/day. Calcium levels were decreased for males and females at doses ≥600/15 mg/kg/day. Magnesium levels were increased for females at doses ≥300/5 mg/kg/day. ALKP activities were increased for males at doses ≥600/15 mg/kg/day. BUN levels were increased for females at 600/15 mg/kg/day; however, no change was evident at 600/60 mg/kg/day. These changes were generally reversible by the end of the recovery period.

Table 60 Changes in clinical chemistry values in dogs dosed orally with VX-770 and VX-809 for 28 days

Table 10.9.3-1: Changes in Clinical Chemistry Values[1] in Dogs Dosed Orally with VX-770 and VX-809 for 28 Days

	Males				Females			
Group	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
VX-770/ VX-809 (mg/kg/day)	**5/ 300**	**15/ 300**	**15/ 600**	**60/ 600**	**5/ 300**	**15/ 300**	**15/ 600**	**60/ 600**
CHOL	-42%***	-31%***	-32%***	-32%***	-25%*	-29%**	-27%**	-29%**
TRIG	-54%***	-39%***	-61%***	-48%***	-	-	-55%***	-59%***
TP	-10%*	-8%*	-8%**	-10%**	–	–	–	–
GLOB	-14%**	-14%**	-14%***	-14%***	–	–	–	–
Cl-	-	-	–	+4%***	–	–	+3%*	+3%*
Ca++	-	-	-4%*	-5%**	-	-	-4%*	-6%***
Mg++	-	-	-	-	+10%*	+11%*	+20%***	+17%***

*Absolute values were statistically significantly different from controls. *=p <0.05
=p<0.01*=p<0.001
[1] Percentage change compared to corresponding control values.
CHOL= Cholesterol; TRIG=Triglyceride; TP=Total Protein GLOB=Globulin Cl=Chloride Ca=Calcium Mg=Magnesium

Table 61 Clinical chemistry parameters in dogs at the end of the 28-day dosing period (continued)

Parameter	Males					Females				
	0/0	300/5	300/15	600/15	600/60	0/0	300/5	300/15	600/15	600/60
ALKP U/L	81	84	78	111 (137%)	140 (173%)	108	128	104	58	127
BUN mg/dL	17	19	17	19	19	14	19	18	23* (164%)	18

Urinalysis: Urine samples for measurement of urinalysis parameters were collected during an approximate 16-hour overnight collection period and were analyzed for all animals pretest and up to 6 dogs/sex/group in Groups 1, 4 and 5 and 4 dogs/sex/group in Groups 2 and 3 at study termination. Animals did not have access to food or water during urine collection.

For urinalysis parameters, there were decreases of sodium and/or sodium/creatinine ratio for males at 600/15 mg/kg/day and males and females at 600/60 mg/kg/day.

Gross Pathology: Necropsy was performed for up to 4 dogs/sex/group after animals had been dosed for at least 28 days and up to 2 dogs/sex/group in Groups 1, 4 and 5 after the 14-day recovery period. Animals were fasted overnight prior to necropsy. A necropsy schedule was established to ensure that approximately equal numbers of males and females from each group were examined at similar times of the day throughout the necropsy period.

Treatment-related gross pathological findings were present in the thymus and prostate. These findings were reversible by the end of the recovery period.

The thymus was slightly to moderately small in a male and a female at 600/15 mg/kg/day and in two males and two females at 600/60 mg/kg/day. Decreased thymic size correlated microscopically with lymphocyte depletion.

The prostate was slightly small in a male at 600/15 mg/kg/day and in two males at 600/60 mg/kg/day. Decreased prostatic size correlated microscopically with reduced secretion and acinar contraction. The observed findings were reversible following the recovery period.

Table 62 Gross pathological findings for dogs that received the combination of VX-809 and VX-770 for 28 days

Organ/Tissue	Males					Females				
	0/0	300/5	300/15	600/15	600/60	0/0	300/5	300/15	600/15	600/60
N =	4	4	3	4	4	4	4	4	4	4
Thymus -small	0	0	0	1	2	0	0	0	1	2
Prostate -small	0	0	0	1	2					

Organ Weights: Organ weights were measured for the adrenal glands, brain, epididymides, heart, kidneys, liver, lung, pituitary, prostate, spleen, testes, thymus, and thyroid with parathyroids.

Thymus weights were decreased for males and females at doses ≥300/5 mg/kg/day. These findings appeared to correlate with a dose-related depletion of lymphocytes in the thymus. Thymus weights were still decreased for males in the 600/15 and 600/60 mg/kg/day groups at the end of the recovery period; however, reversibility appeared to be evident for female drug-treated groups.

Spleen weights were decreased for males and females in the high dose group at 600/60 mg/kg/day, although there were no correlating histopathological findings.

Males in the high dose group at 600/60g/kg/day had decreased testes, prostate and epididymides weights as compared to concurrent controls. These changes potentially represented a slight retardation of sexual maturation in these animals, which was probably associated with treatment-related decreases in body weight gain that occurred during the study. Testes weights were still decreased for males in the 600/15 and 600/60 mg/kg/day groups at the end of the recovery period; however, reversibility appeared to be evident for the prostate and epididymides.

Table 63 Organ weights in dogs that received the combination of VX-809 and VX-770 for 28 days

Organ	Time	Males					Females				
		0/0	300/5	300/15	600/15	600/60	0/0	300/5	300/15	600/15	600/60
Thymus g	Term	12.650	**7.967***	**5.188***	**5.291***	**6.214***	7.966	**5.767**	**5.673**	**4.531**	**4.125**
	Rec	11.867			**7.822**	**8.294**					
Thymus %BW	Term	0.139	**0.099**	**0.066***	**0.070***	**0.080***	0.105	**0.082**	**0.079**	**0.069**	**0.063**
	Rec	0.143			**0.094**	**0.096**					
Thymus %BrW	Term	16.557	**10.684***	**7.284***	**7.350***	**8.315***	10.922	**8.451**	**7.740**	**6.633**	**6.012**
	Rec	13.136			**10.908**	**10.858**					
Spleen g	Term	129.486	107.380	95.128	109.368	**77.023***	99.412	91.728	99.879	91.204	**80.210**
Spleen %BW	Term	1.428	1.309	1 222	1.441	**1.012***	1.314	1.305	1.391	1.391	**1.261**
Spleen %BrW	Term	169.532	144.899	129.083	151.145	**104.762***	138.236	134.432	136.180	134.143	**115.330**
Prostate g	Term	3.377	5.419	3.181	3.208	**1.303***					
Prostate %BW	Term	0.037	0.064	0 041	0.042	**0.017**					
Prostate %BrW	Term	4.452	7.340	4.405	4.381	**1.763**					
Testes g	Term	11.661	11.328	9.406	10.757	**8.354***					
	Rec	13.993			8.387	**9.745**					
Testes %BW	Term	0.129	0.138	0.120	0.142	**0.110**					
	Rec	0.169			0.100	**0.112**					
Testes %BrW	Term	15.341	15.206	13.067	14.922	**11.309**					
	Rec	19.026			11.668	**12.766**					
Epididymides	Term	2.378	2.748	2.648	2.537	**1.820**					

Organ	Time	Males					Females				
		0/0	300/ 5	300/ 15	600/ 15	600/ 60	0/0	300/ 5	300/ 15	600/ 15	600/ 60
g											
Epididymides %BW	Term	0.026	0.033	0.034	0.033	**0.024**					
Epididymides %BrW	Term	3.125	3.687	3.667	3.523	**2.462**					

*p≤0.05
Bold text denotes potential differences between control and drug-treated groups

Histopathology:

Adequate Battery: An adequate panel of organs and tissues was submitted to histopathological examination. After fixation, the tissues and organs from up to 4 dogs/sex/group at termination were routinely processed, embedded in paraffin, cut at a microtome setting of 4-7 microns, mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy. The bones were decalcified. Tissues were shipped for slide preparation to: (b) (4)

Table 9.3.15.4-1: Tissues preserved and examined microscopically

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X	
bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X[a]
brain (medulla, pons, cerebrum, and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
gall bladder		X	X
heart	X	X	X
kidneys	X	X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
liver	X	X	X
lungs (with mainstem bronchi)	X	X	X
lymph nodes (mesenteric and mediastinal)		X	X
mammary gland		X	X[b]
nerve (sciatic)		X	X
optic nerve		X	
ovaries		X	X
pancreas		X	X
pituitary gland	X	X	X
prostate gland	X	X	X
rectum		X	
salivary glands (submandibular)		X	X
skeletal muscle (*Rectus femoris*)-longitudinal and transverse		X	X
skeletal muscle (*m. gastrocnemius*)-longitudinal and transverse		X	
skeletal muscle (*m. psoas)*- longitudinal and transverse		X	
skin (inguinal)		X	X
small intestine (duodenum, ileum, jejunum and Peyer's patches		X	X
spinal cord (cervical)		X	X
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid with parathyroid	X	X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns/cervix)		X	X
vagina		X	X
gross lesions		X	X

[a] Qualitative examination (no differential count).
[b] Females only.

Peer Review: None

Histological Findings: Histopathological findings were evident in the thymus, testes, epididymides, prostate, and gallbladder.

There was a dose related depletion of lymphocytes in the thymus. The severity of lymphoid depletion exceeded control levels in males dosed at ≥300 mg/kg/day VX−809 + ≥ 5 mg/kg/day VX−770 (Groups 3 through 5) and in females at all dose levels. Thymic weights were decreased for all male and female treatment groups.

In the 600/60 mg/kg/day group, 2 of 4 males had increased numbers of multinucleate degenerate germ cells in the testes (moderate vs. minimal) and one of these dogs also had increased sloughed testicular germ cells in the epididymides. The incidence and severity of prostatic acinar contraction and reduced secretion was also greater in this group than in the other groups and this correlated with a decrease in prostatic weight in this group. Given the peripubertal status of the animals, it was likely that these changes represented a slight retardation of sexual maturation in the high dose group animals, which was probably associated with the decreased body weight gain that occurred in these animals during the study. These changes might be an indirect effect of the test article.

The epithelial cells lining the gall bladder contained slightly increased amounts of mucinous secretory product in two Group 5 males and females and in a Group 4 male compared to control animals. This finding was not considered adverse as there was no evidence of structural damage in the gallbladder.

Findings in the kidneys, colon, and cecum were of questionable relationships to treatment.

Table 64 Histopathological findings in dogs at the end of the dosing period

Organ/Tissue	Males					Females				
	0/0	300/ 5	300/ 15	600/ 15	600/ 60	0/0	300/ 5	300/ 15	600/ 15	600/ 60
Thymus										
-decreased lymphocytes	2/4	3/4	3/3	3/4	3/4	2/4	3/4	4/4	4/4	4/4
-slight	2	3	2	1	0	2	1	3	2	3
-moderate	0	0	1	1	2	0	2	1	2	0
-marked	0	0	0	1	1	0	0	0	0	1
-increased apoptosis	0/4	0/4	0/3	0/4	1/4	0/4	1/4	0/4	0/4	0/4
-minimal	0	0	0	0	0	0	1	0	0	0
-slight	0	0	0	0	1	0	0	0	0	0
Testes										
-increased multinucleate germ cell degeneration	2/4	2/4	1/3	2/4	3/4					
-minimal	2	2	1	2	1					
-moderate	0	0	0	0	2					
-immaturity	2/4	0/4	1/3	1/4	2/4					

Organ/Tissue	Males					Females				
	0/0	300/5	300/15	600/15	600/60	0/0	300/5	300/15	600/15	600/60
-minimal	2	0	1	0	1					
-slight	0	0	0	1	1					
Epididymides										
-increased sloughed germ cells, slight	0/4	0/4	0/4	0/4	1/4					
-immaturity (reduced/absent sperm)	3/4	0/4	2/3	1/4	3/4					
Prostate										
-immaturity (reduced secretion/acinar contraction)	3/4	2/4	1/3	2/4	4/4					
-minimal	1	1	0	2	0					
-moderate	1	1	0	0	1					
-marked	1	0	1	0	2					
-severe	0	0	0	0	1					
Gallbladder										
-increased mucinous secretory product, slight	0/4	0/4	0/3	1/4	2/4	0/4	0/4	0/4	0/4	2/4
Kidneys										
-mineral deposits, minimal	2/4	1/4	2/3	1/4	2/4	0/4	0/4	0/4	0/4	2/4
Cecum										
-mucosal edema, slight	0/4	1/4	0/3	0/4	0/4	0/4	0/4	0/4	1/4	1/4
Colon										
-congestion, slight	0/4	0/4	0/3	0/4	1/4	0/4	0/4	0/4	0/4	0/4

Special Evaluation: For potential biomarker analysis, sections of heart, liver, kidneys, and serum were collected and frozen at -70°C. Samples will be shipped to the Sponsor and analyzed by protocol amendment. No data was provided.

Toxicokinetics: Blood samples were obtained for the determination of plasma concentrations of VX−770, VX-809 and the major metabolites of VX−770, VRT−837018 (M1, hydroxymethyl−VX-770) and VRT−842917 (M6, carboxy−VX−770). On Day 1, blood samples for toxicokinetic determinations were obtained from 6 animals/sex/dose group/time point at 0.5, 1, 2, 4, 8, and 24 hours post-dose. On Day 28, blood samples for TK determinations were obtained from all surviving animals predose and 1, 2, 4, 8 and 24 hours post-dose. Blood samples were collected from Group 1 animals at all time points.

The DMPK-NCD group at Vertex Pharmaceuticals Incorporated (Cambridge, MA) conducted the plasma sample analyses using fully validated bioanalytical methods with liquid chromatography/tandem mass spectrometry (LC-MS/MS), which had a lower limit of quantitation (LLOQ) of 2.00 ng/mL. The calibration standard concentrations over the range of 2.00 and 2000 ng/mL were analyzed using a linear regression method. Reverse-phase high performance liquid chromatography was utilized as the separation method and mass spectrometry was used as the detection method in quantitative analysis. Samples were extracted by methyl tertiary-butyl ether (MTBE) using liquid–liquid extraction.

VX-809: Mean C_{max} and $AUC_{0\text{-}24hr}$ values for VX-809 increased more than dose-proportionally as the dose increased from 300 to 600 mg/kg/day on day 1, but increased less than dose-proportionally (non-linear) on day 28. Upon repeated dosing of VX-809, significant accumulation of VX-809 was observed on day 28 compared to day 1 in the 300 mg/kg dose group, but this trend was not observed in the 600 mg/kg dose group. Exposure to VX-809 was lower for the 600/60 mg/kg/day group as compared to the 600/15 mg/kg/day group.

VX770 and metabolites: The mean $AUC_{0\text{-}24hr}$ and C_{max} values of VX-770, VRT-837018 (M1), VRT-842917 (M6) in male and female dogs increased less than dose-proportionally as the VX-770 dose increased from 5 to 60 mg/kg/day on days 1 and 28. For VX-770 the mean C_{max} and $AUC_{0\text{-}24hr}$ values on day 28 were generally higher than values on day 1 suggesting accumulation in the process to achieve steady state exposures. Exposures to VX-770 were relatively comparable for the 300/15 and 600/15 mg/kg/day groups. No accumulation of M1 and M6 were observed upon repeated dosing of VX-770 for 28 days. Systemic exposure of M1 reached a maximum of 48% of VX-770 exposure, whereas systemic exposure of M6 reached a maximum of only 11% of VX-770 exposure, and there were no apparent sex-related differences in the production of either metabolite in dogs.

In general, no sex-related differences (<2-fold systemic exposures or peak plasma concentrations) for VX-809, VX-770, and the metabolites of VX-770, M1 and M6, were observed in any of the dose groups on Day 1 and 28.

Table 65 Toxicokinetic parameters for VX-809 in female and male dogs following once daily oral co-administration of VX-809 and VX-770 for 28 days

Table 10.2-1 Summary of Toxicokinetic Parameters for VX-809 in Female and Male Dogs Following Once Daily Oral Co-administration of VX-809 and VX-770 for 28 Days

Analyte	Study Day	Dose of VX-809 (mg/kg)	Gender							
			Female				Male			
			C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)
VX-809	Day 1	300	37 ±16.3	13.3 ±12.5	357 ±252	3.2	21.1 ±4.90	3.5 ±1.0	148 ±17.7	6.1 ±4.6
		300	24.2 ±6.61	4.3 ±2.9	286 ±143	3.5	20.4 ±9.00	4.0 ±2.8	248 ±12.5	8.8
		600	87.9 ±40.0	6.0 ±2.2	1110 ±602	5.9 ±0.3	50.4 ±21.1	10.7 ±6.5	656 ±24.4	N/D
		600	60.9 ±27.1	6.0 ±2.2	826 ±465	4.4 ±0.9	49.0 ±26.8	5.3 ±2.1	600 ±447	5.1 ±0.5
	Day 28	300	114 ±24.8	2.5 ±1.0	956 ±2140	4.5 ±3.0	70.0 ±45.6	2.8 ±1.5	482 ±329	4.2 ±1.8
		300	98.7 ±35.0	4.5 ±2.5	1090 ±570	4.3 ±0.9	43.7 ±17.2	4.0 ±0.0	388 ±186	7.3 ±3.3
		600	83.3 ±38.7	4.2 ±2.2	976 ±646	6.7 ±3.5	58.8 ±14.3	1.7 ±2.0	503 ±152	9.5 ±8.2
		600	53.5 ±19.6	0.3 ±0.8	459 ±225	12.4 ±12.6	40.0 ±23.5	1.5 ±1.5	316 ±271	13.2 ±11.0

N/D=Not determined

Table 66 Toxicokinetic parameters for VX-770, M1 (VRT-837018), and M6 (VRT-842917) in female and male dogs following once daily oral co-administration of VX-809 and VX-770 for 28 days

Table 10.2-2 Summary of Toxicokinetic Parameters for VX-770, M1 (VRT-837018), M6 (VRT-842917) in Female and Male Dogs Following Once Daily Oral Co-administration of VX-809 and VX-770 for 28 Days

Analyte	Study Day	Dose of VX-770 (mg/kg)	Gender							
			Female				Male			
			C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (µg*hr/mL)	$T_{1/2}$ (hr)
VX-770	Day 1	5	2.05 ±0.812	4.0 ±0.0	24.7 ±4.98	12.4 ±3.6	1.90 ±0.105	4.0 ±0.0	27.2 ±2.54	16.0 ±3.77
		15	3.31 ±1.14	3.5 ±1.0	46.3 ±11.9	21.1 ±15.4	4.20 ±1.29	4.0 ±0.0	58.9 ±21.0	15.4 ±2.33
		15	3.09 ±0.859	8.7 ±7.8	51.9 ±9.88	15.0 ±0.5	3.41 ±0.959	9.3 ±7.5	62.4 ±13.0	133.3 (n=2)
		60	17.9 ±6.31	21.3 ±6.5	272 ±79.4	N/D	10.3 ±6.73	17.3 ±10.3	182 ±115	24.0
	Day 28	5	2.96 ±0.854	4.5 ±2.5	53.6 ±14.6	19.0 ±6.0	2.24 ±1.11	2.3 ±1.3	37.9 ±20.2	20.2 ±1.97
		15	6.31 ±1.78	4.0 ±0.0	115 ±43.0	26.1 ±7.6	5.46 ±2.27	3.0 ±1.7	85.4 ±37.7	15.2 ±0.24
		15	6.43 ±2.78	3.7 ±0.8	102 ±34.7	16.3 ±3.9	7.36 ±1.69	4.0 ±2.2	124 ±29.4	18.5 ±6.11
		60	19.7 ±4.00	4.0 ±3.3	364 ±77.3	28.9 ±7.2	21.0 ±10.8	2.2 ±1.0	291 ±1390	15.3 ±3.40
M1	Day 1	5	1.37 ±0.526	3.5 ±1.0	9.13 ±2.81	6.7 ±2.1	2.04 ±0.385	4.0 ±0.0	13.0 ±0.773	7.22 ±2.02
		15	2.00 ±0.361	3.0 ±1.2	14.1 ±3.80	7.8 ±3.1	2.64 ±1.41	3.5 ±1.0	20.9 ±11.2	10.9 ±2.98
		15	1.89 ±0.522	2.0 ±0.0	14.0 ±1.92	11.8 ±3.8	1.63 ±0.630	2.3 ±0.8	15.5 ±3.74	14.3 ±6.24
		60	4.05 ±2.99	20.3 ±9.0	61.0 ±38.7	19.1 (n=1)	2.46 ±1.58	13.3 ±11.7	41.0 ±29.7	107.2 ±142
	Day 28	5	0.492 ±0.100	2. 0 ±0.0	6.63 ±1.30	32.5 ±10.9	0.521 ±0.308	1.0 ±0.0	5.50 ±3.53	18.4 ±1.89
		15	1.35 ±0.313	2.5 ±1.8	19.6 ±8.72	55.9 ±61.0	1.32 ±0.875	1.0 ±0.0	14.9 ±9.42	15.8 ±1.42
		15	1.92 ±0.771	2.7 ±1.0	21.2 ±7.36	17.5 ±4.7	1.67 ±0.366	2.0 ±0.00	16.7 ±4.36	17.7 ±5.74
		60	3.06 ±1.19	3.2 ±2.6	55.3 ±19.5	51.0 ±11.2	4.50 ±3.10	5.7 ±9.0	68.8 ±68.2	17.8 ±4.94

Table 10.2-2 Summary of Toxicokinetic Parameters for VX-770, M1 (VRT-837018), M6 (VRT-842917) in Female and Male Dogs Following Once Daily Oral Co-administration of VX-809 and VX-770 for 28 Days (Continued)

Analyte	Study Day	Dose of VX-770 (mg/kg)	Gender							
			Female				Male			
			C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/mL)	$T_{1/2}$ (hr)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (ng*hr/mL)	$T_{1/2}$ (hr)
M6	Day 1	5	0.168 ±0.075	4.0 ±0.0	1.79 ±0.681	10.3 ±7.1	0.230 ±0.074.	4.0 ±0.0	3.01 ±0.844	9.9 ±3.5
		15	0.263 ±0.024	4.0 ±0.0	2.76 ±0.724	8.1 ±2.8	0.292 ±0.175	4.0 ±0.0	3.31 ±2.21	12.7 ±6.1
		15	0.278 ±0.159	5.3 ±2.1	3.21 ±1.72	10.0 ±2.4	0.236 ±0.092	5.3 ±2.1	3.22 ±0.127	9.9 ±4.1
		60	0.421 ±0.209	18.7 ±8.3	6.98 ±3.66	N/D	0.246 ±0.097	9.3 ±7.5	4.22 ±1.90	14.9
	Day 28	5	0.069 ±0.018	4.0 ±0.0	0.850 ±0.157	13.0 ±2.3	0.055 ±0.037	3.5 ±1.0	0.701 ±0.522	14.0 ±2.4
		15	0.180 ±0.127	3.5 ±3.4	2.87 ±2.20	15.4 ±2.7	0.090 ±0.055	4.0 ±0.0	1.14 ±0.699	13.3 ±0.5
		15	0.156 ±0.605	4.3 ±2.0	2.32 ±1.06	11.9 ±1.2	0.117 ±0.056	3.7 ±0.8	1.31 ±0.419	10.9 ±3.1
		60	0.234 ±0.122	3.3 ±3.0	3.63 ±1.41	31.1 ±26.0	0.252 ±0.137	4.0 ±0.0	3.86 ±2.62	16.4 ±9.0

N/D=Not determined

Dosing Formulation Analysis: The dose formulations of the test article, VX-770, a 50:49.5:0.5 (b) (4) mixture of VX-770 (API) and Hydroxypropylmethylcellulose Acid Succinate (HPMC-AS) and Sodium Lauryl Sulfate (SLS), in control vehicle [0.5% (w/v) Methylcellulose (MC) with 0.5% (w/v) Sodium Lauryl Sulfate (SLS) and 0.01% (w/v) Simethicone in Water] were analyzed to confirm that the prepared dose formulations were homogeneous and the administered VX- 770 concentrations were appropriate under the study conditions.

The dose formulations of the test article, VX-809 (assumed 100% pure) in control vehicle [0.5% Tween 80 (w/v) + 0.5% Methylcellulose (w/v) + 0.05% (w/v) Simethicone in Water] were also analyzed to confirm that the prepared dose formulations were homogeneous and the administered VX-809 concentrations were appropriate under the study conditions.

For both VX-770 and VX-809, triplicate samples taken from the top, middle and bottom of the dose formulations of low and high concentrations for the study were analyzed on day 1 formulations. The results show that the mixing procedure produced homogeneous suspensions. The concentrations of the triplicate samples at each of the 3 levels (top, middle and bottom) were within ±10% of each other and the mean concentrations of each level were within ±15% of the nominal concentration.

For VX-770, dose confirmations were performed in duplicate on Day 1 (for Group 1 and Groups 3 and 4), and on Day 28 (for all groups). The middle sample results of the homogeneity (for Group 2 and Group 5) also served for concentration verification on Day 1. The concentrations of duplicate samples and triplicate samples (Day 1, Groups 2

and 5) were within ±15% of each other and the mean concentrations were within ±15% of nominal concentrations.

For VX-809, dose confirmations were performed in duplicate on Day 1 (for Group 1) and on Day 28 (for all groups). The middle sample results of the homogeneity (for Groups 2, 3, 4, and 5) also served for concentration verification on Day 1. The concentrations of duplicate samples and triplicate samples (Day 1, Groups 2, 3, 4, and 5) were within ±15% of each other and the mean concentrations were within ±15% of nominal concentrations.

11 Integrated Summary and Safety Evaluation

The Sponsor has proposed Phase 3 clinical trials in CF subjects who are homozygous or heterozygous for the *F508del-CFTR* mutation that will receive the combination of lumacaftor and ivacaftor. Clinical trials VX12-809-103 and VX-12-809-104 will be conducted with CF subjects (≥12 years old) who are homozygous for the *F508del-CFTR* mutation that will receive the combination for 24 weeks. Clinical trial VX12-809-105 with a dosing period of 95 weeks will be conducted with CF subjects who are homozygous for the *F508del-CFTR* mutation that participated in clinical trials VX12-809-103 and VX-12-809-104 or with CF subjects who are heterozygous for the *F508del-CFTR* mutation that participated in Cohort 4 of Study VX-12-809-102. The daily doses of lumacaftor will be either 600 or 800 mg/day administered as 600 mg/day and 400 mg q12hr, respectively. The daily dose of ivacaftor will be 500 mg/day administered as 250 mg q12hr. These clinical trials will use lumacaftor/ivacaftor tablets that are a co-formulation of the active ingredients, lumacaftor (VX-809) and ivacaftor (VX-770), in a single oral dosage form. Each tablet contains 200 mg of lumacaftor and 125 mg of ivacaftor.

In support of the proposed Phase 3 clinical trials with the combination of lumacaftor and ivacaftor, the sponsor submitted supporting nonclinical toxicology studies with lumacaftor alone, ivacaftor alone (see IND 74,633 and NDA 203-188), and the combination of lumacaftor and ivacaftor. For lumacaftor alone, toxicology studies up to 6 months in rats and 12 months in dogs were conducted. For the combination of lumacaftor and ivacaftor, 28-day toxicology studies in rats and dogs and a 3-month toxicology study in rats were conducted.

In Phase I clinical studies with VX-809, the sponsor identified a unique metabolite (VRT-0995096 or M28), which represented 13% of the total parent and metabolites (based on radio-labeling) and was not formed in nonclinical test species. The Sponsor included VRT-0995096 in a 1-month toxicology study with the isolated metabolite, 6-month toxicology study in rats with VX-809, and 3-month toxicology study with the combination of VX-809 and VX-770 to assess its potential toxicity.

Toxicology studies with lumacaftor alone:

In a 26-week toxicology study, Sprague-Dawley rats received VX-809 by oral gavage at doses of 250, 500, or 1000 mg/kg/day in combination with VRT-0995096 at a dose of 25 mg/kg/day. There were no treatment-related deaths. Absolute body weights were

unaffected by treatment for 26 weeks. There were no biologically significant changes of hematology, coagulation, or clinical chemistry parameters. No target organs of toxicity were identified. A number of low incidence findings, primarily confined to the high dose, were observed although relationships to treatment were unclear. Plasma C_{max} and AUC values for VX-809 increased with elevating doses; however, these increases were significantly less than dose proportional. Plasma C_{max} and AUC values for VRT-0995096 were comparable for Group 2, 3, and 4 at each time point suggesting that VX-809 had no effects on exposure to VRT-0995096. The NOAEL was judged to be the high dose at 1000 mg/kg/day VX-809 + 25 mg/kg/day VRT-0995096.

In a 12-month oral (gavage) toxicology study with a 6-month interim sacrifice, dogs received VX-809 at doses of 0, 125, 250, or 500 mg/kg/day. No deaths were attributed to treatment with VX-809. The administration of VX-809 was associated with increases in the incidence/occurrence of abnormal stool in both sexes at ≥125 mg/kg/day (unformed, watery, pale, white and/or yellow) with the highest incidence occurring at 500 mg/kg/day. A slight increase in the incidence/occurrence of vomiting (food) was observed in both sexes at 500 mg/kg/day. Red blood parameters (RBC counts, hemoglobin, and hematocrit) were slightly decreased for males and females at 250 and 500 mg/kg/day during months 3, 6, 9, and 12. Platelet counts were slightly increased for male drug-treated groups and females at 250 and 500 mg/kg/day during months 3, 6, 9, and 12. Slight decreases of absolute reticulocyte counts were observed for males and females at 500 mg/kg/day during months 9 and 12 and also for males and females at 250 mg/kg/day during month 12. These changes would be expected to be monitorable in a clinical setting. Cholesterol, triglyceride, total protein, albumin, and globulin levels were decreased for male and female drug-treated groups at months 3, 6, 9, and 12. Liver weights were increased for male and female drug-treated groups at 6 and 12 months; however, there were no corresponding histopathological findings. Judging the totality of the histopathological findings from the 6-month interim sacrifice and 12-month terminal sacrifice as well as the respective 1-month recovery periods, no treatment-related target organs of toxicity were identified with doses up to 500 mg/kg/day. The NOAEL was identified as the high dose of 500 mg/kg/day. $AUC_{0\text{-}24hr}$ values for males and females at 500 mg/kg/day on day 363 were 750 and 860 µg*hr/mL, respectively. Average steady-state $AUC_{0\text{-}24hr}$ values for males and females at 500 mg/kg/day from days 90 to 363 were 429 and 515 µg*hr/mL, respectively.

In a 3-month oral (gavage) toxicology study, dogs received VX-809 at doses of 0, 125, 250, 500, or 1000 mg/kg/day. At the end of the dosing period, 4 dogs/sex/group were sacrificed. After a 1-month recovery period, an additional 2 dogs/sex/group in the 0, 500, and 1000 mg/kg/day groups were sacrificed. Treatment-related moribund sacrifices occurred for 2 males and 1 female in the 1000 mg/kg/day group. Two dogs developed irregular gait, jerky movements and/or muscle rigidity prior to sacrifice. Irregular gait, trembling, jerky movements, and/or muscle rigidity were observed sporadically in individual animals at 1000 mg/kg/day after approximately 2 months of VX−809 administration (a total of 5 males and 3 females at 1000 mg/kg/day were observed with these findings). There were no histopathological findings that correlated to clinical signs or moribund sacrifices. Histopathological findings were evident for dogs at 1000

mg/kg/day and included the thymus (increased severity of lymphocyte depletion), male reproductive organs (delays of maturation in the testes and prostate and sloughed germ cells in the epididymides), and liver (extramedullary hematopoiesis). Findings were generally reversible by the end of the recovery period with the exception of the epididymides. Findings in the thymus and liver were not considered dose-limiting. The NOAEL was identified 500 mg/kg/day based upon deaths, neurological clinical signs, and histopathological findings in the male reproductive organs at 1000 mg/kg/day. $AUC_{0\text{-}24\ hr}$ values for females and males at 500 mg/kg/day on day 91 were 932 and 897 µg·hr/mL, respectively. These findings were not evident in the 12-month dog study with doses up to 500 mg/kg/day.

Toxicology studies with the combination of lumacaftor and ivacaftor:
In a 28-day oral (gavage) toxicology study, rats received the combination of VX-809 and VX-770 at doses of 0/0, 100/25, 300/50, 1000/50, and 1000/100 mg/kg/day. VX-809 and VX-770 were formulated as separate suspensions for co-administration by oral gavage. Histopathological findings at the end of the dosing period were evident in the kidneys, duodenum, stomach, mediastinal LN, mesenteric LN, and Harderian gland. With the exception of the findings in the kidneys and mesenteric LN, incidences of findings were low. Increased incidences of chronic progressive nephropathy were observed for males in the 1000/50 and 1000/100 mg/kg/day groups. This is a common background finding in rats, particularly males; however, treatment with doses of 1000/50 and 1000/100 mg/kg/day exacerbated the incidence of this finding. Chronic progressive nephropathy has generally been judged to be a rat-specific finding with little or no relevance to humans (Toxicologic Pathology 32:171-80, 2004). Test article-related exacerbation of this finding has been observed with many diverse agents and has little or no relevance to humans. Erosions of the gastric glandular mucosa were present in 2 of 10 females at 1000/50 mg/kg/day, 2 of 10 males at 1000/100 mg/kg/day, and 1 of 10 females at 1000/100 mg/kg/day. This finding was also identified in 1 female at 100/25 mg/kg/day (No. 2503) examined for gross lesions. Minimal to moderate, focal or multifocal erosions of the glandular mucosa occurred without significant inflammatory response. These findings were observed with low incidences and not observed with a dose-response relationship. In discussions with the Medical Officer, based upon the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation, these findings were considered monitorable in a clinical setting. Slight erosion of the duodenal mucosa was identified for one female in the 1000/100 mg/kg/day group. The incidence of free erythrocytes and erythrophagocytosis in the mesenteric LN was increased for males in the 1000/100 mg/kg/day group. This finding was also evident in the mediastinal LN where incidences displayed no relationship to treatment. Treatment with doses of 1000/50 and 1000/100 mg/kg/day increased the incidences of subacute and chronic inflammation in Harderian gland. These findings were rats-specific and have no relevance to humans. The sponsor did not perform histopathological examinations of recovery animals. AUC and C_{max} values for VX-809 increased with elevating dose although they were generally less than dose proportional. AUC and C_{max} values for VX-770 and its metabolites increased in an approximate dose proportional manner from 100/25 to 300/50 mg/kg/day. AUC and C_{max} values for VX-770 and its metabolites at 1000/50 mg/kg/day were lower than values observed at 300/50

mg/kg/day. AUC and C_{max} values for VX-770 and its metabolites at 1000/100 mg/kg/day were approximately equal to values observed at 300/50 mg/kg/day. VX-809 was found to enhance the metabolism of VX-770. The NOAEL was identified as the high dose of 1000/100 mg/kg/day given that observed histopathological findings were either considered irrelevant to humans or of low incidences with little evidence of dose-response relationships and considered monitorable in a clinical setting. $AUC_{0\text{-}24hr}$ values for VX-809 in females and males were 3410 and 2830 µg.hr/mL, respectively. Respective $AUC_{0\text{-}24hr}$ values for VX-770 and its M1 and M6 metabolites in females were 479, 145, and 6.17 µg·hr/mL and in males were 285, 216, and 18.6 µg·hr/mL.

In a 28-day oral (gavage) toxicology study, dogs received the combination of VX-809 and VX-770 at doses of 0/0, 300/5, 300/15, 600/15, and 600/60 mg/kg/day. Combinations of VX-809 and VX-770 were formulated as separate suspensions for co-administration by oral gavage. Following co−administration of VX-770/VX-809, prolonged PR intervals (first degree AV block) were observed in 3/12 and 10/12 dogs in the 600/15, and 600/60 mg/kg/day, respectively. Co−administration of VX-770 and VX-809 also produced early depolarization of supraventricular origin (supraventricular premature complex: SVPC) for 8/12 dogs in the 600/60 mg/kg/day group. Some SVPCs occurred during the inspiratory phase of the respiratory cycle; therefore, early supraventricular depolarization with the P wave in contact with the previous T wave, not buried in the previous T wave, could be resulting from exaggerated respiratory sinus arrhythmia. These ECG changes were not observed with VX-809 alone. SVPCs were increased as compared to VX-770 alone. Histopathological findings were evident in the thymus, testes, epididymides, prostate, and gallbladder. There was a dose-related depletion of lymphocytes in the thymus. These findings were considered monitorable in a clinical setting. The severity of lymphoid depletion exceeded control levels in males dosed at ≥300 mg/kg/day VX−809 + ≥ 5 mg/kg/day VX−770 (Groups 3 through 5) and in females at all dose levels. Thymic weights were decreased for all male and female treatment groups. These findings were not regarded as dose-limiting based upon the high spontaneous background in incidence in the control group. In the 600/60 mg/kg/day group, 2 of 4 males had increased numbers of multinucleate degenerate germ cells in the testes (moderate vs. minimal) and one of these dogs also had increased sloughed testicular germ cells in the epididymides. The incidence and severity of prostatic acinar contraction and reduced secretion was also greater in this group than in the other groups and this correlated with a decrease in prostatic weight in this group. Given the peripubertal status of the animals, it was likely that these changes represented a slight retardation of sexual maturation in the high dose group animals, which was probably associated with the decreased body weight gain that occurred in these animals during the study. These changes might be an indirect effect of the test article. The epithelial cells lining the gall bladder contained slightly increased amounts of mucinous secretory product in males and females at 600/60 mg/kg/day and in one male at 600/15 mg/kg/day compared to control animals. This finding was not considered adverse as there was no evidence of structural damage in the gallbladder. The NOAEL was identified as 600/15 mg/kg/day based upon histopathological findings in the male reproductive organs (testes, epididymides, and prostate) at the high dose of 600/60 mg/kg/day. Findings were reversible at the end of a 14-day recovery period. $AUC_{0\text{-}24hr}$

values for VX-809 in females and males were 976 and 503 µg.hr/mL, respectively. Respective $AUC_{0\text{-}24hr}$ values for VX-770 and its M1 and M6 metabolites in females were 102, 21.2, and 2.32 µg·hr/mL and in males were 124, 16.7, and 1.31 µg·hr/mL.

In a 90-day oral (gavage) toxicology study, rats were dosed once daily by oral gavage with various combinations of VX-809, VX-770, and VRT-0995096 co-formulated as a suspension in the vehicle. Doses of VX-809, VX-770, and VRT-0995096 in Groups 2, 3, 4, and 5 were 1000/100/20, 1000/25/20, 500/25/10, and 500/10/10 mg/kg/day, respectively. Female #2559 in Group 2 (1000/100/20 mg/kg/day) was observed with bilateral posterior subcapsular cataracts of the lens at the end of the 90-day dosing period. This finding was attributed to treatment with VX-770 and judged to be monitorable in a clinical setting, but not reversible. Target organs of toxicity were identified as the stomach, kidneys, thymus, mesenteric LN, and heart. Focal necrosis and/or erosions (minimal to slight) were present in the glandular mucosa of the stomach for all male drug-treated groups and females in Groups 2, 3, and 5. The findings were dose-responsive for male drug-treated groups, but not for female drug-treated groups. Mucosal necrosis and/or erosions were reversible following the recovery period. The squamous epithelium along the limiting ridge of the forestomach showed cystic degeneration (minimal to moderate) in males and females dosed at ≥500/10/10 mg/kg/day VX-809/VX-770/VRT-0995096. Variable numbers of the superficial keratinized cells along the limiting ridge contained small cysts filled with pink material. There was no clear dose relationship in males. Females had an inverse relationship to dose. Epithelial cystic degeneration along the limiting ridge was not reversible. Focal inflammation and edema were variably present. In one male (No. 2038) in Group 2 at 1000/100/20 mg/kg/day, the edema was moderate and diffuse. Based upon discussions with the Medical Officer, findings in the stomach were considered to be monitorable in a clinical setting based upon the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation. Basophilic tubules (minimal) were observed in 6 of 10 males dosed at 1000/100/20 mg/kg/day VX-809/VX-770/VRT-0995096. This finding was also evident for 2 of 10 females in Group 2 and 1 of 10 females in Group 3. The kidneys from recovery animals were not examined microscopically. The sponsor contended that basophilic and dilated tubules are common background findings in rats and the slightly increased incidence observed in terminal sacrifice animals was a known class effect of the test article as this finding was observed at increased incidences in the 28-day toxicology study with rats that received the combination of VX-809 and VX-770. An increased severity of decreased lymphocytes was observed in the thymus for males in Group 2 at 1000/100/20 mg/kg/day. Decreased lymphocytes were observed in the mesenteric LN for one male in Group 2 at 1000/100/20 mg/kg/day. Urothelial hyperplasia in the urinary bladder was observed for one male in Group 2 at 1000/100/20 mg/kg/day. Minimal cardiomyopathy was observed with an increased incidence for 4 of 10 females in Group 2 at 1000/100/20 mg/kg/day. The significance of this finding was unclear as the finding was observed for 1 of 9 control females and 2 of 10 control males. The incidence in male drug-treated groups ranged from 2 of 10 to 5 of 10 with no dose-response relationship. There were no findings that suggested any toxicokinetic interactions between VX-809, VX-770 and it metabolites, or VRT-0995096. Histopathological findings in the stomach,

kidneys, thymus, mesenteric LN, and heart were not considered dose-limiting. The NOAEL was identified at 1000/25/20 mg/kg/day based upon a finding of bilateral, subcapsular cataracts for one animal at 1000/100/20 mg/kg/day. However, based upon the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation, the finding of cataracts for one rat at 1000/100/20 mg/kg/day might be considered an acceptable risk that is monitorable in a clinical setting, but not reversible.

Safety Assessment:
In chronic toxicology studies with VX-809 alone, rats received doses up to 1000 mg/kg/day and dogs received doses up to 500 mg/kg/day. No dose-limiting toxicity or target organs of toxicity were identified in these studies.

Exposure margins for clinical exposures to VX-809 at proposed doses of 600mg/day and 400 mg q12hr (800 mg/day) relative to NOAELs in the 6-month rat and 12-month dog studies with VX-809 alone and the 3-month toxicology study with the combination of VX-809, VX-770, and VRT-0995096 in rats are shown in the table below. Safety margins were ≥1, which were considered adequate.

Table 67 Safety margins for clinical doses of VX-809 at 600 mg/day and 400 mg q12hr (800 mg/day)

Study	NOAEL, mg/kg/day	Sex	AUC µg*hr/mL	Safety margins for proposed clinical doses of VX-809 (Lumacaftor)	
				600 mg/day (300 µg*hr/mL)[a]	400 mg q12hr (372 µg*hr/mL)[a]
6-month Rat with VX-809 alone	1000	M	1300	4.3	3.5
		F	3160	10.5	8.5
12-month Dog with VX-809 alone	500	M	429	1.4	1.1
		F	515	1.7	1.4
3-month Rat with combination	1000 (Group 2)	M	2430	8.1	6.5
		F	3010	10	8.1
3-month Rat with combination	1000 (Group 3) NOAEL	M	2150	7.2	5.8
		F	3400	11.3	9.1

a. From Investigator's Brochure dated April 25, 2013

Exposure margins for clinical exposures to M28 obtained with clinical doses of VX-809 at 600 and 800 mg/day relative to the NOAELs in the 1-month toxicology study with M28 in rats, 6-month toxicology study with VX-809 + M28 in rats, and 3-month toxicology study with the combination of VX-809, VX-770, and VRT-0995096 were shown in the table below. Exposure margins were greater than 1, which were considered sufficient.

Table 68 Safety margins for estimated exposures to VRT-0995096 achieved with clinical doses of VX-809 at 600 mg/day and 400 mg q12hr (800 mg/day) (Assuming M28/VX-809 = 0.15)

Study	NOAEL for VRT-0995096, mg/kg/day	Sex	AUC µg*hr/mL	Safety margins for estimated exposures to VRT-0995096 (M28) achieved with clinical doses of VX-809 (Lumacaftor)	
				600 mg/day VX-809 (M28: 32.1 µg*hr/mL)[a]	400 mg q12hr VX-809 (M28: 28.2 µg*hr/mL)[a]
1-month rat with VRT-0995096	100	M	2910	90.7	103.2
		F	1800	56.1	63.8
6-month Rat with VX-809 + VRT-0995096	25	M	1163	36.2	41.2
		F	712	22.2	25.3
3-month Rat with combination	20 (Group 2)	M	725	22.6	25.7
		F	938	29.2	33.3
3-month Rat with combination	20 (Group 3) NOAEL	M	788	24.6	27.9
		F	875	27.3	31.0

a. From Investigator's Brochure dated April 25, 2013

Toxicology studies with the combination of VX-809 and VX-770 in rats up to 3 months and dogs up to 28 days identified the following findings that were not generally observed with the individual monoproducts:

1. In rats treated with the combination of VX-809 and VX-770, focal necrosis and/or erosions (minimal to slight) were present in the glandular mucosa of the stomach for all male and female drug-treated groups. The findings were dose-responsive for male drug-treated groups, but not for female drug-treated groups. Mucosal necrosis and/or erosions were reversible following the recovery period. The squamous epithelium along the limiting ridge of the forestomach showed cystic degeneration (minimal to moderate) in males and females dosed at ≥500/10/10 mg/kg/day VX-809/VX-770/VRT-0995096. Variable numbers of the superficial keratinized cells along the limiting ridge contained small cysts filled with pink material. There was no clear dose relationship in males. Females had an inverse relationship to dose. Epithelial cystic degeneration along the limiting ridge was not reversible. Focal inflammation and edema were variably present. In one male (No. 2038) in Group 2 at 1000/100/20 mg/kg/day, the edema was moderate and diffuse. Based upon discussions with the Medical Officer, findings in the stomach were considered to be monitorable in a clinical setting based upon the indication for treatment of CF subjects that are homozygous or heterozygous for the F508del-CFTR mutation.

2. Following co−administration of VX-770/VX-809 to dogs , prolonged PR intervals (first degree AV block) were observed in 3/12 and 10/12 dogs in the 600/15, and 600/60 mg/kg/day, respectively. Co−administration of VX-770 and VX-809 also produced early depolarization of supraventricular origin (supraventricular premature complex: SVPC) for 8/12 dogs in the 600/60 mg/kg/day group. Some SVPCs occurred during the inspiratory phase of the respiratory cycle; therefore, early supraventricular

depolarization with the P wave in contact with the previous T wave, not buried in the previous T wave, could be resulting from exaggerated respiratory sinus arrhythmia. These ECG changes were not observed with VX-809 alone. SVPCs were increased as compared to VX-770 alone. These findings were considered monitorable in a clinical setting.

3. In the 600/60 mg/kg/day group, 2 of 4 male dogs had increased numbers of multinucleate degenerate germ cells in the testes (moderate vs. minimal) and one of these dogs also had increased sloughed testicular germ cells in the epididymides. The incidence and severity of prostatic acinar contraction and reduced secretion was also greater in this group than in the other groups and this correlated with a decrease in prostatic weight in this group. Given the peripubertal status of the animals, it was likely that these changes represented a slight retardation of sexual maturation in the high dose group animals, which was probably associated with the decreased body weight gain that occurred in these animals during the study. These changes might be an indirect effect of the test article. The NOAEL was identified as 600/15 mg/kg/day based upon histopathological findings in the male reproductive organs (testes, epididymides, and prostate) at the high dose of 600/60 mg/kg/day. Findings were reversible at the end of a 14-day recovery period.

Lumacaftor (VX-809) has been found to affect the PK of ivacaftor (VX-770). Ivacaftor and its active metabolites, M1 and M6, all reach steady-state within 7 days during ivacaftor monotherapy. When ivacaftor is administered in combination with lumacaftor, the ivacaftor and M1 steady-state levels were decreased by 81% and 72%, respectively, whereas M6 steady-state levels did not exhibit any major change. The marked reduction in the levels of ivacaftor and M1-ivacaftor was likely due to the induction of CYP3A metabolism as ivacaftor was mainly metabolized by this enzyme. This induction was predicted to plateau within 7 days. In combination therapy with lumacaftor and ivacaftor, M6 is the primary form as compared to ivacaftor and M1. This contrasts to ivacaftor monotherapy in which M1 was found to be the primary form.

Toxicology studies with VX-770 alone in rats and dogs, the 28-day toxicology studies with the combination of VX-809 and VX-770 in rats and dogs, and the 3-month toxicology study with the combination of VX-809, VX-770, and VRT-0995096 in rats provide adequate safety margins for exposures to the parent drug, VX-770, and metabolite M1 (VRT-837018) achieved with clinical doses of VX-770 up to 250 mg q12hr (500 mg/day) in the combination as shown in the tables below.

For clinical exposures to M6 (VRT-842917), there was inadequate coverage from the 6-month rat and 12-month dog toxicology studies with VX-770 alone, the 28-day combination toxicology studies with rat and dogs, and the 3-month combination toxicology study with rat as shown in the tables below. However, structures of VX-770 and its two metabolites, M1 and M6, were relatively similar. M1 (VRT-837018) is formed by oxidation on the t-butyl group. M6 (VRT-842917) is formed by carboxylation of the hydroxy-t-butyl M1 metabolite (sequential methyl oxidation to acid). M1 and M6 were more polar than VX-770 and might be expected to be less toxic. Further, the exposure

to M6 in children and adults that received the approved dose of KALYDECO® (Ivacaftor) at 150 mg q12hr alone was higher than observed with VX-770 at 250 mg q12hr in combination with VX-809 at 600 or 800 mg/day.

Table 69 Estimated Safety margins on an AUC basis for the clinical dose of VX-770 and its metabolites, M1 (VRT-837018) and M6 (VRT-842917), with a clinical dose of VX-770 at 250 mg q12hr (combined with VX-809 at 600 mg/day)

Study	NOAEL mg/kg/day (Dose of VX-770)	Parent drug or metabolite	Sex	AUC µg·hr/mL	Exposure margins for VX-770 at 250 q12 hr[a]		
					VX-770 (7.6 µg*hr/mL)	M1 (24.4 µg*hr/mL)	M6 (43.2 µg*hr/mL)
6-month rat with VX-770 alone	50[b]	Ivacaftor	M	445	58.6	-	-
			F	561	73.8	-	-
		M1	M	131	-	5.4	-
			F	75.2	-	3.1	-
		M6	M	38.9	-	-	0.9
			F	14.7	-	-	0.3
12-month dog with VX-770 alone	60[b]	Ivacaftor	M	351	46.2	-	-
			F	254	33.4	-	-
		M1	M	49.4	-	2.0	-
			F	40.4	-	1.7	-
		M6	M	7.3	-	-	0.17
			F	5.5	-	-	0.13
3-month rat combination	25 (Group 3; NOAEL)	Ivacaftor	M	143	18.8	-	-
			F	138	18.2	-	-
		M1	M	35.9	-	1.5	-
			F	22.9	-	0.9	-
		M6	M	5.97	-	-	0.14
			F	2.62	-	-	0.06
3-month rat combination	100 (Group2; Cataracts observed)	Ivacaftor	M	372	49	-	-
			F	653	86	-	-
		M1	M	97.4	-	4	-
			F	65.9	-	2.7	-
		M6	M	17.1	-	-	0.40
			F	6.46	-	-	0.15
28-day rat combination	100	Ivacaftor	M	285[c]	37.5	-	-
			F	479[c]	63.0	-	-
		M1	M	216	-	8.9	-
			F	145	-	5.9	-
		M6	M	18.6	-	-	0.43
			F	6.17	-	-	0.14
28-day dog combination	15	Ivacaftor	M	124	16.3	-	-
			F	102	13.4	-	-
		M1	M	16.7	-	0.7	-
			F	21.2	-	0.9	-
		M6	M	1.31	-	-	0.03
			F	2.32	-	-	0.05

a. From Investigator's Brochure dated April 25, 2013 (2 x $AUC_{0\text{-}12hr}$)
b. see IND 74,633 and NDA 203-188
c. These values are corrected. There was a 10-fold error in converting from ng*hr/mL to µg*hr/mL in reviews dated 2-2-10, 9-24-10, 9-1-11, and 9-28-11 (one error was carried forward into later reviews). This error had no impact on the conclusions of the reviews.

Table 70 Estimated Safety margins on an AUC basis for the clinical dose of VX-770 and its metabolites, M1 (VRT-837018) and M6 (VRT-842917), with a clinical dose of VX-770 at 250 mg q12hr (combined with VX-809 at 400 mg q12hr)

Study	NOAEL mg/kg/day (Dose of VX-770)	Parent drug or metabolite	Sex	AUC µg·hr/mL	Exposure margins for VX-770 at 250 q12 hr[a]		
					VX-770 (5.2 µg*hr/mL)	M1 (19.76 µg*hr/mL)	M6 (38.8 µg*hr/mL)
6-month rat with VX-770 alone	50[b]	Ivacaftor	M	445	85.6	-	-
			F	561	107.9	-	-
		M1	M	131	-	6.6	-
			F	75.2	-	3.8	-
		M6	M	38.9	-	-	1
			F	14.7	-	-	0.4
12-month dog with VX-770 alone	60[b]	Ivacaftor	M	351	67.5	-	-
			F	254	48.9	-	-
		M1	M	49.4	-	2.5	-
			F	40.4	-	2.0	-
		M6	M	7.3	-	-	0.19
			F	5.5	-	-	0.14
3-month rat combination	25 (Group 3; NOAEL)	Ivacaftor	M	143	27.5	-	-
			F	138	26.5	-	-
		M1	M	35.9	-	1.8	-
			F	22.9	-	1.2	-
		M6	M	5.97	-	-	0.15
			F	2.62	-	-	0.07
3-month rat combination	100 (Group2; Cataracts observed)	Ivacaftor	M	372	71.5	-	-
			F	653	125.6	-	-
		M1	M	97.4	-	4.9	-
			F	65.9	-	3.3	-
		M6	M	17.1	-	-	0.4
			F	6.46	-	-	0.2
28-day rat combination	100	Ivacaftor	M	285[c]	54.8	-	-
			F	479[c]	92.1	-	-
		M1	M	216	-	10.9	-
			F	145	-	7.3	-
		M6	M	18.6	-	-	0.5
			F	6.17	-	-	0.2
28-day dog combination	15	Ivacaftor	M	124	23.9	-	-
			F	102	19.6	-	-
		M1	M	16.7	-	0.85	-
			F	21.2	-	1.1	-
		M6	M	1.31	-	-	0.03
			F	2.32	-	-	0.06

a. From Investigator's Brochure dated April 25, 2013 (2 x $AUC_{0\text{-}12hr}$)
b. see IND 74,633 and NDA 203-188
c. These values are corrected. There was a 10-fold error in converting from ng*hr/mL to µg*hr/mL in reviews dated 2-2-10, 9-24-10, 9-1-11, and 9-28-11 (one error was carried forward into later reviews). This error had no impact on the conclusions of the reviews.

Table 71 Safety margins for the clinical dose of VX-770 at 150 mg q12hr

Table 4 Exposure Safety Margins Observed for Ivacaftor and its Metabolites from Ivacaftor Monotherapy Development Program (NDA 203188)

Species	Dose	Mean $AUC_{0\text{-}24hr}$ (µg•hr/mL) [a]	AUC Ratio (A/H)
Rat	NOAEL – 50 mg/kg		-
	Ivacaftor	503 [b]	-
	M1	103 [b]	-
	M6	26.8 [b]	-
Human (Ages 12 and over)	150 mg Ivacaftor q12h	18.4	27
	M1	78.5	1.3
	M6	55.4	0.48
Human (Ages 6 to 11)	150 mg Ivacaftor q12h	36.4	14
	M1	158	0.65
	M6	108	0.25

AUC: Area under the plasma concentration versus time curve over 24 hours; C_{max}: Maximum plasma observed concentration; A: Animal; H: Human; NOAEL: No-observed-adverse-effect limit

[a] Expressed as mean of males and females

[b] Last day of dosing in the rat chronic toxicity study (VX-770-TX-010)

[c] Mean value observed at 150 mg q12h of ivacaftor from Population PK Report (Report G198; NDA 203188 Module 5.3.3.5)

Comments conveyed to the Sponsor regarding nonclinical study findings:

Safety concerns regarding the findings in the stomach from rats treated with the combination of VX-809 and VX-770 were conveyed to the Medical Officer, Dr. Witzmann. The Division provided comments to the Sponsor regarding proposed Phase 3 clinical trials on April 10, 2013. Vertex's responses to these comments are provided below. The Division's comments are included in the boxed *italic* text and Vertex's response is provided in plain text below each comment.

Division Comment 7:

We note that your nonclinical toxicology study in rats treated with combination VX-809 + VX-770 resulted in necrosis and/or erosions in the stomach of test animals at all doses. We view these findings as likely clinically monitorable. Include in your Phase 3 studies specific evaluations to monitor for upper gastrointestinal side effects consistent with stomach erosions.

Vertex Response:
Vertex appreciates the Division's comments regarding the findings in the nonclinical toxicology studies evaluating lumacaftor and ivacaftor treatment in rats. Vertex has reviewed the current clinical data available from the program, reviewed the current safety evaluations in the Phase 3 studies, and has considered potential additional specific evaluations to monitor for upper gastrointestinal side effects consistent with gastric erosions. As part of this process, Vertex also consulted with external experts, including (b) (4) a pediatric gastroenterologist with extensive clinical and clinical research experience in cystic fibrosis from (b) (4) .

Ongoing review of all available clinical data has yielded no gastrointestinal safety signal in humans to date. Safety assessments in Study 103 and Study 104 include adverse events (AEs), serious adverse events (SAEs), symptom-directed physical examinations, serial hemoglobin assessments, and assessments collected within the gastrointestinal domain of the CFQ-R.

Potential specific evaluations for monitoring upper gastrointestinal events consistent with stomach erosion include upper endoscopy and/or fecal occult blood. However, these may pose additional risk to subjects and/or lead to unnecessary evaluation. Risks associated with upper endoscopy include deleterious effects from sedation and analgesia in a patient population with underlying lung disease. For this nonclinical finding that was observed in a single species and which has not resulted in a safety signal within clinical studies, these risks are not outweighed by the benefit of endoscopic surveillance. Serial monitoring of fecal occult blood would likely result in a high positivity rate (e.g., swallowed blood from hemoptysis and/or sinusitis, intestinal inflammation, colitis from antibiotics, and hemorrhoids from constipation) leading to difficulties in detecting a true positive signal related to study drug, unnecessary endoscopies, and/or interruption or premature discontinuation of study drug. Therefore, Vertex believes that adding specific evaluations to monitor for upper gastrointestinal events consistent with stomach erosions in the clinical study protocols for Study 103 and Study 104 is not necessary at this time. However, Vertex agrees that these findings should be considered by investigators, and will provide additional guidance regarding these findings in an addendum to the lumacaftor IB. This addendum is included in this submission and reads as follows:

"Single and repeat oral doses of lumacaftor were well tolerated in rats and dogs, and no significant toxicities were identified that would preclude dosing chronically in humans. In 2 nonclinical studies in rats evaluating lumacaftor in combination with ivacaftor, focal or multi-focal gastric erosions were observed. This nonclinical finding was limited to a single species and has not been associated with a safety signal in the clinical studies thus far. The relevance of this finding for humans is not known. However, investigators should consider further clinical evaluation as appropriate for possible gastric erosions in the event a subject develops compatible signs or symptoms (epigastric abdominal pain and/or an unexplained drop in hemoglobin).

Evaluation:
The Sponsor's response was considered acceptable based given the indication for the treatment of CF subjects who are homozygous or heterozygous for the *F508del-CFTR* mutation.

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

/s/

TIMOTHY W ROBISON
12/10/2013

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	79,521
Supporting document/s:	EDR SD# 18, 23, 92, 100
Sponsor's letter date:	1/28/2009, 5/20/2009, 11/6/2012, 1/11/2013
CDER stamp date:	1/28/2009, 5/20/2009, 11/6/2012, 1/11/2013
Product:	VX-809 (lumacaftor)
Indication:	Cystic Fibrosis
Sponsor:	Vertex Pharmaceuticals
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Angela Ramsey

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

VX-809 (lumacaftor) is in late-stage development by Vertex Pharmaceuticals as a potential treatment for cystic fibrosis in a combination product that also contains the FDA-approved product ivacaftor (VX-770, tradename Kalydeco). The planned clinical dose of VX-809 is 600 mg QD or 400 mg BID in combination with VX-770 at a dose of 250 mg BID. A pre-NDA meeting for the lumacaftor / ivacaftor combination product was held in August 2014 and an NDA submission is projected by the sponsor for November 2014.

This review covers the developmental and reproductive toxicology studies evaluating VX-809 that will be included in the upcoming NDA. Certain studies also evaluated VRT-0995096 (referred to as M28 in this review), a disproportionate human metabolite of VX-809. Developmental and reproductive toxicity studies evaluating ivacaftor were previously reviewed under NDA 203,188.

1.2 Brief Discussion of Nonclinical Findings

In a fertility and early embryonic development study, rats received VX-809 and M28 at doses of 250/20, 500/20, or 1000/20 mg/kg/day. Males were dosed for 28 days before mating and females were dosed from 14 days before mating through gestation day (GD) 7. There were no adverse effects on male fertility, female fertility, or early embryonic viability and development through GD 15. The high-dose level of 1000 mg/kg/day VX-809 and 20 mg/kg/day M28 was therefore considered as the NOAEL in the study.

In an embryo-fetal development study, female rats received VX-809 at doses of 500, 1000 or 2000 mg/kg/day from GD 7 through GD 17. There were no adverse effects in the study with regards to maternal health or embryofetal development and the high-dose of 2000 mg/kg/day was considered as a NOAEL. Exposure to VX-809 in the study increased less than dose-proportionally from 500-2000 mg/kg and was similar on GD 7 and GD 17. The VX-809 $AUC_{0\text{-}24h}$ at the NOAEL was 3,320 ug*h/mL.

In an embryo-fetal development study, female rabbits received VX-809 at doses of 50, 100 or 200 mg/kg/day from GD 7 through GD 19. An excessive level of maternal toxicity was observed at the high-dose, with two premature deaths and four additional does that aborted and were sacrificed. Additional findings at the high-dose included body weight losses (-5% during the dosing period) and decreased food consumption, scant feces, poor grooming, thin body condition, dehydration, and decreased activity. Significant decreases in body weight gain (+1% vs. +5% in controls) were also noted at the mid-dose level. Macroscopic findings at the high-dose were noted in the heart (white bands surrounding the ventricles, pale appearance), lungs (red/mottled lobes), and kidneys (pale appearance). There were no test article-related effects on embryofetal survival or any evidence of teratogenicity in the study. The NOAELs in the study with respect to maternal and embryofetal toxicity were considered as 50 and 200 mg/kg/day,

respectively. Exposure to VX-809 in the study increased less than dose-proportionally from 50-200 mg/kg and was similar on GD 7 and GD 19. The VX-809 AUC_{0-24h} at the NOAELs of 50 mg/kg and 200 mg/kg were 995 and 1950 ug*h/mL, respectively.

In an embryo-fetal development study, female rats received M28 at doses of 200, 400 or 800 mg/kg/day from GD 7 through GD 17. Excessive maternal toxicity was observed at the HD, including four unscheduled deaths (two main study and two satellite animals). Clinical signs included red/clear material around the nose, mouth and/or urogenital area, red vaginal discharge, rales, and pale/cool body. At the HD, maternal body weight gains were 40% lower than controls, a difference that was statistically significant and considered to be adverse. Food consumption was also decreased at the HD. There were no test article-related effects on embryofetal viability but the HD level was associated with a 20% decrease in mean fetal body weights. Skeletal variations were frequently noted in the HD group, particularly decreased ossification of cervical centrum #1, unossified sternebrae #5/6, and 14^{th} rudimentary ribs. Vertebral malformations were noted in five HD pups from three different litters (vertebral agenesis, vertebral centra anomaly, and vertebral anomaly with or without associated rib anomaly) but these were not considered to represent test article-related teratogenicity. Only a single malformation (vertebral anomaly with or without associated rib anomaly) exceeded the historical control range and this finding was confined to a single litter. The MD level of 400 mg/kg/day was considered the NOAEL for embryofetal toxicity based upon decreased fetal body weights observed at 800 mg/kg/day; however, this finding can most likely be attributed to maternal toxicity observed at 800 mg/kg/day. M28 exposure increased less than dose-proportionally from 200 to 800 mg/kg/day and was similar on GD 6 and GD 17. AUC_{0-24h} at the maternal and embryofetal NOAELs was 3360 and 4240 ug*h/mL, respectively.

In a pre- and post-natal development study, F_0 female rats received VX-809/M28 at doses of 250/20, 500/20, and 1000/20 mg/kg/day from GD 6 through lactation day (LD) 21. The development and reproductive performance of F_1 offspring was assessed and the status of F_2 litters was assessed by Cesarean section of F_1 dams at GD 14. There were no test article related deaths nor effects on body weights or food consumption in F_0 dams. There were no test article-related effects on F_0 mating performance, duration of gestation, litter size and fetal viability statistics, or sex ratio. There were no test article-related effects on F_1 survival through weaning, body weights, or food consumption. There were no test article-related effects on pre-weaning or post-weaning functional assessments in F_1 animals. There were no test article-related effects on F_1 sexual maturation, mating performance, fertility, or status of F_2 litters based upon C-section at GD 14. The HD of 1000/20 mg/kg/day was considered as the NOAEL for maternal and F_1 pre-/post-natal toxicity.

2 Drug Information

2.1 Drug

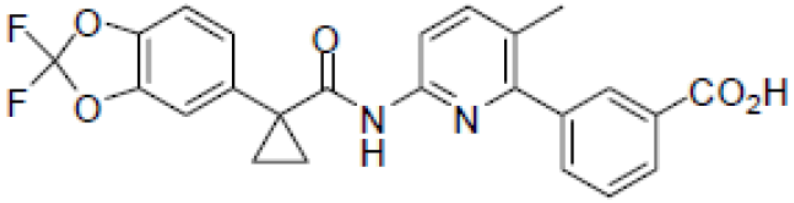
Generic Name: Lumacaftor

Code Name: VX-809

Chemical Name: 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid

Molecular Formula/Molecular Weight: $C_{24}H_{18}F_2N_2O_5$ / 452 g/mole

Structure or Biochemical Description



Pharmacologic Class: Cystic Fibrosis Transmembrane conductance Regulator (CFTR) [redacted] (b) (4) (proposed by sponsor)

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 74,633 and NDA 203,188 (Ivacaftor / Kalydeco / VX-770)
- NDA 206,038 (for Lumacaftor / Ivacaftor combination, rolling submission has begun with CMC module submitted 7/30/2014)

2.7 Previous Clinical Experience

The following table summarizes the lumacaftor clinical development program and was included by the sponsor in the briefing package for the 8/12/2014 pre-NDA meeting.

Table 1. Lumacaftor (VX-809) Clinical Development Program

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Biopharmaceutic Studies							
Bioavailability Studies							
Phase 1 BA	VX08-809-003 (Study 003)	Primary Objectives • Evaluate BA of capsule formulation of lumacaftor relative to suspension formulation administered in Study VX07-809-001 • Assess effect of food on PK of lumacaftor capsule formulation after single dose of 200 mg Secondary Objective Evaluate safety and tolerability of lumacaftor suspension and capsule formulations after single dose administration of 200 mg	Open-label, randomized, single-dose, 2-formulation, 6-sequence, 3-period crossover (Williams Design)	**Lumacaftor** (50-mg capsule or 200-mg suspension) • 200 mg lumacaftor (suspension) in fasted conditions • 200 mg lumacaftor (capsule) in fasted or fed conditions Oral administration	18 subjects Healthy male subjects aged 18 through 60 years, inclusive	Single dose on 3 dosing occasions	Completed; Full
Phase 1 BA	VX13-809-012 (Study 012)	Primary Objective Evaluate effect of food on relative bioavailability of 2 fixed-dose combinations of lumacaftor and ivacaftor tablet formulations Secondary Objective Evaluate safety and tolerability of single, oral doses of 2 fixed-dose combinations of lumacaftor and ivacaftor tablet formulations when administered in fed and fasted conditions	Single-center, randomized, open-label, single dose, 2-part (2-sequence, 2-period per part), crossover	**Lumacaftor/ivacaftor (fixed-dose, film-coated):** 200-mg lumacaftor/125-mg ivacaftor tablet, 200-mg lumacaftor/83-mg ivacaftor tablet **Part A:** 400 mg lumacaftor in combination with 250 mg ivacaftor **Part B:** 600 mg lumacaftor in combination with 250 mg ivacaftor Oral administration	28 subjects (planned) Healthy adult male and female subjects aged 18 to 55 years, inclusive	Single dose on 2 dosing occasions	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Comparative Bioavailability and Bioequivalence Studies							
Phase 1 BA	VX12-809-007 (Study 007)	**Part A** Primary Objective Evaluate relative bioavailability of new tablet formulation (Form 1 High Drug Load) of lumacaftor compared to reference tablet formulation (Form 1) at 2 different doses Secondary Objective Evaluate safety and tolerability of single oral doses of lumacaftor administered as new tablet formulation (Form 1 High Drug Load) compared to reference tablet formulation (Form 1) **Part B** Primary Objective Evaluate relative bioavailability of new tablet coformulation (200-mg lumacaftor/125-mg ivacaftor FDC tablets) compared to codosing as separate tablet formulations (b) (4) of lumacaftor and film-coated ivacaftor tablets) Secondary Objective Evaluate safety and tolerability of single, oral doses of the 200-mg lumacaftor/125-mg ivacaftor FDC coformulation compared to codosing as separate tablet formulations (b) (4) of lumacaftor and film-coated ivacaftor tablets)	Open-label, randomized, single-dose, crossover	**Part A** **Lumacaftor** (b) (4) (b) (4) (b) (4) 200-mg tablet 400 mg or 600 mg lumacaftor in fed conditions Oral administration **Part B** **Lumacaftor and ivacaftor coformulation** (200-mg lumacaftor/125-mg ivacaftor FDC tablets) **Lumacaftor (Form 1):** 200-mg tablet**Ivacaftor (film-coated):** 100-mg and 150-mg tablets 400 mg lumacaftor in combination with 250 mg ivacaftor Oral administration	61 subjects total **Part A:** 30 subjects **Part B:** 31 subjects Healthy male subjects aged 18 to 55 years (inclusive)	**Part A** Single dose on 4 dosing occasions **Part B** Single dose on 2 dosing occasions	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Human Pharmacokinetic (PK) Studies							
Healthy Subject PK and Initial Tolerability Studies							
Phase 1 Safety, tolerability and PK	VX07-809-001 (Study 001)	**Part A: Healthy Males** Primary Objective Evaluate safety and tolerability of single ascending and descending doses of lumacaftor suspension in fasted conditions Secondary Objective Evaluate PK of lumacaftor following administration of single ascending and descending doses of lumacaftor suspension in fasted conditions **Part B: Healthy Females** Primary Objective Evaluate safety and tolerability of single ascending doses of lumacaftor suspension in fasted conditions Secondary Objective Evaluate PK of single ascending doses of lumacaftor suspension in fasted conditions **Part C: Healthy Males** Primary Objective Evaluate effect of food on the safety and tolerability of single doses of lumacaftor suspension Secondary Objective Evaluate effect of food on PK of single doses of lumacaftor suspension **Part D: Healthy Males and Females** Primary Objective Evaluate safety and tolerability of multiple ascending doses of lumacaftor suspension	4-part, 5-panel, randomized, double-blind, single ascending dose, multiple ascending dose, pbo-controlled	**Lumacaftor** (suspension) **Pbo** (suspension) **Part A** Single doses of 25, 75, 200, or 400 mg lumacaftor or pbo **Part B** Single doses of 75, 200, or 400 mg lumacaftor or pbo **Part C** Single dose of 200 mg lumacaftor or pbo **Part D** 50, 100, or 200 mg qd lumacaftor or pbo Oral administration	64 subjects total **Part A:** 16 subjects **Part B:** 9 subjects **Part C:** 9 subjects **Part D:** 30 subjects Healthy male and female subjects aged 18 to 60 years, inclusive	**Part A** single dose on 4 dosing occasions **Part B** single dose on 3 dosing occasions **Part C** single dose on 2 dosing occasions **Part D** once daily for 14 days	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
		administered for 14 days in fed conditions Secondary Objective Evaluate PK of lumacaftor following multiple ascending doses of lumacaftor suspension administered for 14 days in fed conditions					
Phase 1 ADME	VX08-809-004 (Study 004)	Primary Objective Characterize PK, route(s) and rate of elimination and total recovery of lumacaftor and total radioactivity after a single, oral dose of [^{14}C] lumacaftor Secondary Objectives • Profile and identify, if possible, major metabolites of lumacaftor in urine, plasma, and feces following administration of a single oral dose of [^{14}C] lumacaftor • Assess safety and tolerability of lumacaftor	Open-label, single-dose	[^{14}C] **Lumacaftor** (suspension) 200 mg lumacaftor in fasted conditions Oral administration	6 subjects Healthy male subjects aged 18 through 45 years, inclusive	Single dose on 1 dosing occasion	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Patient PK and Initial Tolerability Studies							
Phase 1 PK	VX07-809-002 (Study 002)	Primary Objective Evaluate PK of lumacaftor in pancreatic-insufficient subjects with CF Secondary Objectives • Evaluate safety and tolerability of single oral doses of lumacaftor administered in fasted and fed states to pancreatic-insufficient subjects with CF • Evaluate effect of food on PK of lumacaftor in pancreatic-insufficient subjects with CF	Open-label, randomized, single-dose, 2-period crossover	**Lumacaftor**: 50-mg capsule 200 mg lumacaftor in fasted or fed conditions Oral administration	8 subjects Pancreatic-insufficient male and female adult subjects with CF aged 18 years or older	Single dose on 2 dosing occasions	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase 1 (Part A) and Phase 3 (Part B) PK (pediatric population), safety, tolerability, and efficacy	VX13-809-011 (Study 011)	**Part A** Primary Objective Evaluate PK of multiple doses of lumacaftor in combination with ivacaftor Secondary Objectives • Investigate PK of a lumacaftor metabolite, M28-lumacaftor, and ivacaftor metabolites, M1-ivacaftor and M6-ivacaftor • Evaluate safety and tolerability of multiple doses of lumacaftor in combination with ivacaftor **Part B** Primary Objective Evaluate safety and tolerability of lumacaftor in combination with ivacaftor through Week 24 Secondary Objectives • Evaluate efficacy of lumacaftor in combination with ivacaftor through Week 24 • Evaluate off-drug response after Washout Period (Week 24 to Week 26) • Evaluate PK of lumacaftor, M28-lumacaftor, ivacaftor, M1-ivacaftor, and M6-ivacaftor for lumacaftor in combination with ivacaftor	Open-label, multiple-cohort, multiple-dose, multicenter	**Part A** **Lumacaftor/ivacaftor (fixed-dose, film-coated):** 200-mg lumacaftor/125-mg ivacaftor tablet **Ivacaftor (film-coated):** 125-mg tablet 200 mg lumacaftor q12h in combination with 250 mg ivacaftor q12h Oral administration **Part B** TBD Oral administration	68 subjects total (planned) **Part A:** 12 subjects (planned) **Part B:** 56 subjects (planned) Male and female subjects aged 6 through 11 years (inclusive) with CF who are homozygous for the *F508del-CFTR* mutation	**Part A** Approximately 14 days **Part B** Approximately 24 weeks	Ongoing; Interim CSR for Part A will be submitted with NDA

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Intrinsic Factor PK Studies							
Phase 1 PK (hepatic impairment) and safety	VX13-809-010 (Study 010)	Primary Objective Compare PK of multiple doses of lumacaftor in combination with ivacaftor in subjects with moderate hepatic impairment to PK in matched healthy subjects Secondary Objectives • Compare PK of M28-lumacaftor, following multiple doses of lumacaftor in combination with ivacaftor in subjects with moderate hepatic impairment to PK in matched healthy subjects • Compare PK of M1-ivacaftor and M6-ivacaftor, following multiple doses of lumacaftor in combination with ivacaftor in subjects with moderate hepatic impairment to PK in matched healthy subjects • Assess safety and tolerability of multiple doses of lumacaftor in combination with ivacaftor in subjects with moderate hepatic impairment and in matched healthy subjects	Open-label, multiple-dose, multicenter	**Lumacaftor** (b) (4) 200-mg tablet **Ivacaftor (film-coated):** 100-mg and 150-mg tablets 200 mg lumacaftor q12h in combination with 250 mg ivacaftor q12h Oral administration	12 subjects with hepatic impairment and 11 matched healthy subjects **Group A:** 12 subjects (planned) **Group B:** 12 subjects (planned) **Group A:** Male and female subjects with moderate hepatic impairment (Child Pugh Class B) aged 18 to 65 years inclusive **Group B:** Healthy male and female subjects aged 18 to 65 years matched to subjects in Group A for age, sex, weight, and BMI	10 days	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Extrinsic Factor PK Studies							
Phase 1 PK (DDI)	VX08-809-005 (Study 005)	Primary Objective Assess PK of lumacaftor in combination with ivacaftor in healthy adult subjects Secondary Objective Assess safety and tolerability of lumacaftor in combination with ivacaftor in healthy adult subjects	Randomized, double-blind, pbo-controlled, multiple-dose, 3-treatment period, DDI	**Lumacaftor:** 50-mg capsule **Ivacaftor:** 50-mg tablet **Pbo:** capsules and/or tablets • 200 mg q24h of lumacaftor or pbo • 150 mg q12h of ivacaftor or pbo • 200 mg q24h of lumacaftor in combination with 150 mg q12h of ivacaftor or pbo Oral administration	24 subjects Healthy male and female subjects aged 18 through 55 years (inclusive)	14 days of lumacaftor monotherapy, followed by a washout period of 14 days, followed by 14 days of ivacaftor monotherapy, followed by a washout period of 14 days, followed by 14 days of lumacaftor and ivacaftor combination therapy	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase 1 PK (DDI)	VX10-809-006 (Study 006)	Primary Objective Assess PK of lumacaftor in combination with ivacaftor in healthy adult subjects Secondary Objective Assess safety and tolerability of lumacaftor in combination with ivacaftor in healthy adult subjects	Randomized, double-blind, pbo-controlled, multiple-dose, 3-treatment period, DDI	**Lumacaftor:** 50-mg capsule **Ivacaftor:** 100-mg and 150-mg tablets **Pbo:** capsules and/or tablets **Cohort 1** • 200 mg qd of lumacaftor or pbo • 250 mg q12h of ivacaftor or pbo • 200 mg qd of lumacaftor in combination with 250 mg q12h of ivacaftor or pbo **Cohort 2** • 400 mg qd of lumacaftor or pbo • 150 mg q12h of ivacaftor or pbo • 400 mg qd of lumacaftor in combination with 150 mg q12h of ivacaftor or pbo **Cohort 3** Not conducted Oral administration	72 subjects total (planned) Healthy male and female subjects aged 18 through 55 years, inclusive	14 days of lumacaftor monotherapy, followed by a washout period of 14 days, followed by 14 days of ivacaftor monotherapy, followed by a washout period of 14 days, followed by 14 days of lumacaftor and ivacaftor combination therapy	Data analyses are ongoing; Full CSR will be submitted with NDA

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase 1 PK (DDI)	VX12-809-009 (Study 009)	**Cohorts 1 through 3** Primary Objectives • Assess PK of lumacaftor and ivacaftor in absence and presence of ciprofloxacin in healthy adult subjects • Assess PK of lumacaftor and ivacaftor in absence and presence of itraconazole in healthy adult subjects • Assess PK of lumacaftor and ivacaftor in absence and presence of rifampin in healthy adult subjects Secondary Objectives • Assess safety and tolerability of lumacaftor and ivacaftor in absence and presence of ciprofloxacin in healthy adult subjects • Assess safety and tolerability of lumacaftor and ivacaftor in absence and presence of itraconazole in healthy adult subjects • Assess safety and tolerability of lumacaftor and ivacaftor in absence and presence of rifampin in healthy adult subjects **Cohort 4** Primary Objective • Evaluate effect of long-acting bronchodilators (i.e., indacaterol and tiotropium) in healthy adult subjects treated with lumacaftor in combination with ivacaftor Secondary Objectives • Evaluate effect of short-acting bronchodilators (i.e., albuterol and ipratropium) in healthy adult subjects treated with lumacaftor in combination with ivacaftor	**Cohorts 1 through 3** Open-label, multiple-dose **Cohort 4** Open-label, single-dose	**Lumacaftor:** 200-mg tablets **Ivacaftor (film-coated):** 100-mg and 150-mg tablets **Cohorts 1 through 3** • 200 mg q12h of lumacaftor in combination with 250 mg q12h of ivacaftor • 200 mg q12h of lumacaftor in combination with 250 mg q12h of ivacaftor and 750 mg q12h of ciprofloxacin • 200 mg q12h of lumacaftor in combination with 250 mg q12h of ivacaftor and 200 mg qd of itraconazole • 200 mg q12h of lumacaftor in combination with 250 mg q12h of ivacaftor and 600 mg qd of rifampin **Cohort 4** • 200 mg lumacaftor in combination with 250 mg ivacaftor and short-acting bronchodilator • long-acting	78 subject total (planned) **Cohort 1:** 18 subjects (planned) **Cohort 2:** 18 subjects (planned) **Cohort 3:** 18 subjects (planned) **Cohort 4:** 24 subjects (planned) Healthy male and female subjects aged 18 through 55 years (inclusive)	**Cohort 1 and Cohort 2:** 14 days of lumacaftor and ivacaftor combination therapy followed by 7 days of lumacaftor and ivacaftor combination therapy with ciprofloxacin or itraconazole **Cohort 3:** 14 days of lumacaftor and ivacaftor combination therapy followed by 10 days of lumacaftor and ivacaftor combination therapy with rifampin **Cohort 4:** Single dose on 3 dosing occasions	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
		• Assess safety and tolerability of lumacaftor and ivacaftor in absence and presence of short- and long-acting bronchodilators in healthy adult subjects • Assess PK of lumacaftor and ivacaftor in absence and presence of short- and long-acting bronchodilators in healthy adult subjects		bronchodilator, 200 mg lumacaftor in combination with 250 mg ivacaftor, and short-acting bronchodilator Oral administration			
Human Pharmacodynamic (PD) Studies							
Healthy Subject PD and PK/PD Studies							
Phase 1 PK (thorough QT), safety, and tolerability	VX12-809-008 (Study 008)	**Part A** Primary Objective Evaluate safety and tolerability of multiple ascending doses of lumacaftor administered for 7 days Secondary Objective Evaluate PK of lumacaftor and M28-lumacaftor, following multiple ascending doses of lumacaftor administered for 7 days **Part B** Primary Objective Evaluate effects of a therapeutic and a supratherapeutic dose of lumacaftor in combination with ivacaftor administered for 7 days on the QT/QTc interval Secondary Objectives • Evaluate assay sensitivity (i.e., to evaluate the effect of a positive control, a single, oral, 400-mg dose of moxifloxacin administered on Day 14, on the QT/QTc interval) • Assess effects of a therapeutic dose and a supratherapeutic dose of lumacaftor in combination with ivacaftor on non-QT interval ECG parameters (HR, RR, PR,	Randomized, pbo- and active-controlled, double-blind, parallel, thorough QT	**Lumacaftor** (b) (4) (b) (4) (b) (4) 200 mg tablet **Ivacaftor (film-coated):** 100-mg and 150-mg tablets **Pbo:** lumacaftor-matching tablet and ivacaftor-matching tablets **Part A:** **Cohort 1:** 600 mg lumacaftor qd or pbo **Cohort 2:** 1000 mg lumacaftor qd or pbo **Cohort 3:** 1200 mg lumacaftor qd or pbo **Cohort 4 (optional):** lumacaftor qd (dose TBD) or pbo **Part B:** **Cohort A:** 600 mg lumacaftor qd + 250 mg ivacaftor q12h for 7 days followed by 1000 mg qd lumacaftor qd + 450 mg	205 subject total (planned) **Part A:** 40 subjects (planned) **Part B:** 165 subjects (planned) **Part A:** Healthy male and female subjects aged 18 to 55 years, inclusive **Part B:** Healthy male and female subjects aged 18 to 45 years, inclusive	**Part A:** 7 days **Part B:** Cohorts A and B: 14 days Cohort C: single dose	Data analyses are ongoing; Full CSR will be submitted with NDA

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
		and QRS intervals) • Determine lumacaftor, M28-lumacaftor, ivacaftor, M1-ivacaftor, and M6-ivacaftor plasma concentration-effect relationship for the QT/QTc interval and the magnitude of the relationship, if any exist • Evaluate PK of lumacaftor, M28-lumacaftor, ivacaftor, M1-ivacaftor, and M6-ivacaftor at therapeutic and supratherapeutic doses of lumacaftor in combination with ivacaftor • Evaluate safety and tolerability of therapeutic and supratherapeutic systemic exposure to lumacaftor in combination with ivacaftor		ivacaftor q12h for 7 days **Cohort B:** pbo for 14 days **Cohort C:** 400 mg moxifloxacin (single dose on Day 14) Oral administration			

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Patient PD and PK/PD Studies							
Phase 2a PK, PD	VX08-809-101 (Study 101)	Primary Objective Evaluate safety and tolerability of lumacaftor in subjects with CF who are homozygous for the *F508del-CFTR* mutation Secondary Objectives • Evaluate effect of lumacaftor on biomarkers of CFTR activity, pulmonary function, and patient-reported outcomes in subjects with CF who are homozygous for the *F508del-CFTR* mutation • Evaluate PK of lumacaftor in subjects with CF who are homozygous for the *F508del-CFTR* mutation • Determine a dose of lumacaftor for further clinical study	Randomized, double-blind, pbo-controlled, parallel-group, multiple-dose, dose-finding, multicenter	**Lumacaftor**: 25-mg and 50-mg capsules **Pbo:** lumacaftor-matching capsules **Cohort 1 (Group A)** 25 mg or 50 mg qd of lumacaftor or pbo **Cohort 2 (Group B)** 100 mg or 200 mg qd of lumacaftor or pbo Oral administration	93 subjects Male and female subjects with CF who are homozygous for the *F508del-CFTR* mutation aged 18 years or older	Lumacaftor monotherapy for 28 days	Completed; Full

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase 2 PK, PD	VX09-809-102 (Study 102)	**Cohort 1 (Homozygous)** Primary Objectives • Evaluate safety and tolerability when lumacaftor is administered alone or in combination with ivacaftor • Evaluate effect of lumacaftor administered alone or in combination with ivacaftor on sweat chloride Secondary Objectives • Evaluate effect of lumacaftor administered alone or in combination with ivacaftor on pulmonary function • Evaluate effect of lumacaftor administered alone on sweat chloride • Assess PK of lumacaftor and M28-lumacaftor when lumacaftor is administered alone and in combination with ivacaftor (including M1-ivacaftor and M6-ivacaftor) **Cohort 2 (Homozygous or Heterozygous)** Primary Objectives • Evaluate safety and tolerability when lumacaftor is administered alone or in combination with ivacaftor • Evaluate effect of lumacaftor administered alone or in combination with ivacaftor on sweat chloride Secondary Objectives • Evaluate effect of lumacaftor administered alone or in combination with ivacaftor on pulmonary function • Evaluate effect of increasing doses of lumacaftor administered alone on sweat chloride • Evaluate effect of lumacaftor	Double-blind, pbo-controlled, multiple-dose, dose-finding	**Lumacaftor (Form 1):** 200-mg tablet **Ivacaftor (film-coated):** 100-mg and 150-mg tablets **Lumacaftor/ivacaftor (fixed-dose, film-coated):** 200-mg lumacaftor/125-mg ivacaftor tablet **Pbo:** lumacaftor-matching, ivacaftor-matching, fixed dose lumacaftor/ivacaftor-matching tablets **Cohort 1** Group 1 (Homozygous): 200 mg qd lumacaftor, followed by 200 mg qd of lumacaftor in combination with 150 mg q12h of ivacaftor Group 2 (Homozygous): 200 mg qd of lumacaftor followed 200 mg qd of lumacaftor in combination with 250 mg q12h of ivacaftor Group 3 (Homozygous): pbo **Cohort 2** Group 1(Homozygous): 200 mg qd of lumacaftor followed by 200 mg qd of lumacaftor in combination with 250 mg q12h of ivacaftor Group 2 (Homozygous):	190 subjects total (randomized) **Cohort 1:** 64 subjects **Cohort 2:** 111 subjects **Cohort 3:** 15 subjects **Cohort 4:** 120 subjects (planned) **Cohort 1 and Cohort 3** Subjects with CF who are homozygous for the *F508del-CFTR* mutation aged 18 years or older **Cohort 2** Subjects with CF who are homozygous or heterozygous for the *F508del-CFTR* mutation aged 18 years or older **Cohort 4** Subjects with CF who are heterozygous for the *F508del-CFTR* mutation aged 18 years or older	**Cohort 1** 14 days of lumacaftor monotherapy or pbo followed by 7 days of lumacaftor and ivacaftor combination therapy or pbo **Cohort 2 and Cohort 3** 28 days of lumacaftor monotherapy or pbo followed by 28 days of lumacaftor and ivacaftor combination therapy or pbo **Cohort 4** 56 days of lumacaftor and ivacaftor combination therapy or pbo	Data analyses are ongoing; Full CSR will be submitted with NDA

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
		administered alone or in combination with ivacaftor on CFQ-R score • Assess PK of lumacaftor and M28-lumacaftor when lumacaftor is administered alone and in combination with ivacaftor (including M1-ivacaftor and M6-ivacaftor) **Cohort 3 (Homozygous)** Primary Objectives • Evaluate safety and tolerability when lumacaftor is administered alone or in combination with ivacaftor • Evaluate efficacy of lumacaftor administered alone or in combination with ivacaftor on sweat chloride Secondary Objectives • Evaluate effect of lumacaftor administered alone or in combination with ivacaftor on pulmonary function • Evaluate effect of increasing doses of lumacaftor administered alone on sweat chloride • Evaluate effect of lumacaftor administered alone or in combination with ivacaftor on CFQ-R score • Assess PK of lumacaftor and M28-lumacaftor when lumacaftor is administered alone and in combination with ivacaftor (including M1-ivacaftor and M6-ivacaftor) **Cohort 4 (Heterozygous)** Primary Objectives • Evaluate safety and tolerability of lumacaftor in combination with ivacaftor		400 mg qd of lumacaftor followed by 400 mg qd of lumacaftor in combination with 250 mg q12h of ivacaftor Group 3 (Homozygous): 600 mg qd of lumacaftor followed by 600 mg qd of lumacaftor in combination with 250 mg q12h of ivacaftor Group 4 (Heterozygous): 600 mg qd of lumacaftor followed by 600 mg qd of lumacaftor in combination with 250 mg q12h of ivacaftor Group 5 (Homozygous or Heterozygous): pbo **Cohort 3** Group 1 (Homozygous): 400 mg q12h of lumacaftor followed by 400 mg q12h of lumacaftor in combination with 250 mg q12h of ivacaftor Group 2 (Homozygous): pbo **Cohort 4** Group 1 (Heterozygous): 400 mg q12h of lumacaftor in combination with 250 mg q12h of ivacaftor Group 2 (Heterozygous): pbo			

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
		• Evaluate efficacy of lumacaftor in combination with ivacaftor Secondary Objective • Assess PK of lumacaftor, M28-lumacaftor, ivacaftor, M1-ivacaftor, and M6-ivacaftor		Oral administration			

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Efficacy and Safety Studies							
Controlled Clinical Studies Pertinent to the Claimed Indication							
Phase 3 Efficacy and safety	VX12-809-103 (Study 103)	Primary Objective Evaluate efficacy of lumacaftor in combination with ivacaftor at Week 24 in subjects with CF who are homozygous for the *F508del-CFTR* mutation Secondary Objectives • Evaluate safety of lumacaftor in combination with ivacaftor through Week 24 • Investigate PK of lumacaftor, M28-lumacaftor, ivacaftor, M1-ivacaftor, and M6-ivacaftor	Randomized, double-blind, pbo-controlled, parallel-group, multicenter	**Lumacaftor/ivacaftor (fixed-dose, film-coated):** 200-mg lumacaftor/125-mg ivacaftor tablet and 200-mg lumacaftor/83-mg ivacaftor tablet **Ivacaftor (film-coated):** 125-mg tablet **Pbo (film-coated):** fixed-dose lumacaftor/ivacaftor-matching tablet or ivacaftor-matching tablet • 600 mg lumacaftor qd in combination with 250 mg ivacaftor q12h • 400 mg lumacaftor q12h in combination with 250 mg ivacaftor q12h • lumacaftor pbo q12h in combination with ivacaftor pbo q12h Oral administration	549 subjects Male and female subjects with CF who are homozygous for the *F508del-CFTR* mutation aged 12 years or older	Up to 24 weeks + 5 days	Data analyses are ongoing; Full CSR will be submitted with NDA

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase 3 Efficacy and safety	VX12-809-104 (Study 104)	Primary Objective Evaluate efficacy of lumacaftor in combination with ivacaftor at Week 24 in subjects with CF who are homozygous for the *F508del-CFTR* mutation Secondary Objectives • Evaluate safety of lumacaftor in combination with ivacaftor through Week 24 • Investigate PK of lumacaftor, M28-lumacaftor, ivacaftor, M1-ivacaftor, and M6-ivacaftor	Randomized, double-blind, pbo-controlled, parallel-group, multicenter	**Lumacaftor/ivacaftor (fixed-dose, film-coated):** 200-mg lumacaftor/125-mg ivacaftor tablet and 200-mg lumacaftor/83-mg ivacaftor tablet **Ivacaftor (film-coated):** 125-mg tablet **Pbo (film-coated):** fixed-dose lumacaftor/ivacaftor-matching tablet or ivacaftor-matching tablet • 600 mg lumacaftor qd in combination with 250 mg ivacaftor q12h • 400 mg lumacaftor q12h in combination with 250 mg ivacaftor q12h • lumacaftor pbo q12h in combination with ivacaftor pbo q12h Oral administration	559 subjects Male and female subjects with CF who are homozygous for the *F508del-CFTR* mutation aged 12 years or older	Up to 24 weeks + 5 days	Data analyses are ongoing; Full CSR will be submitted with NDA

Type of Study	Study Identifier (Abbreviation)	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) (Formulation); Dosage Regimen; Route of Administration	Number of Subjects/ Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Uncontrolled Clinical Studies							
Phase 3 Safety and efficacy	VX12-809-105 (Study 105)	Primary Objective **Part A and Part B** Evaluate long-term safety and tolerability of lumacaftor in combination with ivacaftor in subjects with CF, homozygous or heterozygous for the *F508del-CFTR* mutation, who are in the Part A and Part B Treatment Cohorts Secondary Objectives **Part A** • Evaluate long-term efficacy and durability of lumacaftor in combination with ivacaftor for subjects in the Part A Treatment Cohort • Evaluate post-treatment safety and tolerability of lumacaftor in combination with ivacaftor for subjects in the Part A Observational Cohort **Part B** Evaluate long-term efficacy and durability of lumacaftor in combination with ivacaftor for subjects in the Part B Treatment Cohort	Parallel-group, multicenter, rollover	**Lumacaftor/ivacaftor (fixed-dose, film-coated):** 200-mg lumacaftor/125-mg ivacaftor tablet and 200-mg lumacaftor/83-mg ivacaftor tablet **Ivacaftor (film-coated):** 125-mg tablet **Pbo (film-coated):** fixed-dose lumacaftor/ivacaftor-matching tablet or ivacaftor-matching tablet **Part A (Treatment Cohort only):** • 600 mg lumacaftor qd in combination with 250 mg ivacaftor q12h • 400 mg lumacaftor q12h in combination with 250 mg ivacaftor q12h **Part B:** 400 mg lumacaftor q12h in combination with 250 mg ivacaftor q12h Oral administration	1165 subjects (1050 subjects from Studies 103 and 104 and 115 subjects from Study 102 Cohort 4) **Part A:** Male and female subjects with CF who are homozygous for the *F508del-CFTR* mutation aged 12 years or older **Part B:** Male and female subjects with CF who are heterozygous for the *F508del-CFTR* mutation aged 12 years or older	Approximately 96 weeks	Ongoing Interim CSR will be submitted with NDA

2.8 Regulatory Background

VX-809 (lumacaftor) received Breakthrough Therapy designation on 12/7/2012 in combination with ivacaftor (Kalydeco) for the treatment of cystic fibrosis patients homozygous for the *F508del-CFTR* mutation.

Meeting minutes from the Type B meeting held on 2/12/2013 reflect the agreement that while a 6-month transgenic rat carcinogenicity study of VX-809 would be included in an NDA submission, the 2-year rat carcinogenicity study report could be submitted at a later date as a post-marketing requirement (PMR).

A pre-NDA meeting was held on 8/12/2014. The topic of the pharmaceutical classification of lumacaftor and the lumacaftor / ivacaftor combination was discussed. The sponsor indicated that they intend to submit the mouse carcinogenicity study final results to the IND in September 2014 and that the full NDA submission was expected to be completed in November 2014.

This memo provides detailed review of the developmental and reproductive toxicology studies evaluating lumacaftor (VX-809) and its disproportionate human metabolite (VRT-0995096, referred to as M28). These studies will be included in the anticipated NDA submission.

3 Studies Submitted

3.1 Studies Reviewed

- VX-809 and VRT-0995096 [M28] fertility and early embryonic development (FEED) study in rats (VX-809-TX-016)
- VX-809 embryo-fetal development (EFD) study in rats (VX-809-TX-005)
- VX-809 EFD study in rabbits (VX-809-TX-006)
- VRT-0995096 [M28] EFD study in rats (VRT-0995096-TX-008)
- VX-809 and VRT-0995096 [M28] pre- and post-natal development (PPND) study in rats (VX-809-TX-017)

3.3 Previous Reviews Referenced

Nonclinical reviews covering general toxicity studies of lumacaftor, lumacaftor / ivacaftor combination, and metabolite M28 filed 11/2/2012 and 12/10/2013 by Dr. Timothy Robison.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study 102 (see Section **2.7** above) was a phase 2 study which evaluated the ivacaftor / lumacaftor combination in the same dosing regimens as the phase 3 program. Pharmacokinetic (PK) data from that study after 28 days of combination therapy (compared to 6 months in the phase 3 studies 103 and 104) are summarized in the table below. These exposure ($AUC_{0\text{-}24h}$) estimates will be used for the purposes of safety margin calculations in this review. The suitability of these exposure estimates was confirmed with Clinical Pharmacology reviewer Dr. Jianmeng Chen via email on 8/26/2014.

Two different lumacaftor dosing regimens have been employed, 600 mg once daily and 400 mg twice daily (total daily dose 800 mg), each with 250 mg ivacaftor twice daily (500 mg total daily dose). Only a single regimen is intended for approval and marketing, with the sponsor proposing the fixed-dose combination option of 400 mg lumacaftor and 250 mg ivacaftor given twice daily. The potential approved dose regimen will be a review issue determined by the clinical team during the NDA review cycle. For the purposes of this review, the most conservative (i.e., highest) human exposure values will be used for safety margin determinations.

Table 2. Pharmacokinetics of Lumacaftor / Ivacaftor Combination in CF Patients

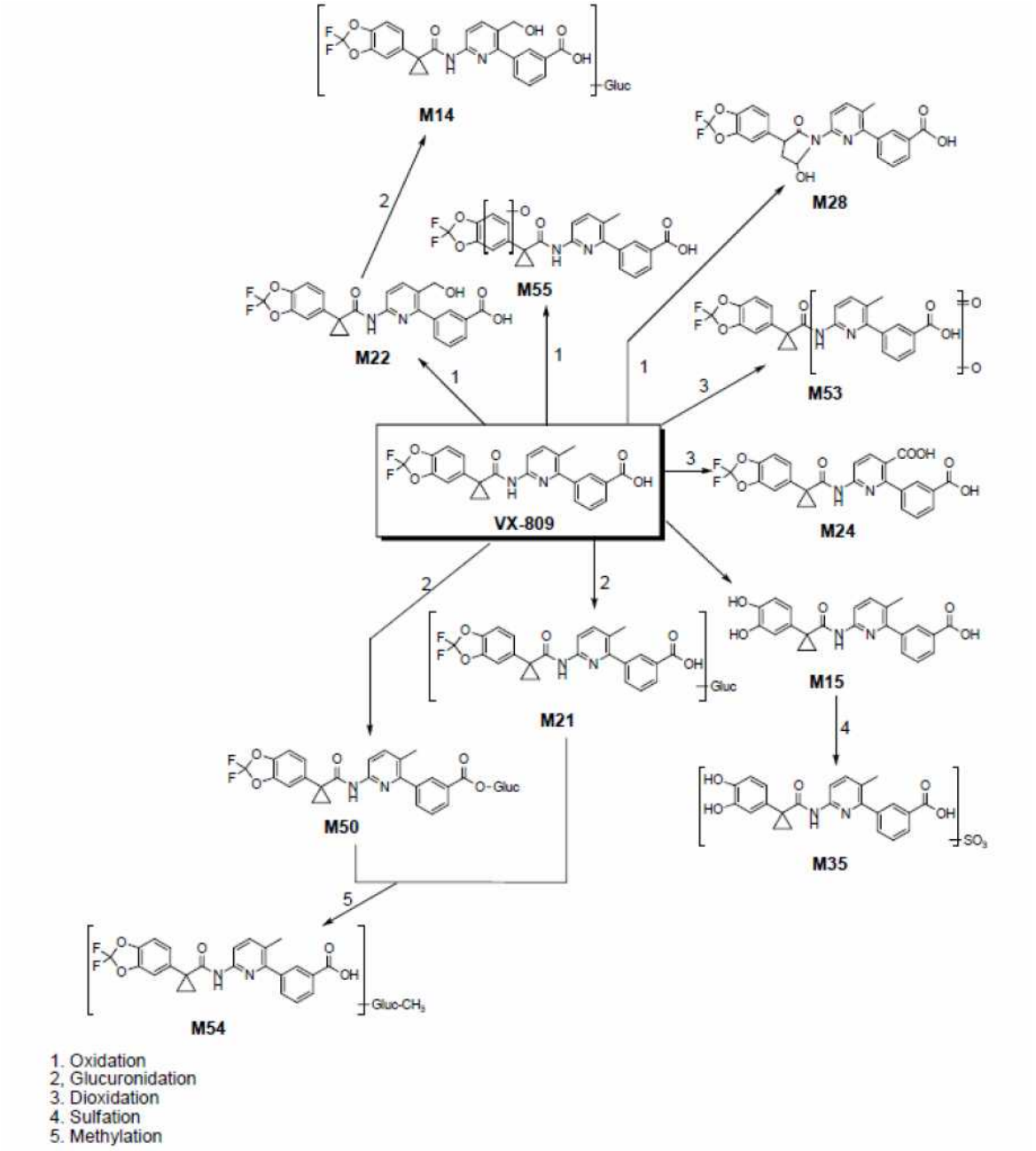
Compound	Exposure at Lumacaftor / Ivacaftor Dose Levels	
	600 mg QD / 250 mg BID	**400 mg BID / 250 mg BID**
Ivacaftor (VX-770)	**7.6** ug*h/mL	5.2 ug*h/mL
Lumacaftor (VX-809)	290 ug*h/mL	**371** ug*h/mL
VRT-0995096 (M28)	**32.1** ug*h/mL	28.2 ug*h/mL

Bold font denotes exposure value to be used for safety margin determinations

Finally, while the nominal daily dose of ivacaftor (500 mg) is greater than in the approved monoproduct label (300 mg), the exposure levels are lower with the combination product due to drug-drug interactions between ivacaftor and lumacaftor. The estimated daily exposure ($AUC_{0\text{-}24h}$) for ivacaftor monotherapy is 18.4 ug*h/mL, compared to 5.2-7.6 ug*h/mL for the combination product.

The absorption, metabolism, and excretion of a single 200 mg dose of [^{14}C] VX-809 was evaluated in Study 004. The major metabolite in plasma was found to be hydroxyl-pyrrolidone VX-809, designated as M28. M28 exposure was 38.5 ng*h/mL, representing 13% of total exposure and 25% of the parent exposure levels. In studies 101 and 102, it was determined that M28 C_{max} and AUC levels increased less than dose-proportionally, resulting in the metabolite representing only 8% of parent VX-809 exposure at the 400 mg BID dose level. A complete diagram of the proposed VX-809 metabolic pathways is shown in the figure below.

Figure 1. Proposed VX-809 Metabolic Pathways



M28 was judged to be a disproportionate human metabolite as it was not formed at relevant levels in the VX-809 rat and dog toxicology studies. Therefore, the sponsor proposed a series of nonclinical studies to evaluate the safety of M28 (also designated VRT-0995096). The proposed plan listed below was considered generally acceptable (see nonclinical review and comments to the sponsor filed by Dr. Timothy Robison 3/10/2010).

- In vitro genetic toxicity studies (Ames and Chromosomal Aberration) with M28 alone
- 28-day study in rats with M28 alone

- 13-week combination study with VX-770 and VX-809 in rats spiked with M28 (species selection was based upon 4-week combination studies with VX-770 and VX-809 in rats and dogs)
- 6-month rat toxicity studies with VX-809 spiked with M28
- Segment I and III developmental and reproductive toxicity studies in rats with VX-809 and spiked with M28
- Segment II toxicity study with M28
- 2-year carcinogenicity study in rats (b) (4)

5.2 Toxicokinetics

Toxicokinetic (TK) analysis was not included in all of the developmental and reproductive toxicology studies reviewed in this memo. TK data from select general toxicology studies of VX-809 and M28 in rats are included in this section and are referenced below in the **Integrated Summary and Safety Evaluation**.

Table 3. 28-day VX-809/VX-770 Rat Study: VX-809 Exposure Data

Day	Sex	VX-809 / VX-770 Dose Level (mg/kg/day)			
		100/25	300/50	1000/50	1000/100
Day 1	M	2130	3430	5960	5990
	F	1830	4520	6940	6300
Day 28	M	971	1950	2320	2830
	F	1180	2900	3650	3410

$AUC_{0\text{-}24h}$ values are shown in ug*h/mL.
NOAEL was considered as the high-dose (Tim Robison review 12/2013)

Table 4. 3-month VX-809/VX-770/M28 Rat Study: VX-809 and M28 Exposure Data

Compound	Day	Sex	VX-809 / VX-770 / M28 Dose Level (mg/kg/day)			
			500/10/10	500/25/10	1000/25/20	1000/100/20
VX-809	Day 1	M	2030	2990	3610	3570
		F	2870	2150	3390	5070
	Day 90	M	2300	2250	2150	2430
		F	3120	3040	3400	3010
M28	Day 1	M	340	344	685	535
		F	476	387	707	828
	Day 90	M	443	430	788	725
		F	507	473	875	938

$AUC_{0\text{-}24h}$ values are shown in ug*h/mL.
NOAEL was considered as the 1000/25/20 dose group (Tim Robison review 12/2013)

Table 5. 3-month VX-809 Rat Study: VX-809 Exposure

Day	Sex	VX-809 / VX-770 Dose Level (mg/kg/day)			
		250	500	1000	2000
Day 1	M	1950	2670	3250	4690
	F	1920	3530	3590	5180
Day 45	M	1140	1610	1760	2160
	F	2100	2690	3190	2680
Day 90	M	1630	3050	2340	3010
	F	2530	3860	4850	5880

$AUC_{0\text{-}24h}$ values are shown in ug*h/mL.
NOAEL was considered as the high-dose (Tim Robison review 11/2012)

Table 6. 6-month VX-809 / M28 Rat Study: VX-809 and M28 Exposure

Compound	Day	Sex	VX-809 / M28 Dose Level (mg/kg/day)		
			250/25	500/25	1000/25
VX-809	Day 1	M	1080	1000	1510
		F	1350	2010	2170
	Day 90	M	853	881	1140
		F	1520	1660	2010
	Day 180	M	943	973	1300
		F	2150	2500	3160
M28	Day 1	M	304	334	323
		F	586	504	546
	Day 90	M	727	733	712
		F	1220	1370	1100
	Day 180	M	765	621	751
		F	1110	1200	1180

$AUC_{0\text{-}24h}$ values are shown in ug*h/mL.
NOAEL was considered as the high-dose group (Tim Robison review 11/2012)

Table 7. 28-day M28 Rat Study: M28 Exposure

Day	Sex	M28 Dose Level (mg/kg/day)		
		25	50	100
Day 1	M	535	1030	2080
	F	571	1480	2830
Day 28	M	868	1200	1800
	F	738	1440	2910

$AUC_{0\text{-}24h}$ values are shown in ug*h/mL.
NOAEL was considered as the high-dose (Tim Robison review 11/2012)

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: VX-809 and VRT-0995096: An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation in Rats

Study no.:	(b) (4) 395097 VX-809-TX-016
Study report location:	EDR SD #100, 1/11/2013
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 5, 2012 (animal receipt) January 4, 2013 (final report signed)
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	VX-809 Lot #11-401 Purity 100% COA present VRT-0995096 ("M28") Lot #A4152-049 Purity 91.8% COA present

Key Study Findings

- In a fertility and early embryonic development (FEED) study, rats received vehicle or VX-809/M28 at 250/20, 500/20, or 1000/20 mg/kg/day. Males were dosed for 28 days before mating and through the mating period. Females were dosed for 14 days before mating, throughout the mating period, and from gestation day (GD) 0 through GD 7.
- There were no test article-related effects on male or female fertility.
- There were no test article-related effects on early embryonic viability or development.
- The high-dose level of 1000 mg/kg/day VX-809 and 20 mg/kg/day M28 was considered as the NOAEL for male and female reproductive toxicity as well as early embryonic toxicity. No toxicokinetic assessment was performed in this study.
- No maternal toxicity was achieved in the study, but the high-dose of 1000 mg/kg is considered an acceptable limit dose.

Methods

Doses:	Vehicle control 250 mg/kg VX-809 / 20 mg/kg M28 (LD) 500 mg/kg VX-809 / 20 mg/kg M28 (MD) 1000 mg/kg VX-809 / 20 mg/kg M28 (HD)
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (400 cps), 0.5% (w/v) Tween-80, 0.05% (w/v) simethicone in deionized water
Species/Strain:	Sexually mature male and virgin female Sprague-Dawley rats [Crl:CD(SD)] Males 9 weeks (278-338 g) at start of dosing Females 10 weeks (198-314 g) at start of dosing
Number/Sex/Group:	25
Satellite groups:	None – no TK analysis in this study
Study design:	Animals housed individually for minimum 10 day acclimation period. Paired in males' home cages until evidence of mating (copulatory plug and/or sperm in vaginal lavage, designated GD 0), after which the animals were returned to individual cages. Estrous cycle determinations began on study Day 4, dosing of females began on Day 14. Males dosed Days 0-63/64 (minimum 28 days before cohabitation); females dosed ≥14 days before cohabitation through GD 7 = 22-32 doses total).
Deviation from study protocol:	Deviations were reviewed and were not judged to affect the interpretation or integrity of the study.

Observations and Results

Mortality

All animals were observed morning and afternoon for mortality and moribundity.

None of the following unscheduled deaths in the study were considered to be test article-related.

- LD male 54945 was found dead prior to the breeding period without any notable macroscopic findings.
- LD male 54987 was found dead after the breeding period with macroscopic evidence of mechanical injury (fractured frontal, parietal and palate bones, dark red contents in trachea, lungs partially collapsed).
- HD female 55755 had no evidence of mating but was euthanized early due to a presumed pregnancy. The animal was determined to be gravid but had no other notable macroscopic findings

- HD female 55674 with no evidence of mating delivered 2 pups (9 additional live fetuses retained in utero) on study day 50 due to mistimed pregnancy.

Clinical Signs

Individual clinical observations were recorded daily in the morning (prior to dosing) and approximately 1 hour post-dose.

Clinical observations that were test article-related included wet or dried, clear or red material around the mouth and eyes. These findings were more frequent in males compared to females.

Table 8. FEED Study: Male Clinical Findings

Finding	Control	250/20	500/20	1000/20
Dried red material around right eye	0	0	0	56/3
Dried red material around left eye	2/1	0	0	61/2
Wet red material around right eye	0	0	0	8/2
Wet red material around left eye	0	0	0	9/2

Total number of occurrences / Number of affected animals

Table 9. FEED Study: Post-dose Observations

Finding	Control	250/20	500/20	1000/20
Males				
Wet red material around mouth	0	6/2	35/13	83/20
Wet clear material around mouth	1/1	9/2	61/13	131/21
Dried red material around mouth	0	3/3	21/10	63/17
Females				
Wet red material around mouth	0	1/1	4/4	4/4
Wet clear material around mouth	0	1/1	4/4	7/5
Dried red material around mouth	0	1/1	0	11/8

Total number of occurrences / Number of affected animals

Body Weight

Male body weights were recorded twice weekly throughout the study. Female body weights were recorded twice weekly until copulation was detected and then on GD 0, 3, 7, 10, 13 and 15.

There were no test article-related effects on body weights in the study. Body weight curves for males and females are shown in the following figures.

Figure 2. FEED Study: Male Body Weights

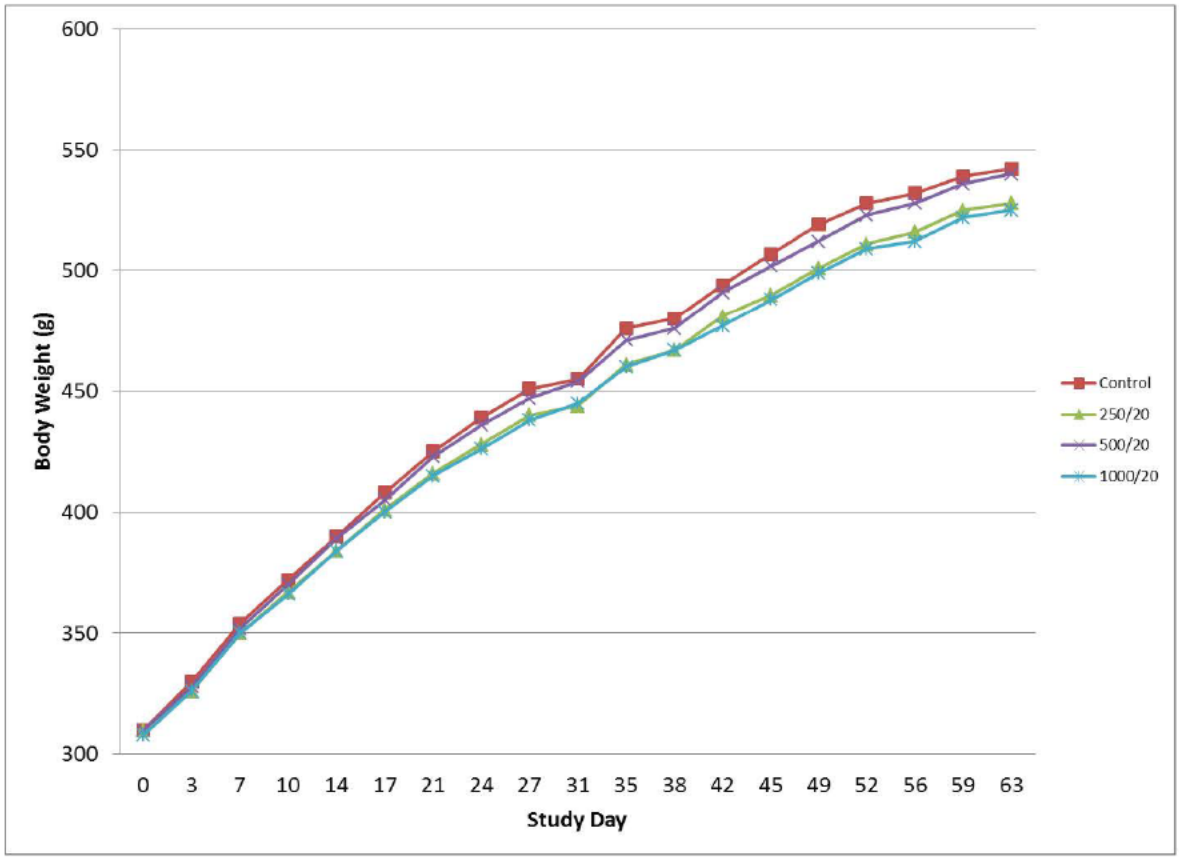
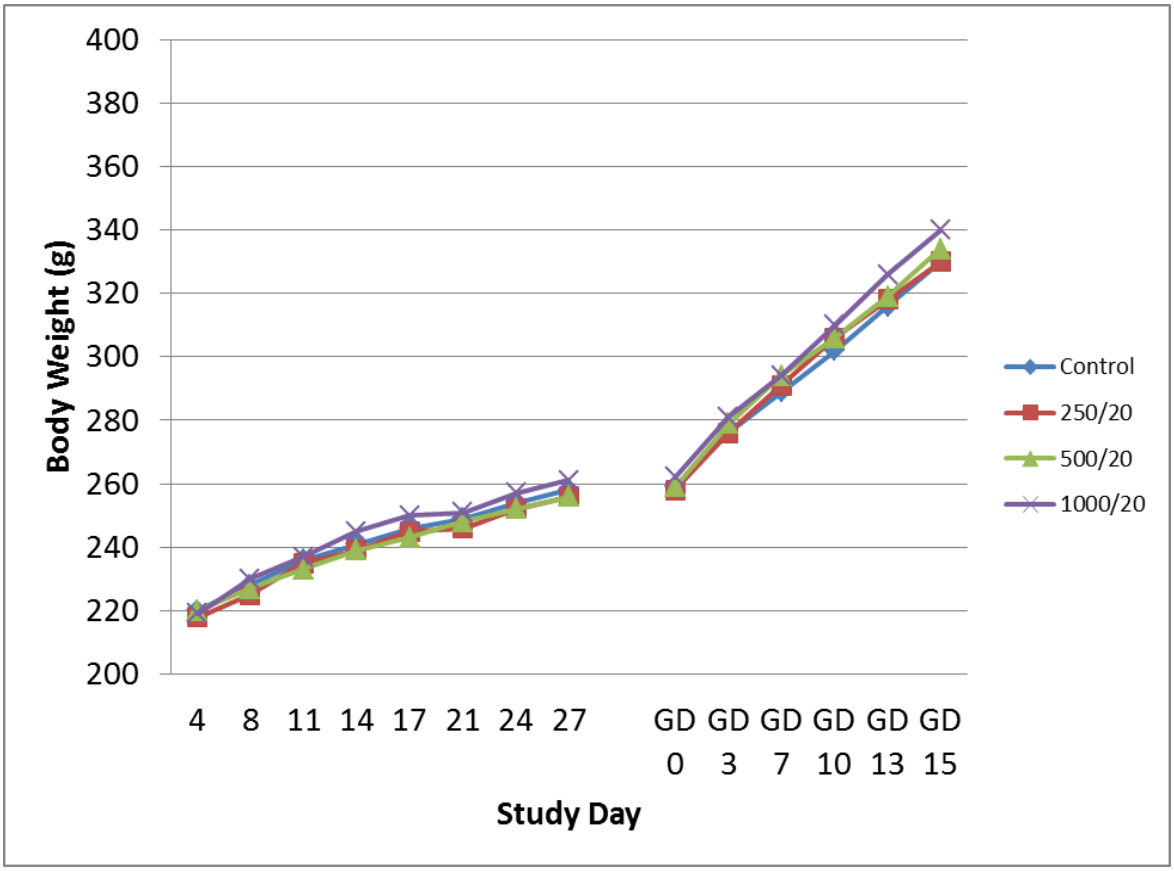


Figure 3. FEED Study: Female Body Weights



Feed Consumption

Individual male and female food consumption was recorded twice weekly until cohabitation but was not collected during the mating period. Female food consumption was also recorded on GD 0, 3, 7, 10, 13 and 15.

There were no test article-related effects on food consumption in the study.

Estrous Cycles

Vaginal lavages were performed daily and the slides were evaluated microscopically to determine the stage of the estrous cycle of each female for 10 days prior to test article administration and continuing until evidence of copulation was observed or until termination of the mating period for females with no evidence of mating.

There were no test article-related effects on estrous cycle length or time to mating in the study. Cycle length was statistically significantly increased at the HD compared to controls, but remained well within the historical control range.

Table 10. FEED Study: Estrous Cycle and Pre-coital Internal Analysis

Parameter	Control	250/20	500/20	1000/20	Historical control data
Estrous cycle length (days)	4.1	4.2	4.2	4.5*	4.2 (3.7-5.2)
Pre-coital interval (days)	3.0	2.3	2.6	2.2	2.9 (2.0-3.9)

Sperm Analysis

Upon euthanasia, the right epididymis was excised for analysis. Sperm motility was assessed in at least 200 (each) motile and non-motile spermatozoa. Sperm morphology (including double heads, double tails, microcephalic or megacephalic) was assessed for 200 spermatozoa per animal from the right epididymis, which was also prepared for possible microscopic histopathological assessment. The left testis and epididymis from each male were weighed, stored frozen, homogenized, and analyzed for determination of homogenization-resistant spermatid count and calculation of sperm production rate. 200 cells per animal were analyzed and sperm production rate was calculated as number of sperm per gram of tissue / 6.1 days (rate of turnover of germinal epithelium).

There were no test article-related effects on sperm morphology, sperm motility, or sperm content in the study (see table below).

Table 11. FEED Study: Sperm Analysis

Parameter	Control	250/20	500/20	1000/20
Sperm motility (%)	86	88	86	87
Epididymis weight (g)	0.68	0.69	0.67	0.67
Epididymis concentration (10^6/g)	546	546	558	553
Testis weight (g)	1.76	1.79	1.78	1.80
Testis concentration (10^6/g)	134	126	123	132
Sperm production rate (10^6/g/day)	22.0	20.7	20.2	21.7
Morphology – normal (%)	99.3	99.6	99.6	99.8

Fertility Parameters

For females that delivered or were euthanized on GD 15 because they were presumed pregnant, laparohysterectomies were performed and the number and location of implantation sites, corpora lutea, early resorptions and viable embryos/fetuses/pups were recorded. Intact fetuses/pups were examined externally, euthanized by a subcutaneous (in the scapular region) or intraperitoneal injection of sodium pentobarbital (as appropriate), and discarded. Uteri of females without macroscopic evidence of implantation were opened and placed in 10% ammonium sulfide solution for detection of early implantation loss. Fertility metrics were calculated as indicated in the footnotes to the tables below.

There were no test article-related effects on male fertility parameters.

Table 12. FEED Study: Male Reproductive Performance

Parameter	Control	250/20	500/20	1000/20
Males paired for mating (#)	25	24	25	25
Male mating index (%)[a]	100	96	100	100
Male fertility index (%)[b]	100	96	96	92
Male copulation index (%)[c]	100	100	96	92

[a] (Evidence of mating or confirmed pregnancy / males used for mating)
[b] (Males siring a litter / males used for mating)
[c] (Males siring a litter / males with evidence of mating)

There were no statistically significant test article-related effects on any female fertility parameter. One MD and two HD females had evidence of mating but were determined to be nongravid, but this slight trend was not considered to represent an adverse effect attributable to VX-809/M28.

Table 13. FEED Study: Female Reproductive Performance

Parameter	Control	250/20	500/20	1000/20
Females paired for mating (#)	25	25	25	25
Female mating index (%)[a]	100	100	100	100
Female fertility index (%)[b]	100	100	96	92
Female conception index (%)[c]	100	100	96	92

[a] (Evidence of mating or confirmed pregnancy / females used for mating)
[b] (Females with confirmed pregnancy / females used for mating)
[c] (Females with confirmed pregnancy / females with evidence of mating or confirmed pregnancy)

There were no test article-related effects on embryofetal parameters quantified upon laparohysterectomies performed on GD 15 (see table below).

Table 14. FEED Study: Summary of Embryonic Data

Parameter	Control	250/20	500/20	1000/20
Number of gravid females	25	24	24	21
Corpora Lutea (litter mean)	15.3	14.7	15.9	16.3
Implantation sites (litter mean)	14.2	13.3	14.3	14.1
Viable embryos (% per litter)[a]	94.7	94.5	94.6	95.8
Early resorptions (% per litter)[b]	5.3	5.7	5.4	4.2
Pre-implantation loss (% per litter)	7.0	9.8	9.6	11.9
Post-implantation loss (% per litter)	5.3	5.7	5.4	4.2

[a] No dead embryos were observed.
[b] No late resorptions were observed, therefore this also represents total resorptions

Necropsy

Animals were euthanized by carbon dioxide asphyxiation, on GD 15 for females with evidence of mating. A gross necropsy was performed on all animals found dead, that delivered, or that survived to the scheduled necropsy and included examination of the external surfaces, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord and the thoracic, abdominal, and pelvic cavities including viscera.

The following organ weights were collected: brain, epididymides, ovaries, pituitary gland, and testes. The following tissues and organs were prepared for microscopic evaluation: coagulating glands, ovaries and oviducts, pituitary gland, prostate gland, seminal vesicles, testes with epididymides and vas deferens, uterus with cervix and vagina, and all gross lesions.

There were no test article-related macroscopic findings or organ weight effects in the study. Given the lack of adverse reproductive effects in the study, microscopic examination was not performed.

Toxicokinetics

No TK analysis was performed in this study.

Dosing Solution Analysis

Vehicle and test article formulations were prepared daily for the first 10 days. Following completion of a concurrent stability study, formulations were prepared weekly and stored refrigerated and protected from light. Samples for homogeneity and concentration analysis were collected from the top, middle, and bottom strata from the first dosing preparations. Additional samples were collected for concentration analysis during Week 5 and at the end of the study.

Analysis of dosing solutions indicated test article concentrations in a range of 97-112% of target for VX-809 and 85-97% of target for M28, which met the threshold for acceptability. Homogeneity results also met the protocol-defined specifications (RSD ≤10%). The test articles were not detected in control dosing solutions.

9.2 Embryonic Fetal Development

Study title: Oral (Gavage) Developmental Toxicity Study of VX-809 in Rats

Study no.:	Test facility # (b) (4) 00446 Sponsor #VX-809-TX-005
Study report location:	EDR SD #23, 5/20/2009
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Protocol signed 5/13/2008 Experiment start date 5/18/2008 Completion of main study 6/13/2008 Final report signed 5/4/2009
GLP compliance:	Yes
QA statement:	Yes, signed 5/4/2009
Drug, lot #, and % purity:	VX-809 Lot P-1625 99.8% purity COA provided

Key Study Findings

- In an oral EFD study, rats received 0 (vehicle control), 500, 1000, or 2000 mg/kg/day VX-809 from GD 7 to GD 17.
- There were no test article-related effects on any parameters evaluated in this study in terms of either maternal or embryofetal toxicity.
- The NOAEL for maternal and fetal toxicity was considered as the high-dose of 2000 mg/kg/day by oral gavage.
- No maternal toxicity was achieved, but the high-dose of 2000 mg/kg is above the limit dose (1000 mg/kg) required in reproductive toxicity studies.
- Exposure to VX-809 (C_{max} and AUC) increased less than dose-proportionally. T_{max} was generally 10 hours and there was no accumulation between GD 7 and 17. $AUC_{0\text{-}24h}$ at the NOAEL was 3,320 ug*h/mL.

Methods

Doses:	0 (vehicle control), 500, 1000, 2000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose 400 cps, 0.5% (w/v) Tween 80, 0.05% simethicone in deionized water
Species/Strain:	Crl:CD (SD) rats (b) (4)
Number/Sex/Group:	25 presumed pregnant females per group ~65 days old at arrival 212-244 g at GD 0
Satellite groups:	3 (control) or 6 (VX-809) per group for TK
Study design:	Dosing on GD 7-17. TK rats sacrificed on GD 18. Main study rat sacrificed and Caesarean-sectioned on GD 21.
Deviation from study protocol:	A number of minor deviations were listed but they were not judged to affect study interpretation.

Observations and Results

Mortality

Animals were observed for viability twice daily.

There were no unscheduled deaths in the study.

Clinical Signs

Animals were observed for clinical signs and general appearance once weekly starting during acclimation. Mating was evaluated daily and confirmed by the presence of spermatozoa in a smear of the vaginal contents and/or a copulatory plug. Additional observations for clinical signs, abortions, premature deliveries and deaths were conducted during the dosing period as follows:

- Before, hourly for the first four hours after dosing, and at the end of the workday for the first three days of dosing
- Pre-dose, approximately 1-2 hours post-dose, and at the end of the workday for the remainder of the dosing period.
- Once daily during the post-dosage period

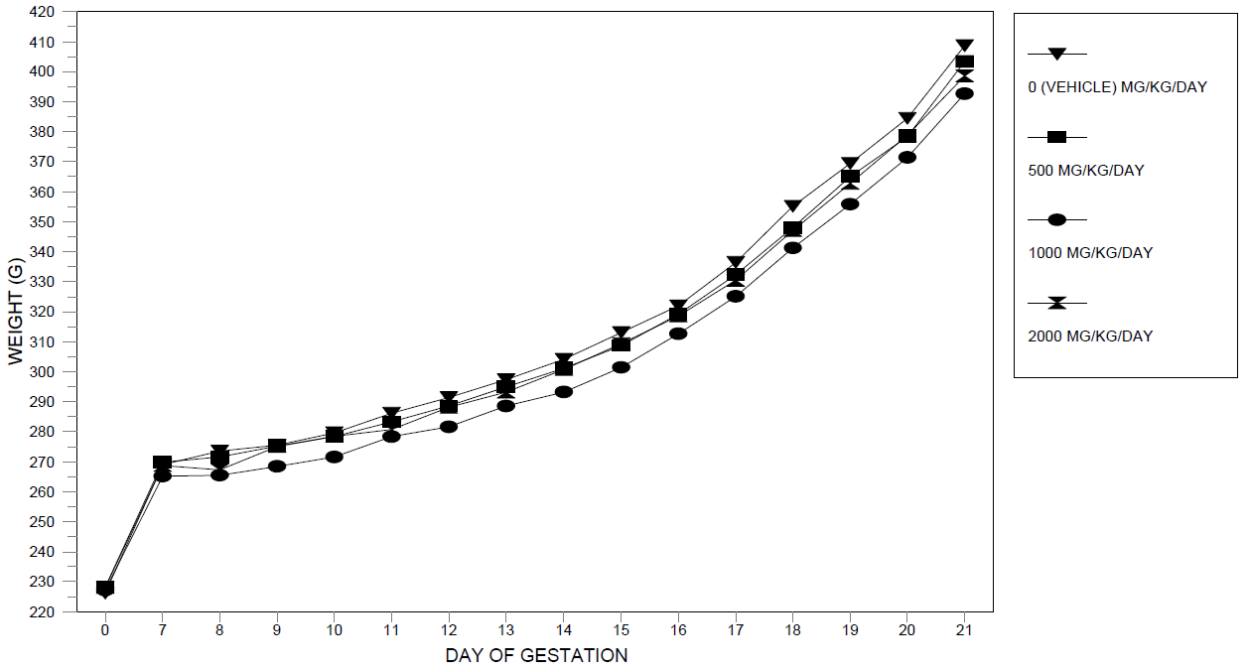
Isolated clinical observations at the high-dose included red perioral substance (3 instances in 3 animals), excess salivation (3/2), and gasping (1/1). These are not considered adverse or conclusively test article-related.

Body Weight

Body weights were recorded at least weekly during the acclimation period, on GD 0 and daily during the dosage and post-dosing periods.

There were no test article related effects on body weights in the study.

Figure 4. VX-809 Rat EFD Study: Body Weights



Feed Consumption

Feed consumption values were recorded on GDs 0, 7, 10, 12, 15, 18 and 21.

There were no test article-related effects on food consumption levels in the study.

Toxicokinetics

Blood samples (0.5 mL) for TK analysis were collected from the jugular vein on GD 7 and 17. Samples were collected from control rats 4 hours after dosing. Samples from VX-809 groups were collected according to the schedule shown in the table below.

Rats Assigned to Satellite Groups II through IV	Day 7 of Presumed Gestation							Day 17 of Presumed Gestation						
		Postdosage Timepoints							Postdosage Timepoints					
	Prior to dosage	30 min.	1 hr.	2 hrs.	4 hrs.	10 hrs.	24 hrs.	Prior to dosage	30 min.	1 hr.	2 hrs.	4 hrs.	10 hrs.	24 hrs.
Three rats	X		X		X		X		X		X		X	
Three rats		X		X		X		X		X		X		X

Samples were placed in K_3EDTA tubes on ice and centrifuged to isolate plasma. Samples were frozen on dry ice, stored at -70°C, and shipped on dry ice to the Sponsor for analysis.

Exposure to VX-809 increased less than dose-proportionally between 500-2000 mg/kg/day in the study. Tmax was generally observed to be 10 hours with the exception of the first day of dosing at the LD (4 hours). No evidence of accumulation over the GD 7-17 dosing period was observed. TK parameters are summarized in the table below.

Table 15. VX-809 Rat EFD Study: Toxicokinetics Parameters

Parameter	Day	VX-809 dose (mg/kg/day)		
		500	1000	2000
C_{max} (ug/mL)	GD 7	114	135	175
	GD 17	120	174	190
T_{max} (h)	GD 7	4.0	10.0	10.0
	GD 17	10.0	10.0	10.0
AUC_{0-24h} (ug*h/mL)	GD 7	1790	2150	3200
	GD 17	1860	2570	3320
AUC/dose	GD 7	3.58	2.15	1.60
	GD 17	3.73	2.57	1.66

Dosing Solution Analysis

Test article suspensions were prepared at least daily and used within 24 hours (room temperature storage). Vehicle solutions were prepared once weekly and stored refrigerated for up to 7 days. While stability for these conditions has previously been established by the Sponsor, it was verified at the low and high-dose concentrations over

3 and 7 days at room temperature, 1-8°C, and -82-70°C. To verify concentration and homogeneity, quadruplicate 1 mL samples were taken from the top, middle, and bottom of the first and last formulations prepared. Samples were analyzed by HPLC/UV (LOQ 40 ug/mL) in duplicate with the remaining samples stored as backup.

Results indicated that concentration, homogeneity, and stability samples fell within 101-113% of the intended levels and met acceptance criteria.

Necropsy

Rats were sacrificed by CO_2 asphyxiation. TK animals were sacrificed at GD 18, examined for pregnancy status, and discarded without further evaluation. Main study animals were sacrificed on GD 21 followed by Caesarean sectioning and gross necropsy of the thoracic, abdominal, and pelvic viscera. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites.

3, 4, 5, and 6 animals were found to be nongravid in the control, LD, MD, and HD, respectively (see table in next section below). There were no test article-related gross observations.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites, early and late resorptions and live and dead fetuses. Placentae were examined for size, color and shape.

All placentae in all groups appeared normal. Cesarean section data is summarized in the table below. There was a slight trend towards fewer pregnant females with increasing VX-809 doses but this was considered incidental (i.e., treatment was started on GD7). VX-809 at doses up to 2000 mg/kg/day had no impact on pre- or post-implantation loss, litter size, or fetal body weight.

Table 16. VX-809 Rat EFD Study: Cesarean Section Data

	VX-809 Dose (mg/kg/day)			
Parameter	**0**	**500**	**1000**	**2000**
Pregnant Animals[a]	22	21	20	19
Corpora Lutea (litter mean)	15.9	16.0	15.5	15.0
# of Implantations (litter mean)	14.8	15.6	14.8	14.6
Early resorptions (litter mean)	0.5	1.0	0.6	0.7
Late resorptions (litter mean)	0.0	0.0	0.0	0.1
Resorbed conceptuses per litter (%)	3.9	6.2	4.5	5.6
Dams with any resorptions (%)	45.4	66.7	40.0	63.2
# of live fetuses (litter mean)[b]	14.2	14.6	14.1	13.7
Sex Ratio (litter mean % male)	52.1	50.3	49.2	51.2
Fetal body weight (litter mean, g)	5.44	5.27	5.34	5.28

There were no statistically significant differences vs. the control group
[a] Number (out of 25) that were found to be pregnant and survived to schedule cesarean section
[b] No dead fetuses so number of live fetuses = litter size

Offspring (Malformations, Variations, etc.)

Each fetus was weighed, examined for sex and gross lesions, and sacrificed by sodium pentobarbital if live. Fetuses were divided approximately in half for either soft tissue (variation of Staples microdissection technique, fixation in Bouin's solution, and heads examined by free-hand sectioning) or skeletal (eviscerated, cleared, and stained with alizarin red S) examinations (see table).

Table 17. VX-809 Rat EFD Study: Fetal Examinations

	VX-809 Dose (mg/kg/day)			
Parameter	**0**	**500**	**1000**	**2000**
Litters evaluated	22	21	20	19
Fetuses evaluated - external	313	307	282	261
Fetuses evaluated – soft tissue	153	149	135	125
Fetuses evaluated – skeletal	160	158	147	136

There were no test article-related external, visceral, or skeletal malformations or variations in pups born to female rats administered up to 2000 mg/kg/day VX-809. Further, no effects on the number of ossification sites were observed.

Study title: Oral (Stomach Tube) Developmental Toxicity Study of VX-809 in Rabbits

Study no.:	Test facility # (b) (4) 00447 Sponsor #VX-809-TX-006
Study report location:	EDR SD #23
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Protocol signed 5/21/2008 Experimental start 5/23/2008 Final sacrifice 6/20/2008 Report signed 5/5/2009
GLP compliance:	Yes
QA statement:	Yes, signed 5/5/2009
Drug, lot #, and % purity:	VX-809 Lot P-1625 99.8% purity COA provided

Key Study Findings

- In an oral EFD study, rabbits received VX-809 at 0 (vehicle control), 50, 100, or 200 mg/kg/day via a stomach tube from GD 7-19.

- Extreme maternal toxicity was observed at the HD of 200 mg/kg/day. There were two premature deaths and four additional does aborted and were sacrificed. Associated clinical signs included scant feces, poor grooming, thin body condition, dehydration, and decreased activity. Significantly reduced food consumption and body weight gains vs. controls were observed in this group. Evidence of toxicity in the MD group included scant feces and a significant reduction in body weight gain over the dosing period vs. controls. The NOAEL for maternal toxicity was considered as 50 mg/kg/day.
- Test article-related gross observations in multiple animals at the HD were noted in the heart (white band surrounding ventricles, pale), lungs (red/mottled lobes), and kidneys (pale appearance).
- There were no test article related changes on pre- or post-implantation loss, litter size, or fetal weight. There were no test article-related external, visceral, or skeletal malformations or variations. The NOAEL for embryofetal toxicity was considered as 200 mg/kg/day.
- Exposure to VX-809 increased with increasing doses from 50-200 mg/kg/day but in a less than dose-proportional manner. $AUC_{0\text{-}24h}$ values were 995, 1200, and 1950 ug*h/mL at the LD, MD, and HD, respectively. T_{max} was in the range of 1-10 hours and no notable accumulation was observed over the GD 7-19 dosing period.

Methods

Doses:	0 (vehicle control), 50, 100, or 200 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	3 mL/kg
Route of administration:	Oral (stomach tube)
Formulation/Vehicle:	0.5% (w/v) methylcellulose 400 cps, 0.5% (w/v) Tween 80, 0.05% simethicone in deionized water
Species/Strain:	Hra:(NZW) SPF rabbits (b) (4) ~6 months old at arrival 2.7-4.0 kg at GD 0.
Number/Sex/Group:	20 timed-mated females per group
Satellite groups:	3/group for TK
Study design:	Dosing from GD 7 to GD 19. TK group sacrifice on GD 20. Main study sacrifice on GD 29.
Deviation from study protocol:	A number of deviations were noted but were not judged by the Reviewer to affect interpretation of study data.

Observations and Results

Mortality

Rabbits were examined for viability twice daily.

Test article-related mortality was observed at the HD. One animal was found dead on GD 24 and another was euthanized for humane reasons on GD 23. These animals had clinical signs beginning on GD 14-15 including scant feces, dehydration, lack of grooming, decreased movement, bradypnea, thin body, and cold to touch body. Both animals lost weight and consumed little feed during and after the dosing period. Gross lesions were noted on the heart (white band area on heart surrounding all ventricles, pale color) of each animal and also the lungs (mottled red appearance) of one rabbit. The animals were each found to be gravid, though one litter consisted entirely of resorptions.

Four additional does aborted on days GD 25-27 and were euthanized. Clinical signs, body weight loss, and low food consumption were similar to the premature deaths described above. Gross findings on the heart (white band on heart surrounding all ventricles, pale color) and lungs (dark red lobes) were each observed in two of the rabbits at necropsy.

The deaths and abortions were attributed to maternal toxicity resulting from administration of VX-809 at the 200 mg/kg/day dose level.

Clinical Signs

Rabbits were observed once during the predose period. During the dosing period rabbits were observed daily before dosing, 1-2 hours post-dose, and at the end of the normal working day. Rabbits were observed once daily during the post-dosing period until sacrifice.

Increased incidences of scant feces were observed in drug-treated groups. Other clinical signs were primarily confined to the high dose group.

Table 18. VX-809 Rabbit EFD Study: Clinical Signs

Dose (mg/kg/day)	0[a]	50	100	200
Clinical Signs[b]				
Scant Feces	3/1	16/2	**25/10****	**74/13****
Ungroomed Coat	35/5	41/6	29/5	**58/11**
Small Feces	0/0	1/1	4/1	**10/7****
Dehydration (Total)	0/0	0/0	4/1	**39/8****
Mild	0/0	0/0	4/1	**33/7****
Moderate	0/0	0/0	0/0	**7/4****
Thin Body Condition	0/0	0/0	0/0	**13/7****
No Feces in Cage Pan	0/0	0/0	**5/3**	**6/6****
Decreased Motor Activity	0/0	0/0	0/0	**11/4****
Mucoid Feces	0/0	0/0	2/1	**5/3**

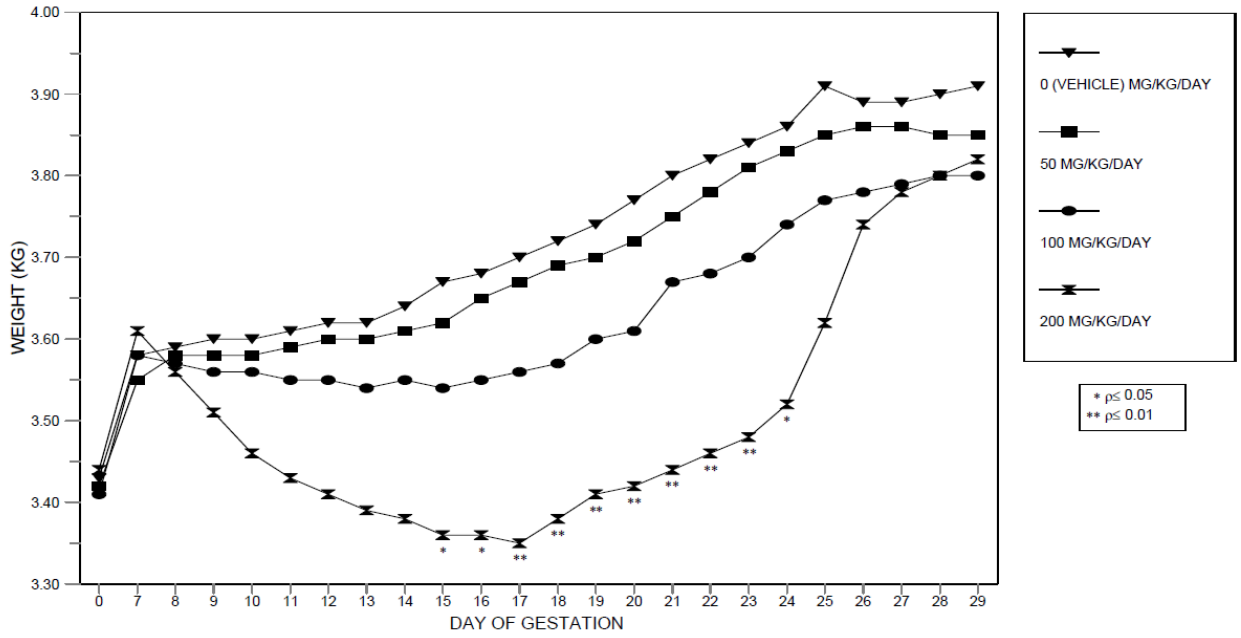
a. Vehicle Control Article
b. Incidence/No. of animals
** Significantly different from the vehicle control group value ($p \leq 0.01$).
Bold/Underlined = Test article-related

Body Weight

Body weights were recorded on GD 0, on arrival at the test facility (GD 1-4) and daily during the dosing and post-dosing period.

Body weight changes are summarized in the figure below. VX-809 was associated with a dose-dependent decrease in body weight gain, including absolute body weight losses at the high dose. Control does gained an average of 5% of their starting body weight over the GD 7-20 period, but body weight gains were statistically significantly lower in the MD and HD groups. MD animals gained just 1% body weight compared to the GD 7 values and the HD group exhibited body weight decreases of 5%. These changes are clearly indicative of maternal toxicity induced by VX-809 at doses ≥100 mg/kg/day.

Figure 5. VX-809 Rabbit EFD Study: Body Weights



Feed Consumption

Individual food consumption was recorded daily beginning upon arrival at the test facility.

Dose-dependent decreases in food consumption were also noted in the study and reached statistical significance for the high-dose. Body weight-normalized food consumption during the dosing period was 40% lower at the HD compared to controls.

Toxicokinetics

0.8-1.0 mL samples were collected on GD 7 and GD 19 via the medial auricular artery and/or marginal ear vein. Time points were pre-dose and 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours post-dose. Samples were placed in K_3EDTA tubes on ice and centrifuged to isolate plasma. Samples were frozen on dry ice, stored at -70˚C, and shipped on dry ice to the Sponsor for analysis.

Exposure to VX-809 increased with increasing doses from 50-200 mg/kg/day but in a less than dose-proportional manner. $AUC_{0\text{-}24h}$ values were 995, 1200, and 1950 ug*h/mL at the LD, MD, and HD, respectively. T_{max} was in the range of 1-10 hours and no notable accumulation was observed over the GD 7-19 dosing period. TK results are summarized in the table below.

Table 19. VX-809 Rabbit EFD Study: Toxicokinetic Parameters

Parameter	Day	VX-809 dose (mg/kg/day)		
		50	100	200
C_{max} (ug/mL)	GD 7	54.5	89.4	113
	GD 19	71.9	86.2	110
T_{max} (h)	GD 7	1.33	2.33	2.00
	GD 19	9.67	2.00	2.33
AUC_{0-24h} (ug*h/mL)	GD 7	619	1270	2040
	GD 19	995	1200	1950
AUC/dose	GD 7	12.39	12.73	10.23
	GD 19	19.88	11.97	9.73

Dosing Solution Analysis

Dosing suspensions were prepared daily and used within 24 hours. Quadruplicate 1 mL samples were collected from the top, middle, and bottom of each dosing formulation from the first and last preparations. Two samples were sent to (b) (4) for analysis by HPLC/UV (LOQ 40 ug/mL) and two were retained as backup. Stability data bracketing the range of concentrations have previously been collected by the Sponsor.

Dosing solution analysis revealed mean test article levels of 87-93% percent of target from the first preparation and 102-104% from the last preparation. These results met the acceptance criteria of <15% difference from the intended concentration. Relative standard deviations from all of the top/middle/bottom samples at each concentration met the acceptance criteria of <5%.

Necropsy

Rabbits were sacrificed via IV administration of 390 mg pentobarbital sodium and 50 mg phenytoin sodium. TK animals were examined for pregnancy status only. Main study animals underwent gross necropsy of the thoracic, abdominal, and pelvic viscera.

Table 20. VX-809 Rabbit EFD Study: Gross Necropsy Findings

Finding	Control	LD	MD	HD
Number examined	*20*	*20*	*20*	*20*
Lung				
All lobes, dark red or mottled	0	0	0	3
Spongy	0	0	0	1
Heart				
White band surrounding all ventricles	0	0	0	4
Fissure, medially down apex	0	0	0	1
Pale	0	0	0	3
Stomach				
Pyloric region, dark brown area	0	0	0	1
Liver				
All lobes, numerous tan areas	0	0	0	1
Kidneys				

Bilateral, pale	1	0	0	2

Includes unscheduled deaths (found dead, human sacrifice, and sacrifice due to abortion)

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The number and distribution of corpora lutea were recorded. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses and early and late resorptions. Placentae were examined for size, shape, and color.

Cesarean section data are summarized in the table below. The 20% rate of abortion at the HD due to maternal toxicity was statistically significantly higher than the control group. While not statistically significant, there was a trend towards higher numbers of late resorptions in the treated group. At the MD and HD, these values exceeded the test facility historical control data (mean 0.1, range 0.0-0.4 per litter). These effects are also likely attributable to the maternal toxicity described above.

Table 21. VX-809 Rabbit EFD Study: Cesarean Section Findings

	VX-809 Dose (mg/kg/day)			
Parameter	**0**	**50**	**100**	**200**
Pregnant Animals (out of 20)	19	19	19	20
Found dead or moribund sacrifice	0	0	0	2
Aborted and sacrificed	0	0	0	**4**
Pregnant and C-sectioned on GD29	19	19	19	14
Corpora Lutea (litter mean)	9.0	9.2	8.7	9.0
# of Implantations (litter mean)	8.6	8.4	8.1	8.6
Early resorptions (litter mean)	0.4	0.2	0.2	0.5
Late resorptions (litter mean)	0.1	0.4	0.6	0.5
Resorbed conceptuses per litter (%)	0.5	0.6	0.8	1.0
Does with any resorptions (%)	26.3	36.8	36.8	57.1
# of live fetuses (litter mean)	8.1	7.7	7.3	7.6
Sex Ratio (litter mean % male)	49.6	45.0	49.5	54.0
Fetal body weight (litter mean, g)	45.15	43.21	44.04	44.20

Bold font denotes statistically significant difference vs. control group

Offspring (Malformations, Variations, etc.)

Fetuses were individually weighed, examined for gross external alterations, sacrificed via IP injection of 390 mg pentobarbital sodium and 50 mg phenytoin sodium, and examined internally to determine sex. Cavitated organs from all fetuses were examined by dissection, brains were examined in situ after a single cross section between the parietal and frontal bones, and examined for skeletal alterations after Alizarin red S staining (see table below).

Table 22. VX-809 Rabbit EFD Study: Fetal Examinations

	VX-809 Dose (mg/kg/day)			
Parameter	**0**	**500**	**1000**	**2000**
Litters evaluated	19	19	19	14
Fetuses evaluated	154	148	138	107

No external or visceral malformations or variations were noted in more than a single fetus. No test article-related skeletal findings were observed. One hyoid finding (angulated ala) was significantly higher in the HD group (10 fetuses / 7 litters) compared to controls (5 fetuses / 3 litters) but was judged to be incidental due to lack of any dose-response and high background incidence (litter mean 18%, range 0-50% in test facility historical control database).

Study title: VRT-0995096 [M28]: An Oral (Gavage) Study of the Effects on Embryo/Fetal Development in Rats

Study no.:	Test facility # (b) (4) 395087 Sponsor #VRT-0995096-TX-008
Study report location:	SD #92 (11/16/2012)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/9/2012
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	VRT-0995096 (metabolite of VX-809) Batch A4152-049 Purity 91.8%

Key Study Findings

- In an embryo-fetal development study, female rats received M28 at doses of 200, 400 or 800 mg/kg/day from GD 7 through GD 17.
- Excessive maternal toxicity was observed at the HD, including four unscheduled deaths (two main study and two satellite animals). Clinical signs included red/clear material around the nose, mouth and/or urogenital area, red vaginal discharge, rales, and pale/cool body.
- At the HD, maternal body weight gains were 40% lower than controls, a difference that was statistically significant and considered to be adverse. Food consumption was also decreased at the HD.
- There were no test article-related effects on embryofetal viability. The HD level was associated with a 20% decrease in mean fetal body weights.
- Skeletal variations were frequently noted in the HD group, particularly decreased ossification of cervical centrum #1, unossified sternebrae #5/6, and 14th rudimentary ribs. Vertebral malformations were noted in five HD pups from three different litters (vertebral agenesis, vertebral centra anomaly, and vertebral

anomaly with or without associated rib anomaly) but these were not considered to represent test article-related teratogenicity.

- The MD level of 400 mg/kg/day was considered as the NOAEL with regards to maternal toxicity. The MD level of 400 mg/kg/day was considered the NOAEL for embryofetal toxicity based upon decreased fetal body weights observed at 800 mg/kg/day; however, this finding can most likely be attributed to maternal toxicity observed at 800 mg/kg/day.
- M28 exposure increased less than dose-proportionally from 200 to 800 mg/kg/day and was similar on GD 6 and GD 17. $AUC_{0\text{-}24h}$ at the NOAELs was 3360 and 4240 ug*h/mL, respectively

Methods

Doses:	0 (hydroxypropylmethylcellulose acetate succinate [a 49% component of the test article (b) (4) formulation] control in the vehicle), 200, 400, 800 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v methylcellulose, 0.5% w/v Tween 80, 0.05% w/v simethicone in deionized water
Species/Strain:	Crl:CD (SD) rats ~14 weeks of age at start of dosing 232-311 g at GD 0.
Number/Sex/Group:	25 females per group
Satellite groups:	8 per group for TK
Study design:	Day of mating evidence designated GD 0. Rats dosed from GD 6 to GD 17. Laparohysterectomy performed on GD 20.
Deviation from study protocol:	Deviations were reviewed but were not judged to affect the interpretation or integrity of the study.

Observations and Results

Mortality

All animals were observed twice daily for mortality and moribundity. In a dose-ranging study, animals in a 1000 mg/kg/day group were euthanized prematurely due to excessive body weight loss.

There were two deaths in the main study 800 mg/kg (HD) group; one animal was euthanized in extremis on GD 16 (#39453) and another was found dead on GD 17 (39513). Both animals were noted to decreased body weights, decreased food consumption, red/clear material around the nose, mouth and/or urogenital area throughout the dosing period, and less frequent red vaginal discharge and rales following dosing between GD10-16. Animal #39453 was also noted with pale/cool body

on GD 16 and necropsy indicated 8 normally developing implantations and 7 early resorptions. Animal #39513 was also noted with unkempt appearance on GD 16 and necropsy indicated 11 dead fetuses (no apparent malformations) and 6 early resorptions. There were two additional premature deaths in the HD toxicokinetic satellite group.

Clinical Signs

Clinical observations were recorded daily from GD 0 to GD 20 (GD 18 for TK animals). An additional observation was made 1 hour post-dose during the dosing phase.

There were not any notable test article-related clinical observations in animals surviving to the scheduled terminations. Post-dose observations that were dose-related are summarized in the table below (numbers include the premature deaths described above).

Table 23. M28 Rat EFD Study: Post-dose Observations

Finding	Control	200	400	800
Body cool	0	0	0	2/2
Rales	0	0	3/2	6/5
Wet red material around nose	0	0	1/1	7/5
Dried red material around nose	0	0	5/4	7/7
Wet red material around mouth	0	2/2	4/4	21/16
Dried red material around mouth	0	19/13	51/20	98/25
Wet clear material around mouth	1/1	2/2	13/8	37/15
Dried clear material around mouth	0	1/1	3/3	7/7

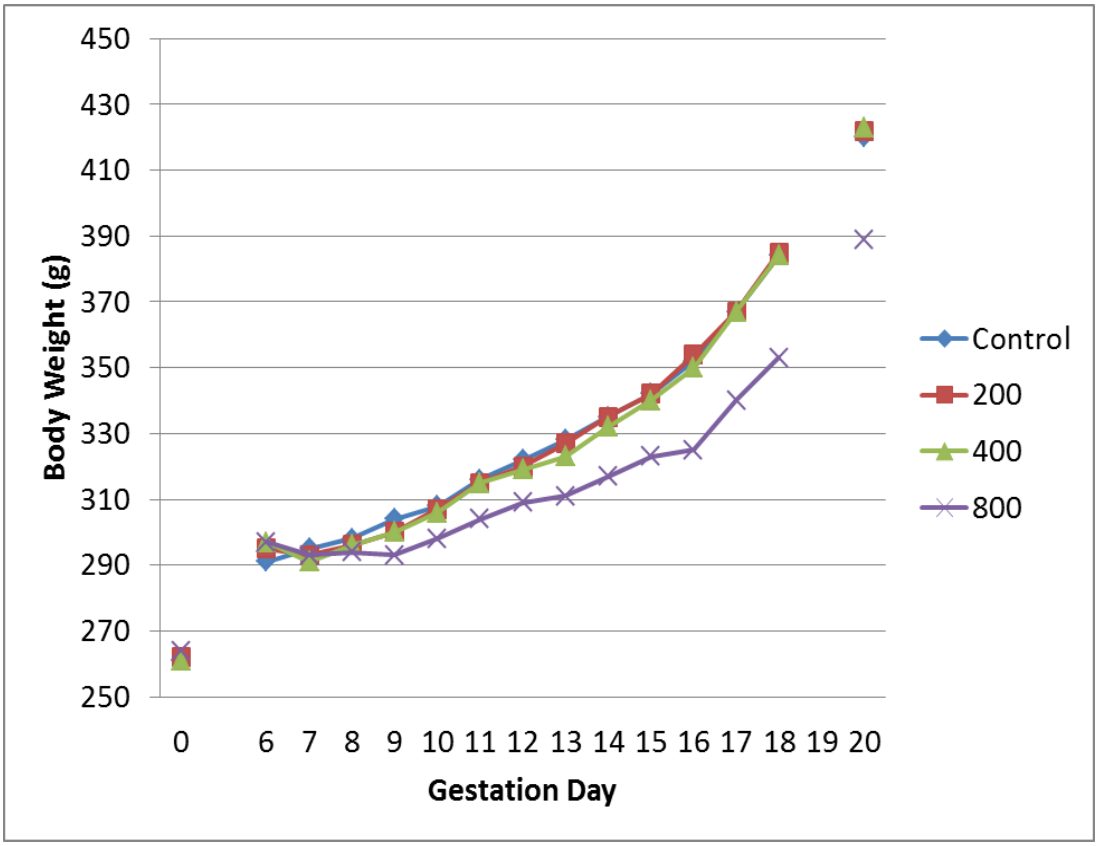
Total number of occurrences / Number of affected animals

Body Weight

Individual maternal body weights were recorded on GD 0, 6-18, and 20 for the main study groups and GD 0 and 6-17 for TK animals. Net body weights (GD 20 body weight minus gravid uterine weight) were also collected at laparohysterectomy.

Body weight gains during the dosing period were statistically significantly lower in the HD group compared to controls. The mean body weight gain in the HD group was 56 grams, 40% lower than the mean 93 gram body weight gain in the control group. Gravid uterine weights (-16%) and net body weight change (-26%) over the course of the study were also significantly reduced in the HD group. LD and MD results were comparable to controls. The effects at the HD were considered adverse.

Figure 6. M28 Rat EFD Study: Body Weights



Feed Consumption

Individual food consumption was recorded on GD 0, 6-18, and 20 for the main study groups only.

Feed consumption was statistically significantly reduced in HD animals (-19% vs. controls) during the dosing period.

Necropsy

Laparohysterectomies and macroscopic examinations were performed blind to treatment group. Surviving females were euthanized on GD 20 via CO_2 inhalation and the thoracic, abdominal, and pelvic cavities were examined.

Gross observations of firm stomach contents (concretion, believed to be solidified test article) were noted in 1 MD and 7 HD females at the scheduled necropsy. There were no test article-related differences in pregnancy status among either main study (1, 2, 0, and 2 nongravid animals for control, LD, MD, and HD, respectively) or TK groups (0, 3, 1, and 0 nongravid animals for control, LD, MD, and HD, respectively).

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The uterus and ovaries were excised and numbers/locations of corpora lutea, fetuses, early and late resorptions, and total implantation sites were recorded. Placentae were also examined. Maternal tissues (including representative samples from the control group) were preserved in 10% neutral-buffered formalin. When applicable, early implantation loss was detected with 10% ammonium sulfide. TK group females were only evaluated for pregnancy status and evidence of intubation error (where applicable for premature deaths).

A numerical increase in post-implantation loss was observed at the HD. This increase was driven by a litter with 60% (3/5) early resorptions, was not statistically significant compared to controls, and remained within the test facility historical control values. Therefore, this observation was not judged to represent an adverse test article-related effect. In addition, the HD level was associated with a statistically significant 20% decrease in mean fetal body weight compared to control. This mean fetal body weight (3.2 grams) remained within the historical control database but is considered attributable to M28, possibly related to the adverse effects on maternal animals manifested in the decreased body weight gains and mortality.

Table 24. M28 Rat EFD Study: Cesarean Section Data

	VRT-0995096 Dose (mg/kg/day)			
Parameter	**0**	**200**	**400**	**800**
Pregnant Animals[a]	24	23	25	21
Corpora Lutea (litter mean)	16.0	16.8	16.7	15.7
# of Implantations (litter mean)	15.4	15.3	15.7	14.7
Early resorptions (litter mean)	0.7	0.7	0.6	0.9
Late resorptions (litter mean)	0.0	0.0	0.0	0.0
Resorbed conceptuses per litter (%)	4.7	4.6	3.4	8.5
Pre-implantation loss (%)	3.3	8.6	5.4	7.0
Post-implantation loss (%)	4.7	4.6	3.4	8.5
# of live fetuses (litter mean)[b]	14.7	14.6	15.2	13.8
Sex Ratio (litter mean % male)	49.8	49.2	49.4	55.5
Fetal body weight (litter mean, g)	4.0	3.9	3.8	**3.2**

Bold font denotes statistically significant difference vs. control group
[a] Number (out of 25) that were found to be pregnant and survived to schedule cesarean section
[b] No dead fetuses so number of live fetuses = litter size

Offspring (Malformations, Variations, etc.)

Viable fetuses were examined externally (including eyes, palate, and external orifices), sexed, weighed, and euthanized via SC sodium pentobarbital injection. All fetuses underwent a visceral examination (modified Stuckhardt and Poppe method) which included confirmation of sex and grading for renal papillae development. Fetal heads were divided into two groups: 1) Bouin's fixative and soft-tissue examination by Wilson sectioning technique and 2) examination by midcoronal slice. Carcasses were eviscerated, fixed in 100% ethyl alcohol, macerated in potassium hydroxide, stained for Alizarin Red S and examined for skeletal malformations and developmental variations.

Several vertebral malformations were noted at the HD, each in a single (and unique) litter. Of these, only the finding of vertebral anomaly with or without associated rib anomaly (3 pups in 1 litter, litter mean incidence 1.8%) exceeded the maximum test facility historical control incidence of 0.7% but was no statistically significantly different vs. the concurrent control group.

On average, skeletal variations were noted in 70% of pups in HD group litters. Statistically significant differences vs. the concurrent control group were noted for decreased ossification of cervical centrum #1, more frequent unossification of sternebrae #5 and/or 6, and increased presence of a 14th rudimentary rib. Numerical increases were also noted at the HD for reduced ossification of the vertebral arches, presence of 27 presacral vertebrae, reduced ossification of the skull, and 14th full rib(s). The rate of each of these variations in HD litters also fell outside of the test facility historical control range.

Results of external, visceral, and skeletal examinations for malformations and variations are summarized in the table below.

Table 25. M28 Rat EFD Study: Fetal Malformations and Variations

Parameter	VXRT-0995096 Dose (mg/kg/day)			
	0	200	400	800
External evaluation (fetuses/litters)	*352/24*	*336/23*	*379/25*	*289/21*
Vertebral agenesis (M)	0	0	0	1/1
Visceral evaluation (fetuses/litters)	*352/24*	*336/23*	*379/25*	*289/21*
Liver – accessory lobules (V)	0	0	0	2/2
Spleen – pale (V)	0	0	0	3/2
Skeletal evaluation (fetuses/litters)	*352/24*	*336/23*	*379/25*	*289/21*
Vertebral centra anomaly (M)	0	0	0	1/1
Vertebral anomaly with or without associated rib anomaly (M)	0	0	0	3/1
Cervical centrum #1 ossified (V)	98/19	65/17	37/19	**10/5**
Sternebrae 5 and/or 6 unossified (V)	10/7	17/9	31/10	**84/15**
14th rudimentary rib(s) (V)	29/12	50/13	69/15	**125/21**
Reduced ossification of the vertebral arches (V)	0	0	0	8/4
27 presacral vertebrae (V)	0	1/1	4/2	6/4
Reduced ossification of the skull (V)	1/1	0	1/1	3/2
14th full rib(s) (V)	0	1/1	1/1	4/3

M: Malformation; V: Variation
Bold font denotes statistically significant difference vs. concurrent control.

The sponsor considered the MD of 400 mg/kg as the NOAEL for embryofetal toxicity based on the group of vertebral malformations and associated variations (27 presacral vertebrae and 14th rudimentary ribs), while attributing the ossification-related skeletal variations to the lower fetal body weights at the HD. The reviewer disagrees that the study demonstrated evidence of test article-related teratogenicity at the 800 mg/kg dose level. Specifically, malformations were generally equally distributed across the groups and the single malformation that exceeded the historical control range (vertebral

anomaly with or without associated rib anomaly) affected only a single litter. These findings were also noted at a dose that was associated with severe maternal toxicity and produced lower fetal body weights and increased incidence of non-adverse skeletal variations. The MD level of 400 mg/kg/day was considered the NOAEL for embryofetal toxicity based upon decreased fetal body weights observed at 800 mg/kg/day; however, this finding can most likely be attributed to maternal toxicity observed at 800 mg/kg/day.

Toxicokinetics

Blood samples (0.5 mL) were collected from the jugular vein into K_3-EDTA tubes on GD 6 and 17 according to the schedule outlined in the table below. Plasma was isolated and shipped to the sponsor for analysis.

Table 26. M28 Rat EFD Study: TK Sampling Schedule

Number of Females	Time of Blood Collection (hours postdose administration)					
	0 (pre-dose)	1	2	4	8	24
3-4[a]/group	X		X		X	
4/group		X		X		X

X = Time of blood collection for 4 females per group.
[a] = Only 3 females/time point were evaluated on gestation day 17 in the 800 mg/kg/day group as female number 39450 was euthanized *in extremis* on gestation day 15.

The test article was detected above the LLOQ of 2 ng/mL in a number of control samples at levels ranging from 2.29-21.7 ng/mL (generally higher concentrations on GD 17 compared to GD 6). The reason for this anomaly was not provided by the sponsor but likely represents ex vivo contamination. The test article concentrations in control samples were generally ≥10,000 times lower than the M28 concentrations in samples from treated animals and were not judged to affect study interpretation. TK results for the M28-treated groups are shown in the table below. Half-life was in the range of 9-16 hours. C_{max} and $AUC_{0\text{-}24h}$ increased less than dose-proportionally and there was not evidence of accumulation over the dosing period.

Table 27. M28 Rat EFD Study: Toxicokinetics

Parameter	Day	VRT-0995096 dose (mg/kg/day)		
		200	400	800
C_{max} (ug/mL)	GD 6	174	235	204
	GD 17	186	210	249
T_{max} (h)	GD 6	1.0	8.0	8.0
	GD 17	1.0	8.0	1.0
$AUC_{0\text{-}24h}$ (ug*h/mL)	GD 6	2810	3970	4340
	GD 17	2590	3360	4240
AUC/dose	GD 6	14.1	9.92	5.43
	GD 17	12.9	8.40	5.30

Dosing Solution Analysis

Test article solutions were prepared at 40, 80, and 160 mg/mL, reflecting the fact that VRT-0995096 comprises 50% of the test article by weight (with 49% HPMC-AS and 1% SLS. Test and control article formulations were prepared daily and used within 5 hours of preparation. The sponsor referenced previous stability data covering 20-200 mg/mL formulations for 24 hours. Samples for homogeneity were collected on GD 6 from the top, middle, and bottom of the formulations. Additional samples were collected from the middle stratum for concentration verification. Analyses were performed via a validated HPLC-UV assay.

All samples met the criteria for homogeneity and concentration verification (85-115% of target). No test article was detected in the samples from the vehicle dosing solution.

9.3 Prenatal and Postnatal Development

Study title: VX-809/VRT-0995096: Oral (gavage) developmental and perinatal/postnatal reproduction study in rats – Final report

Study no.:	Test facility #12-4392 Sponsor #VX-809-TX-017 and #VRT-0995096-TX-010
Study report location:	EDR SD #269 (7/8/2014)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/28/2012 (receipt of animals) 7/2/2012 (start of dosing) 11/15/2012 (final sacrifice) 4/4/2013 (report signed)
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	VX-809 (lumacaftor) Lot #11-401 Purity 100% VRT-0995096 (“M28”) Lot #A3379-274 50% M28: 49% hypromellose acetate succinate (HPMC-AS): 1% sodium lauryl sulfate (SLS) (b) (4) (b) (4) powder Purity 94%

Key Study Findings

- In a pre-natal/post-natal development (PPND) study in Sprague-Dawley rats, F_0 females received vehicle control (0.5% Tween 80, 0.5% methylcellulose, 0.05%

simethicone, 8 mg/mL HPMC-AS in water) or VX-809/M28 at doses of 250/20, 500/20, and 1000/20 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 21. The development and reproductive performance of F_1 offspring was assessed and the status of F_2 litters was assessed by Cesarean section of F_1 dams at GD 14.

- There were no test article related deaths or effects on body weights or food consumption in F_0 dams. Increased observations of alopecia at ≥500/20 mg/kg/day were attributed to the test article but were not considered adverse.
- There were no test article-related effects on F_0 mating performance, duration of gestation, litter size and fetal viability statistics, or sex ratio.
- There were no test article-related effects on F_1 survival through weaning, body weights, or food consumption.
- There were no test article-related effects on pre-weaning (surface righting, mid-air righting, auditory function, visual function) or post-weaning (open field evaluation, locomotor activity, learning and memory) functional assessments in F_1 animals.
- There were no test article-related effects on F_1 sexual maturation, mating performance, fertility, or status of F_2 litters upon C-section at GD 14.
- The HD of 1000/20 mg/kg/day was considered as the NOAEL for maternal and F_1 pre-/post-natal toxicity.
- While overt maternal toxicity was not identified in this study, the HD of 1000 mg/kg/day is generally considered as the limit dose in reproductive toxicity studies and is therefore acceptable.

Methods

Doses:	Vehicle control 250 mg/kg VX-809 / 20 mg/kg M28 (LD) 500 mg/kg VX-809 / 20 mg/kg M28 (MD) 1000 mg/kg VX-809 / 20 mg/kg M28 (HD)
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) Tween 80, 0.5% (w/v) methylcellulose, 0.05% simethicone in water and HPMC-AS-HF (8 mg/mL)
Species/Strain:	Sprague-Dawley CD rats
Number/Sex/Group:	22 time-mated females per group 11-13 weeks old at start of dosing 223-309 g at start of dosing
Satellite groups:	No TK or other satellite groups were included
Study design:	The M28 dose of 25 mg/kg/day has been judged to be acceptable to qualify the metabolite levels formed in human subjects; no toxicity was observed in a 28-day rat study at doses up to 100 mg/kg/day. The HD approximates the 1000/25 mg/kg/day HD in the 6-month general toxicology study and was considered to represent less than the MTD by reviewer Timothy Robison (11/2/2012). F_0 females were dosed from gestation day (GD) 6 through lactation day (LD) 20. GD 0 represented the detection of mating and LD 0 represents the onset of parturition. Observation of F_1 pups (one per sex per litter randomly selected on PND 21) continued through sexual maturity, mating, and establishment of pregnancy (F_1 females sacrificed at GD 14).
Deviation from study protocol:	Deviations were reviewed but were not judged to affect the integrity of the study.

Observations and Results: F_0 Dams

Survival

Viability checks were performed each morning and afternoon, as well as before and after test article administration on dosing days.

Two deaths in the study were not test article-related. A MD female was sacrificed in moribund condition on LD 5 with macroscopic findings suggestive of gavage error. Clinical signs included ocular lacrimation and chromodacryorrhea, excessive salivation, labored breathing, and body cool to touch. Gross necropsy observations included abnormal thoracic cavity contents (severe, clear fluid) and severe adhesion involving

the diaphragm, heart, lungs, and thymus. In addition, a MD female was electively sacrificed on LD 7 due to the single live pup no longer being present.

Clinical Signs
Detailed physical examinations were performed daily beginning on GD 4 (≤30 minutes prior to dosing and ~4 hours post-dose).

The incidence of alopecia of the torso and/or limbs was elevated, generally noted at doses of ≥500/20 mg/kg/day. This finding was not considered adverse.

Body Weight
Individual body weights were recorded on GD 4, 6, 9, 12, 15, 18, 20 and then daily until parturition, and after parturition on LD 1, 4, 7, 10, 14, 18 and 21.

There were no test article-related effects on F0 maternal body weights during the gestational period and after parturition.

Figure 7. PPND Study: F_0 Maternal Body Weights (GD 4-21)

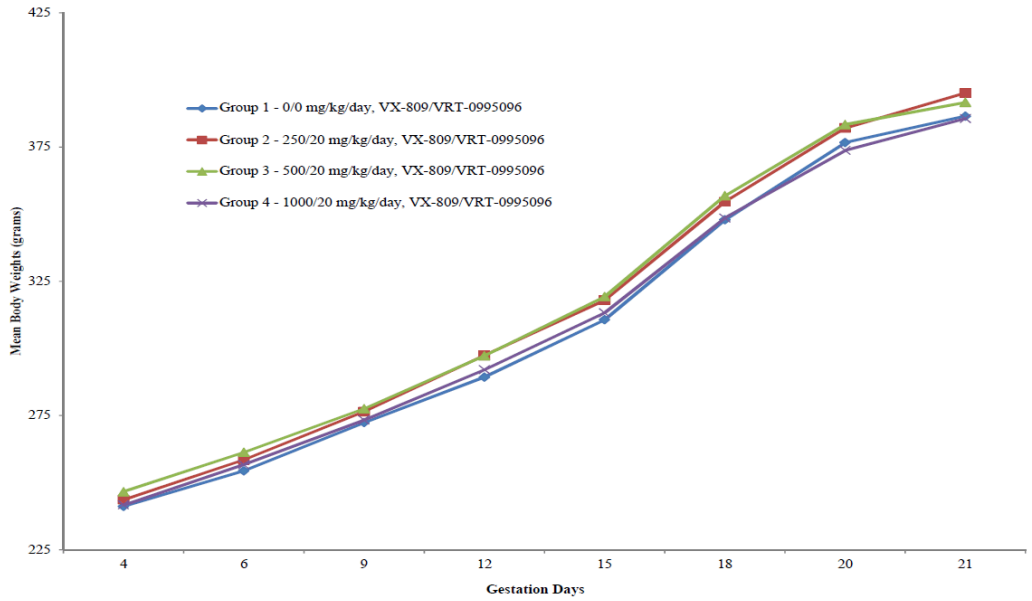
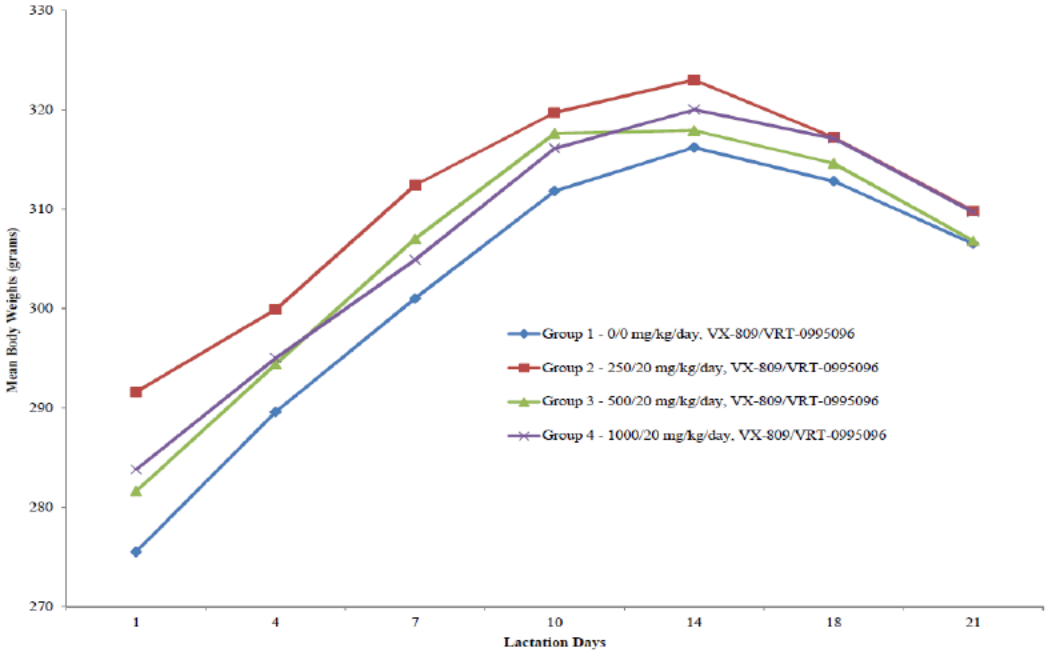


Figure 8. PPND Study: F_0 Maternal Body Weights LD 1-21



Feed Consumption
Individual food consumption was recorded on the days indicated above for body weights.

There were no test article-related effects on food consumption over the GD 4-21 and LD 1-21 time periods.

Natural Delivery and Litter Observations
Examinations for parturition were made three times daily beginning on GD 18. Pregnancy, delivery, and litter/pup data are summarized in the table below.

There were no test article-related effects on mating performance, duration of gestation, litter size and fetal viability statistics, sex ratio, or survival of F_1 pups through weaning.

Table 28. PPND Study: F_0 delivery and litter size data

	VX-809/VRT-0995096 Dose (mg/kg/day)			
Parameter	**0**	**250/20**	**500/20**	**1000/20**
Pregnant Animals[a]	21	22	22	22
Gestation index (%)[b]	100	100	100	100
Duration of gestation (days)	21.8	21.7	21.9	21.9
Number of implantation sites (mean)	13.2	13.4	12.8	13.2
Unaccounted-for implantation sites (%)	4.7	8.1	2.5	8.3
Number of pups delivered (mean)	12.6	12.3	12.5	12.1
Number of pups delivered live (mean)	12.6	12.1	12.4	12.0
Live birth index (%)[c]	99.6	98.5	99.3	99.6

Delivery index (%)[d]	95.0	90.5	96.8	91.4
Viability index (%)[e]	99.2	99.6	98.9	98.1
Lactation index (%)[f]	100	98.6	95.2	99.0
Sex ratio Day 0 (% male)	44.9	52.0	48.5	45.9
Sex ratio Day 21 (% male)	47.1	49.3	47.7	47.1

[a] Number (out of 22) that were found to be pregnant.
[b]All pregnant females completed delivery (# females with live-born / # of pregnant females)
[c]Total # of live-born pups / total number of pups born
[d]Total # of live pups delivered / total # of implantations
[e]Number of pups alive on PND 4 / # of live-born pups. There was no test article-related effect on the number of pups found dead, cannibalized, or found without milk spots during this period.
[f]Number of pups alive at PND 21 weaning / numbers of pups at PND 4 post-cull

Necropsy Observation
F_0 females were euthanized on LD 21 after weaning and a macroscopic postmortem examination was performed. The number of visible implant sites was recorded. Non-pregnant status was confirmed by ammonium sulfide staining. The ovaries, uterus and vagina were preserved for possible further examination.

There were no macroscopic findings at necropsy in the animals surviving to the scheduled sacrifice.

Toxicokinetics
NA

Observations and Results: F_1 Generation

Survival
Litters were observed as soon as possible to record live and dead pup numbers, abnormalities, and sex. Litters were observed twice daily through PND 21. Litters were culled to no more than 10 pups with sexes equalized on post-natal day (PND) 4.

One F_1 female from the HD group was sacrificed on PND 34. Clinical observations included reduced body weight, decreased activity, irregular gait, thinness, paleness, and abdominal distension. There were no macroscopic observations at necropsy and the death is of uncertain relationship to the test articles.

Clinical Signs
Gross physical examinations (abnormalities and sex recorded) were performed on PND 1, 4, 7, and 21. Post-weaning clinical observations were performed twice daily. Physical examinations were conducted twice weekly from weaning through euthanasia (males) or mating (females). Mated females were also examined on GD 0, 7 and 14.

Up to 22 litters per group were examined during the pre-weaning period and 20 animals per sex per group were examined in the post-weaning period. There were no test article-related effects on any parameters evaluated pre-weaning (e.g., number of normal-appearing live pups, pups found dead, or clinical observations). One HD group

female was consistently observed with decreased activity, abnormal gait, and thin/pale appearance starting in the first week of post-weaning observations, leading to euthanasia as noted above.

Body Weight
Individual body weights were recorded on PND 1, 4, 7, 10, 14, 18, 21. Post-weaning male body weights were collected on PND 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, 59, and 63, on the days that sexual maturation was achieved, and then weekly until termination. Post-weaning female body weights were collected on PND 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, 59, and 63, on the days that sexual maturation was achieved, and twice weekly until mating. Mated females were weighed on GD 0, 7 and 14.

There were no effects on pre-weaning or post-weaning body weights of F_1 rats in the study (see figures below).

Figure 9. PPND Study: F_1 male pre-weaning body weights

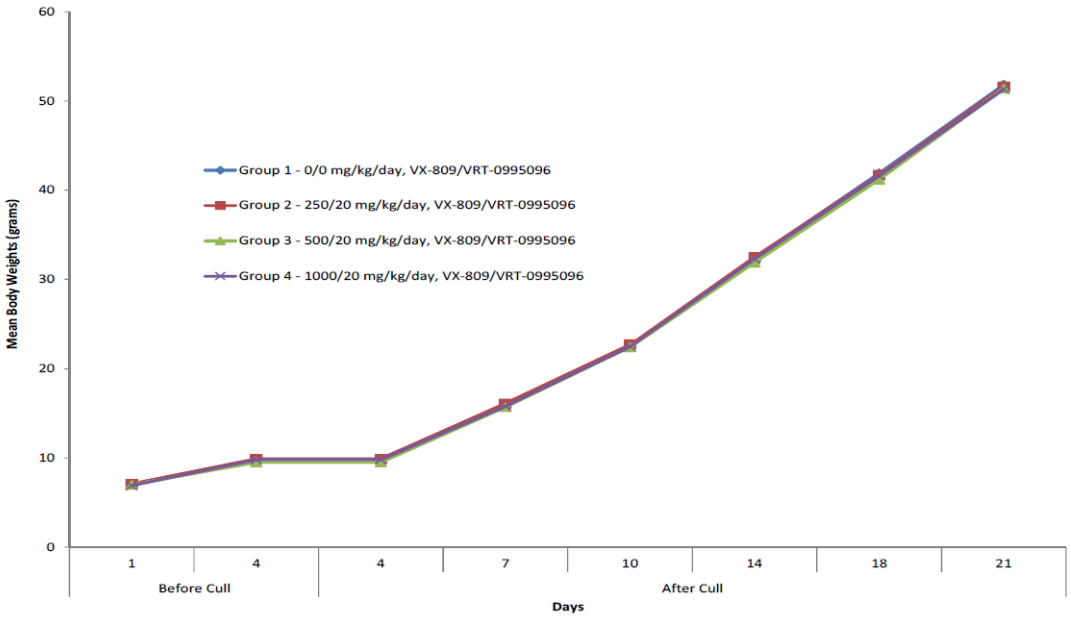


Figure 10. PPND Study: F_1 female pre-weaning body weights

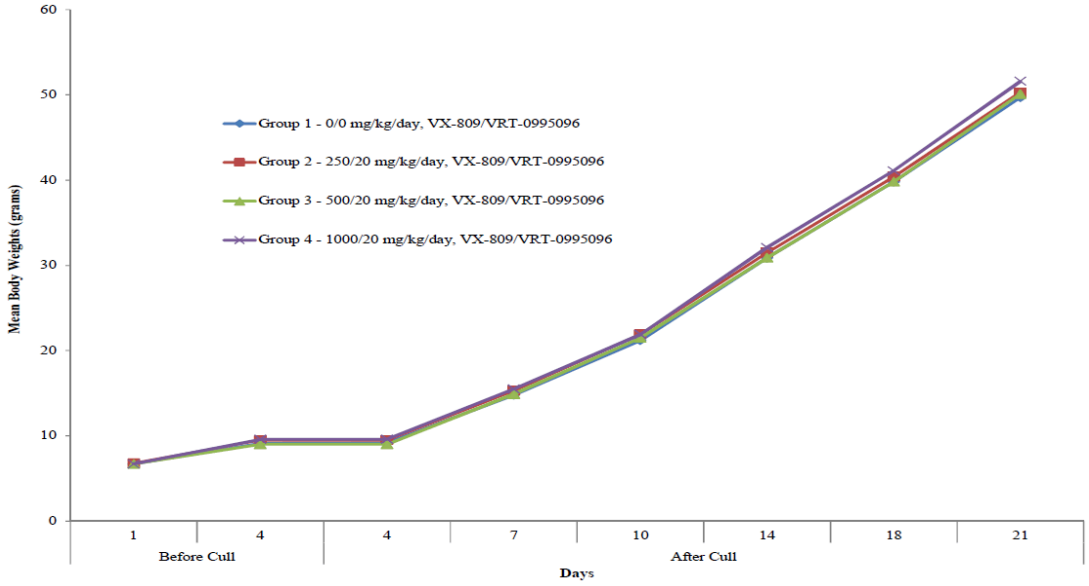


Figure 11. PPND Study: F_1 male post-weaning body weights

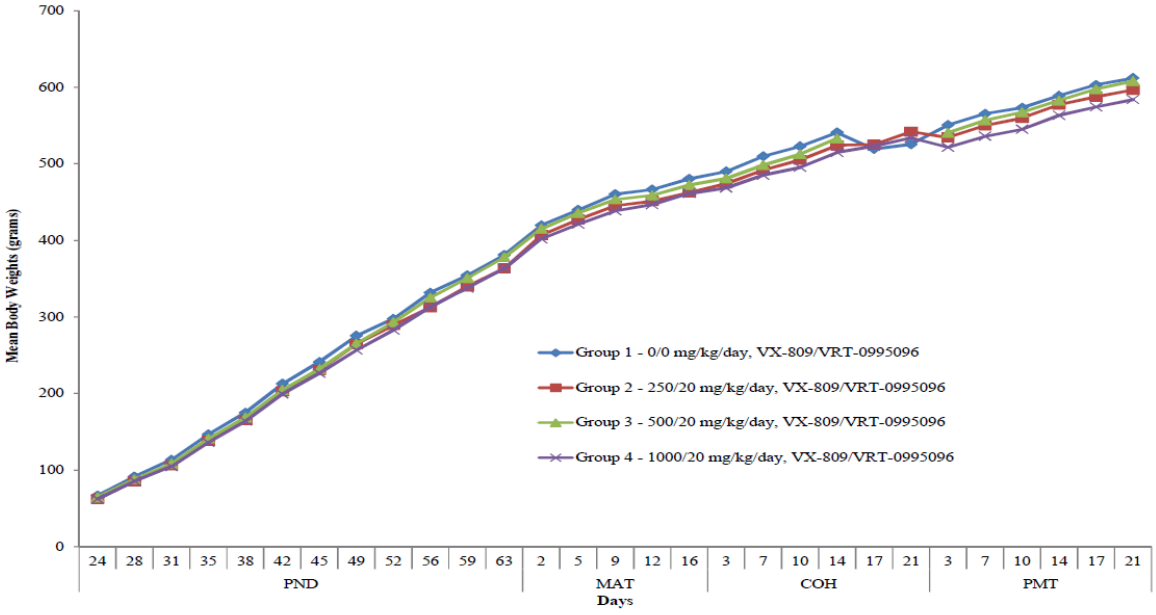
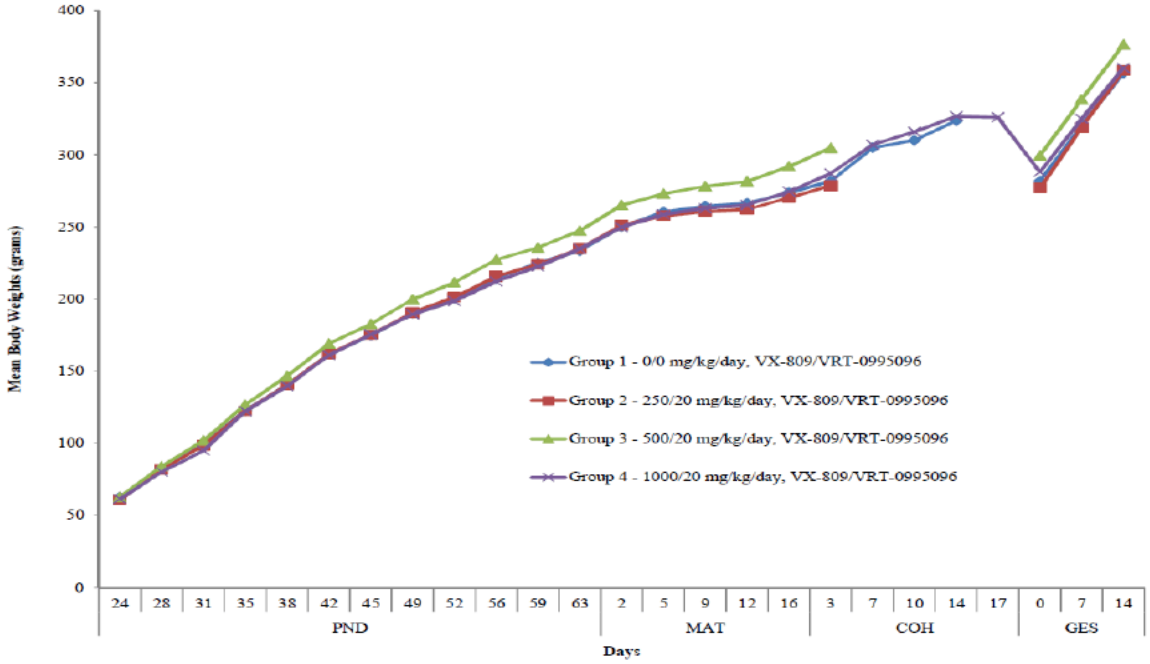


Figure 12. PPND Study: F_1 female post-weaning body weights



Feed Consumption
Measured beginning around PND 35 and continuing until cohabitation. After the cohabitation period, measurements were recorded weekly for males and on GD 4, 7, 11 and 14 for females.

There was no test article-related effect on food consumption in F_1 animals in the study.

Pre-weaning Functional Assessments
Surface righting: from PND 2 until achieved or culled
Mid-air righting: from PND 14 until achieved
Auditory function (startle response to sudden sound): from PND 20
Visual function: PND 20 (pupil closure response of dark-adapted eyes)

There were no test article-related effects on any of the above functional assessments.

Table 29. PPND Study: F_1 pups pre-weaning functional assessments

	VX-809/VRT-0995096 Dose (mg/kg/day)			
Parameter	**0**	**250/20**	**500/20**	**1000/20**
Surface righting (days to acquisition)	3.4	3.3	3.0	3.3
Auditory function (percent passed)	100	100	100	100
Mid-air righting (days to acquisition)	16.2	16.3	16.4	16.0
Visual function (percent passed)	100	100	99.5	99.5

199 to 217 pups tested per group.

Post-weaning Functional Assessments
One male and one female from each litter were selected randomly on PND 21 for subsequent functional, behavioral and reproductive assessments. Grossly abnormal animals, but not normal-appearing runts, were excluded from selection if their long-term survival was judged to be adversely effected. The following parameters were assessed:

- Open-field evaluation: selected weanlings were observed for one minute on PND 22 or 23 for abnormalities of posture, gait, behavior, and/or vocalization.
- Locomotor activity: Spontaneous exploratory activity was measured on PND 28. Activity over a 60-minute period was monitored with an automated system. The number of photobeam breaks per five minute period was recorded.
- Learning and memory: Evaluated from ~8 weeks of age using water-filled Biel maze. Each animal was limited to two trials per day each of a maximum duration of three minutes. A straight line 1.2 meter swim time was recorded on the first day of testing. Days 2-5 (learning) of the test consisted of the entire maze with the time to complete and number of errors recorded. The pups were required to complete the maze again on day 8 (memory).

There were no findings in any males in any treatment group in any of the open-field assessments. All animals were observed with normal gait and posture and did not exhibit vocalization or abnormal behavior. One MD group female and two HD group females exhibited abnormal vocalization upon handling.

There were no test article-related effects on F_1 locomotor activity or learning and memory.

Sexual Maturation
Males were observed daily for preputial separation beginning on PND 38. Females were observed daily for vaginal opening beginning on PND 28. Complete achievement of these developmental milestones was required to be assessed as sexually mature. Daily vaginal smears were taken and the stage of estrous was determined, starting two weeks before cohabitation until evidence of mating or 20-day cohabitation period ended.

There were no test article-related effects on F_1 sexual maturation or estrous cycling.

Reproduction
12-week old females were cohabitated with non-sibling males from the same treatment group, until evidence of mating or for 20 days. Presence of a vaginal plug or observation of sperm in vaginal smears obtained daily was considered evidence of mating and the date of observation was considered as GD 0. Females could be transferred to the cage of a second male after 14 days of cohabitation. Females were euthanized and c-sectioned on GD 14.

There were no statistically significant differences in F_1 male or female mating and fertility parameters in the study.

Table 30. PPND Study: F_1 reproductive performance data

	VX-809/VRT-0995096 Dose (mg/kg/day)			
Parameter	**0**	**250/20**	**500/20**	**1000/20**
Females paired with males (#)	20	20	20	19
Female mating index (%)[a]	95	95	100	100
Female fertility index (%)[b]	100	95	95	95
Males paired with females (#)	22	21	20	21
Male mating index (%)[a]	86	91	100	91
Male fertility index (%)[b]	100	95	95	95
Mean time to mating (days)	2.6	2.6	2.5	3.7

[a]Number mated / number paired
[b]Number pregnant / number mated

Necropsy Observation and Organ Weights
F_1 females were euthanized on GD 14 and a macroscopic postmortem examination was performed. The number of corpora lutea was counted and the number per ovary was recorded. The number of live and dead implantations was recorded for each uterine horn. Non-pregnant status was confirmed by ammonium sulfide staining. The ovaries, uterus and vagina were preserved for possible further examination. F_1 males were sacrificed up to 23 days after the end of the cohabitation period and a macroscopic postmortem examination was performed. The testes, epididymides, prostate, and seminal vesicles with coagulating glands were weighed and preserved.

Table 31. PPND Study: F_1 female Caesarean section data

	VX-809/VRT-0995096 Dose (mg/kg/day)			
Parameter	**0**	**250/20**	**500/20**	**1000/20**
Number of pregnant females	19	18	19	18
Mean number of corpora lutea	15.8	15.4	15.9	15.2
Mean number of implantation sites	14.6	14.7	14.4	13.7
Mean number of live fetuses	13.7	13.7	13.2	12.3
Mean number of dead fetuses	0.9	0.9	1.2	1.4
Pre-implantation loss (%)	4.9	5.5	9.4	10.5
Post-implantation loss (%)	8.3	6.2	8.0	10.7

There were no test article-related observations at gross necropsy and there were no statistically significant differences in male reproductive organ weights between groups.

Dosing Solution Analysis

Samples for homogeneity analysis were collected on Day 1 from the top, middle, and bottom strata of the low- and high-dose solutions. Dose confirmation was performed on all samples on the first (GD 6) and last (LD 20) day of dosing. Previous 7-day stability data was referenced.

Concentration analysis results were within 101-109% of the intended VX-809 concentration and 95-110% of the intended VRT-0995096 concentration. The test

articles were not detected in any control dosing sample. Homogeneity analysis indicated the different strata were within the +/-15% acceptance criteria.

11 Integrated Summary and Safety Evaluation

VX-809 (lumacaftor) is in development as a potential treatment for cystic fibrosis in a combination product that also contains the FDA-approved product ivacaftor (VX-770,). The planned clinical dose of VX-809 is 600 mg QD or 400 mg BID in combination with VX-770 at a dose of 250 mg BID. Vertex completed five nonclinical studies to evaluate the developmental and reproductive toxicity associated with VX-809 (lumacaftor) and its disproportionate human metabolite, M28. VX-809 was studied in EFD studies in rats and rabbits, and M28 was evaluated in an EFD study in rats. VX-809 spiked with M28 was evaluated in FEED and PPND studies in rats.

In a rat FEED study, animals received vehicle or VX-809 and M28 at doses of 250/20, 500/20, or 1000/20 mg/kg/day. Males were dosed for 28 days before mating and females were dosed from 14 days before mating through gestation day (GD) 7. There were no adverse effects on male fertility, female fertility, or early embryonic viability and development through GD 15. The high-dose level of 1000 mg/kg/day VX-809 and 20 mg/kg/day M28 was therefore considered as the NOAEL in the study. No maternal toxicity was achieved in the study, but the high-dose of 1000 mg/kg/day is considered an acceptable limit dose. No TK analysis was performed in this study, but based on prior data, exposure at the NOAEL was expected to be greater than that at clinical doses by a margin of at least 4 (males) – 8 (females) times for VX-809 and about 25 times for M28.

In a rat EFD study, females received VX-809 at doses of 500, 1000 or 2000 mg/kg/day from GD 7 through GD 17. There were no adverse effects in the study with regards to maternal health or embryofetal development and the high-dose of 2000 mg/kg/day was considered as a NOAEL. Exposure to VX-809 in the study increased less than dose-proportionally from 500-2000 mg/kg and was similar on GD 7 and GD 17. The VX-809 $AUC_{0\text{-}24h}$ at the NOAEL was 3,320 ug*h/mL, representing an approximately 9-fold margin to clinical exposures. No maternal toxicity was achieved, but the high-dose of 2000 mg/kg is above the limit dose (1000 mg/kg) required in reproductive toxicity studies.

In a rabbit EFD study, females received VX-809 at doses of 50, 100 or 200 mg/kg/day from GD 7 through GD 19. An excessive level of maternal toxicity was observed at the high-dose, with two premature deaths and four additional does that aborted and were sacrificed. Additional findings at the high-dose included body weight losses (-5% compared to GD 7 value vs. 5% body weight gain for controls) and decreased food consumption, scant feces, poor grooming, thin body condition, dehydration, and decreased activity. Significant decreases in body weight gain (1% of GD 7 starting weight vs. 5% gain in controls) were also noted at the mid-dose level. Macroscopic findings at the high-dose were noted in the heart (white bands surrounding the ventricles, pale appearance), lungs (red/mottled lobes), and kidneys (pale appearance).

There were no test article-related effects on embryofetal survival or any evidence of teratogenicity in the study. Exposure to VX-809 in the study increased less than dose-proportionally from 50-200 mg/kg and was similar on GD 7 and GD 19. At the 50 mg/kg NOAEL for maternal toxicity, VX-809 exposure was about three times higher than at the clinical dose. At the 200 mg/kg NOAEL for embryofetal toxicity, VX-809 exposure was about five times higher than at the clinical dose.

In a rat EFD study, females received M28 at doses of 200, 400 or 800 mg/kg/day from GD 7 through GD 17. Excessive maternal toxicity was observed at the HD, including four unscheduled deaths (two main study and two satellite animals). Clinical signs included red/clear material around the nose, mouth and/or urogenital area, red vaginal discharge, rales, and pale/cool body. At the HD, maternal body weight gains were 40% lower than controls, a difference that was statistically significant and considered to be adverse. Food consumption was also decreased at the HD. There were no test article-related effects on embryofetal viability but the HD level was associated with a 20% decrease in mean fetal body weights. Skeletal variations were frequently noted in the HD group, particularly decreased ossification of cervical centrum #1, unossified sternebrae #5/6, and 14th rudimentary ribs. Vertebral malformations were noted in five HD pups from three different litters (vertebral agenesis, vertebral centra anomaly, and vertebral anomaly with or without associated rib anomaly) but these were not considered to represent test article-related teratogenicity. Only a single malformation (vertebral anomaly with or without associated rib anomaly) exceeded the historical control range and this finding was confined to a single litter. The MD level of 400 mg/kg/day was considered as the NOAEL with regards to maternal and embryofetal toxicity. The adverse decreases in fetal body weights observed at 800 mg/kg/day can most likely be attributed to maternal toxicity observed at 800 mg/kg/day. M28 exposure increased less than dose-proportionally from 200 to 800 mg/kg/day and was similar on GD 6 and GD 17. At the 400 mg/kg NOAEL for maternal toxicity, M28 exposure was about 105 times higher than at the clinical dose. At the 800 mg/kg NOAEL for embryofetal toxicity, M28 exposure was about 132 times higher than at the clinical dose.

In a rat PPND study, F_0 female received VX-809/M28 at doses of 250/20, 500/20, and 1000/20 mg/kg/day from GD 6 through lactation day (LD) 21. The development and reproductive performance of F_1 offspring was assessed and the status of F_2 litters was assessed by Cesarean section of F_1 dams at GD 14. There were no test article related deaths nor any effects on body weights or food consumption in F_0 dams. There were no test article-related effects on F_0 mating performance, duration of gestation, litter size and fetal viability statistics, or sex ratio. There were no test article-related effects on F_1 survival, body weights, or functional assessments. Finally, there were no test article-related effects on F_1 sexual maturation, mating performance, fertility, or status of F_2 litters upon C-section at GD 14. The HD of 1000/20 mg/kg/day was considered as the NOAEL for maternal and F_1 pre-/post-natal toxicity. While there was no apparent toxicity in the study, 1000 mg/kg is considered an acceptable limit dose. No TK analysis was performed in this study, but based on prior data exposure at the NOAEL was expected to be greater than that at clinical doses by a margin of at least 4 (males) – 8 (females) times for VX-809 and about 25 times for M28.

It is noted that the VX-809 / M28 combination studies did not include monoproduct comparison arms, as had been suggested by the division (communication dated 3/19/2010). The purpose of the recommendation was to assist in attributing adverse effects in the combination arms to an individual test article. However, given that no notable toxicity was observed in the combination high-dose arms in either study, the studies were considered acceptable. The combined evaluation of VX-809 and M28 also more closely models the clinical situation.

Overall there was no evidence for developmental or reproductive toxicity attribute to the VX-809 and M28 test articles. All observed adverse findings were attributed to excessive levels of maternal toxicity in the VX-809 rabbit EFD study and M28 rat EFD study. Safety margins between the NOAELs in the developmental / reproductive toxicology studies and the estimated VX-809 and M28 exposures in the clinical setting are summarized in the table below.

Table 32. VX-809 and M28 Safety Margins

Study	VX-809		M28	
	$AUC_{0\text{-}24hr}$ (ug*hr/mL)	Safety Margin	$AUC_{0\text{-}24hr}$ (ug*hr/mL)	Safety Margin
Human (28-day phase 2 study)				
600 mg QD or 400 mg BID VX-809 and 250 mg BID VX-770	371[a]	-	32.1[a]	-
Rat (FEED study)				
1000 / 20 mg/kg VX-809 / M28 (NOAEL for M/F fertility and early embryonic toxicity)	1300[b] (M) 3160[b] (F)	3.5 (M) 8.5 (F)	800[c]	24.9
Rat (EFD Study)				
2000 mg/kg VX-809 (NOAEL for maternal and embryofetal toxicity)	3320	8.9		
Rabbit (EFD Study)				
50 mg/kg (NOAEL for maternal toxicity)	995	2.7		
200 mg/kg (NOAEL for embryofetal toxicity)	1950	5.3		
Rat (Metabolite EFD Study)				
400 mg/kg (NOAEL for maternal toxicity)			3360	105
800 mg/kg (NOAEL for embryofetal toxicity)			4240	132
Rat (PPND Study)				
1000 / 20 mg/kg VX-809 / M28 (NOAEL for maternal and F_1 pre-/post-natal toxicity)	1300-2830[b] (M) 3010-4850[b] (F)	3.5-7.6 (M) 8.1-13.1 (F)	800[c]	24.9

[a] From Study 102 (see **Section 5.1**)
[b] These values from the 6-month general toxicology study are somewhat lower than in other VX-809 toxicity studies; therefore, the safety margins presented here may be conservative. (see **Section 5.2**)
[c] Average M/F Day 28 exposure at 25 mg/kg/day (see **Section 5.2**)

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/s/

ANDREW C GOODWIN

10/16/2014

Review of developmental and reproductive toxicology studies for lumacaftor (VX-809) and a disproportionate human metabolite.

TIMOTHY W ROBISON

10/16/2014

I concur

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	IND 79521, NDA 206038
Supporting document/s:	SD #286 (IND), SD #1 (NDA)
Sponsor's letter date:	9/15/2014 (IND), 11/5/2014 (NDA)
CDER stamp date:	9/15/2014 (IND), 11/5/2014 (NDA)
Product:	Lumacaftor (VX-809)
Indication:	Cystic Fibrosis
Sponsor:	Vertex Pharmaceuticals
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Angela Ramsey

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

VX-809 (lumacaftor) is in late-stage development by Vertex Pharmaceuticals as a potential treatment for cystic fibrosis in a combination product that also contains the FDA-approved product ivacaftor (VX-770, tradename Kalydeco). The planned clinical dose of VX-809 is 600 mg QD or 400 mg BID in combination with ivacaftor at a dose of 250 mg BID. A pre-NDA meeting for the lumacaftor / ivacaftor combination product was held on August 12, 2014 (see minutes dated September 2, 2014) and the rolling submission under NDA 206038 was completed on November 5, 2014.

In the preliminary comments provided ahead of the pre-NDA meeting, the sponsor was requested, if feasible, to submit the results from the six-month Tg.rasH2 transgenic mouse carcinogenicity study to the IND ahead of the estimated November 2014 NDA submission to facilitate review. The sponsor submitted the final study report and datasets on September 15, 2014 and the results are reviewed in this memo and will be presented to the Executive Carcinogenicity Assessment Committee (ECAC) on November 18, 2014. Note that a two-year rat carcinogenicity study is in progress and the division has previously agreed that the results from this study may be submitted as a post marketing requirement (see memo by Dr. Timothy Robison dated July 26, 2013).

1.2 Brief Discussion of Nonclinical Findings

The carcinogenic potential of VX-809 (lumacaftor) was evaluated in a six-month oral gavage carcinogenicity study in Tg.rasH2 transgenic mice. Animals received VX-809 at doses of 200, 700 or 2000 mg/kg/day in males and 200, 500, or 1500 mg/kg/day in females. Negative control groups received vehicle or water and a positive control group received urethane. While transgenic mouse assays are generally considered to solely provide hazard identification, these doses represent approximately two- to six-fold multiples compared to the proposed clinical dose.

VX-809 had no effect on survival in the study. In male animals only, slightly lower body weight gains were noted at the higher doses. Kidney toxicity was observed in high-dose males only, manifested by decreased organ weights and histopathological findings of renal cortex tubular epithelial degeneration and regeneration.

There were no neoplastic findings related to VX-809 treatment in males. However, the combined incidence of Harderian gland adenoma and carcinoma (5/25 animals) was statistically significantly increased in high-dose females compared to the two control groups. However, the control incidence (0/50) was low compared to historical control rate of 3.5% and the combined incidence of adenoma and carcinoma in the HD group did not exceed the historical control range (0-24%). Therefore the Harderian gland finding was not considered to represent a test article-related effect.

The positive control group confirmed the responsiveness of the Tg.rasH2 model to urethane, a known carcinogen. Urethane treatment was associated with acute body weight losses in both sexes and a statistically significant decrease in survival for males. Neoplastic findings included lung adenomas, lung carcinomas, and spleen hemangiosarcomas observed in both sexes at rates generally similar to historical data.

In conclusion, VX-809 was negative for carcinogenicity in males and females in a six-month study in Tg.rasH2 mice.

1.3 Recommendations

The results of the VX-809 six-month Tg.rasH2 mouse carcinogenicity study were presented at the ECAC meeting held on November 18, 2014. Meeting minutes dated November 19, 2014 are appended to this review.

Executive CAC Recommendations and Conclusions

Tg.rasH2 mouse:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in the study.

2 Drug Information

2.1 Drug

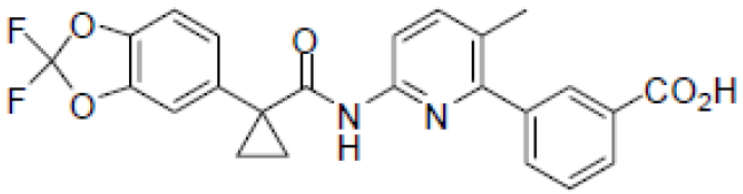
Generic Name: Lumacaftor

Code Name: VX-809

Chemical Name: 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid

Molecular Formula/Molecular Weight: $C_{24}H_{18}F_2N_2O_5$ / 452 g/mole

Structure or Biochemical Description



Pharmacologic Class: Cystic Fibrosis Transmembrane conductance Regulator (CFTR) (b) (4) (proposed by sponsor)

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 74633 and NDA 203188 (ivacaftor / Kalydeco / VX-770)
- NDA 206038 (for lumacaftor / ivacaftor combination)

2.3 Drug Formulation

The formulation of the sponsor's fixed-dose combination tablet containing 200 mg lumacaftor and 125 mg ivacaftor is summarized in the table below. There are no novel excipients and no nonclinical concerns with the daily exposure to these inactive ingredients at the proposed daily dose of 800 mg lumacaftor and 500 mg ivacaftor (4 tablets).

Table 1. Lumacaftor – Ivacaftor Fixed-dose Combination Tablet Formulation

Component	Quality Standard	Component Function	200 mg/125 mg Amount per tablet (mg)	200 mg/125 mg Content (% w/w)
(b) (4)				
Lumacaftor drug substance	In house standard	Active Ingredient	200.00	(b) (4)
Ivacaftor (b) (4)	NDA 203188	Drug product	(b) (4)	(b) (4)
Microcrystalline cellulose	USP/NF	(b) (4)		
Croscamellose sodium	USP/NF			
Sodium lauryl sulfate	USP/NF			
Povidone (b) (4)	USP/NF			
(b) (4)	USP			
	USP/NF			
	USP/NF			
Magnesium stearate (b) (4)	USP/NF			
Film Coat				
(b) (4)	DMF No. (b) (4) USP			
Print Ink				
(b) (4) Black	DMF No. (b) (4)			
Total Tablet Weight			582.5	100.0

(b) (4)

(b) (4)

2.8 Regulatory Background

The sponsor submitted a Special Protocol Assessment (SPA) request on September 24, 2012 for a 6-month carcinogenicity study in Tg.rasH2 transgenic mice. The dose-ranging toxicology studies and the proposed protocol were reviewed by Dr. Jane Sohn (November 2, 2012) and presented to the ECAC on October 30, 2012. A "no

agreement" letter was issued on November 1, 2012 due to incomplete data for the 28-day mouse study. A subsequent SPA request was received on November 16, 2012 and the ECAC provided its concurrence in meeting minutes dated November 30, 2012. The additional information provided by the sponsor was reviewed by Dr. Sohn on November 30, 2012. The committee's recommendations are summarized below.

Executive CAC Recommendations and Conclusions:

- The Committee recommended doses of 0 (vehicle), 200, 500 and 1500 mg/kg/day in females, with the high dose based on deaths at 4000 mg/kg/day, and 0 (vehicle), 200, 700, and 2000 mg/kg/day in males, with the high dose based on the limit dose for the 6-month oral carcinogenicity study in Tgras.H2 mice.
- For transgenic mice, the sponsor should conduct histopathological examination of all tissues from all dose groups.

3 Studies Submitted

3.1 Studies Reviewed

VX-809: 26-week repeated dose oral carcinogenicity study in Tg.rasH2 mice
(b) (4)
Test facility study # (b) (4) 57YX.7G8R (b) (4)
Sponsor project # VX-809-TX-019
Final report dated September 10, 2014

3.3 Previous Reviews Referenced

Nonclinical reviews filed to IND 79521:

- January 3, 2008 by Dr. Timothy Robison
- November 2, 2012 by Dr. Jane Sohn
- November 30, 2012 by Dr. Jane Sohn
- July 26, 2013 by Dr. Timothy Robison

5 Pharmacokinetics/ADME/Toxicokinetics

The following overview of lumacaftor pharmacokinetics in mice is excerpted from the nonclinical review filed by Dr. Jane Sohn November 2, 2012.

- Half-life following intravenous administration in CD-1 mice is 4.28 hours, with distribution into tissues.
- Systemic clearance was low, with clearance of ~1% compared to hepatic blood volume in mice.
- In vitro assessment of plasma protein binding in male CD-1 mouse plasma showed that 99.7-99.8% of VX-809 is bound, compared to 99.3-99.5% in human plasma.

7 Genetic Toxicology

VX-809 was found to be negative for genotoxic potential in an in vitro bacterial reverse mutation assay, in vitro Chinese Hamster Ovary (CHO) cell chromosomal aberration assay, and in vivo mouse micronucleus assay. These studies were previously reviewed by Dr. Timothy Robison (January 3, 2008).

8 Carcinogenicity

Study title: VX-809: 26-WEEK REPEATED DOSE ORAL CARCINOGENICITY STUDY IN Tg.rasH2 MICE

Study no.:	Test facility #(b) (4)57YX.7G8R(b) (4) Sponsor #VX-809-TX-019
Study report location:	EDR SD #286 (9/15/2014)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Study initiation 10/9/2012 First dosing 1/7/2013 Final sacrifice 7/11/2013 Final report dated 9/10/2014
GLP compliance:	Yes, signed statement
QA statement:	Yes, signed statement
Drug, lot #, and % purity:	VX-809 Lot 4281-P54-12-401 99.7% purity
CAC concurrence:	Yes, see nonclinical review by Dr. Jane Sohn and ECAC meeting minutes dated November 30, 2012.

Key Study Findings

- In a six-month oral gavage carcinogenicity study in Tg.rasH2 mice, males received VX-809 at 200, 700, or 2000 mg/kg/day and females received VX-809 at 200, 500, or 1500 mg/kg/day. Two negative control groups received water or vehicle (0.5% methylcellulose (400 cps), 0.5% Tween 80, 0.05% simethicone in deionized water) and a positive control group was administered urethane.
- There were no effects on survival in VX-809 groups compared to the control groups. None of the 10 unscheduled deaths were clearly attributable to the test article; however, three satellite females receiving the HD of 1500 mg/kg/day died of unknown causes in the first week and were replaced.
- Dose-related clinical signs in males and females included hunched posture and rapid / shallow breathing. Additional finding in males included decreased motor activity, hyperactivity, ruffled fur, and swelling.
- Males treated with VX-809 showed a slight (but not statistically significant) trend towards decreased body weight gains at 700 and 2000 mg/kg/day. The reviewer

did not judge the effect to be adverse. VX-809 had no effect on body weights in females.

- The kidneys were identified as a target organ of toxicity in males receiving 2000 mg/kg/day VX-809. Absolute kidney weights were decreased by approximately 10% vs. controls and histopathological findings of cortex tubular degeneration and regeneration were noted.
- Females receiving VX-809 at 1500 mg/kg/day had an increased incidence of Harderian gland adenoma (4/25 = 16%) and carcinoma (1/25 = 4%) with a combined incidence of 5/25 or 20%. This incidence was statistically significantly higher than the vehicle and water control groups (p=0.025 vs. each control group and p=0.004 for trend); however, the combined incidence was within the historical control range (0-24%). There were no notable tumor findings in males at doses up to 2000 mg/kg/day.
- The urethane-treated positive control group exhibited lung adenoma, lung carcinoma and spleen hemangiosarcoma. These findings were generally consistent with historical control data for this known carcinogen.
- Toxicokinetic analysis showed that VX-809 exposure increased less than dose-proportionally from 200 to 1500 (females) or 2000 (males) mg/kg/day and was greater in females vs. males. At Day 178, $AUC_{0\text{-}24hr}$ values at the highest doses tested were 1390 and 2090 ug*hr/mL in males and females, respectively. The exposure levels at the HD correspond to 3.7x and 5.6x margins to the proposed clinical dose.

Adequacy of Carcinogenicity Study

The high-dose of 2000 mg/kg/day in males represents the limit dose for carcinogenicity studies. The high dose of 1500 mg/kg/day was set at approximately one-third the lethal dose of 4000 mg/kg/day in a 28-day dose-ranging study in mice. There were no test article-related body weight changes or other adverse effects suggestive of the MTD being exceeded in females receiving 1500 mg/kg/day VX-809. The ECAC provided its concurrence with the dose levels employed in meeting minutes dated November 30, 2012 and this study to evaluate the carcinogenic potential of VX-809 in Tg.rasH2 mice is considered adequate.

Appropriateness of Test Models

The Tg.rasH2 transgenic mouse strain is an acceptable model for evaluating the carcinogenic potential of pharmaceuticals per ICH *Guidance for Industry S1B Testing for Carcinogenicity of Pharmaceuticals*.

Evaluation of Tumor Findings

- There were no test article-related statistically significant tumor findings in male Tg.rasH2 mice at VX-809 doses up to 2000 mg/kg/day.
- The combined incidence of Harderian gland adenoma and carcinoma was statistically significantly increased in females receiving the highest VX-809 dose of 1500 mg/kg/day compared to the vehicle and water control groups (p=0.025

vs. each control and p=0.004 for trend). However, the control incidence was low compared to historical control rates and the incidences of these neoplasias in the HD group were within the historical control ranges. Therefore the Harderian gland finding was not considered to represent a test article-related effect.

- The urethane-treated positive control group exhibited lung adenoma, lung carcinoma and spleen hemangiosarcoma, which are expected findings in response to exposure to this known carcinogen.
- VX-809 was concluded to be negative for carcinogenicity in this transgenic mouse study.

Methods

Doses:	Vehicle control Deionized water control 1000 mg/kg urethane (positive control administered by i.p. injection on Days 1, 3, 5) *Males* 200 mg/kg/day VX-809 700 mg/kg/day VX-809 2000 mg/kg/day VX-809 *Females* 200 mg/kg/day VX-809 500 mg/kg/day VX-809 1500 mg/kg/day VX-809
Frequency of dosing:	Once daily for 26 weeks
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose (400 cps), 0.5% Tween 80, 0.05% simethicone in deionized water
Basis of dose selection:	Dose selection was based on results of a 28-day study in transgenic mice at 1000, 2000, and 4000 mg/kg/day. Dose-limiting mortality and kidney toxicity (bilateral tubular degeneration) were observed in females only at 4000 mg/kg/day. The high-dose for males was therefore set at the limit dose of 2000 mg/kg. The high-dose for females was set at 1500 mg/kg, approximately one-third the lethal dose. Lower doses were based on AUC spacing.
Species/Strain:	Hemizygous Tg.rasH2 mice (Main cohort) Wild-type CByB6F1 (TK cohort) (b) (4) [redacted]
Number/Sex/Group:	25 (Main cohort vehicle, water, and VX-809) 10 (Main cohort positive control) 14 (TK cohort vehicle and water groups) 38 (TK cohort at each VX-809 dose level)
Age:	7 weeks old at start of dosing 18.1-23.6 grams (males) 15.4-19.7 grams (females)
Animal housing:	Individual housing following randomization
Paradigm for dietary restriction:	None. Ad libitum access to Harlan TEKLAD Global Diet #2018CM (18% protein).
Dual control employed:	See above.
Interim sacrifice:	NA
Satellite groups:	Wild-type TK groups as described above.
Deviation from study protocol:	Three extra 1500 mg/kg female TK animals

were added to replace early deaths on Day 6. The deviation log was reviewed and all deviations were judged to be minor and did not affect the integrity or interpretation of the study.

Observations and Results

Mortality

All animals were observed twice daily at least 6 hours apart for moribundity and mortality.

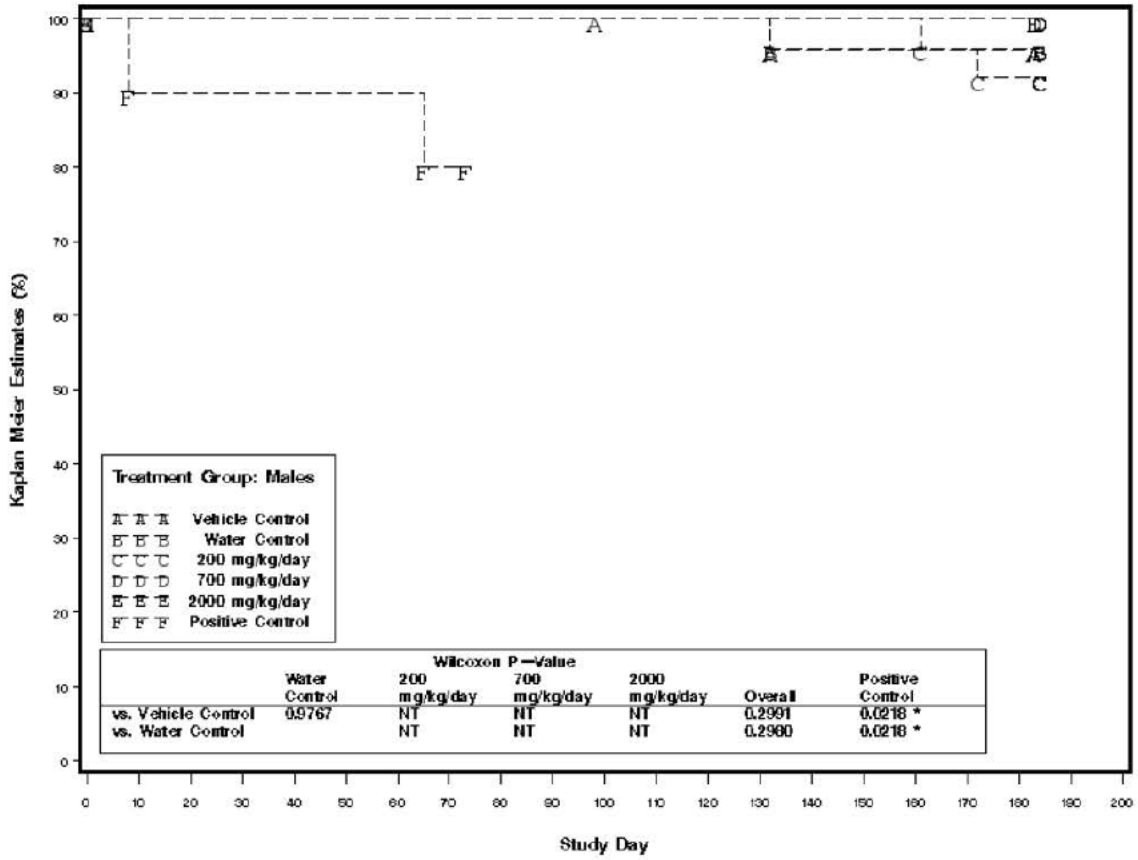
There were six unscheduled deaths in the main study cohort:

- Vehicle control male #2103 – moribund sacrifice Day 132, undetermined cause.
- Vehicle control male #2122 was sacrificed based on injuries and clinical signs following being dropped (Day 98).
- Water control male #2141 – found dead Day 132, undetermined cause.
- 200 mg/kg (LD) VX-809 male #2155 found dead, attributed to primary malignant hemangiosarcoma of the skin on Day 161.
- 200 mg/kg (LD) VX-809 male #2163 found dead Day 172, undetermined cause.
- 1500 mg/kg (HD) VX-809 female #2352 moribund sacrifice Day 161 attributed to primary malignant hemangiosarcoma of the skin.

In addition, among HD TK animals, one male (moribund sacrifice Day 161) and three female (found dead or moribund sacrifice Day 6) did not survive to scheduled necropsy. There was no gross evidence of gavage error but there was no further histopathological examination. Two positive control males also died prior to the scheduled necropsy, one on Day 8 of undetermined cause and one on Day 65 attributed to lung tumors.

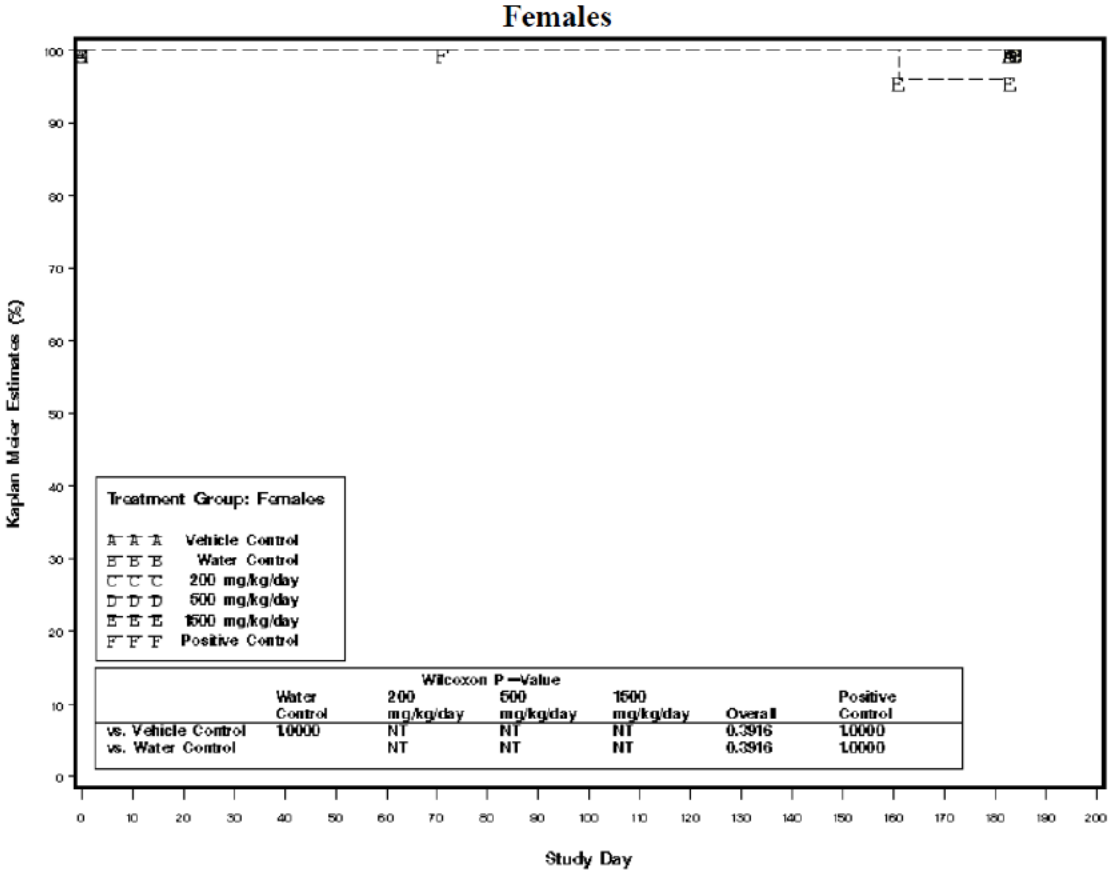
The only statistically significant effect on survival was observed in the male positive control group compared to the two control groups. The test article VX-809 was not associated with any effect on survival in male or females.

Figure 1. 6-month Mouse Carcinogenicity Study: Male Survival



* - statistically significant: NT – Not tested due to non-significant overall comparison across all groups.

Figure 2. 6-month Mouse Carcinogenicity Study: Female Survival



* - statistically significant; NT – Not tested due to non-significant overall comparison across all groups.

Clinical Signs

Cage-side observations were conducted on each day of dosing, within approximately 2 hours of the end of dosing. Detailed physical examinations were performed on Day 1 and weekly throughout the study.

There were no dose-related findings during cage-side observations in males. A single HD female was noted with eye discharge as well as red discoloration and swelling of the nose from Day 156-158. Test article-related findings observed at the detailed physical examinations are summarized in the table below.

Table 2. 6-month Mouse Carcinogenicity Study: Clinical Signs

Finding	Males (mg/kg/day VX-809)				
	Vehicle	Water	200	700	2000
Decreased motor activity	0	0	0	51/10	37/7
Hyperactive	0	0	0	38/5	12/5
Ruffled fur	0	0	2/2	8/4	34/8
Hunched	0	0	0	29/7	9/3
Rapid and shallow breathing	3/2	0	16/9	67/15	81/15

Swelling	1/1	0	0	14/2	46/7
Finding	**Females (mg/kg/day VX-809)**				
	Vehicle	**Water**	**200**	**500**	**1500**
Hunched	0	0	0	5/3	0
Rapid and shallow breathing	0	2/1	2/1	9/6	9/8

Total # of observations / # of affected animals shown.

Consistent with urethane toxicity, positive control animals exhibited prostrate posture, labored / rapid / shallow breathing, ataxia, decreased motor activity, thinness and/or ruffled fur.

Body Weights

Body weights were recorded for main study and TK animals before dosing, weekly through Week 13 and biweekly thereafter.

Body weight curves for males and females were generated by the reviewer based on the sponsor's raw data and are presented in the figures below. There were no consistent statistically significant effects on body weights. Males receiving the 2000 mg/kg HD (and to a lesser extent the 700 mg/kg MD) of VX-809 generally trended towards lower body weights, but this effect was not seen in females receiving the nominally lower HD of 1500 mg/kg/day. Positive control animals of each sex experienced acute body weight decreases but then recovered after cessation of urethane dosing and grew more quickly that the other groups. Differences in absolute body weights compared to control are summarized in the table below.

Table 3. 6-month Mouse Carcinogenicity Study: Body Weights

Parameter	Sex	Control	LD	MD	HD
Body Weight (% difference from controls at Day 183)	M	Vehicle	2	-4	-4
		Water	-1	-7	-7
	F	Vehicle	-4	-3	-2
		Water	-3	-2	-1

Figure 3. 6-month Mouse Carcinogenicity Study: Male Body Weights

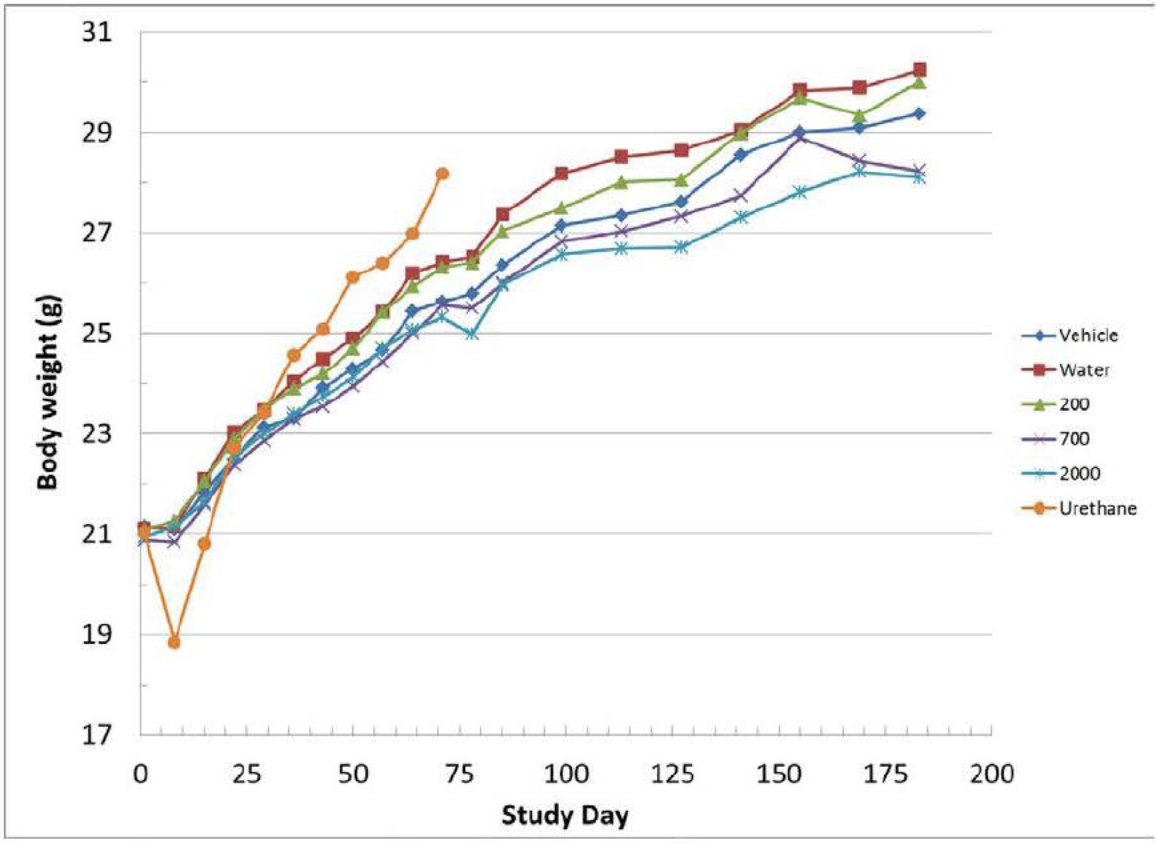
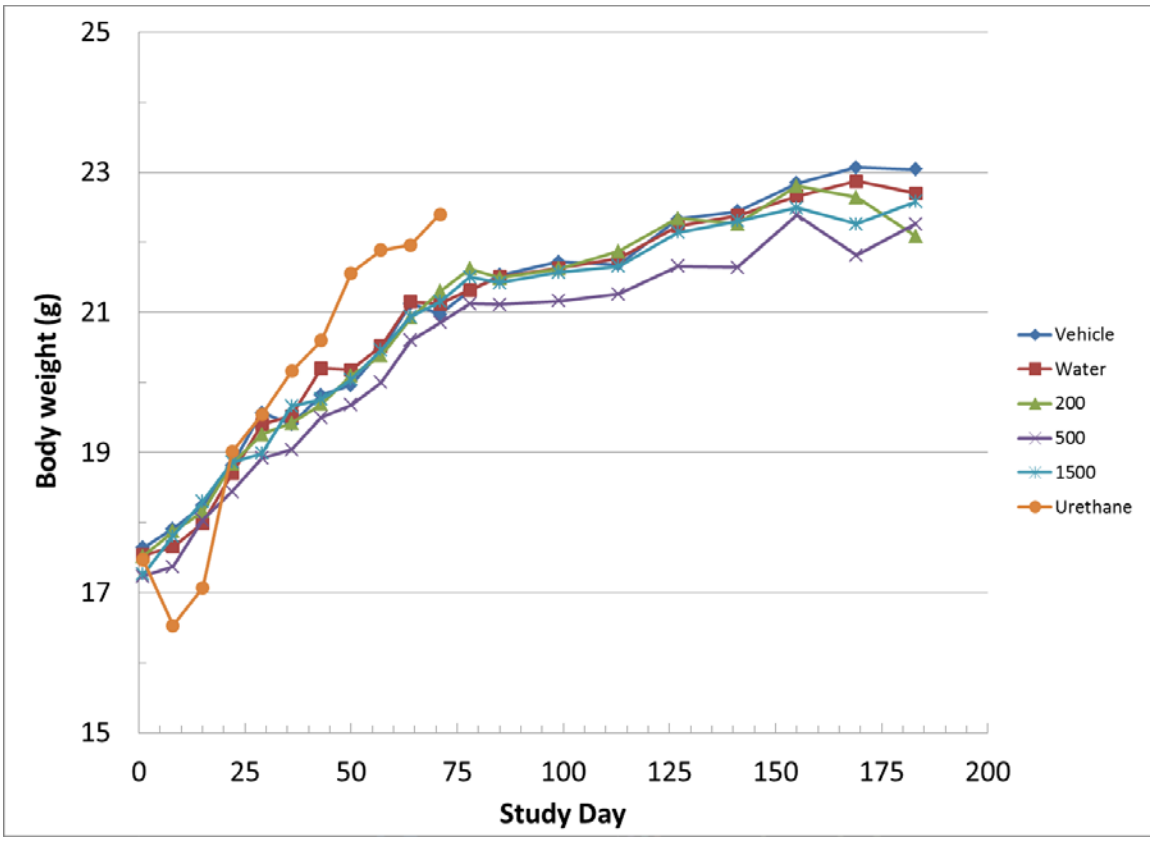


Figure 4. 6-month Mouse Carcinogenicity Study: Female Body Weights



Feed Consumption

Food consumption was recorded for main study animals only on Day 1 and weekly throughout the study.

There were no effects on food consumption in mice receiving VX-809 in the study.

Gross Pathology

Positive control animals were sacrificed on Day 71 (females) or Day 73 (males) and subjected to a full necropsy. A complete necropsy was performed on main study animals sacrificed during Week 26.

There were no notable test article-related gross pathology findings in the study.

Organ Weights

The following organ weights were collected from main study negative control and VX-809 groups: adrenal glands, brain, heart, kidneys, liver, spleen, testes and ovaries.

Test article-related organ weight differences were noted for the kidneys and liver. Kidney weights were up to 11% lower in males receiving VX-809, a difference that was

statistically significant only for the HD group using absolute organ weight values. This observation correlated with the histopathology findings of tubular degeneration / regeneration in the kidneys of the male HD group and was not observed in females. Increased liver organ weights of up to 16% compared to controls were noted, a difference that was generally statistically significant compared to the vehicle and water controls at the HD on both an absolute and body weight-adjusted basis.

Table 4. 6-month Mouse Carcinogenicity Study: Organ Weights

Organ	Sex	Control	LD	MD	HD
Kidney (Absolute)	M	Vehicle	2%	-2%	**-8%**
		Water	-1%	-5%	**-11%**
	F	Vehicle	-3%	-6%	-2%
		Water	-2%	-6%	-2%
Kidney (BW-adjusted)	M	Vehicle	1%	2%	-4%
		Water	0%	0%	-5%
	F	Vehicle	0%	-3%	0%
		Water	0%	-3%	0%
Liver (Absolute)	M	Vehicle	4%	3%	6%
		Water	7%	6%	9%
	F	Vehicle	2%	-1%	**13%**
		Water	1%	-1%	**12%**
Liver (BW-adjusted)	M	Vehicle	1%	**7%**	**10%**
		Water	**7%**	**12%**	**16%**
	F	Vehicle	4%	3%	**15%**
		Water	3%	2%	**14%**

Bold font denotes statistically significant difference vs. the specified control

Histopathology

Organs and tissues listed in the table below were prepared for microscopic examination. The final pathology report was signed December 12, 2013 by Study Pathologist (b) (4)

Table 5. 6-month Mouse Carcinogenicity Study: Histopathology Catalog

Tissues/Organs	
Adrenal glands	Nasal cavity
Aorta	Ovaries
Bone (femur and sternum)	Pancreas
Bone marrow (femur and sternum)	Parathyroid glands
Brain	Pituitary gland
Epididymides	Prostate gland
Esophagus	Salivary gland
Eyes	Sciatic nerve
Gall bladder	Seminal vesicles
Gross lesions	Skeletal muscle (thigh)
Harderian gland	Small intestine (duodenum, jejunum, and ileum)
Heart	
Kidneys	Spinal cord (cervical, thoracic, and lumbar)
Large intestine (cecum, colon, rectum)	
Liver	Spleen
Lungs and bronchi	Stomach
Lymph nodes (mesenteric and mandibular)	Testes
	Thymus
Skin from mammary area (male and female mice)	Thyroid glands
	Trachea
Mammary gland (females only)	Urinary bladder
	Uterus
	Vagina

Peer Review

The peer review pathologist (b) (4) re-examined all tissues form 10% of control and 20% of VX-809 animals, plus all unscheduled deaths. All hyperplasias and neoplasias, all kidneys from males, and all lungs / spleen from positive controls were also reviewed. A signed peer review statement was included in the final study report.

Neoplastic

Tumors observed in vehicle control, water control, and VX-809 treatment groups are summarized in the table below. The sponsor's statistical analysis consisted on one-sided comparisons of VX-809 groups to each of the controls, with significance evaluated at both the $p<0.01$ and 0.05 levels.

Table 6. 6-month Mouse Carcinogenicity Study: Neoplastic Lesions

Site / Neoplasia	Sex	V	H_20	LD[1]	MD[2]	HD[3]	Historical Control Data[4]
Number Examined:		***25***	***25***	***25***	***25***	***25***	
Ear							
Papilloma	M	0	0	0	0	0	0.56% (0-8%)[5]
	F	0	0	0	0	1	0.14% (0-4%)

Harderian Glands							
Adenoma	M	0	0	0	0	0	1.4% (0-8%)
	F	0	0	1	1	**4**[#]	2.8% (0-16%)
Carcinoma	M	0	0	0	0	0	0.14% (0-4%)
	F	0	0	0	0	1	0.70% (0-8%)
Combined	M	0	0	0	0	0	1.5% (0-12%)
	F	0	0	1	1	**5***	3.5% (0-24%)
Liver							
Hepatocellular adenoma	M	0	0	0	2	0	0.28% (0-4%)
	F	0	0	0	0	0	0% (0-0%)
Lungs with Bronchi							
Alveolar-bronchiolar adenoma	M	0	3	3	3	3	10% (0-24%)
	F	4	2	3	2	3	5.8% (0-24%)
Alveolar-bronchiolar carcinoma	M	0	1	0	1	0	0.56% (0-8%)
	F	0	0	0	0	0	1.3% (0-4%)
Combined	M	0	3	3	4	3	12% (0-32%)
	F	4	2	3	2	3	7.7% (0-28%)
Multiple Organs							
Hemangiosarcoma, skin	M	0	0	1	0	0	0.14% (0-4%)
	F	0	1	0	0	1	0.70% (0-8%)
Hemangiosarcoma, spleen	M	0	1	0	2	3	3.7% (0-16%)
	F	0	3	2	1	1	3.7% (0-16%)
Hemangioma, epididymides	M	0	0	0	1	0	0.14% (0-4%)
Hemangiosarcoma, vagina	F	0	0	1	0	1	0.14% (0-4%)
Combined (Whole-Body)	M	0	1	1	3	3	5.9% (NA)
	F	0	4	3	1	3	7.2% (NA)
Stomach							
Papilloma	M	0	0	0	0	0	0.28% (0-4%)
	F	0	0	0	0	1	0.70% (0-4%)
Thymus							
Thymoma	M	0	0	0	1	0	0.14% (0-4%)
	F	0	0	0	0	1	0.56% (0-4%)

[1]LD = 200 mg/kg/day in both males and females
[2]MD = 700 mg/kg/day in males and 500 mg/kg/day in females
[3]HD = 2000 mg/kg/day in males and 1500 mg/kg/day in females
[4]Data displayed represent mean and range from test facility historical control data for 26 studies covering 1420 animals. Note that in this case the data included in the study report was used, not that reported in the (b) (4) publication
[5]Represents historical data for "skin papilloma"
[#]Denoted statistically significant difference in terms of dose-response trend (p=0.013)
*****Denotes statistically significant difference in terms of dose-response trend (p=0.004) and vs. water and vehicle control groups (p=0.025) according to statistical reviewer Dr. Min Min.

Several tumors occurred with singular incidence in male and/or female high dose groups in the study, including ear papilloma, stomach papilloma, and thymus thymoma. These neoplastic lesions occurred at rates that were within historical controls ranges and were not statistically significantly different from either control group. Hepatocellular adenoma was observed in two MD males, an 8% (2/25) incidence that exceeded the

historical control range of 0-4%. However, due to the lack of dose response and there being no statistically significant difference vs. the control groups, this neoplastic finding in the liver was not considered to be test article-related.

The combined incidence of lung adenoma / carcinoma in MD males was 4/25 (16%), consisting of three adenomas and one carcinoma. This incidence was statistically significantly higher than the vehicle control group (0/25, p=0.0237). However, the reviewer did not consider the lung tumors to be a test article-related effect in males. In response to an Information Request, the sponsor clarified that, in the historical data from this test facility, animals presenting with both lung adenoma and carcinoma were scored as carcinoma only. Therefore the historical control range for the combined lung tumor incidence is the sum of the adenoma and carcinoma historical control ranges, or up to 32% and 28% in males and females, respectively. The statistical finding appears to be driven by the unexpected absence of lung neoplasias in the male vehicle control group as compared to historical control data and the concurrent water control group. The incidence of neoplastic lesions in the lungs was 8-16% in all other control and VX-809 groups, with no relationship to dose and well within the expected ranges based on historical control data.

Increased numbers of Harderian gland neoplasias were noted in HD females in the study, with an incidence of 16% (4/25 animals) for adenoma and an additional animal with carcinoma. There were no neoplastic findings in the Harderian glands from the vehicle or water control group animals. The individual incidences of adenoma (16%) and carcinoma (4%) were each at the upper bound of the respective historical control ranges. The tumor findings were reviewed by statistician Dr. Min Min. The statistical review confirmed the statistically significant differences in Harderian gland tumors in females, whether evaluated for dose response (adenoma and combination of adenoma / carcinoma) or in comparison to the control groups (combination of adenoma / carcinoma only). Dr. Min's summary data (from a draft review provided via email on October 31, 2014) is shown in the table below.

Table 7. 6-month Carcinogenicity Study: Statistical Analysis of Harderian Gland Tumors in Females

Organ Name	Tumor Name	Vehicle Cont N=25	200 mg Low N=25	700 mg Med N=25	2000 mg High N=25	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Female									
Harderian_gland	ADENOMA+CARCONOMA	0	1	1	5	0.004	0.500	0.500	0.025
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	20%				
harderian gland	adenoma	0	1	1	4	0.013	0.500	0.500	0.055
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	16%				

Organ Name	Tumor Name	Water Cont N=25	200 mg Low N=25	500 mg Med N=25	1500 mg High N=25	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Harderian_gland	ADENOMA+CARCONOMA	0	1	1	5	0.004	0.500	0.500	0.025
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	20%				
harderian gland	adenoma	0	1	1	4	0.013	0.500	0.500	0.055
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	16%				

As described above for the lung tumors, the sponsor provided clarification regarding the historical control ranges for the combined incidence of Harderian gland adenoma plus carcinoma. The combined historical control incidence for the test facility is up to 24%, which is greater than the combined 20% incidence in this study.. The overall historical control rate for Harderian gland adenoma in female Tg.rasH2 mice at the test facility is 2.8% (range of 0-16%), therefore the incidence of 0/50 in the two control groups in the present study is considered to represent an atypically low event rate. Taking this into account, along with the incidence of adenoma, carcinoma and the combination being within the historical control ranges, the reviewer concludes that the Harderian gland adenoma / carcinoma findings do not represent a test article-related effect.

Hemangiomas or hemangiosarcomas were noted at several sites in all study groups except for the vehicle control group. The combined (whole-body) incidence in this study was somewhat above the historical control average, but was within the historical control

range, lacked any dose-response, and was similar in water control and VX-809 groups. The hemangioma / hemangiosarcoma findings in the study were not considered test article-related.

Animals receiving the positive control exhibited tumors typical of previous experience with urethane in Tg.rasH2 mice. Findings in the present study, as well as results reported in a literature review[1] of 26 studies including 555 animals per sex that was published by the same testing facility, are summarized in the table below.

Table 8. 6-month Mouse Carcinogenicity Study: Positive Control Tumors

Tumor Type (positive control animals)	Male Incidence	Male Historical	Female Incidence	Female Historical
Lung adenoma	9/10	94%	10/10	92%
Lung carcinoma	1/10	43%	2/10	73%
Spleen hemangiosarcoma	6/10	89%	8/10	92%

Lung adenoma incidence closely matched the historical data but lung carcinomas and spleen hemangiosarcomas were observed at a lower rate in the present study compared to the historical reference. Test facility historical control data for urethane-treated animals was not included in the present study report. The incidences of lung adenoma, combined lung adenoma / carcinoma and spleen hemangiosarcoma were all statistically significantly higher in male and female positive control groups compared to each control group.

Non-neoplastic

Non-neoplastic findings in the kidneys (tubular degeneration / regeneration), sternum (peripheral inflammation), lungs (alveolar hyperplasia), and preputial gland (dilation) are noted in the table below.

Table 9. 6-month Mouse Carcinogenicity Study: Non-neoplastic Lesions

Finding	Male					Female				
	V	H_20	200	700	2000	V	H_20	200	500	1500
Kidneys	***25****	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***
Degeneration, cortex, tubule	1	6	5	8	15	0	1	1	0	0
Minimal	1	4	4	7	13	0	1	1	0	0
Mild	0	2	1	1	2	0	0	0	0	0
Regeneration, cortex, tubule	3	10	7	10	18	0	0	0	0	0
Minimal	3	8	7	9	16	0	0	0	0	0
Mild	0	2	0	1	0	0	0	0	0	0
Bone, sternum	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***
Inflammation, peripheral	0	1	0	1	6	2	1	0	1	0

(b) (4)

Minimal	0	0	0	0	4	0	0	0	1	0
Mild	0	1	0	0	0	1	1	0	0	0
Moderate	0	0	0	1	2	1	0	0	0	0
Lungs	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***
Hyperplasia, alveolar epithelium	0	0	0	0	2	0	1	0	0	0
Minimal	0	0	0	0	0	0	1	0	0	0
Mild	0	0	0	0	2	0	0	0	0	0
Preputial glands	***25***	***25***	***25***	***25***	***25***					
Dilation	0	0	0	1	2					
Mild	0	0	0	1	0					
Marked	0	0	0	0	2					

* Denotes number of animals examined per group.

The major target organ of test article-related toxicity in the study was the kidneys in male mice receiving VX-809. Tubular degeneration was noted and was most prevalent in the sub-capsular cortex. According to the study pathologist, the study consisted of dilation of tubules containing sparse amounts of protein and lined with "attenuated or swollen" epithelium. Tubular regeneration was observed adjacent to these lesions in some cases, consisting of basophilia and increased numbers of cells containing hyperchromatic nuclei. The background incidence of this finding was quite high in the water (but not vehicle) control group, and a clear increase in incidence of these findings was only observed at the HD of 2000 mg/kg/day. In response to an Information Request, the sponsor provided recent background data from 6 studies (175 animals per sex) at the test facility. The background rate of degeneration was 9.14% (range 0-32%) in males and 1.71% (range 0-8%) in females. The background rate of regeneration was 8.57% (range 0-32%) in males and 1.71% (range 0-8%) in females. The historical data confirms the male-specific nature of this spontaneous finding and the rates of tubular degeneration/regeneration in the LD and MD male groups in this study (20-40%) are comparable to the upper end of the historical control data.

The test facility has published a review[2] of spontaneous non-neoplastic lesions in 26 Tg.rasH2 mouse carcinogenicity studies including 710 animals per sex. Lung alveolar hyperplasia was more common in males vs. females and the finding was observed in an average of 3.7% of male mice (individual study range 0-12%); therefore this finding was considered to be incidental. The lung hyperplasia was noted in two animals which did not have lung adenoma or carcinoma. Historical data for bone inflammation and preputial gland dilation was not present in the journal article. In response to an Information Request, the sponsor provided the following assessment of the sternum findings:

> *The peripheral inflammation observed in sternum and noted in the report is an extension of the skeletal muscle myopathy typically observed in this strain. Skeletal muscle myopathy is characterized by degeneration, regeneration, necrosis, and inflammation. Skeletal muscle myopathy is a very common spontaneous lesion in Tg.rasH2 mice and*

(b) (4)

can be seen in almost all skeletal muscle. The incidence at the Test Facility is 56-100% in males and 52-100% in females. The incidence of skeletal muscle myopathy in this study, as diagnosed in the thigh, ranged from 92 to 100% in males and 96 to 100% in females (vehicle control, de-ionized water control, and test article treated groups). The inflammation was observed in the muscle, and not the bone. When the sternum is trimmed and processed for microscopic evaluation, variable amount of skeletal muscle remains attached to the periphery of the sternum, thus contributing to the variability in the incidence of skeletal muscle myopathy/inflammation on the sternum. The variability in the trimming procedures could have led to the higher incidence in the high dose males.

Similar findings in the sternum, lungs, and preputial glands were not observed in the 28-day dose-ranging study in mice at doses up to 4000 mg/kg/day or in chronic toxicology studies conducted in rats or dogs. The reviewer does not consider these lesions to be test article-related.

Toxicokinetics

Blood samples for TK analysis were collected from control (vehicle and water) TK animals on Day 1 and Day 178 at 1 and 4 hours post-dose. Blood samples for TK analysis were collected from VX-809 groups 0.5, 1, 2, 4, 8, and 24 hours post-dose on Day 1 and Day 178. Three animals per sex were analyzed at each time point. Plasma samples were shipped to the sponsor for analysis via a validated LC/MS/MS method (LLOQ 2 ng/mL).

Toxicokinetic results calculated from wild-type satellite animals are summarized in the table below. VX-809 was not detected in plasma samples from control group animals. Exposure was slightly higher in females vs. males at 200 mg/kg and higher $AUC_{0\text{-}24hr}$ values were noted for females receiving 1500 mg/kg/day compared to the higher nominal dose of 2000 mg/kg/day for the highest male dose group. AUC and C_{max} increases were less than dose-proportional and no accumulation was observed over the course of the 6-month study. Exposure levels observed in the carcinogenicity study at the high-dose levels are quite comparable to those predicted based on the results of the 4-week dose-ranging study in transgenic mice (see nonclinical SPA reviews filed by Dr. Jane Sohn November 2, 2012 and November 30, 2012).

Table 10. 6-month Mouse Carcinogenicity Study: Toxicokinetics

Study Day	Sex	VX-809 Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ug/mL)	$AUC_{0\text{-}24hr}$ (ug*hr/mL)
Day 1	Male	200	2	60.9	646
		700	2	118	944
		2000	2	138	1350
	Female	200	2	79.2	827
		500	2	138	1460
		1500	2	194	2260
Day 178	Male	200	2	68.7	624
		700	2	99.2	934
		2000	4	114	1390

	Female	200	2	89.6	886
		500	2	148	1310
		1500	2	192	2090

Dosing Solution Analysis

Samples for stability evaluation were collected from the first 200 mg/kg and 2000 mg/kg VX-809 dosing formulations. Homogeneity of VX-809 dosing formulations was assessed by collecting samples from the top, middle, and bottom strata of the first dosing solution preparations in the study. Samples for concentration verification were obtained from the middle strata of the first, six intermediate preparations, and the final dosing solution. A VX-809 concentration in the dosing solutions was measured by HPLC.

VX-809 was not detected in any samples from the vehicle or water control dosing solutions. A single interim formulation of the low-dose (20 mg/mL) failed to meet all of the acceptance criteria (74% of target concentration, RSD 14%). All other dosing solutions met the acceptance criteria including VX-809 concentration at 85-115% of target and ≤10% RSD. The single deviation in the low-dose group was not judged to affect the interpretation of the study data.

11 Integrated Summary and Safety Evaluation

VX-809 (lumacaftor) is a proposed cystic fibrosis transmembrane conductance regulator (CFTR) (b) (4) in development for the treatment of cystic fibrosis (CF) by Vertex Pharmaceuticals. VX-809 is administered as a fixed-dose combination with the approved CFTR potentiator ivacaftor and in 2012 this regimen was granted Breakthrough Designation for the treatment of CF patients homozygous for the ΔF508 mutation. A rolling submission under NDA 206038 was completed on November 5, 2014.

Vertex submitted plans for the assessment of VX-809 carcinogenicity in rats and mice via the special protocol assessment (SPA) process. The ECAC provided its concurrence with the design of a two-year rat study evaluating VX-809 (b) (4) in meeting minutes dated November 1, 2012. In Type B meeting minutes dated March 20, 2013, the Division agreed that the results of the two-year rat carcinogenicity study need not be included in the NDA filing and could be completed as a post-marketing requirement. The ECAC provided its concurrence with the design of the six-month Tg.rasH2 transgenic mouse study in meeting minutes dated November 30, 2012. As agreed to at the pre-NDA meeting (minutes dated September 2, 2014), the sponsor submitted the final study report and datasets to the IND on September 15, 2014 and the results are reviewed in this memo and were presented to the ECAC on November 18, 2014.

In the Tg.rasH2 carcinogenicity study, 25 animals per sex received vehicle, water, or VX-809 by oral gavage for 178 days. VX-809 doses were 200, 700, or 2000 mg/kg/day for males and 200, 500 or 1500 mg/kg/day for females. In addition, 10 animals per sex received urethane via i.p. administration as a positive control.

There were no statistically significant effects of VX-809 on survival in the study. Six unscheduled deaths were noted without relationship to dose (2 vehicle males, 1 water male, 2 LD males, 1 HD female) between Day 98-172 and were attributed to hemangiosarcoma of the skin or undetermined causes. Three early deaths were noted in HD female satellite animals in the first week. There was no gross evidence of gavage error but no further histopathological assessment was conducted and the relationship to VX-809 is uncertain. A trend towards lower body weights was noted in MD and HD males, but there were no statistically significant differences vs. the control groups.

The kidneys were identified as the target organ of test article-related non-neoplastic toxicity. Findings of renal cortex tubular epithelial degeneration and regeneration were observed in HD males only. A corresponding ~10% decrease in kidney organ weights was noted.

The combined incidence of Harderian gland adenoma and carcinoma was statistically significantly increased in HD females compared to either the vehicle or water controls (20% vs. 0%; p=0.025 vs. each control and p=0.004 for trend according to the analysis of statistical reviewer Dr. Min Min). The individual incidence of adenoma (16%) and carcinoma (4%) were each within the historical control range (0-16% and 0-8%, respectively based on 26 studies conducted at the test facility) for the test facility. The combined adenoma plus carcinoma incidence of 20% was also within the historical control data (mean 3.5%, range 0-24%) No Harderian gland neoplastic lesions were noted in the female control groups (combined incidence 0/50, at the lower bound of the historical control ranges noted above). Based on the fact that the HD incidence was within the historical control data and the control group incidence was lower than would be expected, the reviewer did not judge the neoplastic findings in the Harderian gland to be a test article-related effect.

The positive control group was noted with acute body weight losses in both sexes and a statistically significant decrease in survival for males. The responsiveness of the Tg.rasH2 model to urethane, a known carcinogen, was confirmed based on the observation of lung adenomas, lung carcinomas, and spleen hemangiosarcomas in this study.

Toxicokinetic analysis was conducted in a satellite group of wild-type hemizygous mice and was consistent with the previous 28-day dose-ranging study. Exposure to VX-809 increased less than dose-proportionally and there was no evidence of accumulation over the course of the six-month study. VX-809 exposure was higher in females than males.

The results of the Tg.rasH2 mouse carcinogenicity study were presented to the ECAC (meeting minutes dated November 19, 2014 are appended to this review). The committee's conclusions were as follows:

Executive CAC Recommendations and Conclusions

Tg.rasH2 mouse:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms in the study.

In summary, VX-809 was concluded to be negative for carcinogenic potential in a valid six-month Tg.rasH2 transgenic mouse study that was designed with ECAC concurrence. These transgenic mouse studies are generally considered to provide only hazard identification, but as shown in the table below, the exposure margins between the expected human dose and the tested doses were 1.7-3.7 times in males and 2.4-5.6 times in females. No test article-related neoplastic findings were observed in Tg.rasH2 mice receiving VX-809 at doses of up to 2000 mg/kg/day in males and 1500 mg/kg/day in females.

Table 11. Exposure Margins in 6-month Tg.rasH2 Mouse Carcinogenicity Study

Study	Sex	Dose	$AUC_{0\text{-}24hr}$ (ug*hr/mL)	Exposure Margin
Human (28-day phase 2 study #102)	M/F	VX-809 400 mg BID Ivacaftor 250 mg BID	371	-
Tg.rasH2 Mouse (Carcinogenicity study)	M	200 mg/kg/day	624	1.7
		700 mg/kg/day	934	2.5
		2000 mg/kg/day	1390	3.7
	F	200 mg/kg/day	886	2.4
		500 mg/kg/day	1310	3.5
		1500 mg/kg/day	2090	5.6

12 Appendix

Executive Carcinogenicity Assessment Committee (ECAC) meeting minutes dated November 19, 2014.

Executive CAC
Date of Meeting: November 18, 2014

Committee: Paul Brown, Ph.D., OND IO, Acting Chair
Tim McGovern, Ph.D., OND IO, Member
Ron Wange, Ph.D., DMEP, Alternate Member
Timothy Robison, Ph.D., DPARP, Pharm Tox Team Leader
Andrew Goodwin, Ph.D., DPARP, Presenting Reviewer
Min Min, Ph.D., OB/DB-6, Observer (Statistics Reviewer)

Author of Minutes: Andrew Goodwin

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 206038
IND # 79521
Drug Name: Lumacaftor (VX-809)
Sponsor: Vertex Pharmaceuticals

Tg.rasH2 Mouse Carcinogenicity Study Final Results

Lumacaftor (VX-809) is a proposed cystic fibrosis transmembrane conductance regulator (CFTR) in development for the treatment of cystic fibrosis patients homozygous for the ΔF508 mutation. NDA 206038 was submitted on November 5, 2014 seeking approval for a fixed-dose combination product containing lumacaftor and ivacaftor. A two-year rat carcinogenicity study is ongoing and will be completed as a post-marketing requirement.

VX-809 was negative for genotoxic potential in an in vitro bacterial reverse mutation assay, in vitro CHO cell chromosomal aberration assay, and in vivo mouse micronucleus assay.

In a six-month oral gavage carcinogenicity study in Tg.rasH2 mice, males received VX-809 at 200, 700, or 2000 mg/kg/day and females received VX-809 at 200, 500, or 1500 mg/kg/day. Two negative control groups received vehicle (0.5% methylcellulose (400 cps), 0.5% Tween 80, 0.05% simethicone in deionized water) or deionized water and a positive control group was administered urethane.

There were no effects on survival in VX-809 groups compared to the control groups. Dose-related clinical signs in males and females included hunched posture and rapid / shallow breathing. Additional findings in males included decreased motor activity, hyperactivity, ruffled

fur, and swelling. Males treated with VX-809 showed a slight trend towards decreased body weights at 700 and 2000 mg/kg/day (-7% vs. control, not statistically significant).

Females receiving VX-809 at 1500 mg/kg/day had an increased incidence of Harderian gland adenoma (4/25 = 16%) and carcinoma (1/25 = 4%) with a combined incidence of 5/25 or 20%. Data from 26 studies conducted at the test facility indicated historical control incidences in females of 2.8% (range 0-16%), 0.7% (range 0-8%), and 3.5% (range 0-24%) for Harderian gland adenoma, carcinoma, and combined incidence, respectively. Based on the fact that the incidence at the high-dose remained within the historical control range, these findings were not considered test article-related.

There were no notable tumor findings in males at doses up to 2000 mg/kg/day.

The urethane-treated positive control group exhibited lung adenomas, lung carcinomas and spleen hemangiosarcomas. These findings were generally consistent with historical control data for this known carcinogen.

Toxicokinetic analysis showed that VX-809 exposure increased less than dose-proportionally from 200 to 1500 (females) or 2000 (males) mg/kg/day and was greater in females vs. males. At Day 178, $AUC_{0\text{-}24hr}$ values at the highest doses tested were 1390 and 2090 ug*hr/mL in males and females, respectively.

Executive CAC Recommendations and Conclusions

Tg.rasH2 mouse:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms in the study.

Paul Brown, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, DPARP
/TRobison, DPARP
/AGoodwin, DPARP
/LHann, DPARP
/ASeifried, OND IO

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

/s/

ADELE S SEIFRIED
11/19/2014

PAUL C BROWN
11/19/2014

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/s/

ANDREW C GOODWIN
11/20/2014
Review of VX-809 six month Tg.rasH2 carcincogenicity study and ECAC conclusions

TIMOTHY W ROBISON
11/20/2014
I concur

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/s/

ANDREW C GOODWIN
05/05/2015

TIMOTHY W ROBISON
05/05/2015
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206038 **Applicant: Vertex Pharma** **Stamp Date: 11/05/2014**

Drug Name: Orkambi (lumacaftor-ivacaftor) **NDA/BLA Type: 505(b)(1)**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		Project manager will obtain corrected version of one illegible figure in 2.6.2
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

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

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes.

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

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

No nonclinical review issues identified.

Andrew Goodwin, PhD — December 5, 2014
Reviewing Pharmacologist — Date

Timothy Robison, PhD, DABT — December 5, 2014
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

ANDREW C GOODWIN
11/24/2014

TIMOTHY W ROBISON
11/24/2014
I concur

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	IND 79521, NDA 206038
Supporting document/s:	SD #286 (IND), SD #1 (NDA)
Sponsor's letter date:	9/15/2014 (IND), 11/5/2014 (NDA)
CDER stamp date:	9/15/2014 (IND), 11/5/2014 (NDA)
Product:	Lumacaftor (VX-809)
Indication:	Cystic Fibrosis
Sponsor:	Vertex Pharmaceuticals
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Angela Ramsey

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

VX-809 (lumacaftor) is in late-stage development by Vertex Pharmaceuticals as a potential treatment for cystic fibrosis in a combination product that also contains the FDA-approved product ivacaftor (VX-770, tradename Kalydeco). The planned clinical dose of VX-809 is 600 mg QD or 400 mg BID in combination with ivacaftor at a dose of 250 mg BID. A pre-NDA meeting for the lumacaftor / ivacaftor combination product was held on August 12, 2014 (see minutes dated September 2, 2014) and the rolling submission under NDA 206038 was completed on November 5, 2014.

In the preliminary comments provided ahead of the pre-NDA meeting, the sponsor was requested, if feasible, to submit the results from the six-month Tg.rasH2 transgenic mouse carcinogenicity study to the IND ahead of the estimated November 2014 NDA submission to facilitate review. The sponsor submitted the final study report and datasets on September 15, 2014 and the results are reviewed in this memo and will be presented to the Executive Carcinogenicity Assessment Committee (ECAC) on November 18, 2014. Note that a two-year rat carcinogenicity study is in progress and the division has previously agreed that the results from this study may be submitted as a post marketing requirement (see memo by Dr. Timothy Robison dated July 26, 2013).

1.2 Brief Discussion of Nonclinical Findings

The carcinogenic potential of VX-809 (lumacaftor) was evaluated in a six-month oral gavage carcinogenicity study in Tg.rasH2 transgenic mice. Animals received VX-809 at doses of 200, 700 or 2000 mg/kg/day in males and 200, 500, or 1500 mg/kg/day in females. Negative control groups received vehicle or water and a positive control group received urethane. While transgenic mouse assays are generally considered to solely provide hazard identification, these doses represent approximately two- to six-fold multiples compared to the proposed clinical dose.

VX-809 had no effect on survival in the study. In male animals only, slightly lower body weight gains were noted at the higher doses. Kidney toxicity was observed in high-dose males only, manifested by decreased organ weights and histopathological findings of renal cortex tubular epithelial degeneration and regeneration.

There were no neoplastic findings related to VX-809 treatment in males. However, the combined incidence of Harderian gland adenoma and carcinoma (5/25 animals) was statistically significantly increased in high-dose females compared to the two control groups. However, the control incidence (0/50) was low compared to historical control rate of 3.5% and the combined incidence of adenoma and carcinoma in the HD group did not exceed the historical control range (0-24%). Therefore the Harderian gland finding was not considered to represent a test article-related effect.

The positive control group confirmed the responsiveness of the Tg.rasH2 model to urethane, a known carcinogen. Urethane treatment was associated with acute body weight losses in both sexes and a statistically significant decrease in survival for males. Neoplastic findings included lung adenomas, lung carcinomas, and spleen hemangiosarcomas observed in both sexes at rates generally similar to historical data.

In conclusion, VX-809 was negative for carcinogenicity in males and females in a six-month study in Tg.rasH2 mice.

1.3 Recommendations

The results of the VX-809 six-month Tg.rasH2 mouse carcinogenicity study were presented at the ECAC meeting held on November 18, 2014. Meeting minutes dated November 19, 2014 are appended to this review.

Executive CAC Recommendations and Conclusions

Tg.rasH2 mouse:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in the study.

2 Drug Information

2.1 Drug

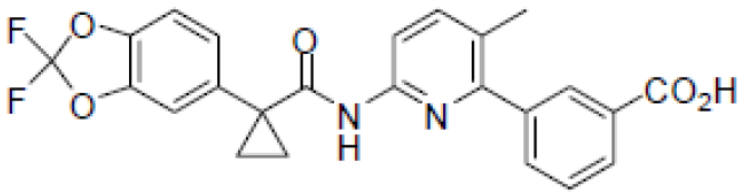
Generic Name: Lumacaftor

Code Name: VX-809

Chemical Name: 3-(6-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid

Molecular Formula/Molecular Weight: $C_{24}H_{18}F_2N_2O_5$ / 452 g/mole

Structure or Biochemical Description



Pharmacologic Class: Cystic Fibrosis Transmembrane conductance Regulator (CFTR) (b) (4) (proposed by sponsor)

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 74633 and NDA 203188 (ivacaftor / Kalydeco / VX-770)
- NDA 206038 (for lumacaftor / ivacaftor combination)

2.3 Drug Formulation

The formulation of the sponsor's fixed-dose combination tablet containing 200 mg lumacaftor and 125 mg ivacaftor is summarized in the table below. There are no novel excipients and no nonclinical concerns with the daily exposure to these inactive ingredients at the proposed daily dose of 800 mg lumacaftor and 500 mg ivacaftor (4 tablets).

Table 1. Lumacaftor – Ivacaftor Fixed-dose Combination Tablet Formulation

Component	Quality Standard	Component Function	200 mg/125 mg	
			Amount per tablet (mg)	Content (% w/w)
(b) (4)				
(b) (4)				
Lumacaftor drug substance	In house standard	Active Ingredient	200.00	(b) (4)
Ivacaftor (b) (4)	NDA 203188	Drug product	(b) (4)	(b) (4)
Microcrystalline cellulose	USP/NF			
Croscamellose sodium	USP/NF			
Sodium lauryl sulfate	USP/NF			
Povidone (b) (4)	USP/NF			
(b) (4)	USP			
	USP/NF			
	USP/NF			
Magnesium stearate (b) (4)	USP/NF			
Film Coat				
(b) (4)	DMF No. (b) (4) USP			
Print Ink				
(b) (4) Black	DMF No. (b) (4)			
Total Tablet Weight			582.5	100.0

(b) (4)

(b) (4)

2.8 Regulatory Background

The sponsor submitted a Special Protocol Assessment (SPA) request on September 24, 2012 for a 6-month carcinogenicity study in Tg.rasH2 transgenic mice. The dose-ranging toxicology studies and the proposed protocol were reviewed by Dr. Jane Sohn (November 2, 2012) and presented to the ECAC on October 30, 2012. A "no

agreement" letter was issued on November 1, 2012 due to incomplete data for the 28-day mouse study. A subsequent SPA request was received on November 16, 2012 and the ECAC provided its concurrence in meeting minutes dated November 30, 2012. The additional information provided by the sponsor was reviewed by Dr. Sohn on November 30, 2012. The committee's recommendations are summarized below.

Executive CAC Recommendations and Conclusions:

- The Committee recommended doses of 0 (vehicle), 200, 500 and 1500 mg/kg/day in females, with the high dose based on deaths at 4000 mg/kg/day, and 0 (vehicle), 200, 700, and 2000 mg/kg/day in males, with the high dose based on the limit dose for the 6-month oral carcinogenicity study in Tgras.H2 mice.
- For transgenic mice, the sponsor should conduct histopathological examination of all tissues from all dose groups.

3 Studies Submitted

3.1 Studies Reviewed

VX-809: 26-week repeated dose oral carcinogenicity study in Tg.rasH2 mice
(b) (4)
Test facility study # (b) (4) 57YX.7G8R (b) (4)
Sponsor project # VX-809-TX-019
Final report dated September 10, 2014

3.3 Previous Reviews Referenced

Nonclinical reviews filed to IND 79521:

- January 3, 2008 by Dr. Timothy Robison
- November 2, 2012 by Dr. Jane Sohn
- November 30, 2012 by Dr. Jane Sohn
- July 26, 2013 by Dr. Timothy Robison

5 Pharmacokinetics/ADME/Toxicokinetics

The following overview of lumacaftor pharmacokinetics in mice is excerpted from the nonclinical review filed by Dr. Jane Sohn November 2, 2012.

- Half-life following intravenous administration in CD-1 mice is 4.28 hours, with distribution into tissues.
- Systemic clearance was low, with clearance of ~1% compared to hepatic blood volume in mice.
- In vitro assessment of plasma protein binding in male CD-1 mouse plasma showed that 99.7-99.8% of VX-809 is bound, compared to 99.3-99.5% in human plasma.

7 Genetic Toxicology

VX-809 was found to be negative for genotoxic potential in an in vitro bacterial reverse mutation assay, in vitro Chinese Hamster Ovary (CHO) cell chromosomal aberration assay, and in vivo mouse micronucleus assay. These studies were previously reviewed by Dr. Timothy Robison (January 3, 2008).

8 Carcinogenicity

Study title: VX-809: 26-WEEK REPEATED DOSE ORAL CARCINOGENICITY STUDY IN Tg.rasH2 MICE

Study no.:	Test facility #(b) (4)57YX.7G8R.BTL Sponsor #VX-809-TX-019
Study report location:	EDR SD #286 (9/15/2014)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Study initiation 10/9/2012 First dosing 1/7/2013 Final sacrifice 7/11/2013 Final report dated 9/10/2014
GLP compliance:	Yes, signed statement
QA statement:	Yes, signed statement
Drug, lot #, and % purity:	VX-809 Lot 4281-P54-12-401 99.7% purity
CAC concurrence:	Yes, see nonclinical review by Dr. Jane Sohn and ECAC meeting minutes dated November 30, 2012.

Key Study Findings

- In a six-month oral gavage carcinogenicity study in Tg.rasH2 mice, males received VX-809 at 200, 700, or 2000 mg/kg/day and females received VX-809 at 200, 500, or 1500 mg/kg/day. Two negative control groups received water or vehicle (0.5% methylcellulose (400 cps), 0.5% Tween 80, 0.05% simethicone in deionized water) and a positive control group was administered urethane.
- There were no effects on survival in VX-809 groups compared to the control groups. None of the 10 unscheduled deaths were clearly attributable to the test article; however, three satellite females receiving the HD of 1500 mg/kg/day died of unknown causes in the first week and were replaced.
- Dose-related clinical signs in males and females included hunched posture and rapid / shallow breathing. Additional finding in males included decreased motor activity, hyperactivity, ruffled fur, and swelling.
- Males treated with VX-809 showed a slight (but not statistically significant) trend towards decreased body weight gains at 700 and 2000 mg/kg/day. The reviewer

did not judge the effect to be adverse. VX-809 had no effect on body weights in females.

- The kidneys were identified as a target organ of toxicity in males receiving 2000 mg/kg/day VX-809. Absolute kidney weights were decreased by approximately 10% vs. controls and histopathological findings of cortex tubular degeneration and regeneration were noted.
- Females receiving VX-809 at 1500 mg/kg/day had an increased incidence of Harderian gland adenoma (4/25 = 16%) and carcinoma (1/25 = 4%) with a combined incidence of 5/25 or 20%. This incidence was statistically significantly higher than the vehicle and water control groups (p=0.025 vs. each control group and p=0.004 for trend); however, the combined incidence was within the historical control range (0-24%). There were no notable tumor findings in males at doses up to 2000 mg/kg/day.
- The urethane-treated positive control group exhibited lung adenoma, lung carcinoma and spleen hemangiosarcoma. These findings were generally consistent with historical control data for this known carcinogen.
- Toxicokinetic analysis showed that VX-809 exposure increased less than dose-proportionally from 200 to 1500 (females) or 2000 (males) mg/kg/day and was greater in females vs. males. At Day 178, $AUC_{0\text{-}24hr}$ values at the highest doses tested were 1390 and 2090 ug*hr/mL in males and females, respectively. The exposure levels at the HD correspond to 3.7x and 5.6x margins to the proposed clinical dose.

Adequacy of Carcinogenicity Study

The high-dose of 2000 mg/kg/day in males represents the limit dose for carcinogenicity studies. The high dose of 1500 mg/kg/day was set at approximately one-third the lethal dose of 4000 mg/kg/day in a 28-day dose-ranging study in mice. There were no test article-related body weight changes or other adverse effects suggestive of the MTD being exceeded in females receiving 1500 mg/kg/day VX-809. The ECAC provided its concurrence with the dose levels employed in meeting minutes dated November 30, 2012 and this study to evaluate the carcinogenic potential of VX-809 in Tg.rasH2 mice is considered adequate.

Appropriateness of Test Models

The Tg.rasH2 transgenic mouse strain is an acceptable model for evaluating the carcinogenic potential of pharmaceuticals per ICH *Guidance for Industry S1B Testing for Carcinogenicity of Pharmaceuticals*.

Evaluation of Tumor Findings

- There were no test article-related statistically significant tumor findings in male Tg.rasH2 mice at VX-809 doses up to 2000 mg/kg/day.
- The combined incidence of Harderian gland adenoma and carcinoma was statistically significantly increased in females receiving the highest VX-809 dose of 1500 mg/kg/day compared to the vehicle and water control groups (p=0.025

vs. each control and p=0.004 for trend). However, the control incidence was low compared to historical control rates and the incidences of these neoplasias in the HD group were within the historical control ranges. Therefore the Harderian gland finding was not considered to represent a test article-related effect.

- The urethane-treated positive control group exhibited lung adenoma, lung carcinoma and spleen hemangiosarcoma, which are expected findings in response to exposure to this known carcinogen.
- VX-809 was concluded to be negative for carcinogenicity in this transgenic mouse study.

Methods

Doses:	Vehicle control Deionized water control 1000 mg/kg urethane (positive control administered by i.p. injection on Days 1, 3, 5) *Males* 200 mg/kg/day VX-809 700 mg/kg/day VX-809 2000 mg/kg/day VX-809 *Females* 200 mg/kg/day VX-809 500 mg/kg/day VX-809 1500 mg/kg/day VX-809
Frequency of dosing:	Once daily for 26 weeks
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose (400 cps), 0.5% Tween 80, 0.05% simethicone in deionized water
Basis of dose selection:	Dose selection was based on results of a 28-day study in transgenic mice at 1000, 2000, and 4000 mg/kg/day. Dose-limiting mortality and kidney toxicity (bilateral tubular degeneration) were observed in females only at 4000 mg/kg/day. The high-dose for males was therefore set at the limit dose of 2000 mg/kg. The high-dose for females was set at 1500 mg/kg, approximately one-third the lethal dose. Lower doses were based on AUC spacing.
Species/Strain:	Hemizygous Tg.rasH2 mice (Main cohort) Wild-type CByB6F1 (TK cohort) (b) (4)
Number/Sex/Group:	25 (Main cohort vehicle, water, and VX-809) 10 (Main cohort positive control) 14 (TK cohort vehicle and water groups) 38 (TK cohort at each VX-809 dose level)
Age:	7 weeks old at start of dosing 18.1-23.6 grams (males) 15.4-19.7 grams (females)
Animal housing:	Individual housing following randomization
Paradigm for dietary restriction:	None. Ad libitum access to Harlan TEKLAD Global Diet #2018CM (18% protein).
Dual control employed:	See above.
Interim sacrifice:	NA
Satellite groups:	Wild-type TK groups as described above.
Deviation from study protocol:	Three extra 1500 mg/kg female TK animals

were added to replace early deaths on Day 6. The deviation log was reviewed and all deviations were judged to be minor and did not affect the integrity or interpretation of the study.

Observations and Results

Mortality

All animals were observed twice daily at least 6 hours apart for moribundity and mortality.

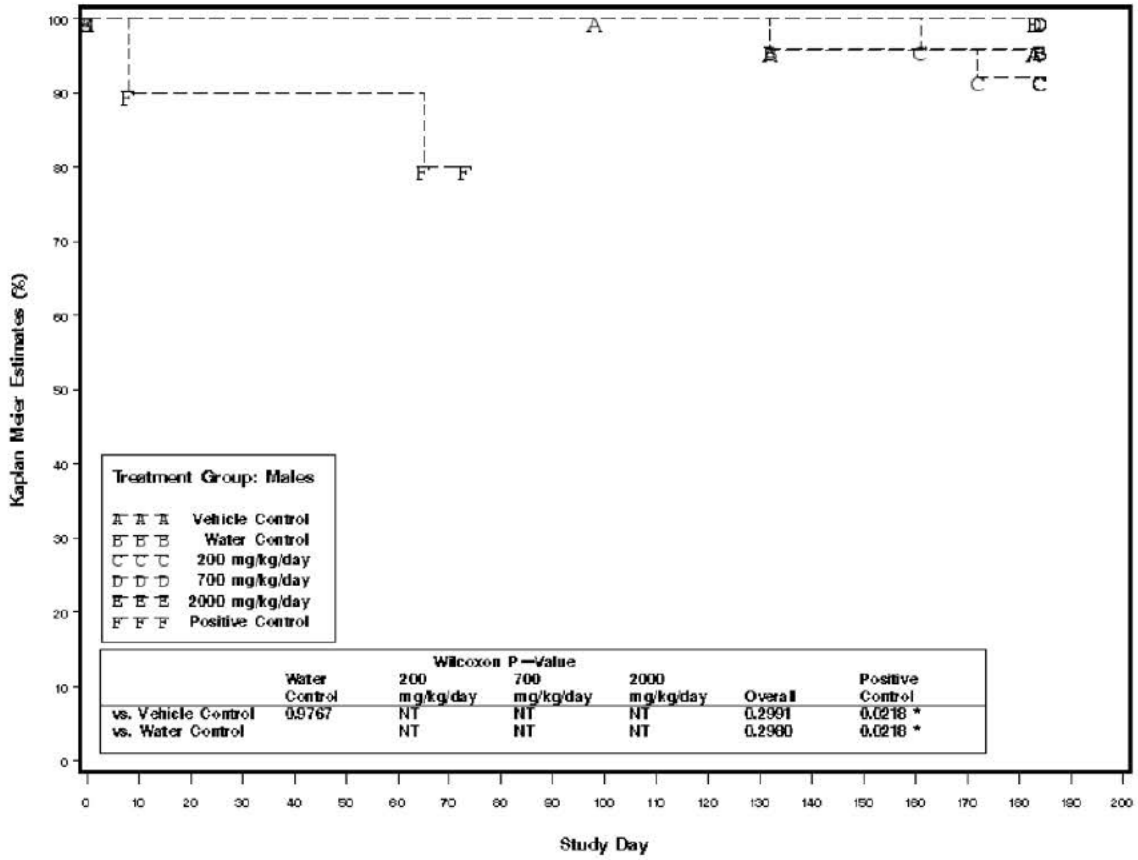
There were six unscheduled deaths in the main study cohort:

- Vehicle control male #2103 – moribund sacrifice Day 132, undetermined cause.
- Vehicle control male #2122 was sacrificed based on injuries and clinical signs following being dropped (Day 98).
- Water control male #2141 – found dead Day 132, undetermined cause.
- 200 mg/kg (LD) VX-809 male #2155 found dead, attributed to primary malignant hemangiosarcoma of the skin on Day 161.
- 200 mg/kg (LD) VX-809 male #2163 found dead Day 172, undetermined cause.
- 1500 mg/kg (HD) VX-809 female #2352 moribund sacrifice Day 161 attributed to primary malignant hemangiosarcoma of the skin.

In addition, among HD TK animals, one male (moribund sacrifice Day 161) and three female (found dead or moribund sacrifice Day 6) did not survive to scheduled necropsy. There was no gross evidence of gavage error but there was no further histopathological examination. Two positive control males also died prior to the scheduled necropsy, one on Day 8 of undetermined cause and one on Day 65 attributed to lung tumors.

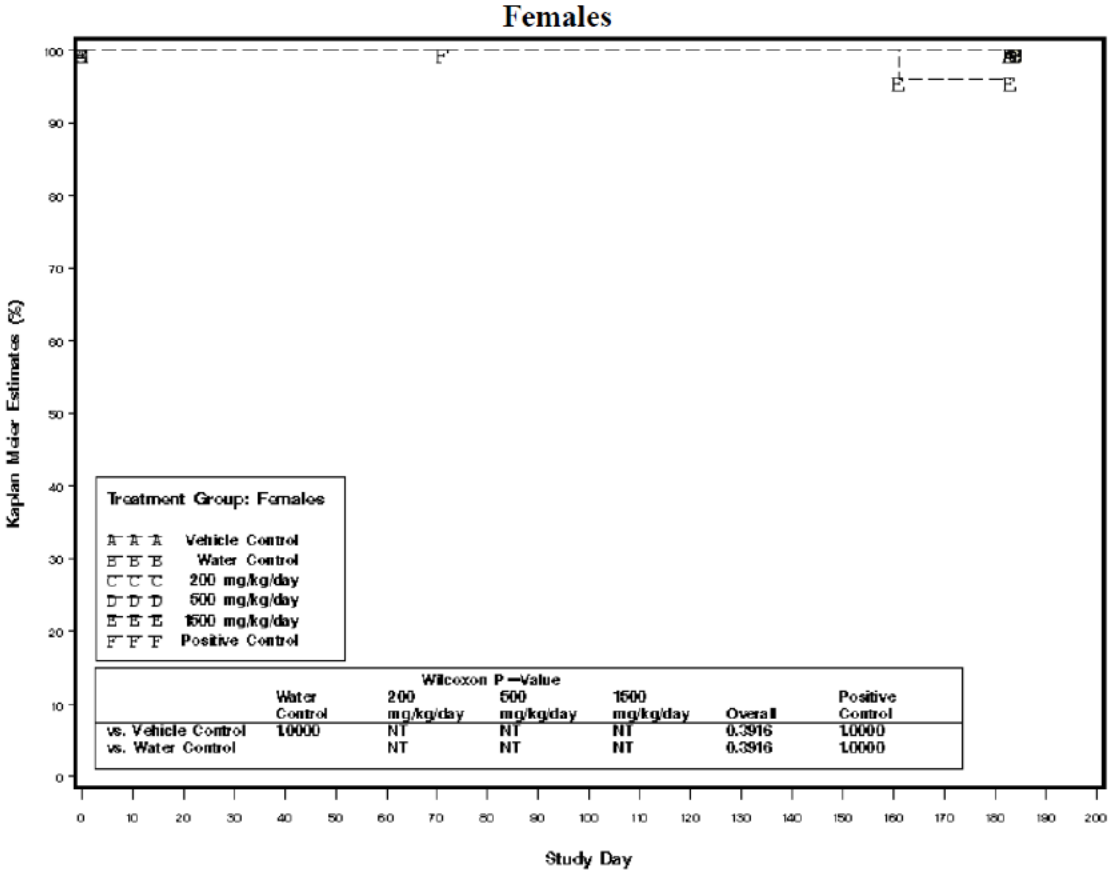
The only statistically significant effect on survival was observed in the male positive control group compared to the two control groups. The test article VX-809 was not associated with any effect on survival in male or females.

Figure 1. 6-month Mouse Carcinogenicity Study: Male Survival



* - statistically significant: NT – Not tested due to non-significant overall comparison across all groups.

Figure 2. 6-month Mouse Carcinogenicity Study: Female Survival



* - statistically significant; NT – Not tested due to non-significant overall comparison across all groups.

Clinical Signs

Cage-side observations were conducted on each day of dosing, within approximately 2 hours of the end of dosing. Detailed physical examinations were performed on Day 1 and weekly throughout the study.

There were no dose-related findings during cage-side observations in males. A single HD female was noted with eye discharge as well as red discoloration and swelling of the nose from Day 156-158. Test article-related findings observed at the detailed physical examinations are summarized in the table below.

Table 2. 6-month Mouse Carcinogenicity Study: Clinical Signs

Finding	Males (mg/kg/day VX-809)				
	Vehicle	Water	200	700	2000
Decreased motor activity	0	0	0	51/10	37/7
Hyperactive	0	0	0	38/5	12/5
Ruffled fur	0	0	2/2	8/4	34/8
Hunched	0	0	0	29/7	9/3
Rapid and shallow breathing	3/2	0	16/9	67/15	81/15

Swelling	1/1	0	0	14/2	46/7
Finding	**Females (mg/kg/day VX-809)**				
	Vehicle	**Water**	**200**	**500**	**1500**
Hunched	0	0	0	5/3	0
Rapid and shallow breathing	0	2/1	2/1	9/6	9/8

Total # of observations / # of affected animals shown.

Consistent with urethane toxicity, positive control animals exhibited prostrate posture, labored / rapid / shallow breathing, ataxia, decreased motor activity, thinness and/or ruffled fur.

Body Weights

Body weights were recorded for main study and TK animals before dosing, weekly through Week 13 and biweekly thereafter.

Body weight curves for males and females were generated by the reviewer based on the sponsor's raw data and are presented in the figures below. There were no consistent statistically significant effects on body weights. Males receiving the 2000 mg/kg HD (and to a lesser extent the 700 mg/kg MD) of VX-809 generally trended towards lower body weights, but this effect was not seen in females receiving the nominally lower HD of 1500 mg/kg/day. Positive control animals of each sex experienced acute body weight decreases but then recovered after cessation of urethane dosing and grew more quickly that the other groups. Differences in absolute body weights compared to control are summarized in the table below.

Table 3. 6-month Mouse Carcinogenicity Study: Body Weights

Parameter	Sex	Control	LD	MD	HD
Body Weight (% difference from controls at Day 183)	M	Vehicle	2	-4	-4
		Water	-1	-7	-7
	F	Vehicle	-4	-3	-2
		Water	-3	-2	-1

Figure 3. 6-month Mouse Carcinogenicity Study: Male Body Weights

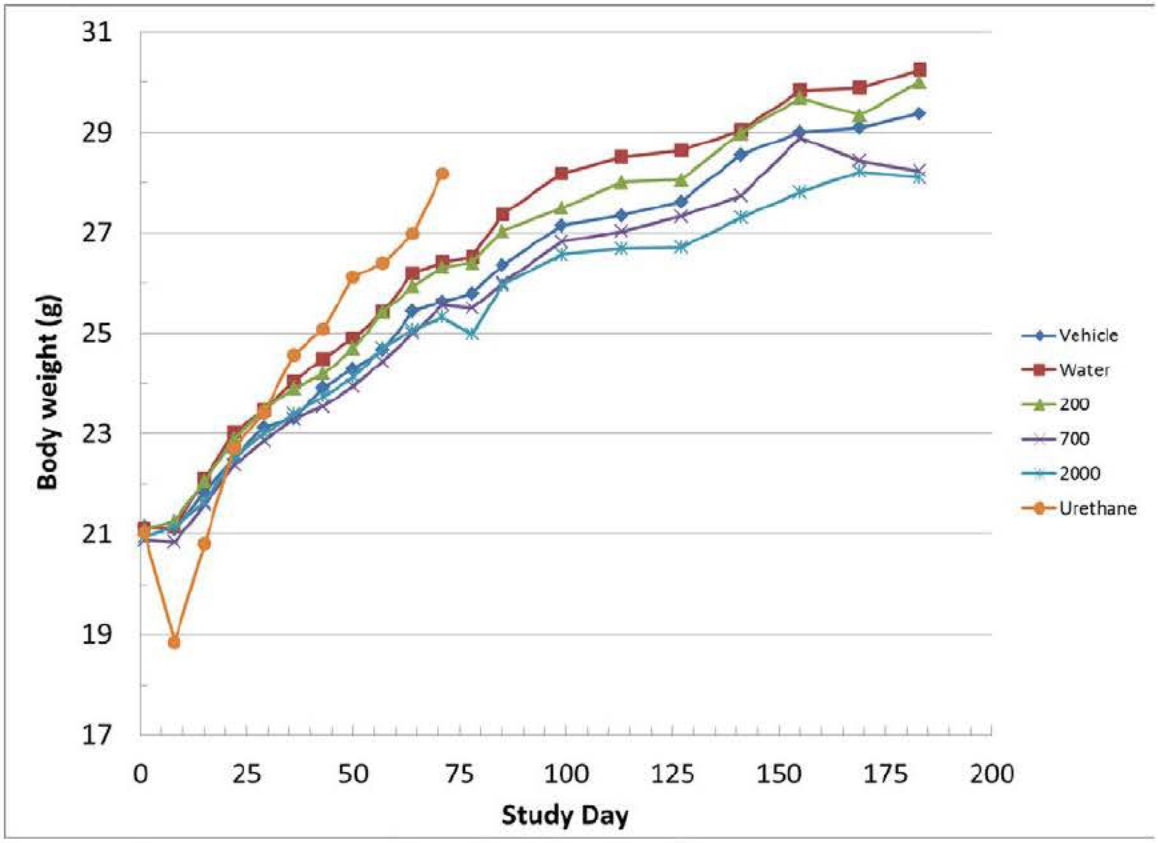
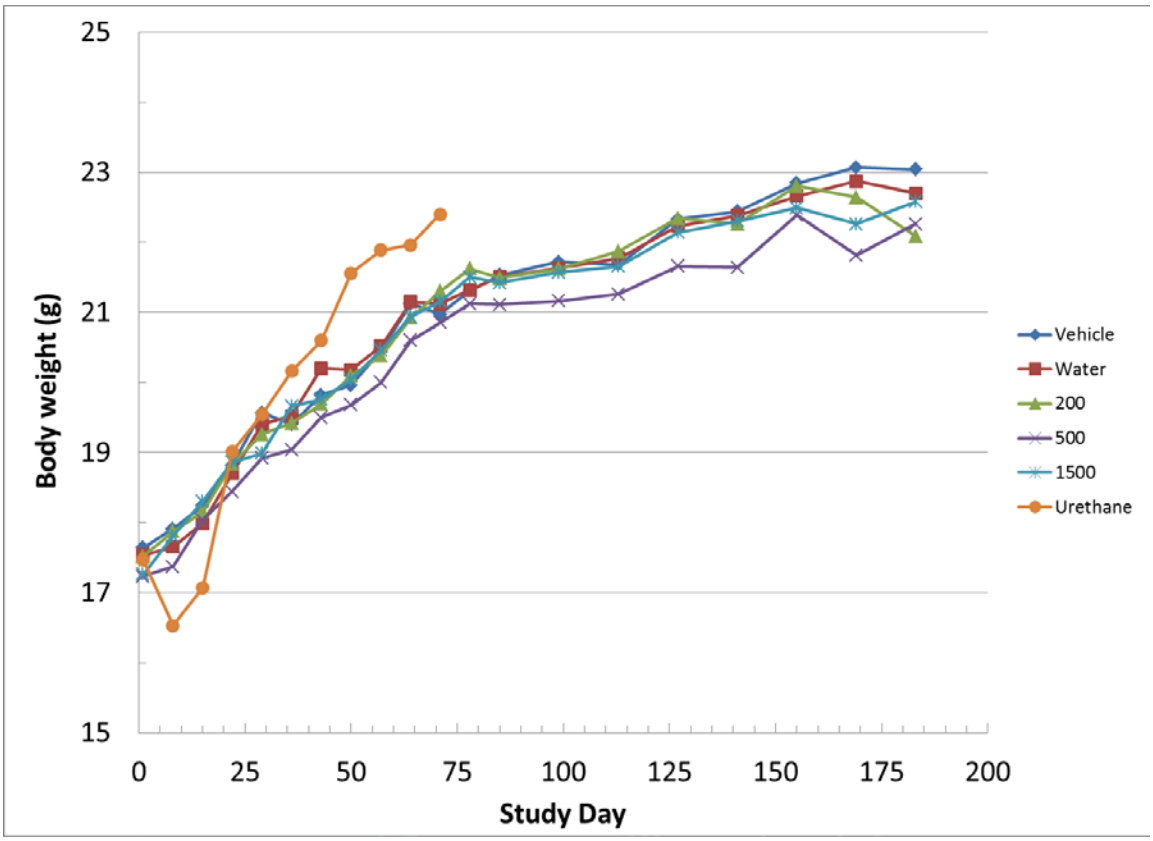


Figure 4. 6-month Mouse Carcinogenicity Study: Female Body Weights



Feed Consumption

Food consumption was recorded for main study animals only on Day 1 and weekly throughout the study.

There were no effects on food consumption in mice receiving VX-809 in the study.

Gross Pathology

Positive control animals were sacrificed on Day 71 (females) or Day 73 (males) and subjected to a full necropsy. A complete necropsy was performed on main study animals sacrificed during Week 26.

There were no notable test article-related gross pathology findings in the study.

Organ Weights

The following organ weights were collected from main study negative control and VX-809 groups: adrenal glands, brain, heart, kidneys, liver, spleen, testes and ovaries.

Test article-related organ weight differences were noted for the kidneys and liver. Kidney weights were up to 11% lower in males receiving VX-809, a difference that was

statistically significant only for the HD group using absolute organ weight values. This observation correlated with the histopathology findings of tubular degeneration / regeneration in the kidneys of the male HD group and was not observed in females. Increased liver organ weights of up to 16% compared to controls were noted, a difference that was generally statistically significant compared to the vehicle and water controls at the HD on both an absolute and body weight-adjusted basis.

Table 4. 6-month Mouse Carcinogenicity Study: Organ Weights

Organ	Sex	Control	LD	MD	HD
Kidney (Absolute)	M	Vehicle	2%	-2%	**-8%**
		Water	-1%	-5%	**-11%**
	F	Vehicle	-3%	-6%	-2%
		Water	-2%	-6%	-2%
Kidney (BW-adjusted)	M	Vehicle	1%	2%	-4%
		Water	0%	0%	-5%
	F	Vehicle	0%	-3%	0%
		Water	0%	-3%	0%
Liver (Absolute)	M	Vehicle	4%	3%	6%
		Water	7%	6%	9%
	F	Vehicle	2%	-1%	**13%**
		Water	1%	-1%	**12%**
Liver (BW-adjusted)	M	Vehicle	1%	**7%**	**10%**
		Water	**7%**	**12%**	**16%**
	F	Vehicle	4%	3%	**15%**
		Water	3%	2%	**14%**

Bold font denotes statistically significant difference vs. the specified control

Histopathology

Organs and tissues listed in the table below were prepared for microscopic examination. The final pathology report was signed December 12, 2013 by Study Pathologist (b) (4)

Table 5. 6-month Mouse Carcinogenicity Study: Histopathology Catalog

Tissues/Organs	
Adrenal glands	Nasal cavity
Aorta	Ovaries
Bone (femur and sternum)	Pancreas
Bone marrow (femur and sternum)	Parathyroid glands
Brain	Pituitary gland
Epididymides	Prostate gland
Esophagus	Salivary gland
Eyes	Sciatic nerve
Gall bladder	Seminal vesicles
Gross lesions	Skeletal muscle (thigh)
Harderian gland	Small intestine (duodenum, jejunum, and ileum)
Heart	
Kidneys	Spinal cord (cervical, thoracic, and lumbar)
Large intestine (cecum, colon, rectum)	
Liver	Spleen
Lungs and bronchi	Stomach
Lymph nodes (mesenteric and mandibular)	Testes
	Thymus
Skin from mammary area (male and female mice)	Thyroid glands
	Trachea
Mammary gland (females only)	Urinary bladder
	Uterus
	Vagina

Peer Review

The peer review pathologist (b) (4) re-examined all tissues form 10% of control and 20% of VX-809 animals, plus all unscheduled deaths. All hyperplasias and neoplasias, all kidneys from males, and all lungs / spleen from positive controls were also reviewed. A signed peer review statement was included in the final study report.

Neoplastic

Tumors observed in vehicle control, water control, and VX-809 treatment groups are summarized in the table below. The sponsor's statistical analysis consisted on one-sided comparisons of VX-809 groups to each of the controls, with significance evaluated at both the $p<0.01$ and 0.05 levels.

Table 6. 6-month Mouse Carcinogenicity Study: Neoplastic Lesions

Site / Neoplasia	Sex	V	H_20	LD[1]	MD[2]	HD[3]	Historical Control Data[4]
Number Examined:		***25***	***25***	***25***	***25***	***25***	
Ear							
Papilloma	M	0	0	0	0	0	0.56% (0-8%)[5]
	F	0	0	0	0	1	0.14% (0-4%)

Harderian Glands							
Adenoma	M	0	0	0	0	0	1.4% (0-8%)
	F	0	0	1	1	**4**[#]	2.8% (0-16%)
Carcinoma	M	0	0	0	0	0	0.14% (0-4%)
	F	0	0	0	0	1	0.70% (0-8%)
Combined	M	0	0	0	0	0	1.5% (0-12%)
	F	0	0	1	1	**5***	3.5% (0-24%)
Liver							
Hepatocellular adenoma	M	0	0	0	2	0	0.28% (0-4%)
	F	0	0	0	0	0	0% (0-0%)
Lungs with Bronchi							
Alveolar-bronchiolar adenoma	M	0	3	3	3	3	10% (0-24%)
	F	4	2	3	2	3	5.8% (0-24%)
Alveolar-bronchiolar carcinoma	M	0	1	0	1	0	0.56% (0-8%)
	F	0	0	0	0	0	1.3% (0-4%)
Combined	M	0	3	3	4	3	12% (0-32%)
	F	4	2	3	2	3	7.7% (0-28%)
Multiple Organs							
Hemangiosarcoma, skin	M	0	0	1	0	0	0.14% (0-4%)
	F	0	1	0	0	1	0.70% (0-8%)
Hemangiosarcoma, spleen	M	0	1	0	2	3	3.7% (0-16%)
	F	0	3	2	1	1	3.7% (0-16%)
Hemangioma, epididymides	M	0	0	0	1	0	0.14% (0-4%)
Hemangiosarcoma, vagina	F	0	0	1	0	1	0.14% (0-4%)
Combined (Whole-Body)	M	0	1	1	3	3	5.9% (NA)
	F	0	4	3	1	3	7.2% (NA)
Stomach							
Papilloma	M	0	0	0	0	0	0.28% (0-4%)
	F	0	0	0	0	1	0.70% (0-4%)
Thymus							
Thymoma	M	0	0	0	1	0	0.14% (0-4%)
	F	0	0	0	0	1	0.56% (0-4%)

[1]LD = 200 mg/kg/day in both males and females
[2]MD = 700 mg/kg/day in males and 500 mg/kg/day in females
[3]HD = 2000 mg/kg/day in males and 1500 mg/kg/day in females
[4]Data displayed represent mean and range from test facility historical control data for 26 studies covering 1420 animals. Note that in this case the data included in the study report was used, not that reported in the (b) (4) publication
[5]Represents historical data for "skin papilloma"
[#]Denoted statistically significant difference in terms of dose-response trend (p=0.013)
*****Denotes statistically significant difference in terms of dose-response trend (p=0.004) and vs. water and vehicle control groups (p=0.025) according to statistical reviewer Dr. Min Min.

Several tumors occurred with singular incidence in male and/or female high dose groups in the study, including ear papilloma, stomach papilloma, and thymus thymoma. These neoplastic lesions occurred at rates that were within historical controls ranges and were not statistically significantly different from either control group. Hepatocellular adenoma was observed in two MD males, an 8% (2/25) incidence that exceeded the

historical control range of 0-4%. However, due to the lack of dose response and there being no statistically significant difference vs. the control groups, this neoplastic finding in the liver was not considered to be test article-related.

The combined incidence of lung adenoma / carcinoma in MD males was 4/25 (16%), consisting of three adenomas and one carcinoma. This incidence was statistically significantly higher than the vehicle control group (0/25, p=0.0237). However, the reviewer did not consider the lung tumors to be a test article-related effect in males. In response to an Information Request, the sponsor clarified that, in the historical data from this test facility, animals presenting with both lung adenoma and carcinoma were scored as carcinoma only. Therefore the historical control range for the combined lung tumor incidence is the sum of the adenoma and carcinoma historical control ranges, or up to 32% and 28% in males and females, respectively. The statistical finding appears to be driven by the unexpected absence of lung neoplasias in the male vehicle control group as compared to historical control data and the concurrent water control group. The incidence of neoplastic lesions in the lungs was 8-16% in all other control and VX-809 groups, with no relationship to dose and well within the expected ranges based on historical control data.

Increased numbers of Harderian gland neoplasias were noted in HD females in the study, with an incidence of 16% (4/25 animals) for adenoma and an additional animal with carcinoma. There were no neoplastic findings in the Harderian glands from the vehicle or water control group animals. The individual incidences of adenoma (16%) and carcinoma (4%) were each at the upper bound of the respective historical control ranges. The tumor findings were reviewed by statistician Dr. Min Min. The statistical review confirmed the statistically significant differences in Harderian gland tumors in females, whether evaluated for dose response (adenoma and combination of adenoma / carcinoma) or in comparison to the control groups (combination of adenoma / carcinoma only). Dr. Min's summary data (from a draft review provided via email on October 31, 2014) is shown in the table below.

Table 7. 6-month Carcinogenicity Study: Statistical Analysis of Harderian Gland Tumors in Females

Organ Name	Tumor Name	Vehicle Cont N=25	200 mg Low N=25	700 mg Med N=25	2000 mg High N=25	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Female									
Harderian_gland	ADENOMA+CARCONOMA	0	1	1	5	0.004	0.500	0.500	0.025
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	20%				
harderian gland	adenoma	0	1	1	4	0.013	0.500	0.500	0.055
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	16%				

Organ Name	Tumor Name	Water Cont N=25	200 mg Low N=25	500 mg Med N=25	1500 mg High N=25	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Harderian_gland	ADENOMA+CARCONOMA	0	1	1	5	0.004	0.500	0.500	0.025
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	20%				
harderian gland	adenoma	0	1	1	4	0.013	0.500	0.500	0.055
	#EXAMINED ANIMALS	(25)	(25)	(25)	(25)	.	.	.	.
	PERCENTAGE	0%	4%	4%	16%				

As described above for the lung tumors, the sponsor provided clarification regarding the historical control ranges for the combined incidence of Harderian gland adenoma plus carcinoma. The combined historical control incidence for the test facility is up to 24%, which is greater than the combined 20% incidence in this study.. The overall historical control rate for Harderian gland adenoma in female Tg.rasH2 mice at the test facility is 2.8% (range of 0-16%), therefore the incidence of 0/50 in the two control groups in the present study is considered to represent an atypically low event rate. Taking this into account, along with the incidence of adenoma, carcinoma and the combination being within the historical control ranges, the reviewer concludes that the Harderian gland adenoma / carcinoma findings do not represent a test article-related effect.

Hemangiomas or hemangiosarcomas were noted at several sites in all study groups except for the vehicle control group. The combined (whole-body) incidence in this study was somewhat above the historical control average, but was within the historical control

range, lacked any dose-response, and was similar in water control and VX-809 groups. The hemangioma / hemangiosarcoma findings in the study were not considered test article-related.

Animals receiving the positive control exhibited tumors typical of previous experience with urethane in Tg.rasH2 mice. Findings in the present study, as well as results reported in a literature review[1] of 26 studies including 555 animals per sex that was published by the same testing facility, are summarized in the table below.

Table 8. 6-month Mouse Carcinogenicity Study: Positive Control Tumors

Tumor Type (positive control animals)	Male Incidence	Male Historical	Female Incidence	Female Historical
Lung adenoma	9/10	94%	10/10	92%
Lung carcinoma	1/10	43%	2/10	73%
Spleen hemangiosarcoma	6/10	89%	8/10	92%

Lung adenoma incidence closely matched the historical data but lung carcinomas and spleen hemangiosarcomas were observed at a lower rate in the present study compared to the historical reference. Test facility historical control data for urethane-treated animals was not included in the present study report. The incidences of lung adenoma, combined lung adenoma / carcinoma and spleen hemangiosarcoma were all statistically significantly higher in male and female positive control groups compared to each control group.

Non-neoplastic

Non-neoplastic findings in the kidneys (tubular degeneration / regeneration), sternum (peripheral inflammation), lungs (alveolar hyperplasia), and preputial gland (dilation) are noted in the table below.

Table 9. 6-month Mouse Carcinogenicity Study: Non-neoplastic Lesions

Finding	Male					Female				
	V	H_20	200	700	2000	V	H_20	200	500	1500
Kidneys	***25****	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***
Degeneration, cortex, tubule	1	6	5	8	15	0	1	1	0	0
Minimal	1	4	4	7	13	0	1	1	0	0
Mild	0	2	1	1	2	0	0	0	0	0
Regeneration, cortex, tubule	3	10	7	10	18	0	0	0	0	0
Minimal	3	8	7	9	16	0	0	0	0	0
Mild	0	2	0	1	0	0	0	0	0	0
Bone, sternum	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***
Inflammation, peripheral	0	1	0	1	6	2	1	0	1	0

(b) (4)

Minimal	0	0	0	0	4	0	0	0	1	0
Mild	0	1	0	0	0	1	1	0	0	0
Moderate	0	0	0	1	2	1	0	0	0	0
Lungs	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***	***25***
Hyperplasia, alveolar epithelium	0	0	0	0	2	0	1	0	0	0
Minimal	0	0	0	0	0	0	1	0	0	0
Mild	0	0	0	0	2	0	0	0	0	0
Preputial glands	***25***	***25***	***25***	***25***	***25***					
Dilation	0	0	0	1	2					
Mild	0	0	0	1	0					
Marked	0	0	0	0	2					

* Denotes number of animals examined per group.

The major target organ of test article-related toxicity in the study was the kidneys in male mice receiving VX-809. Tubular degeneration was noted and was most prevalent in the sub-capsular cortex. According to the study pathologist, the study consisted of dilation of tubules containing sparse amounts of protein and lined with "attenuated or swollen" epithelium. Tubular regeneration was observed adjacent to these lesions in some cases, consisting of basophilia and increased numbers of cells containing hyperchromatic nuclei. The background incidence of this finding was quite high in the water (but not vehicle) control group, and a clear increase in incidence of these findings was only observed at the HD of 2000 mg/kg/day. In response to an Information Request, the sponsor provided recent background data from 6 studies (175 animals per sex) at the test facility. The background rate of degeneration was 9.14% (range 0-32%) in males and 1.71% (range 0-8%) in females. The background rate of regeneration was 8.57% (range 0-32%) in males and 1.71% (range 0-8%) in females. The historical data confirms the male-specific nature of this spontaneous finding and the rates of tubular degeneration/regeneration in the LD and MD male groups in this study (20-40%) are comparable to the upper end of the historical control data.

The test facility has published a review[2] of spontaneous non-neoplastic lesions in 26 Tg.rasH2 mouse carcinogenicity studies including 710 animals per sex. Lung alveolar hyperplasia was more common in males vs. females and the finding was observed in an average of 3.7% of male mice (individual study range 0-12%); therefore this finding was considered to be incidental. The lung hyperplasia was noted in two animals which did not have lung adenoma or carcinoma. Historical data for bone inflammation and preputial gland dilation was not present in the journal article. In response to an Information Request, the sponsor provided the following assessment of the sternum findings:

> *The peripheral inflammation observed in sternum and noted in the report is an extension of the skeletal muscle myopathy typically observed in this strain. Skeletal muscle myopathy is characterized by degeneration, regeneration, necrosis, and inflammation. Skeletal muscle myopathy is a very common spontaneous lesion in Tg.rasH2 mice and*

(b) (4)

can be seen in almost all skeletal muscle. The incidence at the Test Facility is 56-100% in males and 52-100% in females. The incidence of skeletal muscle myopathy in this study, as diagnosed in the thigh, ranged from 92 to 100% in males and 96 to 100% in females (vehicle control, de-ionized water control, and test article treated groups). The inflammation was observed in the muscle, and not the bone. When the sternum is trimmed and processed for microscopic evaluation, variable amount of skeletal muscle remains attached to the periphery of the sternum, thus contributing to the variability in the incidence of skeletal muscle myopathy/inflammation on the sternum. The variability in the trimming procedures could have led to the higher incidence in the high dose males.

Similar findings in the sternum, lungs, and preputial glands were not observed in the 28-day dose-ranging study in mice at doses up to 4000 mg/kg/day or in chronic toxicology studies conducted in rats or dogs. The reviewer does not consider these lesions to be test article-related.

Toxicokinetics

Blood samples for TK analysis were collected from control (vehicle and water) TK animals on Day 1 and Day 178 at 1 and 4 hours post-dose. Blood samples for TK analysis were collected from VX-809 groups 0.5, 1, 2, 4, 8, and 24 hours post-dose on Day 1 and Day 178. Three animals per sex were analyzed at each time point. Plasma samples were shipped to the sponsor for analysis via a validated LC/MS/MS method (LLOQ 2 ng/mL).

Toxicokinetic results calculated from wild-type satellite animals are summarized in the table below. VX-809 was not detected in plasma samples from control group animals. Exposure was slightly higher in females vs. males at 200 mg/kg and higher $AUC_{0\text{-}24hr}$ values were noted for females receiving 1500 mg/kg/day compared to the higher nominal dose of 2000 mg/kg/day for the highest male dose group. AUC and C_{max} increases were less than dose-proportional and no accumulation was observed over the course of the 6-month study. Exposure levels observed in the carcinogenicity study at the high-dose levels are quite comparable to those predicted based on the results of the 4-week dose-ranging study in transgenic mice (see nonclinical SPA reviews filed by Dr. Jane Sohn November 2, 2012 and November 30, 2012).

Table 10. 6-month Mouse Carcinogenicity Study: Toxicokinetics

Study Day	Sex	VX-809 Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ug/mL)	$AUC_{0\text{-}24hr}$ (ug*hr/mL)
Day 1	Male	200	2	60.9	646
		700	2	118	944
		2000	2	138	1350
	Female	200	2	79.2	827
		500	2	138	1460
		1500	2	194	2260
Day 178	Male	200	2	68.7	624
		700	2	99.2	934
		2000	4	114	1390

	Female	200	2	89.6	886
		500	2	148	1310
		1500	2	192	2090

Dosing Solution Analysis

Samples for stability evaluation were collected from the first 200 mg/kg and 2000 mg/kg VX-809 dosing formulations. Homogeneity of VX-809 dosing formulations was assessed by collecting samples from the top, middle, and bottom strata of the first dosing solution preparations in the study. Samples for concentration verification were obtained from the middle strata of the first, six intermediate preparations, and the final dosing solution. A VX-809 concentration in the dosing solutions was measured by HPLC.

VX-809 was not detected in any samples from the vehicle or water control dosing solutions. A single interim formulation of the low-dose (20 mg/mL) failed to meet all of the acceptance criteria (74% of target concentration, RSD 14%). All other dosing solutions met the acceptance criteria including VX-809 concentration at 85-115% of target and ≤10% RSD. The single deviation in the low-dose group was not judged to affect the interpretation of the study data.

11 Integrated Summary and Safety Evaluation

VX-809 (lumacaftor) is a proposed cystic fibrosis transmembrane conductance regulator (CFTR) (b) (4) in development for the treatment of cystic fibrosis (CF) by Vertex Pharmaceuticals. VX-809 is administered as a fixed-dose combination with the approved CFTR potentiator ivacaftor and in 2012 this regimen was granted Breakthrough Designation for the treatment of CF patients homozygous for the ΔF508 mutation. A rolling submission under NDA 206038 was completed on November 5, 2014.

Vertex submitted plans for the assessment of VX-809 carcinogenicity in rats and mice via the special protocol assessment (SPA) process. The ECAC provided its concurrence with the design of a two-year rat study evaluating VX-809 (b) (4) in meeting minutes dated November 1, 2012. In Type B meeting minutes dated March 20, 2013, the Division agreed that the results of the two-year rat carcinogenicity study need not be included in the NDA filing and could be completed as a post-marketing requirement. The ECAC provided its concurrence with the design of the six-month Tg.rasH2 transgenic mouse study in meeting minutes dated November 30, 2012. As agreed to at the pre-NDA meeting (minutes dated September 2, 2014), the sponsor submitted the final study report and datasets to the IND on September 15, 2014 and the results are reviewed in this memo and were presented to the ECAC on November 18, 2014.

In the Tg.rasH2 carcinogenicity study, 25 animals per sex received vehicle, water, or VX-809 by oral gavage for 178 days. VX-809 doses were 200, 700, or 2000 mg/kg/day for males and 200, 500 or 1500 mg/kg/day for females. In addition, 10 animals per sex received urethane via i.p. administration as a positive control.

There were no statistically significant effects of VX-809 on survival in the study. Six unscheduled deaths were noted without relationship to dose (2 vehicle males, 1 water male, 2 LD males, 1 HD female) between Day 98-172 and were attributed to hemangiosarcoma of the skin or undetermined causes. Three early deaths were noted in HD female satellite animals in the first week. There was no gross evidence of gavage error but no further histopathological assessment was conducted and the relationship to VX-809 is uncertain. A trend towards lower body weights was noted in MD and HD males, but there were no statistically significant differences vs. the control groups.

The kidneys were identified as the target organ of test article-related non-neoplastic toxicity. Findings of renal cortex tubular epithelial degeneration and regeneration were observed in HD males only. A corresponding ~10% decrease in kidney organ weights was noted.

The combined incidence of Harderian gland adenoma and carcinoma was statistically significantly increased in HD females compared to either the vehicle or water controls (20% vs. 0%; p=0.025 vs. each control and p=0.004 for trend according to the analysis of statistical reviewer Dr. Min Min). The individual incidence of adenoma (16%) and carcinoma (4%) were each within the historical control range (0-16% and 0-8%, respectively based on 26 studies conducted at the test facility) for the test facility. The combined adenoma plus carcinoma incidence of 20% was also within the historical control data (mean 3.5%, range 0-24%) No Harderian gland neoplastic lesions were noted in the female control groups (combined incidence 0/50, at the lower bound of the historical control ranges noted above). Based on the fact that the HD incidence was within the historical control data and the control group incidence was lower than would be expected, the reviewer did not judge the neoplastic findings in the Harderian gland to be a test article-related effect.

The positive control group was noted with acute body weight losses in both sexes and a statistically significant decrease in survival for males. The responsiveness of the Tg.rasH2 model to urethane, a known carcinogen, was confirmed based on the observation of lung adenomas, lung carcinomas, and spleen hemangiosarcomas in this study.

Toxicokinetic analysis was conducted in a satellite group of wild-type hemizygous mice and was consistent with the previous 28-day dose-ranging study. Exposure to VX-809 increased less than dose-proportionally and there was no evidence of accumulation over the course of the six-month study. VX-809 exposure was higher in females than males.

The results of the Tg.rasH2 mouse carcinogenicity study were presented to the ECAC (meeting minutes dated November 19, 2014 are appended to this review). The committee's conclusions were as follows:

Executive CAC Recommendations and Conclusions

Tg.rasH2 mouse:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms in the study.

In summary, VX-809 was concluded to be negative for carcinogenic potential in a valid six-month Tg.rasH2 transgenic mouse study that was designed with ECAC concurrence. These transgenic mouse studies are generally considered to provide only hazard identification, but as shown in the table below, the exposure margins between the expected human dose and the tested doses were 1.7-3.7 times in males and 2.4-5.6 times in females. No test article-related neoplastic findings were observed in Tg.rasH2 mice receiving VX-809 at doses of up to 2000 mg/kg/day in males and 1500 mg/kg/day in females.

Table 11. Exposure Margins in 6-month Tg.rasH2 Mouse Carcinogenicity Study

Study	Sex	Dose	$AUC_{0\text{-}24hr}$ (ug*hr/mL)	Exposure Margin
Human (28-day phase 2 study #102)	M/F	VX-809 400 mg BID Ivacaftor 250 mg BID	371	-
Tg.rasH2 Mouse (Carcinogenicity study)	M	200 mg/kg/day	624	1.7
		700 mg/kg/day	934	2.5
		2000 mg/kg/day	1390	3.7
	F	200 mg/kg/day	886	2.4
		500 mg/kg/day	1310	3.5
		1500 mg/kg/day	2090	5.6

12 Appendix

Executive Carcinogenicity Assessment Committee (ECAC) meeting minutes dated November 19, 2014.

Executive CAC
Date of Meeting: November 18, 2014

Committee: Paul Brown, Ph.D., OND IO, Acting Chair
Tim McGovern, Ph.D., OND IO, Member
Ron Wange, Ph.D., DMEP, Alternate Member
Timothy Robison, Ph.D., DPARP, Pharm Tox Team Leader
Andrew Goodwin, Ph.D., DPARP, Presenting Reviewer
Min Min, Ph.D., OB/DB-6, Observer (Statistics Reviewer)

Author of Minutes: Andrew Goodwin

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 206038
IND # 79521
Drug Name: Lumacaftor (VX-809)
Sponsor: Vertex Pharmaceuticals

Tg.rasH2 Mouse Carcinogenicity Study Final Results

Lumacaftor (VX-809) is a proposed cystic fibrosis transmembrane conductance regulator (CFTR) in development for the treatment of cystic fibrosis patients homozygous for the ΔF508 mutation. NDA 206038 was submitted on November 5, 2014 seeking approval for a fixed-dose combination product containing lumacaftor and ivacaftor. A two-year rat carcinogenicity study is ongoing and will be completed as a post-marketing requirement.

VX-809 was negative for genotoxic potential in an in vitro bacterial reverse mutation assay, in vitro CHO cell chromosomal aberration assay, and in vivo mouse micronucleus assay.

In a six-month oral gavage carcinogenicity study in Tg.rasH2 mice, males received VX-809 at 200, 700, or 2000 mg/kg/day and females received VX-809 at 200, 500, or 1500 mg/kg/day. Two negative control groups received vehicle (0.5% methylcellulose (400 cps), 0.5% Tween 80, 0.05% simethicone in deionized water) or deionized water and a positive control group was administered urethane.

There were no effects on survival in VX-809 groups compared to the control groups. Dose-related clinical signs in males and females included hunched posture and rapid / shallow breathing. Additional findings in males included decreased motor activity, hyperactivity, ruffled

fur, and swelling. Males treated with VX-809 showed a slight trend towards decreased body weights at 700 and 2000 mg/kg/day (-7% vs. control, not statistically significant).

Females receiving VX-809 at 1500 mg/kg/day had an increased incidence of Harderian gland adenoma (4/25 = 16%) and carcinoma (1/25 = 4%) with a combined incidence of 5/25 or 20%. Data from 26 studies conducted at the test facility indicated historical control incidences in females of 2.8% (range 0-16%), 0.7% (range 0-8%), and 3.5% (range 0-24%) for Harderian gland adenoma, carcinoma, and combined incidence, respectively. Based on the fact that the incidence at the high-dose remained within the historical control range, these findings were not considered test article-related.

There were no notable tumor findings in males at doses up to 2000 mg/kg/day.

The urethane-treated positive control group exhibited lung adenomas, lung carcinomas and spleen hemangiosarcomas. These findings were generally consistent with historical control data for this known carcinogen.

Toxicokinetic analysis showed that VX-809 exposure increased less than dose-proportionally from 200 to 1500 (females) or 2000 (males) mg/kg/day and was greater in females vs. males. At Day 178, $AUC_{0\text{-}24hr}$ values at the highest doses tested were 1390 and 2090 ug*hr/mL in males and females, respectively.

Executive CAC Recommendations and Conclusions

Tg.rasH2 mouse:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms in the study.

Paul Brown, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, DPARP
/TRobison, DPARP
/AGoodwin, DPARP
/LHann, DPARP
/ASeifried, OND IO

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

/s/

ADELE S SEIFRIED
11/19/2014

PAUL C BROWN
11/19/2014

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/s/

ANDREW C GOODWIN
11/20/2014
Review of VX-809 six month Tg.rasH2 carcincogenicity study and ECAC conclusions

TIMOTHY W ROBISON
11/20/2014
I concur