CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

210491Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date:	February 2, 2018
From:	Timothy J. McGovern, PhD, ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA:	210491
Agency receipt date:	June 28, 2017
Drug:	Smydeko (tezacaftor/ivacaftor)
Sponsor:	Vertex Pharmaceuticals, Inc.
Indication:	Treatment of adult and pediatric patients (12 years and older) with cystic fibrosis (CF)
Reviewing Division:	Division of Pulmonary, Allergy, and Rheumatology Products

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

Symdeko is co-packaged as combination tablets of tezacaftor and ivacaftor (containing 100 mg tezacaftor, a new molecular entity, and 150 mg ivacaftor) and ivacaftor 150 mg tablets. The proposed clinical dosing regimen is one combination tablet in the morning and one ivacaftor tablet in the evening. Ivacaftor, a CFTR potentiator, was previously approved by FDA as a monoproduct (Kalydeko, 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. Ivacaftor is also approved in combination with lumacaftor (Orkambi).

The nonclinical program supporting clinical development of Symdeko included a complete program for ivacaftor (conducted in support of NDA 203188), a complete program for tezacaftor, and a program for the drug combination that included primary pharmacology, general toxicology studies up to 3-month duration in rats. Ivacaftor was associated with hepatotoxicity in some studies in rats and mice and cardiac toxicity in dogs; the findings were considered 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.

Tezacaftor was evaluated in general toxicology studies in rats and dogs. The primary drug related findings included decreased body weight and dilated lymphatics in the GI tract. Tezacaftor was not associated with any findings of concern in genetic toxicity studies or carcinogenicity studies. In a battery of reproductive toxicity studies, tezacaftor produced maternal toxicity (reduced body weight and food consumption) but had no effects on fertility and was not teratogenic. Tezacaftor administration did result in reduced rabbit and rat pup weights, and led to early developmental and sexual maturation delays in rat pups.

A 3-month study in rats evaluating the drug combination identified no new toxicities compared to those already associated with the two drugs though the study was not optimally designed to identify synergistic effects.

The Established Pharmacologic Class (EPC) for ivacaftor is "CFTR potentiator". The sponsor proposed an EPC for tezacaftor of (b) (4). Extensive communications regarding the EPC for lumacaftor occurred during the review of Orkambi and, in the end, no EPC was determined. A similar conclusion was made for tezacaftor,

Conclusion: I agree with the Division pharmacology/toxicology conclusion that this NDA can be approved from the pharmacology/toxicology perspective. I have discussed and agree with labeling revisions proposed by the Division.

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/s/

TIMOTHY J MCGOVERN
02/02/2018

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:	210491
Supporting document/s:	1, 21
Applicant's letter date:	June 28, 2017, Dec 13, 2017
CDER stamp date:	June 28, 2017, Dec 13, 2017
Product:	Symdeko (tezacaftor and ivacaftor)
Indication:	Treatment of adult and pediatric patients (12 years and older) with Cystic Fibrosis (CF)
Applicant:	Vertex Pharmaceuticals, Inc.
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products (DPARP)
Reviewer:	L. Steven Leshin, DVM, PhD
Supervisor/Team Leader:	Carol Galvis, PhD
Division Director:	Badrul A. Chowdhury, MD, PhD
Project Manager:	Jessica Lee

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Symdeko is a combination product consisting of 2 small molecule drugs, ivacaftor and tezacaftor (VX-661). It is indicated for the treatment of cystic fibrosis (CF) in patients with the F508del mutation and other mutations in the CF transmembrane conductance regulator (CFTR) gene responsive to tezacaftor/ivacaftor based on in vitro data and/or clinical evidence.

A consult was requested by the CMC Reviewer, Dr. Sukhamaya Bain via email (Attachment 1), to assess the specification limits proposed for the impurities of tezacaftor (VX-661) that were qualified by previous and currently submitted toxicology studies.

There are 35 organic impurities arising from the starting materials, which the Applicant has either identified as an observed impurity or predicts as a potential impurity. A potential impurity may or may not actually appear in the starting materials, intermediates, or final drug substance. These are presented in Table 1, below.

Table 1: Tezacaftor Drug Substance Organic Impurities (from Applicant's Table 1, Module 3.2.S.3.2)

(b) (4)

The proposed specifications of the observed organic drug substance impurities identified in the drug substance, a subset of Table 1 impurities, and qualified by their presence in nonclinical toxicology studies, are presented in Table 2

Table 2: Proposed Specifications for Organic Impurities in Tezacaftor Drug Substance **(from Applicant's Table 1, Module 3.2.S.4.1)**

Since ivacaftor is approved and marketed as Kalydeco, only impurities associated with the manufacture of tezacaftor are reviewed herein. There were no new drug product impurities from combining tezacaftor and ivacaftor drug substances into one tablet. This review covers the toxicology of the observed and potential impurities, with emphasis on the observed impurities and their proposed specifications, since that is the most pertinent and where the most reliable information exists.

1.2 Brief Discussion of Nonclinical Findings

Tezacaftor drug substance impurities, present at levels that required structural and toxicological characterization, were assigned acceptable manufacturing specification limits. The limits set by the applicant were based on levels that were present in nonclinical toxicology studies in which no adverse effects were found. Due to differences in the levels among manufacturing batches, three different nonclinical studies were used to make this assessment.

For other impurities, including starting materials, i.e., potential impurities, the applicant conducted genetic toxicology studies or ICH-M7 *in-silico* assessments using DEREK Nexus (v.5.0.2) and SARAH Nexus (v.2.0.1) software. Except for the clastogenic findings for (b) (4) (attributed to polyploidy rather than clastogenicity), the starting materials and associated potential impurities were negative for mutagenicity and clastogenicity.

Other toxicology studies of starting materials were more appropriate for hazard determination rather than a safety evaluation to determine a safe level of substance (impurity) for an ingested therapeutic product.

1.3 Recommendations

1.3.1 Approvability

Refer to the review dated January 28, 2018 for the pharmacology/toxicology NDA approvability recommendation. There are no pharmacology/toxicology issues with the specifications for impurities.

1.3.2 Additional Non Clinical Recommendations

There are no additional nonclinical recommendations.

1.3.3 Labeling

There are no labeling issues associated with impurities in the drug substance or drug products.

2 Drug Information

2.1 Drug 1

CAS Registry Number: 1152311-62-0

Generic Name: Tezacaftor

Code Name: VX-661, VRT-893661, VRT-0893661

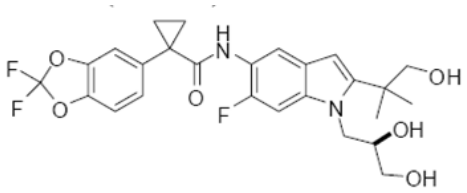
Chemical Name

(R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

Molecular Formula/Molecular Weight

$C_{26}H_{27}F_3N_2O_6$ (free form) / 520.5 Da (free form)

Structure or Biochemical Description



Pharmacologic Class: A pharmaceutical class was not established. Tezacaftor's mechanism of action is acting as a CFTR protein conformational modifier or stabilizer.

2.1 Drug 2

CAS Registry Number: 873054-44-5

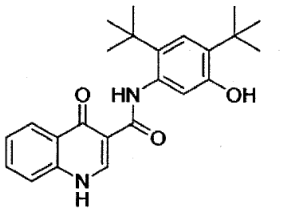
Generic Name: Ivacaftor

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: $C_{24}H_{28}N_20_3$/392.49

Structure or Biochemical Description



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

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 74,633 for ivacaftor (Vertex Pharmaceuticals, Inc.)
IND 108015 for tezacaftor (Vertex Pharmaceuticals, Inc.)
NDA 203188, Kalydeco (ivacaftor), Vertex, approved January 31, 2012
NDA 206038, Orkambi (ivacaftor and lumacaftor), Vertex, approved July 2, 2015

2.3 Drug Formulation

Symdeko consists of co-packaged tablets of the fixed dose combination (FDC) tezacaftor (100 mg)/ivacaftor (150 mg) and a separate ivacaftor tablet (150 mg). The 150 mg ivacaftor tablets are the same as the approved Kalydeco product (NDA 203188). The fixed dose combination tablet is formulated as an immediate-release film coated tablet for oral administration. The composition of the tezacaftor/ivacaftor FDC tablet, consisting of the core tablet and film coat, is provided in Table 1. The drug substances for both drugs were produced as (b) (4). Refer to the CMC review for specific details of the drug manufacture and analytical characteristics.

Table 3: Composition of Tezacaftor/Ivacaftor Fixed Dose Combination Tablet

Component	Quality Standard	Component Function	Amount per tablet (mg)	Content (% w/w)
Core Tablet				
(b) (4)				
(b) (4)				
Ivacaftor				
Ivacaftor drug substance	Internal standard	Active ingredient	150.0	24.61
(b) (4)				
(b) (4)				
Tezacaftor				
Tezacaftor drug substance	Internal standard	Active ingredient	100.0	16.40
(b) (4)				
(b) (4)				
Core Tablet Total				
Film Coat				
(b) (4)				
Total Tablet Weight			**609.6**	**100**
(b) (4)				

2.4 Comments on Novel Excipients

There are no novel excipients in the tezacaftor/ivacaftor FDC tablets.

2.5 Comments on Impurities/Degradants of Concern

The impurities with required structural identification were assigned appropriate specification limits based on ICH Q3A(R2), Q3B(R2), and ICH M7, as described in later sections of this review.

Impurities present in general toxicology studies of VX-661 were qualified for amounts present at dose corresponding with no adverse findings. Since some studies lacked impurity levels for identified impurities, an IR was sent on December 8, 2017 requesting the Applicant to provide the missing information. A response with this information was submitted to the NDA on December 13, 2017. The Applicant also indicated that an older version of the quantification procedures was used for impurity values in the original Certificates of Analysis, and those were rerun with an updated methodology and the new values were provided. The Applicant also indicated that these impurities are not degradation products, so they would not form under normal storage conditions.

Potential impurities and starting materials for the manufacture of tezacaftor were all evaluated in toxicology studies or by chemical structure computational software for mutagenicity following ICH-M7 recommendations. The results of these assessments are also described later in the review.

2.6 Proposed Clinical Population and Dosing Regimen

Symdeko is indicated for the treatment of patients with cystic fibrosis aged 12 years and older who are homozygous for the *F508del* mutation or who have at least one mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that is responsive to tezacaftor/ivacaftor based on *in vitro* data and/or clinical evidence. The recommended oral dose is one tablet (tezacaftor 100 mg/ivacaftor 150 mg) taken in the morning and one tablet (ivacaftor 150 mg) taken in the evening, approximately 12 hours apart. Tablets should be taken with fat-containing food. There are recommendations for dose adjustments for patients with moderate and severe hepatic impairment and if co-administered with drugs that are moderate or strong CYP3A inhibitors.

2.7 Regulatory Background

Impurities of ivacaftor were addressed under NDA 203188. With regards to safety and specification levels of impurities of tezacaftor, a consult request was submitted via email by the CMC Reviewer, Dr Sukhamaya Bain, on September 14, 2017. This was to assess the specifications of the impurities of tezacaftor (VX-661) that were qualified by previous and currently submitted toxicology studies. Impurity levels exceeding ICH recommended values were noted in the initial review of May 14, 2010, and detailed further in the review of June 21, 2010. Until the NDA submission, the impurities had not been addressed by the Applicant, other than an April 28, 2010 response to a CMC information request concerning the drug substance and product testing and specifications.

Under the Quality by Design program, the tezacaftor starting materials, (b) (4) were discussed at the CMC End-of-Phase-2 Type B meeting on July 30, 2015 and agreed to in the FDA meeting minutes on August 11, 2015. There were also assessed for safety by toxicological studies and computer software to detect chemical structures of mutagenicity as described in this review.

3 Studies Submitted

3.1 Studies Reviewed

Study Number/ Location	Study Title
Toxicology: Genetic Toxicology Studies	
VX-661-TX-059 Module 4.2.3.7.7-other	*In Silico* Analysis of Process Intermediates and Impurities in the Synthesis of VX-661 for Mutagenic Potential
	(b) (4)
(b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
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(b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
(b) (4) Module 4.2.3.7.7-other	(b) (4): In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes
	(b) (4)
V (b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
(b) (4) Module 4.2.3.7.7-other	(b) (4): In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes
	(b) (4)
(b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
(b) (4) Amended Final Report Module 4.2.3.7.7-other	(b) (4): In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes
VX-661 and VX-661 Impurity Toxicology Studies	
VX-661-TX-026	Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (pooled treatment group approach)
VX-661-TX-039	Acute Eye Irritation in the Rabbit
VX-661-TX-025	The Bovine Corneal Opacity and Permeability Assay
VX-661-TX-024	EpiSkin™ Skin Irritation Test
VX-661 Impurity Toxicology	
(b) (4)	(b) (4): Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
	(b) (4): EpiSkin™ Skin Irritation Test
	(b) (4): The Bovine Corneal Opacity and Permeability Assay (BCOP)
	(b) (4): Acute Oral Toxicity to the Rat (Acute Toxic Class Method)
	(b) (4) Assessment of Skin Sensitization Potential using the

(b) (4)	Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
	(b) (4): EpiSkin™ Skin Irritation Test
	(b) (4): The Bovine Corneal Opacity and Permeability Assay (BCOP)
	(b) (4): Acute Oral Toxicity to the Rat (Acute Toxic Class Method)
	(b) (4): Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
	(b) (4): In Vitro Skin Corrosivity Test Using the EpiDerm™ Human Skin Model
	(b) (4): The Bovine Corneal Opacity and Permeability Assay (BCOP)
	(b) (4): Acute Oral Toxicity to the Rat (Acute Toxic Class Method)
	(b) (4): EpiSkin™ Skin Irritation Test
	(b) (4): Acute Eye Irritation in the Rabbit

3.2 Studies Not Reviewed

Studies pertaining to impurities related to VX-770 (ivacaftor) manufacture were reviewed previously for the approval of Kalydeco (NDA 203188) and Orkambi (NDA 206038).

VX-770 Impurity Toxicology	
Study Number / Location	**Study Title**
(b) (4)	(b) (4): Bacterial Reverse Mutation Assay
	(b) (4): Bacterial Reverse Mutation Assay
	(b) (4): Bacterial Reverse Mutation Assay
	(b) (4) Bacterial Reverse Mutation Assay

3.3 Previous Reviews Referenced

NDA 203188, Kalydeco (ivacaftor), Vertex, approved January 31, 2012
NDA 206038, Orkambi (ivacaftor and lumacaftor), Vertex, approved July 2, 2015
NDA 210491, December 6, 2017, review of carcinogenicity studies
NDA 210491 January 28, 2018, PharmTox review
IND 108105 PharmTox reviews for tezacaftor (VX-661) include the following:

- May 14, 2010, initial safety review with limit of highest clinical dose
- June 21, 2010, full review of initial submission
- November 5, 2010, support for increasing the clinical dose
- December 19, 2010, support for women in clinical studies
- December 30, 2011, support for 28-day VX661/VX770 combination study
- January 10, 2013, lack of adequate M2 metabolite safety from previous studies
- December 18, 2013, Rat Carcinogenicity SPA, ECAC Recommendations
- November 20, 2014, Mouse Carcinogenicity SPA, ECAC Recommendations
- October 16, 2015, advice on terminating rat carcinogenicity study groups
- July 28, 2016, review of metabolites M2 and M5
- March 14, 2017, review of metabolite M5

4 Pharmacology

Refer to the Pharmacology and Toxicology Review of January 28, 2018.

5 Pharmacokinetics/ADME/Toxicokinetics

Refer to the Pharmacology and Toxicology Review of January 28, 2018.

6 General Toxicology

Impurities in Toxicology Studies of VX-661

Impurities that were qualified by their presence in the toxicology studies conducted during VX-661 development are presented in the following table.

Table 4: Impurities and Levels in Qualifying Toxicology Studies of VX-661.

Study		6-month rat	28-day dog	6-month dog
Study #		VX-661-TX-012	VRT-0893661-TX-011	VX-661-TX-018
NOAEL dose		100 mg/kg/day	250 mg/kg/day	200 mg/kg/day
Drug Substance Manufacture / Year		(b) (4) (2012)	Vertex (2009)	(b) (4) (2013)
Lot		17QB10SDNJ00001	A3613023	19QB10A-50.HQ00002*
Purity (% area of VX-661)		98.7%	97.0	98.6%
RRT	Impurity	% area		
(b) (4)				

(b) (4)

The human equivalent level for the above impurities was calculated and qualified level's for a daily dose was compared with the daily amount at the specification limit. The specification limit was substantially less for each impurity than the amount qualified, which is considered adequate.

Table 5: Specification Limits of Detectable Impurities based on Levels Present in Qualifying Toxicology Studies

Drug Substance Impurity	Tox Study / (NOAEL dose)	Amount Present (% area) / (NOAEL Amount, mg/kg)	Human Equivalent Dose (HED) (mg/kg)	Qualified level* mg/day (Applicant's value)	Specification Amount (%w/w) (mg/day)	Daily amount at Spec. Limit (mg/day)
(b) (4)	VRT-0893661-TX-011 28-day dog (250 mg)	(b) (4)				
	VRT-0893661-TX-011 28-day dog (250 mg)					
	VX-661-TX-018 6-month dog (200 mg)					
	VX-661-TX-018 6-month dog (200 mg)					
	VRT-0893661-TX-011 28-day dog (250 mg)					

(b) (4)

Toxicology Studies of Starting Material

This section is a review of the toxicology studies of the starting materials: (b) (4) was reviewed previously for the approval of Kalydeco. When similar studies were conducted with VX-661, these studies were also reviewed. The following toxicology studies were conducted for most of the 3 starting materials and VX-661:

- Acute Oral Toxicity to the Rat (Acute Toxic Class Method)
- Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse
- EpiSkin™ Skin Irritation Test
- In Vitro Skin Corrosivity Test Using the EpiDerm™ Human Skin Model
- Acute Eye Irritation in the Rabbit
- The Bovine Corneal Opacity and Permeability Assay

Due to the commonality of procedures, since the studies were conducted at the same laboratory, and sometimes with a common control treatment across studies, a brief description of the procedures and analysis is presented followed by a table of the results that includes all the tested compounds.

Acute Oral Toxicity

Compounds	**VX-661**	(b) (4)		
Study Number	No study			
GLP		Yes	Yes	Yes
QA		Yes	Yes	Yes
Test facility	(b) (4)			
Study Initiation		October 27, 2014	October 27, 2014	October 27, 2014
Substance Lot / Purity		nGMP-420-236-01 99.4%	SKF-PD252-14-004T 96.9%	DB6990FP 62.8%

Reviewer's Comment: The acute toxic class method is a stepwise procedure to determine a substance's acute toxicity depending on the mortality and/or the moribund

status of the animals using three animals of a single sex per step. It is described and internationally validated [Schlede et al., 1995. The International Validation Study of the Acute-Toxic-Class Method (Oral). *Arch Toxicol*. **69**, 659-670]. It was developed as a modification of the LD_{50} determination to classify an acute toxic dose and reduce animal numbers for the study. It is not used in usual drug development studies, but for chemical characterization and classification. The initial dose level is selected from one of four fixed levels, 5, 50, 300, and 2000 mg/kg, which is judged to be most likely to produce mortality in at least some of the dosed animals. Chemical category or classification [1-5, or unclassified (no toxicity); Globally Harmonised System (United Nations, 2005) as adopted by European Regulation (EC) 1272/2008] is based on the number of animals surviving the administered dose. When there is no information available, an initial dose level of 300 mg/kg body weight is used. Testing at 5000 mg/kg requires justification and above 5000 mg/kg is prohibited.

Methods

Two groups of three fasted female rats (nulliparous and non-pregnant female RccHan®WIST albino rats, body weight 164 to 192 g, and 8-9 weeks of age) received a single oral gavage dose of the test substance at a dose level of 300 mg/kg, followed by 2000 mg/kg in a second set of animals since the acute (median) lethal oral dose was not attained at 300 mg/kg. Animals were observed for 1 week. There was no control group in these studies. The results are summarized below.

Table 6: Summary of Results for the Acute Oral Toxicity to the Rat (Acute Toxic Class Method)

Compounds	**VX-661**	(b) (4)		
Study Number	No study			
Doses concentrations (administered by oral gavage)		300 mg/kg 2000 mg/kg No control dose	300 mg/kg 2000 mg/kg No control dose	300 mg/kg 2000 mg/kg No control dose
Vehicle		1% methylcellulose	corn oil	1% methylcellulose
Deaths		None	2 at 2000 mg/kg on day 2 See below[1]	1 at 2000 mg/kg at 4 hr post dose See below[2]
Clinical signs		None	See below[1]	See below[2]
Body weight		2000 m/kg: 1 female with BW loss 300 mg/kg:1 female with low BW gain	No effect in survivors	No effect in survivors
Macroscopic findings		None	No abnormalities in survivors	No abnormalities in survivors
Acute median lethal oral dose (LD_{50})		>2000 mg/kg	>2000 mg/kg	>2000 mg/kg
Conclusion		Unclassified	Category 5 > 2000-5000	Category 5 > 2000-5000

1 (b) (4)
Two females at 2000 mg/kg died on Day 2. Clinical signs prior to death included unsteady gait, irregular breathing and piloerection in both animals, elevated gait, decreased activity, cold to the

touch, shallow breathing, partially closed eyelids, reduced body tone and hunched posture in one animal or increased activity, flattened posture and unresponsive in the other animal. These signs developed from approximately two hours after dosing. Both lost body weight. Macroscopic examination in one animal revealed pallor of the liver, kidneys, and spleen; brown fluid in the large and small intestines; and congestion of the lungs and bronchi and subcutaneous tissue. The other animal had a small caecum and stomach and black fluid in the small and large intestines.

Survivors at the 2000 mg/kg dose had clinical signs of decreased activity, hunched posture and piloerection, unsteady gait, elevated gait, body tremor, chin rubbing, salivation and reduced body tone. These signs generally developed from approximately two hours after dosing. Recovery of surviving animals, as judged by external appearance and behavior, was complete by Day 6. No clinical signs were seen in any animal dosed at 300 mg/kg.

2 (b) (4)

One female at 2000 mg/kg died approximately 4 hours after dosing. Clinical signs prior to death comprised unsteady, decreased activity, unresponsive, cold to the touch, irregular and shallow breathing, piloerection, red urine, flattened posture, head tilt and hunched posture. These signs began to develop approximately 30 minutes after dosing. The animal also lost body weight. Macroscopic examination of the animal revealed light brown fluid in the stomach and duodenum and black fluid in the small and large intestines.

Survivors at the 2000 mg/kg dose had clinical signs of red urine seen in all females, decreased activity, hunched posture, and cold to the touch. These signs were first noted approximately 30 minutes after dosing. Recovery as judged by external appearance and behavior was complete by Day 7. No clinical signs were seen in any animal dosed at 300 mg/kg.

Skin Sensitization Potential (local lymph node assay)

Compounds	**VX-661**	(b) (4)		
Study Number	VX-661-TX-026			
GLP	Yes	Yes	Yes	Yes
QA	Yes	Yes	Yes	Yes
Test facility	(b) (4)			
Study Initiation	November 25, 2014	November 25, 2014	November 25, 2014	November 25, 2014
Substance Lot / Purity (%)	17QB10.NJ00004, 98.8%	nGMP-420-236-01 99.4%	SKF-PD252-14-004T 96.9%	DB6990FP for preliminary study 62.8% PTC115-140805 for main study 97.2%

In the local lymph node assay (LLNA), a sensitizing substance is applied to the surface of the ear pinna and induces proliferation of lymphocytes in the lymph nodes draining the application site. Lymph nodes are extracted and cultured with radiolabeled thymidine. The proliferation of lymphocytes is proportional to the dose and potency of the sensitizing substance and is quantified by measuring the incorporation of radiolabeled thymidine. The mean proliferation in each test group is compared to the mean proliferation in the vehicle control to arrive at a Stimulation Index (SI), measure of sensitization potential. A test substance is regarded as a sensitizer if the SI is 3 or more in any of the concentrations tested.

OECD Guideline for Testing of Chemicals No. 429 “Skin Sensitization: Local Lymph Node Assay”. 22 July 2010.

EEC Methods for the determination of toxicity and other health effects. Commission Regulation No. 440/2008. Part B, Method B.42. Skin sensitization: Local Lymph Node Assay. 30 May 2008, as amended in Commission Regulation No. 640/2012 dated 6 July 2012.

Methods

A vehicle trial performed with the various test substances (starting materials) indicated that a thick pale brown/cream suspension formed at 50% w/v in 4:1 v/v acetone:olive oil (AOO) which was satisfactory for dose administration.

Preliminary investigations were performed to ensure the highest concentration to be used on the main study did not result in systemic toxicity or excessive local irritation. The maximum practical concentration for dosing was 50% w/v in acetone:olive oil (4:1 v/v). An assessment of local irritation was performed and an erythema score greater or equal to 3 and/or an increase in ear thickness of greater than 25% at Day 3 or 6 compared with predose would be regarded as irritation, which would preclude the use of that concentration on the main study. The results of the preliminary investigations indicated that 50% w/v was a suitable high concentration for administration in the main phase of the study.

Substances (25 µL) were applied to the dorsal surface of each ear in 4 mice (16.0 to 19.2 g, approximately 8 to 11 weeks of age) per dose group for three consecutive days, including vehicle control and positive control groups. On Day 6, 20 µCi of ^{3}H-methyl thymidine was administered in the tail vein and 5 hours later, the draining auricular lymph nodes were excised and pooled for each experimental group in phosphate buffered saline and prepared for liquid scintillation counting and a Stimulation Index was determined. The Estimated Concentration of Three (EC3, Ryan et al, 2007), the concentration of test substance which would result in a SI of 3, was calculated from the results that included one concentration which had an SI greater than 3 and one concentration which had an SI of less than 3, using the following formula:

$$EC3 = c + \left[\frac{(3-d)}{(b-d)}\right] x\ (a-c)$$

Where a = concentration giving SI greater than 3
b = SI at concentration a
c = concentration giving SI less than 3
d = SI at concentration c.

Table 7: Summary of Results for Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (pooled treatment group approach)

Compounds	**VX-661**	(b) (4)		
Study Number	VX-661-TX-026			
Drug concentrations (main study)	10, 25, or 50% (w/v)	10, 25, and 50% (w/v)	25 or 50% v/v or as supplied	10, 25, or 50% w/v
Vehicle, negative control	dimethylformamide (DMF)	50% v/v in 4:1 v/v acetone:olive oil	50% v/v in 4:1 v/v acetone:olive oil (AOO)	50% w/v dimethylformamide (DMF)
Positive control; effect	50% v/v hexyl cinnamic aldehyde	50% v/v hexyl cinnamic Aldehyde; Slight erythema on day 2 and 3on day 2 and 3	50% v/v hexyl cinnamic aldehyde; Slight to moderate erythema on day 2 and 3	50% v/v hexyl cinnamic Aldehyde; Slight to moderate erythema on day 2 and 3
Mortality	None	None	None	None
Clinical signs	None, dose residue on head	None, dose residue on head	None, dose residue on head	None, dose residue on head; Red staining on the cage floor and bedding post dose for all treated animals. No similar finding for control animals
Dermal reaction	No irritation	No irritation	No irritation	No irritation
Ear thickness	No effect	No effect	No effect	No effect
Body weight	Small loss in 2 mice	Small loss in some mice	Small loss in some mice	Small loss in some mice
Stimulation Index	0.9, 1.4, and 0.9, respectively for doses above Positive control = 11.8	2.2, 1.0, and 1.8, respectively for doses above; Positive control = 15.7	1.4, 2.7, 7.3, respectively for doses above Positive control = 11.7	3.0, 2.5, and 6.9, respectively for doses above Positive control = 11.6
Conclusion	Not a skin sensitizer	Not a skin sensitizer; EC3 > highest concentration tested 50% w/v.	Potential to induce skin sensitization; EC3 = 53.3% v/v.	Potential to induce skin sensitization; EC3 = 10.0% v/v

EpiSkin™ Skin Irritation Test

Compounds	**VX-661**	(b) (4)		
Study Number	VX-661-TX-024			
GLP	Yes	Yes	Yes	Yes
QA	Yes	Yes	Yes	Yes
Test facility	(b) (4)			

	[redacted] (b) (4)			
Study initiation	October 29, 2014	October 29, 2014	October 29, 2014	October 27, 2014
			Note: Signed GLP and QA are on a page indicated corneal assay not Episkin assay, but different date than the actual one for the corneal assay	
Substance Lot / Purity (%)	17QB10.NJ00004 98.8% w/w	nGMP-420-236-01 99.4%	SKF-PD252-14-004T 96.9%	DB6990FP 62.8%

The EpiSkin™ Skin Irritation Test[15 min - 42 hours] uses reconstructed human epidermis skin constructs as a replacement to the in vivo Draize Skin Irritation Test, by the European Centre for the Validation of Alternative Methods (ECVAM) April 27, 2007. EpiSkin™ is a three- dimensional human skin model with reagents including controls provided by a commercial French company. The model consists of normal, human-derived epidermal keratinocytes seeded on a dermal substitute consisting of a collagen type 1 matrix coated with type IV collagen. After 13 days in culture, a multi-layered, highly differentiated model of the human epidermis with a functional multi-layered stratum corneum has formed. Substances are applied to the 3-D skin model and irritant substances that are sufficiently cytotoxic to cause cell damage in the cell layers. A test substance is applied for 15 min to Episkin human epidermis skin constructs, rinsed away, and constructs incubated for 42 h. Cell viability is determined by mitochondrial dehydrogenase activity, assessed by the reduction of MTT (3 (4,5 dimethylthiazol 2 yl) 2, 5 diphenyltetrazolium bromide) to a soluble, colored, formazan product. The prediction model uses the percentage viability values (compared to negative control viability) to identify irritant and non-irritant substances. The test includes acceptance criteria for both negative and positive controls. Preliminary tests include testing for the interfering reduction of MTT by the test substance, interfering solution colorization by the test substance.

After incubation of at least 24 h in maintenance medium, triplicate tissues were dosed for 15 min with the test substance, or negative and positive control at room temperature. Neat test substance powder (10 ± 2 mg) was dispensed over each tissue using glass weighing boats. The tissues were wetted with 5 µL of purified water prior to application of the test substance. After 15 min, each tissue was rinsed with sterile Dulbeccos Phosphate Buffered Saline (DPBS) to remove residual test substance, blotted, then transferred to a well containing maintenance medium and incubated for 42 h at 37°C in a humidified atmosphere of 5% CO2 in air. After 42 h, each insert was transferred to a well containing MTT and incubated for 3 h at 37 °C in a humidified atmosphere of 5%

CO2 in air. At the end of 3 h, the triplicate inserts were blotted, the epidermis was removed from the insert using a biopsy punch, the epidermis separated from the collagen matrix using forceps, and both parts placed in a micro-tube. The tissues were vortexed with acidic isopropanol (0.04 N HCl final concentration) and extracted by storing at 2-8 ºC, protected from light, for 48 - 70 hours, then aliquots were pipetted into 96-well plates and absorbance (optical density, OD) determined.

The viability of each tissue was expressed as a percentage of the mean negative control value. The mean absorbance of the triplicate negative control values should be >0.6 and ≤1.5. The Standard Deviation (SD) value of the % viability should be ≤18. The OD of the positive control is an indicator of the sensitivity of the tissues. The mean viability should be ≤40% of the negative control and the SD ≤18%. If the mean tissue viability was equal to or less than 50% of the negative control value, the sample was classified as Category 2.

Criteria for in vitro interpretation	Globally Harmonized System
Mean tissue viability is ≤ 50 %	Category 2
Mean tissue viability is > 50 %	No category

Table 8: Summary of Results for the EpiSkin™ Skin Irritation Test

Compounds	**VX-661**	(b) (4)		
Study Number	VX-661-TX-024			
Drug concentrations	10 ± 2 mg powder over each tissue, prewetted with 5 µl water	10 ± 2 mg powder over each tissue, prewetted with 5 µl water	10 ± 2 mg powder over each tissue, prewetted with 5 µl water	10 ± 2 mg powder over each tissue, prewetted with 5 µl water
Reduction of MTT	None	None	None	None
Coloring potential of test substance	None	None	None	None
Negative control	Dulbecco's Phosphate Buffered Saline with magnesium and calcium. 100.0 ± 8.1	Dulbecco's Phosphate Buffered Saline with magnesium and calcium. 100.0 ± 8.1	Dulbecco's Phosphate Buffered Saline with magnesium and calcium. 100.0 ± 8.1	Purified Water 3 min: 100.0 ± 1.54 1 hr: 100.0 ± 10.284
Positive control	5% Sodium Dodecyl Sulphate 17.7 ± 5.1	5% Sodium Dodecyl Sulphate 17.7 ± 5.1	5% Sodium Dodecyl Sulphate 17.7 ± 5.1	8.0 N Potassium Hydroxide 3 min: 19.4 ± 0.068 1 hr: 2.4 ± 0.084
Tissue Viability	74.9 ± 2.0%	89.6 ± 4.4%	63.3 ± 5.0%	3 min: 101.9 ± 0.274 1 hr: 94.9 ± 0.508f
Conclusion	Non-irritant to the	Non-irritant to skin	Non-irritant to skin	Non-corrosive to

	skin			skin

Acute Eye Irritation

Table 9 Summary of Results for Acute Eye Irritation in the Rabbit

Compounds	**VX-661**	[redacted] (b) (4)		
Study Number	VX-661-TX-039	No study	No study	[redacted] (b) (4)
GLP	Yes			Yes
QA	Yes			Yes
Test facility	[redacted] (b) (4)			[redacted] (b) (4)
Study initiation	April 4 ,2016			April 4, 2016
Substance Lot / Purity	4313.E.15.2 99.4%			DB6990FP 62.8%

Methods

Testing was conducted in two New Zealand White (Hsdlf:NZW) strain rabbits (3.03 or 3.60 kg, 12 to 52 weeks old) and the response in those animals was such that exposure of a third animal would not affect classification of [redacted] (b) (4), therefore, no further testing was needed.

Before the start of the test, both eyes of the selected test rabbits were examined for evidence of ocular irritation or defect with the aid of a light source from a standard ophthalmoscope. Only animals free of ocular damage were used. Rabbits were tested one after the other. They received buprenorphine (0.01 mg/kg, sc, 60 min prior to testing) to provide systemic analgesia and predose ocular anesthetic (2 drops of 0.5% tetracaine hydrochloride) 5 min prior to application of the test substance. Volume of the test substance was 0.1 mL and this was placed into the conjunctival sac of the right eye. The left eye was left untreated. An initial pain reaction was obtained using a 6 point scale. A second dose of buprenorphine and meloxicam 0.5 mg/kg was administered at 8 hours after application. The eyes were examined at 1, 24, 48, 72 hours and at day 7 and scored according to Draize method.

The data relating to the conjunctivae were designated by the letters A (redness), B (chemosis), and C (discharge), those relating to the iris designated by the letter D and those relating to the cornea by the letters E (degree of opacity) and F (area of cornea involved). For each tissue the score was calculated as follows:

Score for conjunctivae = (A + B + C) x 2

Score for iris = D x 5

Score for cornea = (E x F) x 5

The total scores for the cornea, iris, and conjunctivae for each time point for each rabbit were calculated. The group means of the total scores for each observation were calculated. The highest of these group means (the maximum group mean score) together with the persistence of the reactions enabled classification of the eye irritancy potential. If evidence of irreversible ocular damage is noted, (b) (4) will be classified as corrosive to the eye.

Category for Irreversible Eye Effects

Category	Criteria
Irreversible effects on the eye (Category 1)	If, when applied to the eye of an animal, a substance produces:

	- at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
	- at least in 2 of 3 animals, a positive response of:
	  - cornea opacity ≥3 and/or
	  - iritis >1.5
	- calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test item.

Irritating to eyes (Category 2)	If, when applied to the eye of an animal, a substance produces:

	- at least in 2 of 3 tested animals, a positive response of:
	  - cornea opacity ≥1 and/or
	  - iritis >1, and/or
	  - conjunctival redness ≥2 and/or
	  - conjunctival edema (chemosis) ≥2
	- calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test item, and which fully reverses within an observation period of 21 days.

Within this category (2), an eye irritant is considered mildly irritating to eyes (Category 2B) when the effects listed above are fully reversible within 7 days of observation.

Table 10: Acute Eye Irritation Test Results

Compounds	**VX-661**	(b) (4)		(b) (4)
Study Number	VX-661-TX-039	No study	No study	(b) (4)
Drug concentrations	Single application			0.1 mL 0.69 mg Single application
N	2 rabbits			2 rabbits
Corneal or Iridial effects	No corneal effects Iridial inflammation			None
Conjunctival effects	Moderate conjunctival irritation at 1 h, minimal at 24, 48,			Moderate conjunctival irritation at 1 h, minimal at 24, 48, and

	and 72 h			72 h
7 day exam	Eyes appeared normal			Eyes appeared normal
	Eyes appeared normal at 72 hr			
Modified Kay and Calandra classification system	Maximum group mean score of 11.5 classified as a mild irritant (Class 4 on a 1 to 8 scale)			Maximum group mean score of 8.0 Classified as a mild irritant (Class 4 on a 1 to 8 scale)
Conclusion	VX-661 does not meet the criteria for classification *			(b) (4) does not meet the criteria for classification *
* According to the Globally Harmonized System of Classification and Labelling of Chemicals and to Regulation (EC) No. 1272/2008, relating to the Classification, Labelling and Packaging of Substances and Mixtures				

Bovine Corneal Opacity and Permeability (BCOP)

Compounds	**VX-661**	(b) (4)		
Study Number	VX-661-TX-025			
GLP	Yes	Yes	Yes	Yes
QA	Yes	Yes	Yes	Yes
Test Facility	(b) (4)			
Exp. Start Date	Oct 14, 2014	Oct 14, 2014	Oct 14, 2014	October 14, 2014
Lot number / Purity	17QB10.NJ00004 98.8% w/w	nGMP-420-236-01 99.4%	SKF-PD252-14-004T 96.9%	DB6990FP 62.8% 94.2%

Reviewer's Comment: The Bovine Corneal Opacity & Permeability Assay (BCOP) was developed as an in vitro alternative to the in vivo Draize Eye Irritation test (Pierre Gautheron et al.1992). The BCOP assay uses isolated bovine corneas from freshly slaughtered cattle to determine the potential of a test substance to cause serious eye damage. Corneal opacity and permeability are determined and combined to give an In Vitro Irritancy Score which was used to assign an in vitro irritancy hazard classification category for prediction of the ocular irritation potential of the test substance. The test was conducted in accordance with the OECD Guidelines for the Testing of Chemicals, Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. Guideline 437, 26 July 2013. The BCOP test method was evaluated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), in conjunction with the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM), in 2006 and 2010. The test method can correctly identify chemicals (both substances and mixtures) inducing serious eye damage as well as those not requiring classification for eye irritation or serious eye damage, as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling

of Chemicals (GHS) (UN, 2011), and therefore, was endorsed as scientifically valid for both purposes.

Methods

The bovine eyes were obtained from cattle aged under 30 months, maintained and transported to the laboratory in HBSS containing 1% (v/v) penicillin/streptomycin solution. The eyes were used within 4 hours of slaughter. Eyes were examined and any exhibiting defects were discarded. The cornea was dissected away from surrounding tissue leaving 2 to 3 mm of sclera present around the cornea, rinsed in fresh HBSS plus 1% penicillin/streptomycin solution, then mounted in cornea holders with the endothelial side against the O-ring of the posterior half of the holder. The anterior half of the holder was then positioned on top of the cornea and secured with screws. Both compartments of the holder were filled with HBSS plus 1% penicillin/streptomycin. The holders were then plugged and incubated, in an upright position, overnight at room temperature (approximately 20°C). The next day, the chamber solution was replaced with cMEM, and incubated upright for 60 min at 32°C in a water bath, after which the media was replaced with fresh cMEM and basal opacity measurement obtained using a calibrated opacitometer. Corneas with an opacity value greater than 7 units were discarded. The mean basal opacity value of the corneas was then calculated and three corneas with opacity values close to the mean value were chosen for use as negative control corneas.

Corneas were treated in triplicate with either the test substance, positive control (imidazole) or negative control (0.9% sodium chloride solution). The controls and their results were shared with several studies performed in the same assay. The test substance and the positive control were tested at 20% (w/w) in 0.9% sodium chloride solution. The medium was removed from the anterior compartment of the holder and 750 µL of test substance, negative control or positive control was introduced into the anterior part of the holder, and the anterior compartment was plugged. Each holder was incubated in a horizontal position at 32°C for 4 hours in a water bath. Following incubation, the test substance, positive and negative controls were removed and the epithelial surface of the cornea by washing at least 3 times, the medium was removed from both compartments and re-filled with fresh cMEM. The posterior compartment was re-plugged and the opacity of each cornea measured and recorded. The opacity values obtained at this stage were used in calculating the final In Vitro Irritancy Score. Throughout the assay the corneas were examined for opaque spots or other irregularities. Following the final opacity measurement, the medium was removed from the anterior compartment of the holder, replaced with sodium fluorescein solution, and incubated for 90 min at 32°C in a water bath. Following incubation, the medium in the posterior compartment was mixed and an aliquot was removed for spectrophotometer measurement.

A valid test required that the positive control should elicit an In Vitro Irritancy Score that falls within two standard deviations of the historical mean for the laboratory and the

negative control mean opacity change value should be ≤3.0 and the permeability mean value ≤0.1.

The change in the opacity of each cornea was calculated by subtracting the initial basal opacity from the post-treatment opacity measurement. The mean change in opacity for the negative control corneas was calculated and was subtracted from the change in opacity of each treated cornea to obtain the corrected opacity value and the mean values determined. The corrected permeability value (OD490) of each treated cornea was calculated by subtracting the mean negative control cornea value from the permeability value of each cornea and the mean values determined.

The In Vitro Irritancy Score (IVIS) was calculated using the following formula:

In Vitro Irritancy Score =
Corrected Opacity Value + (15 x Corrected OD490 Value)

The test substance was classified based on the IVIS:

≤3	No Category
>3; ≤55	No prediction can be made
>55	Category 1

If the test substance was not identified as either Category 1 or No Category, additional testing (not part of this study) should be conducted for classification or labeling purposes.

In cases of borderline results in the first testing run, a second testing run will be considered, as well as a third one in case of discordant mean IVIS results between the first two testing runs. A result in the first testing run is considered borderline if the predictions from the 3 corneas are non-concordant, such that:
2 of the 3 corneas give discordant predictions from the mean of all 3 corneas, OR,
1 of the 3 corneas gives a discordant prediction from the mean of all 3 corneas, AND the discordant result is >10 IVIS units from the cut-off threshold of 55.

Table 11: The Bovine Corneal Opacity and Permeability Assay (BCOP) Results

Compounds	**VX-661**	(b) (4)		
Study Number	**VX-661-TX-025**			
Drug concentrations	20% w/w	20% w/w	20% w/w	20% w/w
Negative control	sodium chloride solution, 0.9% Not applicable, Clear corneas	0.9% sodium chloride solution Not applicable, Clear cornea	0.9% sodium chloride solution Not applicable, Clear cornea	sodium chloride solution, 0.9%; Not applicable, Clear cornea
Mean score for Opacity		-0.333	2.333	-0.333
Permeability		0.015	0.018,	0.015

Positive control **Opacity** **Permeability** **Irritancy score**	Imidazole Very opaque corneas 103.333 3.005 148.4 ± 11.1	Imidazole Very opaque corneas 103.333 3.005 148.4 ± 11.1	Ethanol, 21.000 1.284 40.3 ± 5.0	Imidazole Very opaque corneas 103.333 3.005 148.4 ± 11.1,
Test Substance **Opacity** **Permeability** **Irritancy Score**	Small opaque area in 1 cornea, Clear in 2 corneas 6.000 0.002 6.0 ± 3.3,	Clear corneas 1.333 0.018 1.6 ± 1.0	Clear corneas -0.333 -0.005 -0.4 ± 1.0	Slightly opaque corneas 30.000 -0.002 30.0 ± 4.8
Conclusion	No prediction for ocular irritation	No Category	No Category	No prediction for ocular irritation

7 Genetic Toxicology

A summary table of the genetic toxicology studies of VX-661 from the Pharmacology-Toxicology review of January 28, 2018 is provided below for comparison reference for the studies of impurities that follows.

Table 12: Summary of Genetic Toxicology Studies of VX-661

Test Report Number	Species	Dose	Result
Mutagenicity			
Bacteria Reverse Mutation VRT-893661-TX-007	S. Typhimurium, E. coli	up to 5000 µg/plate with or without S9 metabolic activation	**Negative**
Clastogenicity			
***In vitro* Chromosomal Aberrations** VRT-893661-TX-008	Chinese hamster ovary (CHO) cells	up to 5000 µg/mL with or without S9 metabolic activation	**Negative**
***In vivo* Mouse Micronucleus Test** VRT-893661-TX-003	Mouse, male	oral gavage, up to 2000 mg/kg	**Negative**

Table 13: Summary of Genetic Toxicology Studies of VX-661 Drug Substance Starting Materials

Compound Report Number	Species / Strain	Dose	Findings / Conclusion
(b) (4)			
Bacteria Reverse Mutation Assays (b) (4)	*S. typhimurium* and *E. coli*	5- 5000 µg/plate with and without S9 metabolic activation	Negative
(b) (4)			
Bacteria Reverse Mutation Assays (b) (4)	*S. typhimurium* and *E. coli*	5- 5000 µg/plate with and without S9 metabolic activation	Negative
***In vitro* Chromosomal Aberrations** (b) (4)	Human peripheral blood lymphocytes		Negative
(b) (4)			
Bacteria Reverse Mutation Assays (b) (4)	*S. typhimurium* and *E. coli*	0.15-5000 µg/plate with and without S9 metabolic activation	Negative
***In vitro* Chromosomal Aberrations** (b) (4)	Human peripheral blood lymphocytes		Negative
(b) (4)			
Bacteria Reverse Mutation Assays (b) (4)	*S. typhimurium* and *E. coli*	1.0-3335.4 µg/plate with and without S9 metabolic activation	Negative
***In vitro* Chromosomal Aberrations** (b) (4)	Human peripheral blood lymphocytes		Positive, with Increased Polyploidy

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

The four assays of in vitro mutagenesis were conducted by the same contract laboratory at about the same time using similar procedures. Therefore, the studies are described in the single study summary below with indication of results specific for each compound.

Study title: Bacterial Reverse Mutation Test

Study no.: (b) (4)

Study report location: Module 4.2.3.7.6-imp

Conducting laboratory and location: (b) (4)

Date of study initiation: (b) (4) November 25, 2013
October 21, 2014
October 28, 2014
November 6, 2014

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: (b) (4) Lot PT-C07091701-JF13002, Purity 100% (w/w)
(b) (4), Lot nGMP-420-236-0 I, Purity 99.4% (HPLC)
(b) (4): Lot SKF-PD252-14-004T, Purity 96.9% (GC DI)
(b) (4), Lot DB6990FP, Purity 62.8% (HPLC); purity of the free base

Key Study Findings

- (b) (4) starting materials in the manufacture of VX-661 were negative for inducing an increase in revertants in the bacteria reverse mutation test, and therefore, are not considered mutagenic.

Methods

Strains: Salmonella typhimurium, strains TA98, TA100, TA1535, and TA1537, Escherichia coli, strain WP2 uvrA (pKM101)

Concentrations in definitive study: up to 5000 µg/plate

Basis of concentration selection: Toxicity of bacterial culture and the amount of precipitation

Negative control: DMSO

Positive control:

Strain	-S9	+S9
TA98	2 µg/plate 2-nitrofluorene	5 µg/plate Benzo[a]pyrene
TA100	2 µg/plate Sodium azide	5 µg/plate 2-Aminoanthracene

TA1535	2 µg/plate Sodium azide	5 µg/plate 2-Aminoanthracene
TA1537	50 µg/plate 9-Aminoacridine	5 µg/plate Benzo[a]pyrene
WP2 uvr	2 µg/plate 4-Nitroquinoline-1-oxide	10 µg/plate 2-Aminoanthracene

Formulation/Vehicle: DMSO

Incubation & sampling time: First test was without preincubation. The second test used preincubation if negative results found in first test. If a third test was conducted it also was similar to the first test, except more compound concentrations were included. Triplicate plates for each dose concentration and controls were tested. Incubations were approximately 72 hr at 37°C. Incubations were conducted in the presence and absence of liver preparations (S9 mix, commercially obtained) from male Sprague-Dawley rats treated with phenobarbital and 5,6-benzoflavone.

Study Validity

Note that the stability, homogeneity, and concentrations of the test compounds in the vehicle were not conducted. This can obviously impact the validity of the study. Even if doses are prepared from dilutions of a high stock concentration and are appropriately diluted, the lack of verification of the high dose will impact all subsequent doses.

Validity of the study was based on the following procedures:

- The mean of the vehicle control revertant colony numbers for each strain should lie within or close to the current historical control range for the laboratory unless otherwise justified by the Study Director. The historical range is maintained as a rolling record over a maximum of five years.
- The positive control compounds must induce an increase in mean revertant colony numbers of at least twice (three times in the case of strains TA1535 and TA1537, which have relatively low spontaneous reversion rates) that of the concurrent vehicle controls.
- The absence of colonies on sterility check plates confirmed the absence of microbial contamination of the S9 mix, buffer and test substance formulation.
- The viability counts confirmed that the viable cell density of the cultures of the individual organisms exceeded 10^9/mL in all cases, and therefore met the acceptance criteria.
- Data Analysis and Interpretation
  Statistical analyses (Dunnett's test and trend analysis) were incorporated if the results obtained fail to satisfy the criteria for a clear "positive" or "negative"

response, although an equivocal response could be concluded as well. The Applicant also incorporated biological interpretation, claiming that treatment-associated increases in revertant colony numbers below two or three times those of the vehicle controls (as described above) are not considered biologically important.

Results

For most assays, sterility check plates and viability counts confirmed met the acceptance criteria, except where indicated otherwise. The results from negative and positive control plates were as expected with negative controls within or close to the current historical control range.

(b) (4) **Results**

In both the first and second tests, there was no evidence of toxicity following exposure to (b) (4) and no precipitate occurred on any plates containing (b) (4). There were no substantial increases in revertant colony numbers over control counts with any of the tester strains following exposure to (b) (4) at concentration up to 5000 µg/plate in either the presence or absence of S9 mix.

(b) (4) **results**

Toxicity, observed as a reduction in the number of revertant colonies, was observed only in the first test in strain TA1537 at 500 and 5000 µg/plate in the presence of S9 mix. In the first and second tests, precipitate was observed on all plates containing (b) (4) at 1500 and 5000 µg/plate.

There were no substantial increases in revertant colony numbers over control counts for any of the tester strains following exposure to (b) (4) up to 5000 µg/plate in either the presence or absence of S9 mix

(b) (4) **Results**

Toxicity was observed in the first test only in strain TA1535 following exposure to (b) (4) at 500 and 5000 µg/plate in the presence of S9 mix. In the second test, slight toxicity occurred in all examined strains following exposure to (b) (4) at various dose levels depending on strain and presence or absence of S9. In the second test, viability plates indicated poor growth in strains TA98 and WP2 uvrA (pKM101). A third assay was conducted with 10 concentrations of (b) (4) (5 concentrations were used in the second test). Toxicity, observed as a thin or absent background lawn of non-revertant colonies and/or reduction in the number of revertant colonies, was observed in all strains following exposure to (b) (4). Similar to the second test, there were no substantial increases in revertant colony numbers over control counts with any of the tester strains following exposure to (b) (4) at any concentration up to 5000 µg/plate in either the presence or absence of S9 mix.

[redacted] **Results**

Toxicity, observed as a reduction in the number of revertant colonies, was obtained only in strain TA98 following exposure to [redacted] at 3335.4 µg/plate in the presence of S9 mix. Precipitate was observed on all plates containing [redacted] at 3335.4 µg/plate in the absence of S9 mix. A maximum exposure concentration of 3335.4 µg/plate was used in the second test.

In the second assay, results with strains TA98, TA1535, and TA1537 were not obtained due to poor bacterial growth on viability plates. Also, toxicity (observed as a thin background lawn of non-revertant colonies and/or reduction in the number of revertant colonies) occurred at concentrations of 1000.6 and 3335.4 µg/plate in the absence and presence of S9 mix.

A third test was conducted with all strains. Precipitate was observed on all plates containing [redacted] at 3335.4 µg/plate in the absence of S9 mix. No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure to [redacted] at any concentration up to and including 3335.4 µg/plate in either the presence or absence of S9 mix.

An additional test was conducted with strain TA98. No signs of toxicity towards the tester strain were observed following exposure to [redacted] with or without S9. Precipitate was observed on all plates containing [redacted] at 1500 and 5000 µg/plate in the absence or presence of S9 mix. There were no substantial increases in revertant colony numbers over controls counts following exposure to [redacted] up to 5000 µg/plate in either the presence or absence of S9 mix.

7.2 *In Vitro* Assays in Mammalian Cells

The assays of in vitro chromosomal aberration (clastogenicity) were conducted by the same contract laboratory at about the same time using similar procedures. Therefore, the studies are described in the single study report below with indication of results specific for each compound.

Study title: In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes

Study no.: [redacted]

Study report location: Module 4.2.3.7.7-other

Conducting laboratory and location: [redacted]

Date of study initiation: [redacted]: October 21, 2014

	(b) (4) October 28, 2014 November 4, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4), Lot nGMP-420-236-0 I, Purity 99.4% (HPLC) (b) (4), Lot SKF-PD252-14-004T, Purity 96.9% (GC DI) (b) (4), Lot DB6990FP, Purity 94.2% area (HPLC) generic method; 62.8% w/w (HPLC); purity of the free base

Key Study Findings

- One of the starting materials of VX-661 manufacturing: (b) (4) was positive for clastogenicity. Starting materials (b) (4) and (b) (4) were negative for inducing an increase in the frequency of structural chromosome aberrations in human lymphocytes and therefore were not clastogenic.
- Evidence of induced polyploidy also occurred with (b) (4). Since the certificate of analysis indicated a substantial number of impurities associated with the synthesis of (b) (4), which would not be present in the synthesis of VX-661, additional testing would be needed to verify the clastogenic effects of (b) (4). However, since this is a starting material and is not detected in the drug substance or product, further verification with more specific methods for the detection of clastogenicity and polyploidy are not warranted at this time.

Methods

Cell line:	Human lymphocytes pooled in equal amounts from 2 healthy, nonsmoking adult donors

Concentrations in definitive study:

Incubation ± S9	**Concentration of Compound (**µg/mL)
(b) (4)	
3 hr –S9	14, 16, 18, 20, 22, 24, 26, 28, 30
3 hr +S9	40, 60, 80, 100, 120,140, 160
21 hr –S9	10, 11, 12, 13, 14, 15, 16, 18, 20
(b) (4)	
3 hr –S9	50, 60, 70, 80, 90, 100
3 hr +S9	50, 70, 90, 110, 130, 150, 170
21 hr –S9	30, 35, 40, 45, 50, 55, 60
(b) (4)	
3 hr –S9	33.40, 66.79, 100.19, 133.58, 166.98, 200.38, 233.77, 267.17
3 hr +S9	16.70, 33.40, 40.08, 46.75, 53.43, 60.11, 66.79
21 hr –S9	16.70, 33.40, 40.08, 53.43, 66.79, 73.47, 80.15, 86.83, 93.51, 100.19, 113.55

Basis of concentration selection: The concentration causing a decrease in mitotic index of at least 50% (where possible) of the vehicle control value was the highest concentration selected for metaphase analysis.

Negative control: DMSO

Positive control:

Incubation duration	-S9	+S9
	Mitomycin C	Cyclophosphamide
3 hour	0.2 µg/mL	7.5 µg/mL
21 hour	0.1 µg/mL	-

Formulation/Vehicle: Compounds were dissolved in DMSO, but the final volume of DMSO added to the cultures was 1% v/v.

Incubation & sampling time: Lymphocyte cultures were treated approximately 48 hours after commencement of incubation of lymphocyte cultures. Duplicate cultures were prepared for each treatment and concentration (3-hour treatment in the absence and presence of S9 mix, and 21-hour continuous treatment in the absence of S9 mix). Incubations were conducted in the presence and absence of liver preparations (S9 mix, commercially obtained) from male Sprague-Dawley rats treated with phenobarbital and 5,6-benzoflavone.

Mitotic activity was arrested by addition of Colcemid® to each culture at a final concentration of 0.1 µg/mL. Cell suspensions were dropped onto microscope slides, stained with Giemsa and examined for mitotic activity and toxicity.

Study Validity

Note that the stability, homogeneity, and concentrations of the test compounds in the vehicle were not conducted. This can obviously impact the validity of the study. Even if doses are prepared from dilutions of a high stock concentration and are appropriately diluted, the lack of verification of the high dose will impact all subsequent doses. In addition, there were no repeat or confirmation assays performed.

Validity of the study was based on the following procedures:

- One hundred metaphase figures were examined from each culture, except where there were high levels of aberrant cells. Chromosome aberrations were scored according to the classification of the ISCN 2009. Only cells with 44 – 48 chromosomes were analyzed.

- An assay was considered acceptable if the negative and positive control values lie within the current historical control range.
- The test substance is considered to cause a positive response if the following conditions were met:
  - Statistically significant increases (p<0.01) in the frequency of metaphases with aberrant chromosomes (excluding gaps) are observed at one or more test concentration.
  - The increases exceed the vehicle control range of this laboratory, taken at the 99% confidence limit.
  - The increases are reproducible between replicate cultures.
  - The increases are not associated with large changes in pH, osmolality of the treatment medium, or extreme toxicity.
  - Evidence of a concentration-related response is considered to support the conclusion.
- A negative response was determined if no statistically significant increases in the number of aberrant cells above concurrent control frequencies are observed, at any concentration.
- A further statistical evaluation was carried out if the above criteria for a positive or a negative response were not met. The number of aberrant metaphase cells in each test substance group was compared with the vehicle control value using the one-tailed Fisher exact test. Then a Cochran-Armitage test for trend was applied to the control and all test substance groups. If this is significant at the 1% level, the test is reiterated excluding the highest concentration group and this process continued until the trend test was no longer significant. D20's (the minimum concentration (mg/mL) at which aberrations were found in 20% of metaphases) were estimated (where possible) using logistic regression on a log(concentration) scale, allowing the number of control aberrations to be non-zero.

Results

The results from negative and positive control plates were as expected with negative controls within or close to the current historical control range. The results for each test strains are presented in the table below.

Table 14: Summary of In Vitro Mammalian Chromosome Aberration Assays

	3 h without S9	3 h with S9	21 h without S9
(b) (4)			
Mitotic index	51% at 28 µg/mL	50% at 120 µg/mL	48% at 16 µg/mL
Concentration for analysis	18, 24, 28 µg/mL	60, 100, and 120 µg/mL	11, 13, and 16 µg/mL
Chromosomal aberrations	Negative	Negative	Negative
Polyploid or endoreduplicated	Negative	Negative	Negative
(b) (4)			
Mitotic index	52% at 90 µg/mL	49% at 150 and 170 µg/mL	52% at 60 ug/mL

Concentration for analysis	60, 80, and 90 µg/mL	70, 130, and 150 µg/mL	35, 45, and 60 µg/mL
Chromosomal aberrations	Negative	Negative	Negative
Polyploid or endoreduplicated	Negative	Negative	Negative
(b) (4)			
Mitotic index	49 % at 267.17 µg/mL	54 % at 66.79 µg/mL	52% at 80.15 µg/mL
Concentration for analysis	133.58, 233.77, and 267.17 µg/mL	16.70, 60.11, and 66.79 µg/mL	Not conducted since positive results found without S9 in the 3 hr incubation test
Chromosomal aberrations	Positive (including or excluding gaps) at 233.77 and 267.17 µg/mL	Negative	-
Polyploid or endoreduplicated	Increased polyploid metaphase cells	Negative	-

10 Special Toxicology Studies

Study Title: In Silico Analysis of Process Intermediates and Impurities in the Synthesis of VX-661 for Mutagenic Potential

Report Number: VX-661-TX-059
Report Date: May 25, 2017

Key Study Findings

- 102 structures that are either known or potential impurities in the synthesis of VX-661 were analyzed for potential DNA reactivity (mutagenicity) using 2 different *in silico* methods, an expert rule-based method (DEREK Nexus) and a statistical QSAR method (SARAH Nexus).
- 10 were predicted to be mutagenic by both methodologies.
- 31 were equivocal or contrasting results, with 12 considered potentially mutagenic by expert analysis.
- 57 were predicted to be non-mutagenic by both methodologies, supported by expert analysis.
- 4 were outside the domain of applicability of the models used, but based on other similar compounds and expert analysis, there 4 structures were considered non-mutagenic.
- Therefore, 22 structures were predicted likely to be mutagenic in the bacterial reverse mutation test.

Abbreviation	Term
DEREK	Deductive Evaluation of Risk from Existing Knowledge
ICH	International Conference on Harmonisation
SARAH	Lhasa SARAH QSAR Model
QSAR	Quantitative Structure-Activity Relationship
OOD	Outside of Domain
WoE	Weight of Evidence

Methods

Known or potential impurities in the synthesis of VX-661 were analyzed for potential DNA reactivity (mutagenicity) using 2 different *in silico* methods according to the ICH M7 (Step 4) guideline [Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk]. There were 102 structures analyzed. The *in silico* methods were an expert rule-based methodology (DEREK Nexus, v.5.0.2) and a statistical QSAR (SARAH Nexus, v.2.0.1). For DEREK Nexus, the threshold for alert identification was set at the "Equivocal" level (i.e. any compounds containing an alert with a likelihood of equivocal or greater were flagged as POSITIVE). In addition, a custom version of SARAH (SARAH-VERTEX) was used that was derived from SARAH v.2.0.1 by the addition of 144 proprietary Vertex structures to the original SARAH model. All compound structures were obtained either from the Vertex compound database based on their unique Vertex identification code (VRT number) or from publically available sources based on their CAS registry numbers and verified by the Vertex Impurities Strategy Team.

All results were reviewed and assessed by experts in computational and genetic toxicology. Individual compound predictions were compared with experimental data from structural analogues from the public domain and from Vertex's internal compound collection using Leadscope Enterprise (v. 3.4.2-2), and a weight of evidence conclusion was assembled for each compound. "Significant" similarity was defined as Leadscope Tanimoto similarity index ≥0.4. Mutagenicity data for internal analogues was generated as part of the [Vertex] handler safety package.

Results

The following descriptions are taken directly from the Applicant's report with minimal changes.

Agreement on mutagenicity predictions between the 2 methodologies occurred for 67 of the 102 compounds. For 31 compounds, SARAH and DEREK gave discordant results. The structures for 4 compounds were outside the domain of applicability of the SARAH model.

Consensus Positive Predictions

Ten compounds were predicted to be positive by both in silico methodologies. (b) (4) are known mutagens. Similar to (b) (4)

(b) (4) contains (b) (4), which is a known structural alert. (b) (4) contains (b) (4) that would be expected to impart mutagenic activity to this compound. The remaining 7 consensus positives are low molecular weight (b) (4) compounds. These are both well-defined alerting structures for bacterial mutagenicity. Moreover, the query compounds do not contain clear deactivating features. Therefore, these predictions are believed to accurate and are all supported by the experimental literature.

Consensus Negative Predictions

There were 57 compounds that were predicted to be non-mutagenic by both methodologies. These include fragments derivative from (b) (4) VX-661 that do not contain any alerting features (e.g. (b) (4)), derivatives of (b) (4) that do not contain any alerting features (e.g. (b) (4)), close analogues of the parent compound that do not contain any novel or alerting features (e.g. (b) (4)), high molecular weight (b) (4) derivatives of VX-661 that do not contain any alerting features (e.g. (b) (4)) and derivatives (b) (4) of VX-661 that contain an nominally alerting feature that is deactivated by other features of the overall structure such as (b) (4)

In addition, all of the functional groups present in these compounds are covered by experimental data from selected compounds and intermediates tested previously by Vertex. These include (b) (4) and the parent compound, VX-661 all of which are experimentally inactive in GLP bacterial reverse mutation assays. All of these compounds are included in the SARAH-VERTEX training set. Thus, expert analysis of these predictions based on internal testing of close structural analogues supports these negative predictions.

Discordant Predictions

Conflicting results from the 2 methodologies occurred for 31 compounds. Of these, 12 of these structures were assigned a weight of evidence (WoE) positive prediction, and the remaining 19 structures were assigned a WoE negative prediction.

WoE Positive Discordant Predictions

(b) (4) was tested in a 5-strain Ames assay and was found to be mutagenic. Of the remaining 11 structures assigned a WoE positive prediction, 9 were (b) (4) compounds and were predicted to be mutagenic by DEREK."

(b) (4)

These 3 compounds are considered potentially mutagenic. This conclusion was arrived at by a comparison of these structures with tested analogues supporting the

mutagenicity of [redacted]. Although [redacted] is similar to both [redacted] and is experimentally non-mutagenic, the absence of the [redacted] moiety decreases the relevance of this example for read-across analysis.

[redacted]

These two compounds are predicted to be positive for mutagenic activity by DEREK. Comparisons were made of these structures to tested analogues. The most relevant analogue for read-across analysis of these structures is [redacted], which is the only [redacted] in the read-across set. Although this compound was experimentally non-mutagenic, it also has a higher molecular weight and contains [redacted] heavy atoms, which represents an activity cliff for mutagenicity of [redacted]. By comparison, both of the query compounds have [redacted] heavy atoms, resulting in the positive predictions by DEREK, which incorporates heavy atom count as an exclusion criterion for [redacted]. Overall, the strength of the available data set is considered insufficient to reject the positive DEREK prediction.

[redacted]

These 4 compounds are considered potentially mutagenic. Similarity searches against these structures identified a single analogue for this group: [redacted] Although this compound exhibits relatively high overall similarity to the query compounds [redacted], it contains a different structural alert from the query set [redacted] the body of existing data on mutagenicity of [redacted] compounds is considered insufficient to reject the positive DEREK predictions for the 4 compounds.

[redacted]

This compound is predicted to be mutagenic by DEREK based on the presence of the (b) (4) substructure. An equivocal result was obtained from SARAH for this structure. Overall, the existing database did not provide a clear rationale to reject the positive DEREK prediction.

WoE Negative Discordant Predictions

Of the 19 WoE negative structures all but 2 were (b) (4) structurally related to (b) (4) both of which are experimentally non-mutagenic. It should be noted that DEREK incorrectly predicts (b) (4) to be mutagenic. Similarity analysis of structures related to (b) (4) was conducted. (b) (4) All of these structures are predicted to be mutagenic by DEREK and yield an equivocal result from SARAH. Based on the similarity these structures to (b) (4), all 3 compounds are considered unlikely to be mutagenic. (b) (4) likewise have significant similarity to (b) (4) However, because of the high molecular weight of these structures ((b) (4) heavy atoms), all were predicted to be non-mutagenic by DEREK, but gave equivocal results in SARAH. The only other analogue identified in these similarity searches was (b) (4). Based on the similarity to (b) (4) the lack of novel alerting features relative to (b) (4) and the high heavy atom count, these structures are considered unlikely to exhibit mutagenic activity.

Similarity analysis of structures related to (b) (4) was conducted. (b) (4) and is therefore considered to be non-mutagenic. (b) (4) all have very high structural similarity to (b) (4) alert resides in a very similar chemical environment in all of these compounds. Moreover, none of the query structures contain additional alerting features relative to (b) (4). Based on these considerations, (b) (4) are all considered unlikely to exhibit mutagenic activity.

Similarity analysis of structures related to (b) (4) is presented in Table 7. Structural similarity of tested analogues ranges from (b) (4) for VX-661.Table 7 in the model training set, predicts (b) (4) to be non-mutagenic. Based on the absence of known alerting features and the read-across from close structural analogues, (b) (4) is considered unlikely to exhibit mutagenic activity. (b) (4) differs from the parent (b) (4) and does not contain any alerting features relative to VX-661 or any of the other tested analogues. Moreover, (b) (4) does not contain any unclassified or misclassified structural features. Finally, the custom SARAH-VERTEX model, which includes the tested analogues from (b) (4) is the (b) (4) analogue of (b) (4), which is experimentally non-mutagenic and both structures were predicted to

be inactive by DEREK. Based on the absence of any additional alerting features in [redacted] relative to [redacted], the former is considered unlikely to exhibit mutagenic activity.

[redacted] was predicted to be inactive by DEREK, but gave equivocal results in SARAH. The structure does not contain any known alerting features and was considered within the domain of both methodologies. SARAH generated only local hypotheses for this structure, so the rationale for the equivocal call is unclear. Based on the absence of any alerting structural features, [redacted] is considered unlikely to be mutagenic."

Structures outside the QSAR applicability domain

A total of 4 structures evaluated fell outside the domain of applicability (OOD, outside of domain) of the SARAH and SARAH-VERTEX QSAR models, and were considered to have unclassified structural features. Three of these, [redacted], contain a [redacted] functional group. This moiety is not represented in the SARAH training set and no information around its mutagenic potential was identified in the publicly available databases. [redacted] Based on these considerations [redacted] are considered unlikely to exhibit mutagenic activity. [redacted] was considered out of domain for DEREK based on the presence of the [redacted] moiety and for SARAH based on both the [redacted] group and the [redacted] group. The carbon analogue of this structure was considered in domain for DEREK (no unclassified features) but remains OOD for SARAH due to the [redacted] group. [redacted] are uncommon and no toxicology data could be found for this compound class in the public domain. The closest relevant analogue identified was [redacted], which is non-mutagenic. Based on the low general reactivity of the [redacted] group and the absence of any structural alerts for the corresponding carbon analogue (which did not flag as unclassified in DEREK), [redacted] is considered unlikely to exhibit mutagenic activity.

11 Integrated Summary and Safety Evaluation

A large number of impurities of VX-661 manufacturing were assigned specification limits based on studies described in this review. Some impurities were qualified for the levels in the drug substance at the NOAEL dose used in toxicology studies of VX-661. The starting reagents were examined for their potential toxicity in genetic toxicology tests (bacteria reverse mutation (Ames) assay and chromosomal aberration assay with human lymphocytes). Starting materials were also tested in a variety of alternative toxicity tests that included an acute oral toxicity test in the rat, skin sensitization potential using the local lymph node assay in the mouse, EpiSkin™ skin irritation test, in vitro skin irritation test, acute eye irritation in the rabbit, and the bovine corneal opacity and permeability assay. For all impurities, *in silico* computer algorithms were conducted to examine the mutagenic potential of each compound's chemical structure.

For observable impurities in the toxicity studies of VX-661 drug substance, the acceptance criteria were set well below the qualified levels based on the VX-661 NOAEL dose.

For the starting reagents of VX-661 manufacturing; (b) (4) were negative for mutagenic and clastogenic potential. However, (b) (4) was positive for clastogenicity in the in vitro chromosomal aberration assay, although it was not mutagenic in the bacteria reverse mutation assay. There was an increase in polyploidy, which may indicate that (b) (4) is not actually clastogenic. However, additional testing would be needed to verify the potential clastogenic effects of (b) (4). Because this is a starting material and is not detected in the final drug substance, further verification for the potential of clastogenicity and polyploidy are not warranted at this time. The certificate of analysis for (b) (4) also indicated a substantial number of impurities that might account for this effect. However, in the final product, VX-661, these impurities would be limited due to adequate specification limits.

The alternative toxicity studies were not considered useful in characterizing these starting reagent compounds, mainly due to the very large discrepancy between the small amounts that may be present in the drug substance and drug product and the large amounts or doses tested. The studies were all of an acute nature with a short observation time (up to 1 week for some studies). Furthermore, no histopathology was conducted and local effects on the skin and eye are considered of minimal relevance for an orally administered drug. Based on the summary table below, there is little relevant toxicity that can be obtained from these studies concerning the starting materials.

Table 15: Summary of Toxicity Studies of Starting Reagents and VX-661

Compounds	**VX-661**	(b) (4)		
Acute Oral Toxicity: Acute median lethal oral dose	NS	>2000 mg/kg	>2000 mg/kg	>2000 mg/kg

(LD_{50})				
Skin Sensitization Potential (local lymph node assay)	Not a skin sensitizer	Not a skin sensitizer.	Potential to induce skin sensitization	Potential to induce skin sensitization
EpiSkin™ Skin Irritation Test	Non-irritant to the skin	Non-irritant to skin	Non-irritant to skin	Non-corrosive to skin
Acute Eye Irritation	Not meet the criteria for classification	NS	NS	Not meet the criteria for classification
Bovine Corneal Opacity and Permeability	No prediction for ocular irritation	No Category	No Category	No prediction for ocular irritation
NS: no study				

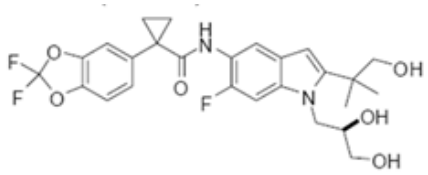
In silico modeling for mutagenicity based on chemical structure using DEREK and SARAH algorithms, found that 22 of 102 structure (including the 35 impurities associated with VX-661 manufacture) were predicted to be mutagenic in the bacterial reverse mutation test. However, only 10 were predicted to be mutagenic by both methods.

The specifications for the organic impurities were set based on the following:

- Existing Ames result or *in-silico* analysis, combined with expert analysis to determine the potential for DNA reactivity as described in ICH M7
- Levels of the impurities in representative batches
- Impurity fate and purge capability under the downstream processing conditions

The specifications set based on toxicity studies and computational structural analysis are appropriate and no further studies need to be conducted at this time.

Table 16: Summary of Impurity Evaluation

Compound	Comments#
Tezacaftor (VX-661)	
VX-661 	• (b) (4) structural alert • **Negative in genetic toxicology standard battery of *in vitro* and *in vivo* assays** • **Negative in carcinogenicity studies: 2-year rat and 6-month transgenic rasH2 mouse** • Threshold of toxicological concern (TTC) (b) (4) ppm for tezacaftor drug substance

14 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

12 Appendix/Attachments

ATTACHMENT 1: Consult Request

From: Bertha, Craig M **Sent:** Thursday, September 14, 2017 6:22 AM
To: Leshin, Lawrence
Cc: Bain, Sam; Lalmansingh, Anika
Subject: FW: NDA 210491- Potential mutagenic impurities in Tezacaftor - PT input needed

Steve, FYI - Other email from Sam about tezacaftor impurities (with attachment),

Craig

Craig M. Bertha
Chemist
FDA/OPQ/ONDP/Div II/Branch IV
White Oak Building 21
Room 2548
Phone 301-796-1646

From: Bain, Sam
Sent: Wednesday, September 13, 2017 2:57 PM
To: Aisida, Bamidele (Florence); Lalmansingh, Anika
Cc: Christner, Donna; Pinto, Julia; Bertha, Craig M
Subject: RE: NDA 210491- Potential mutagenic impurities in Tezacaftor - PT input needed

Oops! Forgot to attach the file!

Anika, I am sending this to you too; as Florence in OOO, and asked people to contact you in her place. Thanks.

From: Bain, Sam
Sent: Wednesday, September 13, 2017 2:54 PM
To: Aisida, Bamidele (Florence)
Cc: Christner, Donna; Pinto, Julia; Bertha, Craig M
Subject: NDA 210491- Potential mutagenic impurities in Tezacaftor - PT input needed

Hi Florence,

Looking at the emails on this NDA, I do not see who the PharmTox reviewers for this NDA are. Could you please forward this message to them?

There are 30 potential impurities in the API that PT would need to evaluate, and we at CMC would need their input. I have organized them in the attached file. Please note that many of these impurities have (b) (4) structural alerts.

Thanks,

Sam

Sukhamaya (Sam) Bain, Ph.D.
Senior Chemistry Reviewer
US Department of Health and Human Services
FDA/CDER/OPQ/ONDP/Division of New Drug API
10903 New Hampshire Avenue, Bldg 75, Rm 5726
Silver Spring, MD 20993
Phone (240) 402-8890

For PT Consult for NDA 210491 Drug Substance (From Sam Bain)

API Impurity	Reason for Seeking PT Input
(b) (4)	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant justifies based upon (b) (4) qualification threshold ((b) (4)%).
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.

(b) (4)	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant refers to toxicity study.
	(b) (4) structural alert; controlled at NMT (b) (4)% in API. Applicant justifies based upon ICH Q3A qualification threshold ((b) (4)%).
	(b) (4) structural alerts. (b) (4) . Purging to NMT TTC ((b) (4) ppm) not demonstrated.
	Impurity in key (b) (4) structural alerts. Not monitored in the API. Purging to NMT TTC not demonstrated.

(b) (4)	Fate product of (b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated. (Please ignore the 's' and the hyphen on top of it.)
	Fate product of (b) (4) structural alerts. Not monitored in the API. Purging to NMT TTC not demonstrated.
	Impurity in (b) (4) structural alerts. Not monitored in the API. Purging to NMT TTC not demonstrated. (Please ignore the 'step' and the hyphen on top of it.)
	Fate product of (b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated. (Please ignore the 's' and the hyphen on top of it.)
	Impurity in (b) (4) structural alerts. Not monitored in the API. Purging to NMT TTC not demonstrated.
	Impurity in (b) (4) tructural alerts. Not monitored in the API. Purging to NMT TTC not demonstrated. (Please ignore the 'step' and the hyphen on top of it.)
	Fate product of (b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated. (Please ignore the 's' and the hyphen on top of it.)
	Impurity in (b) (4) structural alerts. Not monitored in the API. Purging to NMT TTC not demonstrated.

(b) (4)	Fate product of (b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated.
	Fate product of impurity (b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated.
	Fate product of impurity (b) (4) in the starting material (b) (4) structural alert. Monitored in the API as unspecified impurity with a limit of NMT (b) (4) % as per ICH Q3A identification threshold.
	Fate product of impurity (b) (4) in the starting material (b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated.
	Fate product of impurity (b) (4) in the starting material (b) (4) structural alert. Monitored in the API as unspecified impurity with a limit of NMT (b) (4) % as per ICH Q3A identification threshold.
	Fate product of impurity (b) (4) in the starting material (b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated.
	Fate product of impurity (b) (4) in the starting material (b) (4) structural alert. Monitored in the API as unspecified impurity with a limit of NMT (b) (4) % as per ICH Q3A identification threshold. (Please ignore the 'step' and the hyphen on top of it.)
	(b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated.
	(b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated.

(b) (4)	(b) (4) structural alert. Not monitored in the API. Purging to NMT TTC not demonstrated.

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

/s/

LAWRENCE S LESHIN
02/02/2018

CAROL M GALVIS
02/02/2018
I concur.

Pharmacology and Toxicology Secondary Review for NDA 210491

Date: January 31, 2018

To: **NDA 210491**
Symdeko (tezacaftor/ivacaftor combination)
Vertex Pharmaceuticals, Inc.

From: Carol M. Galvis, PhD
Acting Pharmacology and Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products (DPARP)

Recommendation

I concur with the recommendation of Dr. Lawrence S. Leshin that NDA 210491 can be approved from the pharmacology and toxicology perspective (refer to Dr. Leshin's review dated January 29, 2018). The nonclinical pharmacology and toxicology profiles of tezacaftor have been adequately characterized. The nonclinical program supports the tezacaftor/ivacaftor combination and is considered complete.

Background

Vertex Pharmaceuticals submitted a 505(b)(1) New Drug Application to propose Symdeko (tezacaftor/ivacaftor combination) for the treatment of cystic fibrosis (CF) in patients age 12 and older who are homozygous for the *F508del* mutation in the *CFTR* gene or who have at least one mutation that is responsive to tezacaftor/ivacaftor based on in vitro data and/or clinical evidence. Tezacaftor is a CFTR modulator that facilitates cellular processing and trafficking of normal and select mutant forms of CFTR (including *F508del*) to increase the amount of mature CFTR protein delivered 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.

Ivacaftor is currently approved in the US as monotherapy (Kalydeco) for the treatment of CF in patients 2 years of age and older who have one mutation in the CFTR gene that is responsive to the drug based on clinical and/or in vitro data. Ivacaftor is also approved in combination with lumacaftor [another CFTR modulator that facilitates processing and transport of CFTR protein to the cell membrane (Orkambi)] for the treatment of CF patients homozygous for the *F508del* mutation in the *CFTR* gene. In vitro studies demonstrated an additive effect when tezacaftor and ivacaftor were used in combination in that there was increased quantity and function of CFTR at the cell surface, resulting in increased chloride transport (see "Pharmacology" below).

Symdeko is co-packaged as tezacaftor 100mg/ivacaftor 150mg fixed dose combination tablets and ivacaftor 150mg tablets. The proposed clinical dosing regimen for adults and pediatric patients ages 12 and older is one tablet containing 100mg tezacaftor and 150mg ivacaftor in the morning and one tablet containing 150mg ivacaftor in the evening, approximately 12 hours apart.

Since ivacaftor is an approved product and the pharmacology and toxicology profiles have been already characterized, this review will focus on the nonclinical program to support tezacaftor as well as tezacaftor/ivacaftor combination.

Pharmacology

Pharmacology studies assessed the potency and efficacy of tezacaftor on the CFTR-mediated chloride transport from monolayers of human bronchial epithelial (HBE) cells isolated from CF patients that were *F508del*-homozygous. Tezacaftor was shown to increase delivery and amount of functional CFTR protein at the cell surface, resulting in enhanced functional chloride transport. In Ussing chamber electrophysiology studies using a panel of Fisher rat thyroid (FRT) cells expressing mutant CFTR that had demonstrated responsiveness to ivacaftor alone, tezacaftor/ivacaftor combination resulted in increased chloride transport compared to ivacaftor alone. These data supported development of this combination product.

Safety pharmacology studies with tezacaftor did not identify any adverse effects on the central nervous, cardiovascular, or respiratory systems. A gastrointestinal safety pharmacology study found that tezacaftor delays gastric emptying but had no effects on intestinal transit.

Pharmacokinetics/ADME

Three major metabolites of tezacaftor were identified in humans; M1, M2, and M5. M1 is a dehydrogenation metabolite with pharmacological activity similar to tezacaftor. M1 was found in plasma of rats and dogs at levels comparable to humans; therefore, was considered qualified in the toxicity studies conducted with tezacaftor.

M2 is an oxidation product of M1, also with pharmacological activity, but weaker than M1. M2 was identified as a disproportionate human metabolite in that very little was present in plasma from mice, rats, and dogs. Therefore, additional safety studies (genetic toxicology, 28-day subcutaneous toxicity study in dogs, and embryofetal development study in rabbits) were conducted with M2 to further characterize its toxicity profile. M2 was not genotoxic, not teratogenic, and no adverse findings were observed in dogs with treatment up to 28 days (see below). M2 was not tolerated in rodents after subcutaneous administration due to severe skin lesions. Therefore, additional toxicity studies (general toxicology, reproductive and developmental toxicology in rats, or carcinogenicity) with M2 were not conducted.

M5 is a phosphate conjugate of M1 and has no pharmacological activity. M5 was present in rats (but not in dogs) after administration of tezacaftor at levels comparable to humans. Therefore, M5 was considered qualified based on the toxicity studies conducted with tezacaftor in rats.

Established Pharmacologic Class (EPC)
Vertex originally proposed to classify tezacaftor as [redacted](b) (4)". During review of Orkambi (lumacaftor/ivacaftor), there was extensive discussion regarding the proposed classification [redacted](b) (4)" for lumacaftor. The Division did not consider this classification was supported or justified based on the available data. Of note, lumacaftor has no EPC.

A comment was sent to Vertex in the filing letter for this NDA to communicate concerns with the proposed classification for tezacaftor, based on discussions during Orkambi's review. Therefore, a pharmacologic class was not established for tezacaftor.

Toxicology Program
The toxicity profile of tezacaftor was evaluated in rats and dogs. In a 6-month rat toxicity study, animals received 0, 25, 50, or 100 mg/kg/day tezacaftor for up to 6 months. Drug-related findings included decreased body weights and dilated terminal lymphatics in the villi of the jejunum and ileum. This finding was still present after a 2-week recovery period. However, incidence and severity did not increase from the 3-month toxicity study to the 6-month toxicity study. A NOAEL was not identified due to these findings in the gastrointestinal (GI) tract. Additional safety studies were conducted in dogs to characterize this finding.

In dogs, several toxicity studies were conducted up to 1-year duration, including studies with two different drug formulations (a liquid formulation vs. a tablet formulation) to characterize findings in the GI tract. In the 1-year study, dogs received 0, 2, 10, 100, or 200 mg/kg/day tezacaftor for 52 weeks. Dilated lymphatics in the small intestine (jejunum, proximal ileum, and duodenum) were observed at doses ≥10 mg/kg/day. Dilated lymphatics were present mostly at the tip of the villi. There were a few changes in clinical chemistry (decreases in albumin and increases in cholesterol) that may have been associated with this finding in the GI tract. Due to lack of progression over time, partial reversibility after 1 year recovery, and lack of associated toxicities, these findings were eventually judged drug-related but not adverse.

M2 metabolite
A 28-day toxicity study was conducted with M2 metabolite in dogs using the subcutaneous route of administration. There were severe skin lesions identified in this study, but no additional toxicities. This was consistent with findings observed in rats and rabbits (see below under "Reproductive and Developmental Toxicology").

Toxicology with tezacaftor/ivacaftor combination
To support development of the tezacaftor/ivacaftor (TEZ/IVA) combination, a 13-week toxicity study was conducted in rats. Rats received TEZ/IVA at 0/0, 20/80, 40/40, or 80/20 mg/kg/day for 13 weeks. The study design was considered suboptimal in that increasing doses of tezacaftor were used with decreasing doses of ivacaftor, monoproduct control groups were not included, and recovery groups were not included for the two lower tezacaftor dose levels. Therefore, it was not possible to assess any potential synergistic effects. The target organs were identified as kidney (basophilic tubules, inflammatory cell infiltrates, tubular degeneration, and thickening of the basement membranes), small intestine (dilated lymphatics terminals), pancreas (single-cell necrosis of pancreatic exocrine cells), and bone marrow (decreased cellularity of multiple lineages). No new toxicities were identified in this study compared to the toxicities already identified with tezacaftor (discussed above) or ivacaftor.

Reproductive and Developmental Toxicology
No reproductive and developmental studies were conducted with tezacaftor/ivacaftor combination. The reproductive and developmental toxicology assessment of tezacaftor was conducted in rats (fertility and early embryonic development, embryofetal development, and pre- and post-natal development) and rabbits (embryofetal development). In all the studies, tezacaftor resulted in decreased maternal weight and food consumption. There were no effects on male or female fertility at doses up to 100 mg/kg/day. Tezacaftor was not teratogenic in rats or rabbits when administered to pregnant females during organogenesis at doses up to 100 mg/kg/day. In rabbits, there was decreased pup weights at doses of ≥50 mg/kg/day.

In the pre- and post-natal development study in rats (tezacaftor doses of 0, 25, 50, and 100 mg/kg/day), there was severe maternal toxicity at 100 mg/kg/day. At 50 mg/kg/day, there was decreased fetal weight and early developmental delays in pinna detachment, eye opening, and static righting reflex. At 100 mg/kg/day, there were also delays in pup sexual maturation (vaginal opening and preputial separation).

M2 metabolite
To assess reproductive and developmental effects of the M2 metabolite, an embryofetal development study was conducted in rabbits using the subcutaneous route of administration at doses up to 120 mg/kg/day. M2 was not teratogenic in rabbits. However, there was severe skin toxicity observed, which is consistent with findings observed in rats and dogs. No additional reproductive and developmental studies were conducted with M2 due to lack of tolerability in rodents.

Genetic Toxicology and Carcinogenicity
Tezacaftor was not genotoxic in a standard battery of genetic toxicology assays (in vitro Ames and chromosomal aberration assays and in vivo micronucleus

assay). No carcinogenicity studies were conducted with tezacaftor/ivacaftor combination. The carcinogenic potential of tezacaftor was evaluated in a 2-year Sprague-Dawley rat bioassay and a 6-month transgenic Tg.rasH2 study. Tezacaftor was not teratogenic in rats at doses up to 50 or 75 mg/kg/day in males and females, respectively, or in mice at doses up to 500 mg/kg/day.

Comments on metabolites
Regarding metabolites, M1 and M5 were considered qualified for carcinogenicity, since both are present in rats. M2 is present at low levels after oral administration in rodents and is not tolerated using an alternate route (e.g., subcutaneous). After discussions with the Executive Carcinogenicity Assessment Committee, it was determined that additional carcinogenicity studies were not required for M2.

Labeling
Dr. Leshin's review dated January 29, 2018 recommended edits to the proposed product labeling for the following sections: Section 8 Use in Specific Populations (Sections 8.1, 8.2, and 8.4), Section 12 Clinical Pharmacology (only Section 12.1 Mechanism of Action), and Section 13 Nonclinical Toxicology. Regarding Section 12, I defer to the clinical pharmacology and clinical disciplines for final language. I concur with Dr. Leshin's proposed edits for Sections 8 and 13, which are consistent with current labeling practices and with the approved labels for Kalydeco (ivacaftor) and Orkambi (lumacaftor/ivacaftor).

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/s/

CAROL M GALVIS
01/31/2018

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:	210491
Supporting document/s:	1
Applicant's letter date:	June 28, 2017
CDER stamp date:	June 28, 2017
Product:	Symdeko™ (Tezacaftor and Ivacaftor)
Indication:	Cystic Fibrosis (CF)
Applicant:	Vertex Pharmaceuticals Incorporated
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Eleni Salicru, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Jessica Lee, PharmD

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

Vertex Pharmaceuticals Incorporated (Vertex) submitted a 505(b)(1) New Drug Application (NDA) for tezacaftor (VX-661)/ivacaftor (VX-770) combination therapy for the treatment of cystic fibrosis (CF) in patients 12 year of age and older who are homozygous for the F508del mutation or who have at least one mutation in the CF transmembrane conductance regulator (CFTR) gene that is responsive to tezacaftor/ivacaftor, based on in vitro data and/or clinical evidence. Ivacaftor was approved for the treatment of CF on January 31, 2012. The carcinogenic potential of ivacaftor was reviewed under NDA 203188; ivacaftor was not tumorigenic in either 2-year rat or mouse studies. This review will focus on the carcinogenicity evaluation of tezacaftor (VX-661) in a 2-year rat study and a 26-week Tg.rasH2 mouse study. The Executive Carcinogenicity Assessment Committee (ECAC) concurred with the doses and designs of the studies (see Special Protocol Agreements dated December 18, 2013 and November 20, 2014).

1.2 Brief Discussion of Nonclinical Findings

In a 104-week oral (gavage) carcinogenicity study, Sprague-Dawley (SD) male rats (70 per group) received doses of 0, 5, 15, and 50 mg/kg/day VX-661 and SD female rats (70 per group) received doses of 0, 5, 20, and 75 mg/kg/day VX-661. Although there were no drug-related effects on mortality, both male and female groups were terminated early (i.e., Week 103 and Week 101, respectively) because the number of surviving animals in the respective control groups reached ≤20. At the end of the study period, mean body weights for high dose males and females were 5.9% and 17.7% lower, respectively, compared to control animals. There were no statistically significant drug-related tumor findings in male or female rats. The most notable non-neoplastic histopathology findings were dilated lymphatics in the gut-associated lymphoid tissue (GALT) and small intestine (ileum and jejunum). VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) was 180 and 337 mcg*hr/mL, in males and females, respectively, resulting in exposure margins about 2.4-fold and 4.5-fold above the clinical dose.

In a 26-week oral carcinogenicity study, Tg.rasH2 male and female mice received doses of 0, 30, 100, and 500 mg/kg/day VX-661 (25/sex/group) and 1000 mg/kg/day of the positive control urethane (10/sex/group). There was a statistically significant dose response relationship in mortality for high dose males (76% survival) compared to control males (96% survival). There were no statistically significant drug-related tumor findings in male or female mice. The most notable non-neoplastic histopathology findings were in the adrenal glands (cortex hypertrophy, cortex vacuolation, and x-zone degeneration), liver (centrilobular hypertrophy, centrilobular hepatocellular single cell necrosis, subcapsular inflammation, focal hepatocellular necrosis, focal lipid infiltration, and periportal vacuolation), ovaries (decreased corpora lutea), and jejunum (lamina propria vacuolation). VX-661 AUC exposure at the high dose (500 mg/kg/day) was about 93 and 154 mcg*hr/mL in males and females, respectively.

VX-661 is extensively metabolized (mainly by CYP3A4) in humans and nonclinical species. Dehydrogenation of VX-661 leads to the major metabolite M1 (VRT-0996107), which has been detected in rat, dog, monkey and humans. M1 constitutes approximately 38.8% of total systemic exposure in humans. M1 is pharmacologically active with similar potency and efficacy as VX-661. M1 was found to be a major circulating metabolite in rats. Thus, exposures in rats in the rat toxicity studies reached sufficient levels to provide an assessment of its toxic potential in humans. In the 2-year carcinogenicity study with rats, M1 AUC exposure at the high dose of VX-661 (50 mg/kg/day in males and 75 mg/kg/day in females) was 288 and 479 mcg*hr/mL, in males and females, respectively, resulting in exposure margins about 2.5-fold and 4.2-fold above the clinical exposure. Exposure to M1 was quantified in the 26-week Tg.rasH2 mouse carcinogenicity study. Thus, the 2-year rat and 26-week mouse studies provides an adequate assessment of the carcinogenic potential of M1.

Sequential oxidation of M1 forms metabolite M2 (VRT-1189001), which has been detected in humans, rats, and dogs. M2 is a disproportionate human metabolite (i.e., high levels in humans [approximately 35.7% of total systemic exposure] and low levels in rats and dogs). M2 is pharmacologically active but less so than VX-661. The Applicant found that oral administration of M2 to rats, guinea pigs, and dogs had poor bioavailability. Intravenous administration of M2 resulted in deaths in rats. Further, subcutaneous administration was not tolerated in rats. In a 1-month toxicity study, dogs could tolerate a subcutaneous dose that produced an approximate exposure to the expected therapeutic human dose. As with rats, it is likely also for mice that an oral M2 dose group would not achieve relevant exposures, and a subcutaneous administration may not be tolerated, although this has not been attempted. Thus, it was judged that the carcinogenic potential of the M2 metabolite could not be studied. M2 was negative for potential genetic toxicity by the bacterial reverse mutation assay and chromosomal aberration assay with human peripheral blood lymphocytes. In the 2-year carcinogenicity study with rats, M2 AUC exposure at the high dose of VX-661 (50 mg/kg/day in males and 75 mg/kg/day in females) was 20 and 13 mcg*hr/mL, in males and females, respectively, resulting in exposure margins about 0.19 and 0.12 of the clinical exposure. Exposure to M2 was quantified in the 26-week Tg.rasH2 mouse carcinogenicity study. Exposures to M2 in the 2-year rat and 26-week mouse studies were approximately ≤0.2 of the achieved clinical exposure. No further nonclinical assessment of the carcinogenic potential of M2 is required.

M5 (VRT-1074233) was detected in a human mass balance study at >10% of total systemic exposure. M5 is derived from phosphorylation of M1, and can potentially interconvert back to M1. In vitro studies in cultured F508del-HBE showed that M5 was inactive. M5 has not been routinely monitored in nonclinical studies with rats or dogs. The Applicant provided data that M5 is formed in rats from a single dose study, but not in repeat dose studies. It was judged that M5 was formed in sufficient levels in rats to provide an assessment of its toxic potential in humans. Thus, it is reasonable to assume that the 2-year rat study provides an adequate assessment of the carcinogenic potential of M5. M5 has not been studied for potential genotoxicity.

No statistically significant neoplastic findings were observed in male or female SD rats and male or female Tg.rasH2 mice treated with tezacaftor at maximum tolerated doses (MTDs).

The ECAC concurred that both the 2-year rat and 26-week Tg.rasH2 carcinogenicity studies were adequate and that there were no drug-related neoplasms in males or females in either study. The ECAC also concurred that based upon feasibility and the completed carcinogenicity studies in rats and Tg.rasH2 mice, that no further studies were required for the safety qualification of the M1, M2, and M5 metabolites with respect to carcinogenicity.

1.3 Recommendations

The final study results for the 2-year rat and the 26-week Tg.rasH2 mouse carcinogenicity studies were presented to the ECAC on November 21, 2017. The ECAC recommendations and conclusions are included below.

2-Year SD Rat

- The Committee concurred that the study was adequate, noting prior ECAC approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 2-year rat carcinogenicity study in either males or females.

26-Week Tg.rasH2 Mouse

- The Committee concurred that the study was adequate, noting prior ECAC approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 26-week Tg.rasH2 mouse study in either males or females.

Human metabolites >10% of total systemic exposure

- Based upon feasibility and the completed carcinogenicity studies with VX-661 in SD rats and Tg.rasH2 mice, the Committee concurred that no further studies were required for the safety qualification of the M1, M2, and M5 metabolites with respect to carcinogenicity.

1.3.1 Approvability

See Dr. Leshin's Pharmacology and Toxicology Review of NDA 210491.

1.3.2 Additional Non Clinical Recommendations

See Dr. Leshin's Pharmacology and Toxicology Review of NDA 210491.

1.3.3 Labeling

Recommended labeling for carcinogenicity studies is provided at the end of this review.

2 Drug Information

2.1 Drug

CAS Registry Number: 1152311-62-0

Generic Name: tezacaftor

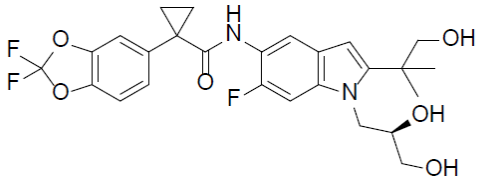
Code Name: VX-661

Chemical Name: 1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1Hindol-5-yl}cyclopropane-1-carboxamide

Molecular Formula: $C_{26}H_{27}N_2F_3O_6$

Molecular Weight: 520.50 g/mol

Structure or Biochemical Description:



Pharmacologic Class: See Dr. Leshin's Pharmacology and Toxicology Review of NDA 210491.

2.2 Relevant INDs, NDAs, BLAs and DMFs

The following are relevant INDs and NDAs by Vertex for tezacaftor and/or ivacaftor:

- IND-074633: KALYDECO® (ivacaftor)
- IND-108105: tezacaftor (VX-661)

- NDA-203188: KALYDECO® (ivacaftor)
- NDA-206038: ORKAMBI® (ivacaftor and lumacaftor)
- NDA-207925: KALYDECO® (ivacaftor)

2.7 Regulatory Background

Under IND 108105, the Sponsor submitted Special Protocol Assessment (SPA) requests for the 2-year carcinogenicity study in rats and the 6-month carcinogenicity

study in Tg.rasH2 transgenic mice. The Division received the Sponsor's original SPA request for the 2-year rat study on November 4, 2013 (Supporting Document Number [SDN] 47). On December 5, 2013 (SDN 52), the Division received an amendment to the 2-year rat study protocol under the SPA, with revisions for the accurate justification of dose selection and the correct proposed doses for the study (i.e., 0, (b) (4) and 50 mg/kg/day). The SPA was reviewed by the ECAC on December 18, 2013, and the ECAC did not concur with the Sponsor's proposed doses. Instead, the ECAC recommended doses of 0, 5, 15, and 50 mg/kg/day in males and 0, 5, 20, and 75 mg/kg/day in females, based on a MTD. The ECAC also suggested that the Sponsor consider testing the major human metabolite VRT-1189001 (M2) in the study, if possible (e.g., at a tolerated dose). See Special Protocol Agreement dated December 18, 2013 for additional details. Of note, carcinogenicity studies to assess the safety qualification of the metabolites were not feasible due to the route of administration.

The Division received the Sponsor's SPA request for the 6-month transgenic mouse study on September 30, 2014 (SDN 80). On October 10, 2014, the SPA was denied because it did not contain the information necessary to support protocol review and dose selection for the proposed carcinogenicity study (i.e., the study report for the 28-day repeat dose oral toxicity and toxicokinetic (TK) study in CByB6F1 mice with a preliminary 5-day range-finding toxicity study). On October 15, 2014 (SDN 84), the Division received the Sponsor's SPA resubmission with the appropriate information. The SPA was reviewed by the ECAC on November 20, 2014 and the ECAC did not concur with the Sponsor's proposed doses of 0, 30, 100, and (b) (4) mg/kg/day. Instead, the ECAC recommended doses of 0, 30, 100, and 500 mg/kg/day based on mortality at 1500 mg/kg/day. See Special Protocol Agreement dated November 20, 2014 for additional details.

3 Studies Submitted

3.1 Studies Reviewed

VX-661: 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice (Sponsor Study Number: VX-661-TX-019; GLP-compliant)

VX-661: A 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Sponsor Study Number: VX-661-TX-020; GLP-compliant)

3.3 Previous Reviews Referenced

NDA 203188

Nonclinical review of GLP-compliant 24-month oral carcinogenicity studies in mice (Sponsor Study Number: VX-770-TX-013) and rats (Sponsor Study Number: VX-770-TX-014) with VX-770 (KALYDECO®; ivacaftor) dated January 3, 2012

IND 108105

Special Protocol Agreement (i.e., ECAC meeting minutes) dated December 18, 2013, for SPA of 2-year carcinogenicity study in rats

Special Protocol Agreement (i.e., ECAC meeting minutes) dated November 20, 2014, for SPA of 6-month carcinogenicity study in Tg.rasH2 mice

Nonclinical review dated October 15, 2015 (response to carcinogenicity update)

8 Carcinogenicity

Study title: VX-661: A 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Study no.:	Conducting laboratory: 863-159 Sponsor: VX-661-TX-020
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 2, 2014 (protocol signed by Study Director)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), lot # 17QB10SD.NJ00003, 99.3% purity
CAC concurrence:	Yes (see Special Protocol Agreement under IND 108105, dated December 18, 2013)

Key Study Findings

- In a 104-week oral (gavage) carcinogenicity study, SD male rats received doses of 0, 5, 15, and 50 mg/kg/day VX-661 and SD female rats received doses of 0, 5, 20, and 75 mg/kg/day VX-661.
- There were no drug-related effects on mortality, but male and female groups were both terminated early (i.e., Week 103 and Week 101, respectively) because the number of surviving animals in the respective control groups reached ≤20.
- At the end of the study period (i.e., Week 103 for males and Week 101 for females) mean body weights for high dose males and females were 5.9% and 17.7% lower, respectively, compared to control animals.
- There were no statistically significant drug-related tumor findings in male or female rats.
- The most notable non-neoplastic histopathology findings were dilated lymphatics in the GALT and small intestine (ileum and jejunum). Additional non-neoplastic lesions were seen in the parathyroid glands, liver, Harderian glands, clitoral glands, ovaries, and sternum bone marrow.
- VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) was 180 and 337 mcg*hr/mL in males and females,

respectively. Exposures to VX-661 metabolites, VRT-0996107 (M1) and VRT-1189001 (M2), were also assessed in rats.

Adequacy of Carcinogenicity Study

- The ECAC concurred with the doses and design of the study (see Special Protocol Agreement dated December 18, 2013).
- The duration of treatment was adequate (i.e., 102 weeks for males and 100 weeks for females).

Appropriateness of Test Models

- The SD rat is considered an acceptable model for 2-year carcinogenicity studies.
- The SD rat achieved high exposures to VX-661 and its M1 metabolite. Exposure to the M2 metabolite was low. Exposure to the M5 metabolite (VRT-1074233) was not measured.

Evaluation of Tumor Findings

- There were no statistically significant drug-related tumor findings in male or female rats.

Methods

Doses:	0, 5, 15 or 50 mg/kg/day (males) 0, 5, 20, or 75 mg/kg/day (females)
Frequency of dosing:	Once daily for up to 102 weeks (males) or 100 weeks (females)
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Vehicle: 0.5% methylcellulose (400 cps) (w/v), 0.5% sodium lauryl sulfate (SLS) (w/v), and 0.01% simethicone (w/v) in deionized water (b) (4) control article: Hypromellose acetate succinate, also known as hydroxyproplymethylcellulose acetate succinate and SLS
Basis of dose selection:	Dose selection was based on the MTD identified from the 3-month (Study No. VX-661-TX-010) and 6-month (Study No. VX-661-TX-012) repeat dose oral toxicity studies in SD rats, in consultation with the ECAC. For males, the recommended high dose (MTD) of 50 mg/kg/day was selected based on lower relative body weights at 100 mg/kg in the 6-month study. The high dose (MTD) of 75 mg/kg/day for females was approximately one-third the lethal dose of 200 mg/kg/day in the 1-month study. Mid- and low-doses in male and female groups were selected to provide an approximately 3-fold dose separation based on systemic exposure.
Species/Strain:	Rat/SD (Crl:CD®[SD])
Number/Sex/Group:	70 (main study) 12 (TK)
Age:	About 8 to 9 weeks of age at start of dosing
Animal housing:	Two to three per cage (same sex) and pair-housed (same sex) in solid bottom cages with nonaromatic bedding in an environmentally controlled room
Paradigm for dietary restriction:	No dietary restrictions. Animals had access ad libitum to Block Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc.) and tap water via an automatic watering system
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	TK (12/sex/group)

Deviation from study protocol: Protocol deviations were presented in the study report and were deemed to have not affected the quality or integrity of the study

Observations and Results

Mortality

Cageside observations for morbidity and mortality were made for all animals, at least twice daily. There were no drug-treated effects on mortality (see Statistical Review and Evaluation for more details). Male and female groups were both terminated early (i.e., Week 103 and Week 101, respectively), because the number of surviving animals in the respective control groups reached ≤20. The number of drug-treated male and female animals that experienced early deaths (i.e., euthanized in extremis, found dead, or died after dosing) was similar across the control and all drug-treated dose groups (see **Table 1**). Survival curves for control and drug-treated male and female groups were similar, as shown in the Kaplan-Meier survival curves below (**Figure 1** and **Figure 2**, respectively).

Table 1 Number of Early Deaths versus Animals Surviving until Terminal Necropsy in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

VX-661 (mg/kg/day) (Males/Females)	Males		Females	
	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]
0/0	50 (71%)	20 (29%)	51 (73%)	19 (27%)
5/5	49 (70%)	21 (30%)	53 (76%)	17 (24%)
15/20	54 (77%)	16 (23%)	47 (67%)	23 (33%)
50/75	45 (64%)	25 (36%)	49 (70%)	21 (30%)

Abbreviations: # = number

Notes:

[1]# of Early Deaths = euthanized in extremis, found dead, or died after dosing

[2]numbers in parentheses represent the percent when compared to the number of main study animals at the start of the study (i.e., 70 animals/sex/group)

Figure 1 Kaplan-Meier Survival Curves for Males in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (FDA Statistical Reviewer's Figure)

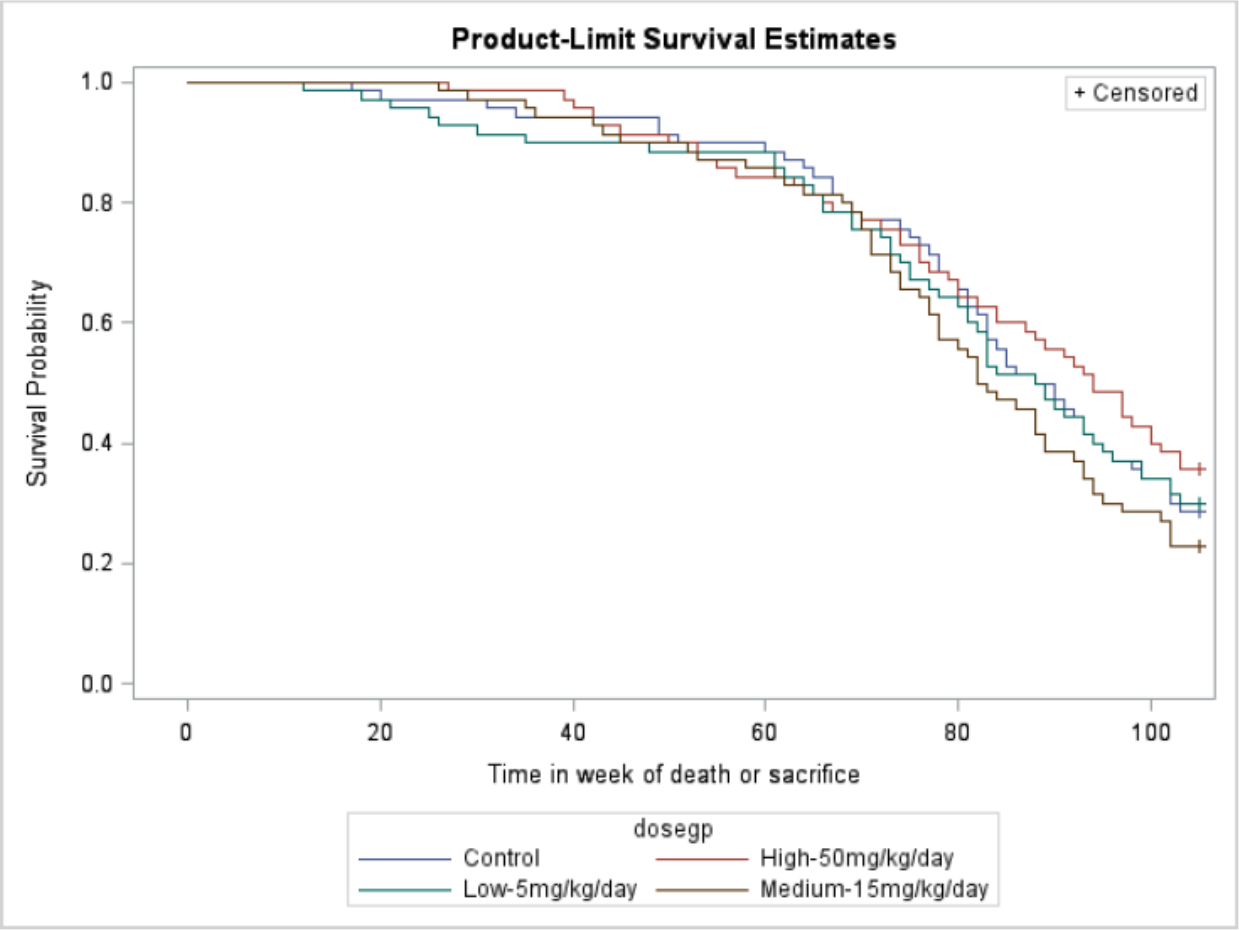
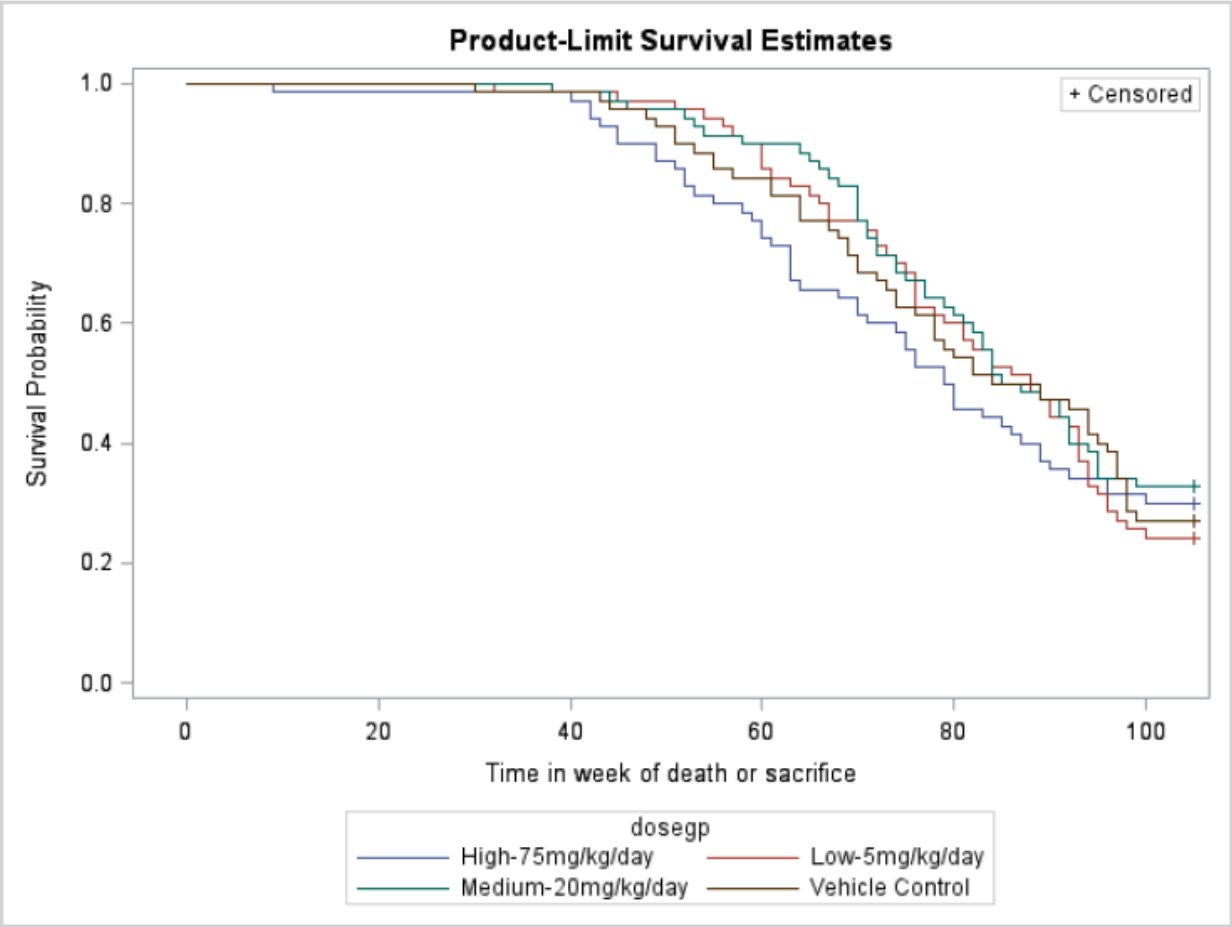


Figure 2 Kaplan-Meier Survival Curves for Females in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (FDA Statistical Reviewer's Figure)



Pituitary tumors were the most common probable cause of death for both male and female animals, although there was no drug-treated difference compared to control animals. In general, there was no probable cause of death that occurred with much greater incidence in male and female drug-treated groups compared to control groups, although a larger number of high dose males than control males died from lymphoid tumors (4/70 versus 1/70, respectively) and skin tumors (3/70 versus 0/70).

Clinical Signs

Detailed clinical observations were performed prior to randomization and generally once weekly during the study (about 4 hours postdose) and included evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior, and the palpation of tissue masses.

Select clinical observations that were seen in a greater number of high dose animals than control animals are shown in **Table 2**, although none of the clinical observations were considered test-article related.

Table 2 Select Clinical Observations with Higher Incidence in the High Dose Group Compared to the Control Group in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Observation	Males (Weeks 1 to 103)				Females (Weeks 1 to 100)			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	5	15	50	0	5	20	75
Number of Animals Observed	70	70	70	70	70	70	70	70
External Appearance								
Discharge, red	6/5	6/6	10/6	13/10	11/6	8/4	9/7	9/6
Ear/portion of ear missing	77/1	292/3	469/6	140/3	0/0	0/0	49/2	50/2
Material around eyes, black	45/10	22/6	70/8	9/5	10/6	129/14	71/16	26/11
Material around nose, red	8/7	17/11	14/8	14/5	10/8	12/6	9/4	28/12
Posture hunched	32/9	18/10	34/12	22/9	12/7	13/7	33/14	40/12
General Status								
Hair discolored, tan	62/7	35/4	68/6	120/8	-	-	-	-
Hair sparse	1030/44	1075/34	1091/38	979/43	2078/41	1783/40	2703/39	3121/46
Hair wet	5/4	10/7	9/8	6/4	3/3	8/5	6/2	17/12
Nodule, 1-5 mm	275/19	344/18	272/16	311/21	48/6	26/1	33/2	2/1
Nodule, 5-20 mm	35/3	33/3	3/2	55/6	-	-	-	-
Nodule, >20 mm	0/0	0/0	0/0	15/3	-	-	-	-

Note: Number of times observed/Total number of animals affected

Body Weights

Animals were weighed at receipt, prior to randomization, weekly for the first 14 weeks, every two weeks until Week 28, every four weeks thereafter for the duration of the study, and on the day of the scheduled terminal necropsy. At Week 103, absolute mean body weights for male drug-treated groups were ≤5.9% relative to the male concurrent control group (see **Table 3**). The body weight curve for the high dose male group separated from the control and lower dose groups, although differences did not exceed 10% (see **Figure 3**). At Week 101, absolute mean body weight for the high dose female group was decreased by 17.7% relative to the female concurrent control group, although mean body weights for the low dose and mid dose female groups were decreased ≤4.3% (see **Table 3**). The body weight curve for the high dose female group separated from the control and lower dose groups with differences exceeding 10% (see **Figure 4**).

Table 3 Body Weight Changes in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Parameter	Males				Females			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	5	15	50	0	5	20	75
Week 1 (grams)	332.5	330.5	331.6	323.0*	212.1	210.4	209.5	196.2*
Week 103 Males or Week 101 Females (grams)	832.7	875.9	804.6	783.8	568.3	544.1	557.9	467.5*
Absolute Body Weight (% change from Week 103 Male or Week 101 Female controls)	0.0	5.2	-3.4	-5.9	0.0	-4.3	-1.8	-17.7

* = statistically significant from control ($p < 0.01$) based on Applicant's group pair-wise comparison's (Levene's/ANOVA-Dunnett's/Welch's)

Figure 3 Mean Body Weights in Males in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

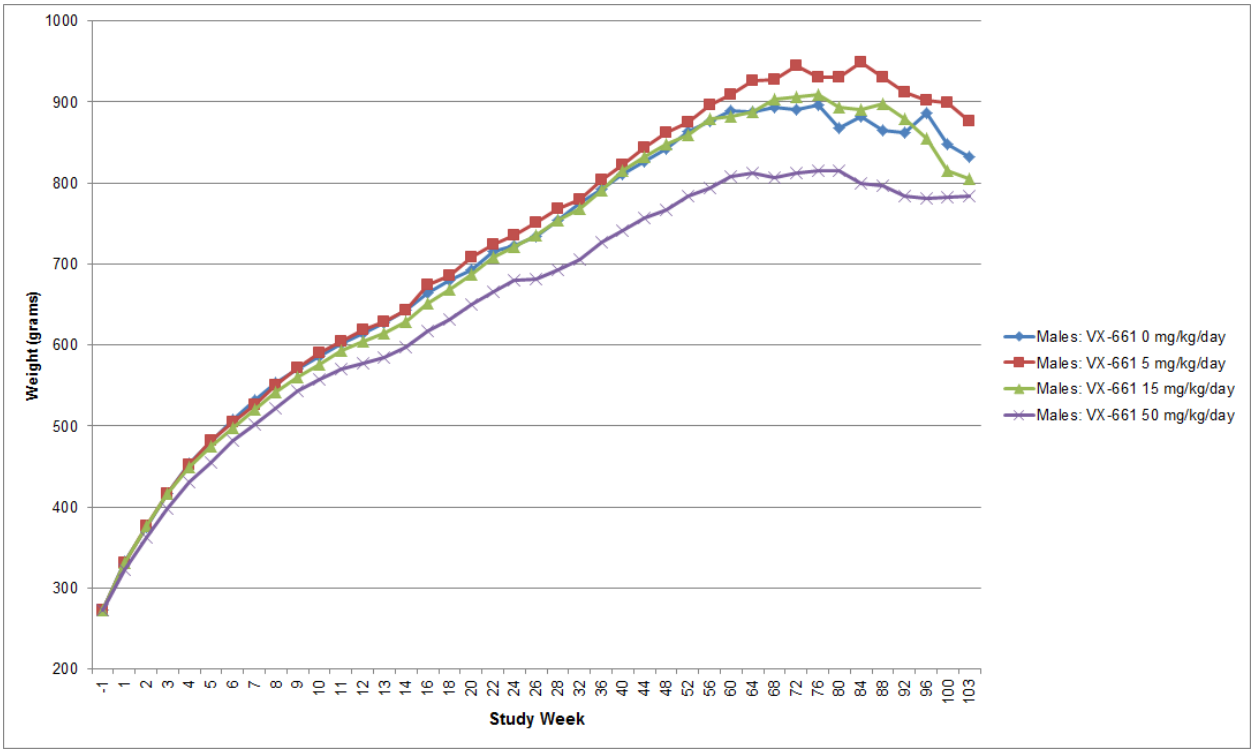
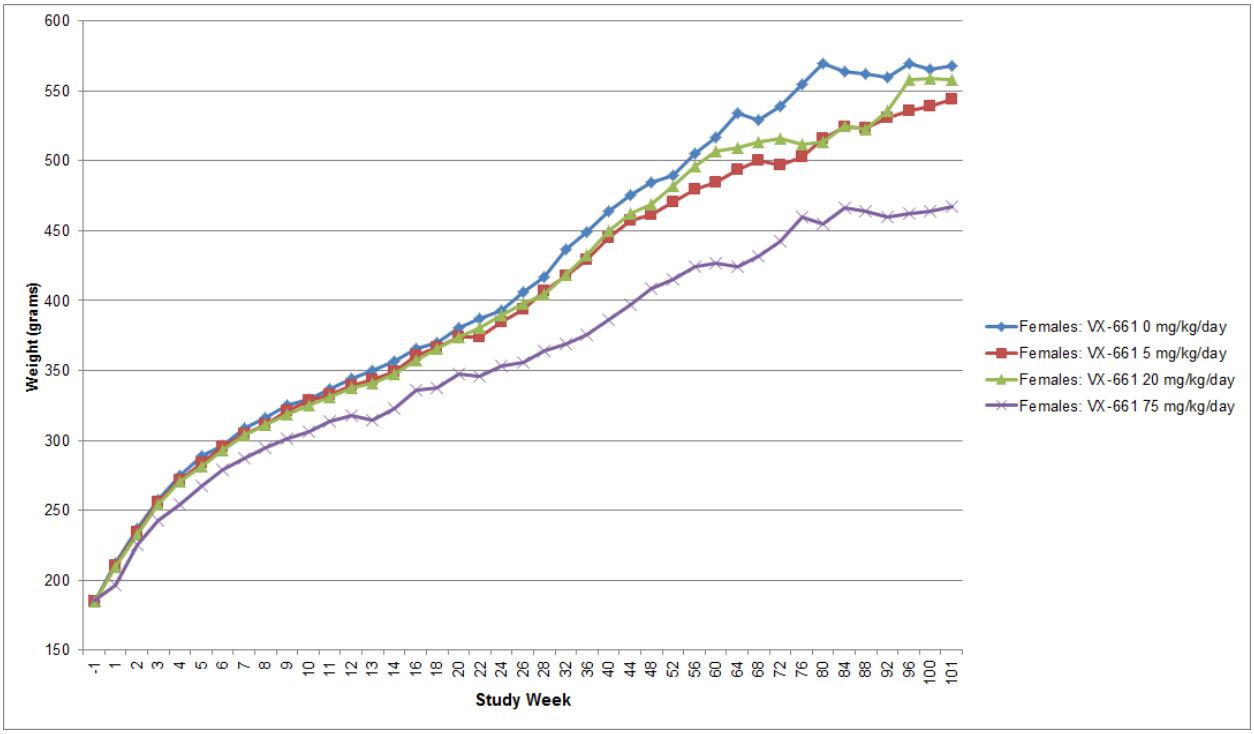


Figure 4 Mean Body Weights in Females in 104-Week Oral (Gavage) Carcinogenicity Study in Rats



Feed Consumption

Animals had access ad libitum to Block Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc.) and tap water via an automatic watering system. Food consumption was measured per cage on main study animals every week for the first 14 weeks, every two weeks until Week 28, and every four weeks thereafter for the duration of the study. As shown in **Figure 5** and **Figure 6**, sporadic decreases in food consumption for drug-treated animals were minimal in magnitude compared to control animals. Overall, there were no evident test-article related effects on food consumption.

Figure 5 Mean Caged Food Consumption in Males in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Figure)

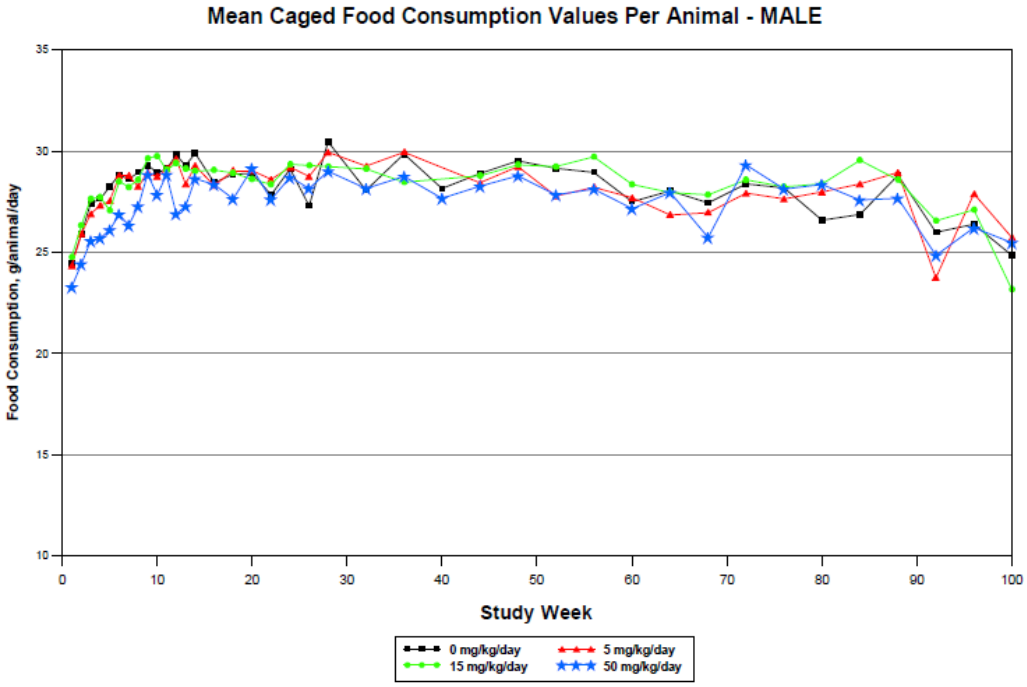
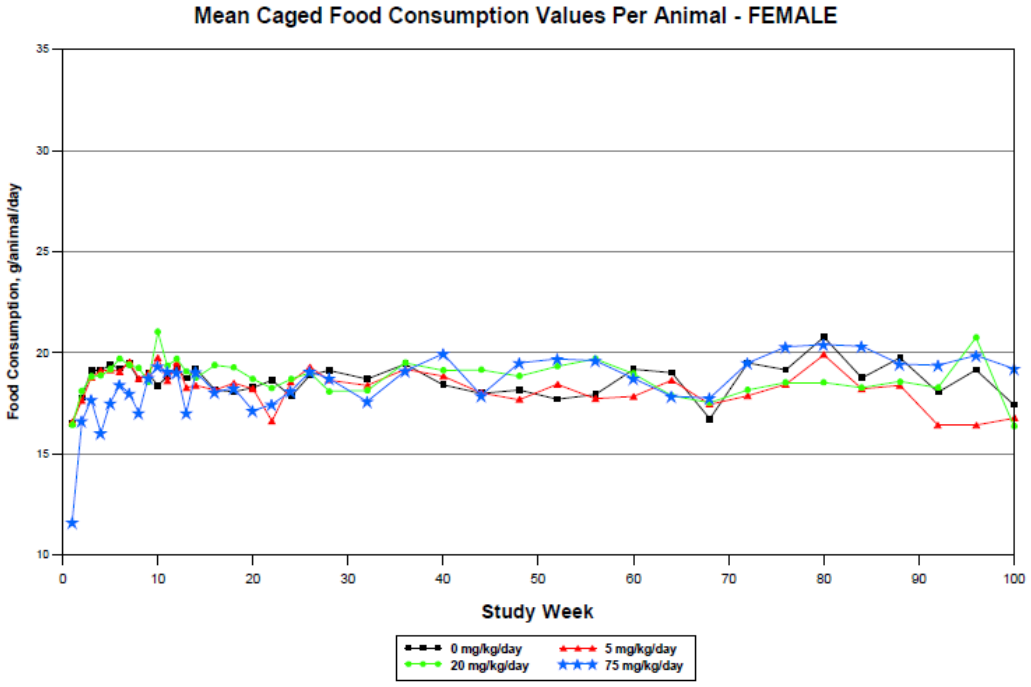


Figure 6 Mean Caged Food Consumption in Females in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Figure)



Gross Pathology

Sacrificed animals were euthanized by carbon dioxide inhalation. Complete necropsy examinations were performed on all main study animals (i.e., animals euthanized in extremis, found dead, and surviving animals). As shown in **Table 4**, there was a dose-dependent increase in the number of males with enlarged spleen in low dose (4/70), mid dose (6/70), and high dose (7/70) groups compared to the control group (1/70). This gross finding is possibly correlated with microscopic findings in the spleen of increased extramedullary hematopoiesis and lymphoma.

Table 4 Gross Pathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Tissue/Organ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
Number of Animals Examined	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
Spleen																
enlarged	1	0	2	2	4	2	5	2	4	0	1	0	1	0	0	1
…mild	0	0	1	1	2	2	2	1	3	0	1	0	0	0	0	1
…moderate	0	0	1	1	1	0	1	1	1	0	0	0	1	0	0	0

Tissue/Organ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
…severe	1	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0

Abbreviations: D = died or euthanized on study; S = scheduled necropsy

Histopathology

As shown in **Table 5**, all organs and tissues from male and female animals in the control and all the drug-treated groups were submitted to histopathological examination (hematoxylin fixed and eosin-stained paraffin tissue sections).

Table 5 Tissues/Organs Collected for Histopathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

Tissue	Collected and Preserved	Microscopic Examination Groups 1 to 4
Adrenal glands	X	X
Aorta	X	X
Bone marrow smear	X	
Bone with bone marrow, femur	X	X
Bone with bone marrow, sternum	X	X
Brain (cerebrum, midbrain, cerebellum, medulla/pons, olfactory bulbs)	X	X
Clitoral gland	X	X
Coagulating glands	X	X
Epididymides	X	X
Esophagus	X	X
Eyes (with optic nerve)	X	X
GALT (Gut-Associated Lymphoid Tissue)	X	X
Gross lesions	X	X
Harderian glands	X	X
Heart	X	X
Joint, tibiofemoral	X	X
Kidneys	X	X
Lacrimal glands, exorbital	X	X
Large intestine, cecum	X	X
Large intestine, colon	X	X
Large intestine, rectum	X	X
Larynx	X	X
Liver	X	X
Lung with bronchi	X	X
Lymph node, mandibular	X	X
Lymph node, mesenteric	X	X

Tissue	Collected and Preserved	Microscopic Examination Groups 1 to 4
Mammary gland (process females only)	X	X
Nose (4 sections)	X	X
Nerve, sciatic	X	X
Ovaries	X	X
Oviduct	X	X
Pancreas	X	X
Pharynx	X	X
Pituitary gland	X	X
Potential target organs	X	X
Preputial gland	X	X
Prostate gland	X	X
Salivary gland, mandibular	X	X
Salivary gland, parotid	X	X
Salivary gland, sublingual	X	X
Seminal vesicles	X	X
Skeletal muscle, biceps femoris	X	X
Skin	X	X
Small intestine, duodenum	X	X
Small intestine, ileum	X	X
Small intestine, jejunum	X	X
Spinal cord, cervical	X	X
Spinal cord, lumbar	X	X
Spinal cord, thoracic	X	X
Spleen	X	X
Stomach, glandular	X	X
Stomach, nonglandular	X	X
Testes	X	X
Thymus	X	X
Thyroid gland (with parathyroid)	X	X
Tissue masses with regional lymph node	X	X
Tongue	X	X
Trachea	X	X
Ureters	X	X

Tissue	Collected and Preserved	Microscopic Examination Groups 1 to 4
Urinary bladder	X	X
Uterus with cervix	X	X
Vagina	X	X
Zymbal's gland (auditory sebaceous gland)	X	X

Peer Review

A pathology peer review was performed by Natasha Neef, BA, VetMB, PhD, DACVP from Vertex and the peer review statement indicates that there was general agreement between the peer review and study pathologists on the evaluation of the data and overall study interpretation.

Neoplastic

Selected neoplastic lesions and tumors identified by histopathology are shown in **Table 6,** although none were considered related to VX-661 treatment. Tumor findings were evaluated separately for males and females. Tumor analysis was performed on combined and malignant neoplasms.[1]

Multicentric Neoplasms: Malignant lymphomas were seen with greater incidence in mid dose males (4.3%) and high dose males (5.8%) compared to control males (1.4%). These increases were not statistically significant (based on either dose response relationship or pairwise comparison of drug-treated groups to the control group).

Adrenal Glands: In the adrenal glands there was a slight increase in the incidence of malignant pheochromocytomas in high dose males (7.2%) compared to control males (1.4%). When combining the incidence of all adrenal pheochromocytomas, the incidence in high dose males (18.8%) was slightly greater than control males (11.4%). This increase was not statistically significant (based on either dose response relationship or pairwise comparison of drug-treated groups to the control group).

Pituitary Gland: Malignant pars distalis carcinoma of the pituitary gland was seen with greater incidence in mid dose females (4.3%) and high dose females (7.1%) compared to control females (1.4%). When combining the incidence of malignant par distalis carcinoma with benign par distalis adenoma the incidence in mid dose females (78.6%) was the same as the control group (78.6%) and that in high dose females (68.6%) was lower, respectively. These increases were not statistically significant (based on either dose response relationship or pairwise comparison of drug-treated groups to the control group).

[1]Brix AE, Hardisty JF, and McConnell EE. 2010. Chapter 28: Combining neoplasms for evaluation of rodent carcinogenesis studies. Cancer Risk Assessment, edited by Ching-Hung Hsu and Todd Stedeford, John Wiley and Sons, Inc.

Table 6 Neoplastic Lesions and Tumors from Histopathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Tissues/Organ Observation	Males				Females			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	**5**	**15**	**50**	**0**	**5**	**20**	**75**
Multicentric Neoplasm n =	3	3	5	5	5	0	4	2
lymphoma, malignant, multicentric	1	0	3	4	2	0	1	0
Adrenal Glands n =	**70**	**70**	**70**	**69**	**70**	**70**	**70**	**70**
pheochromocytoma, benign, primary	7	8	9	8	2	1	0	2
pheochromocytoma, complex, malignant, primary	0	0	0	0	0	0	0	1
pheochromocytoma, malignant, primary	1	1	1	5	1	1	2	0
…combined # of animals =	8	9	10	12	3	2	2	3
Pituitary Gland n =	70	70	70	70	70	70	70	70
adenoma, pars distalis, benign, primary	45	47	41	39	54	57	52	43
carcinoma, pars distalis, malignant, primary	1	0	0	0	1	2	3	5
…combined # of animals =	46	47	41	39	55	59	55	48

Abbreviations: # = number; n = total number examined

Non-Neoplastic

Non-neoplastic toxicities were noted in the GALT, small intestine (ileum and jejunum), parathyroid glands, liver, Harderian glands, clitoral glands, ovaries, and sternum bone marrow (see **Table 7**).

Minimal to mild dilated lymphatics (i.e., lymphatic vessels that are dilated within a tissue or organ) were observed in the GALT, ileum, and jejunum of mid dose and/or high dose males and high dose females compared to control animals. The study report states that the change was characterized by clear cyst-like empty spaces and that there was not an apparent association with other non-neoplastic or neoplastic pathologies or adverse effects. These findings were not associated with mortality. Minimal to mild dilated lymphatics were also noted in the both the 3-month oral gavage and 26-week oral gavage toxicity studies in rats. Neither the incidence nor severity of the dilated lymphatics progressed in this 2-year rat study compared to the 3-month and 26-week rat studies.

Minimal to moderate focal hyperplasia was also seen in the parathyroid glands with increased incidence in low dose, mid dose, and high dose males and females compared to their respective controls.

In the liver, minimal extramedullary hematopoiesis was noted in high dose males and hepatocellular vacuolation was noted in mid dose and high dose males with greater incidence than in control males.

In the Harderian glands, minimal to mild focal hyperplasia was increased in low dose, mid dose, and high dose males and high dose females compared to their respective controls.

In the clitoral glands, minimal to mild dilation was increased in low dose, mid dose, and high dose females compared to control females.

In the ovaries, minimal hematocyst was noted in high dose females (although at a low incidence) compared to control females.

In the bone marrow of the sternum, increased adipocytes (minimal to mild) were noted in low dose, mid dose, and high dose females compared to control females.

Table 7 Non-neoplastic Lesions from Histopathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Tissue/ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
Number of Animals Examined	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
GALT n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
dilated lymphatics	1	0	1	0	3	0	5	6	2	0	0	0	0	0	2	1
…minimal	0	0	1	0	2	0	2	4	1	0	0	0	0	0	1	1
…mild	1	0	0	0	1	0	3	2	1	0	0	0	0	0	1	0
Small Intestine, Ileum n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	48	21
dilated lymphatics	0	0	0	0	0	0	5	6	0	0	0	0	1	0	4	2
…minimal	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1
…mild	0	0	0	0	0	0	5	6	0	0	0	0	0	0	2	1
Small Intestine, Jejunum n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	48	21
dilated lymphatics	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0
…minimal	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
…mild	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Parathyroid Glands n =	48	16	47	21	50	14	41	24	47	17	46	17	42	23	46	21
hyperplasia, focal	1	3	6	9	7	3	6	10	2	1	3	3	4	2	3	6
…minimal	1	1	4	8	3	3	5	7	1	1	2	3	3	1	2	4
…mild	0	0	1	1	4	0	1	2	1	0	1	0	0	1	1	2
…moderate	0	2	1	0	0	0	0	1	0	0	0	0	1	0	0	0
Liver n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21

Tissue/ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
hematopoiesis, extramedullary	0	1	3	0	0	0	4	2	7	3	4	1	2	0	7	2
…minimal	0	1	1	0	0	0	4	2	5	3	2	1	1	0	7	2
…mild	0	0	2	0	0	0	0	0	2	0	2	0	1	0	0	0
vacuolation, hepatocellular	5	6	5	6	10	8	13	14	10	4	7	3	6	11	5	3
…minimal	3	4	5	5	10	8	11	12	10	4	6	3	6	8	4	3
…mild	2	2	0	1	0	0	2	2	0	0	1	0	0	3	1	0
Harderian Glands n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
hyperplasia, focal	3	7	7	7	11	6	11	10	4	3	5	3	2	5	4	7
…minimal	3	6	7	6	10	5	11	9	4	3	5	3	2	5	4	5
…mild	0	1	0	1	1	1	0	1	0	0	0	0	0	0	0	2
Clitoral Glands n =	NA	NA	NA	NA	NA	NA	NA	NA	49	19	53	17	46	22	49	21
dilation	NA	NA	NA	NA	NA	NA	NA	NA	15	11	32	8	27	10	27	17
…minimal	NA	NA	NA	NA	NA	NA	NA	NA	10	3	15	3	20	3	16	8
…mild	NA	NA	NA	NA	NA	NA	NA	NA	5	8	17	5	7	7	8	7
…moderate	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	3	2
Ovaries n =	NA	NA	NA	NA	NA	NA	NA	NA	51	19	53	17	47	23	49	21
hematocyst	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	2	0
…minimal	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	2	0
Bone Marrow, Sternum n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
increased adipocytes	11	1	10	2	13	2	6	1	5	2	8	3	10	10	8	4
…minimal	8	0	8	2	9	2	5	1	3	2	7	3	8	9	6	4
…mild	2	1	2	0	4	0	1	0	2	0	1	0	2	1	2	0
…moderate	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Abbreviations: D = died or euthanized on study; GALT = gut-associated lymphoid tissue; n = number observed; NA = not applicable; S = scheduled necropsy

Toxicokinetics

Blood samples were collected from unfasted TK animals via the sublingual vein at predose, 2, 4, 8, 12, and 24 hours postdose on Days 1, 181 (Week 26), and 363 (Week 52). TK parameters that were determined included C_{max}, T_{max}, and AUC_{0-24} for VX-661 and the metabolites VRT-0996107 (M1) and VRT-1189001 (M2).

As shown in **Table 8** for VX-661, T_{max} for males was 4 hours and for females ranged from 2 to 8 hours, regardless of dose or time interval (i.e., Day 1, Week 26 or Week 52). After repeat exposure in males and females (Week 26 and Week 52), AUC was generally dose proportional between the low dose and mid dose groups and mid dose

and high dose groups. Exposure in females appeared greater than in males. There appeared to be drug accumulation over the time course of dosing in males and females.

Table 8 Summary Toxicokinetics for VX-661 in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

VX-661 Dose (mg/kg/day) M/F	Interval	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (hr*µg /mL)
5/5	Day 1	M	0.605	4.00	4.97
		F	0.834	8.00	13.2
	Week 26	M	1.43	4.00	14.2
		F	1.61	2.00	21.8
	Week 52	M	1.66	4.00	19.2
		F	2.06	2.00	28.3
15/20	Day 1	M	2.24	4.00	20.4
		F	4.14	4.00	55.2
	Week 26	M	5.01	4.00	55.4
		F	8.69	4.00	101
	Week 52	M	6.17	4.00	73.8
		F	8.15	4.00	109
50/75	Day 1	M	10.1	4.00	107
		F	15.4	4.00	241
	Week 26	M	11.6	4.00	134
		F	20.2	8.00	262
	Week 52	M	13.6	4.00	180
		F	29.0	4.00	337
M-Male; F-Female					

As shown in **Table 9** for metabolite VRT-0996107 (M1), T_{max} for males and females ranged from 4 to 12 hours, regardless of dose or time interval (i.e., Day 1, Week 26 or Week 52). After repeat exposure in males and females (Week 26 and Week 52), AUC was generally dose proportional between the low dose and mid dose groups and mid dose and high dose groups. Exposure in males and females appeared similar. There appeared to be metabolite accumulation over the time course of dosing in males and females.

Table 9 Summary Toxicokinetics for Metabolite VRT-0996107 (M1) in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

VX-661 Dose (mg/kg/day) M/F	Interval	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (hr*µg /mL)
5/5	Day 1	M	0.462	4.00	8.54
		F	0.419	12.0	6.73
	Week 26	M	1.51	8.00	29.7
		F	1.41	8.00	28.6
	Week 52	M	1.76	8.00	34.7
		F	1.75	12.0	34.2
15/20	Day 1	M	1.57	12.0	28.7
		F	1.53	12.0	28.0
	Week 26	M	4.10	12.0	84.2
		F	6.28	4.00	125
	Week 52	M	5.29	4.00	106
		F	6.40	4.00	139
50/75	Day 1	M	4.35	12.0	77.8
		F	5.27	12.0	86.4
	Week 26	M	9.80	4.00	199
		F	17.6	8.00	344
	Week 52	M	15.3	8.00	288
		F	26.2	4.00	479
M-Male; F-Female					

As shown in **Table 10** for metabolite VRT-1189001 (M2), T_{max} for males ranged from 2 to 24 hours and for females ranged from 4 to 24 hours, regardless of dose or time interval (i.e., Day 1, Week 26 or Week 52). After repeat exposure in females (Week 26 and Week 52), AUC was generally dose proportional between the low dose and mid dose groups but lower than dose proportional between the mid dose and high dose groups. Exposure in males appeared greater. After repeat exposure in males (Week 26 and Week 52), AUC was generally dose proportional over the dosing range. There appeared to be metabolite accumulation over the time course of dosing in males and females.

Table 10 Summary Toxicokinetics for Metabolite VRT-1189001 (M2) in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

VX-661 Dose (mg/kg/day) M/F	Interval	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (hr*µg /mL)
5/5	Day 1	M	0.0238	4.00	0.449
		F	0.0137	12.00	0.222
	Week 26	M	0.0796	4.00	1.61
		F	0.0430	8.00	0.902
	Week 52	M	0.110	4.00	2.07
		F	0.0638	4.00	1.36
15/20	Day 1	M	0.0907	12.0	1.60
		F	0.0709	12.0	1.20
	Week 26	M	0.278	12.0	5.96
		F	0.233	12.0	4.84
	Week 52	M	0.313	2.00	6.92
		F	0.239	12.0	5.09
50/75	Day 1	M	0.164	12.0	3.10
		F	0.0763	24.0	1.40
	Week 26	M	0.767	24.0	15.9
		F	0.473	8.00	10.4
	Week 52	M	1.06	24.0	20.0
		F	0.624	4.00	13.0
M-Male; F-Female					

Dosing Solution Analysis

Dosing solutions were analyzed for homogeneity (top, middle, and bottom stratum) and concentration (middle stratum) on Day 1 and about monthly thereafter (Days 29, 57, 85, 113, 141, 181, 197, 225, 253, 281, 309, 337, 363, 393, 421, 449, 477, 505, 536, 561, 589, 617, 645, 673, and 701). All samples were found to be homogenous. The calculated concentrations for the control dose level (0 mg/kg/day) were all below the limit of quantitation (<0.0510 mg/mL) and the calculated concentrations for the test article dose levels (5, 15, 20, 50, and 75 mg/kg/day) were all within 10% of their respective nominal concentrations.

Study title: VX-661: 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice

Study no.:	Conducting laboratory: AD80DH.7G8R.(b) (4) Sponsor: VX-661-TX-019
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 18, 2014 (protocol signed by Study Director)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), lot # 19QB10A-50.NJ00001, 98.0% w/w purity
CAC concurrence:	Yes (see Special Protocol Agreement, dated November 20, 2014)

Key Study Findings

- In a 26-week oral carcinogenicity study, Tg.rasH2 mice received doses of 0, 30, 100, and 500 mg/kg/day VX-661 and 1000 mg/kg/day urethane (positive control).
- There was a statistically significant treatment-related increase in mortality for high dose males (76% survival) compared to control males (96% survival).
- There were no statistically significant drug-related tumor findings in male or female mice.
- The most notable non-neoplastic histopathology findings were in the adrenal glands (cortex hypertrophy, cortex vacuolation, and x-zone degeneration), liver (centrilobular hypertrophy, centrilobular hepatocellular single cell necrosis, subcapsular inflammation, focal hepatocellular necrosis, focal lipid infiltration, and periportal vacuolation), ovaries (decreased corpora lutea), and jejunum (lamina propria vacuolation). Additional non-neoplastic findings were noted in the mesenteric and mandibular lymph nodes (lymphoid necrosis), spleen (hemosiderin pigmentation and lymphoid necrosis), and thymus (lymphoid necrosis and increased lymphocytes).
- VX-661 AUC exposure at the high dose (500 mg/kg/day) was about 93 and 154 mcg*hr/mL in males and females, respectively. Exposures to VX-661 metabolites, M1 (VRT-0996107) and M2 (VRT-1189001), were also assessed in mice.

Adequacy of Carcinogenicity Study

- The ECAC concurred with the doses and design of the study (see Special Protocol Agreement dated November 20, 2014).
- The duration of treatment was adequate (i.e., 26 weeks).

- The positive control produced expected increases of neoplastic findings indicating that the mice and study could detect a potential drug-induced neoplastic response.

Appropriateness of Test Models

- The Tg.rasH2 mouse is considered an appropriate model for short-term carcinogenicity assessment. The CByB6F1 mouse is the wild type of the Tg.rasH2 mouse (i.e., same genetic makeup with the omission of the Tg element) and is considered acceptable for the TK portion of the study.
- Wild-type mice achieved high exposures to VX-661 and its M1 metabolite. Exposure to the M2 metabolite was low. Exposure to the M5 metabolite (VRT-1074233) was not measured.

Evaluation of Tumor Findings

- There were no statistically significant drug-related and tumor findings in male or female mice.

Methods

Doses:	See **Table 11** for dose levels in the main cohort and the TK cohort Vehicle control: hydroxypropylmethylcellulose acid succinate in formulation vehicle Positive control: 1000 mg/kg/dose urethane
Frequency of dosing:	Main cohort: vehicle control or VX-661 doses were administered once daily for up to 26 consecutive weeks and the positive control was administered on Days 1, 3, and 5 TK cohort: vehicle control or VX-661 doses were administered either once on Day 1 or once daily for up to 177 days
Dose volume:	10 mL/kg
Route of administration:	Main cohort: vehicle control and VX-661 doses were administered by oral gavage and positive control was administered by intraperitoneal injection TK cohort: vehicle control and VX-661 doses were administered by oral gavage
Formulation/Vehicle:	0.5% methylcellulose, 0.5% SLS, 0.01% simethicone in deionized water
Basis of dose selection:	In consultation with the ECAC, the high dose was selected based on mortality at 1500 mg/kg/day in male and female non-transgenic mice from a 5-day dose ranging study (VX-661-TX-017). The mid and low doses were chosen to provide dose separation based on AUC.
Species/Strain:	Main cohort: hemizygous Tg.rasH2 mice from (b) (4) TK cohort: wild-type CByB6F1 from (b) (4)
Number/Sex/Group:	See **Table 11** for the number of animals/sex/group in the main cohort and the TK cohort
Age:	Tg.rasH2 and CByB6F1 mice were about 8 weeks of age at start of dosing
Animal housing:	During the acclimation period animals were group housed and then were individually housed following randomization, in polycarbonate cages
Paradigm for dietary restriction:	No dietary restrictions. Animals had access ad libitum to Harlan TEKLAD Global Diet #2018CM (Certified 18% Protein Rodent Diet, Harlan TEKLAD, Madison, WI) in meal form, in

	stainless steel rodent feeders, and ad libitum access to drinking water via an automatic watering system
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	TK
Deviation from study protocol:	Protocol deviations were presented in the study report and were deemed to have not affected the outcome or integrity of the study.

Table 11 Study Design in Main Cohort and Toxicokinetic Cohort of 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

Group	Dose Levels (mg/kg/day)[b]	Number of Animals			
		Main Cohort		TK Cohort*	
		Male	Female	Male	Female
1	0[a]	25	25	8	8
2	30	25	25	44	44
3	100	25	25	44	44
4	500	25	25	44	44
5	1000 (urethane)	10	10	-	-
Total		**110**	**110**	**140**	**140**

[a] = Group 1 received the control article.
[b] = Dose levels are expressed as API. A correction factor of 2.00 was used.
* = Extra TK animals (2/sex/group) were used to ensure adequate animals for TK bleeding.

Observations and Results

Mortality

Observations for morbidity and mortality were made for all animals, at least twice daily. As shown in **Table 12**, the percentage of main cohort high dose males surviving until terminal necropsy was 76% compared to 96% for control males. The analysis of the FDA statistical reviewer found a statistically significant treatment-related increase in mortality. Of the six high dose males that had early deaths (found dead or moribund sacrifice), the cause of death was undetermined in 5/6 males and due to marked trachea inflammation in 1/6 males, which did not appear to be related to drug-treatment (see **Table 13**). Kaplan-Meier survival curves for control and drug-treated male and female groups are shown below (**Figure 7** and **Figure 8**, respectively).

Table 12 Number of Early Deaths versus Animals Surviving until Terminal Necropsy in Main Cohort Animals in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

VX-661 (mg/kg/day)	Males		Females	
	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]
0	1 (4%)	24 (96%)	2 (8%)	23 (92%)
30	3 (12%)	22 (88%)	2 (8%)	23 (92%)
100	1 (4%)	24 (96%)	0 (0%)	25 (100%)
500	6 (24%)	19 (76%)	2 (8%)	23 (92%)
1000 (urethane)	0 (0%)	10 (100%)	1 (10%)	9 (90%)

Abbreviations: # = number
Notes:
[1]# of Early Deaths = moribund sacrifice or found dead
[2]numbers in parentheses represent the percent when compared to the number of main study animals at the start of the study (i.e., 25 animals/sex/group for vehicle control and drug-treated groups and 10 animals/sex/group for urethane positive control group)

Table 13 Mortality in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

Sex	Group (Dose)	Mode of Death	Animal Number	Day of Death	Cause of Death
Male	1 (0 mg/kg/day)	Moribund Sacrifice	7607	169	Spinal cord, hemangiosarcoma
	2 (30 mg/kg/day)	Moribund Sacrifice	7635	171	Undetermined
		Natural Death	7647	94	Undetermined
		Natural Death	7650	144	Undetermined
	3 (100 mg/kg/day)	Natural Death	7673	64	Undetermined
	4 (500 mg/kg/day)	Natural Death	7677	117	Undetermined
		Natural Death	7682	52	Undetermined
		Moribund Sacrifice	7686	26	Undetermined
		Natural Death	7688	147	Trachea, inflammation, marked
		Natural Death	7694	22	Undetermined
		Natural Death	7698	93	Undetermined
Female	1 (0 mg/kg/day)	Natural Death	7716	82	Undetermined
		Natural Death	7720	16	Undetermined
	2 (30 mg/kg/day)	Moribund Sacrifice	7742	106	Undetermined
		Moribund Sacrifice	7748	167	Salivary glands, hemangiosarcoma
	4 (500 mg/kg/day)	Moribund Sacrifice	7791	95	Multiple lung lesions
		Natural Death	7793	27	Undetermined

Natural Death = Found Dead

Figure 7 Kaplan-Meier Survival Curves for Males in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (FDA Statistical Reviewer's Figure)

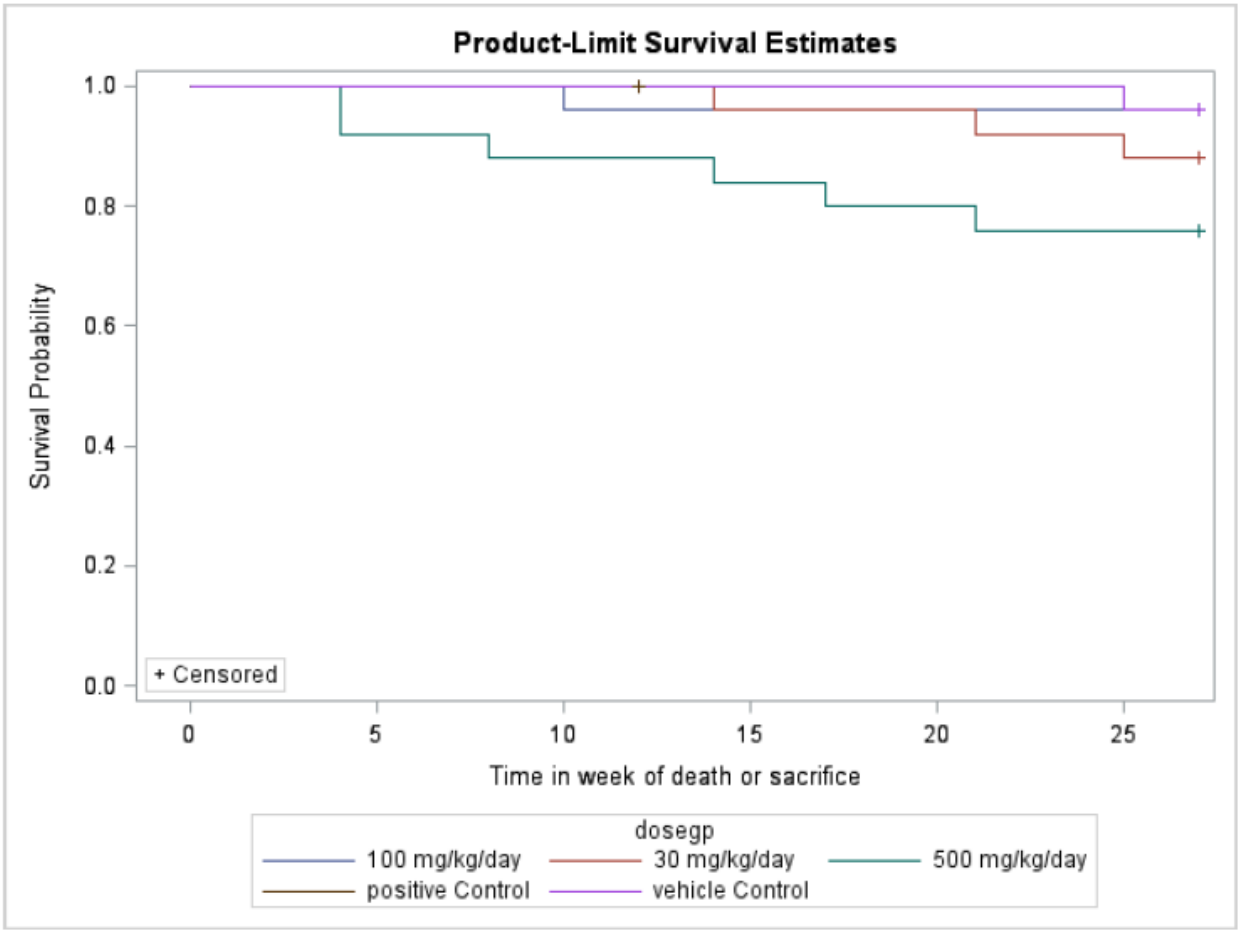
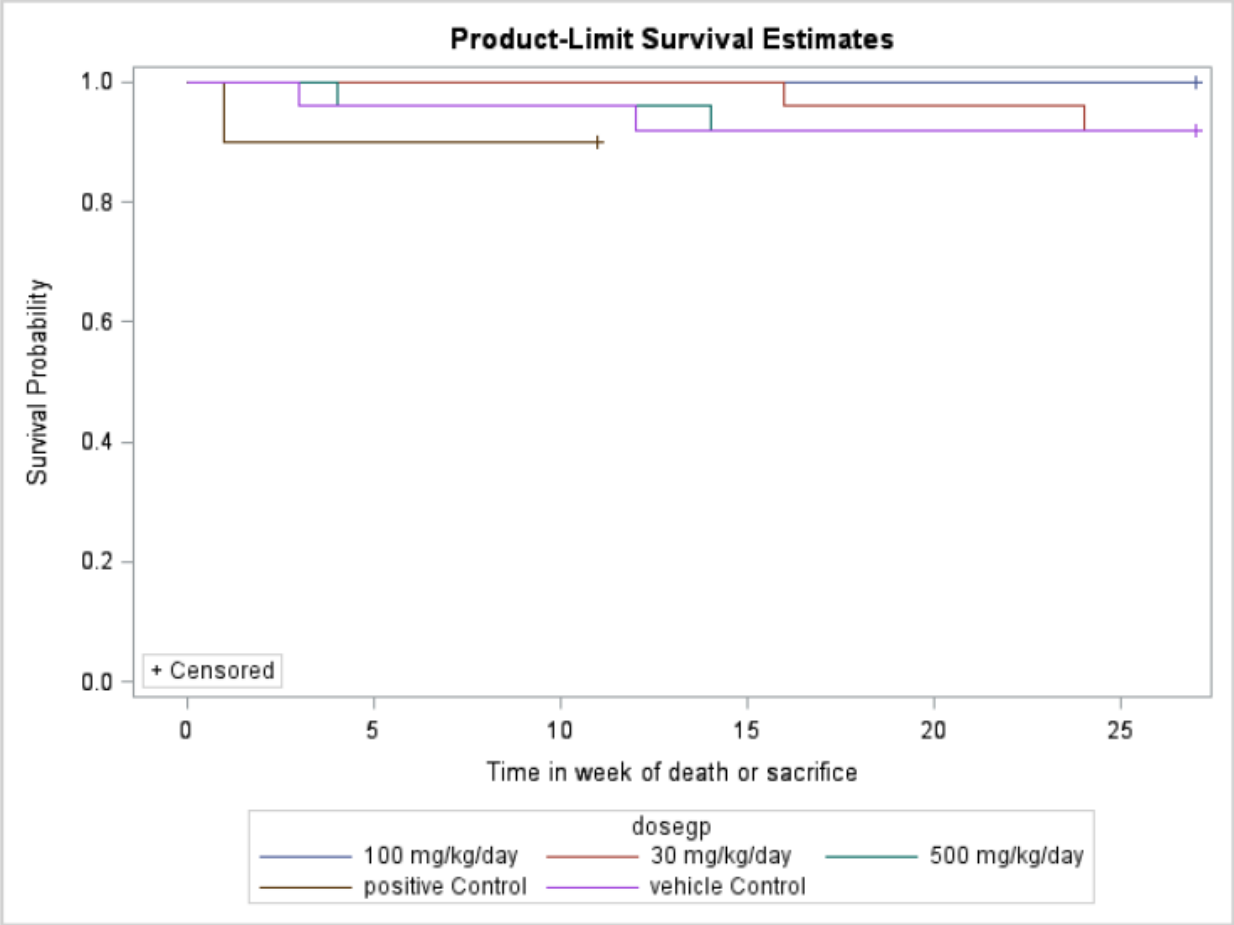


Figure 8 Kaplan-Meier Survival Curves for Females in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (FDA Statistical Reviewer's Figure)



Clinical Signs

Cage side observations were made on main cohort animals on the days of dosing (within 2 hours of the last animal that was dosed in each group). Detailed hands-on observations were made on main cohort animals on Day 1 and weekly thereafter (at the time animals were weighed).

Clinical observations (cage side and hands on) that were seen in a greater number of high dose animals or more times in high dose animals than control animals are shown in **Table 14** and **Table 15**. Notable cage side findings included decreased motor activity, ruffled fur, and hunched posture. Notable hands on findings included thinness, ruffled fur, and hunched posture.

Table 14 Summary of Cage Side Observations in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Observation	Males	Females

	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Decreased Motor Activity	10/5	6/6	9/4	55/18	12/8	11/8	15/4	22/6	33/11	19/10
Days	38 to 158	9 to 171	8 to 131	7 to 136	3 to 5	6 to 182	13 to 180	22 to 180	3 to 182	1 to 5
Ruffled Fur	243/ 24	358/ 25	566/ 25	1221/ 25		44/ 15	106/ 19	92/ 16	335/ 23	
Days	11 to 174	8 to 183	7 to 183	3 to 182		6 to 182	6 to 182	5 to 180	3 to 182	
Hunched	28/7	31/11	25/10	123/22		19/10	31/14	29/6	40/12	
Days	101 to 158	9 to 171	7 to 183	7 to 164		6 to 182	6 to 180	6 to 180	3 to 182	
Labored Breathing				5/4		1/1	4/1		1/1	
Days				21 to 26		16 to 16	121 to 124		182 to 182	
Rapid and Shallow				6/3		3/2	1/1			
Days				49 to 135		15 to 121	130 to 130			
Swelling				1/1			20/1			
Days				26 to 26			147 to 166			

Abbreviations: + = positive control (1000 mg/kg/day urethane)
Note: Number of times observed/Total number of animals affected

Table 15 Summary of Hands On Observations in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Thin	2/1	17/6	1/1	14/8		10/6	22/8	29/13	31/6	
Days	127 to 134	8 to 183	29 to 29	8 to 127		85 to 141	15 to 162	15 to 141	8 to 141	
Ruffled Fur	63/ 18	96/ 18	129/ 21	155/ 23		9/ 4	17/ 9	14/ 8	108/ 19	

Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Days	15 to 183	15 to 183	15 to 183	8 to 183		15 to 134	92 to 183	141 to 183	8 to 183	
Hunched	5/3	8/6	6/3	21/8		8/5	10/6	6/5	18/9	
Days	127 to 162	120 to 183	92 to 183	8 to 176		15 to 141	15 to 141	85 to 141	8 to 183	
Seizures									1/1	
Days									8 to 8	
Rapid and Shallow							1/1		1/1	
Days							106 to 106		183 to 183	
Nodule								12/1	10/1	
Days								36 to 113	50 to 113	
Micro-ophthalmia (small eye)									5/1	
Days									155 to 183	
Exo-phthalmia (bulging eye)									1/1	
Days									127 to 127	
Abnormality (closed left eyelid)									8/1	
Days									134 to 183	

Abbreviations: + = positive control (1000 mg/kg/day urethane)
Note: Number of times observed/Total number of animals affected

Body Weights

Main cohort animals were weighed at pre-dose on Day 1, weekly through Week 13, and biweekly thereafter. TK cohort animals were weighed (for dose volume calculations only) on Day -1 (Day 1 TK mice only) or on Day 1, weekly through Week 13, and

biweekly thereafter (Week 26 TK mice). As shown in **Table 16**, at the end of the study period (i.e., Day 183), mean body weights for VX-661-treated male and female animals were generally similar to control animals, despite a statistical decrease for mid dose males, low dose females, and high dose females. In all cases, the statistically significant decrease represented a percent change from the control that was less than 7% (the change ranged from about 1% to about 6%) so the statistical decreases were not considered related to VX-661 treatment. Male and female mean body weight curves over the course of the study are shown in **Figure 9** and **Figure 10**, respectively.

Table 16 Body Weight Changes in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Parameter	Males				Females			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	30	100	500	0	30	100	500
Day 1 (grams)	22.72	22.68	22.82	21.83	18.41	17.82	18.06	18.03
Day 183 (grams)	25.60	24.34	24.02*	24.30	21.01	19.97*	20.83	20.02*
Absolute Body Weight (% change from Day 183 controls)	0.0	-4.9	-6.2	-5.1	0.0	-5.0	-0.9	-4.7

* = statistically significant from control ($p < 0.05$) based on Applicant's Dunnett's test

Figure 9 Mean Body Weights in Males in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

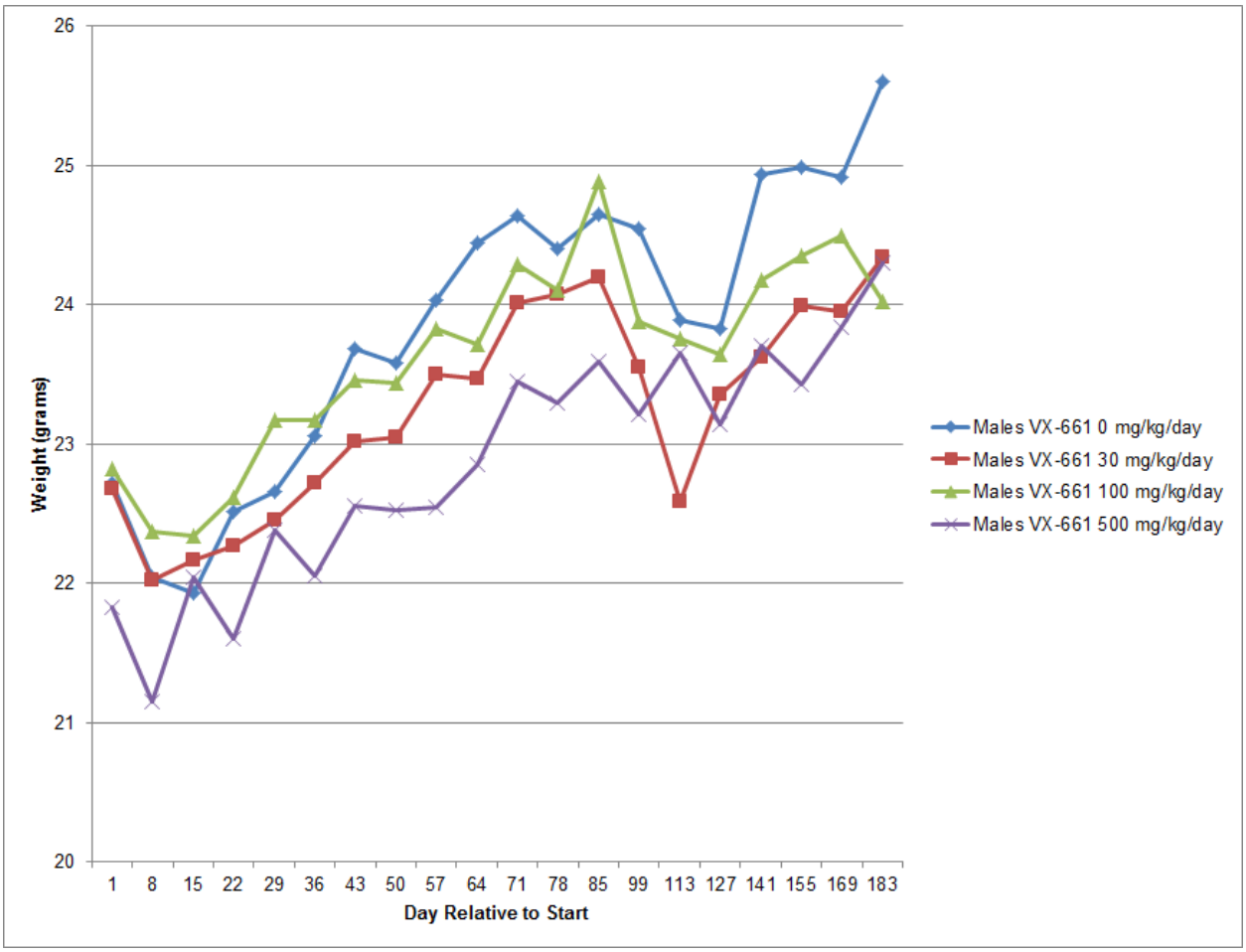
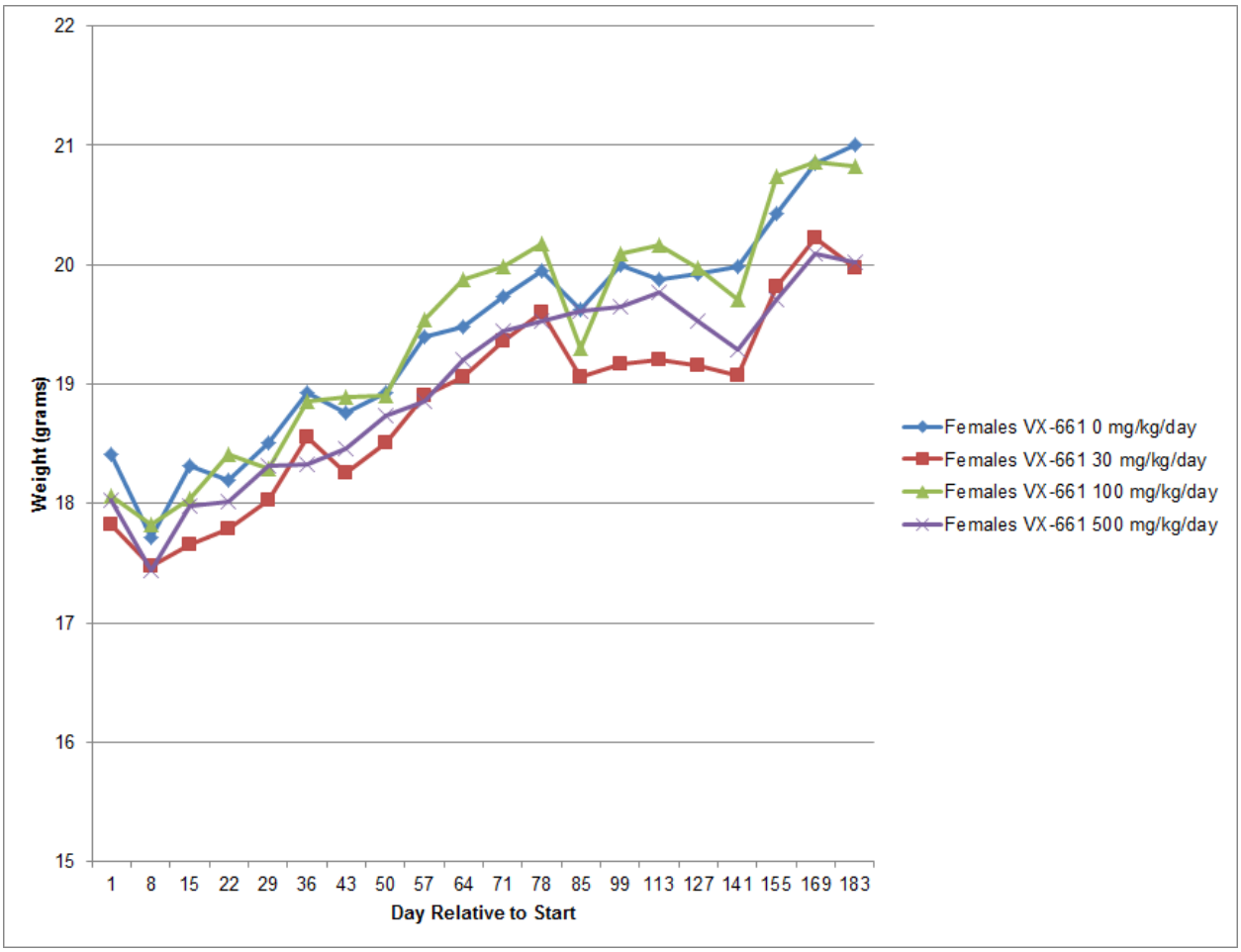


Figure 10 Mean Body Weights in Females in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice



Feed Consumption

Animals had access ad libitum to Harlan TEKLAD Global Diet #2018CM (Certified 18% Protein Rodent Diet, Harlan TEKLAD, Madison, WI) and drinking water via an automatic watering system. Food consumption was measured for main cohort animals on Day 1 and weekly thereafter. As shown in **Figure 11** and **Figure 12**, there were both decreases and increases in food consumption from week to week for drug-treated animals compared to control animals. Overall, there was not an obvious effect of dose on food consumption and there did not appear to be an effect of feed consumption on body weight. Thus, there were no evident drug-related effects on food consumption.

Figure 11 Mean Caged Food Consumption in Males in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

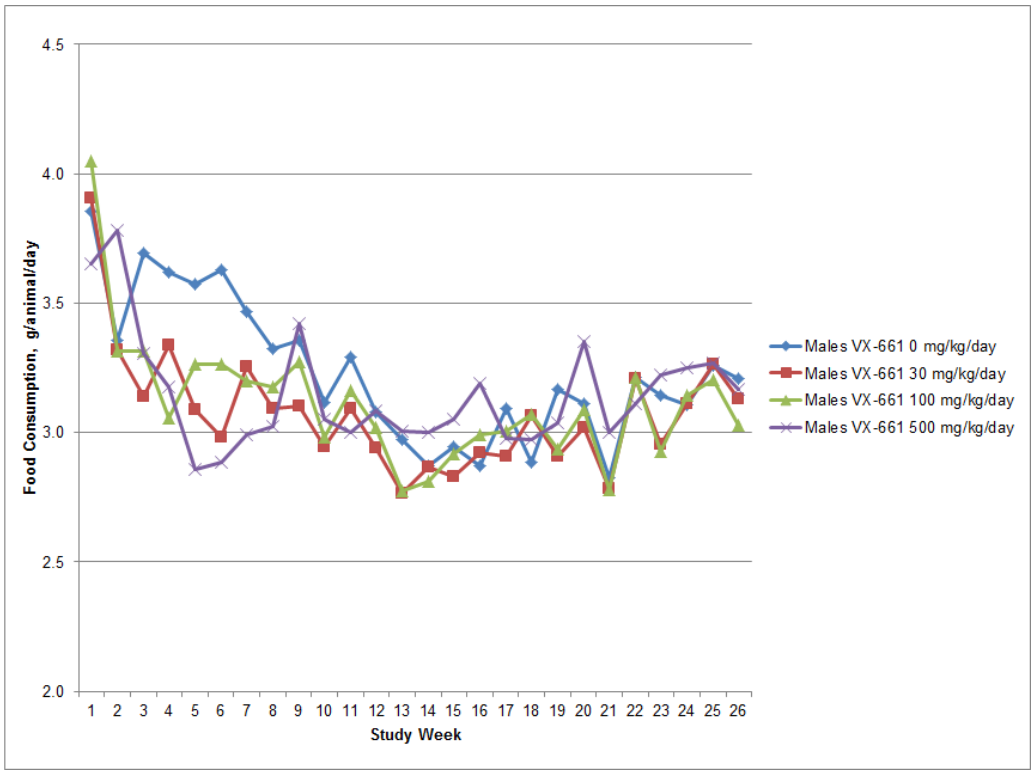
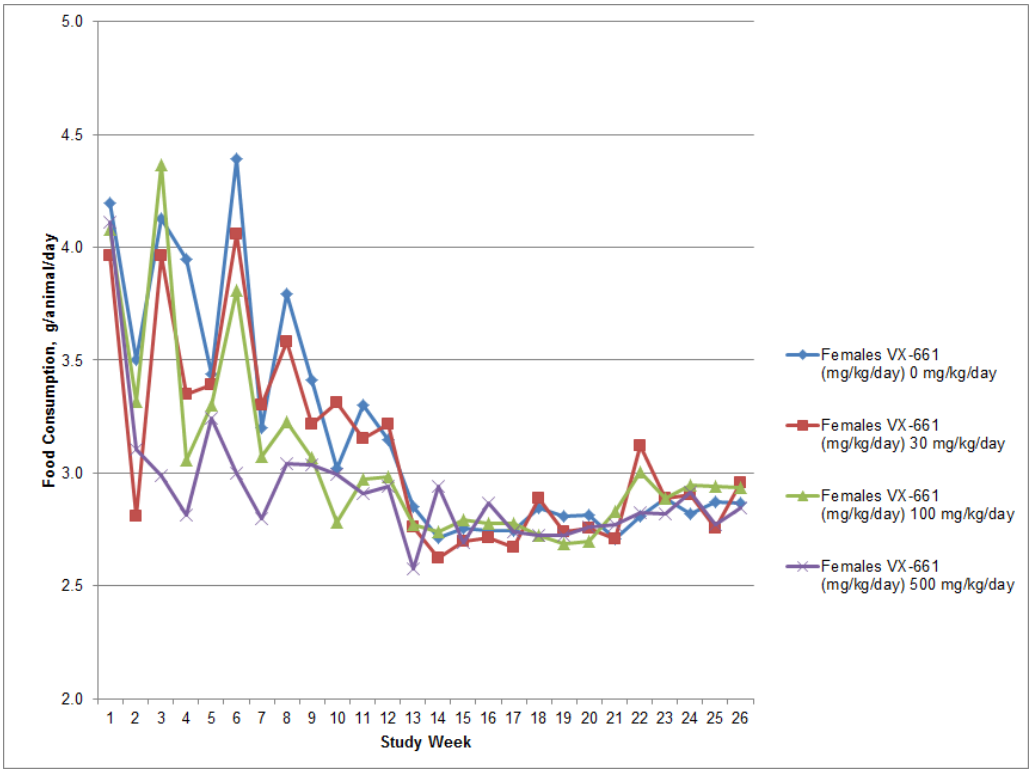


Figure 12 Mean Caged Food Consumption in Females in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice



Gross Pathology

Surviving positive control animals were euthanized by carbon dioxide overdose on Day 78 (males) and Day 76 (females) and subjected to a partial necropsy (lungs and spleen). Surviving main cohort animals were euthanized by carbon dioxide overdose on Day 183 or Day 184 and subjected to a complete necropsy. A complete necropsy was similarly performed on any main cohort early deaths (found dead or moribund sacrifice) and these animals were also evaluated for evidence of gavage error. Surviving TK cohort animals were euthanized by carbon dioxide overdose after completion of their last scheduled blood collection (Days 1-2 or Days 177-178) and no necropsy was performed.

One high dose female (#7788) had a tan nodule on the ventral left lung lobe and a pale right cranial lung lobe, while another high dose female (#7810) had a pale firm right cranial lung lobe, all of which correlated with the microscopic finding of mild focal histiocytic infiltration of the alveoli. One high dose female (#7808) had a mass on the lungs with bronchi that correlated with the microscopic finding of primary malignant alveolar-bronchiolar carcinoma.

Of note, positive control males and females had pale nodule that correlated with the microscopic finding of primary benign alveolar-bronchiolar adenoma.

One high dose male (#7681) had a nodule attached to the pancreas that correlated with the microscopic finding of primary malignant mesentery hemangiosarcoma.

The following gross pathology findings were seen in the spleen and correlated with primary malignant hemangiosarcoma in the spleen: 1) nodule (low dose males, high dose males, positive control males, and positive control females); 2) dark nodule (low dose females); 3) mass (high dose females); and 4) pale mass (low dose males).

One high dose male (#7680) had an enlarged thymus that correlated with the microscopic finding of multicentric malignant lymphoma.

Generally, gross findings for drug-treated male and female mice did not correlate to any treatment-related neoplastic findings.

Table 17 Gross Pathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Tissue/Organ Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Lungs with Bronchi n =	25	25	25	25	10	25	25	25	25	10
nodule	3	0	0	0	0	0	0	0	0	0
nodule, pale	0	0	0	0	0	1	0	0	0	2
nodule, pale, all lobes	0	0	0	0	10	0	0	0	0	7
nodule, tan, left	0	0	0	0	0	0	0	0	1	0
mass	0	0	0	0	0	0	0	0	1	0
pale, firm, right	0	0	0	0	0	0	0	0	1	0
pale, right	0	0	0	0	0	0	0	0	1	0
Pancreas n =	25	25	25	25	0	25	25	25	25	0
nodule	0	0	0	1	0	0	0	0	0	0
Spleen n =	25	25	25	25	10	25	25	25	25	10
nodule	0	4	0	2	8	0	0	0	0	7
nodule, dark	0	0	0	0	0	0	1	0	0	0
mass	0	0	0	0	0	0	0	0	1	0
mass, pale	0	1	0	0	0	0	0	0	0	0
Thymus n =	25	25	25	25	0	25	25	25	25	0
enlarged	0	0	0	1	0	0	0	0	0	0

Abbreviations: + = positive control (1000 mg/kg/day); n = number of animals submitted

Histopathology

The organs and tissues shown in **Table 18** were collected from all main cohort males and females in the vehicle control group and drug-treated groups and were processed, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and submitted for histopathological examination.

Table 18 Tissues/Organs Collected for Histopathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

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	Spinal cord (cervical, thoracic, and lumbar)
Kidneys	Spleen
Large intestine (cecum, colon, rectum)	Stomach
Liver	Testes
Lungs and bronchi	Thymus
Lymph nodes (mesenteric and mandibular)	Thyroid glands
	Trachea
Skin from mammary area (male and female mice)	Urinary bladder
	Uterus
Mammary gland (females only)	Vagina

Peer Review

A pathology peer review was performed by Natasha Neef, BA, VetMB, PhD, DACVP from Vertex and the peer review statement indicates that there was agreement between the peer review and study pathologists on the evaluation of the data and overall study interpretation.

Neoplastic

Neoplastic lesions and tumors identified by histopathology are shown in **Table 19**, although none were considered related to VX-661 treatment. Tumor findings were evaluated separately for males and females. Tumor analysis was performed on combined and malignant neoplasms. When evaluating tumor incidence, the FDA statistical reviewer's analysis took into consideration the statistically significant dose response relationship in early deaths in high dose males compared to control males.

Multicentric Tumors: The incidence of malignant lymphoma was increased in high dose males (4%) compared to control males (0%), which was not statistically significant

(based on either dose response relationship or pairwise comparison of drug-treated groups to the control group). The one male (#7680) in the high dose group with multicentric malignant lymphoma had lymphoma in the aorta, femur bone marrow, sternum bone marrow, heart, kidneys, liver, lungs with bronchi, cervical spinal cord, and thymus.

The incidence of multicentric hemangiosarcoma in males was increased in the low dose group (20%), high dose group (12%), and positive control group (100%) compared to the vehicle control group (4%). Similarly, in females, the incidence was increased in the low dose group (8%), the high dose group (4%), and the positive control group (70%). The findings in both male and female positive control groups were statistically significant but the drug-treated findings were not statistically significant (based on either the dose response relationship or pairwise comparison of drug-treated groups to the control group).

Lungs with Bronchi: Alveolar-bronchiolar adenomas and carcinomas of the lungs with bronchi were statistically increased in both positive control males (100%) and females (90%) in comparison with their respective controls (i.e., 20% for both males and females). This increase was not evident in drug-treated male and female groups.

Table 19 Neoplastic Lesions and Tumors from Histopathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Neoplastic Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Number of Animals on Study	**25**	**25**	**25**	**25**	**10**	**25**	**25**	**25**	**25**	**10**
Number of Animals Completed	**25**	**25**	**25**	**25**	**10**	**25**	**25**	**25**	**25**	**10**
Multicentric Tumors										
lymphoma, malignant, incidental	0	0	0	1	0	0	0	0	0	0
hemangiosarcoma, malignant, primary, fatal or incidental (multiple organs)	1	5	1	3	10*	0	2	0	1	7*
Lungs with Bronchi n =	25	25	25	25	10	25	25	25	25	10
alveolar-bronchiolar adenoma, multiple, benign, primary, incidental	0	0	2	0	10	1	0	0	0	9
alveolar-bronchiolar adenoma, single, benign, primary, incidental	2	0	3	1	0	3	0	2	2	0
alveolar-bronchiolar carcinoma, malignant, primary, incidental	3	0	0	0	3	1	0	0	1	1
…combined animals	5	0	5	1	10*	5	0	2	3	9*

Abbreviations: + = positive control (1000 mg/kg/day); n = number of animals examined
* = statistically significant at <0.000 for pairwise comparison to the control group based on FDA Statistical Reviewer's evaluation.

Non-Neoplastic

Non-neoplastic toxicities were noted in the adrenal glands, liver, ovaries, jejunum, lymph nodes (mesenteric and mandibular), spleen, and thymus (see **Table 20**).

The most notable findings in the adrenal glands included minimal to mild cortex hypertrophy (high incidence in mid dose and high dose males and low incidence in high dose females), minimal to mild cortex vacuolation (high dose males, mid dose females, and high dose females), and minimal increased x-zone degeneration (high dose males).

A notable finding in the liver included centrilobular hypertrophy that ranged from minimal to marked in low dose males, mid dose males, high dose males and minimal in high dose females. Minimal to marked centrilobular hepatocellular single cell necrosis was prominent in mid dose males and high dose males. Of note, in the 3-month oral carcinogenicity range-finding study in mice, the liver was identified as a target organ of toxicity based on minimal foci of hepatocyte necrosis seen in two males and one female treated with 1000 mg/kg/day VX-661 and one female in the 600 mg/kg/day drug-treated group, while this finding was not evident in any control animals. Additional liver findings included subcapsular inflammation (low dose males, mid dose males, high dose males, mid dose females, and high dose females), focal hepatocellular necrosis (low dose males, mid dose males, high dose males, and high dose females), focal lipid infiltration (high dose females), and periportal vacuolation (high dose males). The liver is likely a target organ of toxicity due to VX-661 elimination via hepato-biliary secretion.

In the ovaries decreased corpora lutea were noted at in increased incidence in low dose, mid dose, and high dose females compared to control females.

In the jejunum, lamina propria vacuolation was noted in high dose males (minimal to mild) and high dose females (minimal) and was not evident in control animals.

Lymphoid necrosis was noted in the mesenteric and mandibular lymph nodes of low dose and high dose males at an increased incidence compared to control males.

In the spleen, the most notable findings included minimal to mild hemosiderin pigmentation (low dose, mid dose, and high dose males) and minimal to mild lymphoid necrosis (low dose and high dose males).

In the thymus, the most notable finding was minimal to moderate lymphoid necrosis (high dose males). Although at a low incidence, mild increased lymphocytes were noted in high dose females compared to control females.

Table 20 Non-Neoplastic Lesions and Tumors from Histopathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Number of Animals on Study	25	25	25	25	10	25	25	25	25	10

Number of Animals Completed	**25**	**25**	**25**	**25**	**10**	**25**	**25**	**25**	**25**	**10**
Adrenal Glands n =	25	25	25	25	0	25	25	25	25	0
hypertrophy, cortex	0	0	25	25	0	0	0	0	1	0
…minimal	0	0	24	1	0	0	0	0	0	0
…mild	0	0	1	24	0	0	0	0	1	0
vacuolation, cortex	10	6	4	15	0	3	3	6	6	0
…minimal	7	5	4	8	0	2	3	5	6	0
…mild	3	1	0	7	0	1	0	1	0	0
x-zone degeneration, increased	0	0	0	11	0	0	0	0	0	0
…minimal	0	0	0	11	0	0	0	0	0	0
Liver n =	25	25	25	25	0	25	25	25	25	0
hypertrophy, centrilobular	0	24	24	25	0	0	0	0	22	0
…minimal	0	20	0	0	0	0	0	0	22	0
…mild	0	4	23	1	0	0	0	0	0	0
…moderate	0	0	1	5		0	0	0	0	0
…marked	0	0	0	19	0	0	0	0	0	0
inflammation, subcapsular	0	1	1	4	0	0	0	2	2	0
…minimal	0	1	1	1	0	0	0	2	2	0
…mild	0	0	0	2	0	0	0	0	0	0
…moderate	0	0	0	1	0	0	0	0	0	0
necrosis, hepatocellular, focal	2	3	6	7	0	3	1	0	4	0
…minimal	2	1	4	1	0	1	1	0	3	0
…mild	0	1	2	3	0	1	0	0	0	0
…moderate	0	1	0	2	0	1	0	0	1	0
…marked	0	0	0	1	0	0	0	0	0	0
single cell necrosis, hepatocellular, centrilobular	0	0	7	24	0	0	0	0	0	0
…minimal	0	0	7	14	0	0	0	0	0	0
…mild	0	0	0	7	0	0	0	0	0	0
…moderate	0	0	0	2	0	0	0	0	0	0
…marked	0	0	0	1	0	0	0	0	0	0
infiltration, lipid, focal	0	0	0	0	0	1	0	0	2	0
…minimal	0	0	0	0	0	0	0	0	1	0
…mild	0	0	0	0	0	1	0	0	1	0
vacuolation, periportal	0	0	0	2	0	0	0	0	0	0
…mild	0	0	0	2	0	0	0	0	0	0
Ovaries n =	-	-	-	-	-	25	25	25	25	0
decreased corpora lutea	-	-	-	-	-	4	8	5	19	0
Intestine, Jejunum n =	25	25	25	25	0	25	25	25	25	0
vacuolation, lamina propria	0	0	0	10	0	0	0	0	4	0

…minimal	0	0	0	9	0	0	0	0	4	0
…mild	0	0	0	1	0	0	0	0	0	0
autolysis	0	2	0	3	0	2	0	0	2	0
Lymph Node, Mesenteric n =	25	25	25	25	0	25	25	25	25	0
necrosis, lymphoid	0	1	0	3	0	0	0	0	0	0
…minimal	0	1	0	0	0	0	0	0	0	0
…mild	0	0	0	2	0	0	0	0	0	0
…moderate	0	0	0	1	0	0	0	0	0	0
…missing	0	0	0	1	0	0	0	0	0	0
autolysis	0	0	0	0	0	0	0	0	1	0
Lymph Node, Mandibular n =	25	25	25	25	0	25	25	25	25	0
necrosis, lymphoid	0	2	0	2	0	2	0	0	0	0
…minimal	0	1	0	0	0	1	0	0	0	0
…mild	0	1	0	2	0	1	0	0	0	0
Spleen n =	25	25	25	25	10	25	25	25	25	10
pigmentation, hemosiderin	0	3	1	2	0	0	0	0	0	0
…minimal	0	2	1	1	0	0	0	0	0	0
…mild	0	1	0	1	0	0	0	0	0	0
necrosis, lymphoid	0	1	0	4	0	1	0	0	0	1
…minimal	0	1	0	1	0	0	0	0	0	0
…mild	0	0	0	3	0	1	0	0	0	1
Thymus n =	25	25	25	25	0	25	25	25	25	0
cyst	0	2	1	1	0	0	0	1	0	0
hyperplasia, epithelial	0	0	0	0	0	0	0	2	0	0
…moderate	0	0	0	0	0	0	0	1	0	0
…marked	0	0	0	0	0	0	0	1	0	0
necrosis, lymphoid	0	0	0	5	0	1	0	0	0	0
…minimal	0	0	0	1	0	0	0	0	0	0
…moderate	0	0	0	4	0	1	0	0	0	0
increased lymphocytes	0	0	0	0	0	0	0	0	1	0
…mild	0	0	0	0	0	0	0	0	1	0

Abbreviations: + = positive control (1000 mg/kg/day); n = number of animals examined

Toxicokinetics

Blood samples were collected from the retro-orbital sinus of TK cohort animals, under 70% CO_2/30% O_2 anesthesia. Blood collection times for vehicle control animals were 1 hour postdose on Day 1 and Day 177 (Week 26). For drug-treated animals, blood was collected at predose, 0.5, 1, 2, 4, 8, and 24 hours postdose on Day 1 and Day 177 (Week 26). TK parameters that were determined included C_{max}, T_{max}, and AUC_{0-24} for VX-661 and the metabolites VRT-0996107 (M1) and VRT-1189001 (M2).

As shown in **Table 21** for VX-661, T_{max} for females and males was 0.5 hours for all dose groups on Day 1 and 0.5 hours for the Day 177 (Week 26) low dose and mid dose groups and 1 hour for the Day 177 (Week 26) high dose group. After repeat exposure in females (Day 177 [Week 26]), AUC was generally dose proportional between the low dose and mid dose groups but less than dose proportional between the mid dose and high dose groups. After repeat exposure in males (Day 177 [Week 26]), AUC was generally less than dose proportional between the low dose and mid dose groups and the mid dose and high dose groups. Exposure in females and males was generally comparable. There did not appear to be drug accumulation over the time course of dosing in females or males.

As shown in **Table 21** for metabolite VRT-0996107 (M1), T_{max} for females and males ranged from 2 to 24 hours, regardless of dose or time interval (Day 1 or Day 177 [Week 26]). After repeat exposure in females and males (Day 177 [Week 26]), AUC was generally less than dose proportional between the low dose and mid dose groups and the mid dose and high dose groups. Exposure in females and males was generally comparable. There did not appear to be VRT-0996107 (M1) metabolite accumulation over the time course of dosing in males or females.

As shown in **Table 21** for metabolite VRT-1189001 (M2), T_{max} for females ranged from 4 to 24 hours and for males ranged from 2 to 24 hours, regardless of dose or time interval (Day 1 or Day 177 [Week 26]). After repeat exposure in females and males (Day 177 [Week 26]), AUC was generally less than dose proportional between the low dose and mid dose groups and the mid dose and high dose groups. Exposure in females and males was generally comparable. There appeared to be slight VRT-1189001 (M2) metabolite accumulation over the time course of dosing in males and females.

Table 21 Summary Toxicokinetics for VX-661, Metabolite VRT-0996107 (M1), and Metabolite VRT-1189001 (M2) in Wild-Type CByB6F1 Mice (Applicant's Table)

Analyte	Dose of VX-661 (mg/kg/day)	Sex	Day 1 AUC_{0-t} (µg*h/mL)	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 177 AUC_{0-t} (µg*h/mL)	Day 177 C_{max} (µg/mL)	Day 177 T_{max} (hr)
VX-661	30 (Group 2)	Female	17.7	2.48	0.500	15.1	2.14	0.500
		Male	18.9	2.93	0.500	17.4	2.44	0.500
		Combined*	18.3	2.71	0.500	16.2	2.29	0.500
	100 (Group 3)	Female	62.2	5.60	0.500	54.1	7.46	0.500
		Male	115	9.63	0.500	40.4	6.50	0.500
		Combined*	88.5	7.62	0.500	47.2	6.98	0.500
	500 (Group 4)	Female	255	39.3	0.500	154	16.5	1.00
		Male	302	46.2	0.500	92.6	16.8	1.00
		Combined*	278	42.8	0.500	123	16.6	1.00
VRT-0996107	30 (Group 2)	Female	71.1	4.80	4.00	72.2	4.41	8.00
		Male	82.5	6.33	4.00	90.6	5.76	8.00
		Combined*	76.8	5.57	4.00	81.4	5.08	8.00
	100 (Group 3)	Female	148	9.97	4.00	212	14.4	8.00
		Male	358	25.7	8.00	214	15.1	2.00
		Combined*	253	17.8	6.00	213	14.7	5.00
	500 (Group 4)	Female	578	29.2	24.0	424	27.7	2.00
		Male	576	38.8	24.0	467	34.4	4.00
		Combined*	577	34.0	24.0	446	31.0	3.00
VRT-1189001	30 (Group 2)	Female	3.68	0.260	4.00	4.69	0.288	8.00
		Male	4.61	0.285	4.00	6.02	0.369	8.00
		Combined*	4.14	0.273	4.00	5.36	0.329	8.00
	100 (Group 3)	Female	7.03	0.400	4.00	13.4	0.901	8.00
		Male	16.2	1.04	8.00	14.7	0.985	2.00
		Combined*	11.6	0.720	6.00	14.1	0.943	5.00
	500 (Group 4)	Female	23.4	1.39	24.0	37.9	2.15	8.00
		Male	22.7	1.67	24.0	43.7	2.95	4.00
		Combined*	23.1	1.53	24.0	40.8	2.55	6.00

* Combined is defined as the TK parameters obtained with the pooled data of male and female mice

Dosing Solution Analysis

Dosing solutions were analyzed for homogeneity (top, middle, and bottom stratum) and concentration (middle stratum) on the first and last TK dose formulations for each sex, and from one Week 13 formulation. All dosing formulations were found to be homogenous and met the acceptance criteria of 85 to 115% of the target concentration and ≤10% the relative standard deviation, (b) (4) . A control sample was also analyzed and no test article was detected.

11 Integrated Summary and Safety Evaluation

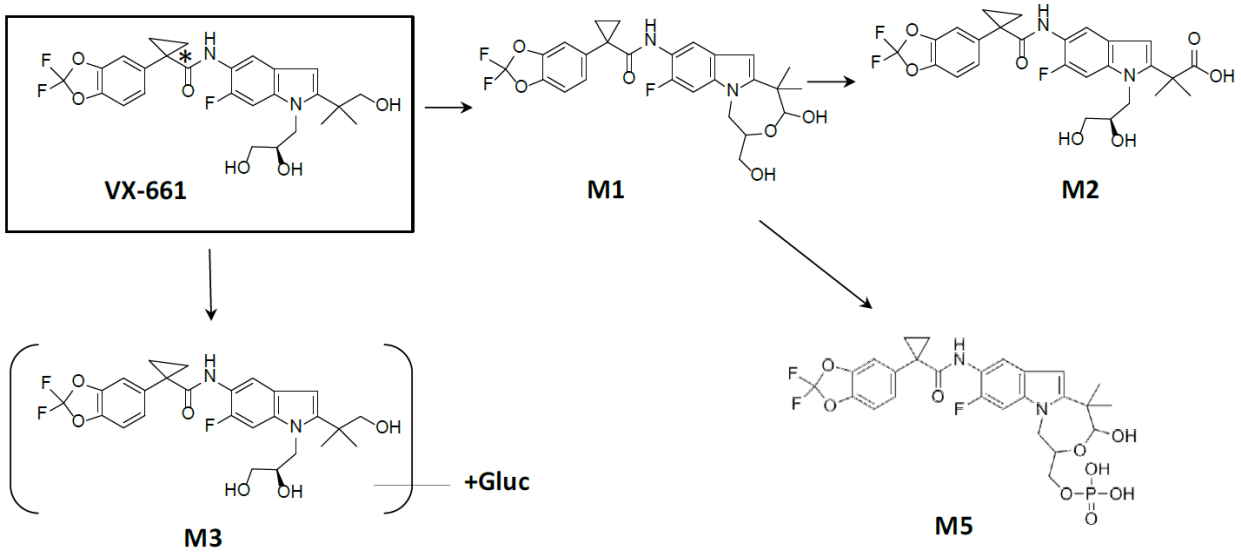
Vertex submitted a 505(b)(1) NDA for tezacaftor (VX-661)/ivacaftor (VX-770) combination therapy for the treatment of CF in patients 12 year of age and older who are homozygous for the F508del mutation or who have at least one mutation in the

CFTR gene that is responsive to tezacaftor (VX-661)/ivacaftor (VX-770), based on in vitro data and/or clinical evidence. Ivacaftor was approved for the treatment of CF on January 31, 2012. The carcinogenic potential of ivacaftor (VX-770) was reviewed under NDA 203188; ivacaftor was not tumorigenic in either 2-year rat or mouse studies.

VX-661 was negative for mutagenicity and clastogenicity in a standard battery of genetic toxicology studies (bacterial reverse mutation assay, in vitro chromosomal aberration assay, and in vivo mouse micronucleus assay).

As shown in **Figure 13**, VX-661 undergoes considerable metabolism (mainly by CYP3A4) in humans and nonclinical species. Dehydrogenation of VX-661 leads to the major metabolite M1 (VRT-0996107). Sequential oxidation of M1 forms metabolite M2 (VRT-1189001), while phosphorylation of M1 forms M5 (VRT-1074233) (which can potentially interconvert back to M1). Metabolites M1 and M2 constitute >10% of total systemic exposure (i.e., approximately 38.8% and 35.7%, respectively) at the proposed clinical dose. M1 is pharmacologically active with similar potency and efficacy as VX-661. M2 is a disproportionate human metabolite (i.e., high levels in humans and low levels in rats and dogs), which is pharmacologically active but less so than VX-661. M5 (VRT-1074233) was detected in a human mass balance study at >10% of total systemic exposure, but is considered pharmacologically inactive.

Figure 13 Proposed Metabolic Pathway of VX-661 in Rats and Humans (Applicant's Figure)



*signifies the position of the radiolabel

The Sponsor conducted a 104-week rat study and 26-week Tg.rasH2 mouse study to assess the carcinogenic potential of VX-661. The ECAC concurred with the doses and design of the studies (see Special Protocol Agreements dated December 18, 2013 and November 20, 2014, respectively).

In a 104-week oral (gavage) carcinogenicity study, SD male rats received doses of 0, 5, 15, and 50 mg/kg/day VX-661 and SD female rats received doses of 0, 5, 20, and 75

mg/kg/day VX-661. There were no drug-related effects on mortality, but male and female groups were both terminated early (i.e., Week 103 and Week 101, respectively) because the number of surviving animals in the respective control groups reached ≤20. At the end of the study period mean body weights for high dose males and females were 5.9% and 17.7% lower, respectively, compared to control animals. There were no statistically significant drug-related tumor findings in male or female rats. The most notable non-neoplastic histopathology findings were dilated lymphatics in the GALT and small intestine (ileum and jejunum). In the GALT, minimal to mild dilated lymphatics were noted with increased incidence in mid dose males, high dose males, and high dose females relative to control animals. In the small intestine (ileum and jejunum) minimal to mild dilated lymphatics were noted with greater incidence in high dose males and females relative to control animals. Neither the incidence or severity of the dilated lymphatics progressed in this 2-year rat study compared to the 3-month and 26-week rat studies. Additional non-neoplastic lesions were seen in the parathyroid glands, liver, Harderian glands, clitoral glands, ovaries, and sternum bone marrow. VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) was 180 and 337 mcg*hr/mL in males and females, respectively. Metabolite M1 AUC exposure at the high dose of VX-661 was about 288 and 479 mcg*hr/mL in males and females, respectively. Metabolite M2 AUC exposure at the high dose of VX-661 was about 20 and 13 mcg*hr/mL in males and females, respectively.

The tezacaftor (VX-661)/ivacaftor (VX-770) dosing regimen will consist of a total daily dose of 100 mg tezacaftor and 300 mg ivacaftor as one tablet of a fixed dose combination of 100 mg tezacaftor/150 mg ivacaftor in the morning and one tablet of 150 mg ivacaftor in the evening. Exposure margins for VX-661, M1 (VRT-0996107), and M2 (VRT-1189001) for the human dose of 100 mg tezacaftor once daily are shown in **Table 22**. VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) results in exposure margins about 2.4-fold and 4.5-fold, respectively, above the clinical exposure achieved with the proposed dosing regimen. Similarly, M1 AUC exposure at the high dose in males and females results in exposure margins about 2.5-fold and 4.2-fold, respectively, above the clinical exposure achieved with the proposed dosing regimen. As expected, since M2 is a disproportionate human metabolite, M2 AUC exposure at the high dose in males and females results in exposure margins about 0.19-fold and 0.12-fold, respectively, above the clinical exposure achieved with the proposed dosing regimen.

Table 22 Clinical Exposure Margins for VX-661, Metabolite VRT-0996107 (M1), and Metabolite VRT-1189001 (M2) Relative to 104-Week Oral (Gavage) Carcinogenicity Study in Rats

VX-661 Exposure Margin for Human Dose of 100 mg Tezacaftor Once Daily:

VX-661 Dose (mg/kg/day) (Males/Females)	VX-661 Week 52 AUC_{0-24hr} (mcg*hr/mL) Males	VX-661 Week 52 AUC_{0-24hr} (mcg*hr/mL) Females	Exposure Margin[1] Males	Exposure Margin[1] Females
50/75	180	337	2.40	4.49
Metabolite VRT-0996107 (M1) Exposure Margin for Human Dose of 100 mg Tezacaftor Once Daily:				
VX-661 Dose (mg/kg/day) (Males/Females)	**M1 Week 52 AUC_{0-24hr} (mcg*hr/mL) Males**	**M1 Week 52 AUC_{0-24hr} (mcg*hr/mL) Females**	**Exposure Margin[1] Males**	**Exposure Margin[1] Females**
50/75	288	479	2.53	4.20
Metabolite VRT-1189001 (M2) Exposure Margin for Human Dose of 100 mg Tezacaftor Once Daily:				
VX-661 Dose (mg/kg/day) (Males/Females)	**M2 Week 52 AUC_{0-24hr} (mcg*hr/mL) Males**	**M2 Week 52 AUC_{0-24hr} (mcg*hr/mL) Females**	**Exposure Margin[1] Males**	**Exposure Margin[1] Females**
50/75	20.0	13.0	0.19	0.12

[1]AUC_{0-24hr} at steady state for 100 mg tezacaftor once daily = 75.1 mcg*hr/mL for VX-661, 114 mcg*hr/mL for M1, and 105 mcg*hr/mL for M2.

In a 26-week oral carcinogenicity study, Tg.rasH2 mice received doses of 0, 30, 100, and 500 mg/kg/day VX-661 and 1000 mg/kg/day urethane (positive control). There was a statistically significant treatment-related increase in mortality for high dose males (76% survival) compared to control males (96% survival). At the end of the study period (i.e., Day 183) mean body weights for VX-661-treated male and female animals were generally similar to control animals. There were no statistically significant drug-related tumor findings in male or female mice. The positive control produced expected increases of neoplastic findings indicating that the mice and study could detect a potential drug-induced neoplastic response. The most notable non-neoplastic histopathology findings were in the adrenal glands (cortex hypertrophy, cortex vacuolation, and x-zone degeneration), liver (centrilobular hypertrophy, centrilobular hepatocellular single cell necrosis, subcapsular inflammation, focal hepatocellular necrosis, focal lipid infiltration, and periportal vacuolation), ovaries (decreased corpora lutea), and jejunum (lamina propria vacuolation). Additional non-neoplastic findings were noted in the mesenteric and mandibular lymph nodes (lymphoid necrosis), spleen (hemosiderin pigmentation and lymphoid necrosis), and thymus (lymphoid necrosis and increased lymphocytes). VX-661 AUC exposure at the high dose (500 mg/kg/day) was about 93 and 154 mcg*hr/mL in males and females, respectively. Metabolite M1 AUC exposure at the high dose of VX-661 (500 mg/kg/day) was about 467 and 424

mcg*hr/mL in males and females, respectively. Metabolite M2 AUC exposure at the high dose of VX-661 (500 mg/kg/day) was about 44 and 38 mcg*hr/mL in males and females, respectively.

The 2-year rat study achieved a greater exposure to M1 relative to the human exposure to M1 associated with the clinical dose of VX-661. Exposure to M1 was quantified in the 26-week Tg.rasH2 mouse carcinogenicity study. Thus, the 2-year rat and 26-week mouse studies provides an adequate assessment of the carcinogenic potential of M1.

Due to M2 being a disproportionate human metabolite, the Applicant evaluated it independently in toxicity studies. Oral administration of M2 to rats, guinea pigs, and dogs had poor bioavailability. Intravenous administration of M2 resulted in deaths in rats. Further, subcutaneous administration was not tolerated in rats. In a 1-month toxicity study, dogs could tolerate a subcutaneous dose that produced an approximate exposure to the expected therapeutic human dose. As with rats, it is also likely for mice that an oral M2 dose group would not achieve relevant exposures, and a subcutaneous administration may not be tolerated, although this has not been attempted. Thus, it was judged that carcinogenic potential of the M2 metabolite could not be studied. M2 possesses no structural alerts for mutagenicity based upon quantitative structure-activity relationship (QSAR) analysis. M2 was negative for potential genetic toxicity by the bacterial reverse mutation assay and the chromosomal aberration assay with human peripheral blood lymphocytes. Exposure to M2 was quantified in the 2-year carcinogenicity study with rats and 26-week Tg.rasH2 mouse carcinogenicity study. Exposures to M2 in the 2-year rat and 26-week mouse studies were approximately ≤0.2 of the achieved clinical exposure. No further nonclinical assessment of the carcinogenic potential of M2 is required.

M5 has not been routinely monitored in nonclinical studies with rats or dogs. The Applicant provided data that M5 is formed in rats from a single dose study, but not in repeat dose studies. It was judged that M5 was formed in sufficient levels in rats to provide an assessment of its toxic potential. Thus, it is reasonable to assume that the 2-year rat study provides an adequate assessment of the carcinogenic potential of M5. M5 has not been studied for potential genotoxicity.

No statistically significant neoplastic findings were observed in male or female SD rats and male or female Tg.rasH2 mice treated with tezacaftor at MTDs.

The ECAC concurred that both the 2-year rat and 26-week Tg.rasH2 carcinogenicity studies were adequate and that there were no drug-related neoplasms in males or females in either study. The ECAC also concurred that based upon feasibility and the completed carcinogenicity studies in rats and Tg.rasH2 mice, that no further studies were required for the safety qualification of the M1, M2, and M5 metabolites with respect to carcinogenicity.

Recommended labeling to describe the results of carcinogenicity studies conducted with tezacaftor and ivacaftor in mice and rats:

Tezacaftor

A two-year study in Sprague-Dawley rats and a (b) (4) study in Tg.rasH2 transgenic mice were conducted to assess the carcinogenic potential of tezacaftor. No evidence of

tumorigenicity was observed in male and female rats at tezacaftor oral doses up to 50 and 75 mg/kg/day (approximately (b) (4) times the MRHD based upon summed AUCs of tezacaftor and its metabolites in males and females, respectively). No evidence of tumorigenicity was observed in male and female Tg.rasH2 transgenic mice at tezacaftor oral doses up to 500 mg/kg/day.

Ivacaftor

Two-year studies were conducted in (CD-1) mice and (Sprague-Dawley) rats to assess (the) carcinogenic potential of ivacaftor. No evidence of tumorigenicity was observed in mice or rats at ivacaftor oral doses up to 200 mg/kg/day and 50 mg/kg/day, respectively (approximately equivalent to (b) (4) times the MRHD, respectively, based on summed AUCs of ivacaftor and its metabolites).

The recommended labeling for ivacaftor is based on the labels for KALYDECO® (ivacaftor) and ORKAMBI® (lumacaftor and ivacaftor). Of note, the mouse and rat strains (CD-1 and Sprague-Dawley, respectively) are not designated in the ORKAMBI® label. Further, the word “the” is not included before “carcinogenic potential” in either the KALYDECO® or ORKAMBI® labels.

(b) (4)

12 Appendix/Attachments

Appendix 1: Special Protocol Agreement (i.e., ECAC meeting minutes) dated December 18, 2013, for SPA of 2-year carcinogenicity study in rats

Appendix 2: Special Protocol Agreement (i.e., ECAC meeting minutes) dated November 20, 2014, for SPA of 6-month carcinogenicity study in Tg.rasH2 mice

APPEARS THIS WAY ON ORIGINAL

Appendix 1

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: December 18, 2013

To: Adel Al-Shyaikh, MSc, RAC	**From:** Adele Seifried
Company: Vertex	OND IO
Fax number: (617) 341-6803	**Fax number**: 301-796-9855
Phone number: (617) 961-1563	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 108,105

Total no. of pages including cover: 5

Comments: **email to adel_al-shaikh@vrtx.com**

Document to be mailed: ☐ YES ☑ NO

Reference ID: 3424313

Executive CAC
Dec 17, 2013

Committee: David Jacobson Kram, OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
David Joseph, Ph.D., Alternate Member, DGIEP
Marcie Wood, Ph.D., Pharm Tox Supervisor, DPARP
L. Steven Leshin, D.V.M., Ph.D., Presenting Reviewer, DPARP

Author of Minutes: L. Steven Leshin (DPARP)

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND 108105
Drug Name: VX-661
Sponsor: Vertex Pharmaceuticals Incorporated

Background
VX-661 is a being developed for the treatment of cystic fibrosis (CF) in patients with the *F508del* mutation in the CF transmembrane conductance regulator gene (CFTR). In pharmacodynamic studies, VX-661 has some efficacy in correcting the tertiary *F508del* CF protein structure and its transport to the surface membrane.

Daily oral dosing toxicity studies of 3-months (Report VX-661-TX-010) and 6-months (Report VX-661-TX-012) duration were submitted to support the dose selection for a 2-year carcinogenicity study in rats. A VX-661 metabolite, VRT-1189001 (M2), was discovered to be present in human plasma at concentrations greater than found in the toxicity study species, and is currently undergoing in vivo toxicological characterization.

In dose range-finding and the 1-month duration toxicity studies (Report VRT-893661-TX-010), deaths and morbidity occurred at doses ≥200 mg/kg/day. In the 6-month study the major limiting toxicity at 100 mg/kg/day was the reduction in mean body weight of approximately 16% in males relative to that of controls at week 26. Reduced mean weight gain of 26% for males and 23% for females was noted during the first 12 weeks of the 6-month study. A similar reduction in mean weight gain was also noted in the 3-month study (-23% and -22% for males and females, respectively). Lower relative weights were associated with a reduction in food consumption during the initial few weeks in both studies; thereafter food consumption was similar to controls.

However, the lower body weights persisted throughout the dosing duration. Clinical and anatomical pathological findings in 3- and 6-month studies were not considered dose-limiting for a 2-year bioassay.

VX-661 was negative for inducing mutations in the bacterial reverse mutation assay, and not clastogenic in both the in vitro Chinese hamster ovary cell chromosomal aberration assay and in vivo mouse micronucleus assay. VRT-1189001 (M2) was also negative for inducing mutations in the bacterial reverse mutation assay and the chromosomal aberration assay with human peripheral blood lymphocytes.

Rat Carcinogenicity Protocol (VX-661-TX-020) and Dose Selection

The proposed doses of VX-661 in the sponsor's protocol were 0 (vehicle), (b) (4) and 50 mg/kg/day administered to both males and females, once daily for 2 years. VX-661 is a (b) (4) (b) (4) formulation consisting of VX-661 in hydroxyproplymethylcellulose acetate succinate (HPMC-AS) and sodium lauryl sulfate (SLS) as 49.5% HPMC-AS/0.5% SLS). The control group will receive the (b) (4) formulation of VX-661 without VX-661 present. The vehicle consists of 0.5% methyl cellulose (400 cps) (w/v), 0.5% sodium lauryl sulfate (SLS) (w/v), and 0.01% simethicone (w/v) in deionized water for all dose groups.

(b) (4)

(b) (4) Therefore, the sponsor selected the dose of 50 mg/kg/day as the high-dose for the 2-year rat bioassay.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee recommended doses of 0, 5, 15, and 50 mg/kg/day in males and doses of 0, 5, 20, and 75 mg/kg/day in females, by oral gavage

  These doses were selected based on a maximum tolerated dose. The high dose of 75 mg/kg/day for females is approximately one-third the lethal dose. For males, the recommended high dose of 50 mg/kg/day was selected based on lower relative body weights at 100 mg/kg in the 6-month study. Mid- and low-doses in male and female groups were selected to provide an approximately 3-fold dose separation based on systemic exposure.

- The Committee suggested that the sponsor consider testing the major human metabolite VRT-1189001 (M2) in this study if possible (e.g., at a tolerated dose).

David Jacobson Kram, Ph.D.
Chair, Executive CAC

Reference ID: 3424313

cc:\

/IND 108105, DPARP
/Marcie Wood, Ph.D., DPARP
/L. Steven Leshin, D.V.M., Ph.D., DPARP
/Angela Ramsey, R.N., M.S.M., DPARP
/A Seifried, 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/

DAVID JACOBSON KRAM
12/18/2013

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Appendix 2

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: December 18, 2013

To: Alissa Minkoff	**From:** Adele Seifried
Company: Vertex	OND IO
Fax number: (617) 341-6803	**Fax number**: 301-796-9855
Phone number: (617) 961-0003	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 108,105

Total no. of pages including cover: 4

Comments: email to alissa_minkoff@vrtx.com

Document to be mailed: ☐ YES ☑ NO

Reference ID: 3661348

Executive CAC
Nov 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
Marcie Wood, Ph.D., Pharm Tox Supervisor, DPARP
L. Steven Leshin, D.V.M., Ph.D., Presenting Reviewer, DPARP

Author of Minutes: L. Steven Leshin (DPARP)

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 6-month Tg.rasH2 mouse carcinogenicity bioassay, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format-Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND 108105
Drug Name: VX-661
Sponsor: Vertex Pharmaceuticals Incorporated

Background

VX-661 is being developed for the treatment of cystic fibrosis (CF) in patients with the *F508del* mutation in the CF transmembrane conductance regulator gene (CFTR). In pharmacodynamic studies, VX-661 has some efficacy in correcting the tertiary *F508del* CF protein structure and its transport to the surface membrane.

A 5-day dose ranging study and a 28-day repeated oral dosing toxicity study (Report VX-661-TX-017) were submitted to support the dose selection for a 6-month carcinogenicity study in Tg.rasH2 mice. In the 5-day dose ranging study, doses were 0 (vehicle control), 500, 750, 1000, 1250, and 1500 mg/kg/day. The vehicle control consisted of the (b) (4) formulation [hydroxyproplymethylcellulose acetate succinate (HPMC-AS) and sodium lauryl sulfate (SLS) as 49.5% HPMC-AS/0.5% SLS] lacking VX-661 in a vehicle consisting of 0.5% methylcellulose, 0.5% sodium dodecyl sulfate, and 0.01% simethicone in deionized water. Mortalities occurred at the dose of 1500 mg/kg/day in both sexes. At 500 mg/kg/day there were no clinical signs. Histopathology was not conducted, and toxicokinetics for doses greater than 750 mg/kg/day were not assessed.

In the 28-day repeated oral dosing study, doses of 0 (vehicle control), 250, 500, and 750 mg/kg/day were administered once daily (using the same vehicle control described above). There

was no effect on body weight, weight gain, or food consumption. Liver function was altered, as reflected in reduced albumin and increase globulin levels, at the high dose. Serum AST and ALT were increased at the mid or high dose. Liver histopathology included focal necrosis, portal inflammation, and excessive centrilobular hypertrophy. There were only minimal differences between sexes in liver toxicity.

VX-661 was negative for inducing mutations in the bacteria reverse mutation assay, and not clastogenic in both the in vitro Chinese hamster ovary cell chromosomal aberration assay and in vivo mouse micronucleus assay. VRT-1189001 (M2), a significant human metabolite, was also negative for inducing mutations in the bacterial reverse mutation assay and negative for clastogenicity in the chromosomal aberration assay with human peripheral blood lymphocytes.

Tg.rasH2 Mouse Carcinogenicity Study Dose Selection

The proposed doses of VX-661 in the sponsor's protocol were 0 (vehicle), 30, 100, and (b) (4) mg/kg/day administered to both males and females, once daily for 6-months using the same control formulation and vehicle as described above. The sponsor considered (b) (4) mg/kg an MTD based on histopathological findings of liver toxicity. Mid and low doses, 100 and 30 mg/kg/day, respectively, were separated by intervals determined from AUC values extrapolated from toxicokinetics of the 28-day and 5-day studies and an additional short-term toxicokinetic study in CByB6F1 mice (wildtypes of Tg.rasH2 mice, Report VX-661-sdpk303292).

Executive CAC Recommendations and Conclusions:

Tg.rasH2 mouse:

- The Committee recommended doses of 0, 30, 100, and 500 mg/kg/day, by oral gavage, based on mortality at 1500 mg/kg/day. Spacing of mid and low doses was based on AUC.

As a point of information, the S1A-S1C carcinogenicity study guidelines are a current topic of an expert working group (EWG) of the International Conference on Harmonization. For further information on the current status of the EWG's activities, and particularly the S1 concept paper, business plan, and regulatory notice document, please visit:
http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html

Paul Brown, Ph.D.
Acting Chair, Executive CAC

cc:\
/IND 108105, DPARP
/Marcie Wood, Ph.D., DPARP
/L. Steven Leshin, D.V.M., Ph.D., DPARP
/Angela Ramsey, R.N., M.S.M., DPARP
/A Seifried, OND IO

Reference ID: 3661348

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/s/

PAUL C BROWN
11/20/2014

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

/s/

ELENI M SALICRU
01/30/2018

TIMOTHY W ROBISON
01/30/2018
I concur. This review replaces an earlier version placed in DARRTS on December 6, 2017.

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:	210491
Supporting document/s:	1, 19, 21, 23, and 28
Applicant's letter date:	June 28, 2017; November 29, 2017; December 13, 2017; January 2, 2018; and January 23, 2018
CDER stamp date:	June 28 ,2017; November 29, 2017; December 13, 2017; January 2, 2018; and January 23, 2018
Product:	Symdeko (tezacaftor and ivacaftor)
Indication:	Treatment of adult and pediatric patients (12 years and older) with Cystic Fibrosis (CF)
Applicant:	Vertex Pharmaceuticals, Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products (DPARP)
Reviewer:	L. Steven Leshin, DVM, PhD
Supervisor/Team Leader:	Carol Galvis, PhD
Division Director:	Badrul A. Chowdhury, MD, PhD
Project Manager:	Jessica Lee

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 210491 are owned by Vertex Pharmaceuticals, Inc. or are data for which Vertex Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 210491 that Vertex Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or

information 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 210491.

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1 Executive Summary

1.1 Introduction

Symdeko is a combination product consisting of 2 small molecule drugs, ivacaftor and tezacaftor (TEZ or VX-661). It is indicated for the treatment of cystic fibrosis (CF) in patients with the F508del and other mutations in the CF transmembrane conductance regulator (CFTR) gene. These mutations result in the absence or deficient function of CFTR protein, an epithelial chloride channel, at the cell surface. Tezacaftor is hypothesized to induce a conformational change in the CFTR mutant molecule that enables its stability and subsequent insertion in the cell membrane and chloride ion conductance function. In Symdeko, the Applicant proposes the therapeutic use of tezacaftor in combination with ivacaftor. Ivacaftor is currently marketed as Kalydeco (IVA or VX-770, NDA 203188 approved in January of 2012), and is a chloride channel potentiator that functions to keep the chloride channel open in patients with the *G551D* mutation in the CFTR gene. A similar combination drug, Orkambi, consisting of ivacaftor and lumacaftor, was approved in July of 2015 (NDA 203068). Lumacaftor, although different in structure from TEZ, has a similar mechanism of action as a conformational modifier to enable stability and function of the defective CFTR protein.

Symdeko is copackaged as a combination tablet containing tezacaftor (100 mg) and ivacaftor (150 mg), and a separate ivacaftor (150 mg) tablet. Each tablet is administered orally once daily 12 hours apart, such that tezacaftor is taken once daily and ivacaftor is taken twice daily (at the approved dosing regimen).

1.2 Brief Discussion of Nonclinical Findings

Pharmacological studies demonstrated that TEZ increases mature CFTR protein in the membrane of human bronchial epithelial cells obtained from patients with mutant F508del CFTR protein. Addition of IVA enhanced conductance of the chloride channel. There is evidence from numerous other CFTR mutants, TEZ enhanced the presence of a mature CFTR protein at the cell surface that is responsive to IVA potentiation of the chloride ion conductance. The effect of TEZ appears to be specific to CFTR protein based on limited data from other mutant proteins. The initial determination of an effective human dose was based on the quantitative data obtained from in vitro studies with human bronchial cells since there was not an appropriate in vivo animal model of CFTR function.

There are 3 major human metabolites of TEZ, identified as M1, M2, and M5. M1 also has pharmacodynamic activity similar to that of TEZ. M2 is much weaker and M5 lacks pharmacological activity. M2 is disproportionate in that its levels in the toxicity studies in rats and dogs were much lower than in clinical studies, requiring specific M2 studies to adequately characterize its safety profile.

General Toxicology Studies

Repeated dose toxicity studies of TEZ were conducted in rat (upt to 6 months) and dogs (up to 1 year). The doses were generally 0, 25, 50, and 100 mg/kg/day in rats and 0, 2, 100, and 200 mg/kg/day in dogs. The major consistent finding was dilated terminal lymphatics of minimal to mild severity in the villi of the jejunum and ileum of males and females of both rats and dogs in the 6-month and 1-year dog studies. The finding was also present at the end of recovery periods of various durations including up to 1-year in dogs, although there was indication of partial recovery at that time.

Studies in the dog found that terminal lymphatic dilation was not caused by the liquid formulation, since a tablet formulation produced the same findings. In all studies, only the lymphatic terminals were dilated, with no dilation along the length of lymphatic vessels and no obstruction within the mesenteric lymph node. There were no significant effects on the limited assessments of blood levels of absorbed nutrients (total protein, glucose, lipids, cholesterol, ions), and no gastrointestinal pathology or signs of adverse function. The mechanism for dilated lymphatic terminals and its associated persistence in the absence of dosing is unknown. Despite this consistent finding, it was not associated with any detrimental health or nutritional effects in initially healthy rats or dogs and is currently considered a nonadverse finding

In TEZ/IVA combination drug studies, TEZ and IVA were administered orally once daily for 1-month to rats and dogs and for 13-weeks to rats. The doses in the pivotal 13-week rat study were TEZ/IVA: 0/0 (vehicle control), 20/80, 40/40, and 80/20 mg/kg/day, respectively. The study design of the 3-month TEZ/IVA combination drug study was problematic in that increasing doses of TEZ were administered with decreasing IVA dose levels. Both compounds have overlapping pharmacodynamic properties, although dissimilar mechanism of action. Without contemporary controls for single drug groups, or recovery groups for all dose levels, it was not possible to evaluate potential synergistic toxicities. Due to design deficiencies, no NOAEL was identified in the study and this study was not useful for addressing safety of the combination product. Despite this situation, no new toxicities were identified when compared to the studies with single drugs.

Genetic Toxicology and Carcinogenicity
TEZ and M2 were not genotoxic in the standard battery of genetic toxicology tests. Carcinogenicity studies found that TEZ did not induce malignancies in a 2-year rat study or in a 6-month study in Tg.rasH2 transgenic mice. M2 was unable to be tested in rodents due to the lack of tolerability from either oral, iv, or sc administration.

Reproduction and Development
TEZ did not affect fertility in males or females and was not teratogenic in rat or rabbits despite severe weight loss and resultant mortality at doses of 50 and 100 mg/kg/day. The disproportional human metabolite M2 was not teratogenic in rabbits. In a pre- and post-natal development study in the rat, in which TEZ was administered through the period of gestation and lactation, pup mortality was increased during the first few days after birth in the 100 mg/kg/day group. There was substantial maternal body weight loss at this dose, reflected in low birth weights and failure to gain weight after birth. At doses of ≥50 mg/kg/day, there were delays in early developmental landmarks (pinna detachment, eye opening, and static righting reflexes), and at 100 mg/kg/day, there were

delays in sexual maturation (vaginal opening and preputial separation). However, there were no effects on F_1 male or female fertility, and no detrimental effects on early embryofetal development in animals from the 25 mg/kg/day dose group. There was no effect of TEZ on learning and memory in passive avoidance testing. TEZ crosses the placenta during pregnancy and was detected in the milk of lactating rats when administered after the first week of lactation.

Conclusions
The characterization of TEZ and its metabolites was adequate to reasonably ensure safety during its clinical development and marketing. There are no outstanding toxicological concerns for TEZ.

1.3 Recommendations

1.3.1 Approvability

The application is recommended for approval from the pharmacology and toxicology perspective.

1.3.2 Additional Non Clinical Recommendations

There are no nonclinical recommendations.

1.3.3 Labeling

The following is the sponsor label from the November 29, 2017 submission (SD-19), January 2, 2018 (SD-23), and January 23, 2018 (SD-28) versions, followed by the reviewer's comments and recommendations. Strikeouts indicate words to be removed, and underlining indicates additions to the label. Labeling was informed by previously approved products, Kalydeco and Orkambi, with an attempt to maintain consistency between labels.

8.1 PREGNANCY

Applicant's Proposed Label (from submission of November 29, 2017)

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
Risk Summary
There are limited and incomplete human data from clinical trials and post-marketing reports on the use of SYMDEKO or its individual components, tezacaftor and ivacaftor, in pregnant women to inform a drug-associated risk. (b) (4) In animal reproduction studies, oral administration of tezacaftor to pregnant rats and rabbits during organogenesis demonstrated no teratogenicity or adverse effects on (b) (4) development at doses that produced maternal exposures (b) (4) up to approximately 3 times the exposure at the maximum recommended human dose (MRHD) in rats and 0.2 times the MRHD in rabbits (based on summed AUCs for tezacaftor and M1

metabolite). [redacted] Oral administration of ivacaftor to pregnant rats and rabbits during organogenesis demonstrated no teratogenicity or adverse effects on [redacted] development at doses that produced maternal exposures up to approximately [redacted] and 16 times the exposure at the MRHD, respectively. No adverse developmental effects were observed after oral administration of either tezacaftor or ivacaftor to pregnant rats from organogenesis through lactation at doses that produced maternal exposures approximately 1 and [redacted] times the exposures at the MRHD, respectively (*see Data*). There are no animal reproduction studies with concomitant administration of tezacaftor and ivacaftor.

The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Data

Animal Data

Tezacaftor

In an embryo-fetal development study in pregnant rats dosed during the period of organogenesis from gestation days 6-17, tezacaftor was not teratogenic and did not affect fetal development or survival at exposures up to 3 times the MRHD (based on summed AUCs for tezacaftor and M1 metabolite at maternal oral doses up to 100 mg/kg/day). In an embryo-fetal development study in pregnant rabbits dosed during the period of organogenesis from gestation days 7-20, tezacaftor was not teratogenic and did not affect fetal development or survival at exposures up to 0.2 times the MRHD (based on summed AUCs for tezacaftor and M1 metabolite at maternal oral doses up to 25 mg/kg/day). In a pre- and postnatal development (PPND) study in pregnant [redacted] rats dosed from gestation day 6 through lactation day 18, [redacted] of approximately 1 times the MRHD (based on summed AUCs for tezacaftor and M1 metabolite). [redacted] Placental transfer of tezacaftor was observed in pregnant rats.

Ivacaftor

In an embryo-fetal development study in pregnant rats dosed during the period of organogenesis from gestation days 7-17, ivacaftor was not teratogenic and did

not affect fetal survival at exposures up to 6 times the MRHD (based on summed AUCs for ivacaftor and its metabolites at a maternal oral dose of 200 mg/kg/day). In an embryo-fetal development study in pregnant rabbits dosed during the period of organogenesis from gestation days 7-(b) (4) ivacaftor was not teratogenic and did not affect fetal development or survival at exposures up to 16 times the MRHD (on an ivacaftor AUC basis at maternal oral doses up to 100 mg/kg/day). In a PPND study in pregnant (b) (4) rats dosed from gestation day 7 through lactation day 20, ivacaftor had no effects on delivery or growth and development of offspring at exposures up to 4 times the MRHD (based on summed AUCs for ivacaftor and its metabolites at maternal oral doses up to 100 mg/kg/day). Decreased fetal body weights were observed at a maternally toxic dose that produced exposures 6 times the MRHD. Placental transfer of ivacaftor was observed in pregnant rats and rabbits.

Reviewer's Labeling Recommendations and Comments:

The label was altered to be consistent with previous combination drug language for Ivacaftor containing products, with some changes in wording for clarity. Changes were made to exposure margins for ivacaftor due to the use of pharmacokinetic data from clinical studies with administration of tezacaftor/ivacaftor combination tablets and ivacaftor tablets, rather than previous studies with ivacaftor alone.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are limited and incomplete human data from clinical trials and post-marketing reports on the use of SYMDEKO or its individual components, tezacaftor and ivacaftor, in pregnant women to inform a drug-associated risk. (b) (4) Although there are no animal reproduction studies with the concomitant administration of tezacaftor and ivacaftor, separate reproductive and developmental studies were conducted with tezacaftor and ivacaftor in pregnant rats and rabbits. In animal reproduction studies, oral administration of tezacaftor to pregnant rats and rabbits during organogenesis demonstrated no teratogenicity or adverse developmental effects ~~on development~~ (b) (4) at doses that produced maternal exposures (b) (4) up to approximately 3 times the exposure at the maximum recommended human dose (MRHD) in rats and 0.2 times the MRHD in rabbits (based on summed AUCs for tezacaftor and M1 metabolite). (b) (4) Oral administration of ivacaftor to pregnant rats and rabbits during organogenesis demonstrated no teratogenicity or adverse developmental effects ~~on development~~ (b) (4) at doses that produced maternal exposures up to approximately (b) (4) 6 and 16 times the exposure at the MRHD, respectively. No adverse

developmental effects were observed after oral administration of either tezacaftor or ivacaftor to pregnant rats from the period of organogenesis through lactation at doses that produced maternal exposures approximately 1 and (b) (4) 4 times the exposures at the MRHD, respectively (*see Data*). ~~There are no animal reproduction studies with concomitant administration of tezacaftor and ivacaftor.~~

The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Data

Animal Data

Tezacaftor

In an embryo-fetal development study in pregnant rats dosed during the period of organogenesis from gestation Days 6-17, tezacaftor was not teratogenic and did not affect fetal development or survival at exposures up to 3 times the MRHD (based on summed AUCs for tezacaftor and M1 metabolite at maternal oral doses up to 100 mg/kg/day). In an embryo-fetal development study in pregnant rabbits dosed during the period of organogenesis from gestation Days 7-20, tezacaftor was not teratogenic and did not affect fetal development or survival at exposures up to 0.2 times the MRHD (based on summed AUCs for tezacaftor and M1 metabolite at maternal oral doses up to 25 mg/kg/day). Lower fetal body weights were observed in rabbits at a maternally toxic dose that produced exposures approximately 0.4 times the MRHD (at a maternal dose of 50 mg/kg/day). In a pre- and postnatal development (PPND) study in pregnant (b) (4) rats dosed from gestation day 6 through lactation day 18, tezacaftor had no adverse developmental effects on pups at an exposure (b) (4) of approximately 1 times the MRHD (based on summed AUCs for tezacaftor and M1 metabolite at a maternal dose of 25 mg/kg/day). Decreased fetal body weights and early developmental delays in pinna detachment, eye opening, and righting reflex occurred at a maternally toxic dose (based on maternal weight loss) that produced exposures approximately 2 times the exposure at the MRHD (based on summed AUCs for tezacaftor and M1 metabolite at a maternal oral dose of 50 mg/kg/day). (b) (4) [redacted] Placental transfer of tezacaftor was observed in pregnant rats.

Ivacaftor

In an embryo-fetal development study in pregnant rats dosed during the period of organogenesis from gestation days 7-17, ivacaftor was not teratogenic and did not affect fetal survival at exposures up to 6 times the MRHD (based on summed AUCs for ivacaftor and its metabolites at a maternal oral dose of 200 mg/kg/day). In an embryo-fetal development study in pregnant rabbits dosed during the period of organogenesis from gestation Days 7-(b) (4)19, ivacaftor was not teratogenic and did not affect fetal development or survival at exposures up to 16 times the MRHD (on an ivacaftor AUC basis at maternal oral doses up to 100 mg/kg/day). In a PPND study in pregnant (b) (4) rats dosed from gestation Day 7 through lactation Day 20, ivacaftor had no effects on delivery or growth and development of offspring at exposures up to 4 times the MRHD (based on summed AUCs for ivacaftor and its metabolites at maternal oral doses up to 100 mg/kg/day). Decreased fetal body weights were observed at a maternally toxic dose that produced exposures 6 times the MRHD. Placental transfer of ivacaftor was observed in pregnant rats and rabbits.

8.2 LACTATION

Applicant's Proposed Label (from submission of November 29, 2017)

8.2 Lactation

Risk Summary

Both tezacaftor and ivacaftor are excreted into the milk of lactating (b) (4) rats. (b) (4)

Data

Tezacaftor

Lacteal excretion of (b) (4) in rats was demonstrated following a single oral dose (30 mg/kg) of ^{14}C-tezacaftor administered 6 to 10 days postpartum to lactating mothers (dams). Exposure (b) (4) in milk were approximately 3 times higher than in plasma (b) (4)

Ivacaftor

Lacteal excretion of (b) (4) in rats was demonstrated following a single oral dose (100 mg/kg) of ^{14}C-ivacaftor administered 9 to 10 days postpartum to lactating mothers (dams). Exposure (b) (4) in milk were approximately 1.5 times higher than in plasma (b) (4)

Reviewer's Labeling Recommendations and Comments:

Wording was altered to be consistent with previous labels for Kalydeco and Orkambi and to convey the lack of avaialable human information. For rodents, the term "dams" was used in place of mothers.

8.2 Lactation

Risk Summary

There is no information regarding the presence of lumacaftor or ivacaftor in human milk, the effects on the breastfed infant, or the effects on milk production. Both tezacaftor and ivacaftor are excreted into the milk of lactating (b) (4) rats (see Data). (b) (4) -The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for SYMDECO and any potential adverse effects on the breastfed child from SYMDECO or from the underlying maternal condition.

Data

Tezacaftor

Lacteal excretion ~~of~~ (b) (4) tezacaftor in rats was demonstrated following a single oral dose (30 mg/kg) of ^{14}C-tezacaftor administered 6 to 10 days postpartum to lactating ~~mothers (dams.).~~ (b) (4) Exposure of ^{14}C-tezacaftor (b) (4) in milk ~~were~~ was approximately 3 times higher than in plasma (b) (4) (based on AUC_{0-24h}).

Ivacaftor

Lacteal excretion of (b) (4) ivacaftor in rats was demonstrated following a single oral dose (100 mg/kg) of ^{14}C-ivacaftor administered 9 to 10 days postpartum to lactating ~~(mothers~~ dams.~~)~~. Exposure of ^{14}C-ivacaftor (b) (4) milk ~~were~~ was approximately 1.5 times higher than in plasma (based on AUC_{0-24}). (b) (4)

8.4 PEDIATRIC USE

Applicant's Proposed Label (from submission of November 29, 2017)

8.4 Pediatric Use

The safety and efficacy of SYMDEKO in patients with CF younger than 12 years of age have not been studied. (b) (4)

Juvenile Animal Toxicity Data

Findings of cataracts were observed in juvenile rats dosed from postnatal Day 7 through 35 with ivacaftor dose levels of 10 mg/kg/day and higher (0.25 times the MRHD based on systemic exposure of ivacaftor and its metabolites (b) (4)

Reviewer's Labeling Recommendations and Comments:

The clinical team revised the clinical dosing information aspects. For the animal toxicity data, the references to (b) (4) were removed. Since there are already cases of pediatric patients with cataracts and this is listed as a warning and precaution, a reference to that section, [see Warnings and Precautions 5.3], would seem appropriate here, however it was not in the Kalydeco label and therefore not included here. The sentence indicating (b) (4) was removed.

8.4 Pediatric Use

SYMDEKO is indicated for the treatment of CF in pediatric patients age 12-17 years who are homozygous for the *F508del* mutation or who have at least one mutation in the *CFTR* gene that is responsive to tezacaftor/ivacaftor based on in vitro data and/or clinical evidence *[see Clinical Pharmacology (12.1) and Clinical Studies (14)].*

Clinical trials included the following CF patients:

- 12 to 17 years of age who are homozygous for the *F508del* mutation *[see Adverse Reactions (6) and Clinical Studies (14)].*
- 12 to 17 years of age who are heterozygous for the *F508del* mutation and a second mutation predicted to be responsive to tezacaftor/ivacaftor *[see Adverse Reactions (6) and Clinical Studies (14)].*

The safety and efficacy of SYMDEKO in patients with CF younger than 12 years of age have not been studied. (b) (4)

Juvenile Animal Toxicity Data

Findings of cataracts were observed in juvenile rats dosed from postnatal Day 7 through 35 with ivacaftor dose levels of 10 mg/kg/day and higher (0.25 times the MRHD based on systemic exposure of ivacaftor and its metabolites. (b) (4)

12.1 MECHANISM OF ACTION

Applicant's Proposed Label (from submission of November 29, 2017)

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Ivacaftor is a CFTR potentiator that (b) (4) the channel-open probability (or gating) of CFTR at the cell surface (b) (4). For ivacaftor to function CFTR protein must be present at the cell surface. Ivacaftor can potentiate the CFTR protein delivered to the cell surface by tezacaftor, leading to a further enhancement of chloride transport than either agent alone. The combined effect of tezacaftor and ivacaftor is increased quantity and function of CFTR at the cell surface, resulting in increases in chloride transport, (b) (4)

CFTR Chloride Transport Assay in Fisher Rat Thyroid (FRT) cells expressing mutant CFTR

(b) (4) the response of mutant CFTR protein to tezacaftor/ivacaftor, chloride transport was determined in Ussing chamber electrophysiology studies using a panel of FRT cell lines transfected with individual *CFTR* mutations. Tezacaftor/ivacaftor increased chloride transport in FRT cells expressing *CFTR* mutations that result in CFTR protein being delivered to the cell surface.

(b) (4)

(b) (4)

Table 4 lists mutations (b) (4) based on (1) a (b) (4) clinical response and/or (2) *in vitro* data in FRT cells, indicating that tezacaftor/ivacaftor increases chloride transport to at least 10% (b) (4)

Table 4: List of *CFTR* Gene Mutations that Produce CFTR Protein Responsive to SYMDEKO

E56K	*R117C*	*F508del**	*S977F*	*F1074L*	*3849+10kbC→T*
P67L	*E193K*	*D579G*	*F1052V*	*D1152H*	
R74W	*L206W*	*711+3A→G*	*K1060T*	*D1270N*	
D110E	*R352Q*	*E831X*	*A1067T*	*2789+5G→A*	
D110H	*A455E*	*S945L*	*R1070W*	*3272-26A→G*	

*A patient must have two copies of the *F508del* mutation or at least one copy of a responsive mutation presented in Table 4 to be indicated.

Reviewer's Labeling Recommendations and Comments:

This section has been an ongoing collaborative effort of recommendations by the pharmtox, clinical, clinical pharmacology, genetic, and electrophysiology reviewers. The pharmtox recommendations are indicated below. Although (b) (4) is not an acceptable drug class to include in the label due to its promotional implications, a recommended drug class such as conformational stabilizer or conformational modifier were unacceptable to the Applicant in previous discussion for the labeling for Orkambi, specifically, the lumicaftor drug substance. Therefore, no drug class is established for tezacaftor. Wording was altered to reduced redundancy and improve clarity of the mechanism of action. At this time this section of the label is still undergoing review, and the pharmtox reviewer defers to the other disciplines for final wording and figure inclusion.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Tezacaftor- (b) (4) acilitates the cellular processing and trafficking of normal (b) (4) mutant forms of CFTR (including F508del-CFTR) to increase the amount of (b) (4) CFTR protein delivered to the cell surface, (b) (4) . Ivacaftor is a CFTR potentiator that (b) (4) facilitates increased chloride transport by potentiating the channel-open probability (or gating) of the CFTR protein at the cell surface (b) (4) . For ivacaftor to function CFTR protein must be present at the cell surface. In combination, ivacaftor can potentiate the CFTR protein delivered to the cell surface by tezacaftor, leading to a further enhancement of chloride transport than either agent alone. The combined effect of tezacaftor and ivacaftor is increased quantity and function of CFTR at the cell surface, resulting in increases in chloride transport, (b) (4) .

CFTR Chloride Transport Assay in Fisher Rat Thyroid (FRT) cells expressing mutant CFTR

(b) (4) the response of mutant CFTR protein to tezacaftor/ivacaftor, chloride transport was determined in Ussing chamber electrophysiology studies using a panel of FRT cell lines transfected with individual *CFTR* mutations. Tezacaftor/ivacaftor increased chloride transport in FRT cells expressing *CFTR* mutations that result in CFTR protein being delivered to the cell surface.

FRT assay was conducted in Kalydeco responsive mutations. The TEZ/IVA incubation has either comparable or increase chloride transport compared to ivacaftor alone. In vitro data are unable to accurately predict any added clinical

benefit of SYMDECO (tezacaftor/ivacaftor combination) over Kalydeco (ivacaftor) alone.

(b) (4)

Table 4 lists mutations (b) (4) based on (1) a (b) (4) clinical response and/or (2) *in vitro* data in FRT cells, indicating that

tezacaftor/ivacaftor increases chloride transport to at least 10% (b) (4)

Table 4: List of *CFTR* Gene Mutations that Produce CFTR Protein Responsive to SYMDEKO					
E56K	*R117C*	*F508del**	*S977F*	*F1074L*	*3849+10kbC→T*
P67L	*E193K*	*D579G*	*F1052V*	*D1152H*	
R74W	*L206W*	*711+3A→G*	*K1060T*	*D1270N*	
D110E	*R352Q*	*E831X*	*A1067T*	*2789+5G→A*	
D110H	*A455E*	*S945L*	*R1070W*	*3272-26A→G*	
*A patient must have two copies of the *F508del* mutation or at least one copy of a responsive mutation presented in Table 4 to be indicated.					

A revised label was resubmitted on January 2, 2018 that incorporated the concepts if not actual wording of the Division's recommendations that were communicated to the Applicant on Decembern 28, 2017. A few additional simplifications are recommended below. The initial paragraph is acceptable from the pharmtox perspective. As noted before, the pharmtox reviewer defers to the other disciplines for final decisions concerning the figure and table, but provided the following edits for clarification for consideration.

12 CLINICAL PHARMACOLOGY
12.1 Mechanism of Action

Tezacaftor facilitates the cellular processing and trafficking of normal and select mutant forms of CFTR (including F508del-CFTR) to increase the amount of mature CFTR protein delivered 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. For ivacaftor to function CFTR protein must be present at the cell surface. Ivacaftor can potentiate the CFTR protein delivered to the cell surface by tezacaftor, leading to a further enhancement of chloride transport than either agent alone. The combined effect of tezacaftor and ivacaftor is increased quantity and function of CFTR at the cell surface, resulting in increases in chloride transport.

CFTR Chloride Transport Assay in Fisher Rat Thyroid (FRT) cells expressing mutant CFTR

(b) (4) the response of mutant CFTR protein to tezacaftor/ivacaftor, chloride transport was determined in Ussing chamber electrophysiology studies using a panel of FRT cell lines transfected with individual *CFTR* mutations that previously were demonstrated responsive to Kalydeco (ivacaftor alone). Tezacaftor/ivacaftor increased chloride transport in FRT cells expressing *CFTR* mutations (listed in Table 4) that result in CFTR protein being delivered to the cell surface.

(b) (4)

Table 4 lists mutations (b) (4) based on (1) a (b) (4) clinical response and/or (2) *in vitro* data in FRT cells, indicating that tezacaftor/ivacaftor increases chloride transport to at least 10% (b) (4)

Table 4: List of *CFTR* Gene Mutations that ~~Produce CFTR Protein~~ Demostrated Responsiveness to SYMDEKO

E56K	*R117C*	*F508del**	*S977F*	*F1074L*	*3849+10kbC→T*
P67L	*E193K*	*D579G*	*F1052V*	*D1152H*	
R74W	*L206W*	*711+3A→G*	*K1060T*	*D1270N*	
D110E	*R352Q*	*E831X*	*A1067T*	*2789+5G→A*	
D110H	*A455E*	*S945L*	*R1070W*	*3272-26A→G*	
*A patient must have two copies of the *F508del* mutation or at least one copy of a responsive mutation presented in Table 4 to be indicated.					

13.1 CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Applicant's Proposed Label (from submission of November 29, 2017)

13 NONCLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Tezacaftor
A 6-month study in Tg.rasH2 transgenic mice and a 2-year study in rats were conducted to assess carcinogenic potential of tezacaftor. No evidence of tumorigenicity was observed (b) (4) oral doses up to (b) (4) mg/kg/day and 75 mg/kg/day, (b) (4) (approximately 2 times the MRHD (b) (4) based on summed AUCs of tezacaftor and its metabolites (b) (4)

Tezacaftor 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.

(b) (4) 100 mg/kg/day, (b) (4) (approximately 3 times the MRHD based on summed AUC of tezacaftor and M1 metabolite).

Ivacaftor
Two-year studies were conducted in mice and rats to assess carcinogenic potential of ivacaftor. No evidence of tumorigenicity was observed in mice or rats at ivacaftor oral doses up to 200 mg/kg/day and 50 mg/kg/day, respectively (approximately equivalent to 2 to 9 times the MRHD, respectively, based on summed AUCs of ivacaftor and its metabolites (b) (4)).

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 9 and 6 times, respectively, 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 6 times the MRHD based on summed AUCs of ivacaftor and its metabolites) 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 6 and 4 times the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites).

Reviewer's Labeling Recommendations and Comments:

The label was altered to indicate that studies were not conducted with the combination drugs, tezacaftor and ivacaftor, but were conducted with the individual drugs in separate studies. Other changes were to present the carcinogenicity studies and fertility studies in a manner consistent for both drugs and consistent with approved ivacaftor products. The reviewer prefers to group the 3 labeling topics of this section by topic then drug rather than separated by drugs then topic, since prescribers are usually looking for information by topic rather than by drug. This is not how the PharmTox community usually handles combination products, so this was not incorporated in this label revision, but it may become necessary as multiple drugs comprise a combination product.

An introductory sentence was included that indicated there were no studies of carcinogenicity, mutagenesis, or impairment of fertility were conducted with the combination of tezacaftor and ivacaftor. Although usually the drug Brand name is used here, in this case the use of Symdeko would be misleading because Symdeko refers to a drug product that includes not only the combination tablet (tezacaftor and ivacaftor), but also a tablet of only ivacaftor.

The exposure margins for the transgenic mouse carcinogenicity study was removed. The impairment of fertility section was simplified to eliminate listings of negative findings. The ivacaftor section was minimally altered to enhance clarity from previous labels.

13 NONCLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with the combination of tezacaftor and ivacaftor, however, separate studies for tezacaftor and for ivacaftor, are described below.

Tezacaftor

A ~~6-month study in Tg.rasH2 transgenic mice and a~~ 2-year study in Sprague-Dawley rats and a 6-month study in Tg.rasH2 transgenic mice were conducted to assess the carcinogenic potential of tezacaftor. No evidence of tumorigenicity from tezacaftor was observed in male and female rats [redacted] oral doses up to [redacted] ~~mg/kg/day~~ and 75 mg/kg/day (approximately 2 and 3 times the MRHD based on summed AUCs of tezacaftor and its metabolites in males and females, respectively) No evidence of tumorigenicity was observed in male and female Tg.rasH2 transgenic mice at tezacaftor doses up to 500 mg/kg/day~~(approximately 2 times the MRHD [redacted], based on summed AUCs of tezacaftor and its metabolites~~ [redacted].

Tezacaftor 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.

There were no effects on male or female [redacted] fertility and early embryonic development in rats at oral tezacaftor doses up to [redacted] -100 mg/kg/day~~,~~ [redacted] (approximately 3 times the MRHD based on summed AUC of tezacaftor and M1 metabolite).

Ivacaftor

Two-year studies were conducted in CD-1 mice and Sprague-Dawley rats to assess the carcinogenic potential of ivacaftor. No evidence of tumorigenicity from ivacaftor was observed in mice or rats at ~~ivacaftor~~ oral doses up to 200 mg/kg/day and 50 mg/kg/day, respectively (approximately equivalent to 2 to 9 times the MRHD, respectively, based on summed AUCs of ivacaftor and its metabolites [redacted]).

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 9 and 6 times, respectively, 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 6 times the MRHD based on summed AUCs of ivacaftor and its metabolites) 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 6 and 4 times the MRHD (b) (4) based on summed AUCs of ivacaftor and its metabolites).

2 Drug Information

2.1 Drug 1

CAS Registry Number: 1152311-62-0

Generic Name: Tezacaftor

Code Name: VX-661, VRT-893661, VRT-0893661

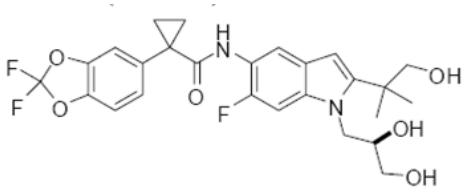
Chemical Name

(R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

Molecular Formula/Molecular Weight

$C_{26}H_{27}F_3N_2O_6$ (free form) / 520.5 Da (free form)

Structure or Biochemical Description



Pharmacologic Class: A pharmaceutical class was not established.

[Note: The Applicant proposed the term (b) (4) However, this term was not considered acceptable, based on discussions of a similarly acting compound, lumacaftor, which is approved as the combination drug Orkambi (ivacaftor and lumacaftor). Considering discussion surrounding class establishment for lumacaftor, no further discussions of pharmaceutical class were undertaken for tezacaftor.]

2.1 Drug 2

CAS Registry Number: 873054-44-5

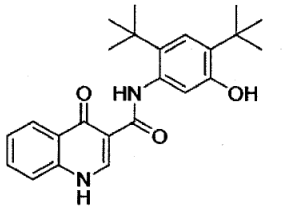
Generic Name: Ivacaftor

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

Structure or Biochemical Description



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

The following code names were commonly used in the submitted study reports:

Related to Ivacaftor (IVA)

VRT-813077, VX-770	early names for ivacaftor
VRT-837018	M1-IVA (also known as hydroxymethyl-ivacaftor)
VRT-842917	M6-IVA (also known as ivacaftor carboxylate)

Related to Tezacaftor (TEZ)

VRT-893661, VX-661	early name for tezacaftor
VRT-0996107	M1-TEZ (also known as dehydrogenation metabolite of tezacaftor)
VRT-1189001	M2-TEZ (also known as tezacaftor carboxylate)
VRT-1074233	M5-TEZ (also known as phosphate conjugate of M2-TEZ)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 74,633 for ivacaftor (Vertex Pharmaceuticals, Inc.)
IND 108,105 for tezacaftor (Vertex Pharmaceuticals, Inc.)
NDA 203188, Kalydeco (ivacaftor), Vertex, approved January 31, 2012
NDA 206038, Orkambi (ivacaftor and lumacaftor), Vertex, approved July 2, 2015

2.3 Drug Formulation

Symdeko consists of a copackaged tablet of the fixed dose combination (FDC) tezacaftor (100 mg)/ivacaftor (150 mg) and a separate ivacaftor tablet (150 mg). The 150 mg ivacaftor tablets are the same as the approved Kalydeco product (NDA203188). The fixed dose combination tablet is formulated as an immediate-release film coated tablet for oral administration. The composition of the tezacaftor/ivacaftor FDC tablet, which consists of the core tablet and film coat, is provided in Table 1.

2.4 Comments on Novel Excipients

There are no novel excipients in the tezacaftor/ivacaftor FDC tablets.

2.5 Comments on Impurities/Degradants of Concern

The impurities due to tezacaftor are at levels that meet ICH specifications or have been qualified in nonclinical toxicology studies. Refer to the separate PharmTox review under NDA 210491.

Table 1: Composition of Tezacaftor/Ivacaftor Fixed Dose Combination Tablet

Component	Quality Standard	Component Function	Amount per tablet (mg)	Content (% w/w)
Core Tablet				
(b) (4)				
Ivacaftor (b) (4)				
Ivacaftor drug substance	Internal standard	Active ingredient	150.0	24.61
(b) (4)				
Tezacaftor (b) (4)				
Tezacaftor drug substance	Internal standard	Active ingredient	100.0	16.40
(b) (4)				
Core Tablet Total			(b) (4)	
Film Coat				
(b) (4)				
Total Tablet Weight			**609.6**	**100**
(b) (4)				

2.6 Proposed Clinical Population and Dosing Regimen

Symdeko is indicated for the treatment of patients with cystic fibrosis aged 12 years and older who are homozygous for the *F508del* mutation or who have at least one mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that is responsive to tezacaftor/ivacaftor based on *in vitro* data and/or clinical evidence. The Applicant proposes if the patient's genotype is unknown, an FDA-cleared cystic fibrosis mutation test should be used to detect the presence of a *CFTR* mutation.

The recommended oral dose is one tablet (tezacaftor 100 mg/ivacaftor 150 mg) taken in the morning and one tablet (ivacaftor 150 mg) taken in the evening, approximately 12 hours apart. Tablets should be taken with fat-containing food. There are recommendations for dose adjustments for patients with moderate and severe hepatic impairment and if co-administered with drugs that are moderate or strong CYP3A inhibitors.

2.7 Regulatory Background

VX-661 was developed under IND 108,105, which was submitted on April 15, 2010. There was no PreIND meeting. Fast Track designation was granted on May 21, 2010.

The overall goal of the VX-661 development program was to investigate the potential of VX-661 as a treatment for CF in adults and children. The clinical program was designed to establish proof-of-mechanism in patients with the *F508del* mutation, followed by larger studies of longer duration to evaluate safety and efficacy data that would support marketing approval.

An initial nonclinical safety review was completed on June 21, 2010. The proposed clinical protocol was considered safe to proceed up to an oral dose of 200 mg due to findings of dilated lymphatics in the gastrointestinal tract villi of unidentified cause and unknown toxicological significance. The systemic exposure was limited to an AUC_{0-24} of 133 µg-h/mL in repeated dosing clinical studies based on these findings in the gastrointestinal tract villi.

Besides the standard nonclinical toxicity studies, specific toxicology studies were conducted during drug development to address the safety and recovery of dilated lymphatics in the gastrointestinal tract villi. The safety of metabolites that were disproportionately elevated in humans compared to the levels in toxicity study animals was also assessed during development. Nonclinical combination studies of VX-661 with VX-770 were also conducted to establish safety and allow clinical studies with the combination drug to proceed.

3 Studies Submitted

3.1 Studies Reviewed

Study Number	Study Title	IND 108105 or NDA Review Date
Pharmacology		
Primary Pharmacology		
D153	Validation of Primary Human Bronchial Epithelia Cultures to Evaluate the Pharmacological Action of CFTR Modulators	June 21 2010
G016	Effects of VRT-893661 on CFTR-Mediated Chloride Secretion in Human Bronchial Epithelia Isolated From Cystic Fibrosis Subjects	June 21 2010
G017	Effects of VRT-893661 on the Trafficking of Mutant P-Glycoprotein and Mutant hERG	June 21 2010
Metabolite M1		
G082	Activity of the VX-661 metabolite M1 (VRT-0996107) in cultured F508del-HBE	June 21 2010
M077	Potency and efficacy assessment of VX-661 and M1 (VRT-0996107) in combination with continuous ivacaftor on chloride transport in bronchial epithelial cells isolated from CF donors homozygous for the *F508del-CFTR* mutation	July 28, 2016
Metabolite M2		
I287	Activity of the VX-661 metabolite M2 (VRT-1189001) in cultured F508del-HBE	July 28, 2016
Metabolite M5		
K300	Activity of VX-661 Metabolite M5 (VRT-1074233) in Cultured F508del-HBE	March 14, 2017
Combination VX661 and VX770		
L204	Effect of VX-661 and ivacaftor on airway surface liquid height and cilia beat frequency in HBE cells isolated from people who are homozygous for the *F508del-CFTR* mutation	Current Review
L205	Effect of VX-661 and ivacaftor on chloride transport in bronchial epithelial cells isolated from CF donors homozygous or heterozygous for the *F508del-CFTR* mutation	Current Review
M078	Assessment of the Combination of **TEZ/IVA Compared to TEZ or IVA** Alone Across CFTR Residual Function Mutations Expressed in Fischer Rat Thyroid Cells: Effects on Processing and Trafficking and Chloride Transport	Current Review
N035	Pharmacological profile of **tezacaftor** and **ivacaftor** in *F508del* homozygous human bronchial epithelial cells	Current Review
N092	Assessment of the C**ombination of TEZ/IVA**	Current Review

	Compared to TEZ or IVA Alone in Fischer Rat Thyroid Cells Expressing CFTR Mutations That Have Been Clinically Proven to be IVA Responsive: Effects on Processing and Trafficking and Chloride Transport	
VRT-0842917-TX-001	FASTPatch® Assay: Effects of One Test Article on Ion Channels Expressed in Mammalian Cells	Current Review
Secondary Pharmacology		
7270-1	Lead Profiling Screen	June 21 2010
7695-1	Spectrum Screen	June 21 2010
G018 VRT-893661-TX-015	Effects of VRT-893661 on Voltage-Gated Sodium Channels	June 21 2010
Safety Pharmacology		
VRT-893661-TX-013	VRT-893661: Oral (gavage) dose neurobehavioral safety pharmacology study in male rats	June 21 2010
VRT-893661-TX-006	Effect of VRT-893661 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	June 21 2010
VRT-893661-TX-009	VRT-893661: Oral (gavag) dose latin square cardiovascular safety pharmacology study in male beagle dogs	June 21 2010
VRT-893661-TX-012	VRT-893661: Oral (gavage) dose respiratory safety pharmacology study in male rats	June 21 2010
VRT-893661-TX-014	Assessment of the Effects of VRT-893661 at 30, 100, or 200 mg/kg in a Four Day Oral (Gavage, Once Daily) Dose Gastric Emptying and Intestinal Transit Study in Male Sprague-Dawley Rats	June 21 2010
ADME		
Method Validation		
F148	Full Validation of Bioanalytical Procedure for the Quantitative Determination of VRT-893661 with D_4-^{13}C-VRT-893661 as an Internal Standard in Rat Plasma	June 21 2010
F151	Full Validation of Analytical Procedure for the Quantitative Determination of VRT-893661 with D_4-^{13}C-VRT -VRT-893661 as Internal Standard in Mouse Plasma	June 21 2010
F157	Full Validation of Analytical Procedure for the Quantitative Determination of VRT-893661 with D_4-^{13}C-VRT -VRT-893661 as Internal Standard in Dog Plasma	June 21 2010
863-105	Captisol in Dulbecco's Phosphate Buffered Saline Full Validation of an HPLC-UV Assay for VRT-1189001 in 20%	July 28, 2016
Absorption		
G015	Pharmacokinetics of VRT-893661 in Male Cynomolgus Monkeys following Single Intravenous Administration	June 21 2010
G029	Pharmacokinetics of VRT-893661 in Male CD-1 Mice Following Intravenous Administration	June 21 2010
G030	Pharmacokinetics of VRT-893661 Following Intravenous and Oral Administration in Male Sprague Dawley Rats	June 21 2010
G031	Pharmacokinetics of VRT-893661 Following Intravenous and Oral Administration in Male Beagle Dogs	June 21 2010

Metabolite M1 and M2		
I248	Pharmacokinetics of VRT-1189001 Following Intravenous, Oral, and Subcutaneous Administration to Male Sprague Dawley Rats	July 28, 2016
Metabolites M2-VX661		
I241	Pharmacokinetics of VRT-1189001 Following Intravenous, Oral, and Subcutaneous Administration in Male Beagle Dogs	July 28, 2016
I260	Bi-directional permeability of VX-661, VRT-0996107, and VRT-1189001 across Caco-2 cell monolayer	July 28, 2016
Distribution		
In vivo		
G032	Kinetic Tissue Distribution of VRT–893661 in Male Sprague Dawley Rats Following Single Oral Administration	June 21 2010
VX-661-DMPK-DM-007	Pharmacokinetics and Tissue Distribution of ^{14}C-VX-661 Following Oral Administration to Rats	June 21 2010
H278	Kinetic Tissue Distribution of VX-661 in Male Sprague Dawley Rats Following Multiple Oral Administration of 30 mg/kg VX-661 Once Daily for 7 Days	June 21 2010
Metabolite M1		
H276	Kinetic Tissue Distribution of VX-661 and its Metabolite, VRT-0996107 in Male Sprague Dawley Rats Following Single Oral Administration of 30 mg/kg VX-661	July 28, 2016
Binding to plasma proteins		
G085	In Vitro Binding of VRT-893661 to Plasma Proteins in Mouse, Rat, Dog, Monkey, and Human Plasma	June 21 2010
Metabolite M1 and others		
I220	In Vitro Binding of VRT-1189001 and VRT-0996107 to Plasma Proteins	July 28, 2016
M108 supercedes Report K303	In Vitro Binding of VX-661 and Its Metabolites to Plasma Proteins	July 28, 2016
Metabolism		
In vivo characterization		
G049	Disposition of [14C] VX-661 Following Oral Administration to Intact and Bile Duct-Cannulated Male Sprague-Dawley Rats	December 30, 2011
G090	The Effect of VRT-8936661 on Hepatic Microsomal Levels of Total Cytochrome P450 and Selected Enzyme activites in Male and Female Sprague-Dawley Rats Following Single Oral Administration at Doses of 30, 200 and 600 mg/kg/per day for 7 days	June 21 2010
G025	Characterization of VRT-893661 Metabolites from In Vitro and In Vivo Matrices	June 21 2010
L246	Metabolite Profile and Identification of Metabolites in Plasma, Urine, Bile,and Feces Samples Obtained from Male Sprague Dawley Rats After Oral (PO) and Intravenous (IV)Administration of VX-661 and [^{14}C]VX-661	June 21 2010
L218	Metabolite Profile and Identification of Metabolites in Plasma, Urine, Bile, and Feces Samples Obtained from Male Beagle Dogs After Oral and Intravenous	June 21 2010

	Administration of VX-661&[14C]VX-661	
CYP Enzymes		
G026	Assessment of VRT-893661 Reversible and Time Dependent Inhibition Potential of Human Cytochrome P450 Isozymes	June 21 2010
G027	In Vitro Metabolic Clearance of VRT-893661 in Cryopreserved Hepatocytes from Rat, Dog, Monkey, and Human	June 21 2010
G028	The *In Vitro* Stability of VRT-893661 in Human Recombinant CYP Isozymes	June 21 2010
G035	Evaluation of Induction Potential of Cytochrome P450 Isoforms by VRT-893661 in Cultured Human Hepatocytes	June 21 2010
Metabolite M1		
H033	Assessment of VRT-0996107 Reversible Inhibition Potential of Human Cytochrome P450 Isozymes	December 30, 2011
H076	The *In Vitro* Stability of VRT-0996107 in Human Recombinant CYP Isozymes and Liver Cytosol	December 30, 2011
H108	Evaluation of Induction of Cytochrome P450 Enzymes Following In Vitro Exposure of Human Hepatocytes to VRT-0996107	December 30, 2011
VX-809 and VX661		
VX-661-DMPK-DM-003 /.VX-809-DMPK-DM-028	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	December 30, 2011
CYP enzymes and M1		
K312	IN VITRO INHIBITION OF HUMAN CYP2B6 AND CYP2C8 ENZYMES BY VRT-0893661 AND ITS METABOLITE VRT-0996107	Current review
CYP enzymes and M2		
I229	In Vitro Inhibition of Human Cytochrome P450 Enzymes by VRT-1189001	Current review
I244	Induction of Cytochrome P450 Enzyme, CYP3A4 Following In Vitro Exposure of Human Hepatocytes to VRT-1189001	Current review
P-glycoprotein		
I265	In Vitro Assessment of VX-661 as a Substrate of P-glycoprotein	Current review
Pgp and metabolites		
I264	In Vitro Assessment of VRT-0996107 as a Substrate of P-glycoprotein	Current review
I307	In Vitro Assessment of VRT-1189001 as a Substrate of P-glycoprotein	Current review
K311	In Vitro Assessment of VX-661 and Metabolites as inhibitors of P-glycoprotein	Current review
Transporters and metabolites		
K304	In Vitro Assessment of VX-661, VRT-0996107 (M1), VRT-1189001 (M2) and VRT-1074233 (M5) as inhibitors of OATP1B1	Current review
OPT-2014-114	Assessment of VERTEX COMPOUNDS AS INHIBITORS OF HUMAN BCRP, OAT1, OAT3 AND OCT2 MEDIATED TRANSPORT	Current review

OPT-2014-138	Assessment of VRT-0893661 AS AN INHIBITOR OF HUMAN OAT3 MEDIATED TRANSPORT	Current review
OPT-2014-145	Assessment of VRT-0893661 AS A SUBSTRATE OF HUMAN BCRP MEDIATED TRANSPORT	Current review
Metabolite M5-661 Studies		
VX13-661-005	A Phase 1, Open-Label, Mass Balance Study to Investigate the Absorption, Metabolism, and Excretion of 14C-VX-661 Following Single Oral Administration to Healthy Male Subjects	March 14, 2017
K247	Metabolite Profile and Identification of Metabolites in Plasma, Urine, and Feces Samples Obtained from Healthy Male Human Subjects after Single Oral Administration of [14C]VX-661	March 14, 2017
To support mouse carcinogenicity dose selection		
J158	Pharmacokinetics of VX-661, VRT-0996107 and VRT-1189001 in Male CD-1 Mice Administered VX-661 500, 750, 1000 or 1500 mg/kg/day Once Daily via Oral (Gavage) for 5 Days	November 20, 2014
J165	Pharmacokinetics of VX-661, VRT-0996107 and VRT-1189101 in Male CD-1 Mice Following Single Oral (Gavage) Administration of 1500 and 3000 mg/kg Doses of VX-661	November 20, 2014
K040	Evaluation of VX-661 as an Inducer of Cyp1a2, Cyp2b10, Cyp3a11, Cyp3a13, Cyp4a10 and Cyp4b1 using Mouse Liver Homogenates from rasH2 mice treated with VX-661 for 5 Days	November 20, 2014
K181	The In Vitro Formation of VX-661 M1 metabolite, VRT-0996107, in Aroclor Induced Rat Liver S9;	November 20, 2014
K219	Pharmacokinetics of VX-661, VRT-0996107 and VRT-1189001 in Male ICR (CD-1) Mice Following Single Oral Administration of 2000 mg/kg Dose of VX-661	November 20, 2014
K230	Evaluation of VX-661 as an Inducer of Cyp1a2, Cyp2b10, Cyp3a11, Cyp3a13, Cyp4a10 and Cyp4b1 using Mouse Liver Homogenates from CD-1 mice treated with VX-661 for 5 Days	November 20, 2014
K234	Pharmacokinetics of VX-661, VRT-0996107 and VRT-1189001 in Male Golden Syrian Hamsters Following Single Oral Administration of 100 mg/kg Dose of VX-661	November 20, 2014
Pharmacokinetics		
H022	Pharmacokinetics of VRT-0996107 Following Single Intravenous or Single Oral Administration to Male Sprague Dawley Rats	June 21, 2010
Metabolite M2-661 Studies		
I247	Pharmacokinetics of VRT-1189001 Following Intravenous Administration to Male CD-1 Mice	July 28, 2016
K320	Pharmacokinetics of VRT-1189001 in Male CD-1 Mice Following a Single Intravenous Dose of 5 mg/kg or a Single Oral Dose of 100 mg/kg	July 28, 2016
K324	Pharmacokinetics of VRT-1189001 in Male Sprague-Dawley Rats Following a Single Intravenous Dose of 1 mg/kg or a Single Oral Dose of 3 mg/kg	July 28, 2016
I241	Pharmacokinetics of VRT-1189001 Following	July 28, 2016

	Intravenous, Oral, and Subcutaneous Administration in Male Beagle Dogs	
Repeated Dose Toxicology		
RAT		
VRT-893661-TX-004	VRT-893661: A 7-day oral range-finding toxicity and toxicokinetic study in rats	June 21, 2010
VRT-893661-TX-010	VRT-893661: A 4-week oral (gavage) toxicity study in rats with a 2 week recovery period	June 21, 2010
VX-661-TX-001	VX-661: A 4-week oral (gavage) toxicity study in rats	
VX-661-TX-010	VX-661: A 3-month oral (gavage) toxicity and toxicokinetic study in rats with a 1-month recovery period	September 1 2017
I122 VX-661-TX-010	Toxicokinetics of VRT-1189001 in Male and Female Sprague Dawley Rats Following Daily Administration of VX-661 via Oral Gavage for 3 Months with a 1-Month Recovery Period	September 1 2017
VX-661-TX-012	VX-661: A 26-Week Oral (Gavage) Toxicity and Toxicokinetic Study in Rats with a 4-Week Recovery Period	September 1 2017
DOG		
VRT-893661-TX-005	VRT-893661: A 7-day oral range-finding toxicity and toxicokinetic study in dogs	June 21 2010
VRT-893661-TX-011	VRT-893661: A 4-week oral toxicity study in dogs with a 2-week recovery period	June 21 2010
VX-661-TX-002	VX-661: A 4-Week oral (Gavage) Toxicity Study in Dogs with a 4-Week Recovery Period	
VX-661-TX-011	VX-661: A 3-month oral (gavage) toxicity and toxicokinetic study in dogs with a 1-month recovery period	September 1 2017
I123 VX-661-TX-011	Toxicokinetics of VRT-1189001 in Male and Female Beagle Dogs Following Daily Administration of VX-661 via Oral Gavage for 3 Months with a 1-Month Recovery Period	September 1 2017
VX-661-TX-015	VX-661: A 28-Day Oral (Gavage) Fed vs. Fasted Investigative and Toxicokinetics Study in Beagle Dogs with a 91-Day Recovery Period	September 1 2017
VX-661-TX-022	VX-661: A 28 Day Oral Toxicity and Toxicokinetics Study in Dogs Followed by 3, 6, 9, and 12 Month Recovery Periods	September 1 2017
VX-661-TX-016	VX-661: A 13-Week Oral (Tablet) Investigative and Toxicokinetic Study in Beagle Dogs (Date of Study Completion 12 September 2013)	September 1 2017
VX-661-TX-018	VX-661: A 26-Week Oral (Gavage) Toxicity Study in Dogs	September 1 2017
VX-661-TX-013	VX-661: A 52-Week Oral (Gavage) Toxicity Study in Dogs	September 1 2017
Combination Drug VX-661 and VX-770		
Rat		
VX-661-TX-004, VX-770-TX-023	VX-661/VX-770: A 4-Week Oral Gavage **Combination** Toxicity Study in Rats with a 2-Week Recovery Period	December 30, 2011
H069 VX-661-TX-004 / VX-770-TX-023	Quantitative Determinations of Concentrations of VX-661, VRT-0996107, VX-770, VRT-837018 and VRT-842917 in Plasma Samples Collected from a 4-Week Oral (Gavage) VX-661 and VX-770 Combination	December 30, 2011

	Toxicity Study in Rats with a 2-Week Recovery Period (May 19 2011 report)	
H065	Quantitative Determination of Concentration of VX-661, VRT-0996107, VX-770, VRT-837018 and VRT-842917 in Plasma Samples Collected from a 4-Week Oral (Gavage) VX-661 and VX-770 Combination Toxicity Study in Dogs with a 2-Week Recovery Period	December 30, 2011
H066 VX-661-TX-004 / VX-770-TX-023	Toxicokinetics of VX-661 and VX-770 and their metabolites in Male and Female Rats Following Once Daily Administration Via Oral Gavage for 28 Consecutive Days	December 30, 2011
VX-661-TX-014	VX-661/ Ivacaftor (VX-770): A 13-Week Gavage Combination Toxicity Study in Rats with a 4-Week Recovery Period	September 1 2017
Dog		
VX-661-TX-003 / VX-770-TX-022	VX-661/VX-770: A 4-Week Oral Gavage Combination Toxicity Study in Dogs with a 2-Week Recovery Period	December 30, 2011
H077 VX-661-TX-003 / VX-770-TX-022	Toxicokinetics of VX-661 and VX-770 and Their Metabolites in Male and Female Dogs Following Once Daily Administration Via Oral Gavage for 28 Consecutive Days	December 30, 2011
H065 VX-661-TX-003 / VX-770-TX-022	Quantitative Determination of Concentration of VX-661, VRT-0996107, VX-770, VRT-837018 and VRT-842917 in Plasma Samples Collected from a 4-Week Oral (Gavage) VX-661 and VX-770 Combination Toxicity Study in Dogs with a 2-Week Recovery Period	December 30, 2011
Metabolite M2-661		
VRT-1189001-TX-001	VRT-1189001: A 28-Day Subcutaneous Toxicity and Toxicokinetics Study in Rats with a 14-Day Recovery Period	July 28, 2016
VRT-1189001-TX-004	VRT-1189001: A 28-Day Subcutaneous Toxicity and Toxicokinetic Study in Beagle Dogs with a 14-Day Recovery Period	July 28, 2016
Genetic Toxicology		
VRT-893661-TX-007	Bacterial Reverse Mutation Assay	June 21 2010
VRT-893661-TX-008	*In Vitro* Mammalian Chromosome Aberration Test	June 21 2010
VRT-893661-TX-003	VRT-893661: Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration	June 21 2010
Metabolite M2		
VRT-1189001-TX-002	Bacterial Reverse Mutation Assay	July 28, 2016
VRT-1189001-TX-003	*In Vitro* Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL)	July 28, 2016
Carcinogenicity		
Rat 2-year		
VX-661-TX-020	A 104-Week Oral (Gavage) Carcinogenicity Study in Rats	December 6, 2017 (statistical review November 22, 2017)
VX-661-TX-010	VX-661: A 3-month oral (gavage) toxicity and toxicokinetic study in rats with a 1-month recovery period	September 1 2017
VX-661-TX-012	VX-661: A 26-Week Oral (Gavage) Toxicity and Toxicokinetic Study in Rats with a 4-Week Recovery	September 1 2017

	Period	
Tg.rasH2 mouse, 6-month		
VX-661-TX-019	VX-661: 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice	December 6, 2017 (statistical review November 22, 2017)
VX-661-SDPK303292	VX-661: 5-Day Repeated Dose Toxicokinetic Study in CByB6F1 Mice	September 1 2017
J158	Pharmacokinetics of VX-661, VRT-0996107 and VRT-1189001 in Male CD-1 Mice Administered VX-661 500, 750, 1000 or 1500 mg/kg/day Once Daily via Oral (Gavage) for 5 Days	September 1 2017
J165	Pharmacokinetics of VX-661, VRT-0996107 and VRT-1189101 in Male CD-1 Mice Following Single Oral (Gavage) Administration of 1500 and 3000 mg/kg Doses of VX-661	September 1 2017
VX-661-TX-017	VX-661: 28-Day Repeated Dose Oral Toxicity and Toxicokinetic Study in CByB6F1 Mice with a Preliminary 5-Day Range-Finding Toxicity Study	September 1 2017
Reproductive and Developmental Toxicology		
Fertility and Early Embryonic Development: Rat		
VX-661-TX-021	VX-661: Study Of Fertility And Early Embryonic Development To Implantation In Rats	Current review
Embryofetal Development		
Rat		
VX-661-TX-006	VX-661: An Oral (Gavage) Dose Range-Finding Developmental Toxicity Study in Rats	Current review
VX-661-TX-008	VX-661: An Oral (Gavage) Embryo/Fetal Development Study in Rats	Current review
H087	Quantitative Determination of Concentration of VX-661 and VRT-0996107 in Plasma Samples Collected from an Oral Embryo/Fetal Developmental Toxicity Study of VX-661 in Sprague-Dawley Rats	Current review
H110	Toxicokinetics of VX-661 and VRT-0996107 in Presumed-Pregnant Female Sprague-Dawley Rats Following Daily Administration via Oral Gavage in an Embryo/Fetal Developmental Toxicity Study	Current review
Rabbit		
VX-661-TX-005	VX-661: Maximum Tolerated Dosage (Gavage) Toxicity and Toxicokinetic Study in Female Rabbits	Current review
VX-661-TX-007	VX-661: An Oral (Gavage) Dose Range-Finding Developmental Toxicity Study in Rabbits	Current review
VX-661-TX-009	VX-661: An Oral (Gavage) Embryo/Fetal Development Study in Rabbits	Current review
H088	Quantitative Determination of Concentration of VX-661 and VRT-0996107 in Plasma Samples Collected from an Oral Embryo/Fetal Developmental Toxicity Study of VX-661 in New Zealand White Rabbits	Current review
H111	Toxicokinetics of VX-661 and VRT-0996107 in Presumed-Pregnant Female New Zealand White Rabbits Following Daily Administration via Oral Gavage in Embryo/Fetal Developmental Toxicity Study	Current review
Metabolite M2		

VRT-1189001-TX-005	VRT-1189001: A 5-Day Subcutaneous Tolerability and Toxicokinetic Study in Non-Gestating Female Rabbits ((b) (4) Study 863-172). Report date: 04 Sep 2014.	Current review
VRT-1189001-TX-006	VRT-1189001: A Subcutaneous Embryo-Fetal Development Study in Rabbits with a Toxicokinetic Evaluation	Current review
Pre-Postnatal Development: Rat		
VX-661-TX-023	VX-661: An Oral (Gavage) Pre- and Postnatal Development Study, Including Maternal Function in Rats	Current Review
SDPK303766	Placental Transfer and Lacteal Excretion of 14C-VX-661 Following Administration of a Single Oral Dose to Pregnant and Lactating Rats	Current Review

3.2 Studies Not Reviewed

The following additional studies were reviewed in a second Pharmacology-Toxicology review for NDA 210491 for the qualification of impurities and setting of appropriate level specifications. Studies of VX-770 and its related impurities that were included in this NDA submission were reviewed previously for the approvals of Kalydeco and Orkambi.

Phamracology/Pharmacokinetics	
Combination VX-809	
K124	VX-809 Directly and specifically interacts with MSD1 domain of CFTR
Toxicology	
VX-661-TX-059 Module 4.2.3.7.7-other	*In Silico* Analysis of Process Intermediates and Impurities in the Synthesis of VX-661 for Mutagenic Potential
	(b) (4)
(b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
	(b) (4)
(b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
(b) (4) Module 4.2.3.7.7-other	(b) (4): In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes
	(b) (4)
(b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
(b) (4) Module 4.2.3.7.7-other	(b) (4): In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes
	(b) (4)
(b) (4) Module 4.2.3.7.6-imp	(b) (4): Bacterial Reverse Mutation Test
(b) (4) Amended Final Report Module 4.2.3.7.7-other	(b) (4): In Vitro Mammalian Chromosome Aberration Test In Human Lymphocytes
VX-661 Local Tolerance	
VX-661-TX-024	VX-661: EpiSkin™ Skin Irritation Test 15 min-42 hours

SD-185, Sept 1 2016	
VX-661-TX-025 SD-185, Sept 1 2016	VX-661: The Bovine Corneal Opacity and Permeability Assay (BCOP)
VX-661 Antigenicity	
VX-661-TX-026 SD-185, Sept 1 2016	VX-661: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
VX-661 Other Toxicology	
VRT-893661-TX-001 SD-185, Sept 1 2016	In Vitro Toxicity Screening of Vertex Compounds in a Rat Hepatoma (H4IIE) Cell Line: 24 Hour Exposure
VX-661-TX-028 SD-185, Sept 1 2016	VX-661: Partition Coefficient
VX-661 Impurity Toxicology	
(b) (4) Module 4.2.3.7.7-other	(b) (4): Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
(b) (4) Module 4.2.3.7.7-other	(b) (4): EpiSkin™ Skin Irritation Test
(b) (4) Module 4.2.3.7.7-other	(b) (4): The Bovine Corneal Opacity and Permeability Assay (BCOP)
(b) (4) Module 4.2.3.7.7-other	(b) (4): Acute Oral Toxicity to the Rat (Acute Toxic Class Method)
(b) (4) Module 4.2.3.7.7-other	(b) (4): Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
(b) (4) Module 4.2.3.7.7-other	(b) (4): EpiSkin™ Skin Irritation Test
(b) (4) Module 4.2.3.7.7-other	(b) (4): The Bovine Corneal Opacity and Permeability Assay (BCOP)
(b) (4) Module 4.2.3.7.7-other	(b) (4): Acute Oral Toxicity to the Rat (Acute Toxic Class Method)
(b) (4) Module 4.2.3.7.7-other	(b) (4): Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Pooled treatment group approach)
(b) (4) Module 4.2.3.7.7-other	(b) (4): In Vitro Skin Corrosivity Test Using the EpiDerm™ Human Skin Model
(b) (4) Module 4.2.3.7.7-other	(b) (4): The Bovine Corneal Opacity and Permeability Assay (BCOP)
(b) (4) Module 4.2.3.7.7-other	(b) (4): Acute Oral Toxicity to the Rat (Acute Toxic Class Method)
(b) (4) Module 4.2.3.7.7-other	(b) (4): EpiSkin™ Skin Irritation Test
(b) (4) Module 4.2.3.7.7-other	(b) (4): Acute Eye Irritation in the Rabbit

3.3 Previous Reviews Referenced

NDA 203188, Kalydeco (ivacaftor), Vertex, approved January 31, 2012
NDA 206038, Orkambi (ivacaftor and lumacaftor), Vertex, approved July 2, 2015

NDA 210491, PharmTox review dated December 6, 2017, review of carcinogenicity studies

IND 108105 nonclinical reviews for tezacaftor (VX-661) include the following:

- May 14, 2010, initial safety review with limit of highest clinical dose
- June 21, 2010, full review of initial submission
- November 5, 2010, support for increasing the clinical dose
- December 19, 2010, support for women in clinical studies
- December 30, 2011, support for 28-day VX661/VX770 combination study
- January 10, 2013, lack of adequate M2 metabolite safety from previous studies
- December 18, 2013, Rat Carcinogenicity SPA, ECAC Recommendations
- November 20, 2014, Mouse Carcinogenicity SPA, ECAC Recommendations
- October 16, 2015, advice on terminating rat carcinogenicity study groups
- July 28, 2016, review of metabolites M2 and M5
- March 14, 2017, review of metabolite M5
- September 1, 2017, review of long-term toxicity studies, and carcinogenicity dosing

4 Pharmacology

4.1 Primary Pharmacology

A detailed pharmacology review was completed on June 21, 2010.

Briefly, tezacaftor (TEZ or VX-661) is a small molecule drug originally developed for the treatment of cystic fibrosis (CF) in patients with the *F508del* mutation in the CF transmembrane conductance regulator gene (CFTR), but this indication was expanded to other mutations that demonstrated positive clinical responses. These mutations result in the absence or deficient function of CFTR protein, an epithelial chloride channel, at the cell surface. In pharmacodynamic studies, TEZ showed efficacy in altering the tertiary *F508del* CF protein structure and enabling its transport to the cell membrane. In January of 2012, the sponsor received approval for ivacaftor (IVA or VX-770, Kalydeco, NDA 203188), a CFTR potentiator that functions to keep the chloride channel open in patients with the *G551D* mutation in the *CFTR* gene. The sponsor proposes therapeutic use of TEZ in combination with Kalydeco, based on the rationale that complementary therapies may produce an enhanced therapeutic effect. Orkambi (NDA 203068) was approved on July 2, 2015, and is a combination of ivacaftor and lumacaftor. Lumacaftor, although different in structure from TEZ, has a similar mechanism of action, enabling a conformational change in the CFTR mutant molecule to aid in its insertion in the membrane and enabling a functional chloride ion channel.

The Applicant provided studies that demonstrated in vitro incubation of F/F (508del/508del) human bronchial epithelial (HBE) cells for 18- to 24-hours (time allowed for *de novo* protein synthesis, processing, and transport to the cell membrane) with 3 μM TEZ, increased amounts of mature CFTR protein 2.4 fold over baseline levels. IVA alone had minimal effect on chloride transport in *F/F-HBE* due to lack of mature CFTR protein at the cell surface. The addition of TEZ increased the amount of mature CFTR protein delivered to the cell surface and potentiated chloride ion transport. These studies supported development of this combination for the proposed indication.

An assay using Fischer rat thyroid (FRT) cells transfected with specific CFTR mutations was developed to readily assess a multitude of CFTR mutations for both protein systhesis and chloride channel conductance. Several CFTR mutants, including residual function, gating defects, and splice mutations, were responsive to TEZ/IVA in this assay (defined by a ≥10 percentage points increase in net chloride transport over baseline as a percentage of normal CFTR activity).

The M1-TEZ metabolite has pharmacological activity similar to TEZ, but M2-TEZ metabolite had about 5-fold less activity. Both are major human metabolites. M5-TEZ, a phase II metabolite of M1-TEZ, was pharmacologically inactive.

4.2 Secondary Pharmacology

Secondary pharmacology studies demonstrated that TEZ effect on protein processing or trafficking was somewhat specific based on limited selection of studies. TEZ did not affect a misfolded mutant protein of hERG that uses a similar trafficking pathway as CFTR or a mutant P-glycoprotein that belongs to the same superfamily of the ATP-binding cassette (ABC) transporters as CFTR. Screening studies in a panel of in vitro receptor, channel, and enzyme binding assays failed to demonstrate significant interaction.

4.3 Safety Pharmacology

There were no significant toxicological findings in a standard battery of safety pharmacology studies. A hERG study testing the effects of TEZ showed a dose-dependent inhibition of I_{Kr}, but was limited by the solubility of TEZ. An assay with M2-TEZ found no dose related effects. A follow-up cardiovascular study in conscious telemetered dogs did not raise any toxicological safety concerns.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetics, ADME, and toxicokinetics are discussed in pharmacology/toxicology reviews of June 21, 2010; January 10, 2013; July 28, 2016; and March 14, 2017. These reviews also describe the toxicology characterization for the safety of metabolite M2, found at disproportionally higher levels in human than in animals.

Absorption
The solubility of TEZ was very low in the pH range of 2 to 9. Early in development an aqueous suspension of TEZ as (b) (4) produced a rapid absorption of orally administered TEZ in mice, rats, rabbits, and dogs with a bioavailability of approximately 40% to 50% in rats and dogs.

TEZ had good permeability in Caco-2 cells but was also a substrate for P-glycoprotein efflux.

Distribution
Distribution studies using radioactive ^{14}C-TEZ demonstrated a rapid distribution across most tissues in rats. The greatest amounts of radioactivity were found in the GI tract, liver, adrenal glands, kidney, pancreas, heart, and lungs, with lowest levels in the brain, eyes, and testes. There was no binding to melanin-containing tissues (skin and/or eyes).

In pregnant rats ^{14}C-TEZ-related radioactivity distributed to various fetal tissues across the placental barrier.

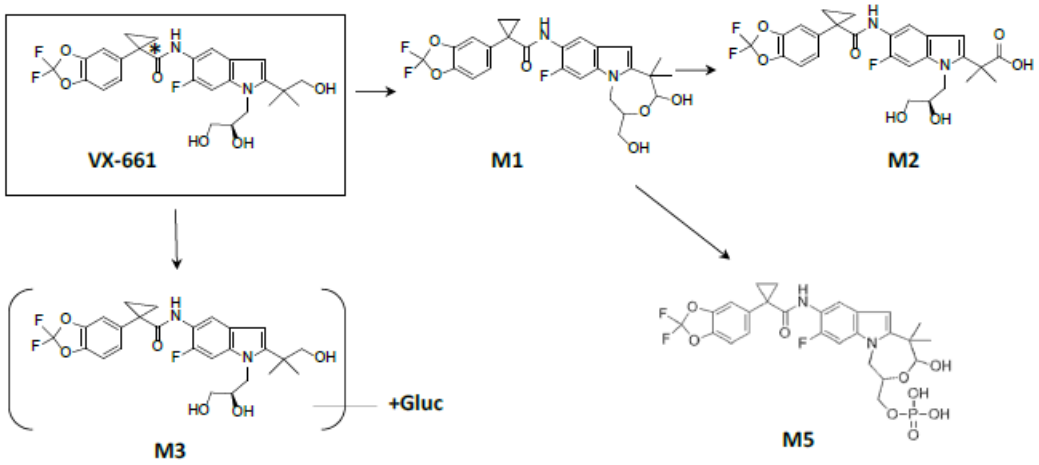
TEZ, and its metabolites (M1-TEZ, M2-TEZ, and M5-TEZ) were highly bound to plasma proteins (>98%) in mouse, rat, dog, monkey, and human. Binding in human plasma was primarily to human serum albumin.

Metabolism

TEZ is metabolized by phase I and phase II pathways. Following administration of ^{14}C-TEZ to rats, most of the circulating radioactivity was associated with unchanged TEZ, M1-TEZ (dehydrogenation metabolite) and M5-TEZ (phosphate conjugate of M1-TEZ). M1 and M2 are both major human metabolites. M2 is a disproportionate human metabolite, with very little detected in mice, rat, and dog plasma. M2 is also the major excreted metabolite in rats. M5-TEZ, a phase II metabolite (phosphate conjugate of M1-TEZ), is also a major metabolite in humans as well as rats, but is poorly permeable through cell membranes.

Figure 1: Proposed Metabolic Pathway of TEZ in Rats and Humans

(from Applicant's Figure 4, Module 2.4, Reports K247and VX13-661-005)



*signifies the position of the radiolabel

A detailed review of the safety assessment of these metabolites was completed on July 28, 2016 and March 14, 2017. The reviews found that all the metabolites have been adequately qualified. A summary is included in section 6.2 below.

CYP Induction and Inhibition

CYP3A4/5 are the main CYP isoforms involved in TEZ metabolism. TEZ did not induce CYP1A2 and CYP2B6 mRNA in human hepatocytes cultured in vitro. At supratherapeutic concentrations (30-100 µM TEZ) CYP3A4 mRNA was also induced. M2-TEZ did not show induction of CYP3A4 mRNA, and M1-TEZ and M5-TEZ showed weak CYP3A4 mRNA induction at 5 and 25 µM, respectively. In a 7-day dosing study in rats there was no effect on Cyp1a1/2, Cyp2b1/2, Cyp2e1, Cyp4a1-3, and UGTs, but there was a variable effect on Cyp3a1. In both Tg.rasH2 mice and CD-1 mice, repeated

oral administration of TEZ, resulted in a significant dose-dependent increase in Cyp2b10 and Cyp3a11 mRNA.

The IC_{50} values for inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were ≥25 µM for TEZ, ≥12 µM for M1-TEZ, and ≥ 75 µM for M2-TEZ and M5-TEZ.

Transporters
TEZ is a substrate for the uptake transporter OATP1B1, but not for OATP1B3. M1-TEZ and M5-TEZ are not substrates for OATP1B1 or OATP1B3. M2-TEZ is a substrate for OATP1B1 and OATP1B3.

TEZ is a substrate for efflux transporters Pgp and BCRP. M1-TEZ is a substrate for Pgp. M2-TEZ may be a substrate for Pgp, but not for BCRP.

TEZ does not significantly inhibit efflux transporters Pgp and BCRP, the hepatic uptake transporter OATP1B3, or renal uptake transporters (OAT1, OAT3, and OCT2). TEZ inhibited OATP1B1 (IC_{50} 3.2 µM). M1-TEZ, M2-TEZ, and M5-TEZ also do not appear to inhibit significantly Pgp, OAT1, OAT3, and OCT2, and appear to inhibit OATP1B1 (IC_{50} 6.2-11.4 µM) and OATP1B3 (IC_{50} 12.9 to 21.5 µM).

Excretion
The primary route of TEZ elimination was metabolism followed by biliary excretion of metabolites in rats and dogs. TEZ was excreted primarily as M2-TEZ in rats, glucuronides of TEZ and glucuronides of M1-TEZ in dogs. TEZ was also excreted in the milk of lactating rats.

5.2 Toxicokinetics

Refer to individual studies in Section 6.2, Repeat-Dose Toxicity.

In summary, when co-administered in combination studies in rats and dogs, systemic exposures to TEZ and IVA were similar to exposures achieved when these compounds were administered individually. The exposure of TEZ increased as the dose increased, and the increase in exposure was dose proportional at lower dose levels and was less than dose proportional at higher doses. There was no significant sex differences in exposure and no consistent accumulation after oral administration in repeated dose studies. However, TEZ exposure was reduced upon repeated oral dosing in mice. This is thought to be attributed to an induction of metabolic enzymes.

6 General Toxicology

Detailed pharmacology/toxicology reviews were completed on June 21, 2010; December 30, 2011; January 10, 2013; July 28, 2016; March 14, 2017; and September 1, 2017. A summary of noteworthy findings from the toxicology studies is included below.

6.2 Repeated Dose

The sponsor conducted studies of VX-661 in rats up to 6-months duration, in dogs up to 12-months duration, and IVA/TEZ combination drug studies in rats and dogs of 1-month duration and in rats of 3-months duration.

Mid-and Long-term Studies with VX-661

3-month rat study

In a 3-month study (Report VX-661-TX-010), rats were administered daily oral doses of 0, 25, 50, or 100 mg/kg/day VX-661. There were no morbidities or mortalities. There was a dose-related reduction in body weight up to 12% in males and 8% in females and up to a 23% reduction in body weight gain over the 91-day dosing period. Food consumption was reduced up to 40% at the high dose and remained lower (≤9% for males, 8-15% for females) than controls throughout the study. Clinical observations included dose-related increased incidence of hair discoloration, hair loss, and piloerection. Some animals in the high dose group exhibited salivation, audible breathing, and vocalization, which were absent in the recovery period. Hematology changes consisted of an increase in reticulocyte counts (up to 172% of control), without significant changes in other red cell parameters. There were reductions in triglycerides in both sexes at the mid and high doses (up to 39% of control values). High dose females also had increases in phosphorus (120%) and alkaline phosphatase (164%). There were reductions in total protein (92%) and albumin (88%) in mid and high dose female groups. There was a dose-related increase in urine volume (up to 314% and 444% for high dose males and females, respectively), slight reductions in specific gravity, and slight alkalinization of urine pH. These changes resolved during the recovery period.

VX-661 treatment produced histopathological effects in the ileum, spleen, and lung. There was an increased incidence of dilated lymphatics in the villi of the ileum in males compared to females and to controls. In the spleen, at the high dose there was an increased incidence of congestion and extramedullary hematopoiesis, which was absent at the end of the recovery period. In the lung, there was a dose-related increased incidence of alveolar histiocytosis in males, and an increased incidence in females that was not dose related. Also, some animals had evidence of hemorrhage and subacute, chronic inflammation. The incidences of lung findings were reduced in recovery animals.

6-month rat study

Overall, the 6-month rat study (Report VX-661-TX-012) had results fairly similar to those of the 3-month study described above. The doses were the same: 0, 25, 50, and 100 mg/kg/day as in the 3-month study. There was a 4-week recovery period, but only for the control and high doses. There were no mortalities. Mean body weight in the high dose groups was reduced 11% in males and 9% in females at week 26 and weight gain was reduced 26% in males and 23% in females for the first 12 weeks compared to controls. Food consumption was also reduced in the high dose during the first two weeks, but then was generally similar to control food intake. VX-661-related clinical observations of hair loss and unkempt appearance occurred in both sexes at 100 mg/kg/day. Hematological and serum chemistry changes were similar to the 3-month study, with less severity. There was a very minimal reduction (-5%) in erythrocyte numbers in males, but no significant change in hemoglobin or hematocrit. Platelets were also reduced in high dose males and females, 83% and 80%, respectively. There was also an increase in reticulocytes at the mid and high doses (up to 160%). Reduction in triglycerides occurred in both sexes at ≥ 25 mg/kg/day, and recovered within the 4-week recovery period. Urine volume was increased with concurrent reductions in urine specific gravity in both sexes at 100 mg/kg/day, which was reversible.

VX-661 treatment appeared to influence some organ weights when expressed relative to body weight, but they were not associated with histopathological changes. Spleen weight was dose-dependently increased in males in the 50 and 100 mg/kg/day dose groups. Mean ovary weight was reduced (and as expressed per brain weight) in the high dose females. There were increases in heart, kidney, and thyroid/parathyroid weights (expressed as % body weight) in male and females in the high dose, an increase in liver weight for the high dose females, but not males, and an increase in weight of lung with bronchi in males but not females. There was a dose-dependent increase in heart weight expressed as percent body weight, which was not statistically different from control at the high dose in either males or females in the 3-month study, but was significantly increased in the 6-month study (124% in males and 121% in female), and not reversed by a 4-week recovery period. Cardiovascular safety studies of VX-661 were negative, but it is doubtful that effects of a slowly progressive change in heart weight would be detected by an acute safety study. Also, there has not yet been a safety study of inotropic effects.

Dilated terminal lymphatics of minimal to mild severity were present in the villi of the jejunum and ileum of males and females in the VX-661 treatment groups, but not in the control groups. This finding was also present in the high dose recovery groups. The incidence and severity did not increase with duration of dosing (3-month vs 6-month studies). The finding is not due to a downstream obstruction, as only the terminals are dilated. Although not analyzed for individual association, the reduction in triglycerides and possibly cholesterol may be related to these findings. While this is unlikely to affect survival of the proposed 2-year rat study, it is significant for human use and has been a major concern of the review team, limiting their dose escalation in clinical studies until there was a more thorough understanding of the clinical significance of dilated gastrointestinal lymphatic terminals. In the lung of males, there was a dose-dependent increase in alveolar histiocytosis of minimal severity. Mononuclear infiltrates were

observed with a fairly high incidence in numerous organs in the control and high dose in males and females, with generally no difference between these groups. The low and mid dose treatment organs were not examined for most tissues and organs. These organs include the epididymides, heart, kidney, liver, lung, pancreas, and prostate. There was no obvious explanation for these findings, which is not usually observed in control animals at the frequency seen here for some organs.

In the 6-month study, there were no significant sex differences in AUC_{0-24} and C_{max} values for VX-661 and VRT-0996107 (M1 metabolite), with only slight differences noted for VRT-1189001 (M2 metabolite) on day 1 only. There was no accumulation of VX-661 on day 90 or 180 compared to day 1. However, there was accumulation of metabolites M1 (VRT-0996107) and M2 (VRT-1189001). The mean AUC_{0-24} ratio of the metabolites to VX-661 ranged from 0.47 to 1.98 for M1 (VRT-0996107) and 0.01 to 0.13 for M2 (VRT-1189001), indicating very little M2 was present in plasma of the rat.

3-Month Dog Study with the Liquid and Tablet Formulations
There were two 3-month repeated dose studies of VX-661 in dogs, one with the previously studied liquid formulation (Report VX-661-TX-011) and one with the clinical tablet formulation (Report VX-661-TX-016). The finding of lymphatic terminal dilation in the small intestinal villi raised the possibility this finding was due to local gastrointestinal drug concentrations which might be absent with different formulations. However, in both studies there was dilation of terminal lymphatics in the small intestinal villi at all VX-661 doses.

In the 3-month study with the liquid formulation, dogs were administered VX-661 at doses of 0, 50, 150, and 450 mg/kg/day for 91 consecutive days. There were no deaths or abnormal clinical observations. Body weight gain was reduced at the mid and high doses, but there were no VX-661-related effects on food consumption. There were 2 important histopathological findings, lymphatic dilation of terminals within the small intestinal villi, and lung inflammation. Minimal to mild lymphatic dilation was evident in the small intestine in both sexes (males, > 150 mg/kg/day; females, all VX-661 dose levels) and present after a 4-week recovery period (only the high dose group was followed for recovery of VX-661 treatment). Due to the absence of VX-661-related clinical signs, clinical pathology findings, and the minimal severity of the dilated lymphatic terminals, this finding was not considered dose-limiting in the dog. Dilated lymphatics were previously observed in the 1-month dog toxicity study (Report VRT-893661-TX-011) also without associated pathological changes. However, it was still a clinical concern and the proposed clinical study was partly designed to address this finding as discussed in previous PharmTox reviews and more extensively in an advice to the sponsor on September 9, 2013. The clinical safety study did not find evidence of intestinal lymphangiectasia with endoscopy.

The other histopathological finding of concern was subacute/chronic lung inflammation of minimal severity that occurred at all doses including controls, but was more frequent with increasing dose of VX-661. Lung inflammation had been observed in the previous rat toxicology studies of 1-, 3-, and 6-month duration, but not in the previous 1-month

dog study. It was not associated with clinical respiratory observations and was not present after a 4-week recovery period. A study NOAEL was not determined due to the dose-dependent increase in lung inflammation. The high dose was 450 mg/kg/day, corresponding to an AUC 1435 µg-hr/mL and C_{max} 94.4 µg/mL. The AUC for the M1 metabolite at this dose was 77.5 µg-hr/mL. For M2, the AUC was 7.3 µg-hr/mL in males and 11.2 µg-hr/mL in females.

In a separate study (Report VX-661-TX-016), dogs were administered an oral tablet formulation of VX-661, once daily for 13 weeks at doses of 0, 5, 25, and 50 mg/kg/day. This study focused on the presence of terminal lymphatic dilation in the small intestine. Lung histopathology was not conducted in this study. Dilated lymphatics of minimal to mild severity occurred at villi tips in the jejunum, ileum, and duodenum in both sexes with a dose-related increase in incidence, although absent in the control and low dose groups. This finding was therefore not dependent on differences between formulations, and indicates it is likely a direct effect of VX-661 on the functional activity of the lymphatic terminals, which is not well understood. Since dilated lymphatic terminals are not considered to be adverse, pending further study, the NOAEL for this finding of VX-661 tablets was 50 mg/kg/day, the highest dose level tested, and equivalent in mg/kg to the low dose in the previously described liquid formulation study. The corresponding VX-66 AUC was 462 µg-hr/mL and C_{max} was 3.3 µg/mL. The AUC for M1 was 41 µg-hr/mL and for M2 was 6.35 µg-hr/mL.

1-month dosing with recovery periods up to 1 year in dogs

To investigate the reversibility of lymphatic dilation in the small intestine, beagle dogs (n=6/sex/dose group) were administered daily oral doses of VX-661 at 100 mg/kg/day with food (fed) or 250 mg/kg/day without food (fasted), or fed and fasted vehicle controls for 28 days (Report X-661-TX-015). Some animals (n=4/sex/dose group) were terminated at 28 days and the other animals (n=2/sex/dose group) were allowed a 91-day recovery period. After 28 days of dosing, minimal to mild dilated lymphatics were present under both feeding regimens. After a 91-day recovery period, dilated lymphatics were still present in the gastrointestinal tract. There were no adverse findings in clinical observations, clinical pathology, or histopathology associated with the dilated lymphatics.

To further investigate the reversibility of lymphatic dilation in the small intestine, additional beagle dogs (n=4 male/group) were administered daily oral doses of VX-661 at 100 mg/kg/day or control article (HPMC-AS) for 28 days (Report VX-661-TX-022). Animals (n=4 males/group) were terminated at 28 days or after 3, 6, 9, or 12 months recovery periods. Dilated lymphatics within the gastrointestinal villi were noted at all necropsy intervals. Partial recovery was noted as the incidence and severity decreased by the 12-month recovery interval.

6- and 12-month dosing toxicity studies in dogs

Dogs were administered VX-661 by oral gavage at doses of 0, 2, 10, 100, or 200 mg/kg, once daily, for 26 weeks (Report VX-661-TX-018) or 52 weeks (Report VX-661-TX-013). In both studies, dilated lymphatics in the small intestines occurred in males and

females at 10, 100, and 200 mg/kg/day, but not at 2 mg/kg/day. The severity and incidence were dose-related. The duodenum was affected at 10, 100, and 200 mg/kg/day whereas the jejunum and proximal ileum (section with GALT) were affected at 100 and 200 mg/kg/day. Dilated lymphatics were present mostly at the tip of the villi and appeared as clusters of variably sized cystic round empty spaces. VX-661-related changes in clinical chemistry parameters included minimal decreases in albumin in both sexes at 200 mg/kg/day and mild increases in cholesterol in males at 200 mg/kg/day and in females at ≥ 100 mg/kg/day. These findings were considered VX-661-related, but not adverse.

VX-661 AUC_{0-24} on day 180 for the dose of 2 mg/kg/day was 6.5 µg-h/mL, and that for 200 mg/kg/day averaged 1248 µg-h/mL, (males, 1530; females, 966 µg-h/mL). On day 364 in the 52-week study, the AUC_{0-24} for the dose of 2 mg/kg/day was 9.1 µg-h/mL, and that for 200 mg/kg/day averaged 1765 µg-h/mL, (males, 1450; females, 2080 µg-h/mL).

Studies with VX-770

VX-770 is an approved drug, Kalydeco (ivcaftor, NDA 203188 approved in Jan 2012), for the treatment of CF, and was developed under IND 74633 also by Vertex. Pertinent to the combination VX-661/VX-770 review, nonclinical studies were conducted with VX-770 in the same species, rat and dogs. The key findings of those studies and are presented below, although were not reviewed for this application.

VX-770: 3-Month Rat Study

In a 3-month repeated oral dosing study (Report VX-770-TX-001) rats were administered doses of 0, 50, 100, 200, or 400 mg/kg/day VX-770. There were VX-770 related deaths at doses of 200 and 400 mg/kg/day associated with decreased activity, food consumption and fecal volume, hunched posture, paleness, labored/rapid breathing, red exudates from the mouth/eyes and unformed/watery stool, prolongation of APTT and PT, increases in ALT, GGT, SDH, and BUN, and reduction in plasma potassium, and an increase in liver weight. Also at these doses histopathologic effects were dilated or cystic lymphatics in the mucosa and epithelial vacuoles the intestinal tract (duodenum, jejunum, ileum); dilated/cystic reticular meshwork in the mesenteric lymph nodes, liver, and mammary tissues; and bone marrow hypocellularity. Doses of 100 and 200 mg/kg resulted in a dose-dependent basophilic epithelial kidney cortex tubules. The NOAEL was 50 mg/kg/day corresponding to an AUC of 98 µg-h/mL for males and 180 µg-h/mL for females. The gastrointestinal cystic lymphatics were only observed at 200 and 400 mg/kg/day doses and these doses were only administered in this one study.

VX-770: 6-Month Rat Study

In a 6-month repeated oral dosing study (Report 06-2028), rats were administered 0, 50, 100, or 150 mg/kg/day VX-770. VX-770-related deaths occurred at 100 and 150 mg/kg/day. There were reductions in body weights of up to -22%, increases of BUN, creatinine, phosphate, and magnesium, and urinalysis changes (increased urine volume, increase in sodium-, potassium-, and chloride-to-creatinine ratio, reduction in

phosphate concentrations, and only in females a reduction in calcium to creatinine ratio). Histopathological effects of VX-770 treatment included exacerbation of signs of chronic progressive nephropathy, cardiomyopathy, and medial coronary artery degeneration. The NOAEL was 50 mg/kg, corresponding to an AUC of 445 µg-h/mL.

VX-770: 12-Month Dog Study

In a 12-month repeated oral dosing study (Report 07-3277) dogs were administered doses of 0, 15, 30, or 60 mg/kg/day of VX-770. The key study findings were increases in incidences of abnormal stool and vomiting, and a higher incidence of supraventricular premature complexes (SVPC) in the 60 mg/kg/day dose group. The NOAEL was identified at 30 mg/kg/day. However, the LOAEL of 60 mg/kg/day was used to determine clinical safety factor since SVPC were considered clinically monitorable. The corresponding AUC averaged ~300 µg-h/mL (females: 254 µg-h/mL; males 351 ug-h/mL).

3-Month Study of VX-661/VX-770 Combination in Rat

VX-661 and VX-770 (ivacaftor) were administered orally once daily to rats for 13 weeks followed by a 4-week recovery period for control and high dose treatment animals. The doses of VX-661/VX-770 were 0/0 (vehicle control), 20/80, 40/40, and 80/20 mg/kg/day, respectively. The study design of this combination drug study was considered problematic in that increasing doses of VX-661 were administered with decreasing VX-770 dose levels. Both compounds have overlapping pharmacodynamic properties, although dissimilar mechanism of action. Without contemporary controls of single drug groups, and the lack of recovery groups for all dose levels, it was not possible to evaluate potential synergistic toxicities.

However, occasionally an association could be established with high doses of VX-661 or VX-770 or with both compounds. There was one drug related death on day 15 in a male in the 80/20 dose group receiving the highest dose of VX-661 with the lowest dose of VX-770 group during the first week. Mean body weight and food consumption was reduced (up to 12%) in the 80/20 dose group. Hematologic changes occurred in both sexes in all VX-661/VX-770 dose groups. These included: decreases in erythrocytes (up to 9%), hemoglobin (up to 8%), and/or hematocrit (up to 8%), and in the 80/20 dose group increases in reticulocytes (up to 74%).

Summary of the Safety Assessment of Metabolites

There were three major drug metabolites found in humans: M1, M2, and M5.

M2 had very poor absorption after oral dosing in the rat and was not tolerated with intravenous or subcutaneous dosing. The dog did tolerate M2 subcutaneous dosing although swelling and discoloration were noted at the site of injection. Skin was the major target of toxicity with microscopic findings of epidermal erosion/ulcer and associated inflammation, edema, and other dermal and/or subcutaneous findings. Exposure-based (AUC) safety margins at the NOAEL in the 28-day repeat-dose toxicity study was approximately 2-fold the expected human M2 exposure. In addition, an

embryo-fetal development study was conducted with M2 in the rabbit. See section 9.2 below.

Metabolites M1 and M5 did not require separate toxicity studies since the animal exposures from VX-661 treatment were approximately similar to the human exposures.

7 Genetic Toxicology

A detailed review of genetic toxicology studies was completed in a pharmacology/toxicology review dated June 21, 2010. TEZ was not mutagenic or clastogenic in a standard battery of genetic toxicity tests. The table below presents a summary of the results.

Table 2: Summary of Genetic Toxicology Studies of VX-661

Test Report Number	Species	Dose	Result
Mutagenicity			
Bacteria Reverse Mutation VRT-893661-TX-007	*S. Typhimurium*, *E. coli*	up to 5000 µg/plate with or without S9 metabolic activation	**Negative**
Clastogenicity			
***In vitro* Chromosomal Aberrations** VRT-893661-TX-008	Chinese hamster ovary (CHO) cells	up to 5000 µg/mL with or without S9 metabolic activation	**Negative**
***In vivo* Mouse Micronucleus Test** VRT-893661-TX-003	Mouse, ICR males	oral gavage, up to 2000 mg/kg	**Negative**

8 Carcinogenicity

A detailed review of the carcinogenicity studies was completed by Dr. Eleni Salicru's on December 6, 2017 and by Dr. Malick Mbodj's (statistical review) on November 22, 2017.

A two-year study in Sprague-Dawley rats and a 26-week study in Tg.rasH2 transgenic mice were conducted to assess the carcinogenic potential of tezacaftor. No evidence of tumorigenicity was observed in male and female rats at tezacaftor oral doses up to 50 and 75 mg/kg/day (approximately equivalent to 1.7 and 2.8 times the MRHD based upon summed AUCs of tezacaftor and its metabolites in males and females, respectively).

No evidence of tumorigenicity was observed in male and female Tg.rasH2 transgenic mice at tezacaftor oral doses up to 500 mg/kg/day.

There was agreement between the Division and CDER's Executive Carcinogenicity Assessment Committee (Exec CAC) regarding study results.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: VX-661: Study of Fertility and Early Embryonic Development to Implantation in Rats

Study no.:	**VX-661-TX-021**
Study report location:	**SD-93 Dec 5, 2014**
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 10, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), Lot 17QB10SD.NJ00003, Purity 99.3%

Key Study Findings

- VX-661 was administered orally to male and female rats (n=25/sex) at doses of 0 (control), 25, 50, or 100 mg/kg/day, by oral gavage. Dosing of males began 28 days prior to pairing for mating and continued through the mating and post-mating period to euthanasia. Dosing of females began 14 days prior to pairing and continued through the mating period to gestation day (GD) 7, with necropsy on GD15.
- There were no significant adverse effects on reproductive performance and fertility in males and females, therefore the NOAEL for male and female fertility was 100 mg/kg/day.
- The maternal NOAEL was 25 mg/kg/day due to the reduction in body weight and weight gain, and food consumption at the 50 and 100 mg/kg/day doses.

Methods

Doses:	0, 25, 50 and 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	*Control article*: (b) (4) (hypromellose acetate succinate/HPMC-AS and SLS) *Vehicle*: 0.5% methylcellulose (400 cps) (w/v), 0.5% sodium lauryl sulfate (SLS) (w/v) and 0.01% simethicone (w/v) in deionized water
Species/Strain:	CD® [Crl:CD®(SD)] rats
Number/Sex/Group:	25/sex/dose group

Satellite groups: None

Study design: For males, dosing began 28 days prior to pairing and continued through mating and post-mating periods until termination (approximately day 64) after the females reached GD15. For females, dosing began 14 days prior to pairing and continued through the mating period to GD7. On dosing day 29 for males and day 15 for females, females were paired to a male in the same treatment group, for up to 21 days. When positive evidence of copulation (copulatory plug or sperm in vaginal lavage occurred, designated GD0) females were housed separately. Females with no evidence of mating, but appeared to be pregnant on the basis of body weight and shape were euthanized.

Group Assignments

Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0	25	25
2	25	25	25
3	50	25	25
4	100	25	25

Deviation from study protocol: Sperm concentration analysis was not conducted for one male (#398) at 100 mg/kg/day. Since this was 1 of 25 animals, there was sufficient information that the absence of one animal would be expected to have minimal affect on the study conclusions.

Due to a scheduling error, several female animals received a single dose after GD7 as indicated in the following table. Since there were no adverse effects of VX-661, this single excess dose was unlikely to affect the study conclusions.

Observations and Results

Mortality

Animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily.

One control female (#416) died on day 10, attributed to a technical error during dosing.

Clinical Signs

A detailed clinical examination was performed each day 4 hours after dosing. Mated females were also given a detailed clinical examination on GD15. Estrous cyclicity was determined from daily vaginal lavage samples obtained until evidence of mating.

Thin appearance occurred in females at all VX-661 dose levels and only in the high dose in males. Other findings in both males and females included low incidences of audible breathing, decreased activity, and hunched posture. These were sometimes observed early in long term repeated dose studies but eventually attenuated.

Body Weight
Body weights of males were obtained twice weekly during treatment until euthanasia. Females were weighed twice weekly at 3- and 4-day intervals prior to and during cohabitation. Confirmed mated females were weighed on GD0, GD4, GD7, GD10, and GD15. Females with no evidence of mating were weighed just prior to euthanasia.

There was a reduction in body weight and body weight gain in males and females at the 50 and 100 mg/kg/day doses compared to control groups. For males receiving 25 mg/kg/day, there was a transient reduction lasting only the first 4 days of dosing (-3.5%, 309 g vs control 320 g). At dosing day 29 (start of mating), males in the high dose group had mean weight -19% less than controls and this was maintained throughout the rest of the study. The weight reduction correlated with reduced food consumption. For females, mean body weight prior to mating was reduced by 8% in the high dose group and by 5% in the mid dose group compared to controls. During the gestation period, reduced body weight occurred only at the 100 mg/kg/dose during the first week (GD4, -7%: and GD7, 5%). Mean body weight change during gestation (GD0 to GD4) was reduced 31% in the high dose group, but then increased to be similar or greater than the control body weight gain for the rest of gestation [GD0 to GD15: control, 81.2 g; 25 mg/kg/day, 85.8 g; 50 mg/kg/day, 90.3 g; 100 mg/kg/day, 92.4 g (+13.8%)].

Feed Consumption
Food consumption was recorded weekly prior to pairing for mating.

Mean food consumption for both males and females was reduced compared to controls in the 50 mg/kg/day and 100 mg/kg/day dose groups. This ranged from maximal reductions in males of -10% to -37%, and -16% and -36% in females, for 50 and 100 mg/kg/day, respectively. There was no effect on mean food consumption for VX-661–treated females during gestation.

Toxicokinetics

Toxicokinetics were not conducted for this study.

Dosing Solution Analysis

The prepared dose formulations from samples collected in weeks 1, 3, 7, and the final preparation were determined to be homogeneous and at appropriate concentrations (between 95.6 and 102.5% for concentrations of 2.5, 5.0, and 10.0 mg/mL corresponding to doses of 25, 50 and 100 mg/kg/day).

Necropsy
Females: On GD15, females were euthanized, and the number of normally developing embryos, resorptions, the total number of implantations, and the number of corpora lutea on each ovary were recorded. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. If no foci were seen, the female was considered nonpregnant. Organs weighed included the gravid uterus and ovaries.

Males: Organs weighed included testes, epididymides, seminal vesicles with coagulating gland, and prostate. The right testis and epididymis were utilized for sperm analysis. Sperm motility was assessed using the automated Hamilton-Thorne Computer Integrated Visual Optical System (IVOS). The right cauda epididymis was separated, weighed, and used for manual (visual) assessment of sperm concentration. Slides were prepared for assessment of sperm morphology from the motility preparations. One male in the 100 mg/kg/day (#398) did not have sperm concentration analysis performed, but this was judged not to affect the final results.

There were no macroscopic findings related to VX-661 treatment.

For females, ovary and uterus with cervix weights were similar in all dose groups. Since male body weight were reduced in the 50 and 100 mg/kg dose group by 6.4% and 29%, respectively, compared to controls, organ weight as a percent of body was used as the comparative measure. There was a statistically significant increase in relative epididymides weight (16%) and relative testes weight (20%) compared to controls, but these changes were judged unlikely to be of toxicological significance.

Fertility Parameters
There was no effect on estrous cyclicity (mean number of estrous cycles and mean cycle length), or copulatory interval. There was no effect on mean number of corpora lutea, uterine implantation sites, viable embryos, resorption sites, or pre- and post-implantation loss.

There was a slight but significant reduction in the mean number of corpora lutea per animal in the high dose group (14.8 vs control group of 17.2). This was also lower than the historical control data mean of 16.9 (range 15.1 to 20.0). The lack of other effects of VX-661 at this dose lead the sponsor to conclude this reduction was not adverse at this dose. The reviewer concurs with this assessment.

Table 3: Fertility and Pregnancy Parameters (From Sponsor's Table 14)

Females				
Dose (mg/kg/day)	**0**	**25**	**50**	**100**
No. Females on Study	**25**	**25**	**25**	**25**
No. Females Paired	24	25	25	25
No. Females Mated	24	25	25	25
No. Pregnant	23	25	24	22
Female Mating Index (%)	100.0	100.0	100.0	100.0
Female Fertility Index (%)	95.8	100.0	96.0	88.0
Female Fecundity Index (%)	95.8	100.0	96.0	88.0
Males				
Dose (mg/kg/day)	**0**	**25**	**50**	**100**
No. Males on Study	**24**	**25**	**25**	**25**
No. Males Paired	24	25	25	25
No. Males Mated	24	25	25	25
No. Males Impregnating a Female	23	25	24	22
Male Mating Index (%)	100.0	100.0	100.0	100.0
Male Fertility Index (%)	95.8	100.0	96.0	88.0
Male Fecundity Index (%)	95.8	100.0	96.0	88.0
Females with Confirmed Mating	22	23	22	25
Mean Copulatory Interval (Days)	3.2	3.4	3.4	3.6

Table 4: Summary of Maternal and Developmental Observations at Uterine Examination (from Sponsor's Table 15)

Dose (mg/kg/day)	**0**	**25**	**50**	**100**
No. Females on Study	25	25	25	25
No. Not Pregnant	2	0	1	3
No. Pregnant	23	25	24	22
No. Died Pregnant	0	0	0	0
No. Females with All Resorptions	0	1	0	0

No. Females with Viable Embryos Day 15 Gestation	21	22	21	22
No. Pregnant Females with No Confirmed Mating Date	2	2	3	0
Corpora Lutea No. per Animal	17.2	15.6	15.7	14.8*
Implantation Sites No. per Animal	14.9	13.55	13.6	13.2
Preimplantation Loss % per Animal	12.46	10.26	12.89	10.25
Viable Embryos No. per Animal	14.3	12.4	13.0	12.4
Postimplantation Loss % per Animal	4.25	11.23	4.57	5.51
Resorptions: Early + Late No. per Animal	0.6	1.1	0.6	0.8
No. – number *p<0.01 vs control				

Sperm Evaluation

There was no effect of VX-661 on sperm counts of the cauda epididymis, concentrations, motility, or % abnormal morphology compared to control males**.**

The percent abnormal sperm in the 100 mg/kg/day dose group was statistically greater than the control group (6.96% vs control group of 2.44%). This was within the range of the laboratory's historical control data (mean 3.31%, range 1.38 to 8.17%). The higher mean values was also attributed to one animal (#398) with an incidence of 49% abnormal sperm. Therefore, this was not considered a drug-related effect.

Table 5: Summary of Sperm Evaluation (from Sponsor's Table 16)

Dose (mg/kg/day)	**0**	**25**	**50**	**100**
N	**25**	**25**	**25**	**24/25**
Sperm % motility	90.8	91.3	92.7	90.6
Total Sperm Count per cauda epididymis x 10^8	2.85	2.75	2.70	2.56*
Sperm Concentration per gram Cauda epididymis x 10^8	9.85	9.42	9.61	9.36
% Abnormal	2.44	3.00	3.30	6.96**
* p<0.05 ** p<0.01				

9.2 Embryonic-Fetal Development

RAT

Study Title: VX-661: An Oral (Gavage) Dose Range-Finding Developmental Toxicity Study in Rats

Study Number: VX-661-TX-006 ([(b) (4)]395071)
Location: SD-41; July 30, 2013

Dose levels for reproduction and fertility studies were based on the results of this dose range-finding study conducted in pregnant rats. VX-661 ([(b) (4)], Lot A3613064) was administered once daily from GD6 to GD17, orally by gavage, at 0, 10, 25, 50, or 100 mg/kg/day (n=8 Crl:CD(SD) rats/dose group). The vehicle was 0.5% methylcellulose and 0.5% sodium lauryl sulfate in deionized water. The control was hydroxypropylmethylcellulose in vehicle. Animals were necropsied on GD21.

In the 100 mg/kg/day group during GD6 to GD18, there were mean reductions in body weight (up to approximately -16%) and weight gain (up to -79%), and food consumption (-36%), with a small, transient reduction in mean body weight gain and food consumption at 50 mg/kg/day. There were no maternal macroscopic necropsy findings related to VX-661 treatment. Fetal weights were severely reduced in only 1 litter in the 100 mg/kg/day dose but mean fetal weights were not different from controls. There were no effects on numbers of corpora lutea, implantation sites, or external fetal morphology.

The high-dose level of 100 mg/kg/day was selected to produce maternal toxicity and the mid- or low-dose level was expected to be the NOAEL for future developmental studies in the rat.

Study Title: VX-661: An Oral (Gavage) Embryo/Fetal Developmental Study in Rats

Study no.:	**VX-661-TX-008**
Study report location:	SD-19, Nov 22 2011
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 28, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), Lot A3613-064, Purity 97.8% and Lot A3613-079, Purity 101.3% (b) (4); composed of Active Pharmaceutical Ingredient VX-661 (50% API) and other pharmaceutically acceptable excipients.

Key Study Findings

- VX-661 was administered orally to pregnant rats once daily from GD6 through GD17 at doses of 0 (vehicle), 25, 50, or 100 mg/kg/day.
- VX-661 was not teratogenic; there was no effect of VX-661 on fetal intrauterine growth and survival, fetal malformations or variations. Therefore, the NOAEL for embryofetal developmental was 100 mg/kg/day, corresponding to an $AUC_{0\text{-}24h}$ exposure of 276 µg-h/mL.
- The NOAEL for maternal toxicity was 25 mg/kg/day, corresponding to an $AUC_{0\text{-}24h}$ exposure of 80.4 µg-h/mL, due to maternal body weight loss and reduced mean body weight gain with corresponding reduced group mean food consumption throughout the dosing period (GD6-18) in the 50 and 100 mg/kg/day groups.
- One animal in the 100 mg/kg/day group had an early delivery of one pup on GD21 and one animal in the 50 mg/kg/day group was euthanized *in extremis* on GD15. Both were attributed to maternal toxicity rather than embryofetal toxicity.

Methods

Doses:	0, 25, 50, and 100 mg/kg/day
Frequency of dosing:	Once daily from gestation days 6 through 17
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Vehicle: 0.5% methylcellulose (MC, 400 cps, w/v) and 0.5% sodium lauryl sulfate (SLS, w/v) hydroxypropylmethylcellulose (hypromellose) acetate succinate,
Species/Strain:	female Crl:CD(SD) rats, approximately 13 weeks old when paired for

breeding and 14 weeks of age at the initiation of dose administration

Number/Sex/Group: 25/dose group

Satellite groups: Toxicokinetics: 8/dose group

Study design:

Group Number	Treatment	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Females: Embryo/Fetal Development Phase ((b) (4)-395073)	Number of Females: Toxicokinetic Phase ((b) (4)-395073T)
1	Control article	0	5	25	4
2	VX-661	25	5	25	8
3	VX-661	50	5	25	8
4	VX-661	100	5	25	8

Group Number	Treatment	Dose Level (mg/kg/day)	Test Article Concentration[a] (mg/mL)	pH[b]
1	Control article[c]	0	0	4.66
2	VX-661[d]	25	5	4.66
3	VX-661[d]	50	10	4.54
4	VX-661[d]	100	20	4.58

[a] = Test article formulation concentrations were calculated as the Active Pharmaceutical Ingredient (API) using a correction factor of 2.0 based on the proportion of API to other pharmaceutical ingredients.

[b] = pH measurement of the first dosing formulations.

[c] = The control article, hydroxypropylmethylcellulose acetate succinate, HF grade (HPMC-AS-HF) was added to the vehicle at the highest concentration within the VX-661 test article groups, 20 mg/mL.

[d] = VX-661 is a 50:49.5:0.5 (b) (4) mixture of VX-661 (API), HPMC-AS-HF, and SLS. The VX-661 (b) (4) powder test article was formulated as a suspension in a vehicle containing 0.5% (w/v) MC and 0.5% (w/v) SLS.

Deviation from study protocol: There were no deviations that affected the interpretation and conclusion of this study.

Observations and Results

Mortality

Animals were checked at least twice daily.

There were 2 mortalities in the study. Female #95966 in the 50 mg/kg/day group was euthanized on GD15 in extremis due to extreme weight loss (32.3%) and reduced food consumption (≤ 14 g/day) that started on dosing day 1 (GD6). Female #95902 in the 100 mg/kg/day group, was euthanized prior to the scheduled necropsy after 1 pup was delivered on GD21.

Clinical Signs
All rats were observed twice daily from GD0 through GD21, prior to dose administration during the dosing period and approximately 8 hours following dosing.

There was a dose-related increased incidence in red material around the mouth noted at 8 hours postdose. Other findings included scabbing and/or hair loss on various body surfaces that occurred throughout the dosing and postdose periods and was not considered toxicologically significant.

Clinical Observations

Dose (mg/kg/day)	0	25	50	100
N	25	25	25	25
Red material around mouth	0	0	5	10
Scabbing and/or hair loss on various body surfaces (forelimbs, hindlimbs, thoracic, abdominal, anogenital, rump, neck, base of tail, and/or inguinal areas)	3	0	11	17

Body Weight
Maternal body weights were recorded on GD0, GD6-18 (daily) and GD21. Group mean gravid uterine weight was calculated and group mean net body weight (the GD 21 body weight minus weight of the uterus and contents) and group mean net body weight change (the GD0-21 body weight change minus the weight of the uterus and contents) were calculated.

Mean body weight (up to -14.8%) and weight gain were reduced in the 100 mg/kg/day group throughout the dosing period (GD6-18) compared to the control group. After dosing stopped (GD18-21), in the high dose group, mean body weight gain increased compared to the control group, partially recovering, but still statistically lower ($p<0.01$) than controls on GD21. There were no VX-661-related effects on group mean gravid uterine weight in the 100 mg/kg/day group when compared to the control group.

In the 50 mg/kg/day group, mean body weight and mean body weight gain were significantly ($p<0.01$) lower than controls during GD6-9 and GD9-12, respectively and resulted in reduced (up to -11.3%) mean body weight gain during GD8-18. During the absence of dosing, mean body gain increased greater than controls and the mid dose mean body weight was similar to control mean body weights on GD21, although the

mean net body weight gain was still lower than mean control values on this day. Mean gravid uterine weight was similar to mean control weight.

Mean body weight and body weight gain in the 25 mg/kg/day dose group were transiently reduced during the dosing days, but then recovered and was similar to controls for the rest of gestation. Mean gravid uterine weight was similar to mean control weight.

Table 6: Body Weight and Body Weight Gain

Dose (mg/kg/day)	**0**	**25**	**50**	**100**
N	**24**	**25**	**24**	**25**
Gestation Day				
0	262	259	262	262
6	296	292	297	296
7				
8	305	293	**290**	**289**
12	326	314	**295**	**294**
17	368	356	**336**	**315**
Body Weight Change	384	373	**352**	**327**
21	442	435	420	**398**
GD0-6	34	33	35	34
GD6-18	58	59	53	33
GD18-21	58	62	**69**	**71**

Feed Consumption

Food consumption was determined on GD0, GD6-18 (daily), and on GD21. Food intake (g/animal/day and g/kg/day) was calculated for the corresponding body weight change intervals.

Group mean food consumption, evaluated as g/animal/day and g/kg/day, in the 50 and 100 mg/kg/day groups was significantly ($p<0.05$ or $p<0.01$) lower than the control group throughout the dosing period (GD6-9, GD9-12, GD12-18, and GD6-18). These reductions were associated with body weight losses or reduced body weight gains. For GD18-21, mean food consumption in the 50 and 100 mg/kg/day groups was either similar to or significantly ($p<0.01$) higher than the control group, and also correlated with the partial recovery from reductions in maternal body weight.

For the 25 mg/kg/day group, mean food consumption was significantly ($p<0.05$) lower than the control group for the initial dosing days (GD6-9) and also correlated with the lower mean body weight gain at this time. Food consumption was similar to controls for the rest of the gestation period.

Table 7: Food Consumption (g/animal/day)

Dose (mg/kg/day)	0	25	50	100
N	**24**	**25**	**24**	**25**
Gestation Day				
0-6	23	22	23	24
6-18	25	23	**19**	**16**
18-21	27	29	31	28

Toxicokinetics

For toxicokinetic evaluation, 4 additional females were administered the control article and 8 additional females/group were administered the test article on a regimen comparable to the embryo/fetal development phase females. Blood samples were collected from each control group female at 8 hours postdose and from 4 rats/test article-treated group at 0 (predose), 2, 4, 8, 12, and 24 hours postdose on GD6 and GD17. All toxicokinetic phase animals were euthanized on GD18, and pregnancy status was determined for each female.

Increased VX-661 doses resulted in an approximately dose-proportional increase in mean AUC_{0-24} for VX-661 on GD6 and GD17, although C_{max} increased in an approximately less-than-dose-proportional manner. There was no accumulation of VX-661 from GD6-17. The AUC_{0-24} of metabolite M1 (VRT-0996107) for all doses of VX-661 was less than the AUC for VX-661 on GD6, but approximately similar to the AUC for VX-661 for all dose groups on GD17.

Table 8: Summary of Toxicokinetic Parameters of VX-661

Dose (mg/kg/day)		25	50	100
Day 6	**C_{max}**	5.8	13.1	19.1
	t_{max}	4.0	8.0	2.0
	AUC_{0-24} (µg-hr/mL)	79.3	**183**	**315**
Day 17	**C_{max}**	7.67	11.1	19.4
	t_{max}	2.0	4.0	8.0
	AUC_{0-24} (µg-hr/mL)	80.4	165	276

Table 9: Summary Toxicokinetic Parameters of M1 (VRT-0996107)

Dose (mg/kg/day)		25	50	100
Day 6	**C_{max}**	2.44	4.77	6.25
	t_{max}	12.0	8.0	12.0

	AUC_{0-24} (µg-hr/mL)	42.7	**86.1**	**128**
Day 17	**C_{max}**	3.92	7.93	16.0
	t_{max}	8.0	8.0	8.0
	AUC_{0-24} (µg-hr/mL)	74.5	168	354

Dosing Solution Analysis

Stability: In a previous study (Zhang, 2010, PPD-0716) VX-661 was found to be stable in the vehicle for 24 hours when stored at 25ºC ± 2ºC at a concentration range of 6 to 60 mg VX-661/mL and that the dosing formulations should be used for dosing immediately following preparation or within 6 hours while maintained stirring on wet ice. Another study ((b) (4)) found that the lower formulation concentrations, 2 to 20 mg VX-661/mL, bracketing those used in this study were stable for at least 6 hours.

Homogeneity: Samples of the formulation concentrations were homogeneous, 104 to 107% of expected levels.

Concentration: Samples of the formulation concentrations were appropriately made with concentrations 103 to 104% of expected levels.

Necropsy
On GD21, a laparohysterectomy was performed. The uteri, placentae, and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

There were no internal macroscopic findings for any VX-661 dose group. Externally, hair loss was noted at necropsy for 1 out of 24 and 5 out of 24 females in the 50 and 100 mg/kg/day groups, respectively, which had been reported in daily clinical observations. With the exception of 1 female each in the control and 50 mg/kg/day groups, all females were determined to be gravid. In the female euthanized *in extremis* on GD15 in the 50 mg/kg/day group, and female that delivered early on GD21 in the 100 mg/kg/day group, there also were no macroscopic findings.

Table 10: Terminal Body Weight, Gravid Uterine Weight, and Net Body Weight

Dose (mg/kg/day)	**0**	**25**	**50**	**100**
n	**24**	**25**	**23**	**25**
Initial body wt	262	259	263	262
Terminal	442	435	420	**398**

body wt				
Gravid uterine wt	111.6 n=23	109.3 n=25	106.0 n=23	106.5 n=24

Net body wt	327.6	325.9	314.3	**292.6**
Net body wt change	67.0	67.0	**51.4**	**30.4**

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
There was no effect of VX-661 on numbers of viable fetuses, post-implantation loss, fetal sex ratios, mean numbers of corpora lutea, implantation sites, mean litter proportions of pre-implantation loss.

Offspring (Malformations, Variations, etc.)

There were no VX-661-related developmental malformations or variations. Findings were not dose related, occurred infrequently and within historical control incidences, and considered to arise spontaneously.

Table 11: Summary of Malformations and Variations

Dose (mg/kg/day)	**0**	**25**	**50**	**100**
Number of litters	24	25	23	**25**
Number of fetuses	374	370	344	388
Total Number of Malformations: [fetuses (liters)]	5 (5)	0	1 (1)	1 (1)
External Malformations [fetuses (liters)]	3 (3)	**0 (0)**	**0 (0)**	**0 (0)**
brachydactyly	1 (1)			
ectodactyly	1 (1)			
bent tail	1 (1)			
External Variations [fetuses, (litters)]	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**
Visceral Malformations [fetuses, (litters)]	**2(2)**	**0 (0)**	**1(1)**	**0 (0)**
Diaphragmatic hermia	1 (1)			
hydrocephaly	1 (1)			
aortic arch/ aorta deviations with no brachiocephalic trunk			1 (1)	

Visceral Variations [fetuses, (litters)]	9 (3)	4 (4)	10 (5)	3 (2)
Incompletely developed renal papilla and/or Distended Ureters	7 (1)	3 (2)	10 (5)	2 (1)
Skeletal Malformations [fetuses, (litters)]	**0 (0)**	**0 (0)**	**0 (0)**	1 (1)
Vertebral and associated rib anomalties				1 (1)
Skeletal Variations [fetuses, (litters)]	27 (18)	27 (15)	39 (18)	16 (11)
Multiple findings	No dose response			

Rabbit

Study Title: VX-661: An Oral (Gavage) Dose Range-Finding Developmental Toxicity Study in Rabbits

Study Number: VX-661-TX-007 ((b) (4)-395072)
Location: SD-41, July 30 2013

Dose levels for the pivotal study were selected based on the results of this dose range-finding study conducted in pregnant rabbits. VX-661 ((b) (4), Lot A3613064) was administered once daily from GD7 to GD20, orally by gavage, at 0 (hydroxypropylmethylcellulose in vehicle), 10, 25, 50, or 100 mg/kg/day (n=5 New Zealand White [Hra(NZW)SPF] rabbits/dose group). The vehicle was 0.5% methylcellulose and 0.5% sodium lauryl sulfate in deionized water. Animals were necropsied on GD29.

The 100 mg/kg/day dose level produced body weight loss (-11.3%) and decreased food consumption, resulting in early termination of this dose group on GD15. Animals in this group were not evaluated further. In the 50 mg/kg/day, there were also 2 animals euthanized *in extremis* on GD20 and GD22 with reduced body weight and food consumption. Animals in the 50 mg/kg/day group that survived to GD29 had body weight losses of 10.6% during the dosing period. Weight gain after dosing was stopped on GD20 and was similar to controls. In the 50 mg/kg/day group animals that survived to termination and the 10 and 25 mg/kg/day dose groups there were no effects of VX-661 on net body weight or gravid uterine weight. Only the 50 mg/kg/day group had reduced mean fetal body weight (-13.8%), but no effect on fetal survival, external malformation, or variations.

For the rabbit embryofetal study, the high dose of 50 mg/kg/day was expected to produce maternal toxicity, while the mid- or low-dose level was expected to be the NOAEL.

Study Title: VX-661: An Oral (Gavage) Embryo/Fetal Development Study in Rabbits

Study no.:	**VX-661-TX-009**
Study report location:	SD-19, Nov 22, 2011
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 8, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), Lot A3613-064, Purity 97.8% and Lot A3613-079, Purity 101.3%

Key Study Findings

- Pregnant rabbits were administered VX-661 by oral gavage at doses of 0, 10, 25, or 50 mg/kg/day from GD7 to GD20, and terminated for uterine and fetal examination on GD29.
- There were no maternal or fetal effects of VX-661 at doses of 10 and 25 mg/kg/day but fetal weights were reduced in the 50 mg/kg/day group with no effect on intrauterine survival, fetal malformations, or variations. The NOAEL for embryofetal developmental was 25 mg/kg/day, corresponding to an $AUC_{0\text{-}24h}$ exposure of 17.4 µg-h/mL.
- The NOAEL for maternal toxicity was also 25 mg/kg/day due to maternal weight loss associated with reduced food consumption and reduced defecation at 50 mg/kg/day. There were two females in this dose group that aborted dead fetuses on GD23 and GD24. This was associated with maternal weight loss.

Methods

Doses:	0, 10, 25, and 50 mg/kg/day
Frequency of dosing:	Once daily from GD7 through 20
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Vehicle: 0.5% methylcellulose and 0.5% sodium lauryl sulfate in deionized water Control group administered 10 mg/mL hydroxypropylmethylcellulose acetate succinate, HF grade [HPMC-AS-HF]) in the above vehicle
Species/Strain:	New Zealand White [Hra:(NZW)SPF] rabbits, 5.5 to 6 months of age at the initiation of dose administration
Number/Sex/Group:	22 pregnant females/dose group

Satellite groups: Toxicokinetics: 4/dose group
Study design: Pregnant females were dosed from GD7 to GD20, and terminated on GD29.

Group Number	Treatment	Dose Level (mg/kg/day)	Test Article Concentration[a] (mg/mL)	pH[b]
1	Control article[c]	0	0	4.91
2	VX-661[d]	10	2	5.03
3	VX-661[d]	25	5	4.77
4	VX-661[d]	50	10	4.72

[a] = Test article formulation concentrations were calculated as the Active Pharmaceutical Ingredient (API) using a correction factor of 2.0 based on the proportion of API to other pharmaceutical ingredients.

[b] = pH measurement of the first dosing formulations.

[c] = The control article, hydroxypropylmethylcellulose acetate succinate, HF grade (HPMC-AS-HF) was added to the vehicle at the highest concentration within the VX-661 test article groups, 10 mg/mL.

[d] = VX-661 is a 50:49.5:0.5 (b) (4) mixture of VX-661 (API), HPMC-AS-HF, and SLS. The VX-661 (b) (4) powder test article was formulated as a suspension in a vehicle containing 0.5% (w/v) MC and 0.5% (w/v) SLS.

Deviation from study protocol: There were no deviations that affected the interpretation and conclusions of the study.

Observations and Results

Mortality

Rabbits were observed twice daily for mortality.

Two females in the 50 mg/kg/day group aborted on GD23 (animal #63214) or GD24 (animal #63218) and were terminated. Female #63214 aborted 5 dead fetuses with no apparent malformations and 2 dead fetuses that were partially cannibalized. This female had dark red discoloration of the mammary glands. Female #63218 aborted 8 late resorptions with no apparent malformations and had an accessory spleen at necropsy. Both females had body weight losses (15.6% and 21.0%, respectively) with concomitant reductions in food consumption (≤5 g feed/day) and increased incidence of decreased defecation that indicated that weight loss with subsequent abortionwere probably due to VX-661. There were no macroscopic findings in these animals.

Clinical Signs

Rabbits were observed twice daily for general changes in appearance and behavior and for signs of toxicity 8 hours after dosing.

In the 50 mg/kg/day dose group there was an increased incidence of a reduction in defecation beginning as early at GD9 and continuing through the dosing period. The incidences in the 10 and 25 mg/kg/day dose groups were similar to controls.

Body Weight
Maternal body weights were recorded on GD0 (by supplier under non-GLP conditions), GD4, GD7-21 (daily), GD24, and GD29.

Mean reductions in body weight occurred in the 50 mg/kg/day group (up to 10.8%) throughout the dosing period (GD7-10, 10-13, 13-21, and 7-21), but there was not a significant reduction in weight related parameters in the 10 and 25 mg/kg/day dose groups. After the cessation of dosing (GD21-29), there was a significant ($p<0.01$) increase in mean body weight gain in the 50 mg/kg/day group compared to the control group. On GD29, the 50 mg/kg/day group had mean body weight reduction of 5.4% compared to the control group. Mean net body weight and gravid uterine weight in the 50 mg/kg/day group were slightly lower (4.6% and 10.8%, respectively; not statistically significant) compared to the control group. The lower group mean gravid uterine weight in this group was due to lower group mean fetal weights.

Feed Consumption
Food consumption was recorded on GD4-29. Food intake was reported as g/animal/day and g/kg/day for the corresponding body weight change intervals. For the embryo/fetal development phase and toxicokinetic phase rabbits that consumed less than 10 g of feed per day for 2 consecutive days, the basal diet was supplemented with kale. Dietary supplementation was discontinued following 2 consecutive days of eating 35 g or more of feed.

Only the 50 mg/kg/day dose group had reduced mean food consumption throughout the dosing period compared to controls for GD 7-10, 10-13, 13-21, and 7-21. For GD21-29, mean food consumption values in this dose group were similar to the mean control values, although for GD24-29 greater food consumption occurred compared to control values.

Toxicokinetics
Blood samples were collected at approximately 0 (predose), 2, 4, 8, 12, and 24 hours postdose on GD7 and GD20. Animals were terminated on GD21 and pregnancy status was determined.

Both C_{max} and $AUC_{0\text{-}24h}$ increased with increasing dose on GD7 and GD20. There was a 4-fold accumulation of VX-661 in the 50 mg/kg/dose group on GD20 compared to GD7, but no accumulation in the 10 and 25 mg/kg/day dose groups.
GD20 maternal maximum mean plasma exposure (C_{max}) and systemic exposure ($AUC_{0\text{-}24hr}$) values were 2.42 µg/mL and 17.4µg·hr/mL, respectively. Metabolite M1 $AUC_{0\text{-}24hr}$ ranged from 1- to 2-fold of that for VX-661 for all doses on either GD7 or GD20.

Table 12: Summary of Toxicokinetic Parameters of VX-661 in Pregnant Rabbits

Dose (mg/kg/day)		10	25	50
Day 7	C_{max}	0.663	1.72	4.00
	t_{max}	3.00	2.50	3.00
	AUC_{0-24} (µg-hr/mL)	3.87	9.86	25.6
Day 20	C_{max}	0.706	2.42	6.63
	t_{max}	2.50	3.00	4.50
	AUC_{0-24} (µg-hr/mL)	4.52	17.4	107

Table 13: Summary Toxicokinetic Parameters of M1 (VRT-0996107) in Pregnant Rabbits

Dose (mg/kg/day)		10	25	50
Day 7	C_{max}	0.853	2.15	4.52
	t_{max}	3.50	4.00	4.00
	AUC_{0-24} (µg-hr/mL)	7.66	18.5	48.7
Day 20	C_{max}	0.929	2.62	7.27
	t_{max}	3.0	4.0	7.0
	AUC_{0-24} (µg-hr/mL)	9.99	33.1	144

Dosing Solution Analysis

Stability: Stability was previously demonstrated if samples were kept on wet ice for up to 6 hours. In this study this was reconfirmed with concentrations 102% and 110% of expected values.

Homogeneity: The dosing formulation solutions were homogenous with values ranging from 98.1% to 103% of expected concentrations and residual standard deviations ranging from 0.45% to 2.2%.

Concentration: The dosing formulation solutions were properly made since concentrations were 103% to 105% of expected values.

Necropsy

Females were terminated on GD29 and laparohysterectomies and macroscopic examinations were performed blind to treatment group. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were

weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

Group mean gravid uterine weights were collected and group mean net body weights (the GD29 body weight exclusive of the weight of the uterus and contents) and group mean net body weight changes (the GD0-29 body weight change exclusive of the weight of the uterus and contents) were calculated, using the data for gravid females at the scheduled laparohysterectomy.

Other than the 2 females that aborted and described previously under Mortality, there were no VX-661-related macroscopic findings on GD29.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Mean fetal body weights in the 50 mg/kg/day group were up to 16.5% lower than the mean control values, with mean fetal weights correlated with the slightly lower mean uterus weight. There was no effect on mean post-implantation loss, live litter size, and fetal sex ratios. There were no effects of VX-661 on intrauterine growth and survival for the 10 and 25 mg/kg/day dose groups. Also, there were no effects of VX-661 at any dose on mean numbers of corpora lutea, number of implantation sites, and the mean pre-implantation loss.

Offspring (Malformations, Variations, etc.)

There were no VX-661-related fetal malformations or variations. The findings were infrequent without dose-dependency, and similar to historical control incidences.

Table 14: Summary of Malformations and Variations

Dose (mg/kg/day)	**0**	**10**	**25**	**50**
Number of litters	**22**	**22**	**20**	**19**
Number of fetuses	**192**	**201**	**188**	**172**
Total Number of Malformations: fetuses (liters)	**2 (2)**	**4 (4)**	**3 (3)**	**1 (1)**
External Malformation: fetuses (liters)	**0 (0)**	**1 (1)**	**1 (1)**	**0 (0)**
gastroschisis		1 (1)		
ectodactyly			1 (1)	
External Variations: fetuses (liters)	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**
Visceral Malformations: fetuses (liters)	**0 (0)**	**0 (0)**	**1 (1)**	**0 (0)**
Malpositioned kidney			1 (1)	
Visceral Variations: fetuses (liters)	74 (22)	71 (22)	61 (20)	50 (19)

Multiple findings	No dose-related effects			
Skeletal Malformations: fetuses (liters)	**3 (3)**	**4 (4)**	**1 (1)**	**1 (1)**
Vertebral anomalties	2 (2)	1 (1)		
Rib anomality	1 (1)	1 (1)		
costal cartilage anomaly		1 (1)		
malaligned and/or fused sternebrae		2 (2)	1 (1)	
vertebral centra anomaly				1 (1)
Skeletal Variations: fetuses (liters)	146 (22)	196 (22)	149 (20)	164 (19)
Multiple findings	No dose-related effects			

Study Title: VRT-1189001: A Subcutaneous Embryo-Fetal Development Study in Rabbits with a Toxicokinetic Evaluation

Study no.: **VRT-1189001-TX-006**
Study report location: SD-185, Sept 1, 2016
Conducting laboratory and location: (b) (4)
Date of study initiation: October 3, 2014
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: VX-661 (VRT-1189001) M2 Metabolite, Lot 130207-012, Purity 99.6%
(Footnote in Certificate of Analysis: RT-1189001 lot ELN 130207-012 was produced by combining two samples of manufacturing lot D-200-69 ((b) (4)) that were kept in separate locations under uncontrolled conditions.)

Key Study Findings

- Pregnant rabbits were administered metabolite VRT-1189001 (M2, a disproportionate human metabolite) of VX-661 subcutaneously at doses of 0, 20, 60, or 120 mg/kg/day from GD7 to GD20, and terminated for uterine and fetal examination on GD29. The vehicle consisted of 20% Captisol in Dulbecco's phosphate buffered saline (PBS), 1X without calcium chloride and magnesium chloride).
- At dose levels of VRT-1189001 ≤60 mg/kg/day, there were no effects on uterine implantation data, fetal sex ratios, fetal body weights, or fetal malformations and variations; thus, the embryofetal developmental NOAEL was 60 mg/kg/day, corresponding to an AUC_{0-t} of 213000 ng-h/mL.

- Due to toxicity at the injection site (discoloration, scabbing, and/or abrasions) in the 120 mg/kg/day group, the dose was reduced to 80 mg/kg/day on GD11, 12, or 13, and eventually stopped on GD16, 17, or 18. Similar but less intense or severe findings were observed in the other dose groups including controls. One animal at 60 mg/kg/day and four animals at 120/80 mg/kg/day were euthanized *in extremis* during the treatment period due to severity of the skin lesions.
- There were no maternal effects at 20 mg/kg/day on gestation body weights, body weight gain, food consumption, or macroscopic findings, thus the NOAEL for maternal toxicity was 20 mg/kg/day corresponding to an AUC_{0-t} of 78000 ng-h/mL.

Methods

Doses:	0 (vehicle), 20, 60, or 120/80 mg/kg/day (A correction factor of 1.04 was used to adjust for purity). The dose of 120 mg/kg/day was reduced to 80 mg/kg/day on GD11, 12, or 13 (due to the stagger-start of the study). [The dose levels in this study were based on data from previous studies which included a 5-day SC tolerability study in rabbits ((b) (4) , Study Number 863-172; Vertex Report VRT 1189001-005). The dose levels tested in the 5-day tolerability study were 20, 40, and 80 mg/kg/day. All dose levels were well tolerated, with no VRT-1189001-related clinical signs or effects on body weights or food consumption. The high dose, 120 mg/kg/day, was expected to represent a 2-3 fold margin above the expected clinical exposure.]
Frequency of dosing:	Once daily from GD7 to GD20
Dose volume:	2 mL/kg
Route of administration:	Subcutaneous, in the scapular region on the back of each animal, with doses rotated between four sites on the right and left side of the scapular and lumbar regions
Formulation/Vehicle:	Vehicle, 20% Captisol in Dulbecco's phosphate buffered saline (PBS), 1X without calcium chloride and magnesium chloride
Species/Strain:	Time-mated female New Zealand White Hra:(NZW)SPF (approximately 6 months of age)
Number/Sex/Group:	22/dose group
Satellite groups:	Toxicokinetics, 4/dose group TK animals received their final dose on GD 16.

Study design:

Group Assignments		
Group Number	Dose Level (mg/kg/day)	Number of Time-mated Females
Main Study		
1	0	22
2	20	22
3	60	22
4	120/80[a,b]	22
Toxicokinetic		
5	0	4
6	20	4
7	60	4
8	120/80[a,c]	4

[a]On GD 11, 12, or 13, the dose level was reduced from 120 to 80 mg/kg/day due to toxicity observed at the injection site.
[b]The last day of dosing was GD 15, 16, or 17.
[c]The last day of dosing was GD 16.

Deviation from study protocol: On GD11, 12, or 13 (due to the stagger-start of the study), the dose volume of the high dose groups was reduced to 1.33 mL/kg, resulting in a dose level of 80 mg/kg/day. Three animals in this group were not dosed for 1 or 2 days (#274, GD16 and 17; #280, GD15 and 16; and #283, GD14). In addition, all main study animals in this high dose group received their final dose on GD15, 16, or 17 (due to the stagger-start of the study). No conclusions were made concerning the effect of VRT-1189001 on embryofetal development at doses greater than 60 mg/kg/day, since the 120/80 mg/kg/day doses were not administered throughout the planned gestation period (through GD20).

Observations and Results

Mortality
All animals were observed twice daily

Six animals were euthanized *in extremis* as presented in the following table.

Table 15: Mortalities in the Rabbit Embryofetal Study of VRT-1189001

Animal number	Day of Euthanization	Dose Group (mg/kg/day)	Reason/Comment

268	GD10	120/80	laceration on the left hindlimb
262	GD19	60	severe lesions at the injection sites; no other necropsy findings
276	GD21	120/80	
277	GD14	120/80	
279	GD13	120/80	
285	GD8	120/80	

Findings at the injection sites included discolorations (purple, red, brown, gray, black), scabbing, and abrasions and were observed in the control and VRT-1189001-treated groups but were most prevalent among animals in the 60 and 120/80 mg/kg/day dose groups. The severity in these findings was also greater in these dose groups resulting in morbidity and subsequent euthanasia and eventually the early termination of dosing in the high dose group on GD15 to GD17. The sponsor did not consider these effects a direct effect of VRT-1189001, but in light of findings of a similar nature in rodent and dog toxicity studies, the reviewer does consider these effects related to VRT-1189001.

Clinical Signs
Animals were observed twice daily from GD7-20 (predose and 4 hours postdose) and once daily from GD21 to GD29. A detailed clinical examination was obtained twice daily during the dosing phase and once daily afterwards until termination.

As noted above, injection site findings included discolorations (purple, red, brown, gray, black), scabbing, and abrasions and were observed in the control and VRT-1189001-treated groups but were most prevalent among animals in the 60 and 120/80 mg/kg/day dose groups.

Body Weight
Body weights were measured on GD0, GD4, daily from GD7 to GD21, GD24, and GD29. Adjusted body weight (GD29 body weight minus gravid uterine weight) and adjusted body weight change (GD0 to 29) were calculated.

There was a dose dependent reduction in body weight and weight gain. At 20 mg/kg/day, there was no effect of VRT-1189001 on gestation body weights or body weight change. At 60 mg/kg/day, the mean body weight gain over GD16 to 19 was statistically lower than the mean control (0.019 kg vs. 0.063 kg in controls). At 120/80 mg/kg/day, significant reductions in mean weight gain relative to mean control occurred from GD13 to 16 (0.015 kg vs. 0.089 kg in controls) and from GD16 to 19 (0.013 kg weight loss vs. a 0.063 kg weight gain in controls). The reduction in weight gain corresponded with low food consumption. Mean body weights in the 60 and 120/80 mg/kg/day dose groups were not different to control means throughout gestation.

Feed Consumption
Food consumption for main study animals was recorded daily but was not statistically analyzed.

There was a dose dependent reduction in food consumption. At 20 mg/kg/day, there was no effect of VRT-1189001 on food consumption. For the 60 mg/kg/day dose group, mean food consumption ranged from 7 to 25% lower than mean control values and were statistically different for GD10 to 13 (-20%), GD13 to 16 (-25%), GD16 to 19 (-19%), and GD7 to 21 (-16%). In the 120/80 mg/kg/day dose group, mean food consumption ranged from 5 to 40% lower than mean control values and were statistically different for GD10 to 13 (-24%), GD13 to 16 (-40%), GD16 to 19 (-34%), and GD7 to 21 (-23% lower). Over the posttreatment period (GD21 to 29), mean food consumption in the 60 and 120/80 mg/kg/day dose groups was 6 and 16% higher than mean control values, respectively.

Toxicokinetics

Blood samples (approximately 1 mL) were collected via the jugular vein at predose and at 1, 2, 4, 8, 12, and 24 hours postdose on GD7 and GD20, except the 120/80 mg/kg/day dose group in wich samples were collected on GD7 and GD16. Since the 120 mg/kg/dose was reduced to 80 mg/kg/day on GD11, and was stopped early, on GD16, the exposure values of this group were not compared with the other groups on GD20.

The increase in VRT-1189001 AUC_{0-t} and C_{max} were approximately dose proportional on GD20 but less than dose proportional on GD7. There was minimal accumulation (ratio of 1.20 to 1.26) over the 14 days of dosing. The elimination half-life ranged from 2.90 to 5.18 hours. The mean clearance and volume of distribution of VRT-1189001 ranged from 0.270 to 0.401 L/h/kg and from 1.45 to 1.96 L/kg, respectively, on GD7 and ranged from 0.269 to 0.286 L/h/kg and 1.22 to 1.54 L/kg, respectively, on GD20 for the 20 and 60 mg/kg/day dose groups.

Table 16: Pharmacokinetic Summary for VRT-1189001 in Pregnant Rabbits

Fold-Increase in VRT-1189001 Exposure Compared to Dose Increase					
Analyte	Treatment	Female			
		GD 7		GD 20	
		AUC_{0-t} (ng*hr/mL)	C_{max} (ng/mL)	AUC_{0-t} (ng*hr/mL)	C_{max} (ng/mL)
VRT-1189001	VRT-1189001 – 20 mg/kg/day	61700	18300	78000	22000
	VRT-1189001 – 60 mg/kg/day	169000	45200	213000	57100
	VRT-1189001 – 120/80 mg/kg/day [O]	302000	47300	189000	44000
Number of fold Increase (60 mg/kg/day vs. 20 mg/kg/day)		2.7	2.5	2.7	2.6
Number of fold Increase (120/80 mg/kg/day [O] vs. 20 mg/kg/day)		4.9	2.6	*	*

* Animals from Group 8 were dosed until GD 16.
[O] The dose level for Group 8 was reduced from 120 to 80 mg/kg/day on GD 11. Therefore, a nominal dose of 120 mg/kg/day and 80 mg/kg/day was considered respectively for GD 7 and GD 16.

Dosing Solution Analysis

Homogeneity: Dose levels 20 and 120 mg/kg/day (10 and 60 mg/mL, respectively) yielded average % recovery ± % relative SD of 97.9 ± 1.135 and 100.8 ± 0.356

Concentration: Dose levels were appropriate since the mean concentrations of the 1st, 3rd, 7th, and last formulations prepared for dosing ranged between 95.8 and 102.5% of nominal with percent RSDs ranging between 0.029 and 1.933.

Necropsy

On GD29, animals were euthanized and subjected to a laparohysterectomy. Parameters examined included gravid uterine weight, location of viable and nonviable fetuses, early and late resorptions for each uterine horn, total number of implantations, and the number of corpora lutea on each ovary. Each fetus was individually examined for external malformations and variations. A diagnostic necropsy was performed on three females at 120/80 mg/kg/day (animal numbers 277, 279, and 285) that were euthanized early per veterinarian recommendation.

In the 60 and 120/80 mg/kg/day dose groups, abrasions/scabbing and abscesses that developed during the dosing period were still evident at GD29. There was no effect of VRT-1189001 at the 20 mg/kg/day dose.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

For the 20 and 60 mg/kg/day doses, there was no effect of VRT-1189001 on numbers of corpora lutea, mean number of implantation sites, viable fetuses, nonviable fetuses, litter size, resorption sites (early, late, and total), pre- and post-implantation losses, mean gravid uterine weights, adjusted GD29 body weights, and adjusted body weight change GD0 to 29.

In the 120/80 mg/kg/day dose group, mean gravid uterine weight was statistically lower than controls (0.463 kg vs control 0.558 kg), although there was no effect on uterine implantations or fetal weights. Mean adjusted GD29 body weight and adjusted weight change GD0 to 29 in the 120/80 mg/kg/day dose group were not different than mean control values.

Table 17: Summary of Pregnancy, Uterine and Ovarian Parameters

Dose (mg/kg/day)	**0**	**20**	**60**	**120/80***
N	**22**	**22**	**22**	**22**
Number not pregnant	1	4	2	3
Number Pregnant	21	18	20	19
Pregnancy Index	95%	81.8%	90.0%	86.4%

(%)				
Mortalities	0	0	1	5
Abortions	0	0	0	0
Litters with viable fetuses on day 29	21	18	19	15

* On GD11, 12, or 13 (due to the stagger-start of the study), the dose volume of the high dose groups was reduced to 1.33 mL/kg, resulting in a dose level of 80 mg/kg/day. Three animals in this group were not dosed for 1 or 2 days. In addition, all main study animals in this high dose group received their final dose on GD15, 16, or 17 (due to the stagger-start of the study). No conclusions were made concerning the effect of VRT-1189001 on embryofetal development at doses greater than 60 mg/kg/day, since the 120/80 mg/kg/day doses were not administered throughout the planned gestation period (through GD20).

Offspring (Malformations, Variations, etc.)

There were no effects of VRT-1189001 on fetal external, visceral, or skeletal examinations.

Table 18: Summary of Malformations and Variations

Dose (mg/kg/day)	**0**	**20**	**60**	**120/80***
Number of litters	**21**	**18**	**19**	**15**
Number of fetuses	**200**	**156**	**183**	**119**
Total Number of Malformations: fetuses (liters)	**10 (8)**	**10 (8)**	**15 (6)**	**4 (4)**
External Malformation: fetuses (liters)	**1 (1)**	**1 (1)**	**4 (2)**	**0 (0)**
abnormal forelimb flexure		1 (1)		
omphalocele			1 (1)	
microphthalmia	1 (1)		2 (1)	
Domws head			1 (1)	
External Variations: fetuses (liters)	0 (0)	0 (0)	0 (0)	0 (0)
Visceral Malformations: fetuses (liters)	**2 (2)**	**0 (0)**	**3 (1)**	**0 (0)**
Gallbladder absent			2 (1)	
Diaphragmatic hernia			1 (1)	
Heart, discontinuous interventricular septum			1 (1)	
hydrocephaly			1 (1)	
Dilated aortic arch	1 (1)			
Gallbladder absent	1 (1)			
Visceral Variations: fetuses (liters)	8 (6)	2 (2)	5 (3)	1 (1)

Multiple findings	No dose-related effects			
Skeletal Malformations: fetuses (liters)	**7 (5)**	**9 (8)**	**8 (5)**	**4 (4)**
Multiple findings	No dose-related effects			
Skeletal Variations: fetuses (liters)	119 (21)	77 (18)	91 (19)	54 (15)
Multiple findings	No dose-related effects			

* On GD11, 12, or 13 (due to the stagger-start of the study), the dose volume of the high dose groups was reduced to 1.33 mL/kg, resulting in a dose level of 80 mg/kg/day. Three animals in this group were not dosed for 1 or 2 days. In addition, all main study animals in this high dose group received their final dose on GD15, 16, or 17 (due to the stagger-start of the study). No conclusions were made concerning the effect of VRT-1189001 on embryofetal development at doses greater than 60 mg/kg/day, since the 120/80 mg/kg/day doses were not administered throughout the planned gestation period (through GD20).

9.3 Prenatal and Postnatal Development

Study Title: VX-661: An Oral (Gavage) Pre- and Postnatal Development Study, Including Maternal Function in Rats

Study no.:	VX-661-TX-023
Study report location:	SD-185, Sept 1, 2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 2, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), Lot 17QB10SD.NJ00003, Purity 99.3%

Key Study Findings

- Rats were administered VX-661 by oral gavage at doses of 0, 25, 50, or 100 mg/kg/day from GD6 to LD (lactation day) 20.
- Dosing of the 100 mg/kg/day females was discontinued on LD17 or 18 due to high F_1 pup mortality. One dam in the 100 mg/kg/day dose group was euthanized *in extremis.* In addition, one pregnant dam in the 50 mg/kg/day dose group was found dead on GD21. A cause of death was not determined. There were no effects of VX-661 on maternal survival at 25 mg/kg/day.
- Maternal toxicity at doses ≥ 50 mg/kg/day included reduced body weights during gestation/lactation, reduced gestation weight gain, associated with reduced food consumption. The maternal NOAEL was 25 mg/kg/day.
- At 100 mg/kg/day, there was increased F1 mortality (decreased litter size), and F_1 pup body weights at birth through to weaning were reduced compared to controls. F_1 clinical signs during lactation included decreased activity, thin appearance, skin cold to touch, and skin discolored purple.

- At 50 mg/kg/day, there was decreased pup weights at birth. Also, fertility of F_1 generation was reduced possibly attributed to alteration of estrous cycle duration (increased cycle length and decrease in number of cycles over the 2-week evaluation period). A dose-response was not determined since dosing in HD animals was discontinued early.
- Developmental delays occurred in the F_1 generation at doses ≥50 mg/kg/day. Preweaning developmental delays included pinna detachment, eye opening, and static righting reflexes. In addition, there were sexual maturation delays (vaginal opening and preputial separation) at 100 mg/kg/day. These effects were often associated with reduced body weights in affected pups.
- Postweaning effects were also noted in pups from the high dose group as motor activity (total distance traveled) was increased and F_1 reproductive effects included lower corpora lutea counts, fewer uterine implantation sites, and fewer viable embryos in the GD13 uterine fertility assessments. F_1 pregnancies in the 100 mg/kg/day dose group had too few offspring to enable conclusions about the potential effects of VX-661 on the F_2 generation. There were no effects of VX-661 on F_1 sperm analysis or male reproductive organ weight.
- There were no effects of VX-661 on macroscopic evaluation of F_0 females and F_1 males or females.
- The NOAEL for F_1 offspring postweaning development, behavior, reproduction, and early pregnancy was 25 mg/kg/day. Toxicokinetics were not conducted in this study.

Methods

Doses:	0, 25, 50, or 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	*Vehicle*: 0.5% methylcellulose (400 cps) (w/v), 0.5% sodium lauryl sulfate (SLS) (w/v), and 0.01% simethicone (w/v) in deionized water *Control Article*: [redacted] (b) (4) control article, Hypromellose acetate succinate (HPMC-AS) and SLS,
Species/Strain:	CD® [Crl:CD®(SD)] rats
Number/Sex/Group:	25 pregnant females/dose group
Satellite groups:	None
Study design:	Dosing began on GD6 and continued through to include LD20, with the exception of animals at 100 mg/kg/day, where treatment ended on LD17 or 18.

Group Assignments

Group Number	Dose Level (mg/kg/day)	Number of Time-mated Females
1	0	25
2	25	25
3	50	25
4[a]	100	25

[a]Beginning on LD 17 or 18, animals in the high dose group were no longer treated.

Deviation from study protocol: Dosing of the 100 mg/kg/day females was discontinued on LD17 or 18 due to high F_1 pup mortality. This eliminated the possible use of this higher dose as a potential NOAEL dose for F1 generation developmental and behavioral NOAEL. However, the number of F1 survivors at this dose was low.
Also, early in the study, some F_0 animals exhibiting adverse effects were replaced with alternative animals as indicated in the following table. These changes were made prior to treatment or due to a technical error during dosing. These animal substitutions were not expected to affect the study interpretation or conclusions.

The following females were replaced with females received in the same shipment of animals but not previously assigned to study. All data for the replaced animals are not reported in the data tables but are maintained in the study data.

Dose Level (mg/kg/day)	Animal Number	Reason for Replacement
0	210	Low body weight gain (GD 6 prior to treatment)
25	229	Physical abnormality(GD 6 prior to treatment)
25	235	Animal did not have access to food for approximately 15 hours (GD 6 prior to treatment)
50	254	Animal was found dead (GD 7 dosing related injury, fluid in the thoracic cavity and perforation of esophagus)
100	277	Animal was found dead (GD 7 dosing related injury fluid in the thoracic cavity and perforation of esophagus)

Deviation from study protocol: There were no deviations that affected the study interpretation and conclusions.

Dosing Solution Analysis

Homogeneity: The dosing formulation solutions were homogeneous. The mean recovery was 97.6 to 98.1% of expected values with relative standard deviations less than 1%.

Concentration: The dosing solutions were prepared at the appropriate concentrations. The mean values of the formulation solutions prepared in weeks 1, 3, 4, and 5 ranged

between 85.1 and 102.5% with residual standard deviations between 0.162 and 2.119%. VX-661 was not detected in the control samples.

Observations and Results

F_0 Dams

F_0 Mortality

There were 2 deaths in the 50 mg/kg/day group and 1 death in the 100 mg/kg/day dose group. In the 50 mg/kg/day dose group, animal #253 died on LD18 attributed to a dosing injury evidenced by fluid in the thoracic cavity and perforation of the esophagus. Animal #270, also in the 50 mg/kg dose group, was found dead on GD21 and was pregnant with 8 fetuses and two resorbing fetuses *in utero*. There were no signs or findings that indicated a cause of death and was considered related to VX-661 treatment. In the 100 mg/kg/day dose group, animal #R277 was euthanized *in extremis* on GD13, after losing 37g of body weight between GD6 to GD10 and eating <2 g/day. This animal was pregnant, and the cause of death although not identified was thought to be related to VX-661 treatment.

F_0 Clinical Observations

At dose levels ≥50 mg/kg/day, there was an increase in number of animals that appeared thin during gestation and/or lactation. There were no effects noted in the 25 mg/kg/day group.

F_0 Body Weights and Body Weight Changes

Mean body weight and body weight gain were reduced in the 50 and 100 mg/kg/day during the gestation and lactation.

Table 19: Summary of Body Weights and Weight Gain in F_0 Dams during Gestation and Lactation

Dose (mg/kg/day)	0	25	50	100
Gestation Period (during dosing)				
Body weight (g and % difference from control)				
GD 0	246	3	-5	-11**
GD 4	271	3	-6	-13**
GD 7	306	2	-6	-14**
GD20	347	0	-7*	-16**
Body Weight Change				
GD6-10	16	6	-108**	-13.5
GD 0-14	26	-2	-18	-44
GD 7-20	43	-11	-9	-26
GD6-20	120	-5	-24**	-48

Lactation Period (during dosing)				
LD0	269	-1	-11**	-18**
LD4	288	1	-6	-15**
LD7	302	0	-6	-12**
LD10	313	1	-4	-12**
LD14	321	0	-3	-11**
LD17	319	3	-4	-9**
LD24 (day of weaning)				
* p≤0.05 ** p≤0.01				

F_0 Food Consumption

Food consumption was reduced in the 50 and 100 mg/kg/day during the gestation and lactation and was associated with reductions in body weight and weight gain.

Table 20: Summary of Food Consumption in F_0 Rats during Gestation and Lactation

Dose (mg/kg/day)	0	25	50	100
Gestation Period (during dosing)				
Gestation Food Consumption (g/animal/day and % change from control)				
GD6-10	19	0	-27**	-49**
GD10-14	20	2	-13	-36**
GD14-17	22	2	-13*	-34**
GD17-20	24	5	-10	-33**
GD6-20	21	3	-16**	-38**
Lactation Period (during dosing)				
Lactation Food Consumption (g/animal/day and % change from control)				
LD0-4	32	-0	-21**	-40**
LD4-7	45	-4	-9	-27**
LD7-10	53	-5	-10	-30**
LD10-14	61	-4	-14**	-45**
LD14-17	63	-0	-36**	-64**
LD17-21	76	-1	-26**	-65**
LD0-21	55	-3	-21**	-43**
* p≤0.05 ** p≤0.01				

Maternal (F_0) Macroscopic Examination

There were no effects of VX-661 on macroscopic findings.

F_1 Generation

F_0 Parturition and F_1 Pup Survival

There was no effect of VX-661 on gestation length, number of pups at birth, number of stillborn pups at birth, gestation index, stillborn index, and uterine implantation scar counts. Mean litter size in the 25 and 50 mg/kg/day dose groups on LD4, LD24, and PND28 was similar to the mean control size. However, litter size in the 100 mg/kg/day group was smaller than that of control (10.2 vs control 12.2 pups/litter, $p<0.05$) due to increased pup mortality (mean pup survival LD4 to LD24: control 99.5% vs 100 mg/kg/day 67.2%). No effect of VX-661 on F_1 pup survival over LD0 to LD4 or LD4 to LD21 was observed at 25 and 50 mg/kg/day. In the 100 mg/kg/day dose group, 15/23 F_0 females (65.2%) with viable pups at birth failed to retain pups to LD21 with 10 of these 15 females (66.7%) lost pups failed to retain viable pups in their litter in the first 4 days, to LD4.

There was no effect of VX-661 on F_1 pup sex ratios and this did not differ during the lactation period.

All F_1 pups selected to continue on study in the control and VX-661-treated groups survived to the scheduled termination in the postweaning period.

Table 21: Summary of F_0 Pregnancies and F_1 Litters

Dose (mg/kg/day)	**0**	**25**	**50**	**100**
F_0 Generation				
N	25	25	25	25
N pregnant	25	25	25	25
N Delivering Litters	25	25	24	23
Mean Gestation Length	21.9	21.8	21.8	22.0
F_1 Generation				
Mean No. Pups at Day 0 (Total Pups Born/Litter)	12.4	12.1	12.0	11.8
Mean No. Liveborn Pups/Litter	12.3	12.1	11.7	11.6
Mean No. Stillborn Pups/Litter	0.1	0.0	0.3	0.2
Mean No. Live Pups/Litter Day 4 (Preculling)	12.2	12.0	11.6	10.2*
Mean No. Live Pups/Litter Day 4 (Postculling)	8.0	7.8	7.9	8.0
Mean No. Live Pups/Litter Day 24 (Weaning) [Litter Size]	8.0	7.8	7.9	5.4
Viability Index (Mean %/Litter)	99.04	99.47	88.17	51.79**
Lactation Index (Mean %/Litter) [Mean pup survival LD-4 to LD 24]	99.50	100.00	100.00	67.19*
* $p\leq0.05$ ** $p\leq0.01$				

Table 22: F_1 Body Weights During Lactation

	Males				Females			
Dose Group (mg/kg/day)	**0**	**25**	**50**	**100**	**0**	**25**	**50**	**100**
N of Litters	25	25	24	23	25	25	24	23
Pup Body Weight (g) at Birth (Day 0)	7.15	7.06	**6.30**	**5.75**	6.78	6.70	6.03	5.55
Day 4 (preculling)	11.60	11.71	**10.11**	**8.83**	11.19	11.23	**9.81**	**8.40**
Day 4 (postculling)	11.60	11.71	**10.11**	**8.81**	11.17	11.28	**9.83**	**8.37**
Day 7	18.42	18.37	**16.12**	**12.96**	17.93	17.75	**15.65**	**12.83**
Day 14	37.73	36.40	**31.92**	**21.47**	36.54	35.67	**31.26**	**20.01**
Day 21	62.01	61.00	**57.19**	**41.63**	59.54	58.63	**55.04**	**40.99**
Day 24 (weaning)	79.28	77.72	**73.28**	**54.27**	75.00	74.10	**69.23**	**52.99**
Day 28	105.82	103.95	**97.41**	**75.95**	96.51	96.15	**89.32**	**71.68**
Bolded number are statistically different than control								

F_1 Pup Detailed Clinical Observations

Only pups in the high dose group exhibited clinical changes. These were decreased activity, thin appearance, skin cold to touch and skin discolored purple. These findings were consistent with a failure to thrive and consistent with the high F_1 pup mortality.

There was no effect of VX-661 on postweaning clinical observations of F_1 pups.

F_1 Pup Macroscopic Examinations Through Weaning

There were no obvious effects of VX-661 in macroscopic examinations of stillborn pups, LD4 culled pups (examined externally), pups found dead during lactation and PND28 unselected pups.

Selection of F_1 Pups for Assessments of Sexual Maturation, Behavioral, and Reproductive Performance

On PND28, a minimum of one male and one female pup from each litter in each group was randomly selected to continue on study for assessment of sexual maturation, and behavioral and reproductive performance to provide a maximum of 25 males and 25 females per dose group. If there were less than 25 litters in a group, additional pups were chosen from randomly selected litters. The remaining offspring from each litter were euthanized and subjected to a necropsy. Due to the high pup mortality observed in the 100 mg/kg/day dose group during lactation, all pups surviving to PND28 (24 males and 19 females) were retained for post weaning study.

F_1 Behavioral and Developmental Indices during the Lactation Period

Static Righting Reflex on LD2

Each pup was tested for a complete righting response within a 15-second time period. Pups that did not exhibit the response initially were retested daily until the response was observed.

Pinna Detachment On LD2
Each pup was observed for unfolding of the pinna. Pups that did not have both pinnae detached on LD 2 were observed daily until detachment was complete.

Cliff Aversion On LD11
Prior to eye opening, each pup was tested for cliff aversion. Pups not perceiving depth by moving away from the edge on LD 11 were retested daily until a response was observed.

Eye Opening On LD13
Each pup was observed for eye opening. The development was considered complete when both eyes were fully open. Pups not achieving this landmark on LD13 were observed daily until both eyes were opened.

Air Drop Righting Reflex On LD16
Each pup was tested for air drop righting. On LD16, each pup was tested for air drop rightings recorded as present for those pups that were able to turn over in the air and land upright on all four legs when dropped from a height of approximately 30 cm. Any pup that did not respond on LD16 was tested on a daily basis until the response was observed.

Neuropharmacological Evaluation
On LD21, each pup was given a neuropharmacological evaluation. Parameters evaluated were comparable to those outlined by Irwin (1968).

Auditory Response
Each pup was evaluated at LD22 for auditory (Preyer's) observed for movement of the ears in response to a sound emitted from a Galton whistle at a distance of 25 cm. Three trials were performed.

In the 50 and 100 mg/kg/day groups, there were statistically significant delays in mean age to pinna detachment, eye opening, and static righting response relative to controls. There were no differences from controls in cliff aversion, air drop righting, and auditory response in the 50 and 100 mg/kg/day F_1 pups. There was no effect of VX-661 in the 25 mg/kg/day dose group compared to controls.

Table 23: Developmental Landmarks (Mean Day of Occurrence)

Dose (mg/kg/day)		**0**	**25**	**50**	**100**
N (pups)		25	25	24	23
	Initial Test Day				
Preweaning					
Physical Development					
Pinna detachment	PND 2	2.4	2.7	3.1**	3.3**
Static righting response	PND 2	2.3	2.7	2.8**	3.3**

Eye opening,	PND 13	14.2	14.3	15.0**	17.1**
Postweaning					
Sexual maturation					
Vaginal opening	PND 28	31.2	31.3	31.6	34.1**
Preputial separation	PND 35	41.8	42.6	43.0	46.6**
* p≤0.05 ** p≤0.01					

Sexual Maturation

Vaginal Opening

Beginning on PND28 of age, F_1 female pups were examined for the presence of vaginal opening. Pups that did not demonstrate vaginal opening were examined daily until this landmark was observed. A body weight was measured and recorded on the day each animal achieved this landmark.

Preputial Separation

Beginning on PND35 of age, F_1 male pups were examined for preputial separation. Pups that did not show complete retraction of the penile prepuce were examined daily until this landmark was observed. A body weight was measured and recorded on the day each animal achieved this landmark.

Sexual maturation of F_1 females and males were delayed in the 100 mg/kg/day dose group. The mean age for vaginal opening and preputial separation, 34.1 days and 46.6 days respectively, were statistically longer than the mean control values in days, respectively. Sexual maturation in the 25 and 50 mg/kg/day dose group was similar to that of controls. Mean body weights of F_1 female pups in the 50 and 100 mg/kg/day dose groups at vaginal opening were statistically lower than controls by 7% and 9%, respectively. However in males, body weights at preputial separation were not different than controls.

Measurement of Motor Activity

Motor activity of each pup was assessed at 37 days of age using a Hamilton Kinder Motor Monitor System equipped with an electronic analyzer-recorder. The test period was 20 minutes (four 5 minute intervals per animal). Movement was recorded by 16 photocell sensors. A sensor check was conducted daily during the testing period. Basic movements, fine movements, rearing counts, and total distance were recorded.

The 100 mg/kg/day dose group exhibited an increase in total distance traveled for each recording intervals over the 20-minute testing period compared to controls. There was no effect of VX-661 at 25 and 50 mg/kg/day on motor activity. The 100 mg/kg/day animals were similar to controls in their habituation to the novel environment of the testing chamber over time, so the biological and toxicological significance of this finding is not known.

Table 24: Summary of Motor Activity of F_1

Summary Mean Total Distance Traveled (cm) – Control versus 100 mg/kg/day dose group						
	F1 Males			F1 Females		
Interval (min)	Control	100 mg/kg/day	% difference	Control	100 mg/kg/day	% difference
0-5	2690.7	2956.7[a]	10	2861.4	3230.5[b]	13
5-10	2179.5	2551.8[b]	17	2258.6	2635.1[a]	17
10-15	1823.3	2115.2	16	1740.5	2281.4[b]	31
15-20	1437.0	1820.4[a]	27	1380.4	1922.6[a]	39
0-20	8130.6	9444.1[b]	16	8240.9	10069.6[b]	22
[a] Significantly different from controls; $p<0.05$ [b] Significantly different from controls; $p<0.01$						

Step-through Passive Avoidance Test
Learning and memory were evaluated using the step-through passive avoidance test. Testing initiated between 70 and 85 days of age for each selected rat. The test was conducted in a fully automated, computerized system consisting of light and dark compartments separated by a mechanical door. In each trial, animals moving to the dark compartment were shocked. Animals were considered to have learned the appropriate response (i.e., not to leave the light compartment) if they did not pass into the dark compartment for two consecutive 3 minute trials. Animals were evaluated for a maximum of five trials on the day of testing.

There was no effect of VX-661 on learning and memory from the passive avoidance testing. In both male and female F_1 animals, the incidence of passive animals (i.e., successfully passing the test) was higher than controls in each of the treated groups and the differences were statistically significant in the 50 and 100 mg/kg/day dose groups. Likewise, the incidence of animals passing the test after the minimum of three trials in the treated groups was greater than controls for both sexes and in some instances, these differences too were statistically significant. Therefore in this testing paradigm the treated animal responded better than the controls. However, the toxicological significance of these results based on this single test is unclear.

Reproduction in the F_1 Generation

Premating, Pairing and Postmating Periods
Mean F_1 body weights for both males and females in the low dose group were comparable to controls throughout the F_1 reproductive study. Mean growth rates for all dose groups were similar to controls in the premating period. The actual mean body weights in the 50 and 100 mg/kg/day dose group were statistically lower than controls due to the lower body weights in these groups at selection on PND28. For males, the mean body weight was similar to controls over the pairing and postmating periods (week 8 to 13).

Estrous Cycle Determination

F_1 females were examined daily by vaginal lavage to establish estrous cyclicity from two weeks prior to pairing until evidence of copulate or the cohabitation period ended.

There was no effect of VX-661 on estrous cyclicity of the F_1 animals. The 50 mg/kg/day group, mean cycle length at 5.9 days differed statistically from the control 4.7 days and the mean number of cycles over the 2-week period at 1.8 cycles differed statistically from the 2.2 cycles in the controls, but the high dose group was not different than control values for mean cycle length (5.3 days) and cycles in the 2 week period (2.0 cycles). The applicant suggests that due to "the limitations inherent in the 100 mg/kg/day group" (early termination of maternal treatment on LD17 or 18 and small number of litters providing offspring to continue on study) the lack of a dose response at the high dose cannot be properly evaluated and it's possible the effect observed on cyclicity at the 50 mg/kg/dose is VX-661 related. The reviewer agrees with this assessment.

F_1 Breeding Procedures (Reproductive/Fertility Assessment)
When the selected F_1 animals were at least 80 days of age, males and females of the same treatment group were placed together in the cage of the male at a ratio of 1:1 for mating. The maximum pairing period was 20 days. The day on which positive evidence of copulation was observed was considered GD0. All females with no confirmed mating date that appeared to be nonpregnant on the basis of body weight and shape were euthanized 13 days after the last scheduled pairing day and examined. Females with unconfirmed mating dates that appeared to be pregnant on the basis of body weight and shape were euthanized as identified to prevent delivery in the cage and loss of the litter.

There was no effect of VX-661 on reproductive performance indices of the F_1 animals. Mating indices in the treated groups ranged from 88.0% to 96.0% and were comparable to the 100% in controls. The mean Copulatory Interval ranged from 2.2 to 3.7 days and was comparable to the 2.3 days in the control. Fecundity indices were 96.0, 95.8, 77.3, and 94.1% in the control, 25, 50, and 100 mg/kg/day dose groups, respectively, and were not statistically different. Fertility indices (ratio of pregnant to number paired) of 92% and 84.2% in the 25 and 100 mg/kg/day dose group were comparable to the 96% in controls. The 68% fertility index in the 50 mg/kg/day group differed statistically from the 96% in controls, but was also outside the range of recent historical control data for the laboratory (range: 76% to 100%).

In the 50 mg/kg/day dose group, besides the longer estrous duration and fewer estrous cycles noted above, both the fecundity and fertility indices were lower, 77.3% and 68%, respectively, than controls and the high dose group which was similar to controls.

Gestation
During gestation body weights and weight gain for the F_1 females in the 25 and 50 mg/kg/day dose groups were similar to controls. In the 100 mg/kg/day dose group,

weight gain was also similar to the other groups and controls, although mean body weights throughout gestation were significantly lower (8 to 9%) than controls.

F_1 Pup Macroscopic Examinations
There were no obvious effects of VX-661 in macroscopic examinations of stillborn pups, LD culled pups (examined externally), pups found dead during lactation and PND28 unselected pups.

Uterine and Ovarian Examinations
On GD13, each F_1 female was euthanized by carbon dioxide inhalation, followed by exsanguination, and immediately subjected to a necropsy with emphasis placed on congenital anomalies and uterine examination. The total number of normally developing embryos, resorptions, and the total number of implantations, and the number of corpora lutea on each ovary were obtained.

Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. If no foci were seen, the female was considered nonpregnant.

There were 24, 23, 17 and 16 pregnant females in the control, 25, 50 and 100 mg/kg/day dose groups, respectively, providing 23, 22, 16, and 16 GD13 litters, respectively for evaluation. One female in each of the control, 25 and 50 mg/kg/day groups was pregnant with no confirmed mating date. There was no effect of VX-661 at 25 and 50 mg/kg/day from GD13 on uterine implantations in the F_1 animals. The mean number of corpora lutea, uterine implantation sites, viable embryos and resorption sites per animal and mean post-implantation loss indices in these groups were comparable to controls. The mean pre-implantation loss index was 7.56% and 15.58% in the 25 and 50 mg/kg/day dose groups respectively, and did not differ statistically from the mean control value of 8.62%. Mean pre-implantation loss in the 50 mg/kg/day dose group at 15.58% was outside the range of recent historical control data for the laboratory [Appendix Y: mean of all study values 8.94% (range of individual study values of 5.9 to11.9%)], but considering the variability in this parameter, the toxicological significant of this increase was unclear particularly since the mean number of corpora lutea was reduced in the high dose group. VX-661 at doses of 25 and 50 mg/kg/day had no effects on the mean number of corpora lutea, uterine implantation sites, viable embryos and resorption sites per animal and mean post-implantation loss. There were increases in pre-implantation loss in the 50 and 100 mg/kg/day dose groups. Mean post-implantation loss and mean number of resorption sites in the 100 mg/kg/day group were comparable to controls.

At 100 mg/kg/day, there was a decrease in number of corpora lutea, that may have contributed to fewer uterine implantation sites and fewer viable embryos in this group. The mean pre-implantation loss at 20.20% in the 100 mg/kg/day group was also increased relative to controls at 8.62%. Mean post-implantation loss and mean number of resorption sites in the 100 mg/kg/day group were comparable to controls.

Table 25: F_1 Pregnancy Summary

Dose (mg/kg/day)	0	25	50	100
N	25	25	25	19
Estrous Cyclicity				
Mean Cycle Length (Days)	4.7	5.0	5.9*	5.3
No. of Cycles (Count)	2.2	2.0	1.8*	2.0
Animals with No Cycles	0	1	1	0
Mating				
Copulatory Interval (Days)	2.3	3.7	2.2	3.3
No. of Females with Confirmed	24	23	21	17
No. of Pregnant Females	24	23	17	16
Female Mating Index (%)	100.0	96.0	88.0	89.5
Female Fertility Index (%)	96.0	92.0	68.0*	84.2
Female Fecundity Index (%)	96.0	95.8	77.3	94.1
Pregnancy				
Mean No. Corpora Lutea	18.0	18.0	17.1	15.8*
Mean No. Implantation Sites	16.4	16.6	14.5	12.7**
Mean % Preimplantation Loss	8.62	7.56	15.58	20.20
Mean No. Viable Embryos	15.7	15.7	13.8	12.0**
Mean % Postimplantation Loss	5.01	5.68	5.26	4.51
Mean No. Early + Late	0.7	0.9	0.8	0.7

* p≤0.05
** p≤0.01

Termination of F_1 Males

After the cohabitation period, the F_1 males were individually housed until completion of the uterine examination, then necropsied with emphasis placed on congenital anomalies and sperm analysis.

There was no effect of VX-661 on sperm analyses of the F_1 animals that consisted of mean percent sperm motility, mean caudal epididymal sperm count, mean sperm concentration per gram caudal epididymal tissue and percent abnormal sperm.

F_1 Sperm Evaluation

There was no effect of VX-661 on sperm analyses of the F_1 animals that consisted of mean percent sperm motility, mean caudal epididymal sperm count, mean sperm concentration per gram caudal epididymal tissue, and percent abnormal sperm.

F_1 Macroscopic Examinations

There were no effects of VX-661 on macroscopic examinations of the F_1 animals.

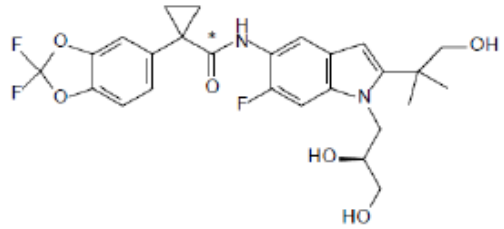
F_1 Organ Weights

There were no effects of VX-661 on F_1 male reproductive organ weights. An increase in relative seminal vesicle weights and decrease in testes weight in the 100 mg/kg/day group, but this was due to a significant reduction (-9%) in body weight of the animals compared to controls.

Study Title: Placental Transfer and Lacteal Excretion of 14C-VX-661 Following Administration of a Single Oral Dose to Pregnant and Lactating Rats

Study no.:	VX-661-TX-023
Study report location:	Module
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 03, 2015
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	^{14}C-VX-661, Lot 157-079-000, Purity >98.3% radiopurity, >98.8% chemical purity, 58.4 mCi/mmol

Structure:



* signifies the position of the radiolabel

Key Study Findings

- Radioactively labelled ^{14}C-VX-661 administered orally to pregnant rats on GD13 or GD18 was detected in fetal tissues. Therefore, it was determined that VX-661 crosses the placenta.
- Radioactively labelled ^{14}C-VX-661 was detectable in milk of lactating rats for up to 72 hours postdose.

Methods

A single oral dose (target 30 mg/kg) of ^{14}C-VX-661 was administered by gavage to pregnant and lactating Sprague Dawley rats (Hsd:Sprague Dawley SD, 242 to 330 g at

Group	Number of Female Animals	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1[a]	7	Oral	30	5	Blood and Carcasses for QWBA
2[b]	7	Oral	30	5	Blood and Carcasses for QWBA
3[c]	21	Oral	30	5	Blood and Milk

QWBA Quantitative whole-body autoradiography.
Note: The dose was approximately 100 µCi/kg.
a Animals were dosed on Day 13 of gestation.
b Animals were dosed on Day 18 of gestation.
c Animals were dosed 6 to 10 days postpartum.

dosing).

Groups 1 and 2 animals were dosed at GD13 and GD18, respectively. Group 3 animals were dose between 6 and 10 days postpartum. Doses stability and concentration were determined on the day of dosing by comparing pre and post measurement of aliquots of ^{14}C-VX-661.

For quantitative whole body autoradiography, one animal/group/time point was prepared for at 1, 2, 4, 8, 24, 48, and 72 hours postdose. Milk was collected from 3 animals/time point at 1, 2, 4, 8, 24, 48, and 72 hours postdose. Litters were culled to 8 pups 4 days postpartum, and to 4 pups the day before milk collection. On the day of collection, the remaining 4 pups were removed from the mothers approximately 4 hours before collection of milk. Animals received a subcutaneous injection of oxytocin before milking to stimulate lactation. The animals were anthestized and milk was collected by a specially constructed milking machine. Following milking, blood was collected. Pups were sacrificed and not examined.

Autoradiography obtained tissue concentrations were interpolated from each standard curve as nCi/g and then converted to ng equivalents/g on the basis of VX-661 specific activity. Blood, plasma, and milk samples were quantitated by a liquid scintillation counter.

Results

^{14}C-VX-661 was stable during the time of preparation and administration and dose formulation was homogenous. Animals received a mean dose of 30.4 mg/kg (109 µCi/kg).

Following oral administration to pregnant rats (Groups 1 and 2), the highest concentrations of radioactivity in blood and plasma in animals dosed on Gestation Day 13 were 6250 and 8930 ng equivalents ^{14}C-VX-661/g, respectively, observed at 2 hours postdose. The highest concentrations of radioactivity in blood and plasma in animals dosed on Gestation Day 18 were 6710 and 9380 ng equivalents ^{14}C-VX-661/g, respectively, also observed at 2 hours postdose. After reaching maximum

concentrations, blood and plasma radioactivity levels in both groups generally declined through 72 hours postdose. Radioactivity in fetal blood was quantifiable through 72 hours postdose in animals dosed on Gestation Day 18, suggesting that placental transfer of radioactivity had occurred.

Tissue Distribution of Radioactivity

For animals dosed on Gestation Day 13, ^{14}C-VX-661-derived radioactivity was distributed to most of the maternal tissues by 1 hour postdose. Most tissues reached maximum concentration by the 2-hour collection time point. The maternal tissues showing the highest maximum concentrations of radioactivity included liver, adrenal gland, pancreas, fat (brown), stomach mucosa, kidney cortex, harderian gland, and kidney. The maternal tissues with the lowest C_{max} values were bone, brain medulla, and spinal cord. Radioactivity was cleared from most maternal tissues by 72 hours postdose. In the whole fetuses, the radioactivity was quantifiable from 1 through 48 hours and was below the limit of quantitation at 72 hours, suggesting placental transfer of radioactivity had occurred.

For animals dosed on Gestation Day 18, ^{14}C-VX-661-derived radioactivity was distributed to most of the maternal tissues by 1 hour postdose. Most tissues reached maximum concentration by the 8-hour collection time point. The maternal tissues showing the highest maximum concentrations of radioactivity included liver, harderian gland, pancreas, fat (brown), adrenal gland, stomach mucosa, kidney cortex, and cecum. The maternal tissues with the lowest C_{max} values were brain medulla, spinal cord, and bone. Radioactivity was not cleared from most maternal tissues by 72 hours postdose. In fetal tissues, radioactivity was distributed across different tissues through 72 hours postdose, although expressed as a ratio of plasma, tissue uptake was negligible or low. However, there was a large increase in tissue/plasma ratio during the 72 hours postdose period in mammary gland (20.6), ovaries (14.2), and preputial gland (38.4), indicative of uptake and sequestering of radioactivity.

Distribution of ^{14}C-VX-661 into Milk

After oral administration to lactating rats, the peak mean concentrations of radioactivity in blood and plasma were 5520 and 8290 ng equivalents ^{14}C-VX-661/g, respectively, observed at 2 hours postdose. The peak mean concentration of radioactivity in milk was 12900 ng equivalents ^{14}C-VX-661/g, observed at 8 hours postdose. After reaching maximum concentrations, the radioactivity in blood, plasma, and milk generally declined through 72 hours postdose. Radioactivity was quantifiable in milk from 1 through 72 hours postdose, and the exposure (AUC_{0-t}) to ^{14}C-VX-661 in milk was approximately 3-fold greater than plasma exposure. Mean milk to plasma concentration ratios of radioactivity after oral dosing to lactating rats ranged from 0.432 at 1 hour to 8.78 at 72 hours, suggesting lacteal excretion of radioactivity increased over time.

Figure 2: Mean concentrations of radioactivity in blood, plasma, and milk at specified times after a single oral administration of ^{14}C-VX-661 to

female rats approximately 10 days postpartum (Group 3, 30 mg/kg) (from applicant's Figure 3)

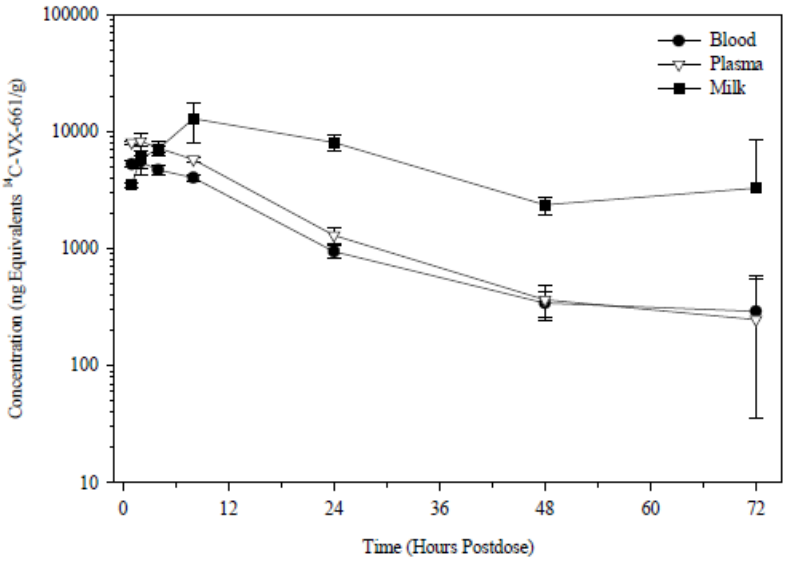


Table 26: Pharmacokinetic parameters for radioactivity in blood, plasma, and milk collected from pregnant or lactating female rats after a single oral administration of ^{14}C-VX-661 (Groups 1, 2, and 3, 30 mg/kg), (from Applicant's Table 8)

Matrix	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	AUC_{0-t} (ng eq·hours/g)	$AUC_{0-\infty}$ (ng eq·hours/g)
		Group 1			
Blood	2	6250	14.4	119000	123000
Plasma	2	8930	12.2	165000	168000
		Group 2			
Blood	2	6710	17.7	148000	158000
Plasma	2	9380	15.6	199000	208000
		Group 3			
Blood	2	5520	15.9	98700	105000
Plasma	2	8290	13.3	138000	143000
Milk	8	12900	33.4	420000	579000[a]

eq Equivalents ^{14}C-VX-661.
a The percentage of $AUC_{0-\infty}$ obtained by extrapolation was >20%; therefore, $AUC_{0-\infty}$ should be interpreted with caution.

11 Integrated Summary and Safety Evaluation

Pharmacology
Pharmacology studies assessed the potency and efficacy of TEZ on the cystic fibrosis transmembrane conductance regulator (CFTR)-mediated chloride secretion from monolayers of human bronchial epithelia (HBE) isolated from the lungs of defective CFTR ion from *F508del*-homozygous CF patients. TEZ increases the delivery and amount of functional CFTR protein at the cell surface, resulting in enhanced functional chloride ions at the membrane surface.

Ivacaftor is a CFTR potentiator that functions to keep the chloride channel open in CF patients with the *G551D* mutation. The sponsor proposes that the combination of tezacaftor and ivacaftor provide an enhanced effect. This was confirmed in in vitro studies using HBE cells from *F508del*-homozygous subjects, whereby the combination of tezacaftor and ivacaftor resulted in increased amount of mature CFTR protein delivered to the cell surface and potentiation of chloride transport as compared to ivacaftor alone.

In safety studies, there were no effects on neurobehavioral parameters and motor activity. Cardiovascular safety studies found that TEZ inhibited hERG I_{Kr} current in a dose-dependent manner, but the maximal dose was limited by solubility to 10 µM that resulted in 16.6% inhibition. In conscious dog studies, oral administration of VX-661 at 250 mg/kg, but not 75 mg/kg (next lower dose) increased arterial blood pressure to 25% and reduced QTc intervals up to 8% during the 6 to 14 hr postdose period. There were no effects at any dose on body temperature, pulse pressure, heart rate, PR interval, or QRS duration. A respiratory study found acute increases in respiratory rate and decreases in tidal volume following administration of 60 and 200 mg/kg, but no change in overall ventilatory capacity (minute volume). However, repeated dose toxicology studies did not reveal any acute respiratory effects. In gastrointestinal safety studies, TEZ was shown to delay gastric emptying of a charcoal test meal in the 100 and 200 mg/kg dose groups, but had no significant effect on intestinal transit.

Pharmacokinetics
In rats and dogs, VX-661 has an oral bioavailability of approximately 50% in rats and up to 77-95% in dogs. It is highly bound to plasma proteins, >98% all species. It has a half-life of approximately 1.2 to 4 hours. TEZ is primarily metabolized by CYP3A. There was no detectable inhibition or induction with CYP450 isoenzymes. The qualitative metabolic profile of TEZ is similar in rats and dogs after oral administration of the compound, and in rat, dog, monkey, and human liver in vitro preparations.

There are 3 major metabolites identified in human plasma; M1, M2, and M5. M1 and M5 are found at comparable levels in the rat or dog, but M2 is a disproportionate metabolite found substantial greater levels in humans than in rats or dogs. Therefore, separate studies were necessary to assess the safety of M2. Since this metabolite was not tolerated when administered to rats, studies were conducted in dogs by subcutaneous administration. M2 produced skin lesions at the site of administration, but no novel

toxicities that weren't present in previous studies. There was a 2-fold safety margin at the NOAEL dose.

General toxicology
The toxicology program was conducted in rats (up to 6 months duration) and dogs (up to 1 year duration). In all studies of rats and dogs, a consistent finding was dilated lymphatic terminals in the gastrointestinal villi of the small intestine (jejunum, ileum, and sometimes the duodenum). This was of concern in early short term studies due to weight loss in most rats and dogs, although they recovered during or after treatment. However, there were no signs of associated adverse findings. The dilation was localized to the terminals, there was no dilation along the length of the lymphatics, and no signs of obstruction in the mesenteric lymph nodes.

Longer term studies in either rats or dogs did not show any changes in serum chemistries and there were no adverse clinical signs associated with dilated lymphatic terminals. Although a NOAEL was determined in dogs for this finding, the recovery period in the rat studies was not long enough to completely eliminate the terminal lymphatic dilation. Additional studies to determine recovery in dogs, found that a 28-day treatment resulted in persistence of the dilated lymphatic terminals for up to a year after treatment was discontinued, with indications at this time of partial recovery. Since there were no clinical signs, no progressive histopathology, and no changes in clinical chemistries, hematology, or urinalysis, and the animal appeared clinically healthy, this finding was eventually considered non-adverse and safety margins were extended to the maximal dose administered in the long term toxicity studies. The mechanism producing this effect is undetermined at present. A clinical endoscopy safety study was conducted early in the drug development program to determine if signs of intestinal lymphangiectasia were present in treatment subjects, but there were no signs indicative of this finding.

Unfortunately a 3-month toxicity study in rats dosed with both VX-661 and VX-770 was poorly designed and it was not possible to adequately assess synergistic effects or to determine a NOAEL. Although sometimes a finding would be dose related to either TEZ or IVA, the fact that there was no contemporary control groups for each compound, made the effects attributed to one or the other drug more tenuous, and required reliance on previously conducted single drug studies that used a different set of dose levels. Therefore, the 3- month rat combination study only assessed any new toxicities and an increase in severity of the toxicities compared to the previous 1-month rat combination studies. There were no new toxicities that developed with the 3-month treatment regimens. The severity of observed toxicities in the various treatment groups appeared to be within the realm of previously characterized TEZ or IVA toxicity profiles. Therefore, despite the low safety margin for a TEZ /IVA clinical dose of 100/150 mg/day, based on AUC values from the rat doses of 20/80 to 80/20 mg/kg/day the available nonclinical data were considered adequate to support clinical dosing.

Genetic Toxicology

VX-661 and M2 had no potential for mutagenesis or clastogenesis when tested in a standard battery of genetic toxicology tests.

Carcinogenicity
VX-661 did not induce tumors when tested in a 2-year carcinogenicity study in rat and in a 6-month Tg.rasH2 transgenic mice.

Fertility and Early Embryonic Development
In a rat study of fertility and early embryonic development, there were no significant adverse effects on reproductive performance and fertility in males and females. The maternal NOAEL was the low dose due to the reduction in body weight and weight gain, and food consumption at the 50 and 100 mg/kg/day doses. Thus, in the presence of weight loss prior to mating there was no effect on fertility or pregnancy.

Embryo-Fetal Development
Embryofetal studies were conducted in rats and rabbits. TEZ was administered orally by gavage to pregnant rats once daily from gestation days (GD) 6 through 17 at doses up to 100 mg/kg/day. At doses ≥50 mg/kg/day, mean body weight loss and reduced mean body weight gain corresponding to reduced group mean food consumption occurred throughout the dosing period (gestation days 6-18). One animal in the 100 mg/kg/day group had an early delivery of one pup on GD21. One animal in the 50 mg/kg/day group was euthanized *in extremis* on GD15 due to substantial weight loss and reduced food consumption. The 50 and 100 mg/kg/day dose groups also had a dose-related increased incidence of red material around the mouth, which was not commonly noted in previous general toxicity studies. Despite these effects there was no effect of TEZ on fetal intrauterine growth and survival. There were no TEZ -related fetal malformations and variations. The NOAEL for maternal toxicity was the low dose of 25 mg/kg/day and the NOAEL for embryofetal developmental was 100 mg/kg/day. In the presence of severe maternal weight loss in rats, there was no effect on embryofetal development.

Pregnant rabbits were administered TEZ by oral gavage at doses up to 50 mg/kg/day from GD7 to GD20, and terminated for uterine and fetal examination on GD29. There were no maternal or fetal effects of VX-661 at doses of 10 and 25 mg/kg/day. However, at 50 mg/kg/day, there was maternal weight loss and reduced food consumption, and associated reduced defecations. Two females in the 50 mg/kg day group aborted dead fetuses on GD23 and GD24, in association with body weight reductions compared to controls. On GD29, fetuses in the 50 mg/kg/day had lower mean weights than the control groups, but there were no effects on fetal intrauterine growth and survival. There were no effects of VX-661 on fetal malformations or variations. The NOAEL for both maternal and embryofetal developmental toxicity was the mid dose of 25 mg/kg/day, due to decreased body weights.

Metabolite M2 (VRT-1189001) Effects on Rabbit Embryo-Fetal Development
Pregnant rabbits were administered metabolite M2, a disproportionate human metabolite of TEZ, subcutaneously at doses of up to 120 mg/kg/day from GD7 to GD20,

and terminated for uterine and fetal examination on GD29. The vehicle consisted of 20% Captisol in Dulbecco's phosphate buffered saline (PBS), 1X without calcium chloride and magnesium chloride. Due to toxicity at the injection site (discoloration, scabbing, and/or abrasions) in the 120 mg/kg/day group, the dose was reduced to 80 mg/kg/day on GD 11, 12, or 13, and eventually stopped on GD 16, 17, or 18. Similar but less intense or severe findings were observed in the other dose groups, including controls.

One animal at 60 mg/kg/day and four animals at 120/80 mg/kg/day were euthanized *in extremis* during the treatment period due to severity of the skin lesions. There were no maternal effects at 20 mg/kg/day on gestation body weights, body weight gain, food consumption, or macroscopic findings. At doses ≤60 mg/kg/day, there were no effects on uterine implantation data, fetal sex ratios, fetal body weights, or fetal malformations and variations. The NOAEL for maternal toxicity was 20 mg/kg/day and the NOAEL for embryofetal development toxicity was 60 mg/kg/day.

The M2 metabolite had toxic effects at the site of administration as described in previous general toxicity studies. Despite this local toxicity, there was no effect on embryofetal development and M2 was not teratogenic at these doses.

Pre- and Postnatal Development Study in Rats

Rats were administered VX-661 by oral gavage at doses up to 100 mg/kg/day from GD6 to LD (lactation day) 20. Dosing of the 100 mg/kg/day females was discontinued on LD 17 or 18 due to high F_1 pup mortality. One female in the 100 mg/kg/day dose group was euthanized *in extremis.* In addition, one female in the 50 mg/kg/day dose group was found dead on GD21, with undetermined cause of death. There were no effects of TEZ on survival and growth at the 25 mg/kg/day dose. At doses ≥ 50 mg/kg/day, TEZ -related effects on F_0 females included lower body weights during gestation/lactation, lower gestation weight gain, and lower food consumption. In addition, at 100 mg/kg/day, TEZ -related effects on F_0 females included increased incidence in thin appearance. The substantial maternal body weight loss was reflected in F_1 low birth weights, failure to gain weight and a failure to thrive. F_1 pup mortality was increased in the early days following birth in the 100 mg/kg/day group. At this dose level, F_1 clinical signs during lactation included decreased activity, thin appearance, skin cold to touch and skin discolored purple. In a separate study, TEZ was detected in the milk of lactating rats. However, it is unclear if mortality was due to TEZ associated suckling deficiency, nutrient deficiency, or some other effect.

There were no effects of TEZ on sexual maturation, behavioral assessments (passive avoidance and motor activity) or GD 13 uterine implantation data for the F_1 generation at doses of 25 and 50 mg/kg/day. However, at 50 mg/kg/day, the fertility index was reduced with effects noted on estrous cyclicity (increased cycle length and decrease in number of cycles over the 2-week evaluation period). Further, F_1 animals at ≥50 mg/kg/day had preweaning developmental delays (pinna detachment, eye opening, and static righting reflexes), and at 100 mg/kg/day, delays in sexual maturation (vaginal

opening and preputial separation). These effects were often associated with reduced body weights in affected pups.

Motor activity was increased as reflected in the total distance traveled. Reproductive effects of F_1 included lower corpora lutea counts, fewer uterine implantation sites and fewer viable embryos in the GD 13 uterine assessments for fertility. F_1 pregnancies in the 100 mg/kg/day dose group had too few offspring to enable conclusions about the potential effects of TEZ on the F_2 generation. There were no effects of TEZ on F_1 sperm analysis or male reproductive organ weight and no effect on macroscopic evaluation of F_0 females and F_1 males or females. The NOAEL for F_0 females was 25 mg/kg/day. The NOAEL for F_1 offspring postweaning development, behavior, and reproduction and early pregnancy was also 25 mg/kg/day.

Since there were few offspring in the 100 mg/kg/day group, interpretation of the results from this group were inconclusive regarding embryofetal development of the F_2 generation. This carried over into the 50 mg/kg/day dose group findings as well, when significant effects noted at this dose (potentially dose-dependent), were not confirmed because high dose findings were similar to control values. It was not possible to distinguish between random data outliers in the 50 mg/kg/day group or inadequate fetal numbers in the 100 mg/kg/day dose groups.

Animal:Human Exposure Margins

Human Exposure

For determination of exposure margins, human exposure values of TEZ were obtained from Clinical Report VX13-661-103 in which 15 cystic fibrosis patients (8 males, 7 females) with F/F mutatation in Group 2 received the proposed therapeutic dose of 100 mg TEZ (q24h) and 300 mg IVA (150 mg q12h). The relevant exposure AUC_{0-24} for TEZ, M1, and M2 were summed to provide a total exposure estimate. The table of exposure margins for the fertility and reproductive and development findings are presented below. Both the fertility and early embryonic developmental study and the pre- and postnatal development study did not collect corresponding toxicokinetic data. The Applicant provided the source of the values used during labeling discussions which were appropriate and are presented in the table below.

Table 27: Summary of Human Exposure

	TEZ		
	TEZ (µg-h/mL)	**M1-TEZ (µg-h/mL)**	**M2-TEZ (µg-h/mL)**
CF patients* AUC_{0-24}	**85.9**	**127**	**121**
Total	**334**		
*from Clinical Report VX13-661-103; Blood samples were obtained on dosing day 29 of a 12-week study. Day 85 PK parameters were similar to day 29 results.			

Table 28: Animal:Human Exposure Ratios for TEZ

Study	NOAEL (mg/kg/day)	VX-611+ M1+M2 AUC (µg-h/mL)		Exposure Margin*	
		M	F	M	F
General Toxicology					
6-Month Rat	**100**	**700**	**876**	**2.1**	**2.6**
1-Year Dog	**200**	**1545**	**2261**	**4.6**	**6.8**
3-Month Rat combination (VX-661/770)	**VX661 80/ /VX770 20**	**682**	**814**	**2.0**	**2.4**
Carcinogenicity					
2-year Rat	**M: 50, F: 75**	**668**	**829**	**2.0**	**2.5**
Fertility and Early Embryonic Development					
Rat, Maternal	25	-	188	**0.6[a]**	
Female Fertility	100	-	880 (630)[a]	**2.6[a] (3.0)[a]**	
Rat, Paternal	25	232[a]	-	**0.7[b]**	
Male Fertility	100	703 (650)[b]	-	**2.1[b]**	
Embryofetal Development					
Rat, Maternal	25	-	154	**0.5**	
Embryofetal	100	-	630	**1.9[c] (2.9)[c]**	
Rabbit, Maternal	25	-	50	**0.15**	
Embryofetal	25	-	50	**0.15**	
M2 Metabolite					
Rabbit, Maternal	20	-	78	**0.6**	
Embryofetal	60	-	213	**1.8**	
Pre- and Postnatal Development					
Rat, Maternal;	25	**-**	154[d]	**0.5 (0.7)[e]**	
F_1, Postnatal Growth and Survival	25	**-**	154[d]	**0.5 (0.7)[e]**	
F_1, Early Physical Development	50	**-**	333[d]	**1.0 (1.5)[e]**	
F_1, Fertility	50	**-**	333[c]	**1.0 (1.5)[d]**	

* based on human AUC_{0-24} 334 ug-h/mL for the sum of VX-661, M1 and M2 metabolites from Clinical Report VX13-661-103

[a] Values for females were obtained from the rat embryofetal study VX-661-TX-008. The applicant values differs since M2 was excluded from the human exposure due to the absence of M2 TK quantification in the rat study. There is minimal biological s gnificance in the exposure values.

[b] Values for males came from 6-month rat study VX-661-TX-012 since TK was not conducted for the fertility and early embryonic development study. TK values for the same dose levels in studies of 1-, 3-, and 6-months duration were generally similar, and the 6-month study included determination of M1 and M2 metabolites. The applicant eliminated the M2 values form their caculations, resulting in a minimal difference in exposure margin that has negligible biological significance.

[c] Value obtained from rat embryofetal developmental study VX-661-TX-008 at 100 mg/kg/day dose, divided by sum of TEZ + M1 + M2. Applicant's value is larger 2.9 vs .9 due to the elimination of M2 from the total human exposure since M2 was not quantitated in the rat developmental study.

[d] Values from GD 17 of the rat embryofetal development study VX-661-TX-008. Although a different dose 30 mg was used in the study VX-661-TX-023 of VX-661 of X-661 distribution into tissues including milk, blood concentrations of VX-661 in that study were approximately expected from interpolation of values in VX-661-TX-008 transfer of VX-661.

[e] The applicant also used values from the embryofetal study VX-661-TX-008, but resulted in an exposure margin of 0.73 and 1.56, for doses of 25 and 50 mg/kg/day, respectively, due to exclusion of M2 in the human PK assessment since M2 was not determined in the embryofetal study. Both are within the clinical therapeutic range ith negligible biological significance between the values.

In summary, the nonclinical program for Symdeko is complete and adequate to support commercial therapeutic use for the treatment of cystic fibrosis. There are no outstanding nonclinical issues at this time.

12 Appendix/Attachments

Not applicable

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

/s/

LAWRENCE S LESHIN
01/29/2018

CAROL M GALVIS
01/29/2018
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:	210491
Supporting document/s:	1
Applicant's letter date:	June 28, 2017
CDER stamp date:	June 28, 2017
Product:	Symdeko™ (Tezacaftor and Ivacaftor)
Indication:	Cystic Fibrosis (CF)
Applicant:	Vertex Pharmaceuticals Incorporated
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Eleni Salicru, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Jessica Lee, PharmD

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Vertex Pharmaceuticals Incorporated (Vertex) submitted a 505(b)(1) New Drug Application (NDA) for tezacaftor (VX-661)/ivacaftor (VX-770) combination therapy for the treatment of cystic fibrosis (CF) in patients 12 year of age and older who are homozygous for the F508del mutation or who have at least one mutation in the CF transmembrane conductance regulator (CFTR) gene that is responsive to tezacaftor/ivacaftor, based on in vitro data and/or clinical evidence. Ivacaftor was approved for the treatment of CF on January 31, 2012. The carcinogenic potential of ivacaftor was reviewed under NDA 203188; ivacaftor was not tumorigenic in either 2-year rat or mouse studies. This review will focus on the carcinogenicity evaluation of tezacaftor (VX-661) in a 2-year rat study and a 26-week Tg.rasH2 mouse study. The Executive Carcinogenicity Assessment Committee (ECAC) concurred with the doses and designs of the studies (see Special Protocol Agreements dated December 18, 2013 and November 20, 2014).

1.2 Brief Discussion of Nonclinical Findings

In a 104-week oral (gavage) carcinogenicity study, Sprague-Dawley (SD) male rats (70 per group) received doses of 0, 5, 15, and 50 mg/kg/day VX-661 and SD female rats (70 per group) received doses of 0, 5, 20, and 75 mg/kg/day VX-661. Although there were no drug-related effects on mortality, both male and female groups were terminated early (i.e., Week 103 and Week 101, respectively) because the number of surviving animals in the respective control groups reached ≤20. At the end of the study period, mean body weights for high dose males and females were 5.9% and 17.7% lower, respectively, compared to control animals. There were no statistically significant drug-related tumor findings in male or female rats. The most notable non-neoplastic histopathology findings were dilated lymphatics in the gut-associated lymphoid tissue (GALT) and small intestine (ileum and jejunum). VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) was 180 and 337 mcg*hr/mL, in males and females, respectively, resulting in exposure margins about 2.4-fold and 4.5-fold above the clinical dose.

In a 26-week oral carcinogenicity study, Tg.rasH2 male and female mice received doses of 0, 30, 100, and 500 mg/kg/day VX-661 (25/sex/group) and 1000 mg/kg/day of the positive control urethane (10/sex/group). There was a statistically significant dose response relationship in mortality for high dose males (76% survival) compared to control males (96% survival). There were no statistically significant drug-related tumor findings in male or female mice. The most notable non-neoplastic histopathology findings were in the adrenal glands (cortex hypertrophy, cortex vacuolation, and x-zone degeneration), liver (centrilobular hypertrophy, centrilobular hepatocellular single cell necrosis, subcapsular inflammation, focal hepatocellular necrosis, focal lipid infiltration, and periportal vacuolation), ovaries (decreased corpora lutea), and jejunum (lamina propria vacuolation). VX-661 AUC exposure at the high dose (500 mg/kg/day) was about 93 and 154 mcg*hr/mL in males and females, respectively.

VX-661 is extensively metabolized (mainly by CYP3A4) in humans and nonclinical species. Dehydrogenation of VX-661 leads to the major metabolite M1 (VRT-0996107), which has been detected in rat, dog, monkey and humans. M1 constitutes approximately 38.8% of total systemic exposure in humans. M1 is pharmacologically active with similar potency and efficacy as VX-661. M1 was found to be a major circulating metabolite in rats. Thus, exposures in rats in the rat toxicity studies reached sufficient levels to provide an assessment of its toxic potential in humans. In the 2-year carcinogenicity study with rats, M1 AUC exposure at the high dose of VX-661 (50 mg/kg/day in males and 75 mg/kg/day in females) was 288 and 479 mcg*hr/mL, in males and females, respectively, resulting in exposure margins about 2.5-fold and 4.2-fold above the clinical exposure. Exposure to M1 was quantified in the 26-week Tg.rasH2 mouse carcinogenicity study. Thus, the 2-year rat and 26-week mouse studies provides an adequate assessment of the carcinogenic potential of M1.

Sequential oxidation of M1 forms metabolite M2 (VRT-1189001), which has been detected in humans, rats, and dogs. M2 is a disproportionate human metabolite (i.e., high levels in humans [approximately 35.7% of total systemic exposure] and low levels in rats and dogs). M2 is pharmacologically active but less so than VX-661. The Applicant found that oral administration of M2 to rats, guinea pigs, and dogs had poor bioavailability. Intravenous administration of M2 resulted in deaths in rats. Further, subcutaneous administration was not tolerated in rats. In a 1-month toxicity study, dogs could tolerate a subcutaneous dose that produced an approximate exposure to the expected therapeutic human dose. As with rats, it is likely also for mice that an oral M2 dose group would not achieve relevant exposures, and a subcutaneous administration may not be tolerated, although this has not been attempted. Thus, it was judged that the carcinogenic potential of the M2 metabolite could not be studied. M2 was negative for potential genetic toxicity by the bacterial reverse mutation assay and chromosomal aberration assay with human peripheral blood lymphocytes. In the 2-year carcinogenicity study with rats, M2 AUC exposure at the high dose of VX-661 (50 mg/kg/day in males and 75 mg/kg/day in females) was 20 and 13 mcg*hr/mL, in males and females, respectively, resulting in exposure margins about 0.19 and 0.12 of the clinical exposure. Exposure to M2 was quantified in the 26-week Tg.rasH2 mouse carcinogenicity study. Exposures to M2 in the 2-year rat and 26-week mouse studies were approximately ≤0.2 of the achieved clinical exposure. No further nonclinical assessment of the carcinogenic potential of M2 is required.

M5 (VRT-1074233) was detected in a human mass balance study at >10% of total systemic exposure. M5 is derived from phosphorylation of M1, and can potentially interconvert back to M1. In vitro studies in cultured F508del-HBE showed that M5 was inactive. M5 has not been routinely monitored in nonclinical studies with rats or dogs. The Applicant provided data that M5 is formed in rats from a single dose study, but not in repeat dose studies. It was judged that M5 was formed in sufficient levels in rats to provide an assessment of its toxic potential in humans. Thus, it is reasonable to assume that the 2-year rat study provides an adequate assessment of the carcinogenic potential of M5. M5 has not been studied for potential genotoxicity.

No statistically significant neoplastic findings were observed in male or female SD rats and male or female Tg.rasH2 mice treated with tezacaftor at maximum tolerated doses (MTDs).

The ECAC concurred that both the 2-year rat and 26-week Tg.rasH2 carcinogenicity studies were adequate and that there were no drug-related neoplasms in males or females in either study. The ECAC also concurred that based upon feasibility and the completed carcinogenicity studies in rats and Tg.rasH2 mice, that no further studies were required for the safety qualification of the M1, M2, and M5 metabolites with respect to carcinogenicity.

1.3 Recommendations

The final study results for the 2-year rat and the 26-week Tg.rasH2 mouse carcinogenicity studies were presented to the ECAC on November 14, 2017. The ECAC recommendations and conclusions are included below.

2-Year SD Rat

- The Committee concurred that the study was adequate, noting prior ECAC approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 2-year rat carcinogenicity study in either males or females.

26-Week Tg.rasH2 Mouse

- The Committee concurred that the study was adequate, noting prior ECAC approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 26-week Tg.rasH2 mouse study in either males or females.

Human metabolites >10% of total systemic exposure

- Based upon feasibility and the completed carcinogenicity studies with VX-661 in SD rats and Tg.rasH2 mice, the Committee concurred that no further studies were required for the safety qualification of the M1, M2, and M5 metabolites with respect to carcinogenicity.

1.3.1 Approvability

See Dr. Leshin's Pharmacology and Toxicology Review of NDA 210491.

1.3.2 Additional Non Clinical Recommendations

See Dr. Leshin's Pharmacology and Toxicology Review of NDA 210491.

1.3.3 Labeling

Recommended labeling for carcinogenicity studies is provided at the end of this review.

2 Drug Information

2.1 Drug

CAS Registry Number: 1152311-62-0

Generic Name: tezacaftor

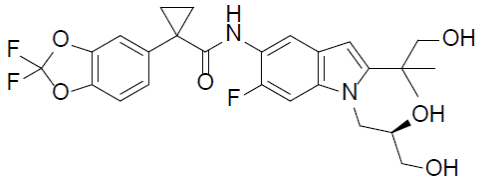
Code Name: VX-661

Chemical Name: 1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1Hindol-5-yl}cyclopropane-1-carboxamide

Molecular Formula: $C_{26}H_{27}N_2F_3O_6$

Molecular Weight: 520.50 g/mol

Structure or Biochemical Description:



Pharmacologic Class: See Dr. Leshin's Pharmacology and Toxicology Review of NDA 210491.

2.2 Relevant INDs, NDAs, BLAs and DMFs

The following are relevant INDs and NDAs by Vertex for tezacaftor and/or ivacaftor:

- IND-074633: KALYDECO® (ivacaftor)
- IND-108105: tezacaftor (VX-661)

- NDA-203188: KALYDECO® (ivacaftor)
- NDA-206038: ORKAMBI® (ivacaftor and lumacaftor)
- NDA-207925: KALYDECO® (ivacaftor)

2.7 Regulatory Background

Under IND 108105, the Sponsor submitted Special Protocol Assessment (SPA) requests for the 2-year carcinogenicity study in rats and the 6-month carcinogenicity

study in Tg.rasH2 transgenic mice. The Division received the Sponsor's original SPA request for the 2-year rat study on November 4, 2013 (Supporting Document Number [SDN] 47). On December 5, 2013 (SDN 52), the Division received an amendment to the 2-year rat study protocol under the SPA, with revisions for the accurate justification of dose selection and the correct proposed doses for the study (i.e., 0, (b) (4) and 50 mg/kg/day). The SPA was reviewed by the ECAC on December 18, 2013, and the ECAC did not concur with the Sponsor's proposed doses. Instead, the ECAC recommended doses of 0, 5, 15, and 50 mg/kg/day in males and 0, 5, 20, and 75 mg/kg/day in females, based on a MTD. The ECAC also suggested that the Sponsor consider testing the major human metabolite VRT-1189001 (M2) in the study, if possible (e.g., at a tolerated dose). See Special Protocol Agreement dated December 18, 2013 for additional details. Of note, carcinogenicity studies to assess the safety qualification of the metabolites were not feasible due to the route of administration.

The Division received the Sponsor's SPA request for the 6-month transgenic mouse study on September 30, 2014 (SDN 80). On October 10, 2014, the SPA was denied because it did not contain the information necessary to support protocol review and dose selection for the proposed carcinogenicity study (i.e., the study report for the 28-day repeat dose oral toxicity and toxicokinetic (TK) study in CByB6F1 mice with a preliminary 5-day range-finding toxicity study). On October 15, 2014 (SDN 84), the Division received the Sponsor's SPA resubmission with the appropriate information. The SPA was reviewed by the ECAC on November 20, 2014 and the ECAC did not concur with the Sponsor's proposed doses of 0, 30, 100, and (b) (4) mg/kg/day. Instead, the ECAC recommended doses of 0, 30, 100, and 500 mg/kg/day based on mortality at 1500 mg/kg/day. See Special Protocol Agreement dated November 20, 2014 for additional details.

3 Studies Submitted

3.1 Studies Reviewed

VX-661: 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice (Sponsor Study Number: VX-661-TX-019; GLP-compliant)

VX-661: A 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Sponsor Study Number: VX-661-TX-020; GLP-compliant)

3.3 Previous Reviews Referenced

NDA 203188

Nonclinical review of GLP-compliant 24-month oral carcinogenicity studies in mice (Sponsor Study Number: VX-770-TX-013) and rats (Sponsor Study Number: VX-770-TX-014) with VX-770 (KALYDECO®; ivacaftor) dated January 3, 2012

IND 108105

Special Protocol Agreement (i.e., ECAC meeting minutes) dated December 18, 2013, for SPA of 2-year carcinogenicity study in rats

Special Protocol Agreement (i.e., ECAC meeting minutes) dated November 20, 2014, for SPA of 6-month carcinogenicity study in Tg.rasH2 mice

Nonclinical review dated October 15, 2015 (response to carcinogenicity update)

8 Carcinogenicity

Study title: VX-661: A 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Study no.:	Conducting laboratory: 863-159 Sponsor: VX-661-TX-020
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 2, 2014 (protocol signed by Study Director)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), lot # 17QB10SD.NJ00003, 99.3% purity
CAC concurrence:	Yes (see Special Protocol Agreement under IND 108105, dated December 18, 2013)

Key Study Findings

- In a 104-week oral (gavage) carcinogenicity study, SD male rats received doses of 0, 5, 15, and 50 mg/kg/day VX-661 and SD female rats received doses of 0, 5, 20, and 75 mg/kg/day VX-661.
- There were no drug-related effects on mortality, but male and female groups were both terminated early (i.e., Week 103 and Week 101, respectively) because the number of surviving animals in the respective control groups reached ≤20.
- At the end of the study period (i.e., Week 103 for males and Week 101 for females) mean body weights for high dose males and females were 5.9% and 17.7% lower, respectively, compared to control animals.
- There were no statistically significant drug-related tumor findings in male or female rats.
- The most notable non-neoplastic histopathology findings were dilated lymphatics in the GALT and small intestine (ileum and jejunum). Additional non-neoplastic lesions were seen in the parathyroid glands, liver, Harderian glands, clitoral glands, ovaries, and sternum bone marrow.
- VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) was 180 and 337 mcg*hr/mL in males and females,

respectively. Exposures to VX-661 metabolites, VRT-0996107 (M1) and VRT-1189001 (M2), were also assessed in rats.

Adequacy of Carcinogenicity Study

- The ECAC concurred with the doses and design of the study (see Special Protocol Agreement dated December 18, 2013).
- The duration of treatment was adequate (i.e., 102 weeks for males and 100 weeks for females).

Appropriateness of Test Models

- The SD rat is considered an acceptable model for 2-year carcinogenicity studies.
- The SD rat achieved high exposures to VX-661 and its M1 metabolite. Exposure to the M2 metabolite was low. Exposure to the M5 metabolite (VRT-1074233) was not measured.

Evaluation of Tumor Findings

- There were no statistically significant drug-related tumor findings in male or female rats.

Methods

Doses:	0, 5, 15 or 50 mg/kg/day (males) 0, 5, 20, or 75 mg/kg/day (females)
Frequency of dosing:	Once daily for up to 102 weeks (males) or 100 weeks (females)
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Vehicle: 0.5% methylcellulose (400 cps) (w/v), 0.5% sodium lauryl sulfate (SLS) (w/v), and 0.01% simethicone (w/v) in deionized water (b) (4) control article: Hypromellose acetate succinate, also known as hydroxyproplymethylcellulose acetate succinate and SLS
Basis of dose selection:	Dose selection was based on the MTD identified from the 3-month (Study No. VX-661-TX-010) and 6-month (Study No. VX-661-TX-012) repeat dose oral toxicity studies in SD rats, in consultation with the ECAC. For males, the recommended high dose (MTD) of 50 mg/kg/day was selected based on lower relative body weights at 100 mg/kg in the 6-month study. The high dose (MTD) of 75 mg/kg/day for females was approximately one-third the lethal dose of 200 mg/kg/day in the 1-month study. Mid- and low-doses in male and female groups were selected to provide an approximately 3-fold dose separation based on systemic exposure.
Species/Strain:	Rat/SD (Crl:CD®[SD])
Number/Sex/Group:	70 (main study) 12 (TK)
Age:	About 8 to 9 weeks of age at start of dosing
Animal housing:	Two to three per cage (same sex) and pair-housed (same sex) in solid bottom cages with nonaromatic bedding in an environmentally controlled room
Paradigm for dietary restriction:	No dietary restrictions. Animals had access ad libitum to Block Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc.) and tap water via an automatic watering system
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	TK (12/sex/group)

Deviation from study protocol: Protocol deviations were presented in the study report and were deemed to have not affected the quality or integrity of the study

Observations and Results

Mortality

Cageside observations for morbidity and mortality were made for all animals, at least twice daily. There were no drug-treated effects on mortality (see Statistical Review and Evaluation for more details). Male and female groups were both terminated early (i.e., Week 103 and Week 101, respectively), because the number of surviving animals in the respective control groups reached ≤20. The number of drug-treated male and female animals that experienced early deaths (i.e., euthanized in extremis, found dead, or died after dosing) was similar across the control and all drug-treated dose groups (see **Table 1**). Survival curves for control and drug-treated male and female groups were similar, as shown in the Kaplan-Meier survival curves below (**Figure 1** and **Figure 2**, respectively).

Table 1 Number of Early Deaths versus Animals Surviving until Terminal Necropsy in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

VX-661 (mg/kg/day) (Males/Females)	Males		Females	
	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]
0/0	50 (71%)	20 (29%)	51 (73%)	19 (27%)
5/5	49 (70%)	21 (30%)	53 (76%)	17 (24%)
15/20	54 (77%)	16 (23%)	47 (67%)	23 (33%)
50/75	45 (64%)	25 (36%)	49 (70%)	21 (30%)

Abbreviations: # = number

Notes:

[1]# of Early Deaths = euthanized in extremis, found dead, or died after dosing

[2]numbers in parentheses represent the percent when compared to the number of main study animals at the start of the study (i.e., 70 animals/sex/group)

Figure 1 Kaplan-Meier Survival Curves for Males in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (FDA Statistical Reviewer's Figure)

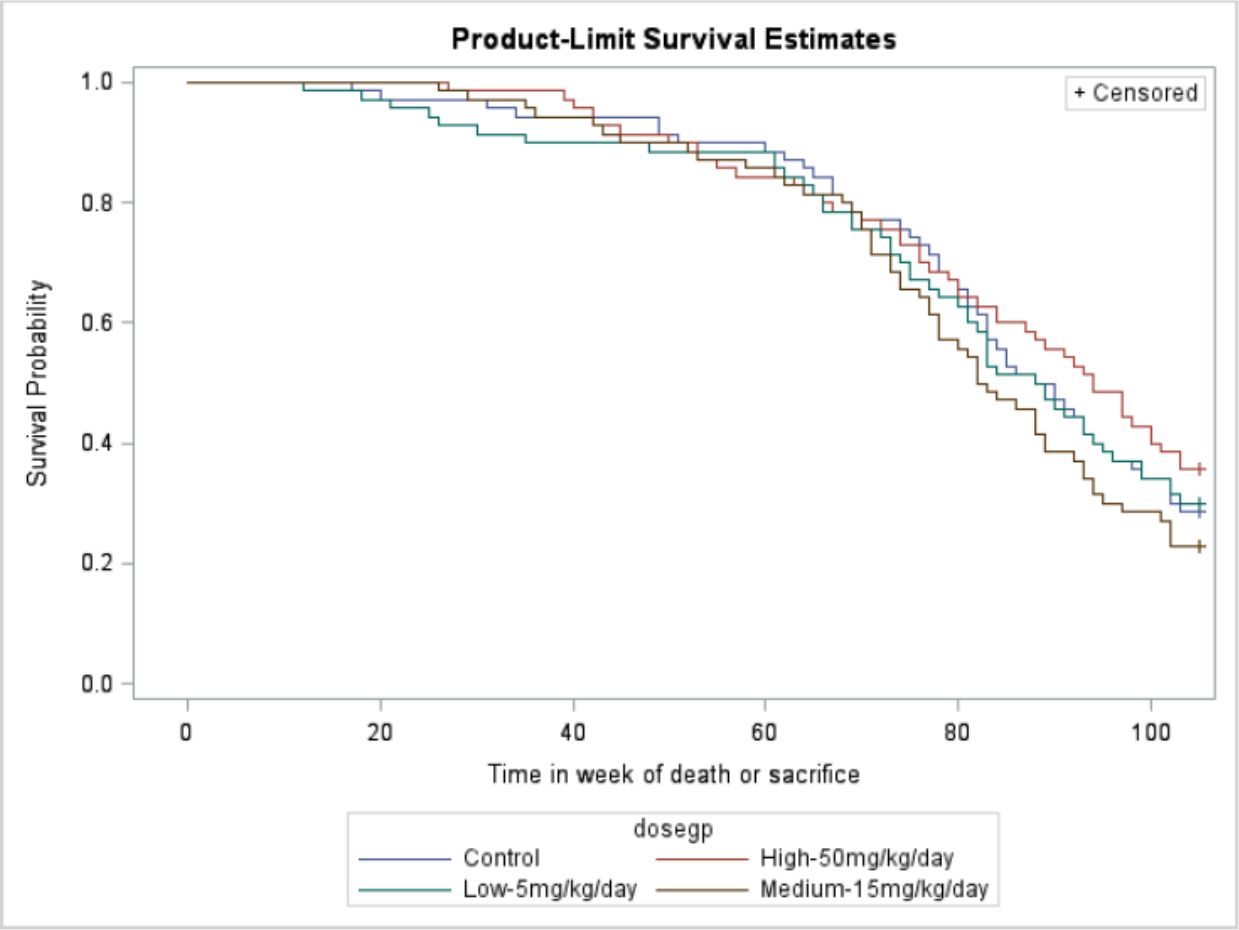
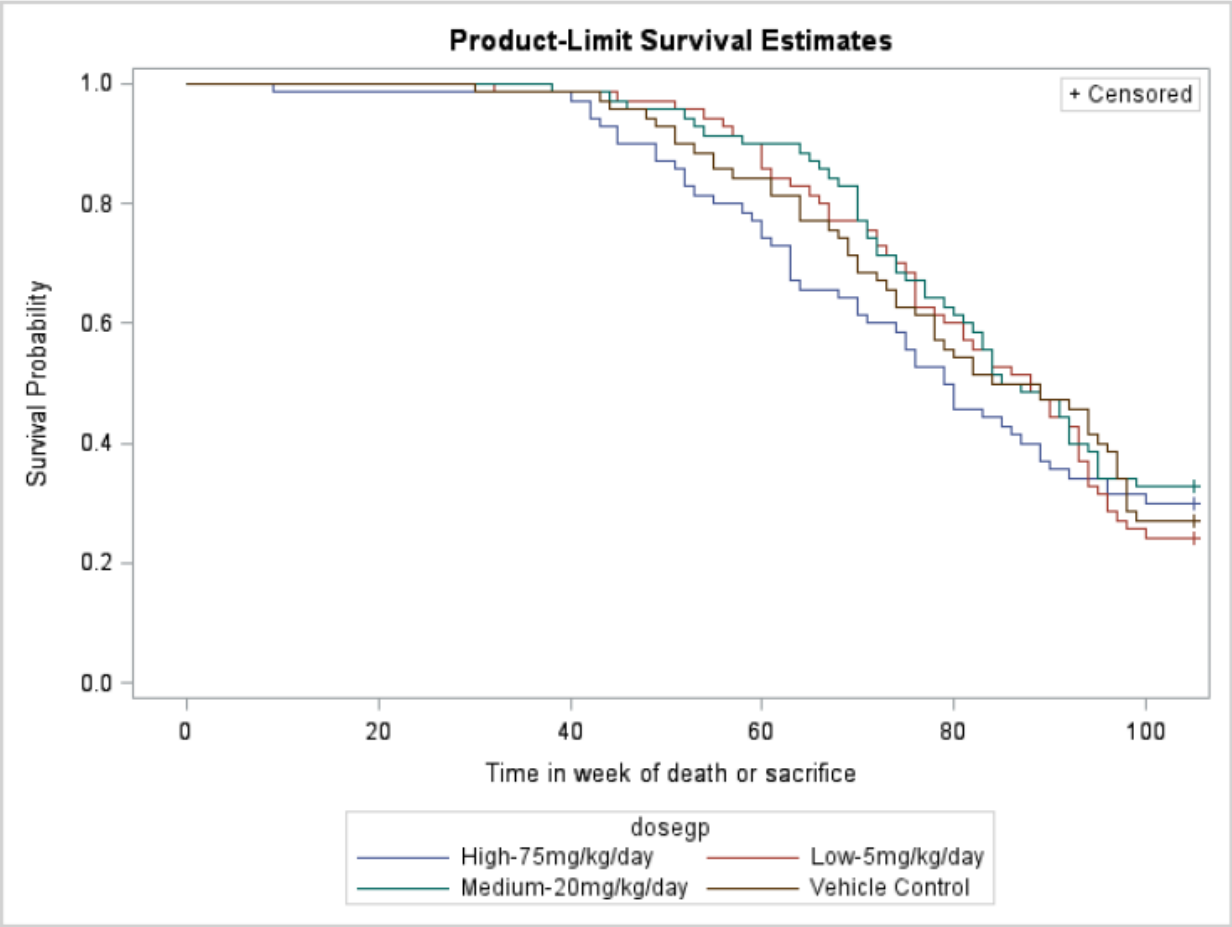


Figure 2 Kaplan-Meier Survival Curves for Females in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (FDA Statistical Reviewer's Figure)



Pituitary tumors were the most common probable cause of death for both male and female animals, although there was no drug-treated difference compared to control animals. In general, there was no probable cause of death that occurred with much greater incidence in male and female drug-treated groups compared to control groups, although a larger number of high dose males than control males died from lymphoid tumors (4/70 versus 1/70, respectively) and skin tumors (3/70 versus 0/70).

Clinical Signs

Detailed clinical observations were performed prior to randomization and generally once weekly during the study (about 4 hours postdose) and included evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior, and the palpation of tissue masses.

Select clinical observations that were seen in a greater number of high dose animals than control animals are shown in **Table 2**, although none of the clinical observations were considered test-article related.

Table 2 Select Clinical Observations with Higher Incidence in the High Dose Group Compared to the Control Group in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Observation	Males (Weeks 1 to 103)				Females (Weeks 1 to 100)			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	5	15	50	0	5	20	75
Number of Animals Observed	70	70	70	70	70	70	70	70
External Appearance								
Discharge, red	6/5	6/6	10/6	13/10	11/6	8/4	9/7	9/6
Ear/portion of ear missing	77/1	292/3	469/6	140/3	0/0	0/0	49/2	50/2
Material around eyes, black	45/10	22/6	70/8	9/5	10/6	129/14	71/16	26/11
Material around nose, red	8/7	17/11	14/8	14/5	10/8	12/6	9/4	28/12
Posture hunched	32/9	18/10	34/12	22/9	12/7	13/7	33/14	40/12
General Status								
Hair discolored, tan	62/7	35/4	68/6	120/8	-	-	-	-
Hair sparse	1030/44	1075/34	1091/38	979/43	2078/41	1783/40	2703/39	3121/46
Hair wet	5/4	10/7	9/8	6/4	3/3	8/5	6/2	17/12
Nodule, 1-5 mm	275/19	344/18	272/16	311/21	48/6	26/1	33/2	2/1
Nodule, 5-20 mm	35/3	33/3	3/2	55/6	-	-	-	-
Nodule, >20 mm	0/0	0/0	0/0	15/3	-	-	-	-

Note: Number of times observed/Total number of animals affected

Body Weights

Animals were weighed at receipt, prior to randomization, weekly for the first 14 weeks, every two weeks until Week 28, every four weeks thereafter for the duration of the study, and on the day of the scheduled terminal necropsy. At Week 103, absolute mean body weights for male drug-treated groups were ≤5.9% relative to the male concurrent control group (see **Table 3**). The body weight curve for the high dose male group separated from the control and lower dose groups, although differences did not exceed 10% (see **Figure 3**). At Week 101, absolute mean body weight for the high dose female group was decreased by 17.7% relative to the female concurrent control group, although mean body weights for the low dose and mid dose female groups were decreased ≤4.3% (see **Table 3**). The body weight curve for the high dose female group separated from the control and lower dose groups with differences exceeding 10% (see **Figure 4**).

Table 3 Body Weight Changes in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Parameter	Males				Females			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	5	15	50	0	5	20	75
Week 1 (grams)	332.5	330.5	331.6	323.0*	212.1	210.4	209.5	196.2*
Week 103 Males or Week 101 Females (grams)	832.7	875.9	804.6	783.8	568.3	544.1	557.9	467.5*
Absolute Body Weight (% change from Week 103 Male or Week 101 Female controls)	0.0	5.2	-3.4	-5.9	0.0	-4.3	-1.8	-17.7

* = statistically significant from control ($p < 0.01$) based on Applicant's group pair-wise comparison's (Levene's/ANOVA-Dunnett's/Welch's)

Figure 3 Mean Body Weights in Males in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

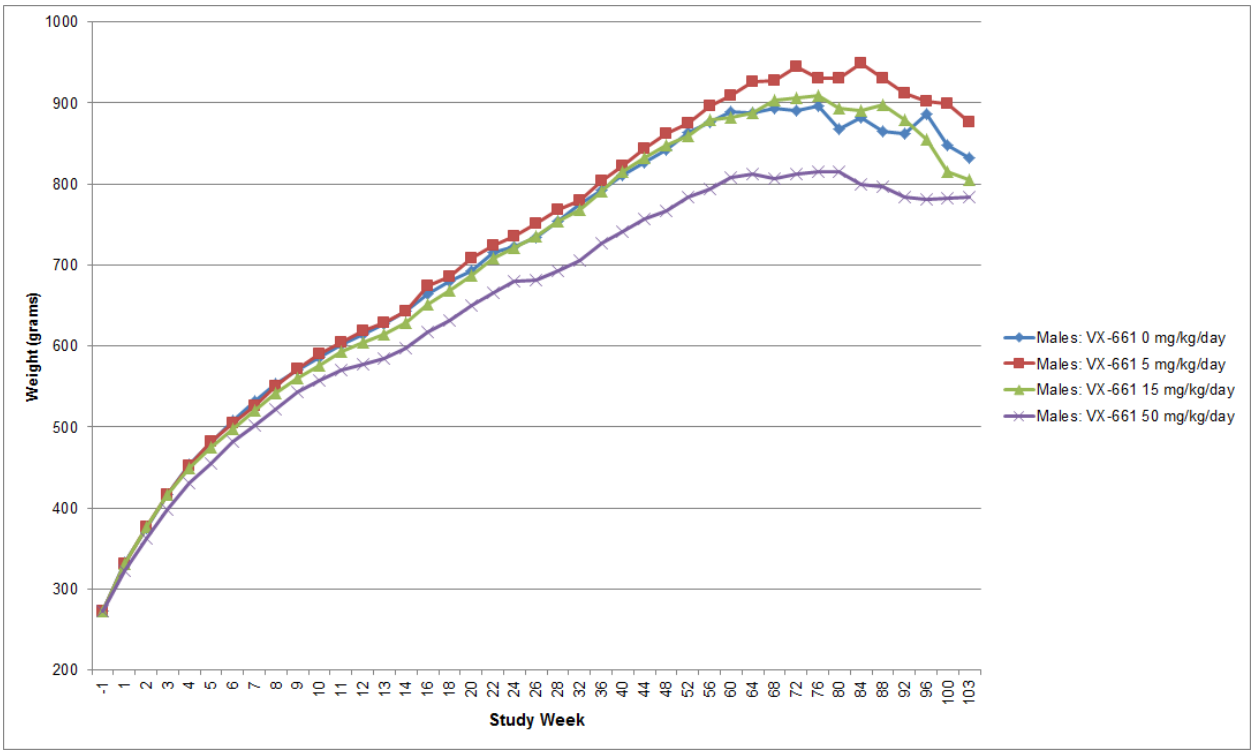
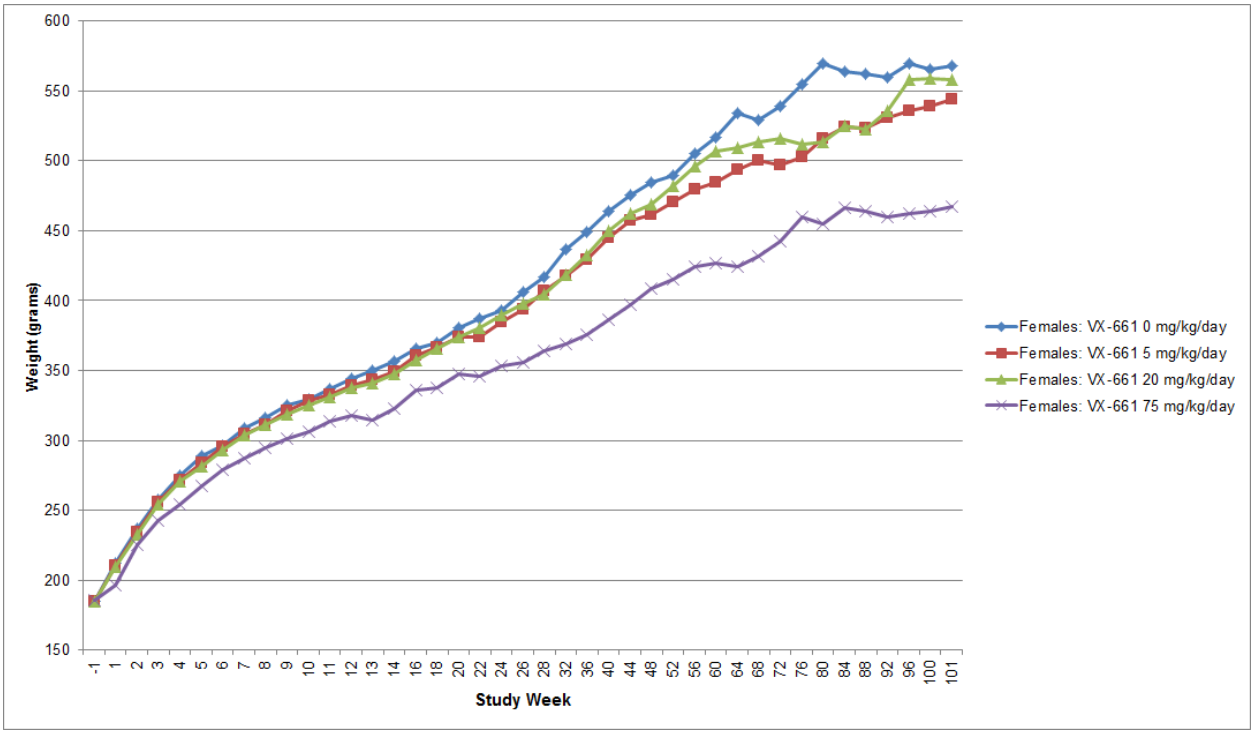


Figure 4 Mean Body Weights in Females in 104-Week Oral (Gavage) Carcinogenicity Study in Rats



Feed Consumption

Animals had access ad libitum to Block Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc.) and tap water via an automatic watering system. Food consumption was measured per cage on main study animals every week for the first 14 weeks, every two weeks until Week 28, and every four weeks thereafter for the duration of the study. As shown in **Figure 5** and **Figure 6**, sporadic decreases in food consumption for drug-treated animals were minimal in magnitude compared to control animals. Overall, there were no evident test-article related effects on food consumption.

Figure 5 Mean Caged Food Consumption in Males in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Figure)

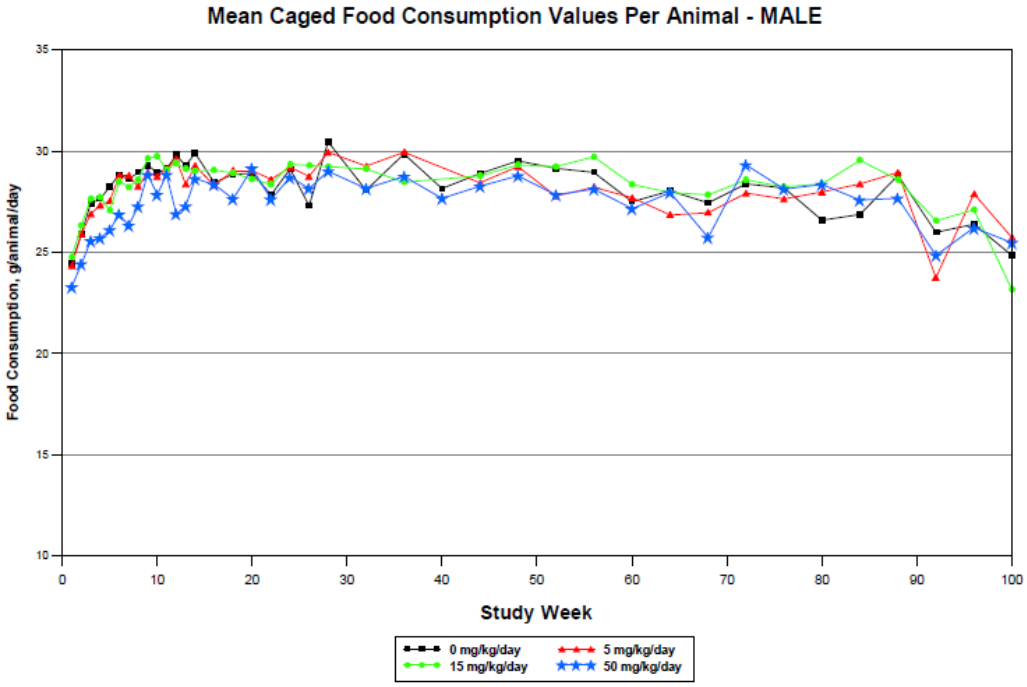
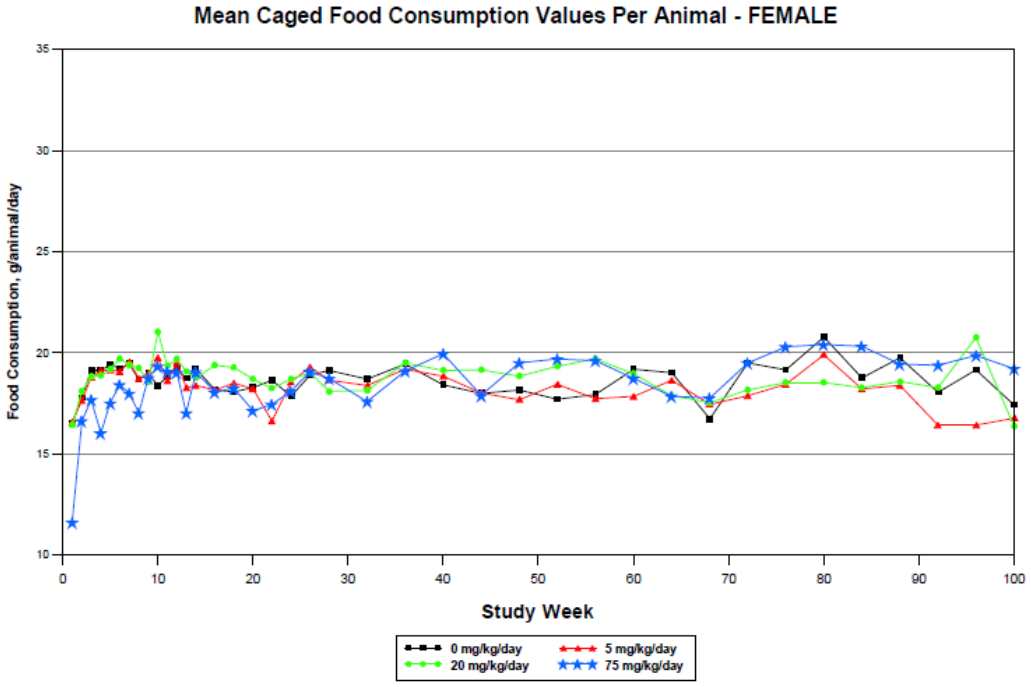


Figure 6 Mean Caged Food Consumption in Females in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Figure)



Gross Pathology

Sacrificed animals were euthanized by carbon dioxide inhalation. Complete necropsy examinations were performed on all main study animals (i.e., animals euthanized in extremis, found dead, and surviving animals). As shown in **Table 4**, there was a dose-dependent increase in the number of males with enlarged spleen in low dose (4/70), mid dose (6/70), and high dose (7/70) groups compared to the control group (1/70). This gross finding is possibly correlated with microscopic findings in the spleen of increased extramedullary hematopoiesis and lymphoma.

Table 4 Gross Pathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Tissue/Organ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
Number of Animals Examined	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
Spleen																
enlarged	1	0	2	2	4	2	5	2	4	0	1	0	1	0	0	1
…mild	0	0	1	1	2	2	2	1	3	0	1	0	0	0	0	1
…moderate	0	0	1	1	1	0	1	1	1	0	0	0	1	0	0	0

Tissue/Organ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
…severe	1	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0

Abbreviations: D = died or euthanized on study; S = scheduled necropsy

Histopathology

As shown in **Table 5**, all organs and tissues from male and female animals in the control and all the drug-treated groups were submitted to histopathological examination (hematoxylin fixed and eosin-stained paraffin tissue sections).

Table 5 Tissues/Organs Collected for Histopathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

Tissue	Collected and Preserved	Microscopic Examination Groups 1 to 4
Adrenal glands	X	X
Aorta	X	X
Bone marrow smear	X	
Bone with bone marrow, femur	X	X
Bone with bone marrow, sternum	X	X
Brain (cerebrum, midbrain, cerebellum, medulla/pons, olfactory bulbs)	X	X
Clitoral gland	X	X
Coagulating glands	X	X
Epididymides	X	X
Esophagus	X	X
Eyes (with optic nerve)	X	X
GALT (Gut-Associated Lymphoid Tissue)	X	X
Gross lesions	X	X
Harderian glands	X	X
Heart	X	X
Joint, tibiofemoral	X	X
Kidneys	X	X
Lacrimal glands, exorbital	X	X
Large intestine, cecum	X	X
Large intestine, colon	X	X
Large intestine, rectum	X	X
Larynx	X	X
Liver	X	X
Lung with bronchi	X	X
Lymph node, mandibular	X	X
Lymph node, mesenteric	X	X

Tissue	Collected and Preserved	Microscopic Examination Groups 1 to 4
Mammary gland (process females only)	X	X
Nose (4 sections)	X	X
Nerve, sciatic	X	X
Ovaries	X	X
Oviduct	X	X
Pancreas	X	X
Pharynx	X	X
Pituitary gland	X	X
Potential target organs	X	X
Preputial gland	X	X
Prostate gland	X	X
Salivary gland, mandibular	X	X
Salivary gland, parotid	X	X
Salivary gland, sublingual	X	X
Seminal vesicles	X	X
Skeletal muscle, biceps femoris	X	X
Skin	X	X
Small intestine, duodenum	X	X
Small intestine, ileum	X	X
Small intestine, jejunum	X	X
Spinal cord, cervical	X	X
Spinal cord, lumbar	X	X
Spinal cord, thoracic	X	X
Spleen	X	X
Stomach, glandular	X	X
Stomach, nonglandular	X	X
Testes	X	X
Thymus	X	X
Thyroid gland (with parathyroid)	X	X
Tissue masses with regional lymph node	X	X
Tongue	X	X
Trachea	X	X
Ureters	X	X

Tissue	Collected and Preserved	Microscopic Examination Groups 1 to 4
Urinary bladder	X	X
Uterus with cervix	X	X
Vagina	X	X
Zymbal's gland (auditory sebaceous gland)	X	X

Peer Review

A pathology peer review was performed by Natasha Neef, BA, VetMB, PhD, DACVP from Vertex and the peer review statement indicates that there was general agreement between the peer review and study pathologists on the evaluation of the data and overall study interpretation.

Neoplastic

Selected neoplastic lesions and tumors identified by histopathology are shown in **Table 6,** although none were considered related to VX-661 treatment. Tumor findings were evaluated separately for males and females. Tumor analysis was performed on combined and malignant neoplasms.[1]

Multicentric Neoplasms: Malignant lymphomas were seen with greater incidence in mid dose males (4.3%) and high dose males (5.8%) compared to control males (1.4%). These increases were not statistically significant (based on either dose response relationship or pairwise comparison of drug-treated groups to the control group).

Adrenal Glands: In the adrenal glands there was a slight increase in the incidence of malignant pheochromocytomas in high dose males (7.2%) compared to control males (1.4%). When combining the incidence of all adrenal pheochromocytomas, the incidence in high dose males (18.8%) was slightly greater than control males (11.4%). This increase was not statistically significant (based on either dose response relationship or pairwise comparison of drug-treated groups to the control group).

Pituitary Gland: Malignant pars distalis carcinoma of the pituitary gland was seen with greater incidence in mid dose females (4.3%) and high dose females (7.1%) compared to control females (1.4%). When combining the incidence of malignant par distalis carcinoma with benign par distalis adenoma the incidence in mid dose females (78.6%) was the same as the control group (78.6%) and that in high dose females (68.6%) was lower, respectively. These increases were not statistically significant (based on either dose response relationship or pairwise comparison of drug-treated groups to the control group).

[1]Brix AE, Hardisty JF, and McConnell EE. 2010. Chapter 28: Combining neoplasms for evaluation of rodent carcinogenesis studies. Cancer Risk Assessment, edited by Ching-Hung Hsu and Todd Stedeford, John Wiley and Sons, Inc.

Table 6 Neoplastic Lesions and Tumors from Histopathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Tissues/Organ Observation	Males				Females			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	**5**	**15**	**50**	**0**	**5**	**20**	**75**
Multicentric Neoplasm n =	3	3	5	5	5	0	4	2
lymphoma, malignant, multicentric	1	0	3	4	2	0	1	0
Adrenal Glands n =	**70**	**70**	**70**	**69**	**70**	**70**	**70**	**70**
pheochromocytoma, benign, primary	7	8	9	8	2	1	0	2
pheochromocytoma, complex, malignant, primary	0	0	0	0	0	0	0	1
pheochromocytoma, malignant, primary	1	1	1	5	1	1	2	0
…combined # of animals =	8	9	10	12	3	2	2	3
Pituitary Gland n =	70	70	70	70	70	70	70	70
adenoma, pars distalis, benign, primary	45	47	41	39	54	57	52	43
carcinoma, pars distalis, malignant, primary	1	0	0	0	1	2	3	5
…combined # of animals =	46	47	41	39	55	59	55	48

Abbreviations: # = number; n = total number examined

Non-Neoplastic

Non-neoplastic toxicities were noted in the GALT, small intestine (ileum and jejunum), parathyroid glands, liver, Harderian glands, clitoral glands, ovaries, and sternum bone marrow (see **Table 7**).

Minimal to mild dilated lymphatics (i.e., lymphatic vessels that are dilated within a tissue or organ) were observed in the GALT, ileum, and jejunum of mid dose and/or high dose males and high dose females compared to control animals. The study report states that the change was characterized by clear cyst-like empty spaces and that there was not an apparent association with other non-neoplastic or neoplastic pathologies or adverse effects. These findings were not associated with mortality. Minimal to mild dilated lymphatics were also noted in the both the 3-month oral gavage and 26-week oral gavage toxicity studies in rats. Neither the incidence nor severity of the dilated lymphatics progressed in this 2-year rat study compared to the 3-month and 26-week rat studies.

Minimal to moderate focal hyperplasia was also seen in the parathyroid glands with increased incidence in low dose, mid dose, and high dose males and females compared to their respective controls.

In the liver, minimal extramedullary hematopoiesis was noted in high dose males and hepatocellular vacuolation was noted in mid dose and high dose males with greater incidence than in control males.

In the Harderian glands, minimal to mild focal hyperplasia was increased in low dose, mid dose, and high dose males and high dose females compared to their respective controls.

In the clitoral glands, minimal to mild dilation was increased in low dose, mid dose, and high dose females compared to control females.

In the ovaries, minimal hematocyst was noted in high dose females (although at a low incidence) compared to control females.

In the bone marrow of the sternum, increased adipocytes (minimal to mild) were noted in low dose, mid dose, and high dose females compared to control females.

Table 7 Non-neoplastic Lesions from Histopathology in 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Tissue/ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
Number of Animals Examined	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
GALT n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
dilated lymphatics	1	0	1	0	3	0	5	6	2	0	0	0	0	0	2	1
…minimal	0	0	1	0	2	0	2	4	1	0	0	0	0	0	1	1
…mild	1	0	0	0	1	0	3	2	1	0	0	0	0	0	1	0
Small Intestine, Ileum n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	48	21
dilated lymphatics	0	0	0	0	0	0	5	6	0	0	0	0	1	0	4	2
…minimal	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1
…mild	0	0	0	0	0	0	5	6	0	0	0	0	0	0	2	1
Small Intestine, Jejunum n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	48	21
dilated lymphatics	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0
…minimal	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
…mild	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Parathyroid Glands n =	48	16	47	21	50	14	41	24	47	17	46	17	42	23	46	21
hyperplasia, focal	1	3	6	9	7	3	6	10	2	1	3	3	4	2	3	6
…minimal	1	1	4	8	3	3	5	7	1	1	2	3	3	1	2	4
…mild	0	0	1	1	4	0	1	2	1	0	1	0	0	1	1	2
…moderate	0	2	1	0	0	0	0	1	0	0	0	0	1	0	0	0
Liver n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21

Tissue/ Observation	Males								Females							
	VX-661 (mg/kg/day)								VX-661 (mg/kg/day)							
	0 D	0 S	5 D	5 S	15 D	15 S	50 D	50 S	0 D	0 S	5 D	5 S	20 D	20 S	75 D	75 S
hematopoiesis, extramedullary	0	1	3	0	0	0	4	2	7	3	4	1	2	0	7	2
…minimal	0	1	1	0	0	0	4	2	5	3	2	1	1	0	7	2
…mild	0	0	2	0	0	0	0	0	2	0	2	0	1	0	0	0
vacuolation, hepatocellular	5	6	5	6	10	8	13	14	10	4	7	3	6	11	5	3
…minimal	3	4	5	5	10	8	11	12	10	4	6	3	6	8	4	3
…mild	2	2	0	1	0	0	2	2	0	0	1	0	0	3	1	0
Harderian Glands n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
hyperplasia, focal	3	7	7	7	11	6	11	10	4	3	5	3	2	5	4	7
…minimal	3	6	7	6	10	5	11	9	4	3	5	3	2	5	4	5
…mild	0	1	0	1	1	1	0	1	0	0	0	0	0	0	0	2
Clitoral Glands n =	NA	NA	NA	NA	NA	NA	NA	NA	49	19	53	17	46	22	49	21
dilation	NA	NA	NA	NA	NA	NA	NA	NA	15	11	32	8	27	10	27	17
…minimal	NA	NA	NA	NA	NA	NA	NA	NA	10	3	15	3	20	3	16	8
…mild	NA	NA	NA	NA	NA	NA	NA	NA	5	8	17	5	7	7	8	7
…moderate	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	3	2
Ovaries n =	NA	NA	NA	NA	NA	NA	NA	NA	51	19	53	17	47	23	49	21
hematocyst	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	2	0
…minimal	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	2	0
Bone Marrow, Sternum n =	50	20	49	21	54	16	45	25	51	19	53	17	47	23	49	21
increased adipocytes	11	1	10	2	13	2	6	1	5	2	8	3	10	10	8	4
…minimal	8	0	8	2	9	2	5	1	3	2	7	3	8	9	6	4
…mild	2	1	2	0	4	0	1	0	2	0	1	0	2	1	2	0
…moderate	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Abbreviations: D = died or euthanized on study; GALT = gut-associated lymphoid tissue; n = number observed; NA = not applicable; S = scheduled necropsy

Toxicokinetics

Blood samples were collected from unfasted TK animals via the sublingual vein at predose, 2, 4, 8, 12, and 24 hours postdose on Days 1, 181 (Week 26), and 363 (Week 52). TK parameters that were determined included C_{max}, T_{max}, and AUC_{0-24} for VX-661 and the metabolites VRT-0996107 (M1) and VRT-1189001 (M2).

As shown in **Table 8** for VX-661, T_{max} for males was 4 hours and for females ranged from 2 to 8 hours, regardless of dose or time interval (i.e., Day 1, Week 26 or Week 52). After repeat exposure in males and females (Week 26 and Week 52), AUC was generally dose proportional between the low dose and mid dose groups and mid dose

and high dose groups. Exposure in females appeared greater than in males. There appeared to be drug accumulation over the time course of dosing in males and females.

Table 8 Summary Toxicokinetics for VX-661 in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

VX-661 Dose (mg/kg/day) M/F	Interval	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (hr*µg /mL)
5/5	Day 1	M	0.605	4.00	4.97
		F	0.834	8.00	13.2
	Week 26	M	1.43	4.00	14.2
		F	1.61	2.00	21.8
	Week 52	M	1.66	4.00	19.2
		F	2.06	2.00	28.3
15/20	Day 1	M	2.24	4.00	20.4
		F	4.14	4.00	55.2
	Week 26	M	5.01	4.00	55.4
		F	8.69	4.00	101
	Week 52	M	6.17	4.00	73.8
		F	8.15	4.00	109
50/75	Day 1	M	10.1	4.00	107
		F	15.4	4.00	241
	Week 26	M	11.6	4.00	134
		F	20.2	8.00	262
	Week 52	M	13.6	4.00	180
		F	29.0	4.00	337
M-Male; F-Female					

As shown in **Table 9** for metabolite VRT-0996107 (M1), T_{max} for males and females ranged from 4 to 12 hours, regardless of dose or time interval (i.e., Day 1, Week 26 or Week 52). After repeat exposure in males and females (Week 26 and Week 52), AUC was generally dose proportional between the low dose and mid dose groups and mid dose and high dose groups. Exposure in males and females appeared similar. There appeared to be metabolite accumulation over the time course of dosing in males and females.

Table 9 Summary Toxicokinetics for Metabolite VRT-0996107 (M1) in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

VX-661 Dose (mg/kg/day) M/F	Interval	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (hr*µg /mL)
5/5	Day 1	M	0.462	4.00	8.54
		F	0.419	12.0	6.73
	Week 26	M	1.51	8.00	29.7
		F	1.41	8.00	28.6
	Week 52	M	1.76	8.00	34.7
		F	1.75	12.0	34.2
15/20	Day 1	M	1.57	12.0	28.7
		F	1.53	12.0	28.0
	Week 26	M	4.10	12.0	84.2
		F	6.28	4.00	125
	Week 52	M	5.29	4.00	106
		F	6.40	4.00	139
50/75	Day 1	M	4.35	12.0	77.8
		F	5.27	12.0	86.4
	Week 26	M	9.80	4.00	199
		F	17.6	8.00	344
	Week 52	M	15.3	8.00	288
		F	26.2	4.00	479
M-Male; F-Female					

As shown in **Table 10** for metabolite VRT-1189001 (M2), T_{max} for males ranged from 2 to 24 hours and for females ranged from 4 to 24 hours, regardless of dose or time interval (i.e., Day 1, Week 26 or Week 52). After repeat exposure in females (Week 26 and Week 52), AUC was generally dose proportional between the low dose and mid dose groups but lower than dose proportional between the mid dose and high dose groups. Exposure in males appeared greater. After repeat exposure in males (Week 26 and Week 52), AUC was generally dose proportional over the dosing range. There appeared to be metabolite accumulation over the time course of dosing in males and females.

Table 10 Summary Toxicokinetics for Metabolite VRT-1189001 (M2) in 104-Week Oral (Gavage) Carcinogenicity Study in Rats (Applicant's Table)

VX-661 Dose (mg/kg/day) M/F	Interval	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24hr} (hr*µg /mL)
5/5	Day 1	M	0.0238	4.00	0.449
		F	0.0137	12.00	0.222
	Week 26	M	0.0796	4.00	1.61
		F	0.0430	8.00	0.902
	Week 52	M	0.110	4.00	2.07
		F	0.0638	4.00	1.36
15/20	Day 1	M	0.0907	12.0	1.60
		F	0.0709	12.0	1.20
	Week 26	M	0.278	12.0	5.96
		F	0.233	12.0	4.84
	Week 52	M	0.313	2.00	6.92
		F	0.239	12.0	5.09
50/75	Day 1	M	0.164	12.0	3.10
		F	0.0763	24.0	1.40
	Week 26	M	0.767	24.0	15.9
		F	0.473	8.00	10.4
	Week 52	M	1.06	24.0	20.0
		F	0.624	4.00	13.0
M-Male; F-Female					

Dosing Solution Analysis

Dosing solutions were analyzed for homogeneity (top, middle, and bottom stratum) and concentration (middle stratum) on Day 1 and about monthly thereafter (Days 29, 57, 85, 113, 141, 181, 197, 225, 253, 281, 309, 337, 363, 393, 421, 449, 477, 505, 536, 561, 589, 617, 645, 673, and 701). All samples were found to be homogenous. The calculated concentrations for the control dose level (0 mg/kg/day) were all below the limit of quantitation (<0.0510 mg/mL) and the calculated concentrations for the test article dose levels (5, 15, 20, 50, and 75 mg/kg/day) were all within 10% of their respective nominal concentrations.

Study title: VX-661: 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice

Study no.:	Conducting laboratory: AD80DH.7G8R.(b) (4) Sponsor: VX-661-TX-019
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 18, 2014 (protocol signed by Study Director)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	VX-661 (b) (4), lot # 19QB10A-50.NJ00001, 98.0% w/w purity
CAC concurrence:	Yes (see Special Protocol Agreement, dated November 20, 2014)

Key Study Findings

- In a 26-week oral carcinogenicity study, Tg.rasH2 mice received doses of 0, 30, 100, and 500 mg/kg/day VX-661 and 1000 mg/kg/day urethane (positive control).
- There was a statistically significant treatment-related increase in mortality for high dose males (76% survival) compared to control males (96% survival).
- There were no statistically significant drug-related tumor findings in male or female mice.
- The most notable non-neoplastic histopathology findings were in the adrenal glands (cortex hypertrophy, cortex vacuolation, and x-zone degeneration), liver (centrilobular hypertrophy, centrilobular hepatocellular single cell necrosis, subcapsular inflammation, focal hepatocellular necrosis, focal lipid infiltration, and periportal vacuolation), ovaries (decreased corpora lutea), and jejunum (lamina propria vacuolation). Additional non-neoplastic findings were noted in the mesenteric and mandibular lymph nodes (lymphoid necrosis), spleen (hemosiderin pigmentation and lymphoid necrosis), and thymus (lymphoid necrosis and increased lymphocytes).
- VX-661 AUC exposure at the high dose (500 mg/kg/day) was about 93 and 154 mcg*hr/mL in males and females, respectively. Exposures to VX-661 metabolites, M1 (VRT-0996107) and M2 (VRT-1189001), were also assessed in mice.

Adequacy of Carcinogenicity Study

- The ECAC concurred with the doses and design of the study (see Special Protocol Agreement dated November 20, 2014).
- The duration of treatment was adequate (i.e., 26 weeks).

- The positive control produced expected increases of neoplastic findings indicating that the mice and study could detect a potential drug-induced neoplastic response.

Appropriateness of Test Models

- The Tg.rasH2 mouse is considered an appropriate model for short-term carcinogenicity assessment. The CByB6F1 mouse is the wild type of the Tg.rasH2 mouse (i.e., same genetic makeup with the omission of the Tg element) and is considered acceptable for the TK portion of the study.
- Wild-type mice achieved high exposures to VX-661 and its M1 metabolite. Exposure to the M2 metabolite was low. Exposure to the M5 metabolite (VRT-1074233) was not measured.

Evaluation of Tumor Findings

- There were no statistically significant drug-related and tumor findings in male or female mice.

Methods

Doses:	See **Table 11** for dose levels in the main cohort and the TK cohort Vehicle control: hydroxypropylmethylcellulose acid succinate in formulation vehicle Positive control: 1000 mg/kg/dose urethane
Frequency of dosing:	Main cohort: vehicle control or VX-661 doses were administered once daily for up to 26 consecutive weeks and the positive control was administered on Days 1, 3, and 5 TK cohort: vehicle control or VX-661 doses were administered either once on Day 1 or once daily for up to 177 days
Dose volume:	10 mL/kg
Route of administration:	Main cohort: vehicle control and VX-661 doses were administered by oral gavage and positive control was administered by intraperitoneal injection TK cohort: vehicle control and VX-661 doses were administered by oral gavage
Formulation/Vehicle:	0.5% methylcellulose, 0.5% SLS, 0.01% simethicone in deionized water
Basis of dose selection:	In consultation with the ECAC, the high dose was selected based on mortality at 1500 mg/kg/day in male and female non-transgenic mice from a 5-day dose ranging study (VX-661-TX-017). The mid and low doses were chosen to provide dose separation based on AUC.
Species/Strain:	Main cohort: hemizygous Tg.rasH2 mice from (b) (4) TK cohort: wild-type CByB6F1 from (b) (4)
Number/Sex/Group:	See **Table 11** for the number of animals/sex/group in the main cohort and the TK cohort
Age:	Tg.rasH2 and CByB6F1 mice were about 8 weeks of age at start of dosing
Animal housing:	During the acclimation period animals were group housed and then were individually housed following randomization, in polycarbonate cages
Paradigm for dietary restriction:	No dietary restrictions. Animals had access ad libitum to Harlan TEKLAD Global Diet #2018CM (Certified 18% Protein Rodent Diet, Harlan TEKLAD, Madison, WI) in meal form, in

	stainless steel rodent feeders, and ad libitum access to drinking water via an automatic watering system
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	TK
Deviation from study protocol:	Protocol deviations were presented in the study report and were deemed to have not affected the outcome or integrity of the study.

Table 11 Study Design in Main Cohort and Toxicokinetic Cohort of 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

Group	Dose Levels (mg/kg/day)[b]	Number of Animals			
		Main Cohort		TK Cohort*	
		Male	Female	Male	Female
1	0[a]	25	25	8	8
2	30	25	25	44	44
3	100	25	25	44	44
4	500	25	25	44	44
5	1000 (urethane)	10	10	-	-
Total		**110**	**110**	**140**	**140**

[a] = Group 1 received the control article.
[b] = Dose levels are expressed as API. A correction factor of 2.00 was used.
* = Extra TK animals (2/sex/group) were used to ensure adequate animals for TK bleeding.

Observations and Results

Mortality

Observations for morbidity and mortality were made for all animals, at least twice daily. As shown in **Table 12**, the percentage of main cohort high dose males surviving until terminal necropsy was 76% compared to 96% for control males. The analysis of the FDA statistical reviewer found a statistically significant treatment-related increase in mortality. Of the six high dose males that had early deaths (found dead or moribund sacrifice), the cause of death was undetermined in 5/6 males and due to marked trachea inflammation in 1/6 males, which did not appear to be related to drug-treatment (see **Table 13**). Kaplan-Meier survival curves for control and drug-treated male and female groups are shown below (**Figure 7** and **Figure 8**, respectively).

Table 12 Number of Early Deaths versus Animals Surviving until Terminal Necropsy in Main Cohort Animals in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

VX-661 (mg/kg/day)	Males		Females	
	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]	# of Early Deaths[1, 2]	# at Terminal Necropsy[2]
0	1 (4%)	24 (96%)	2 (8%)	23 (92%)
30	3 (12%)	22 (88%)	2 (8%)	23 (92%)
100	1 (4%)	24 (96%)	0 (0%)	25 (100%)
500	6 (24%)	19 (76%)	2 (8%)	23 (92%)
1000 (urethane)	0 (0%)	10 (100%)	1 (10%)	9 (90%)

Abbreviations: # = number
Notes:
[1]# of Early Deaths = moribund sacrifice or found dead
[2]numbers in parentheses represent the percent when compared to the number of main study animals at the start of the study (i.e., 25 animals/sex/group for vehicle control and drug-treated groups and 10 animals/sex/group for urethane positive control group)

Table 13 Mortality in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

Sex	Group (Dose)	Mode of Death	Animal Number	Day of Death	Cause of Death
Male	1 (0 mg/kg/day)	Moribund Sacrifice	7607	169	Spinal cord, hemangiosarcoma
	2 (30 mg/kg/day)	Moribund Sacrifice	7635	171	Undetermined
		Natural Death	7647	94	Undetermined
		Natural Death	7650	144	Undetermined
	3 (100 mg/kg/day)	Natural Death	7673	64	Undetermined
	4 (500 mg/kg/day)	Natural Death	7677	117	Undetermined
		Natural Death	7682	52	Undetermined
		Moribund Sacrifice	7686	26	Undetermined
		Natural Death	7688	147	Trachea, inflammation, marked
		Natural Death	7694	22	Undetermined
		Natural Death	7698	93	Undetermined
Female	1 (0 mg/kg/day)	Natural Death	7716	82	Undetermined
		Natural Death	7720	16	Undetermined
	2 (30 mg/kg/day)	Moribund Sacrifice	7742	106	Undetermined
		Moribund Sacrifice	7748	167	Salivary glands, hemangiosarcoma
	4 (500 mg/kg/day)	Moribund Sacrifice	7791	95	Multiple lung lesions
		Natural Death	7793	27	Undetermined

Natural Death = Found Dead

Figure 7 Kaplan-Meier Survival Curves for Males in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (FDA Statistical Reviewer's Figure)

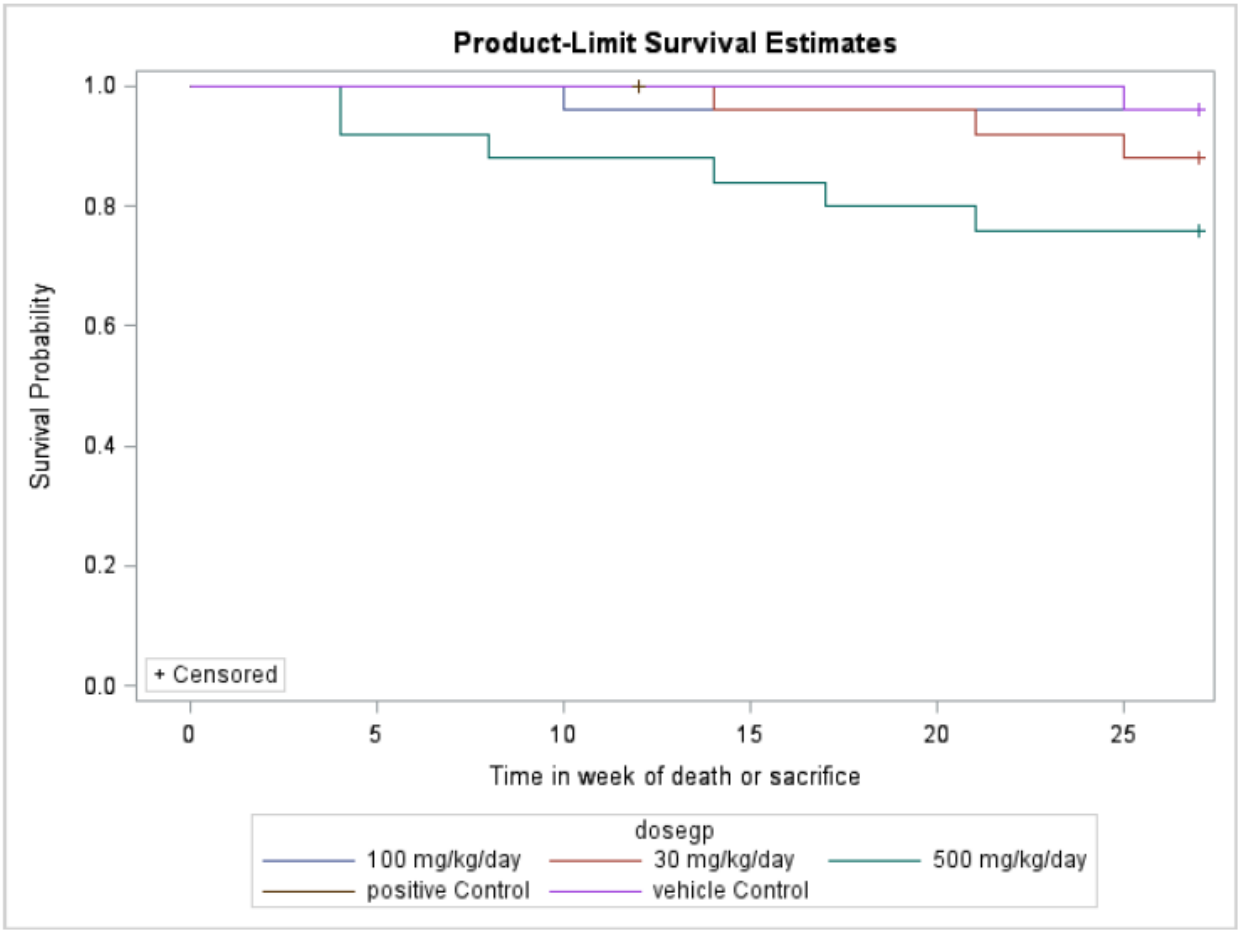
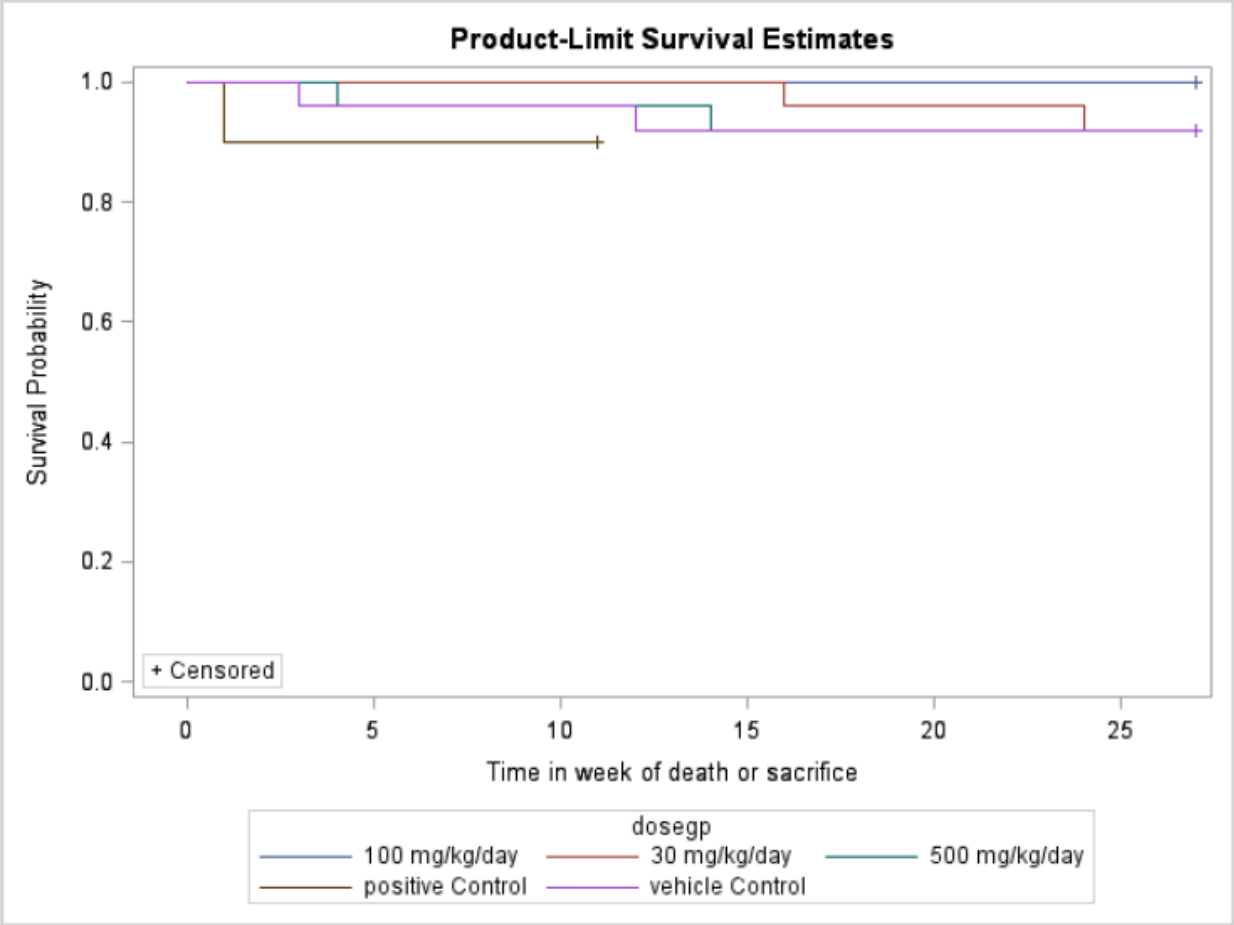


Figure 8 Kaplan-Meier Survival Curves for Females in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (FDA Statistical Reviewer's Figure)



Clinical Signs

Cage side observations were made on main cohort animals on the days of dosing (within 2 hours of the last animal that was dosed in each group). Detailed hands-on observations were made on main cohort animals on Day 1 and weekly thereafter (at the time animals were weighed).

Clinical observations (cage side and hands on) that were seen in a greater number of high dose animals or more times in high dose animals than control animals are shown in **Table 14** and **Table 15**. Notable cage side findings included decreased motor activity, ruffled fur, and hunched posture. Notable hands on findings included thinness, ruffled fur, and hunched posture.

Table 14 Summary of Cage Side Observations in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Observation	Males	Females

	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Decreased Motor Activity	10/5	6/6	9/4	55/18	12/8	11/8	15/4	22/6	33/11	19/10
Days	38 to 158	9 to 171	8 to 131	7 to 136	3 to 5	6 to 182	13 to 180	22 to 180	3 to 182	1 to 5
Ruffled Fur	243/ 24	358/ 25	566/ 25	1221/ 25		44/ 15	106/ 19	92/ 16	335/ 23	
Days	11 to 174	8 to 183	7 to 183	3 to 182		6 to 182	6 to 182	5 to 180	3 to 182	
Hunched	28/7	31/11	25/10	123/22		19/10	31/14	29/6	40/12	
Days	101 to 158	9 to 171	7 to 183	7 to 164		6 to 182	6 to 180	6 to 180	3 to 182	
Labored Breathing				5/4		1/1	4/1		1/1	
Days				21 to 26		16 to 16	121 to 124		182 to 182	
Rapid and Shallow				6/3		3/2	1/1			
Days				49 to 135		15 to 121	130 to 130			
Swelling				1/1			20/1			
Days				26 to 26			147 to 166			

Abbreviations: + = positive control (1000 mg/kg/day urethane)
Note: Number of times observed/Total number of animals affected

Table 15 Summary of Hands On Observations in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Thin	2/1	17/6	1/1	14/8		10/6	22/8	29/13	31/6	
Days	127 to 134	8 to 183	29 to 29	8 to 127		85 to 141	15 to 162	15 to 141	8 to 141	
Ruffled Fur	63/ 18	96/ 18	129/ 21	155/ 23		9/ 4	17/ 9	14/ 8	108/ 19	

Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Days	15 to 183	15 to 183	15 to 183	8 to 183		15 to 134	92 to 183	141 to 183	8 to 183	
Hunched	5/3	8/6	6/3	21/8		8/5	10/6	6/5	18/9	
Days	127 to 162	120 to 183	92 to 183	8 to 176		15 to 141	15 to 141	85 to 141	8 to 183	
Seizures									1/1	
Days									8 to 8	
Rapid and Shallow							1/1		1/1	
Days							106 to 106		183 to 183	
Nodule								12/1	10/1	
Days								36 to 113	50 to 113	
Micro-ophthalmia (small eye)									5/1	
Days									155 to 183	
Exo-phthalmia (bulging eye)									1/1	
Days									127 to 127	
Abnormality (closed left eyelid)									8/1	
Days									134 to 183	

Abbreviations: + = positive control (1000 mg/kg/day urethane)
Note: Number of times observed/Total number of animals affected

Body Weights

Main cohort animals were weighed at pre-dose on Day 1, weekly through Week 13, and biweekly thereafter. TK cohort animals were weighed (for dose volume calculations only) on Day -1 (Day 1 TK mice only) or on Day 1, weekly through Week 13, and

biweekly thereafter (Week 26 TK mice). As shown in **Table 16**, at the end of the study period (i.e., Day 183), mean body weights for VX-661-treated male and female animals were generally similar to control animals, despite a statistical decrease for mid dose males, low dose females, and high dose females. In all cases, the statistically significant decrease represented a percent change from the control that was less than 7% (the change ranged from about 1% to about 6%) so the statistical decreases were not considered related to VX-661 treatment. Male and female mean body weight curves over the course of the study are shown in **Figure 9** and **Figure 10**, respectively.

Table 16 Body Weight Changes in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Parameter	Males				Females			
	VX-661 (mg/kg/day)				VX-661 (mg/kg/day)			
	0	30	100	500	0	30	100	500
Day 1 (grams)	22.72	22.68	22.82	21.83	18.41	17.82	18.06	18.03
Day 183 (grams)	25.60	24.34	24.02*	24.30	21.01	19.97*	20.83	20.02*
Absolute Body Weight (% change from Day 183 controls)	0.0	-4.9	-6.2	-5.1	0.0	-5.0	-0.9	-4.7

* = statistically significant from control ($p < 0.05$) based on Applicant's Dunnett's test

Figure 9 Mean Body Weights in Males in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

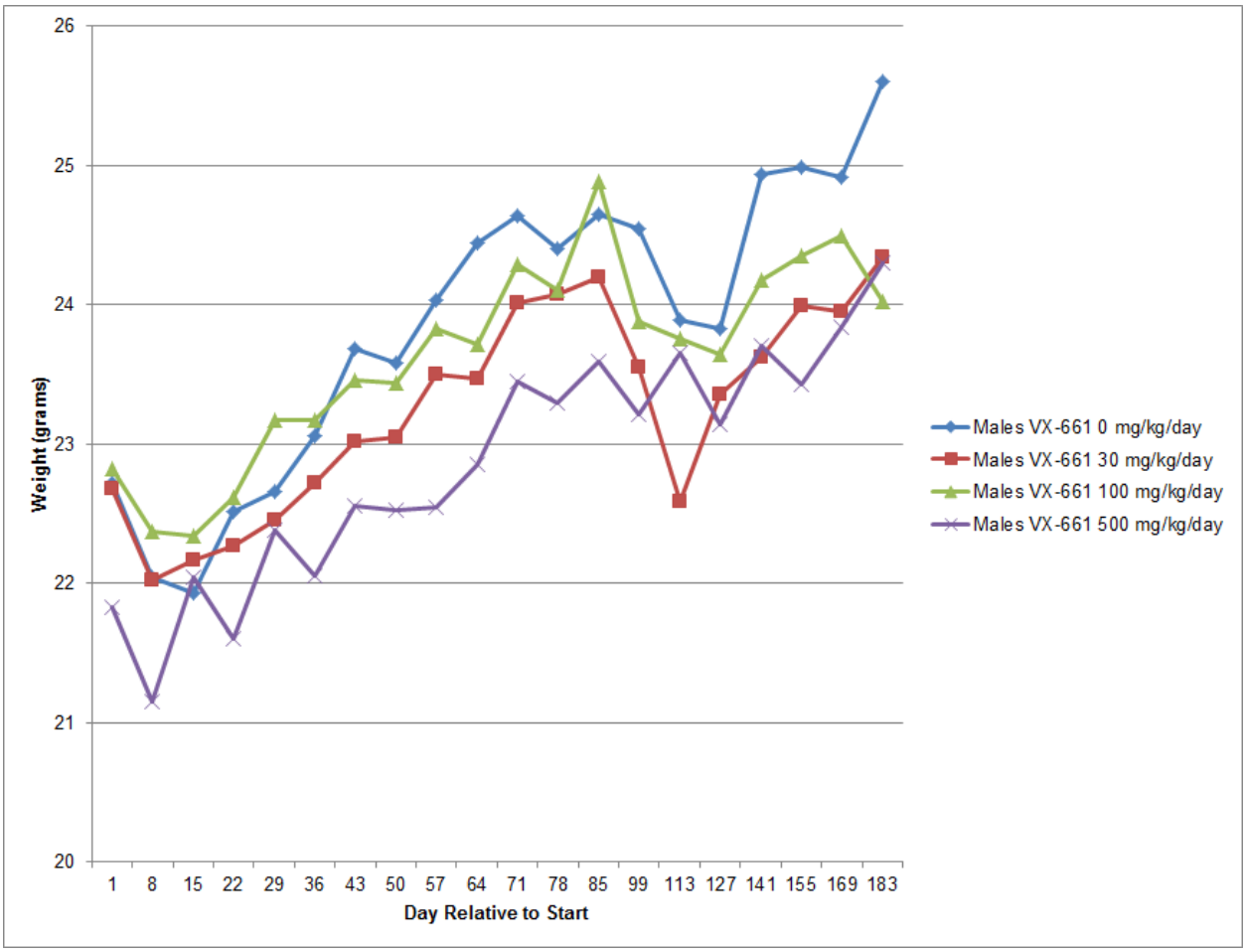
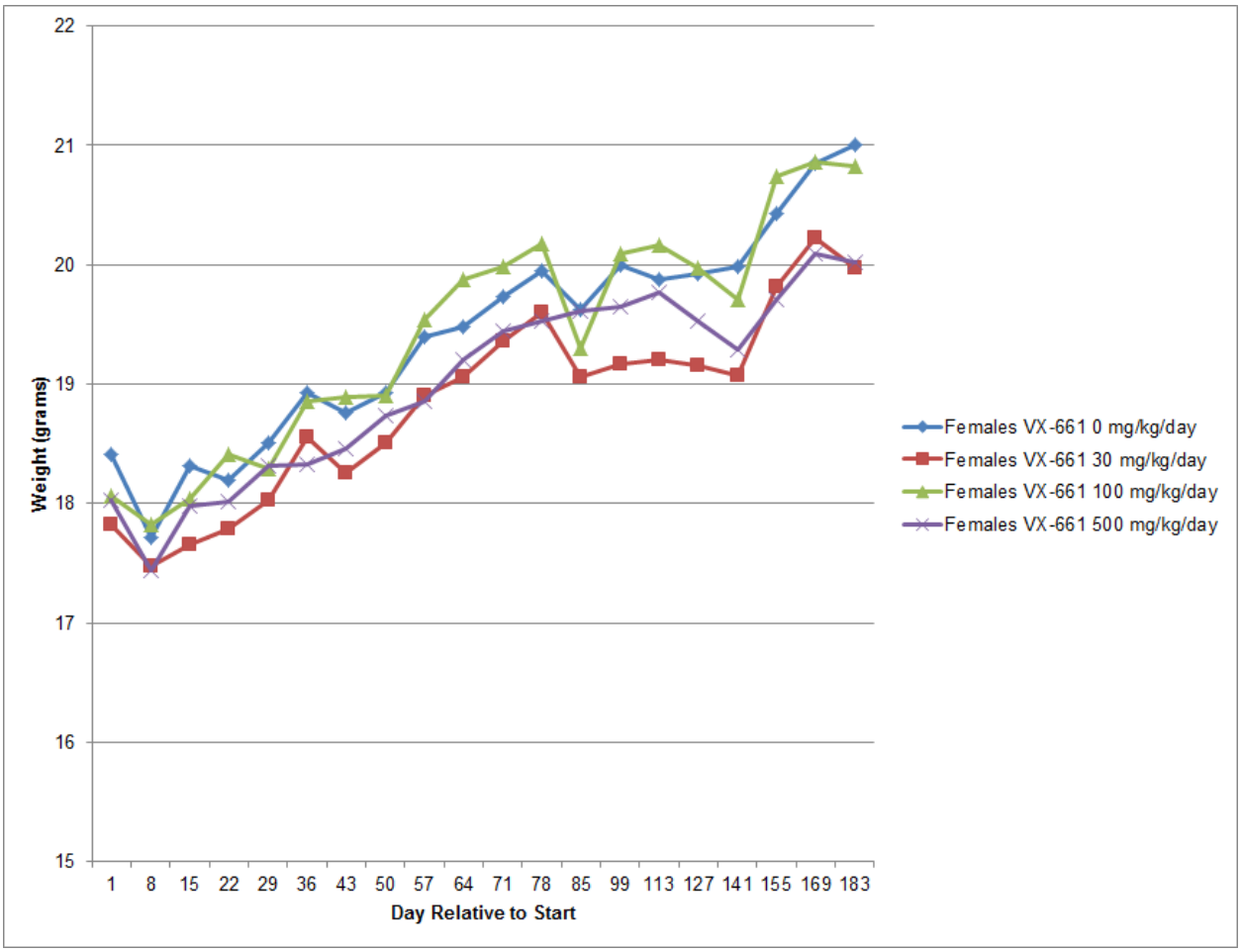


Figure 10 Mean Body Weights in Females in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice



Feed Consumption

Animals had access ad libitum to Harlan TEKLAD Global Diet #2018CM (Certified 18% Protein Rodent Diet, Harlan TEKLAD, Madison, WI) and drinking water via an automatic watering system. Food consumption was measured for main cohort animals on Day 1 and weekly thereafter. As shown in **Figure 11** and **Figure 12**, there were both decreases and increases in food consumption from week to week for drug-treated animals compared to control animals. Overall, there was not an obvious effect of dose on food consumption and there did not appear to be an effect of feed consumption on body weight. Thus, there were no evident drug-related effects on food consumption.

Figure 11 Mean Caged Food Consumption in Males in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

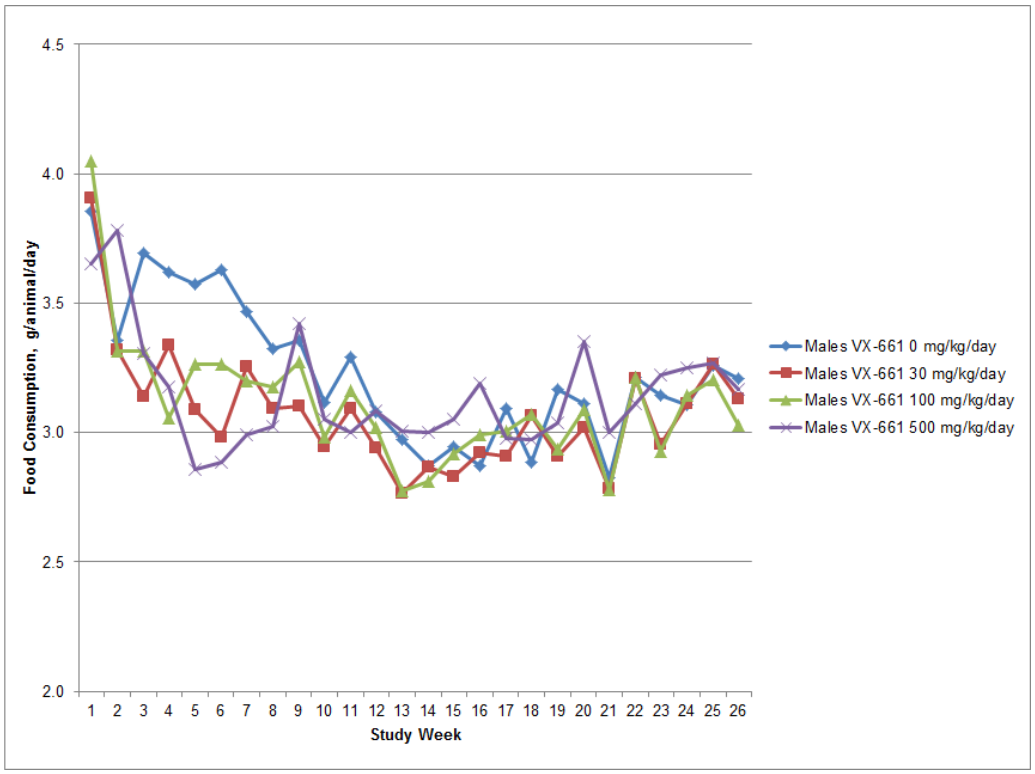
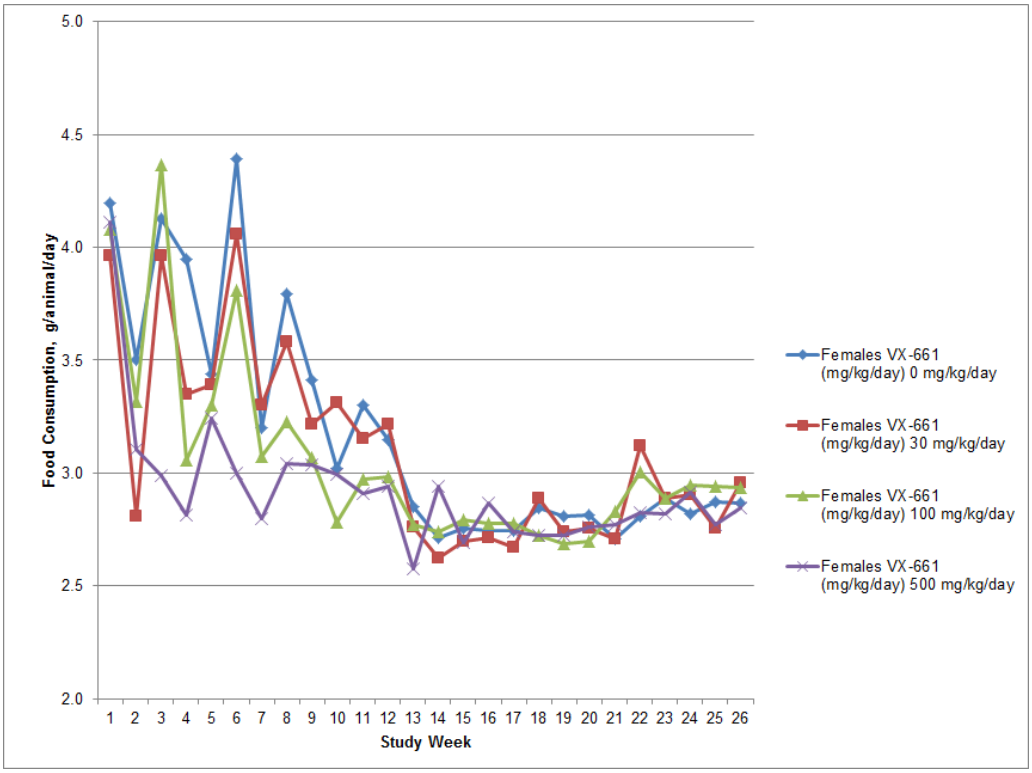


Figure 12 Mean Caged Food Consumption in Females in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice



Gross Pathology

Surviving positive control animals were euthanized by carbon dioxide overdose on Day 78 (males) and Day 76 (females) and subjected to a partial necropsy (lungs and spleen). Surviving main cohort animals were euthanized by carbon dioxide overdose on Day 183 or Day 184 and subjected to a complete necropsy. A complete necropsy was similarly performed on any main cohort early deaths (found dead or moribund sacrifice) and these animals were also evaluated for evidence of gavage error. Surviving TK cohort animals were euthanized by carbon dioxide overdose after completion of their last scheduled blood collection (Days 1-2 or Days 177-178) and no necropsy was performed.

One high dose female (#7788) had a tan nodule on the ventral left lung lobe and a pale right cranial lung lobe, while another high dose female (#7810) had a pale firm right cranial lung lobe, all of which correlated with the microscopic finding of mild focal histiocytic infiltration of the alveoli. One high dose female (#7808) had a mass on the lungs with bronchi that correlated with the microscopic finding of primary malignant alveolar-bronchiolar carcinoma.

Of note, positive control males and females had pale nodule that correlated with the microscopic finding of primary benign alveolar-bronchiolar adenoma.

One high dose male (#7681) had a nodule attached to the pancreas that correlated with the microscopic finding of primary malignant mesentery hemangiosarcoma.

The following gross pathology findings were seen in the spleen and correlated with primary malignant hemangiosarcoma in the spleen: 1) nodule (low dose males, high dose males, positive control males, and positive control females); 2) dark nodule (low dose females); 3) mass (high dose females); and 4) pale mass (low dose males).

One high dose male (#7680) had an enlarged thymus that correlated with the microscopic finding of multicentric malignant lymphoma.

Generally, gross findings for drug-treated male and female mice did not correlate to any treatment-related neoplastic findings.

Table 17 Gross Pathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Tissue/Organ Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Lungs with Bronchi n =	25	25	25	25	10	25	25	25	25	10
nodule	3	0	0	0	0	0	0	0	0	0
nodule, pale	0	0	0	0	0	1	0	0	0	2
nodule, pale, all lobes	0	0	0	0	10	0	0	0	0	7
nodule, tan, left	0	0	0	0	0	0	0	0	1	0
mass	0	0	0	0	0	0	0	0	1	0
pale, firm, right	0	0	0	0	0	0	0	0	1	0
pale, right	0	0	0	0	0	0	0	0	1	0
Pancreas n =	25	25	25	25	0	25	25	25	25	0
nodule	0	0	0	1	0	0	0	0	0	0
Spleen n =	25	25	25	25	10	25	25	25	25	10
nodule	0	4	0	2	8	0	0	0	0	7
nodule, dark	0	0	0	0	0	0	1	0	0	0
mass	0	0	0	0	0	0	0	0	1	0
mass, pale	0	1	0	0	0	0	0	0	0	0
Thymus n =	25	25	25	25	0	25	25	25	25	0
enlarged	0	0	0	1	0	0	0	0	0	0

Abbreviations: + = positive control (1000 mg/kg/day); n = number of animals submitted

Histopathology

The organs and tissues shown in **Table 18** were collected from all main cohort males and females in the vehicle control group and drug-treated groups and were processed, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and submitted for histopathological examination.

Table 18 Tissues/Organs Collected for Histopathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

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	Spinal cord (cervical, thoracic, and lumbar)
Kidneys	Spleen
Large intestine (cecum, colon, rectum)	Stomach
Liver	Testes
Lungs and bronchi	Thymus
Lymph nodes (mesenteric and mandibular)	Thyroid glands
	Trachea
Skin from mammary area (male and female mice)	Urinary bladder
	Uterus
Mammary gland (females only)	Vagina

Peer Review

A pathology peer review was performed by Natasha Neef, BA, VetMB, PhD, DACVP from Vertex and the peer review statement indicates that there was agreement between the peer review and study pathologists on the evaluation of the data and overall study interpretation.

Neoplastic

Neoplastic lesions and tumors identified by histopathology are shown in **Table 19**, although none were considered related to VX-661 treatment. Tumor findings were evaluated separately for males and females. Tumor analysis was performed on combined and malignant neoplasms. When evaluating tumor incidence, the FDA statistical reviewer's analysis took into consideration the statistically significant dose response relationship in early deaths in high dose males compared to control males.

Multicentric Tumors: The incidence of malignant lymphoma was increased in high dose males (4%) compared to control males (0%), which was not statistically significant

(based on either dose response relationship or pairwise comparison of drug-treated groups to the control group). The one male (#7680) in the high dose group with multicentric malignant lymphoma had lymphoma in the aorta, femur bone marrow, sternum bone marrow, heart, kidneys, liver, lungs with bronchi, cervical spinal cord, and thymus.

The incidence of multicentric hemangiosarcoma in males was increased in the low dose group (20%), high dose group (12%), and positive control group (100%) compared to the vehicle control group (4%). Similarly, in females, the incidence was increased in the low dose group (8%), the high dose group (4%), and the positive control group (70%). The findings in both male and female positive control groups were statistically significant but the drug-treated findings were not statistically significant (based on either the dose response relationship or pairwise comparison of drug-treated groups to the control group).

Lungs with Bronchi: Alveolar-bronchiolar adenomas and carcinomas of the lungs with bronchi were statistically increased in both positive control males (100%) and females (90%) in comparison with their respective controls (i.e., 20% for both males and females). This increase was not evident in drug-treated male and female groups.

Table 19 Neoplastic Lesions and Tumors from Histopathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

Neoplastic Observation	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Number of Animals on Study	**25**	**25**	**25**	**25**	**10**	**25**	**25**	**25**	**25**	**10**
Number of Animals Completed	**25**	**25**	**25**	**25**	**10**	**25**	**25**	**25**	**25**	**10**
Multicentric Tumors										
lymphoma, malignant, incidental	0	0	0	1	0	0	0	0	0	0
hemangiosarcoma, malignant, primary, fatal or incidental (multiple organs)	1	5	1	3	10*	0	2	0	1	7*
Lungs with Bronchi n =	25	25	25	25	10	25	25	25	25	10
alveolar-bronchiolar adenoma, multiple, benign, primary, incidental	0	0	2	0	10	1	0	0	0	9
alveolar-bronchiolar adenoma, single, benign, primary, incidental	2	0	3	1	0	3	0	2	2	0
alveolar-bronchiolar carcinoma, malignant, primary, incidental	3	0	0	0	3	1	0	0	1	1
…combined animals	5	0	5	1	10*	5	0	2	3	9*

Abbreviations: + = positive control (1000 mg/kg/day); n = number of animals examined
* = statistically significant at <0.000 for pairwise comparison to the control group based on FDA Statistical Reviewer's evaluation.

Non-Neoplastic

Non-neoplastic toxicities were noted in the adrenal glands, liver, ovaries, jejunum, lymph nodes (mesenteric and mandibular), spleen, and thymus (see **Table 20**).

The most notable findings in the adrenal glands included minimal to mild cortex hypertrophy (high incidence in mid dose and high dose males and low incidence in high dose females), minimal to mild cortex vacuolation (high dose males, mid dose females, and high dose females), and minimal increased x-zone degeneration (high dose males).

A notable finding in the liver included centrilobular hypertrophy that ranged from minimal to marked in low dose males, mid dose males, high dose males and minimal in high dose females. Minimal to marked centrilobular hepatocellular single cell necrosis was prominent in mid dose males and high dose males. Of note, in the 3-month oral carcinogenicity range-finding study in mice, the liver was identified as a target organ of toxicity based on minimal foci of hepatocyte necrosis seen in two males and one female treated with 1000 mg/kg/day VX-661 and one female in the 600 mg/kg/day drug-treated group, while this finding was not evident in any control animals. Additional liver findings included subcapsular inflammation (low dose males, mid dose males, high dose males, mid dose females, and high dose females), focal hepatocellular necrosis (low dose males, mid dose males, high dose males, and high dose females), focal lipid infiltration (high dose females), and periportal vacuolation (high dose males). The liver is likely a target organ of toxicity due to VX-661 elimination via hepato-biliary secretion.

In the ovaries decreased corpora lutea were noted at in increased incidence in low dose, mid dose, and high dose females compared to control females.

In the jejunum, lamina propria vacuolation was noted in high dose males (minimal to mild) and high dose females (minimal) and was not evident in control animals.

Lymphoid necrosis was noted in the mesenteric and mandibular lymph nodes of low dose and high dose males at an increased incidence compared to control males.

In the spleen, the most notable findings included minimal to mild hemosiderin pigmentation (low dose, mid dose, and high dose males) and minimal to mild lymphoid necrosis (low dose and high dose males).

In the thymus, the most notable finding was minimal to moderate lymphoid necrosis (high dose males). Although at a low incidence, mild increased lymphocytes were noted in high dose females compared to control females.

Table 20 Non-Neoplastic Lesions and Tumors from Histopathology in 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice

	Males					Females				
	VX-661 (mg/kg/day)				+	VX-661 (mg/kg/day)				+
	0	30	100	500	+	0	30	100	500	+
Number of Animals on Study	25	25	25	25	10	25	25	25	25	10

Number of Animals Completed	**25**	**25**	**25**	**25**	**10**	**25**	**25**	**25**	**25**	**10**
Adrenal Glands n =	25	25	25	25	0	25	25	25	25	0
hypertrophy, cortex	0	0	25	25	0	0	0	0	1	0
…minimal	0	0	24	1	0	0	0	0	0	0
…mild	0	0	1	24	0	0	0	0	1	0
vacuolation, cortex	10	6	4	15	0	3	3	6	6	0
…minimal	7	5	4	8	0	2	3	5	6	0
…mild	3	1	0	7	0	1	0	1	0	0
x-zone degeneration, increased	0	0	0	11	0	0	0	0	0	0
…minimal	0	0	0	11	0	0	0	0	0	0
Liver n =	25	25	25	25	0	25	25	25	25	0
hypertrophy, centrilobular	0	24	24	25	0	0	0	0	22	0
…minimal	0	20	0	0	0	0	0	0	22	0
…mild	0	4	23	1	0	0	0	0	0	0
…moderate	0	0	1	5		0	0	0	0	0
…marked	0	0	0	19	0	0	0	0	0	0
inflammation, subcapsular	0	1	1	4	0	0	0	2	2	0
…minimal	0	1	1	1	0	0	0	2	2	0
…mild	0	0	0	2	0	0	0	0	0	0
…moderate	0	0	0	1	0	0	0	0	0	0
necrosis, hepatocellular, focal	2	3	6	7	0	3	1	0	4	0
…minimal	2	1	4	1	0	1	1	0	3	0
…mild	0	1	2	3	0	1	0	0	0	0
…moderate	0	1	0	2	0	1	0	0	1	0
…marked	0	0	0	1	0	0	0	0	0	0
single cell necrosis, hepatocellular, centrilobular	0	0	7	24	0	0	0	0	0	0
…minimal	0	0	7	14	0	0	0	0	0	0
…mild	0	0	0	7	0	0	0	0	0	0
…moderate	0	0	0	2	0	0	0	0	0	0
…marked	0	0	0	1	0	0	0	0	0	0
infiltration, lipid, focal	0	0	0	0	0	1	0	0	2	0
…minimal	0	0	0	0	0	0	0	0	1	0
…mild	0	0	0	0	0	1	0	0	1	0
vacuolation, periportal	0	0	0	2	0	0	0	0	0	0
…mild	0	0	0	2	0	0	0	0	0	0
Ovaries n =	-	-	-	-	-	25	25	25	25	0
decreased corpora lutea	-	-	-	-	-	4	8	5	19	0
Intestine, Jejunum n =	25	25	25	25	0	25	25	25	25	0
vacuolation, lamina propria	0	0	0	10	0	0	0	0	4	0

…minimal	0	0	0	9	0	0	0	0	4	0
…mild	0	0	0	1	0	0	0	0	0	0
autolysis	0	2	0	3	0	2	0	0	2	0
Lymph Node, Mesenteric n =	25	25	25	25	0	25	25	25	25	0
necrosis, lymphoid	0	1	0	3	0	0	0	0	0	0
…minimal	0	1	0	0	0	0	0	0	0	0
…mild	0	0	0	2	0	0	0	0	0	0
…moderate	0	0	0	1	0	0	0	0	0	0
…missing	0	0	0	1	0	0	0	0	0	0
autolysis	0	0	0	0	0	0	0	0	1	0
Lymph Node, Mandibular n =	25	25	25	25	0	25	25	25	25	0
necrosis, lymphoid	0	2	0	2	0	2	0	0	0	0
…minimal	0	1	0	0	0	1	0	0	0	0
…mild	0	1	0	2	0	1	0	0	0	0
Spleen n =	25	25	25	25	10	25	25	25	25	10
pigmentation, hemosiderin	0	3	1	2	0	0	0	0	0	0
…minimal	0	2	1	1	0	0	0	0	0	0
…mild	0	1	0	1	0	0	0	0	0	0
necrosis, lymphoid	0	1	0	4	0	1	0	0	0	1
…minimal	0	1	0	1	0	0	0	0	0	0
…mild	0	0	0	3	0	1	0	0	0	1
Thymus n =	25	25	25	25	0	25	25	25	25	0
cyst	0	2	1	1	0	0	0	1	0	0
hyperplasia, epithelial	0	0	0	0	0	0	0	2	0	0
…moderate	0	0	0	0	0	0	0	1	0	0
…marked	0	0	0	0	0	0	0	1	0	0
necrosis, lymphoid	0	0	0	5	0	1	0	0	0	0
…minimal	0	0	0	1	0	0	0	0	0	0
…moderate	0	0	0	4	0	1	0	0	0	0
increased lymphocytes	0	0	0	0	0	0	0	0	1	0
…mild	0	0	0	0	0	0	0	0	1	0

Abbreviations: + = positive control (1000 mg/kg/day); n = number of animals examined

Toxicokinetics

Blood samples were collected from the retro-orbital sinus of TK cohort animals, under 70% CO_2/30% O_2 anesthesia. Blood collection times for vehicle control animals were 1 hour postdose on Day 1 and Day 177 (Week 26). For drug-treated animals, blood was collected at predose, 0.5, 1, 2, 4, 8, and 24 hours postdose on Day 1 and Day 177 (Week 26). TK parameters that were determined included C_{max}, T_{max}, and $AUC_{0\text{-}24}$ for VX-661 and the metabolites VRT-0996107 (M1) and VRT-1189001 (M2).

As shown in **Table 21** for VX-661, T_{max} for females and males was 0.5 hours for all dose groups on Day 1 and 0.5 hours for the Day 177 (Week 26) low dose and mid dose groups and 1 hour for the Day 177 (Week 26) high dose group. After repeat exposure in females (Day 177 [Week 26]), AUC was generally dose proportional between the low dose and mid dose groups but less than dose proportional between the mid dose and high dose groups. After repeat exposure in males (Day 177 [Week 26]), AUC was generally less than dose proportional between the low dose and mid dose groups and the mid dose and high dose groups. Exposure in females and males was generally comparable. There did not appear to be drug accumulation over the time course of dosing in females or males.

As shown in **Table 21** for metabolite VRT-0996107 (M1), T_{max} for females and males ranged from 2 to 24 hours, regardless of dose or time interval (Day 1 or Day 177 [Week 26]). After repeat exposure in females and males (Day 177 [Week 26]), AUC was generally less than dose proportional between the low dose and mid dose groups and the mid dose and high dose groups. Exposure in females and males was generally comparable. There did not appear to be VRT-0996107 (M1) metabolite accumulation over the time course of dosing in males or females.

As shown in **Table 21** for metabolite VRT-1189001 (M2), T_{max} for females ranged from 4 to 24 hours and for males ranged from 2 to 24 hours, regardless of dose or time interval (Day 1 or Day 177 [Week 26]). After repeat exposure in females and males (Day 177 [Week 26]), AUC was generally less than dose proportional between the low dose and mid dose groups and the mid dose and high dose groups. Exposure in females and males was generally comparable. There appeared to be slight VRT-1189001 (M2) metabolite accumulation over the time course of dosing in males and females.

Table 21 Summary Toxicokinetics for VX-661, Metabolite VRT-0996107 (M1), and Metabolite VRT-1189001 (M2) in Wild-Type CByB6F1 Mice (Applicant's Table)

Analyte	Dose of VX-661 (mg/kg/day)	Sex	Day 1			Day 177		
			AUC_{0-t} (µg*h/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t} (µg*h/mL)	C_{max} (µg/mL)	T_{max} (hr)
VX-661	30 (Group 2)	Female	17.7	2.48	0.500	15.1	2.14	0.500
		Male	18.9	2.93	0.500	17.4	2.44	0.500
		Combined*	18.3	2.71	0.500	16.2	2.29	0.500
	100 (Group 3)	Female	62.2	5.60	0.500	54.1	7.46	0.500
		Male	115	9.63	0.500	40.4	6.50	0.500
		Combined*	88.5	7.62	0.500	47.2	6.98	0.500
	500 (Group 4)	Female	255	39.3	0.500	154	16.5	1.00
		Male	302	46.2	0.500	92.6	16.8	1.00
		Combined*	278	42.8	0.500	123	16.6	1.00
VRT-0996107	30 (Group 2)	Female	71.1	4.80	4.00	72.2	4.41	8.00
		Male	82.5	6.33	4.00	90.6	5.76	8.00
		Combined*	76.8	5.57	4.00	81.4	5.08	8.00
	100 (Group 3)	Female	148	9.97	4.00	212	14.4	8.00
		Male	358	25.7	8.00	214	15.1	2.00
		Combined*	253	17.8	6.00	213	14.7	5.00
	500 (Group 4)	Female	578	29.2	24.0	424	27.7	2.00
		Male	576	38.8	24.0	467	34.4	4.00
		Combined*	577	34.0	24.0	446	31.0	3.00
VRT-1189001	30 (Group 2)	Female	3.68	0.260	4.00	4.69	0.288	8.00
		Male	4.61	0.285	4.00	6.02	0.369	8.00
		Combined*	4.14	0.273	4.00	5.36	0.329	8.00
	100 (Group 3)	Female	7.03	0.400	4.00	13.4	0.901	8.00
		Male	16.2	1.04	8.00	14.7	0.985	2.00
		Combined*	11.6	0.720	6.00	14.1	0.943	5.00
	500 (Group 4)	Female	23.4	1.39	24.0	37.9	2.15	8.00
		Male	22.7	1.67	24.0	43.7	2.95	4.00
		Combined*	23.1	1.53	24.0	40.8	2.55	6.00

* Combined is defined as the TK parameters obtained with the pooled data of male and female mice

Dosing Solution Analysis

Dosing solutions were analyzed for homogeneity (top, middle, and bottom stratum) and concentration (middle stratum) on the first and last TK dose formulations for each sex, and from one Week 13 formulation. All dosing formulations were found to be homogenous and met the acceptance criteria of 85 to 115% of the target concentration and ≤10% the relative standard deviation, (b) (4) . A control sample was also analyzed and no test article was detected.

11 Integrated Summary and Safety Evaluation

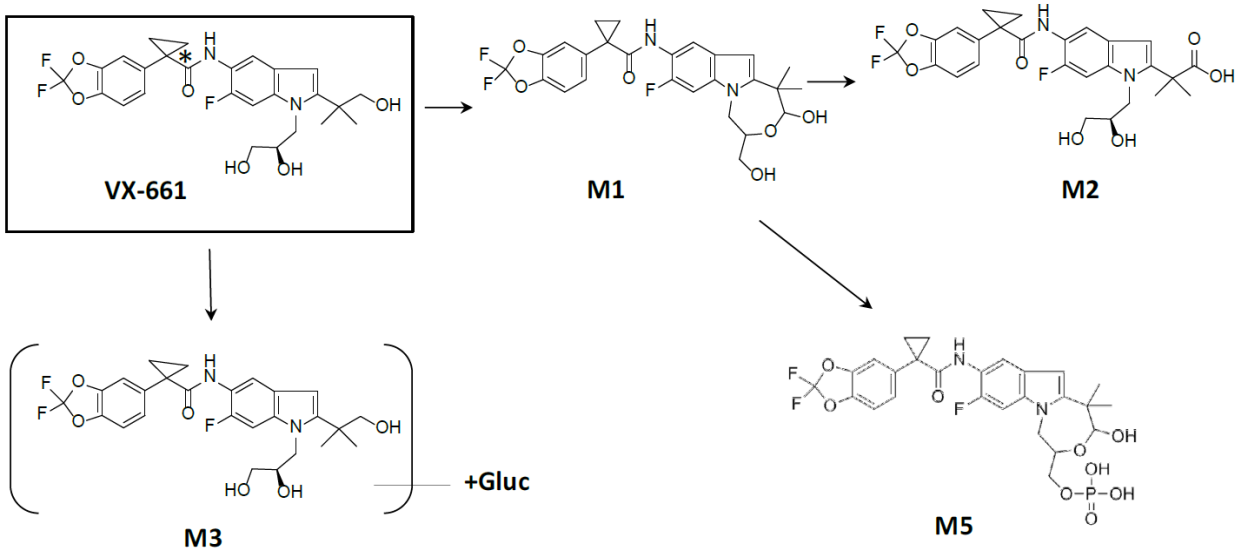
Vertex submitted a 505(b)(1) NDA for tezacaftor (VX-661)/ivacaftor (VX-770) combination therapy for the treatment of CF in patients 12 year of age and older who are homozygous for the F508del mutation or who have at least one mutation in the

CFTR gene that is responsive to tezacaftor (VX-661)/ivacaftor (VX-770), based on in vitro data and/or clinical evidence. Ivacaftor was approved for the treatment of CF on January 31, 2012. The carcinogenic potential of ivacaftor (VX-770) was reviewed under NDA 203188; ivacaftor was not tumorigenic in either 2-year rat or mouse studies.

VX-661 was negative for mutagenicity and clastogenicity in a standard battery of genetic toxicology studies (bacterial reverse mutation assay, in vitro chromosomal aberration assay, and in vivo mouse micronucleus assay).

As shown in **Figure 13**, VX-661 undergoes considerable metabolism (mainly by CYP3A4) in humans and nonclinical species. Dehydrogenation of VX-661 leads to the major metabolite M1 (VRT-0996107). Sequential oxidation of M1 forms metabolite M2 (VRT-1189001), while phosphorylation of M1 forms M5 (VRT-1074233) (which can potentially interconvert back to M1). Metabolites M1 and M2 constitute >10% of total systemic exposure (i.e., approximately 38.8% and 35.7%, respectively) at the proposed clinical dose. M1 is pharmacologically active with similar potency and efficacy as VX-661. M2 is a disproportionate human metabolite (i.e., high levels in humans and low levels in rats and dogs), which is pharmacologically active but less so than VX-661. M5 (VRT-1074233) was detected in a human mass balance study at >10% of total systemic exposure, but is considered pharmacologically inactive.

Figure 13 Proposed Metabolic Pathway of VX-661 in Rats and Humans (Applicant's Figure)



*signifies the position of the radiolabel

The Sponsor conducted a 104-week rat study and 26-week Tg.rasH2 mouse study to assess the carcinogenic potential of VX-661. The ECAC concurred with the doses and design of the studies (see Special Protocol Agreements dated December 18, 2013 and November 20, 2014, respectively).

In a 104-week oral (gavage) carcinogenicity study, SD male rats received doses of 0, 5, 15, and 50 mg/kg/day VX-661 and SD female rats received doses of 0, 5, 20, and 75

mg/kg/day VX-661. There were no drug-related effects on mortality, but male and female groups were both terminated early (i.e., Week 103 and Week 101, respectively) because the number of surviving animals in the respective control groups reached ≤20. At the end of the study period mean body weights for high dose males and females were 5.9% and 17.7% lower, respectively, compared to control animals. There were no statistically significant drug-related tumor findings in male or female rats. The most notable non-neoplastic histopathology findings were dilated lymphatics in the GALT and small intestine (ileum and jejunum). In the GALT, minimal to mild dilated lymphatics were noted with increased incidence in mid dose males, high dose males, and high dose females relative to control animals. In the small intestine (ileum and jejunum) minimal to mild dilated lymphatics were noted with greater incidence in high dose males and females relative to control animals. Neither the incidence or severity of the dilated lymphatics progressed in this 2-year rat study compared to the 3-month and 26-week rat studies. Additional non-neoplastic lesions were seen in the parathyroid glands, liver, Harderian glands, clitoral glands, ovaries, and sternum bone marrow. VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) was 180 and 337 mcg*hr/mL in males and females, respectively. Metabolite M1 AUC exposure at the high dose of VX-661 was about 288 and 479 mcg*hr/mL in males and females, respectively. Metabolite M2 AUC exposure at the high dose of VX-661 was about 20 and 13 mcg*hr/mL in males and females, respectively.

The tezacaftor (VX-661)/ivacaftor (VX-770) dosing regimen will consist of a total daily dose of 100 mg tezacaftor and 300 mg ivacaftor as one tablet of a fixed dose combination of 100 mg tezacaftor/150 mg ivacaftor in the morning and one tablet of 150 mg ivacaftor in the evening. Exposure margins for VX-661, M1 (VRT-0996107), and M2 (VRT-1189001) for the human dose of 100 mg tezacaftor once daily are shown in **Table 22**. VX-661 AUC exposure at the high dose (50 mg/kg/day in males and 75 mg/kg/day in females) results in exposure margins about 2.4-fold and 4.5-fold, respectively, above the clinical exposure achieved with the proposed dosing regimen. Similarly, M1 AUC exposure at the high dose in males and females results in exposure margins about 2.5-fold and 4.2-fold, respectively, above the clinical exposure achieved with the proposed dosing regimen. As expected, since M2 is a disproportionate human metabolite, M2 AUC exposure at the high dose in males and females results in exposure margins about 0.19-fold and 0.12-fold, respectively, above the clinical exposure achieved with the proposed dosing regimen.

Table 22 Clinical Exposure Margins for VX-661, Metabolite VRT-0996107 (M1), and Metabolite VRT-1189001 (M2) Relative to 104-Week Oral (Gavage) Carcinogenicity Study in Rats

VX-661 Exposure Margin for Human Dose of 100 mg Tezacaftor Once Daily:

VX-661 Dose (mg/kg/day) (Males/Females)	VX-661 Week 52 AUC_{0-24hr} (mcg*hr/mL) Males	VX-661 Week 52 AUC_{0-24hr} (mcg*hr/mL) Females	Exposure Margin[1] Males	Exposure Margin[1] Females
50/75	180	337	2.40	4.49
Metabolite VRT-0996107 (M1) Exposure Margin for Human Dose of 100 mg Tezacaftor Once Daily:				
VX-661 Dose (mg/kg/day) (Males/Females)	**M1 Week 52 AUC_{0-24hr} (mcg*hr/mL) Males**	**M1 Week 52 AUC_{0-24hr} (mcg*hr/mL) Females**	**Exposure Margin[1] Males**	**Exposure Margin[1] Females**
50/75	288	479	2.53	4.20
Metabolite VRT-1189001 (M2) Exposure Margin for Human Dose of 100 mg Tezacaftor Once Daily:				
VX-661 Dose (mg/kg/day) (Males/Females)	**M2 Week 52 AUC_{0-24hr} (mcg*hr/mL) Males**	**M2 Week 52 AUC_{0-24hr} (mcg*hr/mL) Females**	**Exposure Margin[1] Males**	**Exposure Margin[1] Females**
50/75	20.0	13.0	0.19	0.12

[1]AUC_{0-24hr} at steady state for 100 mg tezacaftor once daily = 75.1 mcg*hr/mL for VX-661, 114 mcg*hr/mL for M1, and 105 mcg*hr/mL for M2.

In a 26-week oral carcinogenicity study, Tg.rasH2 mice received doses of 0, 30, 100, and 500 mg/kg/day VX-661 and 1000 mg/kg/day urethane (positive control). There was a statistically significant treatment-related increase in mortality for high dose males (76% survival) compared to control males (96% survival). At the end of the study period (i.e., Day 183) mean body weights for VX-661-treated male and female animals were generally similar to control animals. There were no statistically significant drug-related tumor findings in male or female mice. The positive control produced expected increases of neoplastic findings indicating that the mice and study could detect a potential drug-induced neoplastic response. The most notable non-neoplastic histopathology findings were in the adrenal glands (cortex hypertrophy, cortex vacuolation, and x-zone degeneration), liver (centrilobular hypertrophy, centrilobular hepatocellular single cell necrosis, subcapsular inflammation, focal hepatocellular necrosis, focal lipid infiltration, and periportal vacuolation), ovaries (decreased corpora lutea), and jejunum (lamina propria vacuolation). Additional non-neoplastic findings were noted in the mesenteric and mandibular lymph nodes (lymphoid necrosis), spleen (hemosiderin pigmentation and lymphoid necrosis), and thymus (lymphoid necrosis and increased lymphocytes). VX-661 AUC exposure at the high dose (500 mg/kg/day) was about 93 and 154 mcg*hr/mL in males and females, respectively. Metabolite M1 AUC exposure at the high dose of VX-661 (500 mg/kg/day) was about 467 and 424

mcg*hr/mL in males and females, respectively. Metabolite M2 AUC exposure at the high dose of VX-661 (500 mg/kg/day) was about 44 and 38 mcg*hr/mL in males and females, respectively.

The 2-year rat study achieved a greater exposure to M1 relative to the human exposure to M1 associated with the clinical dose of VX-661. Exposure to M1 was quantified in the 26-week Tg.rasH2 mouse carcinogenicity study. Thus, the 2-year rat and 26-week mouse studies provides an adequate assessment of the carcinogenic potential of M1.

Due to M2 being a disproportionate human metabolite, the Applicant evaluated it independently in toxicity studies. Oral administration of M2 to rats, guinea pigs, and dogs had poor bioavailability. Intravenous administration of M2 resulted in deaths in rats. Further, subcutaneous administration was not tolerated in rats. In a 1-month toxicity study, dogs could tolerate a subcutaneous dose that produced an approximate exposure to the expected therapeutic human dose. As with rats, it is also likely for mice that an oral M2 dose group would not achieve relevant exposures, and a subcutaneous administration may not be tolerated, although this has not been attempted. Thus, it was judged that carcinogenic potential of the M2 metabolite could not be studied. M2 possesses no structural alerts for mutagenicity based upon quantitative structure-activity relationship (QSAR) analysis. M2 was negative for potential genetic toxicity by the bacterial reverse mutation assay and the chromosomal aberration assay with human peripheral blood lymphocytes. Exposure to M2 was quantified in the 2-year carcinogenicity study with rats and 26-week Tg.rasH2 mouse carcinogenicity study. Exposures to M2 in the 2-year rat and 26-week mouse studies were approximately ≤0.2 of the achieved clinical exposure. No further nonclinical assessment of the carcinogenic potential of M2 is required.

M5 has not been routinely monitored in nonclinical studies with rats or dogs. The Applicant provided data that M5 is formed in rats from a single dose study, but not in repeat dose studies. It was judged that M5 was formed in sufficient levels in rats to provide an assessment of its toxic potential. Thus, it is reasonable to assume that the 2-year rat study provides an adequate assessment of the carcinogenic potential of M5. M5 has not been studied for potential genotoxicity.

No statistically significant neoplastic findings were observed in male or female SD rats and male or female Tg.rasH2 mice treated with tezacaftor at MTDs.

The ECAC concurred that both the 2-year rat and 26-week Tg.rasH2 carcinogenicity studies were adequate and that there were no drug-related neoplasms in males or females in either study. The ECAC also concurred that based upon feasibility and the completed carcinogenicity studies in rats and Tg.rasH2 mice, that no further studies were required for the safety qualification of the M1, M2, and M5 metabolites with respect to carcinogenicity.

Recommended labeling to describe the results of carcinogenicity studies conducted with tezacaftor and ivacaftor in mice and rats:

Tezacaftor

A two-year study in Sprague-Dawley rats and a (b) (4) study in Tg.rasH2 transgenic mice were conducted to assess the carcinogenic potential of tezacaftor. No evidence of

tumorigenicity was observed in male and female rats at tezacaftor oral doses up to 50 and 75 mg/kg/day (approximately equivalent to (b) (4) times the MRHD based upon summed AUCs of tezacaftor and its metabolites in males and females, respectively). No evidence of tumorigenicity was observed in male and female Tg.rasH2 transgenic mice at tezacaftor oral doses up to 500 mg/kg/day.

Ivacaftor

Two-year studies were conducted in (CD-1) mice and (Sprague-Dawley) rats to assess (the) carcinogenic potential of ivacaftor. No evidence of tumorigenicity was observed in mice or rats at ivacaftor oral doses up to 200 mg/kg/day and 50 mg/kg/day, respectively (approximately equivalent to (b) (4) times the MRHD, respectively, based on summed AUCs of ivacaftor and its metabolites).

The recommended labeling for ivacaftor is based on the labels for KALYDECO® (ivacaftor) and ORKAMBI® (lumacaftor and ivacaftor). Of note, the mouse and rat strains (CD-1 and Sprague-Dawley, respectively) are not designated in the ORKAMBI® label. Further, the word “the” is not included before “carcinogenic potential” in either the KALYDECO® or ORKAMBI® labels.

(b) (4)

12 Appendix/Attachments

Appendix 1: Special Protocol Agreement (i.e., ECAC meeting minutes) dated December 18, 2013, for SPA of 2-year carcinogenicity study in rats

Appendix 2: Special Protocol Agreement (i.e., ECAC meeting minutes) dated November 20, 2014, for SPA of 6-month carcinogenicity study in Tg.rasH2 mice

APPEARS THIS WAY ON ORIGINAL

Appendix 1

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: December 18, 2013

To: Adel Al-Shyaikh, MSc, RAC	**From:** Adele Seifried
Company: Vertex	OND IO
Fax number: (617) 341-6803	**Fax number**: 301-796-9855
Phone number: (617) 961-1563	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 108,105

Total no. of pages including cover: 5

Comments: **email to adel_al-shaikh@vrtx.com**

Document to be mailed: ☐ YES ☑ NO

Reference ID: 3424313

Executive CAC
Dec 17, 2013

Committee: David Jacobson Kram, OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
David Joseph, Ph.D., Alternate Member, DGIEP
Marcie Wood, Ph.D., Pharm Tox Supervisor, DPARP
L. Steven Leshin, D.V.M., Ph.D., Presenting Reviewer, DPARP

Author of Minutes: L. Steven Leshin (DPARP)

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND 108105
Drug Name: VX-661
Sponsor: Vertex Pharmaceuticals Incorporated

Background
VX-661 is a being developed for the treatment of cystic fibrosis (CF) in patients with the *F508del* mutation in the CF transmembrane conductance regulator gene (CFTR). In pharmacodynamic studies, VX-661 has some efficacy in correcting the tertiary *F508del* CF protein structure and its transport to the surface membrane.

Daily oral dosing toxicity studies of 3-months (Report VX-661-TX-010) and 6-months (Report VX-661-TX-012) duration were submitted to support the dose selection for a 2-year carcinogenicity study in rats. A VX-661 metabolite, VRT-1189001 (M2), was discovered to be present in human plasma at concentrations greater than found in the toxicity study species, and is currently undergoing in vivo toxicological characterization.

In dose range-finding and the 1-month duration toxicity studies (Report VRT-893661-TX-010), deaths and morbidity occurred at doses ≥200 mg/kg/day. In the 6-month study the major limiting toxicity at 100 mg/kg/day was the reduction in mean body weight of approximately 16% in males relative to that of controls at week 26. Reduced mean weight gain of 26% for males and 23% for females was noted during the first 12 weeks of the 6-month study. A similar reduction in mean weight gain was also noted in the 3-month study (-23% and -22% for males and females, respectively). Lower relative weights were associated with a reduction in food consumption during the initial few weeks in both studies; thereafter food consumption was similar to controls.

However, the lower body weights persisted throughout the dosing duration. Clinical and anatomical pathological findings in 3- and 6-month studies were not considered dose-limiting for a 2-year bioassay.

VX-661 was negative for inducing mutations in the bacterial reverse mutation assay, and not clastogenic in both the in vitro Chinese hamster ovary cell chromosomal aberration assay and in vivo mouse micronucleus assay. VRT-1189001 (M2) was also negative for inducing mutations in the bacterial reverse mutation assay and the chromosomal aberration assay with human peripheral blood lymphocytes.

Rat Carcinogenicity Protocol (VX-661-TX-020) and Dose Selection

The proposed doses of VX-661 in the sponsor's protocol were 0 (vehicle), (b) (4) and 50 mg/kg/day administered to both males and females, once daily for 2 years. VX-661 is a (b) (4) (b) (4) formulation consisting of VX-661 in hydroxyproplymethylcellulose acetate succinate (HPMC-AS) and sodium lauryl sulfate (SLS) as 49.5% HPMC-AS/0.5% SLS). The control group will receive the (b) (4) formulation of VX-661 without VX-661 present. The vehicle consists of 0.5% methyl cellulose (400 cps) (w/v), 0.5% sodium lauryl sulfate (SLS) (w/v), and 0.01% simethicone (w/v) in deionized water for all dose groups.

(b) (4)

(b) (4) Therefore, the sponsor selected the dose of 50 mg/kg/day as the high-dose for the 2-year rat bioassay.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee recommended doses of 0, 5, 15, and 50 mg/kg/day in males and doses of 0, 5, 20, and 75 mg/kg/day in females, by oral gavage

  These doses were selected based on a maximum tolerated dose. The high dose of 75 mg/kg/day for females is approximately one-third the lethal dose. For males, the recommended high dose of 50 mg/kg/day was selected based on lower relative body weights at 100 mg/kg in the 6-month study. Mid- and low-doses in male and female groups were selected to provide an approximately 3-fold dose separation based on systemic exposure.

- The Committee suggested that the sponsor consider testing the major human metabolite VRT-1189001 (M2) in this study if possible (e.g., at a tolerated dose).

David Jacobson Kram, Ph.D.
Chair, Executive CAC

Reference ID: 3424313

cc:\

/IND 108105, DPARP
/Marcie Wood, Ph.D., DPARP
/L. Steven Leshin, D.V.M., Ph.D., DPARP
/Angela Ramsey, R.N., M.S.M., DPARP
/A Seifried, OND IO

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/s/

DAVID JACOBSON KRAM
12/18/2013

APPEARS THIS WAY ON ORIGINAL

Appendix 2

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: December 18, 2013

To: Alissa Minkoff	**From:** Adele Seifried
Company: Vertex	OND IO
Fax number: (617) 341-6803	**Fax number**: 301-796-9855
Phone number: (617) 961-0003	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 108,105

Total no. of pages including cover: 4

Comments: email to alissa_minkoff@vrtx.com

Document to be mailed: ☐ YES ☑ NO

Reference ID: 3661348

Executive CAC
Nov 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
Marcie Wood, Ph.D., Pharm Tox Supervisor, DPARP
L. Steven Leshin, D.V.M., Ph.D., Presenting Reviewer, DPARP

Author of Minutes: L. Steven Leshin (DPARP)

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 6-month Tg.rasH2 mouse carcinogenicity bioassay, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format-Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND 108105
Drug Name: VX-661
Sponsor: Vertex Pharmaceuticals Incorporated

Background

VX-661 is being developed for the treatment of cystic fibrosis (CF) in patients with the *F508del* mutation in the CF transmembrane conductance regulator gene (CFTR). In pharmacodynamic studies, VX-661 has some efficacy in correcting the tertiary *F508del* CF protein structure and its transport to the surface membrane.

A 5-day dose ranging study and a 28-day repeated oral dosing toxicity study (Report VX-661-TX-017) were submitted to support the dose selection for a 6-month carcinogenicity study in Tg.rasH2 mice. In the 5-day dose ranging study, doses were 0 (vehicle control), 500, 750, 1000, 1250, and 1500 mg/kg/day. The vehicle control consisted of the (b) (4) formulation [hydroxyproplymethylcellulose acetate succinate (HPMC-AS) and sodium lauryl sulfate (SLS) as 49.5% HPMC-AS/0.5% SLS] lacking VX-661 in a vehicle consisting of 0.5% methylcellulose, 0.5% sodium dodecyl sulfate, and 0.01% simethicone in deionized water. Mortalities occurred at the dose of 1500 mg/kg/day in both sexes. At 500 mg/kg/day there were no clinical signs. Histopathology was not conducted, and toxicokinetics for doses greater than 750 mg/kg/day were not assessed.

In the 28-day repeated oral dosing study, doses of 0 (vehicle control), 250, 500, and 750 mg/kg/day were administered once daily (using the same vehicle control described above). There

was no effect on body weight, weight gain, or food consumption. Liver function was altered, as reflected in reduced albumin and increase globulin levels, at the high dose. Serum AST and ALT were increased at the mid or high dose. Liver histopathology included focal necrosis, portal inflammation, and excessive centrilobular hypertrophy. There were only minimal differences between sexes in liver toxicity.

VX-661 was negative for inducing mutations in the bacteria reverse mutation assay, and not clastogenic in both the in vitro Chinese hamster ovary cell chromosomal aberration assay and in vivo mouse micronucleus assay. VRT-1189001 (M2), a significant human metabolite, was also negative for inducing mutations in the bacterial reverse mutation assay and negative for clastogenicity in the chromosomal aberration assay with human peripheral blood lymphocytes.

Tg.rasH2 Mouse Carcinogenicity Study Dose Selection

The proposed doses of VX-661 in the sponsor's protocol were 0 (vehicle), 30, 100, and (b) (4) mg/kg/day administered to both males and females, once daily for 6-months using the same control formulation and vehicle as described above. The sponsor considered (b) (4) mg/kg an MTD based on histopathological findings of liver toxicity. Mid and low doses, 100 and 30 mg/kg/day, respectively, were separated by intervals determined from AUC values extrapolated from toxicokinetics of the 28-day and 5-day studies and an additional short-term toxicokinetic study in CByB6F1 mice (wildtypes of Tg.rasH2 mice, Report VX-661-sdpk303292).

Executive CAC Recommendations and Conclusions:

Tg.rasH2 mouse:

- The Committee recommended doses of 0, 30, 100, and 500 mg/kg/day, by oral gavage, based on mortality at 1500 mg/kg/day. Spacing of mid and low doses was based on AUC.

As a point of information, the S1A-S1C carcinogenicity study guidelines are a current topic of an expert working group (EWG) of the International Conference on Harmonization. For further information on the current status of the EWG's activities, and particularly the S1 concept paper, business plan, and regulatory notice document, please visit:
http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html

Paul Brown, Ph.D.
Acting Chair, Executive CAC

cc:\
/IND 108105, DPARP
/Marcie Wood, Ph.D., DPARP
/L. Steven Leshin, D.V.M., Ph.D., DPARP
/Angela Ramsey, R.N., M.S.M., DPARP
/A Seifried, OND IO

Reference ID: 3661348

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/s/

PAUL C BROWN
11/20/2014

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/s/

ELENI M SALICRU
12/06/2017

TIMOTHY W ROBISON
12/06/2017
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 210491 **Applicant: Vertex Pharmaceutical Inc.** **Stamp Date: June 28, 2017**

Drug Name: Symdeko (tezacaftor/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		Studies of both tezacaftor and ivacaftor are included. Nonclinical studies of ivacaftor have been reviewed previously for the approval of Kalydeco (ivacaftor) and will not be reviewed here unless they were not previously reviewed and provide new safety information.
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		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) 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		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Labeling will be addressed later in the review cycle. The nonclinical aspects of the label are incorporated and generally appropriate. The proposed pharmacologic class of tezacaftor (b) (4) will be discussed internally during the review. A comment will be sent to the sponsor regarding this (see below).
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			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.

Not Applicable

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

- Pharmacologic Class
  As discussed extensively during the review of Orkambi (NDA 206038), we consider the proposed pharmacologic class of (b) (4) to be unacceptable. Submit any new data or other justification that address our concerns with the proposed established pharmacologic class for tezacaftor. In addition, summarize available data or other justification to support describing the pharmacological activity of tezacaftor as (b) (4) as stated in Section 12.1 of your draft labeling. The final pharmacologic class will be a review issue.

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

/s/

LAWRENCE S LESHIN
08/07/2017

CAROL M GALVIS
08/07/2017
I concur.