CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

216386Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Date: March 8, 2023

From: Lois M. Freed, Ph.D.
Director, Division of Pharmacology/Toxicology-Neuroscience
Office of Neuroscience

Subject: NDA 216386 (Zavzpret, zavegepant)

NDA 216386 was submitted by the sponsor (Pfizer) on March 9, 2022, to support marketing approval of zavegepant for the acute treatment of migraine with or without aura in adults. Zavegepant is to be administered by intranasal spray at a maximum recommended human dose of 10 mg/day. At this dose, plasma C_{max} and AUC are 12.98 ng/mL and 32.99 ng*hr/mL, respectively.

A full battery of nonclinical studies was conducted to support clinical development and an NDA. These studies were reviewed under the NDA by Dr. Elizabeth Khoury (Review and Evaluation of Pharmacology/Toxicology, NDA 216386, Elizabeth Khoury, Ph.D., March 8, 2023). Dr. Khoury also references previous reviews of nonclinical studies of zavegepant completed under INDs.

Based on the review, Dr. Khoury has concluded that the nonclinical data support approval of the NDA.

This memo summarizes selected findings from the nonclinical studies.

Pharmacology

The results of in vitro and in vivo studies characterize zavegepant as a calcitonin gene-related peptide (CGRP) receptor antagonist (IC_{50} of 0.04 nM), with selectivity for CGRP compared to other members of the calcitonin receptor family (adrenomedullin 1 and 2, calcitonin, and amylin). In vitro binding affinity to mouse, rat, and rabbit (strains not specified) CGRP receptors were markedly lower compared to human (IC_{50}'s of 140000, 25000, and 8700 nM, respectively), whereas binding affinity to the cynomolgus monkey CGRP receptor was similar

to human (IC_{50} of 0.06 nM). Therefore, monkey was the only pharmacologically relevant species for on-target toxicities.

PK/ADME

PK/ADME studies of zavegepant were conducted in mouse, rat, rabbit, and cynomolgus (and marmoset) monkey using various routes of administration.

In rabbit, absolute bioavailability following intranasal administration of zavegepant (0.3-3 mg/kg) was low but increased with dose (12.5-30.2%). Following intranasal administration of ^{14}C-zavegepant to rats, plasma:olfactory bulb and plasma:trigeminal nerve radioactivity ratios were 500:1 and 180:1, respectively, at 0.5 hours postdose. In rat, brain levels of zavegepant were <LLOQ at 0.083 and 0.25 hours after a 1 mg/kg intravenous bolus, suggesting minimal transport of zavegepant across the blood brain barrier.

Following intravenous and subcutaneous administration, zavegepant was the primary drug-related material in circulation in rat, cynomolgus monkey, and human. No major human metabolites were detected.

Toxicology

Intranasal toxicity studies of zavegepant were conducted in Sprague Dawley rat (4-, 13-, and 26-week) and cynomolgus monkey (4-, 13-, and 39-week). Because systemic exposure was low ("generally <2%") following IN dosing, additional studies were conducted using subcutaneous administration to increase systemic exposure (4- and 13-week in rat; 4-week in monkey). Safety margins were based on interspecies comparisons of dose normalized to nasal surface area (local toxicity) or plasma exposures (systemic toxicity).

Rat: zavegepant was administered at doses of 0, 1.875, 4.375, and 6.25 mg/day in the 4-week intranasal study, doses of 0, 18.8, 28, and 37.5 mg/day in the 13-week intranasal study, and doses of 0, 6, 9.9, and 12.5 mg/day in the 26-week study. Local toxicity (nasal cavity epithelium degeneration/regeneration, subacute inflammation) was observed at all doses in the 13-week study, which resulted in lower doses being used in the 26-week study. At the highest dose tested in the 26-week study, safety margins for local and systemic toxicity were approximately 14- and 50-fold, respectively, compared to human at the maximum recommended human dose (MRHD) of 10 mg/day.

In the subcutaneous studies, zavegepant was administered at doses of 0, 7.5, 25, and 75 mg/kg/day for 4 weeks and at doses of 0, 2, 12, and 20 mg/kg/day for 13 weeks. Injection site toxicity (e.g., chronic inflammation, epidermal necrosis/ulceration, myofiber degeneration, pustule, epidermal hyperplasia, fibrosis, skeletal muscle degeneration and hemorrhage) was observed at all

doses in both studies. At the highest dose tested in the 13-week study, plasma exposures were approximately 2600-fold that in humans at the MRHD.

Monkey: zavegepant was administered at doses of 0, 7.5, 17, and 25 mg/day in the 4-week intranasal study, at doses of 0, 18, 27, and 36 mg/day in the 13-week study, and at doses of 0, 18, 36, and 45 mg/day in the 39-week study. There were no clear drug-related local or systemic toxicity in these studies. At the highest dose tested in the 39-week study, safety margins for local and systemic toxicity were approximately 12- and 8-fold, respectively.

In the 4-week subcutaneous study, zavegepant was administered at doses of 0, 5, 15, and 37.5 mg/kg/day. Injection site toxicity (e.g., subcutaneous inflammation, fibrosis, degeneration/regeneration, necrosis hemorrhage; myofiber degeneration/regeneration) was observed at all doses. At the highest dose tested, plasma exposures were approximately 1500-fold that in humans at the MRHD.

Reproductive and developmental toxicology

A standard battery of reproductive and developmental toxicology studies was conducted for zavegepant in Sprague Dawley rat and New Zealand White rabbit. The studies were conducted using subcutaneous administration to increase systemic exposure. Toxicokinetic analysis was included in the embryofetal development study in rabbit and the first pre- and postnatal development study in rat.

In the fertility and early embryonic development study in rat, zavegepant was administered to males and females at doses of 0, 5, 15, and 25 mg/kg/day prior to mating and continuing in females through gestation day (GD) 7. No adverse effects on fertility or reproductive performance parameters were observed. Plasma exposures at the highest dose tested were approximately 2800 times that in humans at the MRHD.

In the embryofetal development studies, zavegepant was administered to rats (0, 10, 20, and 40 mg/kg/day) and rabbits (0, 20, 40, and 60 mg/kg/day) during the period of organogenesis (GDs 6-17 and GDs 7-19, respectively). No adverse effects on embryofetal development were observed in either species. Plasma exposures (AUC) at the highest doses tested in rat and rabbit were approximately 4000 times that in humans at the MRHD.

Two pre- and postnatal development studies were conducted in rat. In both studies, zavegepant was administered at doses of 0, 5, 10, and 20 mg/kg/day from GD 6 to lactation day (LD) 20. In the first study, there were increases in postimplantation loss, live birth index, and pup deaths at the low and mid doses, and decreases in fertility endpoints in the F_1 generation at the low dose. However, plasma exposures increased (less then dose-proportionally) with dose.

Plasma exposure (AUC) at the highest dose tested was 84200 ng*hr/mL on LD20. Because of these findings and, according to the sponsor, that values for some parameters were “lower” or “in the higher range” of historical data, the study was repeated.

In the second study, no adverse effects on pre- and postnatal development endpoints were observed. Based on data from the first pre- and postnatal development study, plasma exposure at the highest dose tested was approximately 2500 times that in humans at the MRHD.

Genetic toxicology

Zavegepant was negative for mutagenicity and clastogenicity in an adequate battery of genetic toxicology (Ames, in vitro chromosomal aberration in Chinese hamster ovary cells, and in vivo rat micronucleus) assays.

Carcinogenicity

The carcinogenic potential of zavegepant was assessed in a 26-week intranasal carcinogenicity study in Tg.rasH2 mouse (0, 0.3, 0.8, and 2.5 mg/day) and an 85/96-week intranasal carcinogenicity study in Sprague Dawley rat (0 [saline], 0 [vehicle], 2, 9, and 18.8 mg/kg/day). Protocols for both studies were submitted under IND as Special Protocol Assessment requests and reviewed by the division and the Executive CAC. The doses tested are consistent with the Executive CAC recommendations (Meeting Minutes, dated September 3, 2020 [mouse] and May 9, 2019 [rat]).

No drug-related increase in tumors was observed in either species.

In the rat study, plasma exposures (AUC), highest at the mid dose in both males and females, are approximately 140 times that in humans at the MRHD.

Conclusions and Recommendations

The nonclinical data support approval of zavegepant for the proposed indication, with appropriate labeling. A PMR for a juvenile animal toxicology study is recommended, under PREA, to support clinical development for treatment of migraine in the pediatric population.

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/s/

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03/08/2023 01:06:04 PM

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	216386
Supporting document:	SD 1
Applicant's letter date:	March 09, 2022
CDER stamp date:	March 09, 2022
Product:	ZAVZPRET/Zavegepant
Indication:	Acute migraine
Applicant:	Pfizer
Clinical Review Division:	Division of Neurology 2
Reviewer:	Elizabeth S. Khoury, PhD
Team Leader:	David L. Carbone, PhD
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1 Executive Summary

1.1 Introduction

BHV-3500 (zavegepant) was developed by Pfizer for the treatment of migraine in adults. BHV-3500 is a small molecule competitive antagonist of the human calcitonin gene-related peptide (CGRP) receptor. BHV-3500 is to be administered as a 10 mg nasal spray once daily for up to 8 days per month.

1.2 Brief Discussion of Nonclinical Findings

In vitro primary pharmacology studies indicated similar affinity of BHV-3500 for human and cynomolgus monkey CGRP receptors; however, affinity was approximately 10,000-fold lower for dog, rabbit, and rat CGRP. An in vivo pharmacology study indicated suppression of CGRP-induced facial blood flow in monkeys without vasoconstrictive effects on coronary or cerebral arteries (as measured by laser doppler). In rat, BHV-3500 showed minimal distribution to the CNS, and there were no CNS (FOB) effects in a safety pharmacology study in rat. There were no effects of BHV-3500 on cardiovascular or respiratory safety pharmacology endpoints in cynomolgus monkey or Sprague Dawley (SD) rat, respectively. The IC_{50} for hERG inhibition in transfected HEK293 cells was in excess of 30 µM.

There was no systemic toxicity associated with daily SC administration of BHV-3500 at doses up to 20 mg/kg for 13 weeks in rat or up to 37.5 mg/kg for 28 days in monkey. Intranasal toxicology studies up to 6- and 9-months' duration were conducted in male and female SD rat and cynomolgus monkey, respectively. The primary toxicity following IN administration in rat consisted of olfactory and respiratory epithelium degeneration and regeneration in the 13-week repeat dosing study which resolved over a 4-week recovery period. The NOAEL for IN administration in rat was 12.5 mg/day. The primary toxicities in monkeys consisted of decreased body weight gain and olfactory and respiratory epithelium degeneration and regeneration. The NOAEL for IN administration in monkey was 45 mg/day.

BHV-3500 was not mutagenic or clastogenic in a standard genotoxicity battery. A 26-week study in Tg.rasH2 mouse and a 2-year study in SD rat were conducted to assess the carcinogenic potential of BHV-3500. Both studies were negative for carcinogenicity.

Reproductive and developmental toxicology studies were conducted in SD rat and New Zealand White rabbit. There were no reproductive or developmental toxicities at doses resulting in drug exposures at least 2,000-fold higher than human exposure (i.e., AUC) at the proposed clinical dose. A juvenile animal toxicology study was submitted during the review cycle (November 2022) and was, therefore, not reviewed.

1.3 Recommendations

1.3.1 Approvability

BHV-3500 (zavegepant) is approvable from a nonclinical perspective.

1.3.2 Additional Nonclinical Recommendations

A juvenile animal toxicology study in rodents was submitted in November 2022, during the review cycle. This study should be a PMR to allow sufficient time for a review.

1.3.3 Labeling

8.1 Pregnancy

Risk Summary

There are no adequate data on the developmental risk associated with the use of ZAVZPRET in pregnant women. (b) (4) [redacted] –No adverse developmental effects were observed following subcutaneous administration of zavegepant to pregnant animals at doses associated with plasma exposures higher than those used clinically (see Data).

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. The estimated rate of major birth defects (2.2 to 2.9%) and miscarriage (17%) among deliveries to women with migraine are similar to rates reported in women without migraine.

Clinical Considerations
Disease-Associated Maternal and/or Embryo/Fetal Risk
Published data have suggested that women with migraine may be at increased risk of preeclampsia and gestational hypertension during pregnancy.

Data
Animal Data

(b) (4) [redacted]

(b) (4)

Subcutaneous administration of zavegepant (0, 10, 20, 40 mg/kg/day) to pregnant rats or rabbits (0, 20, 40, 60 mg/kg/day) during the period of organogenesis resulted in no adverse effects on embryofetal development. Plasma exposures (AUC) at the highest doses tested were approximately 4000 times that in humans at the maximum recommended human dose (MRHD) of 10 mg/day.

Subcutaneous administration of zavegepant (0, 5, 10, 20 mg/kg/day) to rats through gestation and lactation resulted in no effects on pre- or postnatal development. Plasma exposures (AUC)at the highest dose tested were approximately 2500 times that in humans at the MHRD.

Section 12.1 Mechanism of Action
Zavegepant is a calcitonin gene-related peptide receptor antagonist.

Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis
Intranasal (IN) administration of zavegepant to Tg.rasH2 mice (0, 0.3, 0.8, or 2.5 mg/day) for 26 weeks (b) (4) resulted in no (b) (4) (b) (4) evidence of drug- (b) (4) tumors (b) (4) Plasma exposure (AUC) at the highest doses tested were approximately 140 times that in humans at the maximum recommended human dose (MRHD) of 10 mg (b) (4)

Mutagenesis
Zavegepant was negative in *in vitro* (bacterial reverse-mutation, chromosomal aberration in Chinese hamster ovary cells) and *in vivo* (rat micronucleus) assays.

Impairment of Fertility
Subcutaneous administration of zavegepant (0, 5, 15, or 25 mg/kg/day) to male and female rats prior to and during mating and continuing in females to gestation day (b) (4) -7 resulted in (b) (4) no adverse effects on fertility or reproductive performance (b) (4) Plasma- (b) (4) exposures (AUC) at the highest dose tested were approximately (b) (4) times that in humans at (b) (4) MHRD.

2 Drug Information

2.1 Drug

Generic Name: zavegepant

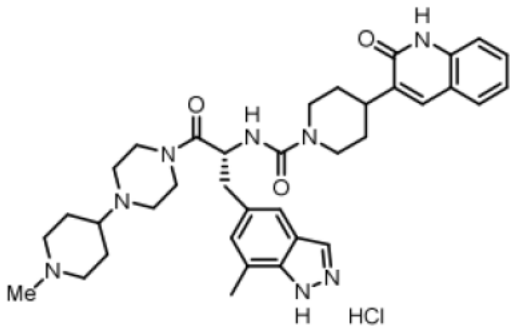
Code Names: BHV-3500, BMS-742413

Proprietary Name: ZAVZPRET

Chemical Name: (R)-N-(3-(7-methyl-1H-indazol-5-yl)-1-(4-(1-methylpiperidin-4-yl) piperazine-1-yl)-1-oxopropan-2-yl)-4-(2-oxo-1,2-dihydroquinolin-3-yl)piperidine-1-carboxamide hydrochloride

Molecular Formula/Molecular Weight:
HCL salt: $C_{36}H_{46}N_8O_3.HCl$ / 675.28 g/mol
Free base: $C_{36}H_{46}N_8O_3$ / 638.80 g/mol

Structure or Biochemical Description:



(Sponsor's Figure)

Pharmacologic Class: Calcitonin gene-related peptide (CGRP) receptor inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 134120 zavegepant (intranasal) for acute migraine, DN2
IND [redacted] (b) (4)
IND [redacted] (b) (4)

2.3 Drug Formulation

The clinical formulation is an intranasal spray ([redacted] (b) (4) mg/mL: dose volume of [redacted] (b) (4) µL) in a single-dose delivery device.

2.4 Comments on Novel Excipients

The drug product contains succinic acid. According to the Inactive Ingredients Database, succinic acid is in FDA-approved intravenous, intramuscular, and oral drug products but not in an intranasal drug product. This excipient was present in vehicle and drug formulations used in the pivotal nonclinical (safety pharmacology, repeat-dose toxicity, carcinogenicity, and reproductive/developmental toxicology) studies at 14- to 39-fold higher than MHRD, and therefore, qualified.

2.5 Comments on Impurities/Degradants of Concern

Four [redacted] (b) (4) impurities were identified. The sponsor has imposed limits of NMT [redacted] (b) (4) % for each of these impurities. This is acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Adults with acute migraine. The proposed dose is 10 mg/day (intranasal).

2.7 Regulatory Background

- August 1, 2017, pIND WRO
- March 16, 2020, EOP2 nonclinical preliminary comments
- May 29, 2020, Type B WRO
- February 12, 2021, Type C Meeting WRO
- February 2021 Agreed iPSP

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

- In vitro efficacy in human neuroblastoma (SK-N-MC) cells
- In vivo efficacy in monkey (facial blood flow assay)

Safety Pharmacology

- Secondary pharmacology binding assays
- In vitro assessments on hERG/IKr channels, sodium channels and purkinje fiber action potentials
- In vivo telemetered assessment of cardiovascular function in monkeys (IN, SC)
- In vivo respiratory and central nervous system batteries in rats

ADME

- Validation of HPLC/MS methods for measuring BHV-3500 in rat, rabbit, and monkey plasma
- Standard PK parameters in mouse, rat, rabbit, dog, and monkey
- In vitro serum stability, protein binding, plasma stability, blood cell partitioning
- In vitro inhibition of human ABC, SLC, NTCP, OAT2v1, OATP1B1, OATP 1B3, OATP2B1 transporters
- In vitro and in vivo metabolite profiling in rat, monkey, and human
- In vitro inhibition and induction of human CYP enzymes

Toxicology

- Single dose studies in mouse, rat, and monkey (intranasal)
- Repeat dose studies:
    - Mouse CBYB6F1 hybrid: 1-week, 4-week IN
    - SD Rat: 1-week (IN, SC), 2 week (SC), 4-week (IN, SC), 13-week (IN, SC), 26-week (IN)
    - Beagle dog: 2-week, 13-week Oral and Sublingual
    - Cynomolgus monkey: 1-week (IN, SC), 4-week (IN), 13-week (IN), 39-week (IN)

- In vitro Ames and chromosomal aberration assays; in vivo rat micronucleus assay (SC)
- Fertility study in rat (SC), embryofetal development studies in rat and rabbit (SC), pre and postnatal development in rat (SC)
- 26-week carcinogenicity study in TgRasH2 mice (IN); 2-year carcinogenicity study in rats(IN)
- Dose-range finding juvenile animal toxicology study in rat (SC)
- Local tolerance study in rat (IV)
- Ocular irritation test
- Phototoxicity test
- Repeat dosing in rat for impurity qualification (13-week, IN)

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Review of carcinogenicity range-finding studies and the protocol for the 26-week Tg.RasH2 mouse carcinogenicity study submitted to IND 134120 (Edmund Nesti, PhD, September 3, 2020).

Review of Tg.RasH2 mouse and 2-year rat carcinogenicity studies by CDER Division of Biometrics (Malick Mbodj, Ph.D., July 28, 2022).

Review of Tg.RasH2 mouse and 2-year rat carcinogenicity studies by the Executive Carcinogenicity Assessment Committee (CAC; September 29, 2022).

4 Pharmacology

4.1 Primary and Secondary Pharmacology

BHV-3500 is a small molecule competitive antagonist of the human calcitonin gene-related peptide (CGRP) receptor. BHV-3500 exhibited high in vitro binding affinity for the human CGRP receptor (IC_{50} = 0.04 nM; K_i = 23 pM); similar binding affinity was observed to the CGRP receptors in cynomolgus (IC_{50} = 0.06 nM) and marmoset (IC_{50} = 0.13 nM) monkeys. In vitro binding affinity was substantially lower to the CGRP receptors in the other animal species tested (IC_{50} values of 500, 600, 8,700, 25,000, and 140,000 nM for beagle dog, guinea pig, rabbit, rat, and mouse, respectively). No vasoconstriction was observed in in vitro preparations of human coronary and intracranial arteries at concentrations up to 10 and 3 µM, respectively. Subcutaneous (SC) administration of BHV-3500 (0.03 mg/kg) in monkeys resulted in 53-80% reduction in facial blood flow at plasma concentrations of 16-19 ng/mL. Potential off-target effects were assessed with an in vitro panel of 38 receptors, enzymes, ion channels, and transporters; 10 µM BHV-3500 inhibited α1 adrenergic receptor and α2 adrenergic receptor by 76% and 86%, respectively.

4.3 Safety Pharmacology

The IC_{50} values for hERG/IKr and SCN5A channels in stably transfected HEK293 cells were in excess of 30 µM. There were no drug-related effects on action potentials in isolated rabbit purkinje fibers incubated with up to 30 µM BHV-3500. CNS and respiratory safety pharmacology assessments were incorporated into the 28-day SC toxicity study in rat. There were no drug effects on the functional observational battery (FOB) or plethysmography parameters following SC administration of up to 75 mg/kg BHV-3500.

CV safety pharmacology was assessed in dedicated single-dose studies in telemetered cynomolgus monkeys. There were no drug-related effects on ECG parameters following administration of 0 and 3.0 mg/kg BHV-3500 by SC injection (3/sex) in an exploratory study. In a second study, BHV-3500 (0, 5, 15, 37.5 mg/kg SC) was administered to 4 telemetered male cynomolgus monkeys using a Latin square design with a 3-day washout period between doses. Cardiovascular parameters were assessed 24 h prior to and after dosing; there were no drug effects.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Summary:

BHV-3500 PK parameters were evaluated in SD rats, balb/c mice, New Zealand white rabbits, beagle dogs, cynomolgus monkeys, and marmoset monkeys. Subcutaneous (SC) administration resulted in greater than 85% bioavailability in dogs, marmosets,

cynomolgus monkeys, balb/c mice, and SD rats. Bioavailability following intranasal (IN) administration in rabbits ranged from 12.5 to 30%.

Table 2 Summary of Pharmacokinetic Parameters of Zavegepant Across Species

Study Design	Species/Strain	Route/Dose (mg/kg)	Animals per group (M/F)	C_{max} (µM)	T_{max} (h)	AUC (0-24) (µg•h/mL	T-HALF (h)	CLTp (mL/min/kg)	Vss (L/kg)	F (%)
Single-dose PK	Dog / Beagle	IV / 1	3 (M)	11 ± 3.1	NA	2.5 ± 0.68	3.8 ± 1.9	11 ± 3	0.82 ± 0.023	NA
		SC / 1	3 (M)	0.95 ± 0.12	0.75 ± 0.25	2.9 ± 0.46	3.6 ± 0.2	NA	NA	117 ± 18
Single-dose PK	Monkey / Marmoset	IV / 1	2 (M)	11 ± 11	NA	7.2 ± 6.4	3.8 ± 3.9	3.6 ± 4.1	0.52 ± 0.59	NA
		SC / 1	3 (M)	2.3 ± 2.1	0.58 ± 0.14	7.4 ± 1.4	3.6 ± 1.3	3.6 ± 0.7	0.8 ± 0.14	115
Single-dose PK	Monkey / Cynomolgus	IV / 1	3 (M)	26 ± 5.8	NA	3.8 ± 0.30	6.4 ± 2.1	6.9 ± 0.61	0.61 ± 0.10	NA
		SC / 1	3 (M)	1.5 ± 0.36	0.5	3.2 ± 0.71	4.4 ± 0.33	NA	NA	85 ± 15
		PO / 10	2 (M)	0.037 ± 0.021	0.67 ± 1.2	0.10 ± 0.056	1.6 ± 0.27	NA	NA	0.25 ± 0.1
Single-dose PK	Balb/c Mouse	IV / 1	9 (M)	5.9	NA	2.2	1.8	12	0.44	NA
		SC / 1	9 (M)	3.9	0.25	3.8	1.2	NA	NA	176
		PO / 10	9 (M)	0.12	0.50	0.31	2.3	NA	NA	1.4

NA = not applicable
Source: BHV-3500-NCPK101 Report[11]
Source: Tables 2.6.5.2-A, D, and E

(Sponsor's Table)

Table 3 Summary of Pharmacokinetic Parameters of Zavegepant Across Species

Study Design	Species/Strain	Route/Dose (mg/kg)	Animals per group (M/F)	C_{max} (µM)	T_{max} (h)	AUC (0-24) (µg•h/mL	T-HALF (h)	CLTp (mL/min/kg)	Vss (L/kg)	F (%) (range)
Single-dose PK	Rat / Sprague-Dawley	IV / 1	3 (M)	14 ± 1.2	NA	7.7 ± 0.42	19 ± 2.9	3.2 ± 0.17	0.8 ± 0.13	NA
		SC / 1	3 (M)	3.4 ± 0.22	0.67 ± 0.29	8.0 ± 1.2	15 ± 4.1	NA	NA	91
Single-dose PK	Rabbit / New Zealand White	IV / 0.5	3 (M)	11 ± 1.3	NA	1.9 ± 0.50	5.7 ± 4.2	7.1 ± 1.8	0.63 ± 0.57	NA
		IN / 0.3	3 (M)	0.12 ± 0.069	0.25 ± 0.083	0.13 ± 0.071	1.1 ± 0.18	NA	NA	12.5 ± 6.5 (5.1 to 28)
		IN / 1	3 (M)	0.55 ± 0.26	0.22 ± 0.048	0.67 ± 0.32	1.0 ± 0.22	NA	NA	18.8 ± 8.9 (6.9 to 36)
		IN / 3	3 (M)	2.0 ± 1.0	0.56 ± 0.39	3.2 ± 1.1	2.2 ± 2.4	NA	NA	30.2 ± 10.8 (14 to 54)

NA = not applicable
Source: BHV-3500-NCPK101 Report[11]
Source: Tables 2.6.5.2-B and C

(Sponsor's Table)

Protein binding ranged from 49 to 84% in rats, dogs, marmoset, cynomolgus monkey, and human plasma. In a separate in vitro study, 0.1 to 10 µg/ml BHV-3500 resulted in 73% plasma protein binding in rabbit. BHV-3500 does not partition into blood cells in rat, dog, monkey, or human (blood/plasma ratio range 0.94 to 1). In tissue distribution studies in non-pigmented rats using radiolabeled BHV-3500, the highest concentrations

of BHV-3500 were detected in urinary bladder and small intestine contents, with maximal concentrations reached at 1 and 2 hours post dose, respectively. Minimal uptake was seen in bladder tissue or intestinal mucosa. In pigmented (Lister Hooded) rats, drug uptake was highest in intestine, kidney cortex, choroid plexus, and spinal nerve; brain to plasma ratios were 0.013 and 0.011 at 5 and 15 minutes, respectively.

Unchanged parent drug was the major component in plasma, urine, and feces of rats and monkeys. An N-desmethyl metabolite (BMS-750873) was identified following SC administration of BHV-3500 in male rats. BHV-3500 was primarily metabolized by CYP3A4 and CYP2D6 in in vitro enzyme assays. BHV-3500 was metabolically stable in human primary hepatocytes, liver microsomes, and olfactory microsomes.

BHV-3500 is primarily excreted intact (40% after IV administration in cynomolgus monkeys). In studies in bile duct-cannulated rat, the majority of BHV-3500 was excreted renally (34%) and to a lesser extent through biliary elimination (6%). In tissue distribution studies in rat using radiolabeled BHV-3500, 63% of the dose was accounted for in feces and 25% was accounted for in urine.

BHV-3500 was a weak inhibitor of CYP3A4 (EC_{20} and EC_{60} > 50 µM), OATP1B1 and OATP1B3 (both IC_{50} > 40 µM) and a more potent inhibitor of OCT2, MATE1, and MATE2-K, with IC_{50} values of 2.15, 0.158, and 4.96 µM, respectively. BHV-3500 is a substrate for transporters P-gp, MATE1, MATE2-K.

6 General Toxicology

6.1 Single-Dose Toxicity

Acute toxicity was assessed in rat and monkey. Injection site toxicity was seen at all doses in SD rats (3/sex/group) administered single SC doses of 60, 100, or 150 mg/kg BHV-3500 SC (no control), resulting in early euthanasia of 2 HDF. There were no adverse effects in female SD rats (5/group) administered single oral doses up to 6,000 mg/kg, or cynomolgus monkeys (1/sex/group) administered single SC doses up to 30 mg/kg.

6.2 Repeat-Dose Toxicity

Mouse *(Summarized from review by Edmund Nesti):*
There were no adverse effects in mice (CBYB6F1 hybrid) following intranasal (IN) administration of 0, 0.3, 0.6, and 1.5 mg/day BHV-3500 for 7 days. Daily IN administration of 0, 1.5, 2, and 2.5 mg BHV-3500 for 28 days resulted in minimal to mild nasal epithelial degeneration and regeneration at 1.5 and 2.5 mg/day. Additional findings consisted of approximately 2.5-fold increases in AST and ALT at all doses in females; however, there were no corresponding histological or gross findings.

Rat:

Repeat-dose studies in rat assessed toxicity following SC or IN administration of BHV-3500. SC administration studies were conducted in male and female SD rats (7-day, 10-day, 14-day, 28-day, 13-week). Injection site toxicity was present at all doses in each study (2-150 mg/kg/day). There was no systemic toxicity associated doses of up to 20 mg/kg for 13 weeks.

Day	Group Number	Dose Level (mg/kg/day)	Sex	T_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{last} (h)	C_{last} (ng/mL)	$AUC_{(last)}$ (h*ng/mL)	$AUC_{(all)}$ (h*ng/mL)	$AUC_{(inf)}$ (h*ng/mL)
1	2	2	M	1.00	3960	NA	24.00	14.8	11000	11000	NA
			F	0.50	2940	0.94	8.00	17.2	7130	7260	7150
1	3	12	M	1.00	8120	3.57	24.00	108	56900	56900	57500
			F	1.00	6850	3.51	24.00	85.2	47400	47400	47800
1	4	20	M	2.00	9630	3.78	24.00	193	86200	86200	87200
			F	2.00	8510	3.73	24.00	156	74700	74700	75600
39	2	2	M	1.00	2630	4.09	24.00	57.9	19600	19600	20000
			F	0.50	2110	NA	24.00	34.9	10400	10400	NA
39	3	12	M	2.00	5830	5.26	24.00	470	92100	92100	95700
			F	4.00	5500	NA	24.00	202	67400	67400	NA
39	4	20	M	2.00	8030	8.01	24.00	1330	116000	116000	131000
			F	2.00	7850	5.29	24.00	456	84900	84900	88300
91	2	2	M	4.00	2010	NA	24.00	61.7	27500	27500	NA
			F	1.00	2090	NA	24.00	39.5	12900	12900	NA
91	3	12	M	4.00	5830	NA	24.00	632	89100	89100	NA
			F	4.00	6490	NA	24.00	236	80500	80500	NA
91	4	20	M	2.00	9140	9.09	24.00	1690	124000	124000	147000
			F	2.00	8650	4.76	24.00	497	111000	111000	114000
NA= Not applicable.											

(Sponsor's Table; 13-week SC rat toxicokinetic data)

Toxicity associated with IN administration was assessed in three non-GLP studies of 1-week duration as well as GLP-compliant studies of 28 days, and 13 and 26 weeks. In the 1-week studies, adverse effects were only seen at the site of administration and consisted of atrophy, erosion, inflammation, necrosis, and ulceration in nasal cavity olfactory epithelium, single cell necrosis and inflammation in nasal cavity respiratory epithelium, and neutrophilic exudate in the nasal cavity.

Study title: BHV-3500: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Sprague-Dawley Rats

Study no.:	1016-3751
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 11, 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, 6D16168, 99.9%

Methods

Doses:	0, 1.875, 4.375, 6.25 mg/day
Frequency of dosing:	Daily
Route of administration:	IN
Dose volume:	12.5 µL per nostril
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, water
Species/Strain:	SD rat
Number/Sex/Group:	Terminal 10/sex/group; Recovery 5/sex Control and HD
Age:	6 weeks
Weight:	198-285 g Males; 160-225 g Females
Satellite groups:	TK Control 3/sex/group; BHV-3500 3/sex/group/timepoint
Unique study design:	None
Deviation from study protocol:	All deviations were minor and did not affect study integrity.

Observations and Results

Mortality and Clinical Signs

Clinical observations and mortality checks were conducted twice daily. No mortality was observed. Salivation, sneezing, and rubbing muzzle on the cage were the most frequently observed clinical signs and occurred at all doses after administration.

Body Weights

Body weight was assessed weekly. There were no drug-related changes in body weight or body weight gain relative to controls.

Food Consumption

Food consumption was assessed weekly. High-dose males (HDM), high-dose females (HDF), mid-dose males (MDM) and mid-dose females (MDF) had slight decreases in food consumption in the second half of the dosing period.

Ophthalmoscopy

Indirect (funduscopic) and biomicroscopic (slit lamp) examinations were conducted prior to the initiation of dosing and at the end of the dosing period (Week 4). All exams were conducted by a board-certified veterinary ophthalmologist. There were no BHV-3500-related findings.

ECG

Not assessed.

Hematology, Clinical Chemistry, and Urinalysis

Clinical pathology parameters were assessed at the end of the dosing period. There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology and Organ Weights

There were no drug-related gross findings or effects on organ weights

Histopathology

Adequate Battery: Yes

Abnormal findings	Larynx	Seminal vesicles
Adrenals	Liver (2 lobes)	Skeletal muscle (thigh)
Animal identification [1]	Lungs with bronchi [4]	Skin, subcutis & mammary gland (inguinal) [8]
Aorta (thoracic)	Lymph nodes (mandibular and mesenteric)	Spinal cord (cervical)
Brain [2]	Nasal cavities (4 levels) [4]	Spleen
Cecum	Olfactory bulb	Sternum with marrow
Colon	Optic nerves [5,7]	Stomach
Duodenum	Ovaries	Testes [7]
Epididymides	Pancreas	Thymus
Esophagus	Pharynx	Thyroids with parathyroids [5]
Eyes [7]	Pituitary	Tongue
Femur with marrow	Prostate	Trachea
Heart [3]	Rectum	Urinary bladder
Ileum	Salivary gland (mandibular)	Uterus [6]
Jejunum	Sciatic nerve	Vagina
Kidneys		

(Sponsor's Table)

Signed Pathology Report: Yes
Peer Review: No
Findings: There were no drug-related findings.

Special Evaluation

None

Toxicokinetics

TK parameters were assessed on Days 1 and 28. T_{max} ranged from 0.25 to 1 h. C_{max} and AUC were similar in males and females; however, there was no clear dose/exposure relationship.

Day	Dose (mg/kg)	Males		Females	
		C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (ng*h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (ng*h/mL)
1	1.875	1220	2420	1160	1530
	4.375	1670	3740	2210	3800
	6.25	1510	3300	1460	3280
28	1.875	366	933	817	1230
	4.375	1320	3090	1190	2650
	6.25	1130	3770	1230	2550

Dosing Solution Analysis

All dosing formulations were within 4% of nominal.

Study title: A 13-week Intranasal Toxicity Study followed by a 28-Day recovery period in Sprague Dawley Rats

Study no.:	1016-3891
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, 6D16168, 93.5% BHV-3500, A021800003, 92.5%

Methods

Doses:	0, 18.8, 28, 37.5 mg/kg
Frequency of dosing:	Daily
Route of administration:	Intranasal
Dose volume:	15-39 microliter per nostril
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, water
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	10/sex/group; Recovery 5/sex (Control and HD)
Age:	7 weeks old
Weight:	Beginning of experiment - Males: 217-290g, Females: 178-247g
Satellite groups:	TK; 3/sex (control); 9/sex/group (BHV-3500)
Unique study design:	None
Deviation from study protocol:	No significant deviations from study protocol.

Observations and Results

Mortality and Clinical Signs

Mortality and cage-side observations were recorded twice daily. There were no drug-related mortalities or adverse effects

Body Weights

Body weight was assessed weekly. On Day 91, body weight was decreased at all doses, up to 6 and 8% in males and females, respectively.

Food Consumption

LDF, MDM, HDM, and HDF had a small decrease in food consumption that resolved in HD animals in the recovery period.

Ophthalmoscopy

Funduscopic and biomicroscopic exams were conducted by a board-certified veterinary ophthalmologist prestudy and at Week 13. No drug-related ophthalmologic changes were detected.

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected from the abdominal aorta in fasted rats at the time of necropsy. Urine was collected overnight prior to necropsy. There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology and Organ Weights

There were no drug-related gross pathology findings or effects on organ weights.

Histopathology

Adequate Battery: Yes

Adrenal Glands	Lungs with Bronchi	Skin & Subcutis
Aorta	Lymph Node, Mandibular	Skin &Subcutis/ Mammary Gland
Brain	Lymph Node, Mesenteric	Spinal Cord, Cervical
Cecum	Nasal Cavities	Spleen
Colon	Olfactory Bulb	Sternum & Marrow
Duodenum	Optic Nerves	Stomach
Epididymides	Ovaries	Testes
Esophagus	Pancreas	Thymus
Eyes	Pharynx	Thyroid & Parathyroid
Femur & Marrow	Pituitary Gland	Tongue
Heart	Prostate Gland	Trachea
Ileum	Rectum	Trigeminal Pathway
Jejunum	Salivary Gland, Mandibular	Urinary Bladder
Kidneys	Sciatic Nerve	Uterus
Larynx	Seminal Vesicles	Vagina
Liver	Skeletal Muscle	

Signed Pathology Report: Yes
Peer Review: No

Histological Findings:

Histopathology findings consisted of degeneration/regeneration of the nasal epithelium, erosions, and inflammation and hemorrhage in the nasal cavity at all doses. These findings were determined to be adverse in HDM due to increased (i.e., moderate) severity of degeneration/regeneration of the nasal epithelium

Text Table 6: Group Incidence and Severity of BHV-3500-related Microscopic Changes in Main Scheduled Euthanasia

Sex	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose Level (mg/kg/day)	0	18.8	28	37.5	0	18.8	28	37.5
Number of Animals	10	10	10	10	10	10	10	10
Nasal Cavity								
Degeneration/Regeneration epithelium	**0**	**9**	**10**	**10**	**0**	**6**	**7**	**10**
Minimal	0	7	6	3	0	5	5	7
Mild	0	2	4	6	0	1	2	3
Moderate	0	0	0	1	0	0	0	0
Erosion	**0**	**0**	**2**	**1**	**0**	**0**	**0**	**0**
Minimal	0	0	0	0	0	0	0	0
Mild	0	0	2	1	0	0	0	0
Inflammation, subacute, epithelium	**0**	**2**	**6**	**10**	**0**	**0**	**1**	**2**
Minimal	0	2	6	9	0	0	1	2
Mild	0	0	0	1	0	0	0	0
Hemorrhage	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**
Minimal	0	0	0	1	0	0	0	0
Mild	0	0	1	0	0	0	0	0

(Sponsor's Table)

Special Evaluation

None

Toxicokinetics

T_{max} ranged from 0.25 to 1 h. There was a secondary peak of absorption 1 to 4 h after T_{max} that was likely the result of delayed GI absorption of BHV-3500 due to high dose volumes. There was no consistent relationship between dose and exposure (i.e., C_{max} and AUC) across sex or timepoint.

Day	Group Number	Dose Level (mg/kg/day)	Sex	T_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{last} (h)	C_{last} (ng/mL)	$AUC_{(last)}$ (h*ng/mL)
1	2	18.8	M	0.50	1020	NA	24.00	43.2	2290
			F	0.50	460	NA	24.00	0.527	1420
1	3	28.0	M	0.50	2570	NA	24.00	10.3	5200
			F	0.50	1510	NA	24.00	7.19	4660
1	4	37.5	M	0.50	3010	NA	24.00	12.3	6450
			F	0.50	2760	NA	24.00	8.06	4440
39	2	18.8	M	2.00	1470	NA	24.00	139	5180
			F	1.00	964	4.21	24.00	2.20	1870
39	3	28.0	M	0.25	1550	NA	24.00	262	6060
			F	0.50	1150	NA	24.00	21.6	3210
39	4	37.5	M	0.25	1220	NA	24.00	106	3820
			F	0.25	968	NA	24.00	41.2	1720
91	2	18.8	M	0.50	1310	NA	24.00	19.5	4050
			F	0.50	668	NA	24.00	4.33	1790
91	3	28.0	M	1.00	2590	3.68	24.00	17.8	9220
			F	0.50	2600	NA	24.00	33.4	5910
91	4	37.5	M	1.00	4540	NA	24.00	108	15700
			F	1.00	3450	4.93	24.00	22.5	7570

NA= Not applicable.

(Sponsor's Table)

Dosing Solution Analysis

Drug formulations were within -2.3% to 5.2% of nominal concentrations.

Study title: A 26-week Intranasal Toxicity Study followed by a 28-Day recovery period in Sprague Dawley Rats

Study no.:	1016-3901
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 6, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, Batch A011810655, 92.1% purity; BHV-3500, A021800003, 92.5% purity

Methods

Doses:	0, 6, 9.9, 12.5 mg/day (6.8, 11.2, 14.2 mg/kg)
Frequency of dosing:	Daily
Route of administration:	Intranasal
Dose volume:	15-25 microliter per nostril
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, water
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	20/sex/group; Recovery: 5/sex/group (control and HD)
Age:	7 weeks old
Weight:	Males: 217-290g, Females: 178-247g
Satellite groups:	TK: 3/sex (control); 9/sex/group (BHV-3500)
Deviation from study protocol:	No significant deviations from study protocol.

Observations and Results

Mortality

Animals were monitored twice daily. One CM and one MDM were found dead for which a definitive cause of death was not determined; however according to the pathology report, based on findings of marked, diffuse congestion in the lungs and the accumulation of vehicle solution in the larynx and pharynx, death was considered likely due to acute inhalation of vehicle. One MDM was found dead, with hematopoietic neoplasms affecting multiple organs including brain, pituitary, kidney, liver, lymph nodes, spleen, adrenals, and nasal tissue; these findings were not drug-related.

Clinical Signs

Detailed clinical observations were conducted prior to the initiation of dosing and then weekly over the dosing period. Cage-side observations were conducted twice daily. There were no adverse drug effects.

Body Weights

Animals were weighed weekly. On Day 182, body weight was decreased 8, 9, and 4% in LDM, MDM, and HDM, respectively (i.e., no dose response); however, body weight and body weight gain was comparable to controls after the recovery period. There were no drug effects on body weight in females.

Food Consumption

Food consumption was assessed weekly. There were no significant effects on food consumption.

Ophthalmoscopy

Ophthalmologic exams were conducted prior to the initiation of dosing and once near Week 26 using a funduscope and biomicroscope. There were no drug-related findings.

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected from the abdominal aorta at the time of the scheduled necropsy after overnight fasting. Urine was collected overnight prior to scheduled necropsy. There were no drug effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology

No drug-related findings.

Organ Weights

No drug-related findings.

Histopathology

Adequate Battery: Yes

Abnormal findings	Kidneys	Skin, subcutis & mammary
Adrenals	Liver (2 lobes)	gland (inguinal) [8]
Animal identification [1]	Lungs with bronchi [4]	Spinal cord (cervical) [11]
Aorta (thoracic)	Lymph nodes (mandibular and	Spleen
Brain [2]	mesenteric)	Sternum with marrow
Cecum	Nasal cavities (4 levels) [4, 9]	Stomach
Colon	Olfactory bulb [2]	Testes [7]
Duodenum	Optic nerves [5,7]	Thymus
Epididymides	Ovaries	Thyroids with parathyroids [5]
Esophagus	Pancreas	Tongue
Eyes [7]	Pituitary	Trachea
Femur with marrow	Prostate	Trigeminal pathway [10]
Heart [3]	Rectum	Urinary bladder
Ileum	Salivary gland (mandibular)	Uterus [6]
Jejunum	Sciatic nerve	Vagina
Larynx and Pharynx	Seminal vesicles	
	Skeletal muscle (thigh)	

(Sponsor's Table)

Signed pathology report: Yes
Peer Review: No

Histological Findings: There were no drug-related findings.

Special Evaluation

None

Toxicokinetics

TK parameters were evaluated on Day 1, Week 13 (Day 91), and Week 26 (Day 182). No sex differences in C_{max} and AUC were observed. Decreases in C_{max} and AUC on

Days 1, 92, and 182 indicated possible induction of metabolic pathways. There was no dose-exposure relationship.

Day	Group Number	Dose Level (mg/animal/day)	Sex	T_{max} (h)	C_{max} (ng/mL)	T_{last} (h)	C_{last} (ng/mL)	$t_{1/2}$ (h)	$AUC_{(last)}$ (h*ng/mL)
1	2	6.0	M	0.25	1490	24.00	3.69	NA	3070
			F	0.50	1460	24.00	4.81	NA	3280
1	3	9.9	M	1.00	1870	24.00	8.16	NA	4790
			F	0.50	2700	24.00	8.17	NA	5150
1	4	12.5	M	0.50	2610	24.00	13.7	NA	6820
			F	1.00	2080	24.00	3.71	NA	4600
91	2	6.0	M	1.00	696	24.00	6.28	NA	1700
			F	1.00	846	24.00	6.30	6.06	1840
91	3	9.9	M	1.00	983	24.00	12.7	NA	2790
			F	1.00	954	24.00	3.07	5.83	1580
91	4	12.5	M	0.25	588	24.00	3.70	7.44	1300
			F	1.00	866	24.00	4.04	7.71	1740
182	2	6.0	M	0.50	659	24.00	3.58	NA	1330
			F	0.50	704	24.00	5.04	NA	1620
182	3	9.9	M	0.50	886	24.00	6.39	NA	1920
			F	0.50	967	24.00	4.19	4.02	2720
182	4	12.5	M	0.50	832	24.00	6.70	NA	2170
			F	0.50	767	24.00	2.96	NA	1170
NA = Not Applicable.									

(Sponsor's Table)

Dosing Solution Analysis

All drug formulation concentrations were within -6.6 to +7.2% of nominal.

Dog:

Two studies (14-day and 13-week) were conducted in which the toxicity of both oral (capsule) and sublingual (tablet) formulations were assessed. Dosing in both studies was 0 or 50 mg/kg BHV-3500 orally and sublingually. There were no adverse effects in either study.

Monkey:
Repeat-dose studies in cynomolgus monkey assessed toxicity following SC or IN administration of BHV-3500. Daily SC dosing for 7 and 28 days resulted in injection site toxicity at all doses (5 to 40 mg/kg/day). There was no systemic toxicity.

Day	Dose (mg/kg)	Males		Females	
		C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (ng*h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (ng*h/mL)
1	5	7400	8130	5600	6620
	15	13800	21600	11800	29700
	37.5	12400	47800	17000	50500
28	5	5240	7260	4940	5980
	15	9810	23600	11900	30000
	37.5	14100	51100	15800	52100

A 7-day dose range finding (DRF) study was conducted in 1/sex/group in which daily IN doses of 1.125, 2.625, and 3.75 (no control) mg/kg BHV-3500 were administered; there were no adverse drug effects.

Study title: A 28-Day Intranasal Toxicity Study Followed by a 14-Day Recovery Period in Cynomolgus Monkeys

Study no.: 1016-3813
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: May 8, 2017
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BHV-3500, 6D16168, 99.9%

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Methods

Doses:	0, 2.5, 5.8, 8.3 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	IN
Dose volume:	33 µL/kg (half per nostril)
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, water
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/sex/group; Recovery: 2/sex/group (Control and HD)
Age:	2 years 8 months to 5.5 years old
Weight:	2.4-3.2 kg females; 2.2-3.6 kg males
Satellite groups:	
Unique study design:	n/a
Deviation from study protocol:	No significant deviations from study protocol.

Observations and Results

Mortality and Clinical Signs

Mortality checks and clinical observations were conducted twice daily. There was no mortality or adverse clinical signs.

Body Weights

Body weight was assessed weekly. No drug-related effects were observed.

Food Consumption

Not assessed.

Ophthalmoscopy

Direct, indirect, and slit-lamp examinations were conducted prior to the initiation of dosing and at Week 4. There were no drug-related effects.

ECG

Standard ECG parameters were assessed prior to the initiation of dosing and at Week 4; there were no drug-related effects.

Hematology, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected from fasted animals prior to the initiation of dosing and during Week 4. There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology and Organ Weights

There were no drug-related gross findings or effects on organ weights.

Histopathology

Adequate Battery: Yes

Abnormal findings	Jejunum	Skeletal muscle (thigh)
Adrenals	Kidneys	Skin, subcutis & mammary gland (thoracic) [7]
Animal identification [1]	Liver (2 lobes)	Spinal cord (cervical)
Aorta (thoracic)	Lungs (2 lobes) with bronchi [4]	Spleen
Brain [2]	Lymph nodes (mandibular and mesenteric)	Sternum with marrow
Cecum	Nasal cavities [4, 9]	Stomach
Colon	Optic nerves [6, 8]	Testes [6]
Duodenum	Ovaries	Thymus
Epididymides	Pancreas	Thyroids with parathyroids [8]
Esophagus	Pituitary	Tongue
Eyes [6]	Prostate	Trachea
Femur with marrow	Rectum	Urinary bladder
Gallbladder	Salivary gland (mandibular)	Uterus [5]
Heart [3]	Sciatic nerve	Vagina
Ileum	Seminal vesicles	

(Sponsor's Table)

Signed and Dated Pathology Report: Yes
Peer Review: No

Histological Findings: There were no drug-related findings.

Special Evaluation

None

Toxicokinetics

TK parameters were assessed on Days 1 and 28. T_{max} ranged from 0.25 to 0.5 h. Increases in C_{max} and AUC were dose proportional. No sex differences in exposure or accumulation were observed. C_{max} and AUC at the NOAEL of 8.3 mg/kg/day were 67.5 ng/mL and 121 h*ng/mL, respectively, in males and 107 ng/mL and 142 h*ng/mL, respectively, in females.

Day	Dose (mg/kg)	Males		Females	
		C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (ng*h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (ng*h/mL)
1	2.5	57.9	61.9	20.4	26.2
	5.8	84.7	116	77.9	93.4
	8.3	95.7	140	118	158
28	2.5	30.9	56.9	22.3	54
	5.8	51.2	79.6	46.6	73.2
	8.3	67.5	121	107	142

Dosing Solution Analysis

Dose formulation concentrations were within 10% of nominal.

Study title: A 13-Week Intranasal Toxicity Study Followed by a 28-Day Recovery Period in Cynomolgus Monkeys

Study no.:	1017-3603
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 9, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, 6D16168, 93.5%

Methods

Doses:	0, 6, 9, 12 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	IN
Dose volume:	24-48 µL/kg (half per nostril)
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, water
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	4/sex/group; Recovery 2/sex/group (Control and HD)
Age:	3-4 years old
Weight:	2.1-2.8 kg females; 2.2-3.4 kg males
Satellite groups:	None
Unique study design:	n/a
Deviation from study protocol:	No significant deviations from study protocol.

Observations and Results

Mortality and Clinical Signs

Mortality checks and clinical observations were conducted twice daily. No mortality was observed. No adverse effects were observed.

Body Weights

Body weight was recorded weekly. There were no drug-related changes in body weight or body weight gain

Food Consumption

Not assessed.

Ophthalmoscopy

Direct, indirect, and slit-lamp examinations were conducted prestudy and at Week 13. No drug-related changes in ophthalmic assessments were detected.

ECG

Standard ECG was conducted prestudy and at Week 13. There were no drug-related changes in ECG parameters.

Hematology, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected prestudy and during Weeks 13 and 17 (recovery). There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology

There were no drug-related gross findings.

Organ Weights

There were increases in the weight of adrenal glands, thyroid/parathyroid, and ovaries and decreases in spleen and thymus in BHV-3500 groups; however, the changes were not dose-related, and there were no histopathological correlates.

Text Table 6 Noteworthy Organ Weight Differences* from Controls (Absolute and Relative to Body or Brain) of Main Scheduled Euthanasia

		Males			Females		
Group		**2**	**3**	**4**	**2**	**3**	**4**
Dose Level (mg/kg/day)		6	9	12	6	9	12
Number of Animals		4	4	4	4	4	4
Terminal Body Weight (kg)		+1.63	-8.94	-6.50	+12.37	-4.12	+9.28
Adrenals							
	Absolute	-18.95	+0.11	+19.98	+22.18	+18.50	+27.71
	% body	-20.42	+9.73	+28.29	+7.71	+22.80	+16.64
	% brain	-10.37	+6.99	+25.26	+34.57	+18.99	+29.74
Spleen							
	Absolute	+7.48	-6.34	-26.17	-2.78	-26.92	-30.07
	% body	+9.13	+5.00	-19.99	-13.32	-22.61	-34.02
	% brain	+19.49	+2.48	-21.37	+5.85	-24.58	-29.16
Thymus							
	Absolute	-49.06	-42.66	-41.50	+4.38	-46.04	-27.98
	% body	-50.85	-37.89	-38.28	-9.98	-43.82	-32.50
	% brain	-44.69	-38.41	-39.29	+12.34	-44.78	-28.29
Thyroid/Parathyroid							
	Absolute	+21.64	+14.25	+39.15	+41.10	-0.42	+40.52
	% body	+15.54	+29.24	+48.31	+22.04	-0.04	+28.87
	% brain	+37.47	+23.90	+48.42	+48.91	-1.37	+42.92
Ovaries							
	Absolute	-	-	-	+107.60	+12.93	+62.27
	% body	-	-	-	+76.02	+15.23	+41.06
	% brain	-	-	-	+118.28	+15.27	+61.49

*Mean organ weights and terminal body weights were presented as percent difference from mean control values.

(Sponsor's Table)

Histopathology

Adequate Battery: Yes

Abnormal findings	Jejunum	Skeletal muscle (thigh)
Adrenals	Kidneys	Skin, subcutis & mammary gland (thoracic) [7]
Animal identification [1]	Liver (2 lobes)	Spinal cord (cervical) [11]
Aorta (thoracic)	Lungs (2 lobes) with bronchi [4]	Spleen
Brain [2]	Lymph nodes (mandibular and mesenteric)	Sternum with marrow
Cecum	Nasal cavities (4 levels) [4, 9]	Stomach
Colon	Optic nerves [6, 8]	Testes [6]
Duodenum	Ovaries	Thymus
Epididymides	Pancreas	Thyroids with parathyroids [8]
Esophagus	Pituitary	Tongue
Eyes [6]	Prostate	Trachea
Femur with marrow	Rectum	Urinary bladder
Gallbladder	Salivary gland (mandibular)	Uterus [5]
Heart [3]	Sciatic nerve	Vagina
Ileum	Seminal vesicles	Trigeminal pathway [10]
Larynx and pharynx		

(Sponsor's Table)

Signed and Dated Pathology Report: Yes
Peer Review: No

Histological Findings
Findings consisted of minimal to mild inflammation, luminal exudate, erosion, and mucous cell hyperplasia in the nasal cavity at all doses, including control. The incidence of these findings decreased in recovery.

Text Table 8 Incidence and Severity of Microscopic Findings in the Nasal Cavity of the Main Animals

	Males				Females			
Groups	1	2	3	4	1	2	3	4
Dose Level (mg/kg/day)	0	6	9	12	0	6	9	12
Nasal Cavity #	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Inflammation, Mixed Cells, Mucosa	**3**	**1**	**1**	**3**	**0**	**4**	**3**	**3**
Minimal	0	1	1	3	0	2	2	3
Mild	3	0	0	0	0	2	1	0
Exudate, Luminal	**2**	**0**	**1**	**3**	**0**	**1**	**0**	**1**
Minimal	2	0	1	3	0	1	0	1
Erosion	**4**	**1**	**1**	**2**	**3**	**3**	**1**	**1**
Minimal	4	1	1	2	3	3	1	1
Hyperplasia, Mucous Cell	**1**	**2**	**2**	**1**	**1**	**1**	**3**	**1**
Minimal	1	0	1	1	0	1	3	1
Mild	0	2	1	0	1	0	0	0

#=Number of animals examined.

(Sponsor's Table)

Toxicokinetics

No sex differences in plasma exposure (C_{max} and AUC) were observed. Reductions in AUC on Days 41 and 91 suggested induction of metabolic pathways.

Day	Group Number	Dose Level (mg/kg/day)	Sex	T_{max}* (h)	C_{max} (ng/mL)	T_{last}* (h)	C_{last} (ng/mL)	$t_{1/2}$ (h)	$AUC_{(last)}$ (h*ng/mL)	$AUC_{(inf)}$ (h*ng/mL)
1	2	6	M	0.50	84.2	12.00	0.895	NR	148	NR
			F	0.38	71.2	12.00	0.879	1.45	136	223
1	3	9	M	0.50	109	12.00	1.50	NR	118	NR
			F	0.25	178	18.00	2.00	NR	223	NR
1	4	12	M	0.38	169	12.00	0.993	3.53	220	255
			F	0.38	359	12.00	1.11	2.15	426	498
41	2	6	M	0.38	85.1	24.01	0.767	NR	162	NR
			F	0.39	74.3	24.00	1.48	NR	113	NR
41	3	9	M	0.50	94.4	24.00	1.77	NR	150	NR
			F	0.40	180	24.00	1.83	NR	257	NR
41	4	12	M	0.38	126	24.00	1.32	13.1	184	250
			F	0.38	146	24.00	1.23	11.7	212	259
91	2	6	M	0.25	58.7	18.00	1.03	NR	108	NR
			F	0.50	83.6	23.99	0.985	NR	138	NR
91	3	9	M	0.50	70.7	18.00	0.751	NR	107	NR
			F	0.28	203	18.00	1.04	NR	216	NR
91	4	12	M	0.50	108	12.00	1.72	6.06	153	131
			F	0.50	119	18.00	0.717	NR	188	NR

*Values represented as Median to reflect the actual sampling timepoint.
NR = Not reportable.

(Sponsor's Table)

Dosing Solution Analysis
Dose formulation concentrations were within 10% of nominal.

Study title: BHV-3500: A 39-Week Intranasal Toxicity Study Followed by a 28-Day Recovery Period

Study no.:	1016-3923
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 4, 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, A021800213, 93.6% purity

Methods

Doses:	0, 18, 36, 45 mg/day (5.8-4.9, 11.6-9.7, 14.5-12.2 mg/kg/day)
Frequency of dosing:	Daily
Route of administration:	Intranasal
Dose volume:	19.5-58.1 microliter per kg
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, water
Species/Strain:	Monkey/Cynomolgus
Number/Sex/Group:	4/sex/group; Recovery: 2/sex/group
Age:	7 weeks old
Weight:	2.6 kg [M], 2.5 kg [F]
Satellite groups:	None
Deviation from study protocol:	No significant deviations from study protocol.

Observations and Results

Mortality and Clinical Signs

Mortality checks and clinical observations were conducted twice daily. No drug-related morbidity was observed. Clinical observations were conducted cage-side twice daily. Non-adverse mild-to-moderate salivation was observed across all drug groups during the dosing period but not in recovery; these findings are not considered adverse.

Body Weights

Body weight was assessed weekly for all animals. No BHV-3500-related weight changes were observed.

Food Consumption

Not assessed.

Ophthalmoscopy

Direct, indirect (funduscopic), and slit lamp examinations were conducted prestudy and during Weeks 13 and 39. No drug-related ophthalmological findings were observed.

ECG

ECG parameters were assessed prestudy and during Weeks 13 and 39; there were no drug-related effects.

Hematology, Clinical Chemistry, and Urinalysis

Blood and urine were collected prestudy and during Weeks 13, 39, and 43 (recovery). There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology

No drug-related macroscopic changes were observed.

Organ Weights

There were no drug-related effects on organ weights

Histopathology

Adequate Battery: Yes

Abnormal findings	Jejunum	Skeletal muscle (thigh)
Adrenals	Kidneys	Skin, subcutis & mammary gland (thoracic) [7]
Animal identification [1]	Liver (2 lobes)	Spinal cord (cervical) [11]
Aorta (thoracic)	Lungs (2 lobes) with bronchi [4]	Spleen
Brain [2]	Lymph nodes (mandibular and mesenteric)	Sternum with marrow
Cecum	Nasal cavities (4 levels) [4, 9]	Stomach
Colon	Optic nerves [6, 8]	Testes [6]
Duodenum	Ovaries	Thymus
Epididymides	Pancreas	Thyroids with parathyroids [8]
Esophagus	Pituitary	Tongue
Eyes [6]	Prostate	Trachea
Femur with marrow	Rectum	Urinary bladder
Gallbladder	Salivary gland (mandibular)	Uterus [5]
Heart [3]	Sciatic nerve	Vagina
Ileum	Seminal vesicles	Trigeminal pathway [10]
Larynx and Pharynx		
Olfactory bulb [2]		

(Sponsor's table)

Signed and Dated Pathology Report: Yes
Peer Review: No

Histological Findings: Nasal cavity findings in all groups, including controls, consisted of minimal to moderate hyperplasia, squamous metaplasia and keratinization of the respiratory and transitional epithelia, hyperplasia of mucous cells, and inflammation of the transitional epithelium and lamina propria. Minimal lumen exudate, inflammation of the nasolacrimal duct were also observed in the nasal cavity. In the larynx, minimal inflammation was observed in the respiratory epithelium. In the pharynx, minimal-to-mild inflammation and hyperplasia was observed in the respiratory epithelium. Because findings were present in controls, it cannot be ruled out that these changes are related to the vehicle ((b) (4) mM succinic acid and (b) (4) % (w/v) dextrose in water). The incidence

and severity of these findings were decreased at the end of the recovery period but were not completely reversed.

Special Evaluation

None

Toxicokinetics

TK analyses were conducted on samples collected on Days 1, 120, and 273. No sex differences in plasma C_{max} or AUC. No accumulation was observed from Day 1 to Day 273 in males or females. Dose-exposure relationships were inconsistent across doses. At the NOAEL of 45 mg/day, C_{max} was 210 ng/mL and 176 ng/mL and AUC_{last} was 263 h* ng/mL and 264 h*ng/mL in males and females, respectively.

Text Table 7: Summary BHV-3500 Toxicokinetic Parameters for Male and Female Cynomolgus Monkeys Following Intranasal Administration of BHV-3500 on Day 273

Day	Dose Level (mg/animal/ day)	Sex		T_{max*} (h)	C_{max} (ng/mL)	T_{last*} (h)	$AUC_{(last)}$ (h*ng/mL)	$t_{1/2}$ (h)
273	18	M	Mean	0.50	45.4	24.01	78.3	1.14
			SD	0.01	37.5	9.01	44.3	NC
273	18	F	Mean	0.50	40.3	18.00	82.9	NR
			SD	0.00	19.4	6.94	48.0	NC
273	36	M	Mean	0.38	108	24.00	178	NC
			SD	0.14	86.6	0.01	108	NC
273	36	F	Mean	0.38	130	24.00	200	5.56
			SD	0.14	50.9	6.00	73.7	3.94
273	45	M	Mean	0.50	210	24.00	263	NR
			SD	0.13	167	0.02	137	NC
273	45	F	Mean	0.50	176	24.00	264	NR
			SD	0.13	71.8	0.01	97.2	NC

*Values represented as median to reflect the actual sampling timepoint.
M= Male; F = Female; NR = Not reported; NC = Not calculated.

(Sponsor's Table)

Dosing Solution Analysis

Dosing solution concentrations were within 4.4% of nominal.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay with BHV-3500

Study no.:	45234 MMO
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	19 June 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BVH-3500 Batch No.: 6D16168, purity 99.9%

Methods

Strains:	Salmonella typhimurium TA 1535, TA 100, TA 102, TA 1537, TA 98
Concentrations in definitive study:	156.3, 312.5, 625, 1250, 2500, and 5000 ug/plate
Basis of concentration selection:	Toxicity in preliminary test at highest dose
Negative control:	Water (vehicle)
Positive control:	Without S9 mix: Sodium azide (NAN3), 9-Aminoacridine (9AA), 2-Nitrofluorene (2NF), Mitomycin C (MMC) With S9 mix: 2-Antramine (2AM), Benzo(a)pyrene (BAP)
Formulation/Vehicle:	Water.
Incubation & sampling time:	48-72 hours

Study Validity

Bacterial strain selection is adequate (i.e., OECD compliant). Positive controls produced responses consistent with criteria for positive response (revertant ratios ranging from 4 to 56.8). Dose selection was adequate based upon use of the limit dose (i.e., 5000 µg/plate).

Results

BHV-3500 was negative in an adequately conducted Ames assay.

7.2 In Vitro Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test with BHV-3500 in Cultured Human Lymphocytes

Study no.:	435235
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4) chemical analysis conducted at (b) (4) (b) (4)
Date of study initiation:	19 June 2017
GLP compliance:	No – storage conditions for the stock formulation samples taken for chemical analysis were not properly documented. This was not considered to have affected study adequacy.
QA statement:	Yes
Drug, lot #, and % purity:	BVH-3500 Batch No. 6D16168, potency 93.2%, purity 99.9%

Methods

Cell line:	Human lymphocytes
Concentrations in definitive study:	31.3, 62.5, 125, 250, 500 µg/mL
Basis of concentration selection:	Preliminary toxicity study
Negative control:	Water
Positive control:	Mitomycin C 2 and 3 ug/mL and Cyclophosphamide 12/5 and 25 ug/ml
Formulation/Vehicle:	water
Incubation & sampling time:	3 and 20 h, with and without S9

Results

BHV-3500 was negative in an adequately conducted (i.e., OECD compliant) in vitro chromosomal aberration study.

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

Study title:

Study no:	44787
Study report location:	4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 June 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, batch number: 6D16168, 99.9%

Methods

Doses in definitive study:	0, 25, 50, 75 mg/kg/day;
Frequency of dosing:	QD, 2 doses
Route of administration:	SC
Dose volume:	5mL/kg
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose USP in sterile water
Species/Strain:	SD rats
Number/Sex/Group:	5 males per group, 5 groups
Satellite groups:	None
Basis of dose selection:	HD was a maximum tolerated dose based on adverse local reactions.
Negative control:	vehicle
Positive control:	Cyclophosphamide by oral route at 15 mg/kg

Results

Animals were administered drug for 2 days and then euthanized 18-24 h after the last dose. Positive control group was euthanized 18-24 h after one dose. Bone marrow smears were prepared, and number of micronucleated polychromatic erythrocytes were counted in 4000 polychromatic erythrocytes. BHV-3500 was negative for clastogenicity in an adequately conducted in vivo rat micronucleus assay.

8 Carcinogenicity

Study title: 26-Week Carcinogenicity Study of BHV-3500 by Intranasal Administration in CByB6F1/Tg rasH2 Hemizygous Mice

Study no.:	20216831
Study report location:	EDR 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 10, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, Lot A21800213, 93.6%
Executive CAC concurrence:	Yes

Methods

Doses:	0 (saline), 0 (vehicle), 0.3, 0.8, 2.5 mg/day BHV-3500; 75 mg/day N-Nitrosomethylurea (NMU)
Frequency of dosing:	Daily BHV-3500; once on Day 1 NMU
Dose volume:	5 µL/nostril/day; 10 mL/kg NMU IP
Route of administration:	Intranasal (Saline, Vehicle, BHV-3500); IP (NMU)
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, USP, in sterile water for injection, USP, adjusted to pH 5.8 to 6.2
Basis of dose selection:	HD is a maximum feasible dose based on dose volume and viscosity
Species/Strain:	TgRasH2 mice (Taconic Biosciences, Germantown, NY)
Number/Sex/Group:	25/sex/group (main); 15/sex/group (NMU)
Age:	9 weeks at initiation of dosing
Animal housing:	Animals were single housed. Lab Diet Certified CR Rodent Diet 5 CR4 was provided *ad libitum* throughout the study.
Paradigm for dietary restriction:	None
Dual control employed:	Yes
Interim sacrifice:	None
Satellite groups:	TK; 3/sex/group (control); 18/sex/group (BHV-3500)
Deviation from study protocol:	No significant deviations

Key Study Findings

BHV-3500 was no tumorigenic.

Observations and Results

Mortality

Animals were monitored twice daily for mortality or signs of morbidity. There were no BHV-3500-related effects on survival. Mortality was increased in the positive control group treated with NMU (Group 6); the most frequent cause of death was malignant lymphoma.

Text Table 23
Mortality Incidence and Survival

Group No.	Dose Level (mg/day)	Males		Females	
		Mortality	Survival	Mortality	Survival
1	0[a]	1/25	96%	2/25	92%
2	0[b]	2/25	92%	2/25	92%
3	0.3	0/25	100%	1/25	96%
4	0.8	0/25	100%	1/25	96%
5	2.5	3/25	88%	1/25	96%
6	NMU[c]	13/15	13%	13/15	13%

Note: Unscheduled euthanasias were attributed to accidental injuries.
[a] Control article (50 mM succinic acid and 1.25% (w/v) anhydrous dextrose, USP in Sterile Water for Injection, USP, adjusted to pH 5.8 to 6.2).
[b] Saline control.
[c] 7.5 mg/mL N-Nitrosomethylurea (NMU) in citrate-buffered saline (pH 4.5) as a single 75 mg/dose on Day 1 only.

(Sponsor's Table)

Text Table 24
Causes of Death (Found Dead and Unscheduled Euthanasia)
Based on Microscopic Findings

	Males						Females					
Group	**1**	**2**	**3**	**4**	**5**	**6[a]**	**1**	**2**	**3**	**4**	**5**	**6[a]**
Dose (mg/day)	**0**	**0**	**0.3**	**0.8**	**2.5**	**75**	**0**	**0**	**0.3**	**0.8**	**2.5**	**75**
Test Material	**CA**	**SC**	**TA**	**TA**	**TA**	**NMU**	**CA**	**SC**	**TA**	**TA**	**TA**	**NMU**
Number of Animals[b]	**1**	**2**	**0**	**0**	**3**	**13**	**2**	**2**	**1**	**1**	**1**	**13**
Undetermined	-	1	-	-	-	-	-	1	1	-	-	-
Neoplastic:												
Adenocarcinoma, mammary gland	-	-	-	-	-	-	-	-	-	-	-	1
Adenocarcinoma, salivary gland	-	-	-	-	-	-	-	-	-	-	-	1
Bronchioalveolar carcinoma	-	-	-	-	-	-	2	1	-	-	1	1
Hemolymphoreticular tumors: Lymphoma, malignant	-	-	-	-	-	11	-	-	-	1	-	7
Hemangiosarcoma, nasal cavity	-	-	-	-	-	1	-	-	-	-	-	-
Hemangiosarcoma, thoracic cavity	1	-	-	-	-	-	-	-	-	-	-	-
Hemangiosarcoma, skin	-	-	-	-	1	-	-	-	-	-	-	-
Hemangiosarcoma, spleen	-	1	-	-	-	-	-	-	-	-	-	-
Squamous cell carcinoma, skin	-	-	-	-	-	1	-	-	-	-	-	-
Squamous cell carcinoma, stomach	-	-	-	-	1	-	-	-	-	-	-	1
Squamous cell carcinoma, uterus	-	-	-	-	-	-	-	-	-	-	-	1

(Sponsor's Table)

Clinical Signs and Palpable Masses

Detailed clinical observations were conducted weekly; there were no BHV-3500-related clinical signs. Clinical signs in the positive control group consisted of breathing abnormalities, dehydration, abnormal gait, ungroomed fur, thin appearance, skin papules, decreased activity, cold to touch, and hunched posture.

Body Weights

Animals were weighed weekly. There were no drug effects on body weight.

Food Consumption

Food consumption was recorded weekly. Food consumption was increased in all treated groups compared to controls on Days 1-8.

Hematology

Not evaluated.

Gross Pathology

There were no BHV-3500-related pathology findings at either unscheduled or scheduled necropsies. Masses, enlargements, or nodules in lymph nodes, spleen, thymus, lung, skin, and stomach were observed in the positive control group; these findings correlated with histopathology findings including malignant lymphoma in multiple organs, squamous cell papilloma/carcinoma in the skin and stomach, and bronchioloalveolar adenoma in the lung.

Histopathology

Signed Pathology Report: Yes
Peer Review: Yes

Non-neoplastic findings:
Epithelial eosinophilic globules, mixed cell inflammation of the lamina propria, and degeneration/atrophy of the olfactory epithelium, characterized as minimal-to-mild in severity, occurred in all drug-treated groups (see table below).

Text Table 27
Summary of BHV-3500-related Non-Neoplastic Microscopic Findings

	Males						Females					
Group	**1**	**2**	**3**	**4**	**5**	**6[a]**	**1**	**2**	**3**	**4**	**5**	**6[a]**
Dose (mg/day)	**0**	**0**	**0.3**	**0.8**	**2.5**	**75**	**0**	**0**	**0.3**	**0.8**	**2.5**	**75**
Test Material	**CA**	**SC**	**TA**	**TA**	**TA**	**NMU**	**CA**	**SC**	**TA**	**TA**	**TA**	**NMU**
No. Animals Examined	**25**	**25**	**25**	**25**	**25**	**15**	**25**	**25**	**25**	**25**	**25**	**15**
Nasal cavity (No. Examined)	25	25	25	25	25	15	25	25	25	25	25	15
Eosinophilic globules, epithelial	(3)[b]	(10)	(23)	(22)	(23)	(2)	(17)	(12)	(24)	(25)	(24)	(9)
Minimal	3	10	22	21	21	2	16	12	15	13	12	7
Mild	-	-	1	1	2	-	1	-	8	12	12	1
Moderate	-	-	-	-	-	-	-	-	1	-	-	1
Inflammation, mixed cell, lamina propria	(0)	(0)	(6)	(14)	(20)	(1)	(0)	(0)	(11)	(23)	(19)	(0)
Minimal	-	-	6	10	20	1	-	-	11	18	18	-
Mild	-	-	-	4	-	-	-	-	-	5	1	-
Hyperplasia, respiratory epithelium	(0)	(0)	(0)	(0)	(4)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Minimal	-	-	-	-	2	-	-	-	-	-	-	-
Mild	-	-	-	-	2	-	-	-	-	-	1	-
Degeneration/atrophy, olfactory epithelium	(0)	(0)	(0)	(1)	(20)	(0)	(0)	(0)	(0)	(0)	(21)	(0)
Minimal	-	-	-	1	17	-	-	-	-	-	2	-
Mild	-	-	-	-	3	-	-	-	-	-	19	-

CA = Control Article; SC = Saline Control; TA = BHV-3500; NMU = Positive control (N-Nitrosomethylurea); Dash (-) indicates absence of finding in group.
[a] NMU (Positive Control) animals. Dosed only once on Day 1 via intraperitoneal injection.
[b] Numbers in parentheses represent the number of animals with the finding.

(Sponsor's table)

Neoplastic findings:
Based on review by the CDER Division of Biometrics (Malick Mbodj, Ph.D., July 28, 2022), there were no statistically significant increases in tumor incidence in drug-treated mice.

Toxicokinetics

TK analysis was conducted on Days 1 and 28 in satellite animals. Plasma C_{max} and AUC were generally dose proportional, with no consistent differences between males and females. Plasma C_{max} was observed 1-2 hours post dose at both timepoints. No accumulation was observed after 28 days of dosing.

Text Table 25
Toxicokinetic Parameters for BHV-3500 in Male and Female Mice

Day	Gender	Dose (mg/day)	AUCtlast (ng*h/mL)	Cmax (ng/mL)	tmax (h)	tlast (h)	RAUC (RATIO)
1	Male	0.3	235	108	2	6	NA
		0.8	894	704	1	6	NA
		2.5	3910	1690	2	24	NA
	Female	0.3	666	489	1	6	NA
		0.8	749	385	1	6	NA
		2.5	2540	1200	1	6	NA
28	Male	0.3	215	135	0	6	0.918
		0.8	362	152	2	6	0.404
		2.5	7680	6080	1	6	1.96
	Female	0.3	577	172	2	6	0.866
		0.8	599	231	1	6	0.799
		2.5	2120	953	1	24	0.834

NA = Not applicable.

(Sponsor's Table)

Dosing Solution Analysis

Concentrations for dose formulations were within acceptable limits (90-110%).

Study title: 2-year intranasal carcinogenicity study of BHV3500 in rats

Study no.:	277000100101
Study report location:	4.2.3.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 9, 2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, A021800213, 99.9%
CAC concurrence:	Yes

Evaluation of Tumor Findings

BHV-3500 was not tumorigenic.

Methods

Doses:	0 (saline), 0 (vehicle), 2, 9, 18.8 mg/kg/day BHV-3500
Frequency of dosing:	Daily
Dose volume:	14.3-61.9 µL
Route of administration:	Intranasal
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, USP, in sterile water for injection, USP, adjusted to pH 5.8 to 6.2
Basis of dose selection:	Doses were recommended by the Executive CAC based on increased incidence and severity of histopathological findings in nasal mucosa at higher doses in 3-month DRF.
Species/Strain:	Rat/SD
Number/Sex/Group:	65/sex/group
Age:	7-8 weeks at initiation of dosing
Animal housing:	Single housed, temp 15.2 to 29.9ºC, humidity 19.5 to 84.2%
Paradigm for dietary restriction:	None
Dual control employed:	Yes
Interim sacrifice:	None
Satellite groups:	TK 6/sex/group BHV-3500 (2,9,18.8 mg/kg); 3/sex/group saline and vehicle control
Deviation from study protocol:	Study was terminated at Week 84 in females and Weeks 95-96 in males due to mortality in the vehicle group.

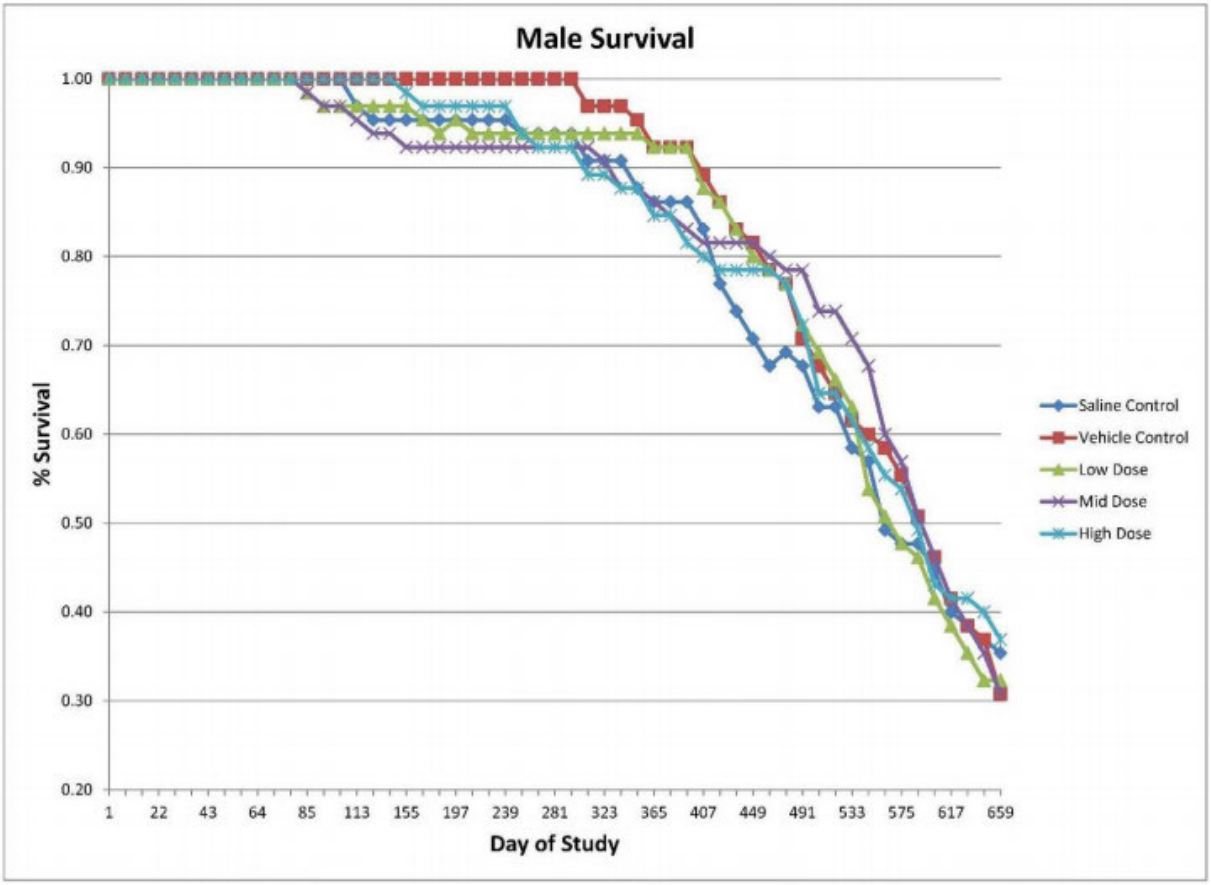
Observations and Results

Mortality

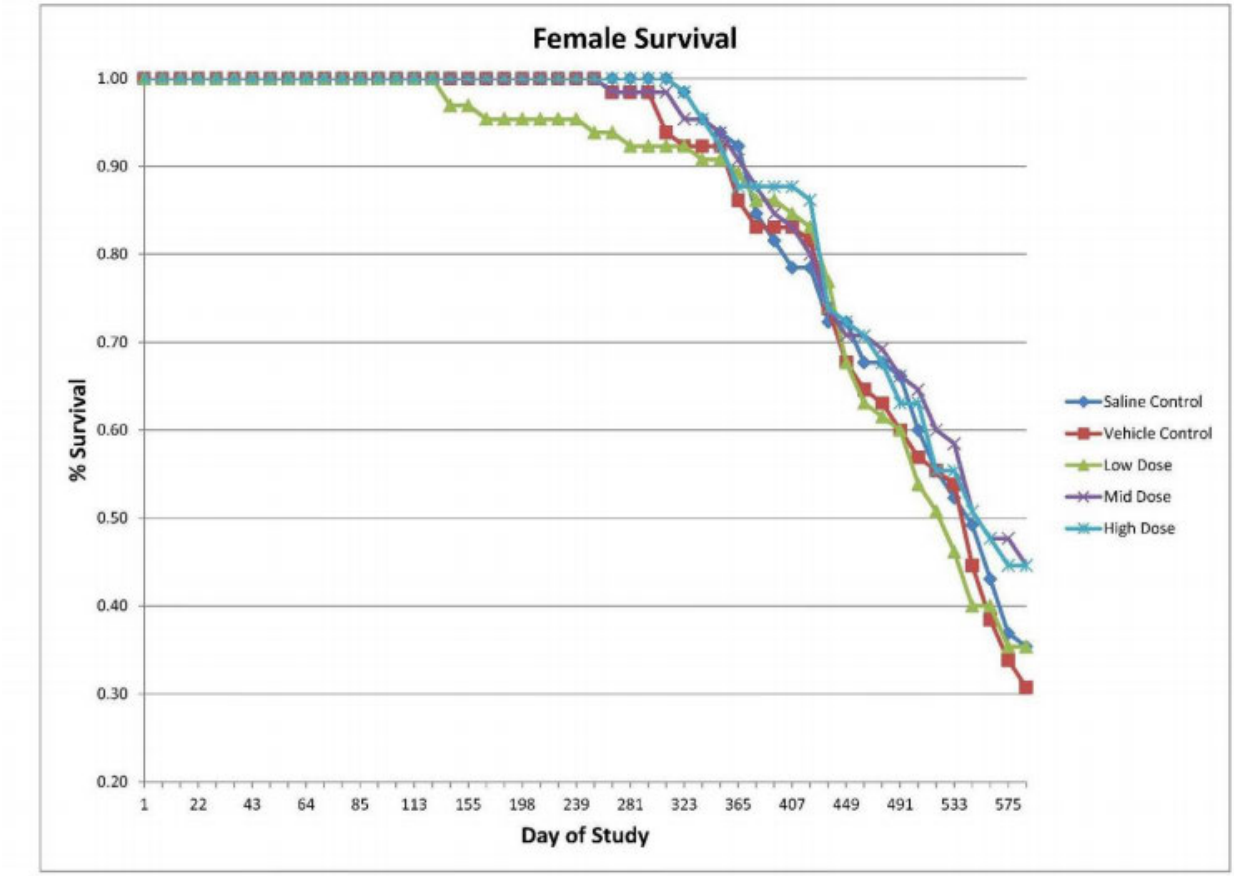
There were no drug-related effects on survival. The vehicle control groups dropped to ≤ 20 rats at Week 84 for females and Week 95-96 in males, resulting in early termination of the study. Causes of death varied without pattern or dose relationship, with the most common being pituitary adenoma.

Table 1. Survival Rates

Group	Males	Females
Saline	35% (23/65)	35% (23/65)
Vehicle	28% (18/65)	31% (20/65)
Low dose (2 mg/kg/day)	31% (20/65)	35% (23/65)
Mid dose (9 mg/kg/day)	31% (20/65)	42% (27/65)
High dose (18.8 mg/kg/day)	34% (22/65)	45% (29/65)



(Sponsor's Figure)



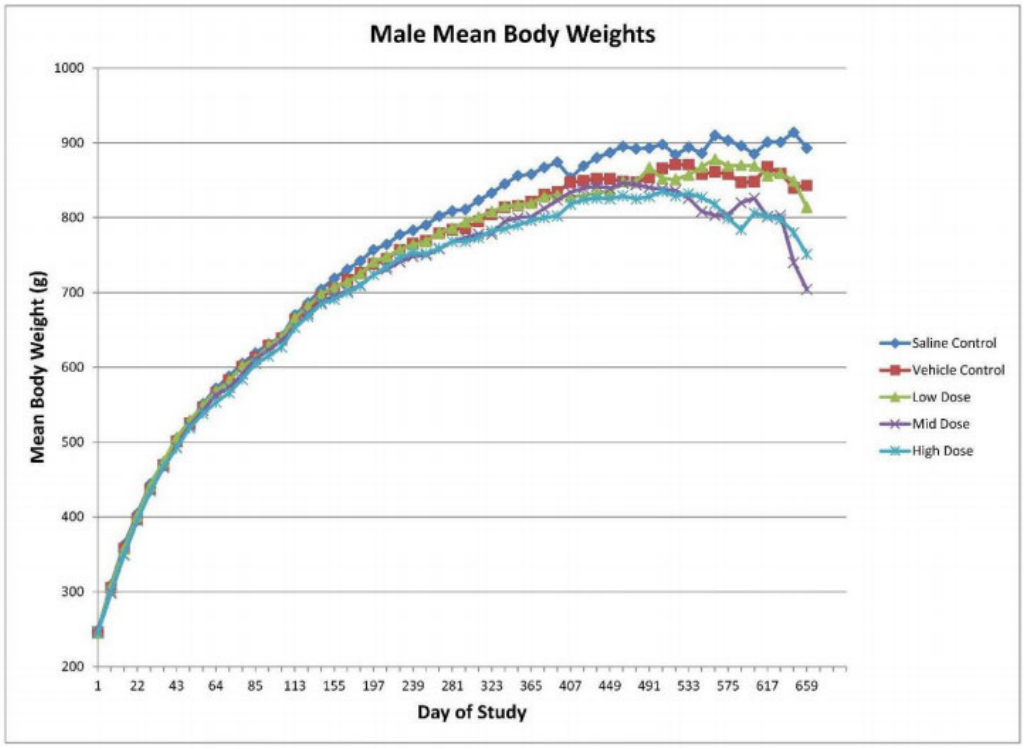
(Sponsor's Figure)

Clinical Signs

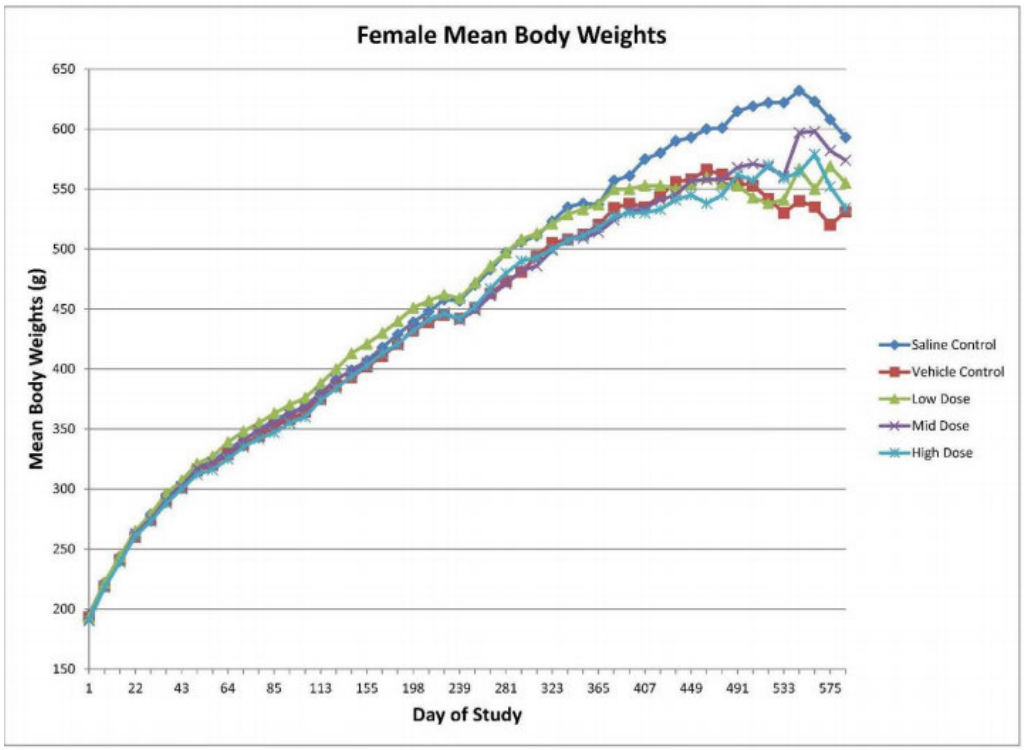
There were no clear drug-related clinical signs. Redness around nose fur was increased in HD males compared to saline controls but not compared to vehicle controls.

Body Weights

Starting at Week 45, body weight was decreased consistently in MD and HD males compared to saline control (2-21% and 4-16%, respectively). Body weight gain was also decreased in MD and HD males compared to saline control (27% and 20%, respectively; see sponsor's figure) on Day 659. There were no drug-related changes in body weight in females (see sponsor's figure).



(Sponsor's Figure)



(Sponsor's Figure)

Food Consumption

Starting Week 2, HD males showed a small (<10%) but consistent decrease in food consumption compared to saline control.

Gross Pathology

Tissue collection for pathology and histology were adequate (see sponsor's table). No drug-related findings were observed.

Text Table 2 – Tissue Collection

Animal identification[1]	Gland, seminal vesicle (paired)	Ovary (paired)
Artery, aorta	Gland, thyroid (paired)	Pancreas
Bone, femur	Gland, Zymbal (paired)[2]	Small intestine, duodenum
Bone, sternum	Gross lesion (if any)	Small intestine, ileum
Bone marrow, femur	Heart	Small intestine, jejunum
Bone marrow, sternum	Kidney (paired)	Skin, ventral abdomen
Brain	Large intestine, cecum	Spinal cord, cervical
Cervix	Large intestine, colon	Spinal cord, lumbar
Epididymis (paired)	Large intestine, rectum	Spinal cord, thoracic
Esophagus	Liver	Spleen
Eye (paired)	Lung	Stomach
Gland, adrenal (paired)	Lymph node, mandibular	Testis (paired)
Gland, Harderian (paired)	Lymph node, mesenteric	Thymus
Gland, mammary (females)	Mass (if any)	Trachea
Gland, parathyroid (paired)[2]	Muscle, skeletal	Urinary bladder
Gland, pituitary	Nasal cavity (4 levels)	Uterus
Gland, prostate	Nerve, optic (paired)	Vagina
Gland, salivary (paired)	Nerve, sciatic	Bone marrow smear (femur)[3]

1 Tail with tattoo; collected but not processed.
2 Due to size, this organ was only evaluated when present in a normal section.
3 Bone marrow smears were prepared at scheduled necropsy only.

(Sponsor's Table)

Histopathology

Signed Pathology Report: Yes

Peer Review: No

Non-neoplastic Findings:
No drug-related microscopic non-neoplastic findings were noted.

Neoplastic

The most common tumor observed (across all groups) were in the pituitary gland and mammary gland.

Based on review by the CDER Division of Biometrics and Executive CAC (September 29, 2022), daily IN BHV-3500 (0, 2, 9, 18.8 mg/kg/day) resulted in no statistically significant dose-related increase in tumors in males or females. There was a statistically significant increase in thyroid gland c-cell adenoma in MDM compared to vehicle-treated rats (Table 2 below taken from Dr. Mbodj); however Executive CAC concluded they were not drug related.

Table 2: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons Treated Groups and Control Group in Rats

Sex	Organ Name	Tumor Name	0 mg Vehicle Cont (N=65) P - Trend	0 mg Saline Cont (N=65) P -VC vs. SC	2 mg Low (N=61) P - VC vs. L	9 mg Med (N=59) P - VC vs. M	18.8 mg High (N=65) P - VC vs. H
Male	brain	astrocytoma, malignant	1/65 (33) 0.0300@	0/65 (31) NC	0/51 (21) 1.0000	0/52 (23) 1.0000	4/65 (34) 0.1873
Sex	Organ Name	Tumor Name	0 mg Vehicle Cont (N=65) P - Trend	0 mg Saline Cont (N=65) P -VC vs. SC	2 mg Low (N=61) P - VC vs. L	9 mg Med (N=59) P - VC vs. M	18.8 mg High (N=65) P - VC vs. H
	gland, thyroid	c-cell adenoma	0/65 (33) 0.4633	1/65 (31) NC	0/45 (16) NC	3/45 (19) 0.0438*	0/65 (32) NC

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
*: Statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively.
@: Not statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively.

Table 3. Thyroid gland tumor incidence

Organ/Tissue	Finding	Male					Female				
		Saline (N= 65)	Vehicle (N = 65)	LD - 2 mg/kg (N = 45)	MD - 9 mg/kg (N = 45)	HD - 18.8 mg/kg (N = 65)	Saline (N= 65)	Vehicle (N = 65)	LD - 2 mg/kg (N = 42)	MD - 9 mg/kg (N = 38)	HD - 18.8 mg/kg (N = 65)
GLAND, THYROID	ADENOMA, C-CELL, BENIGN	1 (1.5%)			3 (6.7%)		1 (1 5%)			1 (2.6%)	
	ADENOMA, FOLLICULAR CELL, BENIGN	4 (6.2%)	1 (1.5%)	1 (2.2%)	1 (2 2%)	1 (1.5%)		1 (1.5%)		1 (2.6%)	
	CARCINOMA, C-CELL, MALIGNANT							1 (1.5%)			
	LYMPHOMA, MALIGNANT	1 (1.5%)									

Toxicokinetics

Plasma exposures (C_{max} and AUC) were similar to values obtained in repeat-dose toxicity studies in the same species. Similar to previous studies, C_{max} and AUC was

lower at the HD than the MD at Week 26 in both males and females (see sponsor's table). Sex differences in exposure were observed (2.2-2.4x higher in males compared to females) but was not consistent across doses or timepoints. Accumulation ratios ranged widely between 0.57 and 11.9 which differs from the pivotal 6-month rodent study in which accumulation ratios were all less than 1.

Day/ Week	Sex	Dose (mg/kg/day)	t_{max} (hr)	C_{max} (ng/mL)	AUC_{0-4} (hr*ng/mL)	AUC_{TAU} (hr*ng/mL)	AUC_{last} (hr*ng/mL)	t_{last} (hr)	$t_{1/2}$ (hr)
Day 1	F	2	1	141	212	NA	212	4	NA
		9	1	545	884	1144	1144	24	NA
		18.8	1	804	1063	1442	1442	24	NA
	M	2	0.5	88.9	153	163	153	4	0.90
		9	0.5	671	1386	1775	1775	24	NA
		18.8	1	1210	2585	3293	3293	24	3.82
Week 26	F	2	1	695	1105	1296	1296	24	NA
		9	1	2191	3574	4746	4746	24	NA
		18.8	1	1146	1556	2075	2075	24	NA
	M	2	1	923	1816	2770	2770	24	3.73
		9	1	3690	7829	10519	10519	24	NA
		18.8	1	875	1467	2260	2260	24	4.02

(Sponsor's Table)

Dosing Solution Analysis

Concentrations for dose formulations were within acceptable limits (90-110%) except for LD formulations at Week 78 which were 112% of the intended value.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A Fertility and Early Embryonic (SEG I) Subcutaneous Toxicity Study in Sprague-Dawley Rats

Study no.: 1016-3931
Study report location: 4.2.3.5.1
Conducting laboratory and location: (b) (4)
Date of study initiation: February 9, 2018
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BHV-3500, A0218003, 99.9%

Methods

Doses:	0, 5, 15, 25 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	3 mL/kg
Route of administration:	SC
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, USP, in sterile water for injection, USP, adjusted to pH 5.8 to 6.2
Species/Strain:	Rat/ Sprague Dawley
Number/Sex/Group:	24/sex/group
Satellite groups:	n/a
Study design:	Males were treated for 28 days prior to mating and continued until necropsy. Females were treated starting 14 days prior to mating, during mating, and to gestational day 7.
Deviation from study protocol:	No major deviations from study protocol.

Observations and Results

Mortality and Clinical Signs

Cage side observation and mortality checks were conducted twice daily. Mortality occurred in 1 MDM and 1 HDM; both deaths were attributed to severe injection site toxicity. Drug-related clinical signs consisted of injection site toxicity (discharge, fur staining, skin discoloration), and wounds with and without discharge.

Body Weight

Body weight was recorded twice weekly. There was a dose-dependent decrease in body weight and body weight gain in males from Day 28 to 63. No drug-related changes in body weight were observed in females.

Text Table 5: Mean Absolute Body Weight and Mean Body Weight Changes Percentage Difference from the Control Group

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose Level BHV-3500	0	5	15	25	0	5	15	25
Number of Animals	24	24	24/23	24/23	24/20	24/23	24/22	24/23
Body Weight								
Start of Dosing (Day -1)								
Absolute (g)	364	365	366	368	260	272	270	**275**
%Diff	-	+0.30	+0.61	+0.98	-	+4.54	+3.82	+5.92
Pre-mating (males: Day 28; females: Day 14)								
Absolute (g)	538	533	**504**	**488**	293	**310**	**306**	**310**
%Diff	-	-1.01	-6.39	-9.33	-	+5.84	+4.51	+5.94
End of Dosing (males: Day 63; females: GD7)								
Absolute (g)	658	635	**577**	**538**	340	358	351	348
%Diff	-	-3.49	-12.3	-18.1	-	+5.17	+3.17	+2.31
Body Weight Change								
Start of Dosing (Day -1 to 3) Absolute (g)	15.0	15.3	14.6	**20.1**	6.2	9.3	10.5	10.2
%Diff	-	+1.95	-2.51	+34.3	-	+51.4	+70.3	+65.5
Pre-mating (males: Day 24-28; females: Day 10-14)								
Absolute (g)	22.0	19.6	**14.0**	**8.4**	10.6	11.5	9.9	9.2
%Diff	-	-10.8	-36.4	-61.6	-	+8.24	-7.06	-13.7
End of Dosing (males: Day 59-63; females: GD4-7)								
Absolute (g)	9.6	6.8	**4.3**	**3.7**	12.7	11.9	11.4	11.0
%Diff	-	-29.6	-55.5	-61.4	-	-6.54	-10.5	-13.4

%Diff: difference percentage when compared to the control group.
Significant difference when compared to Group 1 when in **Bold**.

(Sponsor's Table)

Food Consumption

Food consumption was measured twice weekly. There was a dose-dependent decrease in male food consumption at Day 28 of the premating period (LDM: -1.4%, MDM: -5.9%, HDM: -11.4%) compared to controls.

Toxicokinetics

Not evaluated; the sponsor bridged to TK data from the 28-day study in which SC administration was assessed (Study 1016-3761).

Dosing Solution Analysis

Drug formulations were within -1.2% to 3% of target concentrations.

Necropsy

There were no drug-related changes in estrous cycle or mating indices in males or females. No drug-related effects on organ weights were observed.

There were no adverse effects on male or female fertility, or early embryonic development. The NOAEL for fertility and early embryonic development was 25 mg/kg/day. Due to injection site toxicity and inflammation in MD and HD rats, 5 mg/kg/day SC was the NOAEL for local toxicity.

Text Table 8: Group Means Maternal Performance Summary Results

Group	1	2	3	4
Dose Level (mg/kg/day)	**0**	**5**	**15**	**25**
Total Corpora Lutea	19.6	19.1	19.9	18.8
Total Implantation Sites	18.2	15.6	17.7	17.1
Pre-Implantation Loss (%)	6.8	**19.2**	10.6	8.4
Post-Implantation Loss (%)	7.8	7.9	8.1	4.9
Total Early resorptions	1.5	1.3	1.5	0.9
Live Embryos	16.7	14.1	16.2	16.2
Dead Embryos	0.0	0.0	0.0	0.0
Total Embryos	16.7	14.2	16.2	16.2
Live Embryos Index (%)	92.2	92.1	91.9	95.1

Significant difference when compared to Group 1 when in **Bold** ($p \leq 0.05$, Dunn).

(Sponsor's Table)

Text Table 9: Male Reproductive Assessments Group Mean Summary

Group	1	2	3	4
Dose Level (mg/kg/day)	**0**	**5**	**15**	**25**
Motility (% of males)*	100	100	100	100
Sperm Concentration (million/gram of epididymis)	636	623	631	622
Abnormal Sperm (%)	7.1	6.4	7.1	8.7

*Total percentage of moderate number of motile sperm, large number of motile sperm, some wave motion, and fast wave motion observed (Grades 3, 4, 5, and 6, respectively)

(Sponsor's Table)

9.2 Embryofetal Development

Study title: A subcutaneous range-finding embryo-fetal developmental toxicity study in Sprague Dawley Rats (2016-3851)

Study no.:	2016-3851
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 20, 2017
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BHV-3500, 6D16168, 93.1% as is, 99.8%

corrected

A non-GLP dose range-finding study was conducted in which pregnant SD rats (6/group) were administered 0, 10, 25, and 50 mg/kg/day BHV-3500 SC from GD6 through GD17. There were no mortalities in this study. Swelling at injection site was observed at the MD (1/6) and HD (5/6). Post-implantation loss was increased at all doses (Control: 6.72%, LDF: 15.62%, MDF: 17.6%, HDF: 23.6%). There was one total litter loss in 1 HDF. Due to swelling at the injection site and increased post-implantation loss, the NOAEL was the MD of 25 mg/kg/day.

Study title: A subcutaneous embryo-fetal development toxicity study (SEGII) in Sprague-Dawley rats

Study no.:	1016-3861
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 5, 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, 6D16168, 93.1%

Methods

Doses:	0, 10, 20, 40 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	3 mL/kg
Route of administration:	SC
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, USP, in sterile water for injection, USP, adjusted to pH 5.8 to 6.2
Species/Strain:	Rat/SD
Number/Sex/Group:	24 dams/group
Satellite groups:	N/A
Study design:	Dosed daily from GD6 to GD17; dams euthanized on GD21
Deviation from study protocol:	No major deviations from study protocol.

Observations and Results

Mortality and Clinical Signs

Mortality and clinical signs were evaluated twice daily. There was no mortality. Clinical signs consisted of injection site toxicity (skin inflammation, fur staining, skin scabs, swelling, soft) at all doses. There were tremors in 1 MDF and 1 HDF on GD12.

Body Weight

Body weight was recorded every 3 days beginning on GD0. BHV-3500 administration resulted in a dose-dependent decrease in body weight and body weight gain from GD9 onward. At the end of the study, body weight gain was reduced 0.61%, 6% and 10% in LDF, MDF, and HDF, respectively, compared to controls. When body weight and body weight gain were corrected for uterine weight, there was a dose-dependent decrease in body weight gain (LDM: 7%, MDM: -29.3%, HDM: -33.7%) compared to controls.

Food Consumption

Food intake was assessed every three days. There were no drug-related food consumption changes.

Toxicokinetics

Not evaluated.

Dosing Solution Analysis

Drug formulations ranged from -1.9 to 4% of nominal.

Necropsy

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

No BHV-3500-related changes were observed in any uterine parameters, including implantation sites, pre- and post-implantation loss, live male fetuses, live female fetuses, dead fetuses, early or late resorptions, or gravid uterus weight. There were no significant differences in the ratio of male to female fetuses.

Maternal Macroscopic Findings

Macroscopic observations were confined to injection-related findings (skin scab, skin wound, subcutis) and were observed across all BHV-3500 treated groups.

Offspring (Malformations, Variations, etc.)

No BHV-3500-related external, internal, or skeletal malformations were observed.

The NOAEL for embryofetal toxicity was the HD (40 mg/kg/day).

Study title: BHV-3500: A Subcutaneous Range-finding Teratology Study (SEG II) in New Zealand White Rabbits

Study no.:	2016-3874
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 7, 2017
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BHV-3500, 6D16168, 93.1%

In a non-GLP DRF study, pregnant rabbits (5/group) were administered 0, 20, 40, and 60 mg/kg/day BHV-3500 by SC injection from GD7 to GD19. There was one HD death; irregular epicardia surface was observed in this animal, suggesting inflammation or fibrosis of the epicardium as a possible cause of death. Pre-implantation loss was increased and live fetuses per litter and number of implantation sites were reduced in HDF. Sex ratio showed a dose-dependent decrease in percentage of males compared to controls (Control: 67.7%, LD: 57.8%, MD: 47.1%, HD: 43.3%). There was also a dose-dependent increase in post-implantation loss. There were no drug effects on fetal weight, placental weight, or malformations.

	Control (0 mg/kg/day)	**LD (BHV-3500 20 mg/kg/day)**	**MD (BHV-3500 40 mg/kg/day)**	**HD (BHV-3500 60 mg/kg/day)**
Corpora Lutea	13	11	11.8	13.3
Total Implantation Sites	12.7	10.3	11	10
Empty Implantation Sites	0	0	0	0
Live Male Fetuses	8.3	5.8	5	4
Live Female Fetuses	4	4.3	5.6	5
Sex Ratio (% Males)	67.7	57.8	47.1	43.3
Total Live Fetuses	12.3	10	10.6	9
Dead Fetuses	0	0	0	0
Early Resorptions	0.3	0.3	0.4	1
Late Resorptions	0	0	0	0
Sum of Resorptions	0.3	0.3	0.4	1
Pre-implantation Loss (%)	2.2	6.6	6.4	27.2
Post Implantation Loss (%)	2.4	2.5	3.9	7.7
Gravid Uterus Weight (g)	0.6	0.61	0.58	0.56

Study title: A subcutaneous embryo-fetal development toxicity study (SEGII) in pregnant New Zealand white rabbits

Study no.:	1018-1364
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 29, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, A021800213 purity: 99.9%

Methods

Doses:	0, 20, 40, 60 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	3 mL/kg
Route of administration:	SC
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, USP, in sterile water for injection, USP, adjusted to pH 5.8 to 6.2
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	22 does/group
Satellite groups:	TK: 3/group
Study design:	Rabbits treated daily from GD7 to GD19 and euthanized on GD29
Deviation from study protocol:	No major deviations from study protocol.

Observations and Results

Mortality and Clinical Signs

Clinical observations and mortality checks were conducted daily. Three deaths (1 MD and 2 HD) occurred, for which a cause of death was not identified. The MDF was observed to have severe inappetence and decreased fecal output (GDs 2-7) as well as slight decrease in body weight prior to death on GD9. One HDF was noted as having weakness, labored breathing decreased activity, and laying on cage floor on GD9 (recovered the same day) but was found dead on GD18. There were no notable findings upon necropsy except inflammation at injection sites. The second HDF showed no clinical signs prior to death. Drug-related effects cannot be ruled out.

Clinical observations were conducted on gestational days 0, 5, 7, 13, 16, 20, 23, 26, and 29. Clinical signs in surviving does (skin inflammation, fur staining, skin scabs, swelling) were consistent with local toxicity from drug administration.

Body Weight

Body weights were recorded on gestational days 0, 5, 7, 13, 16, 20, 23, 26, and 29. There were no drug-related body weight changes in pregnant does.

Food Consumption

Food consumption was measured once daily starting GD5. There were no drug-related effects on food consumption.

Toxicokinetics

Increases in systemic exposure (C_{max} and AUC) were less than dose proportional; there was no indication of drug accumulation.

Text Table 6: Mean Plasma BHV-3500 Toxicokinetic Parameters in Pregnant Female New Zealand White Rabbits Following Subcutaneous Injection at Selected Dose Levels on Gestation Day 7 and Day 19.

Gestation Day	Group Number	Dose Level (mg/kg/day)	Sex	T_{max}* (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{last}* (h)	C_{last} (ng/mL)	$AUC_{(last)}$ (h*ng/mL)	$AUC_{(inf)}$ (h*ng/mL)
7	2	20	F	1.00	22800	0.812	6.00	317	40000	46500
7	3	40	F	1.00	36000	0.807	6.00	696	78900	79800
7	4	60	F	2.00	47100	0.840	6.00	2160	143000	120000
19	2	20	F	0.52	41700	0.746	24.00	185	76700	73200
19	3	40	F	1.00	42600	NR	24.00	75.5	125000	NR
19	4	60	F	1.50	48500	3.11	24.00	60.3	140000	115000

*Values represented as Median to reflect the actual sampling timepoint.
NR = Not reported.

(Sponsor's Table)

Dosing Solution Analysis

Drug formulations ranged from -2.4 to 6% of nominal.

Necropsy

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

No BHV-3500-related changes in corpora lutea, number of implantation sites, live or dead fetuses, sex ratio, resorptions, pre- or post-implantation losses or gravid uterine weight were observed.

Offspring (Malformations, Variations, etc.)

No drug-related external or visceral malformations were observed.

9.3 Prenatal and Postnatal Development

Study title: A Subcutaneous Pre- and Post-Natal Reproductive and Developmental Toxicity (SEG III) Including Maternal Functions Study in Sprague-Dawley Rats

Study no.:	1016-3941
Study report location:	EDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 8, 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, 6D16168, 99.9%

Methods

Doses:	0, 5, 10, 20 mg/kg/day BHV-3500
Frequency of dosing:	Daily
Dose volume:	3 mL/kg
Route of administration:	SC
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, USP, in sterile water for injection, USP, adjusted to pH 5.8 to 6.2
Species/Strain:	SD rats
Number/Sex/Group:	22 females/group
Satellite groups:	TK: 6/group
Study design:	Dosing was from GD6 through PND20. F_1 offspring observed for first 11 weeks of life, 2 weeks premating, and 1 to 3 weeks of mating, 3.5 of gestation. F_2 generation observed through PPD4.
Deviation from study protocol:	No significant deviations from protocol.

Observations and Results

F_0 Dams

Survival:	No drug-related mortality.
Clinical signs:	No drug-related clinical observations.
Body weight:	No drug-related changes in body weight.
Food consumption:	No drug-related changes in food consumption.
Uterine content:	BHV-3500 at LD and MD had higher post-implantation loss and lower live birth index. There was no effect at the HD. LD and MD dams had fewer live pups, and the total number of dead pups in the LD and MD groups was significantly increased.
Necropsy observation:	There was a dose-dependent increase in scabs at the injection site of BHV-3500-treated dams. A modest increase in bilateral pale discoloration of the liver was observed in HD dams (6/19) compared to control dams (2/21).
Toxicokinetics:	T_{max} ranged from 2 to 4 hours. C_{max} and AUC were slightly less than dose proportional. Accumulation ratios ranged from 0.97 to 1.09 indicating no accumulation of BHV-3500. C_{max} and AUC at the high dose (20 mg/kg/day) was 7170 ng/mL and 84200 h*ng/mL, respectively.
Dosing Solution Analysis	All formulations were within -2.3 to 2.7% target concentration.

F_1 Generation

Survival:	No BHV-3500-related changes in viability or survival rate.
Clinical signs:	No BHV-3500-related clinical observations.
Body weight:	There were no differences in body weight or weight gain in males. In females (post-weaning), increased absolute body weight and body weight gain that continued into adult life (8.4 to 14.8% increase in body weight at GD0) were observed at all doses. Body weight was comparable across F_1 females during gestation and lactation periods.
Food consumption:	There were no drug-effects on food consumption in males. In females, all doses resulted in higher food consumption between PPD28-31. food consumption was elevated through PPD59 and returned to levels comparable to control females in the pre-mating period (PPD77 to 80).
Physical development:	There were no drug effects on physical development.
Neurological assessment:	No BHV-3500-related changes in functional observation battery. Motor activity and E-water maze performance were comparable to control rats.
Reproduction:	Male and female fertility indices were lower than the control animals. However, the fertility indices for the control group were lower than historical range. Sperm parameters (motility, count, abnormal %) and estrous cycle were not affected by BHV-3500.
Other:	

F_2 Generation

Survival:	No BHV-3500-related changes on viability.
Body weight:	No BHV-3500-related changes on body weight or weight gain.
External evaluation:	No BHV-3500-related macroscopic findings.
Male/Female ratio:	Not evaluated.

Due to control fertility indices outside of historical control range in the F_1 generation, this study is considered inadequate.

Study title: A Developmental and Perinatal/Postnatal Reproduction Study of BHV-3500 by Subcutaneous Injection in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	20254547
Study report location:	EDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 12, 2020
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500, A021800213, 93.6%

Methods

Doses:	0, 5, 10, 20 mg/kg/day BHV-3500
Frequency of dosing:	Daily
Dose volume:	3 mL/kg
Route of administration:	SC
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, USP, in sterile water for injection, USP, adjusted to pH 5.8 to 6.2
Species/Strain:	SD rats
Number/Sex/Group:	F_0: 22 females/group; F_1: 22/sex/group
Satellite groups:	None
Study design:	Daily SC injections were administered from GD6 through PPD20.
Deviation from study protocol:	No significant deviations from protocol.

Observations and Results

F_0 Dams

Survival:	There was no drug-related mortality. 1 LDF and 2 MDF were euthanized between GD23 and LD15 due to difficulties in parturition or dosing error.
Clinical signs:	Dose-related injection site toxicity (skin flaking, scabbing, swelling, and bruising at the injection site) was observed during the gestation and lactation periods.
Body weight:	No BHV-3500-related changes in body weight or body weight gain during gestation or lactation were observed.
Food consumption:	There were no drug-related changes in food consumption.
Uterine content:	There were no drug effects on litter parameters (live birth index, gestation index, % live male pups, implantation sites, post-implantation loss).
Necropsy observation:	Scabs at the injection sites were observed at the LD (1/22), MD (5/22) and HD (11/22).
Toxicokinetics:	Not evaluated.
Dosing Solution Analysis	All formulations were within -2.3 to 2.7% target concentration.

F_1 Generation

Survival:	There were no BHV-3500-related effects on survival.
Clinical signs:	There were no BHV-3500-related clinical observations in preweaning or postweaning periods.
Body weight:	HD male pup weight was reduced by 5% at PPD 21. HD female pups had a 5-9% decreased weight through PPD 7 to 18 but not at the final preweaning timepoint of PPD 21 (0.59% decrease). F_1 Males from the maternal HD group had a 5% decrease in body weight at PPD 106. F_1 Females had no drug-related changes in body weight in post-weaning, mating, gestation, or lactation periods.
Food consumption:	There were no BHV-3500-related effects on food consumption.
Physical development:	There were no BHV-3500-related effects on reflexes or developmental parameters. Balano-preputial separation and vaginal patency were not altered by BHV-3500.
Neurological assessment:	No drug-related effects were observed on the functional observational battery at PPD 60. There were no drug effects on motor activity (PPD60) or the Morris water maze (PPDs 66 to 88) performance.
Reproduction:	BHV-3500 did not affect estrous cycling, mating index or fertility index (% successfully mated animal of all animals and % pregnancies of all mated animals). There were no BHV-3500-related effects on parturition in F_1 females.
Other:	n/a

F_2 Generation

Survival:	No BHV-3500-related effects on viability index or mortality were observed.
Body weight:	There was no effect of BHV-3500 on body weight.
External evaluation:	There were no drug-related external findings.
Male/Female ratio:	Not evaluated.

BHV-3500 administration from GD6 through LD20 at 5, 10, and 20 mg/kg/day SC was well tolerated in F_0, did not result in adverse reproductive or fertility outcomes in F_0 or F_1, and did not result in developmental toxicity in F_1 or F_2 generations. The NOAEL for maternal and reproductive and developmental toxicity was 20 mg/kg/day.

Study title: A 2-week Dose Range-Finding Intranasal Juvenile Toxicity Study of BHV-3500 in Sprague Dawley Rats. (Study # 00803506)

Study no.:	00803506
Study report location:	EDR 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 19, 2018
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BHV-3500, A021800213, 93.6%

A non-GLP study in SD rat (8/sex/group) was conducted in which daily IN doses of 0, 18.8, 28.1, and 37.5 mg/kg/day BHV-3500 were administered from PND24 to PND37. No mortality or drug-related clinical signs, body weight, or food consumption changes were observed. There were no drug-related findings observed at necropsy. Histopathology was not evaluated. BHV-3500 did not show a dose-exposure relationship consistently between sexes or across time points.

Text Table 12
Summary of Toxicokinetics

Dose (mg/kg/day)	t_{max} (hr)	C_{max} (ng/mL)	AUC_{INF} (hr*ng/mL)	t_{last} (hr)
PND 24				
Males				
18.8	1	321	522	6
28.1	1	285	NA	24
37.5	1	431	569	6
Females				
18.8	1	182	472	24
28.1	1	387	821	6
37.5	1	156	486	24
PND 37				
Males				
18.8	1	804	1672	24
28.1	1	388	905	24
37.5	1	342	691	24
Females				
18.8	1	166	495	24
28.1	1	404	901	24
37.5	1	377	1011	24

NA means not available; no terminal phase

(Sponsor's Table)

10 Special Toxicology Studies

Study title: A GLP Neutral Red Uptake Phototoxicity Assay of BHV-3500 in BALB/c 3T3 Mouse Fibroblasts (Study 20242252)
BHV-3500 (up to 100 µg/mL) did not indicate phototoxic potential in mouse fibroblasts.

Study title: Single-dose ocular irritation study in rabbits (Study DM06031)
Ocular administration of a single dose of BMS-742413 (BHV-3500; up to 20 mg/animal) did not result in adverse effects in male rabbits.

Study title: Epiocular™ eye irritation test (EIT) for identifying chemicals not requiring classification and labeling for eye irritation or serious eye damage (Study #: 21AC28.015091)
BHV-3500 (50 mg in 20 µL culture medium for 6 hours) did not affect cell viability and was not considered an eye irritant.

Study title: Intravenous Local Tolerance Study of BHV-3500 in Rats (Study # 293600100101)

BHV-3500 (0 or 1 mg/animal by IV bolus injection) administered to male SD rats (5/group) in vehicle ((b) (4) mM succinic acid and (b) (4) % (b) (4) dextrose) did not result in mortality or adverse clinical signs, injection site toxicity, or macroscopic or microscopic findings. All microscopic findings in the skin of the injection site were associated with route of administration and were present in controls as well as BHV-3500-treated rats.

Study title: Repeated dose (13-week) intranasal impurity (b) (4) qualifying toxicity study of BHV-3500 in Sprague Dawley Rats

Study no.:	VLL/0621/G/T093
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 15, 2021
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BHV-3500 0.25% cocktail ELS-499-042-19 98.9% BHV-3500 0.5% cocktail ELS-499-046-20 97.9%

Methods

Doses:	reference article: 12.5 mg/animal BHV-3500, low dose test article:12.5 mg/animal BHV-3500 with low impurity (b) (4) high dose test article: 12.5 mg/animal BHV-3500 with high impurity (b) (4)
Frequency of dosing:	Daily
Route of administration:	Intranasal
Dose volume:	25 microliter per nostril
Formulation/Vehicle:	(b) (4) mM succinic acid and (b) (4) % (w/v) (b) (4) dextrose, water
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	10/sex/group
Age:	6-9 weeks
Satellite groups:	TK; 3/sex control; 9/sex/group BHV-3500
Deviation from study protocol:	No significant deviations from study protocol.

Results

No mortality or test article-related clinical signs were observed. Body weight was decreased in all (test and reference) groups and was similar in magnitude to those observed in rodent and nonrodent studies with BHV-3500. No clinical pathology changes were observed. No drug-related changes were observed in organ weights,

gross pathology, or histopathological findings. There were no systemic toxicities of [redacted] (b) (4)

11 Integrated Summary and Safety Evaluation

BHV-3500 is a competitive calcitonin gene-related peptide (CGRP) receptor antagonist intended to treat acute migraine. The proposed clinical dose is 10 mg administered by nasal spray once daily for up to 8 days per month.

Pharmacology

The primary pharmacology battery characterized BHV-3500 as is a competitive antagonist of the human CGRP receptor. Ligand displacement studies indicated that BHV-3500 has high affinity for human, cynomolgus monkey, and marmoset CGRP receptors (IC_{50} = 0.94, 0.06, and 0.13 nM, respectively) but very low affinity for dog, guinea pig, rabbit rat, and mouse CGRP receptors (IC_{50} = 500, 600, 8,700, 25,000, and 140,000 nM, respectively) . In vivo, IV injection of 10 µg/kg BHV-3500 resulted in 53-80% suppression of αCGRP-induced facial blood flow in monkeys, associated with a plasma C_{max} of 16 to 19 ng/mL. Safety pharmacology studies did not indicate potential cardiovascular, respiratory, or neurological toxicity following acute BHV-3500 administration. Secondary pharmacology studies indicated that BHV-3500 (10 µM) inhibited binding at α1 and α2 adrenergic receptors by 76% and 86%, respectively; however, there were no in vivo findings typically associated with adrenergic inhibition (i.e., hypertension and bradycardia) in nonclinical studies.

General Toxicology

Single and repeat-dose general toxicity studies with IN, SC, or orally administered BHV-350 were conducted in CByB6F1 mice, SD rats, beagle dogs, and cynomolgus monkeys. Based on nasal surface area, NOAELs in the chronic toxicity studies were 14x (rats) and 12x (monkeys) higher than MRHD.

Mouse

No adverse effects were associated with daily IN administration of BHV-3500 in mouse for 1 or 4 weeks with doses up to a maximum feasible dose of 2.5 mg/kg. In a 28-day study, daily IN administration of 0, 1.5, 2, and 2.5 mg BHV-3500 resulted in 2.3 to 2.5x increases in AST and ALT but no correlating histopathology findings.

Rat

There were no adverse effects associated with single IN doses up to 6.25 mg in rat; however, a single SC dose of 150 mg/kg resulted in premature euthanasia due to injection site toxicity. There were no adverse effects associated with single oral doses up to 6000 mg/kg.

The primary toxicity following daily SC dosing for 28 days (0, 7.5, 25, and 75 mg/kg) or 13 weeks (0, 2, 12, 20 mg/kg) was injection site toxicity for which no NOEL was established. However, such findings are not relevant given that the intended route of administration is IN. No systemic toxicity was observed in either study; therefore, the NOAEL for systemic toxicity in rats administered BHV-3500 by SC injection for 13 weeks is 20 mg/kg.

The primary toxicity associated with daily IN administration of 0, 18.8, 28, and 37.5 mg/kg BHV-3500 for 13 weeks was degeneration and regeneration of olfactory epithelium in the nasal cavity at all doses. However, there were no adverse effects following daily IN administration of 0, 6, 9.9, or 12.5 mg/day for 26 weeks. Therefore, the NOAEL for nasal cavity toxicity in rat following IN administration of BHV-3500 is 12.5 mg/day.

Dog

Repeat oral administration studies (14-day and 13-week) were conducted in dogs to support development of oral formulations of BHV-3500: sublingual (SL) oral disintegrating tablets (ODT, 50 mg/day) and soft gel oral capsules (50 mg/day). No adverse effects were observed with either dose or formulation

Monkey

There were no adverse effects associated with single IN doses up to 30 mg/kg in monkey.

The primary toxicity associated with daily SC administration for 28 days (5, 15, 37.5 mg/kg) was injection site toxicity for which no NOEL was established. However, these findings are not relevant as the intended route of administration is IN.

Daily IN administration was assessed in 28-day and 13- and 39-week studies. There were no adverse effects in the 28-day study at doses up to 8.3 mg. The primary toxicity associated with daily IN administration up to 12 mg/day (13 weeks) and 45 mg/day (39 weeks) was nasal inflammation which occurred at doses. However, similar findings were also observed in control groups in both studies.

Genetic Toxicology and Carcinogenicity

BHV-3500 was negative in Ames, chromosomal aberration, and rat bone marrow micronucleus assays. Based on a review by the CDER Division of Biometrics and Executive CAC (September 29, 2022, meeting minutes), intranasal BHV-3500 did not induce tumor formation in the 26-week study in TgRasH2 mice (0, 0.3, 0.8, 2.5 mg/day) or in the 2-year study in SD rats (0, 2, 9, 18.8 mg/kg/day).

Reproductive and Developmental Toxicity

There were no adverse effects associated with daily SC administration of 0, 5, 15, 25 mg/kg/day BHV-3500 in the rat fertility and early embryonic development study.

There were no adverse effects associated with daily SC administration of 0, 10, 25, 50 mg/kg/day in the rat embryofetal development (EFD) study. There were also no adverse effects associated with daily SC administration of 0, 20, 40, 60 mg/kg/day BHV-3500 in the rabbit EFD study.

There were no adverse effects associated with daily SC administration of 0, 5, 10, and 20 mg/kg/day SC BHV-3500 in the rat pre- and postnatal development (PPND) studies. The first study (0, 5, 10, 20 mg/kg/day SC) indicated decreases in male and female fertility indices in the F_1 generation; however, the control groups were also lower than the historical control range. For this reason, the study was considered inadequate, and a second PPND study was conducted. In the second PPND study (0, 5, 10, and 20 mg/kg/day SC), no drug-effects on fertility indices/reproductive measures were seen in the F_0 or F_1 generations.

Juvenile Toxicity

A dose range-finding study in juvenile SD rat did not indicate any adverse events following daily IN administration of 0, 18.8, 28.1, and 37.5 mg/kg BHV-3500.

Conclusion

The nonclinical data support approval of BHV-3500. It is recommended that a juvenile animal toxicity study be completed in the post marketing period.

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Executive CAC Final Study Minutes
Date of Meeting: September 27, 2022

Committee: Karen Davis Bruno, PhD, OND IO, Chair
Paul Brown, PhD, OND IO, Member
Tim McGovern, PhD, OND IO, Member
Ron Wange, PhD, OND IO, Member
Ijeoma Uzoma, PhD, DPT-II, Pharm/Tox. Alternate Member
Lois Freed, PhD, DPT-N, Pharm/Tox. Division Director
Elizabeth Khoury, PhD, DPT-N, Presenting Reviewer

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

Application Type and Number(s): NDA 216386
Drug Name: Zavegepant/BHV-3500
Sponsor: Biohaven Pharmaceuticals

Background
BHV-3500 (zavegepant) is an intranasally administered, small molecule inhibitor of the human calcitonin gene-related peptide (CGRP) receptor and is intended to treat acute migraine. BHV-3500 was negative in in vitro (Ames, mammalian chromosomal aberration) and in vivo (mouse micronucleus) assays. The Executive CAC provided dose recommendations for both the 26-week Tg.rasH2 mouse study (September 3, 2020) and the 2-year carcinogenicity study in Sprague Dawley rat (May 9, 2019). For the 26-week Tg.rasH2 mouse study, dose selection was based on maximum feasible dose (2.5 mg) for intranasal administration. For the 2-year rat study, dose selection was based on increased incidence and severity of histopathological findings in nasal mucosa at intranasal doses greater than 18.8 mg/kg in a 3-month dose range-finding study.

Mouse Carcinogenicity Study
Male and female TgRasH2 mice (25/sex/group) were administered BHV-3500 at intranasal doses of 0 (saline), 0.3, 0.8, and 2.5 mg/day for 26 weeks. The positive control group (25/sex) was administered a single IP injection of 75 mg/day MNU on Day 1. BHV-3500 did not result in statistically significant effects on mortality or tumor formation. The positive control produced expected tumor responses.

Rat Carcinogenicity Study
Male and female SD rats (65/sex/group) were administered BHV-3500 at intranasal doses of 0 (saline), 0 (vehicle), 2, 9, and 18.8 mg/kg/day. The study was terminated at Week 84 in males and Weeks 95-96 in females due to reduced survival (≤ 20/sex/group) in control groups. There were no statistically significant drug-related effects on mortality or tumor formation.

Executive CAC Conclusions

Mouse:

- The Committee concluded that the 26-week carcinogenicity study in Tg.rasH2 mouse was adequate and negative for drug-related neoplasms.

Rat:

- The Committee concluded that the 2-year carcinogenicity study in Sprague Dawley rat was adequate and negative for drug-related neoplasms.

Karen Davis Bruno, PhD
Chair, Executive CAC

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