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RESEARCH**

APPLICATION NUMBER:

216578Orig1s000

NON-CLINICAL REVIEW(S)



PHARMACOLOGY/TOXICOLOGY LABELING REVIEW

Date:	05/02/23
NDA #	NDA 216578 (SDN 1, eCTD 0001)
Sponsor:	Astellas Pharma
Drug/Indication:	Fezolinetant (or ESN 364) for the treatment of moderate to severe vasomotor symptoms (VMS) associated with menopause
Reviewer:	Miyun Tsai-Turton, PhD, MS

Background

NDA 216578 is a 505(b)(1) application submitted by Astellas Pharma US for fezolinetant (ESN364; proposed tradename VEOZAH) for the treatment of moderate to severe vasomotor symptoms (VMS) associated with menopause. Fezolinetant is a small molecule neurokinin 3 (NK3) receptor antagonist. It is the first-in class product where its mechanism of action is to block neurokinin B (NKB) binding on the KNDy neuron to modulate neuronal activity in the thermoregulatory center.

Astellas Pharma is seeking approval of the following dosing regimen:

- **The recommended dose is 45 mg orally once daily with or without food.**

The nonclinical review of fezolinetant was presented in a separate review dated November 22, 2022. The current document is for labeling only.

Labeling

See pharm/tox proposed edits below.

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/s/

MIYUN M TSAI-TURTON
05/02/2023 09:30:18 AM

ANDREA BENEDICT
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I concur with the review and recommendations of Dr. Tsai-Turton.

**PHARMACOLOGY/TOXICOLOGY
REVIEW ADDENDUM**

NDA #:	216578 (IND 130277)
Supporting Document #:	SDN 1; eCTD 0001 SDN 55; eCTD 0055
Received Date:	SDN 1: June 22, 2022 SDN 55: March 20, 2023
PDUFA Goal Date:	May 22, 2023
Sponsor:	Astellas Pharma Inc.
Drug:	Fezolinetant
Proposed Tradename:	VEOZAH
Pharmacologic Class:	Neurokinin 3 receptor antagonist
Indication:	Moderate to severe vasomotor symptoms (VMS) associated with menopause
Primary Reviewer:	Andrea Benedict, PhD for Miyun Tsai-Turton, PhD, MS
Secondary Reviewer:	Kimberly Hatfield, PhD
Pharm/Tox Division:	Division of Pharm/Tox for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine (DPT-RPURN)
Clinical Review Division:	Division of Urology, Obstetrics and Gynecology (DUOG)
Recommendation on Regulatory Action:	Approval
Review Date:	April 20, 2023

Regulatory Background:

On June 22, 2022, Astellas Pharma Global Development, Inc (Astellas) submitted NDA 216578 for ESN364 (fezolinetant), 45 mg oral tablet, for the proposed indication of treatment of moderate to severe vasomotor symptoms (VMS) associated with menopause.

On August 22, 2022, the Division of Urology, Obstetrics and Gynecology (DUOG) submitted an internal consult request to the Division of Oncology III (DO III) regarding a potential clinical safety signal of malignancy in women taking fezolinetant. In a pooled consideration of the Phase 3 clinical trials (i.e., 2693-CL-0301 2693-CL-0302, and 2693-CL-0304), a potential signal of dose-dependent malignancy imbalance was identified for fezolinetant which included malignancies of several origins. The DO III review team clarified that assessment of biological plausibility of malignancy should be conducted by Division of Pharm/Tox for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine (DPT-RPURN) supporting the primary clinical review division (DUOG). An Addendum Pharm/Tox review was conducted including the available nonclinical information and a review of the currently published literature, which did not identify a

biological mechanism that would indicate an increased risk of malignancy with fezolinetant itself or antagonism of NK3R (refer to the NDA 216578 Nonclinical review, dated January 27, 2023).

On March 2, 2023, the Cross Discipline Safety Advisor from the Office of Surveillance and Epidemiology (OSE) and co-chair of a Drug Safety Team covering DUOG products, Dr. Baoguang Wang, requested that the DPT-RPURN team supporting DUOG conduct an additional assessment of biological plausibility of fezolinetant as a potential cancer promoter. Specifically, information was requested regarding whether fezolinetant met any of the criteria of the key characteristics of carcinogens as presented in the International Agency for Research on Cancer (IARC) monograph¹.

In addition to internal Pharm/Tox evaluation of this request (as discussed in the review below), the Division also sent a request to the Applicant to provide information to address the biological plausibility of fezolinetant as a potential cancer promoter based on the IARC monograph defined key characteristics of carcinogens (refer to the Information Request, dated March 10, 2023, and the Applicant's Response to Information Request, dated March 20, 2023).

Nonclinical Additional Assessment of the Biological Plausibility of Malignancy:

This review discusses the additional assessment of biological plausibility of malignancy, including the IARC monograph defined key characteristics of carcinogens, conducted by DPT-RPURN. For details regarding the general Pharmacology/Toxicology information submitted to support the nonclinical safety of fezolinetant, refer to the NDA 216578 Nonclinical reviews of Drs. Miyun Tsai-Turton and Andrea Benedict, dated November 22, 2022.

IARC Monograph: Key Characteristics of Carcinogens: The IARC Monograph defines 10 key characteristics of carcinogens (Table 10.1 below, excerpted from the IARC Monograph).

Table 10.1. Key characteristics of carcinogens

1. Is electrophilic or can be metabolically activated to electrophiles
2. Is genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

¹ Smith MT. Key characteristics of carcinogens. In: Baan RA, Stewart BW, Straif K, editors. Tumour Site Concordance and Mechanisms of Carcinogenesis. Lyon (FR): International Agency for Research on Cancer; 2019. (IARC Scientific Publications, No. 165.) Chapter 10. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK570335/>.

Reviewer Comments: *The following sections provide information for fezolinetant to address each of the 10 key characteristics of carcinogens. Information from the FDA Pharm/Tox internal review and the Applicant's responses will be noted separately under each of the 10 key characteristics or noted as concurrence when information did not differ in the FDA Pharm/Tox and Applicant's review.*

1. Is fezolinetant electrophilic or can be metabolically activated to electrophiles?

FDA Pharm/Tox Review: The Pharm/Tox team consulted our internal Quality Drug Substance Team and the Division of Applied Regulatory Science (DARS) Computational Toxicology Consultation Service (CTCS) to provide information on these chemical attributes of fezolinetant and its main metabolite ES259564 (See Appendix 1).

The internal FDA chemistry analysis confirmed that there were no structural alerts detected. Fezolinetant was negative for bacterial mutagenicity and other genotoxicity and carcinogenicity endpoints in (Q)SAR predictive models. Computational modeling indicated that there was no evidence of mutagenic potential by direct-acting mutagenic mechanism or indirectly via metabolic activation. Structural comparison modeling identified two candidates, simmiparib (cancer therapy in clinical development) and sitagliptin (approved for type 2 diabetes). Neither compound with structural similarity appears to indicate a critical cancer-risk concern.

Applicant Response/Concurrence: The Applicant's chemistry analysis also indicated that there were no structural alerts detected. The Applicant stated that fezolinetant and its metabolites are not electrophilic and that computational structural-activity models assessing potential for DNA reactivity did not indicate a structural alert for fezolinetant.

2. Is fezolinetant genotoxic?

FDA Pharm/Tox Review with Applicant Concurrence: No.

Fezolinetant and its main metabolite ES259564 did not induce gene mutations or chromosomal aberrations in a standard battery of in vitro and in vivo genetic toxicology studies, including:

Fezolinetant negative in:

- In vitro Ames bacterial reverse mutation assay (Study 8276182)
- In vitro chromosomal aberration assay (Study 8276184)
- In vivo micronucleus assay in rats (Study 8276179)

ES259564 negative in:

- In vitro Ames bacterial reverse mutation assay (Study 8390041)
- In vitro chromosomal aberration assay (Study 8390040)

3. Does fezolinetant alter DNA repair or cause genomic instability?

FDA Pharm/Tox Review: No data on the effects of fezolinetant on DNA repair or genomic instability were identified.

Applicant Response/Concurrence: No additional tests on DNA repair or genomic instability were conducted since fezolinetant and ES259564 showed no genotoxic potential.

4. Does fezolinetant induce epigenetic alterations?

FDA Pharm/Tox Review: No data on the effects of fezolinetant on epigenetic alterations were identified.

Applicant Response/Concurrence: No additional tests on epigenetic alterations were conducted since fezolinetant and ES259564 showed no genotoxic potential.

5. Does fezolinetant induce oxidative stress?

FDA Pharm/Tox Review: No data on the effects of fezolinetant on oxidative stress were identified. There was no indication from the repeat-dose toxicity studies in the rat or monkey that fezolinetant induced inflammation, which is a read-out that reactive oxygen species (ROS)-related inflammation was not produced.

Applicant Response: The Applicant concurred that no ROS-related inflammation was evident in the repeat-dose toxicity studies in the rat or monkey. Additionally, in vitro ROS production assays did not indicate that fezolinetant induced oxidative stress.

6. Does fezolinetant induce chronic inflammation?

FDA Pharm/Tox Review with Applicant Concurrence: No chronic inflammation was observed in the repeat-dose toxicity studies or carcinogenicity studies with fezolinetant.

7. Is fezolinetant immunosuppressive?

FDA Pharm/Tox Review with Applicant Concurrence: No immunosuppressive effects were observed in the repeat-dose toxicity studies or carcinogenicity studies with fezolinetant.

8. Does fezolinetant modulate receptor-mediated effects?

FDA Pharm/Tox Review/Applicant Concurrence: Yes.

- Fezolinetant is a neurokinin 3 (NK3) receptor antagonist (intended mechanism of action).
- There is no evidence that fezolinetant has the potential to modulate off-target receptor-mediated effects.
- There was no evidence from the literature that NK3 receptor antagonism induces or promotes tumorigenesis based on the published literature review conducted by FDA Pharm/Tox (refer to the NDA 216578 Nonclinical review, dated January 27, 2023) or by the Applicant.

9. Does fezolinetant cause immortalization?

FDA Pharm/Tox Review/Applicant Concurrence: No.

10. Does fezolinetant alter cell proliferation, cell death or nutrient supply?

FDA Pharm/Tox Review: There was no indication of altered cell death or nutrient supply for fezolinetant or NK3R antagonism from the available nonclinical information.

Findings from the repeat-dose toxicity studies in the rat and monkey and 2-year female rat and 6-month transgenic (Tg.rasH2) mouse carcinogenicity studies did not indicate a signal for proliferative effects with fezolinetant.

Conclusions from the 2-year carcinogenicity study in female rats and 6-month Tg.rasH2 mouse: Fezolinetant was not carcinogenic in both a 2-year female rat bioassay up to the highest-dose tested of 100 mg/kg/day and a 26-week Tg.rasH2 mouse bioassay up to the highest-dose tested of 450 mg/kg/day. These findings indicate that there was no evidence of drug-related carcinogenicity at 186-fold and 47-fold the human AUC₂₄ at the human therapeutic dose of 45 mg, in the female rat and Tg.rasH2 mouse, respectively. For additional details, refer to the carcinogenicity study review dated October 28, 2022 by Dr. Tsai-Turton.

Findings relevant to the effects of fezolinetant on proliferation from the 2-year female rat carcinogenicity study (Study 8375970):

- *Sex-steroid sensitive tissues:* There was a dose-related decrease in the incidence and severity of squamous cell metaplasia in the cervix and hyperplasia in the mammary gland. These findings were consistent with the effect of fezolinetant antagonism of the NK3 receptor influencing the downstream signaling of KNDy neurons to gonadotropin releasing hormone (GnRH) neurons, modulating the GnRH pulse frequency and consequently the release of LH. Antagonists of luteinizing hormone-releasing hormone (LHRH), also known as GnRH, are a well-established means of treating sex-steroid dependent disorders². Therefore, the blockade of the NK3 receptor, and subsequent suppression of gonadotropin secretion, likely played a role in reducing the hyperplastic effects in the sex-steroid sensitive tissues in the rodent studies.
- *Thyroid:* There was a small increased incidence of follicular cell hyperplasia of the thyroid in mid-dose (MD; 30 mg/kg/day) (4/100) and high-dose (HD; 100 mg/kg/day) (5/100) females. Benign follicular cell adenoma was slightly increased at the HD (9/100 HD vs 3/100 for controls) but did not meet the criteria for a positive tumor finding and fell within historical control range (negative findings, as concurred by the FDA statical review team and the FDA's Executive Carcinogenicity Assessment Committee [ECAC]).
- *Thymus:* There was an apparent increase in incidence of benign and malignant thymomas and thymoma-related mortality in the MD and HD groups of the 2-year female rat carcinogenicity study.
 - Based on the FDA Pharm/Tox review and FDA statistical analysis, with concurrence from the ECAC, thymoma findings were determined to be not related to drug treatment based on:
 - Lack of statistical significance (trend or pairwise tests) and
 - Incidence falling within historical control ranges for the conducting laboratory and the published literature. Additionally, thymomas are

² Engel JB, Schally AV. Drug insight: clinical use of agonists and antagonists of luteinizing-hormone-releasing hormone. Nat Clin Pract Endocrinol Metab 2007; 3:157-67

a more prevalent spontaneous finding in female Wistar Han rats (~6-15%) compared to other rat strains (0.1-2%) in 104-week studies^{3,4,5}. All benign thymomas displayed a lymphocyte-rich morphological pattern, which has been shown to be specific to Han Wistar rats³. Malignant thymomas have been shown to often be the cause of death and early mortality when they spontaneously occur in rats.

Applicant Response: There was no evidence that fezolinetant has the potential to alter cell proliferation, cell death or nutrient supply based on nonclinical data [Study 8276179; Study 8297361; Study 8331724; 8276020; Study 8297388; Study 8331280; Study 8375970; Study 8375967]. In addition, the in vitro target selectivity assay revealed that fezolinetant does not interact with VEGFR-2 kinase [Study 100002171]; therefore, the possibility of altered nutrient supply is considered to be low.

Pharmacology/Toxicology Conclusion:

Pharm/Tox review of the available nonclinical information, Applicant's responses, and additional chemistry assessments of fezolinetant did not identify:

- Structural alerts relevant to DNA-reactivity,
- Positive carcinogenicity signal in the nonclinical bioassays,
- Potential mechanisms of tumorigenesis or tumor promoter activity with fezolinetant or its metabolites.

Based on the currently available nonclinical information provided by the Applicant and in the published literature, no biological mechanism was identified that indicated an increased risk of malignancy or tumor promoter activity with fezolinetant or through NK3R antagonism.

³ Moore RR et al. Comparative Incidences and Biological Outcomes for Thymoma in Various Rat Strains in National Toxicology Program Studies. Toxicol Pathol. 2019. 47(7): 833–841.

⁴ Blankenship B et al. Findings in Historical Control Harlan RCCHan™:WIST Rats from 104-week Oral Gavage Studies. Toxicol Pathol. 2016. 44(7) 947-961

⁵ Tomonari Y et al. Thymomas and Associated Hyperplastic Lesions in Wistar Hannover Rats. Toxicol Pathol. 2019. 47(2) 129-137.

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**PHARMACOLOGY/TOXICOLOGY
REVIEW ADDENDUM**

NDA #:	216578 (IND 130277)
Supporting Document #:	SDN 1; eCTD 0001
Received Date:	June 22, 2022
PDUFA Goal Date:	February 22, 2023
Sponsor:	Astellas Pharma Inc.
Drug:	Fezolinetant
Proposed Tradename:	VEOZAH
Pharmacologic Class:	Neurokinin 3 receptor antagonist
Indication:	Moderate to severe vasomotor symptoms (VMS) associated with menopause
Primary Reviewer:	Andrea Benedict, PhD for Miyun Tsai-Turton, PhD, MS
Secondary Reviewer:	Kimberly Hatfield, PhD
Pharm/Tox Division:	Division of Pharm/Tox for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine (DPT-RPURN)
Clinical Review Division:	Division of Urology, Obstetrics and Gynecology (DUOG)
Recommendation on Regulatory Action:	Approval
Review Date:	January 27, 2023

Regulatory Background:

On June 22, 2022, Astellas Pharma Global Development, Inc (Astellas) submitted NDA 216578 for ESN364 (fezolinetant), 45 mg oral tablet, for the proposed indication of treatment of moderate to severe vasomotor symptoms (VMS) associated with menopause.

On August 22, 2022, the Division of Urology, Obstetrics and Gynecology (DUOG) submitted an internal consult request to the Division of Oncology III (DO III) regarding a potential clinical safety signal of malignancy in women taking fezolinetant. In a pooled consideration of the Phase 3 clinical trials (i.e., 2693-CL-0301 2693-CL-0302, and 2693-CL-0304), a potential signal of dose-dependent malignancy imbalance was identified for fezolinetant which included malignancies of several origins (i.e., squamous cell carcinoma of the skin, squamous cell carcinomas of the oral cavity, basal cell carcinomas, bone cancer, colon cancer, endometrial adenocarcinomas, malignant melanoma in situ, non-small cell carcinoma of the lung, apocrine cancer of breast, and invasive cancer of breast) as well as 1 case of unspecified lung neoplasm and 1 case of keratoacanthoma, a neoplasm with high malignant potential.

DUOG's consult requested specific advice on: 1) whether these cases of malignancies of various primary sites represent a signal for general malignancy with this product and how to characterize the strength of the signal (i.e., weak, moderate, or strong), 2) whether there is a potential biologic mechanism by which an NK3R antagonist could lead to malignancy, and 3) if this malignancy signal is of potential concern to patients that take this medication, did DO III have recommendations for wording of the related warnings and precautions in labeling.

In response to item #2, the DO III review team clarified that assessment of biological plausibility of malignancy should be conducted by Pharmacology/Toxicology reviewers of the primary clinical review division (i.e., DUOG).

Nonclinical Assessment of the Biological Plausibility of Malignancy:

This review discusses the assessment of biological plausibility of malignancy conducted by the Division of Pharm/Tox for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine (DPT-RPUM). Refer to the NDA 216578 Nonclinical reviews of Drs. Miyun Tsai-Turton and Andrea Benedict, dated November 22, 2022, for details regarding the Pharmacology/Toxicology information submitted to support the nonclinical safety of fezolinetant.

Indication-related Mechanism of Action:

Fezolinetant is a New Molecular Entity (NME) neurokinin 3 (NK3) receptor antagonist. The intended mechanism of action of fezolinetant is to block neurokinin B (NKB) binding on the kisspeptin/neurokinin B/dynorphin (KNDy) neurons in the thermoregulatory center in the hypothalamus. KNDy neurons signal to the thermoregulatory center of the brain and modulate the output to heat dissipating mechanisms in response to input from peripheral temperature sensing neurons. By antagonizing NKB/NK3-mediated signal, fezolinetant modulates KNDy neuronal activity in the thermoregulatory center resulting in attenuated vasomotor symptoms associated with menopause.

Findings from Nonclinical Carcinogenicity Studies:

Fezolinetant was found not carcinogenic in both a 2-year female rat bioassay at doses up to 100 mg/kg/day (186X MRHD) and a 26-week Tg.rasH2 mouse bioassay at doses up to 450 mg/kg/day (47X MRHD). While some neoplastic changes were noted in the female rat, including increased incidence of benign follicular adenomas in the thyroid with increased fezolinetant dose (trend only; p-value = 0.0094) and increased incidence of the combination of ovarian epithelial tumors (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma) in mid-dose females only (pair-wise only; p=0.0132; 30 mg/kg/day; 75X MRHD), these findings were not determined to be toxicologically relevant by statistical analysis and considered negative findings for carcinogenicity. For additional details, refer to the carcinogenicity study review dated 10-28-2022 by Dr. Tsai-Turton.

Non-neoplastic histopathological findings in the ovary and vagina in mice, and ovary, uterus, cervix, and mammary gland in female rats were considered related to the pharmacologic action of fezolinetant. Of note, in the female rat, there was a dose-related decrease in the incidence and severity of squamous cell metaplasia in the cervix and hyperplasia in the mammary gland. These findings were consistent with the effect of fezolinetant antagonism of the NK3 receptor influencing the downstream signaling of

KNDy neurons to gonadotropin releasing hormone (GnRH) neurons, modulating the GnRH pulse frequency and consequently the release of LH. Antagonists of luteinizing hormone-releasing hormone (LHRH), also known as GnRH, are a well-established means of treating sex-steroid dependent disorders¹. Therefore, the blockade of the NK3 receptor, and subsequent suppression of gonadotropin secretion, likely played a role in reducing the hyperplastic effects in the sex-steroid sensitive tissues in the rodent studies.

Genetic Toxicology:

Fezolinetant was not mutagenic based on a standard battery of in vitro and in vivo genotoxicity studies.

Literature References Related to NK3R Antagonist Risk of Malignancy:

- Antagonists of the NK3R have been suggested to decrease LH and testosterone in a dose-dependent manner², which may reduce proliferative effects in the sex-steroid sensitive tissues.
- In a mouse model of bone invasion by human oral squamous cell carcinoma, a selective NK3R antagonist reduced the osteoclastic bone destruction associated with invasion of oral squamous cell carcinoma³. These findings suggested that selective NK3R antagonism suppressed oral squamous cell carcinoma tumorigenesis in the osteolytic bone destruction associated with oral squamous cell carcinoma invasion.
- No references could be located that outlined any specific drug class-related risk of malignancy from treatment with NK3R antagonists.

Pharmacology/Toxicology Conclusion:

Review of the nonclinical mutagenicity and carcinogenicity studies conducted with fezolinetant did not indicate a potential risk or mechanism for drug-related genetic toxicity or tumorigenesis. Information from the published literature did not indicate an increased risk or any theoretical mechanism for a risk of malignancy with NK3R antagonism.

Based on the nonclinical information provided by the Applicant and available in the published literature, no biological mechanism was identified that indicated an increased risk of malignancy with fezolinetant or NK3R antagonism.

¹ Engel JB, Schally AV. Drug insight: clinical use of agonists and antagonists of luteinizing-hormone-releasing hormone. Nat Clin Pract Endocrinol Metab 2007; 3:157-67

² Pari M, Ballard, TM, and Ratnub H. Tachykinin neurokinin 3 receptor antagonists: a patent review (2005 – 2010), Expert Opin. Ther. Patents 2011; 21(5):637-655

³ Obata K, et al. Role of Neurokinin 3 Receptor Signaling in Oral Squamous Cell Carcinoma. Anticancer Research. 2017; 37: 6119-6123

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 216578
Supporting document/s: [SDN 1 \(eCTD 0001\)](#)
Applicant's letter date: 06/22/2022
CDER stamp date: 06/22/2022
Product: Fezolinetant tablet
(Proposed tradename: VEOZAH)
Indication: Moderate to severe vasomotor symptoms (VMS)
associated with menopause
Applicant: Astellas Pharma Inc.
Review Division: Division of Urology, Obstetrics, and Gynecology
Reviewer: Miyun Tsai-Turton, PhD, MS
Supervisor/Team Leader: Andrea Benedict, PhD
Tertiary Reviewer: Mukesh Summan, PhD, DABT
(Acting) Division Director: Christina Chang, MD
Project Manager: Samantha Bell

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 216578 are owned by Astellas Pharma US., Inc., or are data for which Astellas Pharma US., Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 216578 that Astellas Pharma US., Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 216578.

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1 Executive Summary

1.1 Introduction

NDA 216578 is a 505(b)(1) application submitted by Astellas Pharma US for fezolinetant (ESN364; proposed tradename VEOZAH) as 45 mg tablets for the treatment of moderate to severe vasomotor symptoms (VMS) associated with menopause. Fezolinetant is a small molecule, neurokinin 3 (NK3) receptor antagonist. It is a first-in class product where its mechanism of action is to block neurokinin B (NKB) binding to the kisspeptin/neurokinin B/dynorphin (KNDy) neurons to modulate neuronal activity in the thermoregulatory center in the hypothalamus. KNDy neurons signal to the thermoregulatory center of the brain and modulate the output to heat dissipating mechanisms in response to input from peripheral temperature sensing neurons. By antagonizing NKB/NK3-mediated signal, fezolinetant modulates NKDy neuronal activity in the thermoregulatory center resulting in attenuated vasomotor symptoms associated with menopause.

The clinical program includes 18 completed studies: There are 11 Phase 1 studies, 4 Phase 2 studies, and 3 Phase 3 studies, comprising 3682 participants across these studies. The nonclinical program includes pharmacodynamic (PD), pharmacokinetic (PK), and repeat dose toxicity (up to 26 weeks in rats and 39 weeks in monkeys), reproductive and developmental toxicology (in rats and rabbits), genotoxicity, and carcinogenicity studies (in transgenic mice and rats). In January 2022, the Sponsor met the Agency for a pre-NDA meeting under IND 130277 and it was established that the nonclinical package and nonclinical data supporting the mechanism of action of fezolinetant appeared to be adequate to support an NDA submission (see more details in the 01/31/22 pharm/tox memo and the 02/24/22 Meeting Minutes in DARRTS). The sponsor submitted pre- and post-natal developmental toxicity study and carcinogenicity studies under IND 130277 (eCTD 0115) these studies were reviewed in the NDA.

1.2 Brief Discussion of Nonclinical Findings

In vitro **pharmacology** studies showed that fezolinetant is a potent antagonist of the human (h)NK3 receptor. Fezolinetant has a similar affinity for human and monkey NK3 receptors and 8-fold less for rat NK3 receptors. The major metabolite, ES00259564 (i.e., approximately 50% of the total fezolinetant-related plasma [AUC] exposure), showed no significant activity at off-target receptors, with a 20-fold weaker hNK3 receptor binding affinity as compared to fezolinetant. *In vivo* pharmacology studies demonstrated fezolinetant attenuated hot flash-like symptoms in ovariectomized (OVX) rats (a nonclinical model of postmenopause). These *in vivo* studies demonstrated that OVX rats exhibited several typical menopause symptoms: hyperphagia, increased body weight, significantly decreased plasma estradiol levels, increased LH and FSH levels, and significantly increased tail skin temperature. Increased neuronal activity in the MnPO (median preoptic nucleus) hypothalamic neurons was also observed in these OVX rats. Repeated oral administration of fezolinetant in OVX rats reduced hyperphagia and weight gain, reduced plasma LH levels, without affecting estradiol or

FSH levels, and attenuated hot flash-like symptoms. Fezolinetant also inhibited the activation of MnPO neurons. Additionally, in intact female rats and monkeys, fezolinetant was found to lower the circulating sex hormones LH, estradiol, and progesterone, but not FSH or testosterone, demonstrating that antagonism of the NK3 receptor inhibits LH secretion in female animals with functioning ovaries. Therefore, fezolinetant antagonism of the NK3 receptor appears to affect the downstream signaling of KNDy neurons to gonadotropin releasing hormone (GnRH) neurons, modulating the GnRH pulse frequency and consequently the release of LH.

Pharmacokinetic studies showed that fezolinetant was rapidly absorbed and widely distributed to the tissues. The bioavailability of fezolinetant was 91.9 to 115% in rats and 74.6-103% in monkeys. The highest levels of fezolinetant in tissues were associated with the kidney cortex, clitoris, uveal tract/retina, adrenal gland (cortex and medulla) and liver. CYP1A2 was the major CYP isozyme implicated in the metabolism of fezolinetant. One oxidated metabolite (ES00259564) was detected in cryopreserved hepatocytes from rats, monkeys, and humans, suggesting that human-specific fezolinetant metabolites were not formed. Fezolinetant and its metabolite were excreted in urine and feces. Additionally, fezolinetant-derived components can be transferred to the infant tissues via breast milk.

Repeated dose toxicity studies were conducted in rats up to 26 weeks and in monkeys up to 39 weeks.

- In the **26-week rat study** (Study No 8331724), fezolinetant was administered orally by daily gavage to sexually mature female rats at a dose of 0 (vehicle), 30, 100, or 200 mg/kg. No adverse effects on in-life parameters, clinical pathology, or microscopic changes were noted. Uterine atrophy and epithelial mucification of the vagina and cervix, attributed to exaggerated pharmacological effects of fezolinetant, did not affect the general health of the animals, and were not adverse. The NOAEL was the high-dose of 200 mg/kg/day, with $AUC_{24} = 569000 \text{ ng}\cdot\text{h/mL}$ (148X MRHD of 45 mg with $AUC_{24} = 3855 \text{ ng}\cdot\text{h/mL}$ obtained from clinical Study 2693-PD-0010). This study was originally reviewed under IND 130277 SN 14 (2-year rat carcinogenicity SPA). The PT review can be found in DARRTS dated 12/05/17 under IND 130277.
- In the **39-week monkey study** (Study No 8331280), fezolinetant was administered orally by daily gavage to sexually mature female monkeys at a dose of 0, 10, 25, or 40 mg/kg. High doses of fezolinetant resulted in weight loss and microscopic findings (hyperplasia of lymphocytes in lymph nodes, spleen, and lung, as well as perivascular inflammation in the kidney). Decreased platelet counts (at 40 mg/kg/day in the 39-week study) due to decreased production in bone marrow resulted in hemorrhage and regenerative anemia. This was recoverable. The NOAEL was the mid-dose of 25 mg/kg/day, with $AUC_{24} = 166000 \text{ ng}\cdot\text{h/mL}$ (43X MRHD). The PT review can be found in DARRTS dated 07/01/19 under IND 130277.

Genotoxicity studies were conducted in vitro (AMES assay, up to 5000 $\mu\text{g}/\text{plate}$ and chromosomal aberration assay in cultured human lymphocytes, up to 358.4 $\mu\text{g}/\text{mL} = 1$

mmol/L), and in vivo (rats, up to 300 mg/kg/day). Fezolinetant had no potential to induce gene mutation and chromosomal aberrations in vitro and in vivo.

Carcinogenicity studies were done in rats and transgenic mice. The final study reports were presented at the 10/25/22 Executive Carcinogenicity Assessment (ECAC) meeting and both studies were considered negative. Both ECAC meeting minutes and final study review are in DARRTS dated 10/27/22 and 10/28/22, respectively.

- In the **2-year carcinogenicity study in female rats** (0, 10, 30, and 100 mg/kg/day for 104 weeks), fezolinetant had increased incidences of benign follicular cell adenomas in the thyroid (common tumor, by trend analysis only, $p=0.0094$). Additionally, the combination of ovarian epithelial tumors (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma) was noted in the mid-dose group when compared to the vehicle control ($p\text{-value} = 0.0132$ for common or rare tumors), without corresponding statistically significant dose response relationship regardless of the tumor classification (rare or common). Therefore, no toxicologically significant, drug-related tumors were identified in female rats. At the high dose of 100 mg/kg/day, $AUC_{24} = 718000 \text{ ng}\cdot\text{h/mL}$ at Week 26 (186X MRHD).
- In the **6-month carcinogenicity study in transgenic mice** (0, 50, 150, and 450 mg/kg/day) in male and female transgenic mice, fezolinetant did not induce neoplasms. At the high dose of 450 mg/kg/day, $AUC_{24} = 183000 \text{ ng}\cdot\text{h/mL}$ at Week 26 (47X MRHD).

Reproductive and developmental toxicity studies were conducted in rats and rabbits.

- In the **fertility rat study** in female rats, fezolinetant did not affect fertility at doses up to 100 mg/kg/day, though there was altered estrous cyclicity and reduced reproductive organ weight, which were exaggerated pharmacological effects of fezolinetant.
- In the **embryo-fetal development toxicity rat study** (0, 25, 50, and 100 mg/kg/day by oral gavage from GD 6-17), embryo-lethality was seen at a dose of 100 mg/kg/day, with maternal $AUC_{24} = 494600 \text{ ng}\cdot\text{h/mL}$ (128X MRHD). Surviving fetuses at this dose showed no teratogenic findings (no developmental variations or abnormalities). Litter loss was regarded as an exaggerated pharmacological effect of fezolinetant (e.g., ↓ LH levels (inhibiting LH surge), ↓ estradiol levels during the follicular phase), ↓ progesterone levels during the luteal phase). The NOAEL was 100 mg/kg/day for dams. The NOAEL was 50 mg/kg/day for embryo-fetal development (with maternal $AUC_{24} = 238700 \text{ ng}\cdot\text{h/mL}$, 62X MRHD). The PT review can be found in DARRTS dated 05/13/16 under IND 130277.
- In the **embryo-fetal developmental toxicity rabbit study** (0, 45, 75, and 125 mg/kg/day by oral gavage from GD 7-19), the pregnant animals showed reduced body weight gain and food consumption at doses of fezolinetant $\geq 45 \text{ mg/kg/day}$. At 125 mg/kg/day, with maternal $AUC_{24} = 670200 \text{ ng}\cdot\text{h/mL}$ (174X MRHD), abortion or total litter loss were observed and an increase in mean percentage postimplantation loss, as well as early resorption and low gravid uterine weights, were noted. At doses of 75 or 125 mg/kg/day, fezolinetant was associated with

reduced fetal weights and a dose-dependent increase in the percentage of late resorptions. There were no fetal malformations at doses up to 125 mg/kg per day. No maternal NOAEL was identified as decreased body weight gains and food consumption were noted in all doses. The fetal NOAEL for embryo-fetal development in rabbits was 45 mg/kg/day (with maternal $AUC_{24} = 60600$ ng•h/mL, 16X MRHD). The PT review can be found in DARRTS dated 05/13/16 under IND 130277.

- In the **pre- and post-natal developmental toxicity rat study** (0, 10, 30, and 100 mg/kg/day by oral gavage from GD6 to LD21), delayed parturition and embryo-lethality were observed at 100 mg/kg/day (with maternal $AUC_{24} = 788000$ ng•h/mL, 204X MRHD). In addition, F₁ males at 100 mg/kg per day showed decreases in mating and fertility associated with incomplete balanopreputial separation. However, F₁ females did not show effects on either mating or fertility at any doses. The NOAEL for maternal toxicity and embryotoxicity was 30 mg/kg/day ($AUC_{24} = 140,000$ ng•h/mL on LD 21, 36X MRHD), based on delayed parturition and in utero embryo-lethality for 4 HD females. The NOAEL for F1 generation development was 100 mg/kg/day (F1 females, $AUC_{24} = 788000$ ng•h/mL on LD 21, 204X MRHD) and 10 mg/kg/day (F1 males, $AUC_{24} = 42500$ ng•h/mL on LD 21, 11X MRHD), based on the changes in fertility mainly due to incomplete balanopreputial separation in MD and HD males. See Section [12 Appendix/Attachments](#) for study details.

1.3 Recommendations

1.3.1 Approvability

Pharm/Tox supports an approval of fezolinetant for VMS associated with menopause.

1.3.2 Additional Nonclinical Recommendations

None.

1.3.3 Labeling

The labeling will be reviewed in a separate review.

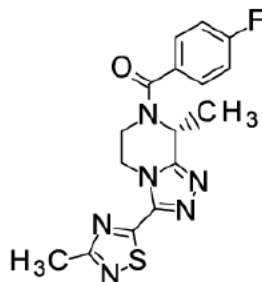
2 Drug Information

2.1 Drug

CAS Registry Number	1629229-37-3
Generic Name	Fezolinetant
Code Name	ESN364
Chemical Name	(4-Fluorophenyl)[(8R)-8-methyl-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-

Molecular Formula/Molecular Weight

dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]methanone

C₁₆H₁₅FN₆OS, 658.39Structure or Biochemical Description
Pharmacologic Class

Neurokinin 3 (NK3) receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 130277 (hot flashes), IND (b) (4) (polycystic ovarian syndrome), IND (b) (4) (uterine fibroids)

2.3 Drug Formulation

Fezolinetant tablets 30 mg are (b) (4) tablets containing 30 mg active ingredient (phase 3 tablet formulation). Fezolinetant tablets 45 mg are round light red film-coated tablets containing 45 mg active ingredient (to-be-marketed tablet formulation). Formulation compositions of the 30 and 45 mg tablets are presented in the table below.

Compositions of Fezolinetant Tablets 30 mg and 45 mg

Component	Function	Reference to Quality Standard	Quantity (mg/tablet)	
			30 mg	45 mg
Fezolinetant	Active ingredient	In-house	30.0	45.0
Mannitol	(b) (4)	USP	(b) (4)	
Hydroxypropyl Cellulose		NF		
Low-Substituted Hydroxypropyl Cellulose		NF		
Microcrystalline Cellulose		NF		
Magnesium Stearate		NF		
Total			(b) (4)	

NF: National Formulary, USP: United States Pharmacopeia, N/A: Not applicable

(b) (4)

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

No concern on impurities.

Drug substance

Impurities in fezolinetant include drug substance process-related impurities, mutagenic impurities, degradants, residual solvents, and elemental impurities. All the impurities were controlled adequately in the drug substance per ICH guidances. See [Module 3.2.S.3.2 Impurities](#).

Drug product

- **Process impurities:** Drug substance process impurities (b) (4) and the (b) (4) were monitored in three primary stability batches. All the test results confirmed that no increases of these process impurities were seen during (b) (4) and storage of fezolinetant tablets. Since these impurities were (b) (4), it is not included in the drug product specification proposed in [Module 2.3.P.5.1 Specifications](#), per ICHQ6A.
- **Degradation products:** No degradation products were observed at the level above the identification threshold during the manufacturing and storage of the drug product, per ICHQ3B(R2).
- **Mutagenic impurities:** All known mutagenic impurities of fezolinetant were controlled in the drug substance, as described in [Modules 2.3.S.2.6 Manufacturing Process Development](#) (pages 10-11) and [Module 2.3.S.3.2.2 Mutagenic Impurities](#) (pages 5-9). There were no additional impurities in the drug product which need to be assessed for mutagenicity. Hence, no further mutagenic evaluation was conducted for the drug product, per ICHM7.
- (b) (4)
(b) (4)
(b) (4).
- **Nitrosamine impurities:** There were no contamination risks of nitrosamine impurities ([Module 2.3.P.5.6.2.3 Nitrosamine Impurities](#), page 20).
- **Elemental impurities:** All the elemental impurities, considering the administration route, were sufficiently below 30% of PDE, per ICHQ3D ([Module 2.3.P.5.6.2.2 Elemental Impurities](#), page 20).

2.6 Proposed Clinical Population and Dosing Regimen

The recommended dose of fezolinetant is 45 mg orally once daily with or without food in the general population of individuals with moderate to severe VMS associated with menopause.

2.7 Regulatory Background

Date	Type of Submission	Notes
March 2016	IND submission	The PT review is in DARRTS dated 05/31/16. This PT review covered three INDs (b) (4) (uterine fibroids), (b) (4) (polycystic ovarian syndrome), and 130277 (hot flashes). The 13-week toxicity studies in rats and monkeys and embryo-fetal development studies in rats and rabbits were reviewed by Dr. Kimberly Hatfield.
March 2017	Type B EOP2 meeting request	There were nonclinical questions regarding review of repeat dose toxicity studies, submissions of SPAs for carcinogenicity studies, and evaluation of one major metabolite ES00259564. The PT memo is in DARRTS dated 03/08/17 under IND 130277.
October 2017	SPA submission for 2-year carcinogenicity rat study	Both PT review and ECAC meeting minutes are in DARRTS dated 12/05/17 under IND 130277.
December 2017	2-year SPA follow up	Sponsor's response to ECAC 12/05/17 meeting minutes with regards to the recommended doses, increased group size due to single sex, and the need for only one vehicle control group. The PT memo is in DARRTS dated 01/22/18 under IND 130277.
October 2018	SPA submission for 6-month carcinogenicity transgenic mouse study	Both PT review and ECAC meeting minutes are in DARRTS dated 11/28/18 and 11/29/18, respectively. Another PT memo addressing the final agreed SPA is in DARRTS dated 02/11/19 under IND 130277.
April 2019	Type B EOP2 meeting	There were nonclinical questions with regards to their nonclinical program. The Sponsor was requested to address the PD/PK profile of the major metabolite, ES00259564, and to submit the 39-week monkey study report for review prior to the initiation of the Phase 3 clinical trial. The PT review is in DARRTS dated 04/26/19 and the meeting minutes is in DARRTS also dated 05/16/19 under IND 130277.
May 2019	Submission of the 26-week rat and 39-week monkey studies	The Sponsor submitted both 26-week rat and 39-week monkey studies for review. The Division agreed that main human metabolite was adequately addressed in these studies and these studies supported the proposed duration of the Phase 3 clinical trial. The PT review is in DARRTS dated 07/01/19 under IND 130277.
October 2021	Submission of Seg III repro rat study and carcinogenicity studies	The Sponsor submitted carcinogenicity study reports and pre- and post-natal developmental study report under IND 130277 eCTD 0115. The carcinogenicity studies and the pre-postnatal developmental study are being reviewed under the current NDA submission.
January 2022	Type B pre-NDA meeting	The Agency agreed that the completed nonclinical studies submitted in the briefing package appeared to be adequate to submit the NDA. New nonclinical (non-pivotal) studies discussed in the briefing package that were not previously reviewed will also be discussed in the NDA review. The PT review is in DARRTS dated 01/31/22 and the meeting minutes is in DARRTS also dated 02/24/22 under IND 130277.

3 Studies Submitted

3.1 Studies Reviewed

See Tables below. Highlighted studies (in gray) were not submitted previously under INDs but included in this NDA review cycle. All other studies have been previously submitted under IND 130277.

Pharmacology

Primary PD Studies					
Study Title	Previously Submitted	Previously Reviewed*	Test System	Study No.	Testing Facility
In Vitro Cell-Based Functional Assay on Human Neurokinin 3 Receptor – Agonist and Antagonist activity of ESN-364 on the Human NK3 Receptor.	IND 130277 eCTD 0048	Yes	In vitro	ESN364-AEQ-001	Ogeda SA
In Vitro Radioligand Assay on Cloned Human Neurokinin 3 Receptor - Binding Affinity of ESN-364 at the human NK3 receptor	IND 130277 eCTD 0048	Yes	In vitro	ESN364-RBL-001	Ogeda SA
In Vitro Radioligand Assay on cloned Monkey and Rat Neurokinin 3 Receptor Orthologs – Binding Affinity of ESN-364 at the monkey and rat NK3 receptors	IND 130277 eCTD 0048	Yes	In vitro	ESN364-RBL-002	Ogeda SA
In Vitro Target selectivity Assay on Human NK1 and NK2 Receptors - Binding Affinity of ESN-364 at the cloned human NK1 and NK2 Receptors	IND 130277 eCTD 0048	Yes	In vitro	ESN364-RBL-003	Ogeda SA
In Vitro Target selectivity Assay on GnRH Axis – Binding Affinity of ESN-364 at the cloned human GnRH, GPR54, NPFF1, NPFF2 and KOP (OP2) Receptors	IND 130277 eCTD 0048	Yes	In vitro	ESN364-RBL-004	Ogeda SA
In Vitro Radioligand Assay on Cloned Human, Rat and Monkey Neurokinin 3 Receptors - Binding Affinity of ESN-564 (ES259564), the metabolite of ESN-364, at the human, rat and monkey NK3 receptors	IND 130277 eCTD 0048	Yes	In vitro	ESN364-RBL-005	Ogeda SA
Effects of ESN364 on Activation of c-fos Expression in Median Preoptic Nucleus Neurons in Ovariectomized Rats	IND NON-RESPONSIVE eCTD 0055	Yes	Rat (oral)	2693-PH-0001	Astellas Pharma Inc.
Effect of ESN364 on Hot Flash like Symptoms and Plasma Levels of Estradiol, Luteinizing Hormone, and Follicle-stimulating Hormone in Ovariectomized Rats	IND NON-RESPONSIVE eCTD 0055	Yes	Rat (oral)	2693 PH 0002	Astellas Pharma Inc.

*PT reviews can be found in DARRTS dated 09/21/16 under IND NON-RESPONSIVE and 04/04/17 under IND NON-RESPONSIVE. However, **only summary was provided in these reviews. It is unclear if the actual study reports were reviewed.**

Secondary PD Studies					
Study Title	Previously Submitted	Previously Reviewed*	Test System	Study No.	Testing Facility
Pharmacokinetics Pharmacodynamics	IND 130277 eCTD 0048	No	In Vivo	ESN364-gnrh001	Ogeda SA

ESN-364 Effect on Circulating LH Levels in Castrate Male Rats Following Single Oral Administration					
Efficacy Study ESN-364 Effect on Pulsatile LH Secretion and FSH Levels in Ovariectomized Female Rats Following Single Oral Administration	IND 130277 eCTD 0048	No	In Vivo	ESN364-gnrh047	Ogeda SA
Pharmacokinetics-Pharmacodynamics ESN-364 Impact on Estradiol Levels throughout the Estrus Cycle in Female Rats Following Chronic Oral Administration for 10 Days	IND 130277 eCTD 0048	No	In Vivo	ESN364-chronic010	Ogeda SA
Pharmacokinetic Study of ESN364 (ES256364), (b) (4) and NON-RESPONSIVE Following Intravenous and Oral Administration in Castrated Cynomolgus Monkey	IND 130277 eCTD 0048	No	Monkey (IV and oral)	49404-17-147	(b) (4)
In Vitro Pharmacology and ADME-Tox: Study of ES00256364	IND 130277 eCTD 0048	No	In vitro	10002171	
In Vitro Pharmacology and ADME-Tox: Study of Two Compounds	IND 130277 eCTD 0048	No	In Vitro	100018051	

*There is no documentation of these studies being reviewed.

Safety Pharmacology Studies					
Study Title	Previously Submitted	Previously Reviewed*	Test System	Study No.	Testing Facility
ESN-364: Electrocardiographic investigations using radiotelemetry in the conscious cynomolgus monkey following oral (gavage) administration	IND 130277 Opening IND, eCTD 0048	No	Monkey (oral)	8276021	(b) (4)
ESN-364: Effects on General Activity, Behavior and Body Temperature in the Rat Following Oral Administration	IND 130277 Opening IND, eCTD 0048	No	Rat (oral)	8276186	
ESN-364: Measurement of Respiratory Parameters in the Freely Moving Conscious Rat Using Whole Body Plethysmography	IND 130277 Opening IND, eCTD 0048	No	Rat (oral)	8276187	
Evaluation of the effect of (b) (4) and ES-256364 on cardiac electrophysiological and mechanical parameters of isolated rabbit hearts.	IND 130277 eCTD 0048	No	Ex vivo (rabbit)	20120123-1	
Evaluation of the effect of ESN-364 on the human delayed rectifier current using human embryonic kidney (HEK) 293 cells transfected with the human ether-a-gogo- related gene (hERG)	IND 130277 eCTD 0048	No	In vitro	20121203-1	
ESN-364: Effects on hERG Tail Current Recorded from Stably Transfected HEK-293 Cells	IND 130277 eCTD 00115	No	In vitro	8447894	

*There is no documentation of these studies being reviewed.

Pharmacokinetics

PK Studies: Absorption					
Study Title	Previously Submitted	Previously Reviewed*	Test System	Study No.	Testing Facility
Blood and Plasma Concentrations and Excretion after a Single Oral	IND NON-RESPONSIVE eCTD 0055	No	Monkey (oral)	2693-me-0014	(b) (4)

Administration of [¹⁴ C]ESN364 to Cynomolgus Monkeys					
ESN-364: Pharmacokinetic Study by Intravenous (Bolus) and Oral Administration to Female Rats	--	No	Rat (IV and oral)	2693-me-0020	(b) (4)
ESN-364: Pharmacokinetic Study by Intravenous (Bolus) and Oral Administration to Female Cynomolgus Monkeys	--	No	Monkey (IV and oral)	2693-me-0021	

*There is no documentation of these studies being reviewed.

PK Studies: Distribution					
Study Title	Previously Submitted	Previously Reviewed*	Test System	Study No.	Testing Facility
[¹⁴ C]ESN364: Tissue Distribution and Excretion of Radioactivity in the Rat	IND 130277 eCTD 0048	No	Rat (oral)	8355672	(b) (4)
In Vitro Plasma Protein Binding of ESN364 in Mice, Rats, Rabbits, Cynomolgus Monkeys and Humans	IND 130277 eCTD 0055	No	In vitro	2693-me-0012	
Transfer of Radioactivity into Fetuses and Breast Milk in Rats after a Single Oral Administration of [¹⁴ C]ESN364	IND 130277 eCTD 00115	No	Rat (oral)	2693-me-0019	

*There is no documentation of these studies being reviewed.

PK Studies: Metabolism					
Study Title	Previously Submitted	Previously Reviewed*	Test System	Study No.	Testing Facility
Hepatocyte Stability of Test Agent	IND 130277 eCTD 0048	No	In vitro	Cyp0720-r1	(b) (4)
Quantitative Metabolite Profiling After a Single Oral Administration of [¹⁴ C]ESN364 to Rats and Humans	IND 130277 eCTD 0055	No	Rat, human (oral)	2693-me-006	
Investigation of Chiral Inversion of the Unchanged Drug after Oral Administration of ESN364 to Rats, Cynomolgus Monkeys, and Humans	IND 130277 eCTD 00115	No	Rat, monkey, human (oral)	2693-me-0015	
Quantitative Metabolite Profiling after a Single Oral Administration of [¹⁴ C]ESN364 to Rats and Cynomolgus Monkeys	IND 130277 eCTD 00115	No	Rat, monkey (oral)	2693-me-0017	

*There is no documentation of these studies being reviewed.

PK Studies: Excretion					
Study Title	Previously Submitted	Previously Reviewed*	Test System	Study No.	Testing Facility
Excretion after a Single Oral Administration of [¹⁴ C]ESN364 to Rats	IND 130277 eCTD 0055	No	Rat (oral)	2693-me-0013	(b) (4)

*There is no documentation of these studies being reviewed.

Toxicology

General Toxicology

Repeat-Dose Toxicity Studies					
Study Title	Previously Submitted	Previously Reviewed	Test System	Study No.	Testing Facility (b) (4)
ESN-364: Maximum tolerated dose (MTD) followed by a 14-day fixed dose oral (gavage) administration toxicity study in the cynomolgus monkey	--	No [§]	Monkey (oral)	8276019	
ESN-364: 5-week oral (gavage) administration toxicity study in the cynomolgus monkey with a 4-week recovery phase	IND 130277 Opening IND, eCTD 0048	No [§]	Monkey (oral)	8276020	
ESN 364: 7 Day Oral (Gavage) Administration Range-finding Study in the Rat	--	No [§]	Rat (oral)	8276178	
ESN-364: 28 Day Oral (Gavage) Administration Toxicity Study in the Rat with Micronucleus Assessment	IND 130277 Opening IND, eCTD 0028	No [§]	Rat (oral)	8276179	
ESN-364: 13 Week Oral (Gavage) Administration Toxicity Study in the Rat	IND 130277 Opening IND, eCTD 0048	Yes* **	Rat (oral)	8297361	
ESN-364: 13-week oral (gavage) administration toxicity study in the female cynomolgus monkey with a 4-week recovery phase	IND 130277 Opening IND, eCTD 0048	Yes*	Rat (oral)	8297388	
ESN364: 39-week oral (gavage) administration toxicity study in the sexually mature female cynomolgus monkey with a 4-week recovery phase	IND 130277 eCTD 0040	Yes***	Monkey (oral)	8331280	
ESN364: 26-Week Oral (Gavage) Toxicity Study in Female Rats	IND 130277 eCTD 0040	Yes** ***	Rat (oral)	8331724	

[§] There is no documentation of these studies being reviewed.

* PT reviews under IND 130277 in DARRTS dated 05/31/16.

** PT (SPA) review under IND NON-RESPONSIVE in DARRTS dated 12/05/17 when reviewing the 2-year rat SPA.

*** PT review under IND NON-RESPONSIVE in DARRTS dated 07/01/19.

Genotoxicity

Genotoxicity Studies					
Study Title	Previously Submitted	Previously Reviewed	Test System	Study No.	Testing Facility (b) (4)
Reverse mutation in five histidine-requiring strains of Salmonella typhimurium	IND 130277 Opening IND, eCTD 0028	Yes*	In vitro	8276182	
Induction of chromosome aberrations in cultured human peripheral blood lymphocytes	IND 130277 Opening IND, eCTD 0028	Yes*	In vitro	8276184	
ESN-364: 28 Day Oral (Gavage) Administration Toxicity Study in the Rat with Micronucleus Assessment	IND 130277 Opening IND, eCTD 0028	Yes*	Rat (oral)	8276179 **	

* PT reviews under IND 130277 in DARRTS dated 05/31/16. All genotoxicity studies were determined to be "negative" in the review.

** Analysis was conducted in the 4-week repeat toxicity study in rats (Study No. 826179)

Carcinogenicity

Carcinogenicity Studies					
Study Title	Previously Submitted	Previously Reviewed	Test System	Study No.	Testing Facility
104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with ESN364 in Female Wistar Han Rats	IND 130277 eCTD 0115 and eCTD 0142 (tumor.xpt file)	No	Rat (oral)	8375967	(b) (4)
A 1-Week Oral Gavage Toxicity and Toxicokinetic Study with ESN364 in Mice	IND 130277 eCTD 0028	Yes*	Mouse (oral)	8375968	
A 4-Week Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study with ESN364 in 001178-W (Wild Type) RasH2 Mice	IND 130277 eCTD 0028	Yes*	Wild-type RasH2 mouse (oral)	8378969	
A 26-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with ESN364 in 001178-T (Hemizygous) and 001178-W (Wild Type) RasH2 mice	IND 130277 eCTD 0115 and eCTD 0142 (tumor.xpt file)	No	Transgenic mouse (oral)	8375970	

* PT (SPA) review under IND NON-RESPONSIVE in DARRTS dated 11/28/18 when reviewing the 6-month tg mouse SPA.

Reproductive and Developmental Toxicity

Fertility and Early Embryonic Developmental Studies (Segment I)					
Study Title	Previously Submitted	Previously Reviewed	Test System	Study No.	Testing Facility
Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with ESN-364 in Female Rats	IND 130277 eCTD 0048	No	Rat (oral)	8331725	(b) (4)

Embryo-Fetal Developmental Studies (Segment II)					
Study Title	Previously Submitted	Previously Reviewed	Test System	Study No.	Testing Facility
ESN-364: Oral (Gavage) Range-Finding study of Embryo-Fetal Development in the Rabbit	--	No	Rabbit (oral)	8297363	(b) (4)
ESN-364: Oral (Gavage) Study of Embryo-Fetal Development in the Rabbit	IND 130277 Opening IND, eCTD 0048	Yes*	Rabbit (oral)	8297364	
ESN-364: Oral (Gavage) Range-Finding Study of Embryo-Fetal Development in the Rat	--	No	Rat (oral)	8297365	
ESN-364: Oral (Gavage) Study of Embryo-Fetal Development in the Rat	IND 130277 Opening IND, eCTD 0048	Yes*	Rat (oral)	8297366	

* PT reviews under IND 130277 in DARRTS dated 05/31/16.

Pre- and postnatal Developmental Studies (Segment III)					
Study Title	Previously Submitted	Previously Reviewed	Test System	Study No.	Testing Facility
ESN-364: Oral (Gavage) Dose Range Finding Study of Pre- and Postnatal Development in the Rat	IND 130277 eCTD 0055	No	Rat (oral)	8381968	(b) (4)
ESN-364: Oral (Gavage) Study of Pre- and Postnatal Development in the Rat	IND 130277 eCTD 0115	No	Rat (oral)	8382261	

Others

Others

Other Toxicity Studies					
Study Title	Previously Submitted	Previously Reviewed	Test System	Study No.	Testing Facility
Mechanistic					
EEG Assessment of ESN364 Administered by Oral Gavage to Female Wistar Rats	IND 130277 Opening IND, eCTD 0048	Yes*	Rat (oral)	8306209	(b) (4)
Dependence					
ESN-364: Bioanalysis, Clinical Observations, and Formulation Assessments in Support of Drug Abuse Potential Testing Using the Self-Administration Model	IND 130277 eCTD 0115	No	Rat (IV bolus)	8416427	(b) (4)
ESN-364: Investigation of the Potential to Induce Physical Dependency in the Rat following 4 Weeks of Oral Administration	IND 130277 eCTD 0115	No	Rat (oral)	8416428	
ESN-364: Assessment of Potential Self-Administration in Rats	IND 130277 eCTD 0115	No	Rat (IV bolus)	8416429	
Metabolite: Genotoxicity					
ES259564: In Vitro Human Lymphocyte Chromosome Aberration Assay	IND 130277 eCTD 0029	No	In vitro	8390040	(b) (4)
ES259564: Bacterial Reverse Mutation Assay	IND NON-RESPONSIVE eCTD 0029	No	In Vitro	8390041	
Impurities					
Assessment of DNA Reactive (Mutagenic) Impurities in Fezolinetant	--	No	In Vitro	210013	Astellas Pharma Inc. (b) (4)
A 13-week Repeated Oral Dose Toxicity Study of (b) (4) (impurity of ESN364) in Female Cynomolgus Monkeys	IND 130277 eCTD 0115	No	Monkey (oral)	501-158	
Phototoxicity					
ESN-364: In Vitro Balb/c 3T3 Neutral Red Uptake Phototoxicity Assay	IND 130277 eCTD 0029	No	In vitro	8390039	(b) (4)

* PT reviews under IND 130277 in DARRTS dated 05/31/16.

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Review	Reviewer	DARRTS	Notes
Under IND 130277			
PT review under IND (b) (4) 130277	Kim Hatfield	05/31/16	The 28-day rat study, 13-week rat study and 13-week monkey study, embryofetal rat and rabbit studies, genotoxicity studies were submitted.
PT memo	Miyun Tsai-Turton	03/08/17	EOP 2
SPA review	Miyun Tsai-Turton	12/05/17	2-year rat carcinogenicity study SPA
PT memo	Miyun Tsai-Turton	01/22/18	2-year rat SPA
SPA review	Miyun Tsai-Turton	11/28/18	6-month transgenic mouse carcinogenicity study SPA
PT memo	Miyun Tsai-Turton	02/11/19	6-month tg mouse SPA

PT review	Miyun Tsai-Turton	07/01/19	The 26-week rat and 39-week monkey studies were reviewed.
PT memo	Miyun Tsai-Turton	01/31/22	Pre-NDA
Under NDA 216578			
Carcinogenicity studies	Miyun Tsai-Turton	10/28/22	ECAC meeting was held on 10/25/22.

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action

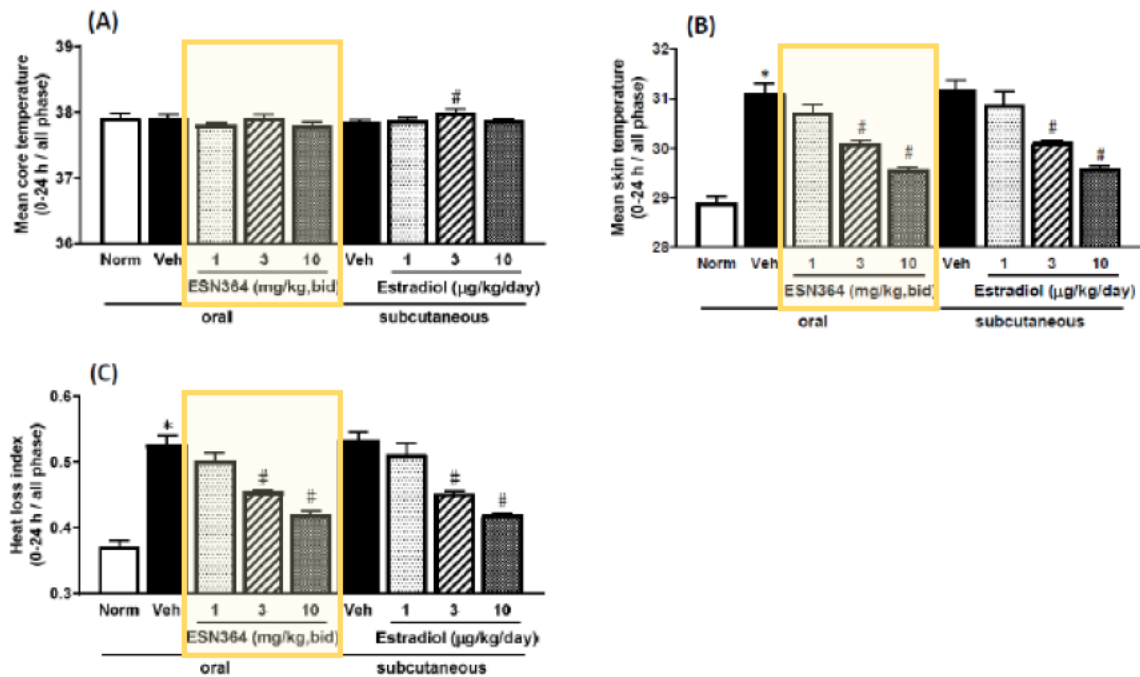
Fezolinetant blocks neurokinin B (NKB)/NK3 binding at the KND γ (kisspeptin, neurokinin B, and dynorphin) neuron to modulate neuronal activity in the thermoregulatory center in the hypothalamus.

Primary PD Studies		
Study No.	Study Aim	Findings
ESN364-RBL-001 hNK3R	In vitro radioligand assay on cloned human neurokinin 3 receptor (i.e., hNK3R)	Fezolinetant fully displaced the specific binding of [^{3} H]-SB222200 (neurokinin 3 receptor ligand) to the hNK3 receptor in a concentration dependent manner, with a K_i = 19.9 - 22.1 nmol/L.
ESN364-RBL-002 mNK3R and rNK3R	In vitro radioligand assay on cloned monkey and rat neurokinin 3 receptor orthologs (i.e., mNK3R and rNK3R)	Fezolinetant had a similar affinity for the hNK3 and mNK3 receptors and lesser potency for the rNK3 receptor. - Fezolinetant displaced the specific binding of [^{3}H]-SB222200 to the mNK3 receptor in a concentration dependent manner (K_i = 26.9 - 34.7 nmol/L). - Fezolinetant also displaced the specific binding to [^{125}I]His, MePhe7-neurokinin B to the rNK3 receptor in a concentration dependent manner (K_i = 183 - 379 nmol/L).
ESN364-RBL-003 hNK1R and hNK2R	In vitro target selectivity assay on human NK1 and NK2 receptors	Fezolinetant had poor affinity binding for both hNK1 and hNK2 receptors. - Fezolinetant (up to 30 μM) failed to displace > 50% of the specific binding to the radioligands, [^{3}H]-Substance P and [^{125}I]-Neurokinin A, at the hNK1 and hNK2 receptors, respectively. - Fezolinetant had > 450X selectivity for the hNK3 receptor in comparison to hNK1 and hNK2 receptors (K_i > 10 μM).
ESN364-RBL-004 GnRH axis (off target receptors)	In vitro target selectivity assay on GnRH axis	Fezolinetant had poor affinity binding for GnRH axis-related, off-target receptors. Fezolinetant (30 μ M) displaced less than 50% of the binding for G-protein coupled receptors, such as GPR54 (kisspeptin neuropeptide), NPFF1 and NPFF2 (neuropeptide FF; FLFQPQRFa), or KOP (k-opioid), and 53% for GnRH (gonadotropin-releasing hormone), with K_i > 10 μ M.
ESN364-AEQ-001 As an antagonist	In vitro cell-based functional assay on human neurokinin 3 receptor	Fezolinetant was a full and potent antagonist on hNK3 receptor and had no agonist activity on this receptor. Fezolinetant fully inhibited neurokinin A at hNK3 with IC_{50} = 12.9 - 22.0 nmol/L.

ESN364-RBL-005 metabolite	In vitro radioligand assay of ES00259564, the metabolite of fezolinetant	The affinity of the ES00259564 metabolite to the three tested orthologue NK3 receptors was approximately 20X weaker than affinity of fezolinetant, with $K_i = 372 - 431$ nmol/L for hNK3, 4617 - 4683 nmol/L for rNK3, and 385 - 578 nmol/L for mNK3.
2693-PH-0002 Rat, oral administration @ 1, 3, 10 mg/kg fezolinetant (bid) for 8 days	Effects of fezolinetant on hot flash-like symptoms in ovariectomized (OVX) rats	OVX rats showed a significant decrease in plasma estradiol level and increases in plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels. These OVX rats also exhibited hot flash-like symptoms, with a significantly increased tail skin temperature but no effect on core temperature was exhibited (Figure 1 and Figure 2). Fezolinetant (3 and 10 mg/kg, bid, po) given for 8 days significantly reduced plasma LH levels, and decreased these increases in tail skin temperature without affecting core temperature, suggesting that fezolinetant attenuated hot flash-like symptoms in OVX rats (Figure 1 and Figure 2).
2693-PH-0001 Rat, oral administration @ 10 mg/kg fezolinetant (bid) for 7 days	Effects of fezolinetant on activation of c-fos expression in MnPO neurons in ovariectomized rats	OVX rats showed a significant increase in c-fos expression (a marker of neuronal activity) in MnPO neurons in the brain (Figure 3). Fezolinetant (10 mg/kg, bid, po) given for 7 days significantly inhibited the c-fos expression in MnPO neurons (Figure 3).

Figure 1 PD Study No. 2693-PH-0002. Effect of Fezolinetant on Hot Flash-like Symptoms in OVX Rats

Effect of Fezolinetant on Core (A), Tail Skin (B) Temperature and Heat Loss Index (C) in OVX Rats



Data represent the mean $\pm$ SEM of 7 animals in 1 and 3 $\mu\text{g/kg/day}$ estradiol groups, and 8 animals in other groups. Fezolinetant (ESN364), estradiol or vehicles were administered for 8 days. Heat loss index was calculated using the following formula: $(\text{tail skin temperature} - \text{room temperature}) / (\text{core temperature} - \text{room temperature})$.

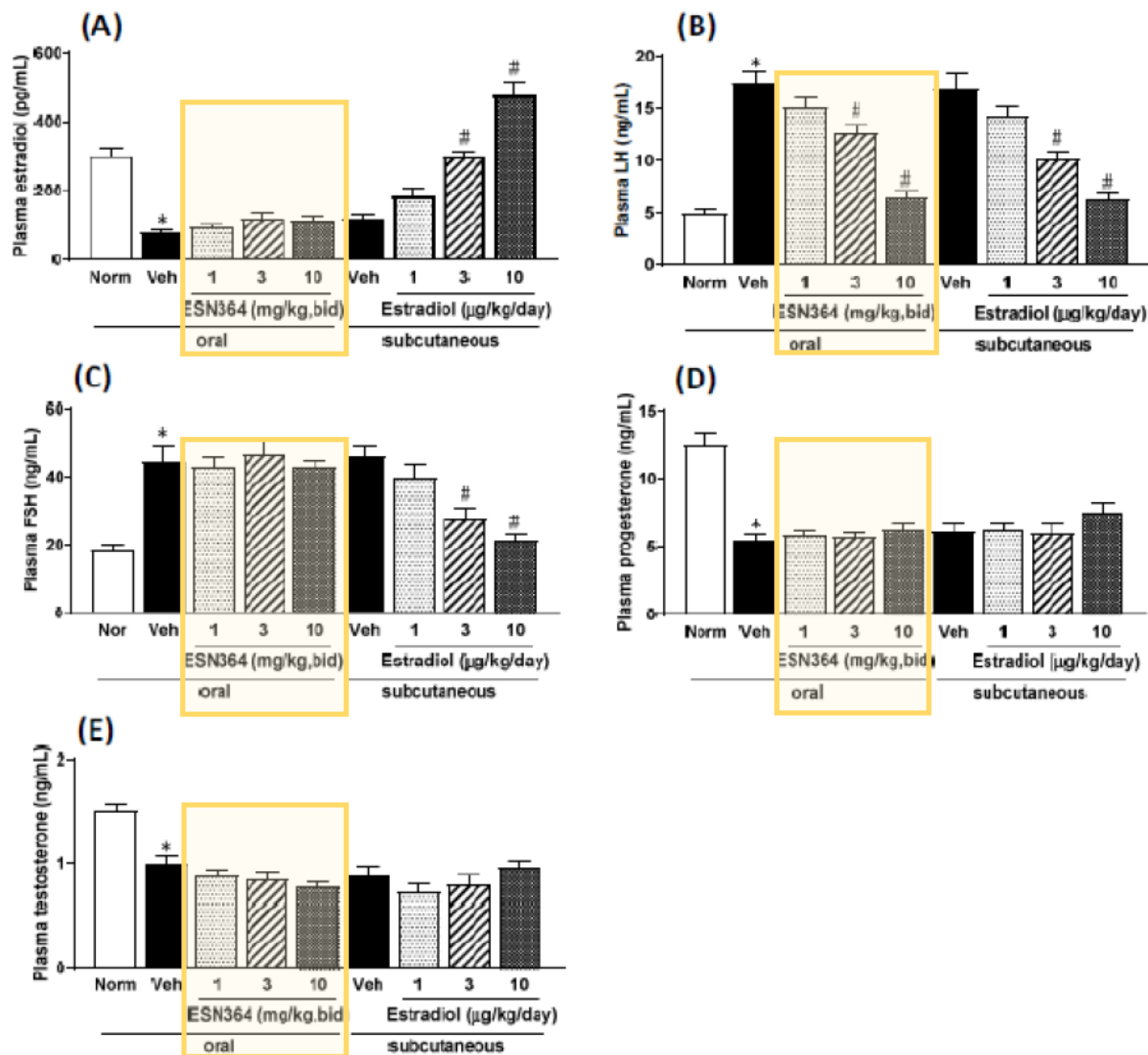
* $P < 0.05$ vs normal group (Student's t-test), # $P < 0.05$ vs vehicle group (Dunnett's multiple comparison test).

ESN364: fezolinetant; Norm: normal; Veh: vehicle

Source: [Study 2693-PH-0002](#)

Figure 2 PD Study No. 2693-PH-0002. Effect of Fezolinetant on Hormones in OVX Rats

Effect of Fezolinetant on Plasma Levels of (A) Estradiol, (B) LH, (C) FSH, (D) Progesterone and (E) Testosterone in OVX Rats



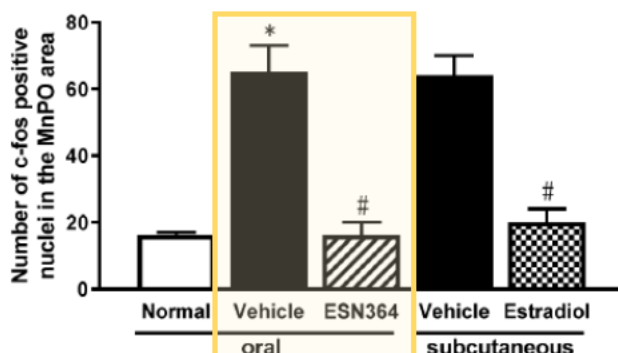
Data represent the mean $\pm$ SEM of 8 animals per group. Fezolinetant (ESN364), estradiol or vehicles were administered for 8 days. * $P < 0.05$ vs normal group (Student's t-test), # $P < 0.05$ vs vehicle group (Dunnnett's multiple comparison test).

ESN364: fezolinetant; FSH: follicle stimulating hormone; LH: luteinizing hormone; Norm: normal; Veh: vehicle

Source: [Study 2693-PH-0002](#)

Figure 3 PD Study No. 2693-PH-0001. Effect of Fezolinetant on c-fos Expression in OVX Rats

Effect of Fezolinetant on the Number of c-fos-positive Nuclei in the MnPO Area of OVX Rats



Data represent the mean $\pm$ SEM of 5 animals in the subcutaneous vehicle group and 6 animals in other groups. Fezolinetant (ESN364; 10 mg/kg, bid, po), estradiol (3 μ g/kg/day, sc) or vehicles were administered for 1 week.

* $P < 0.05$ vs normal group (Student's t-test), # $P < 0.05$ vs vehicle group (Student's t-test).

ESN364: fezolinetant; MnPO: median preoptic nucleus.

Source: [Study 2693-PH-0001](#)

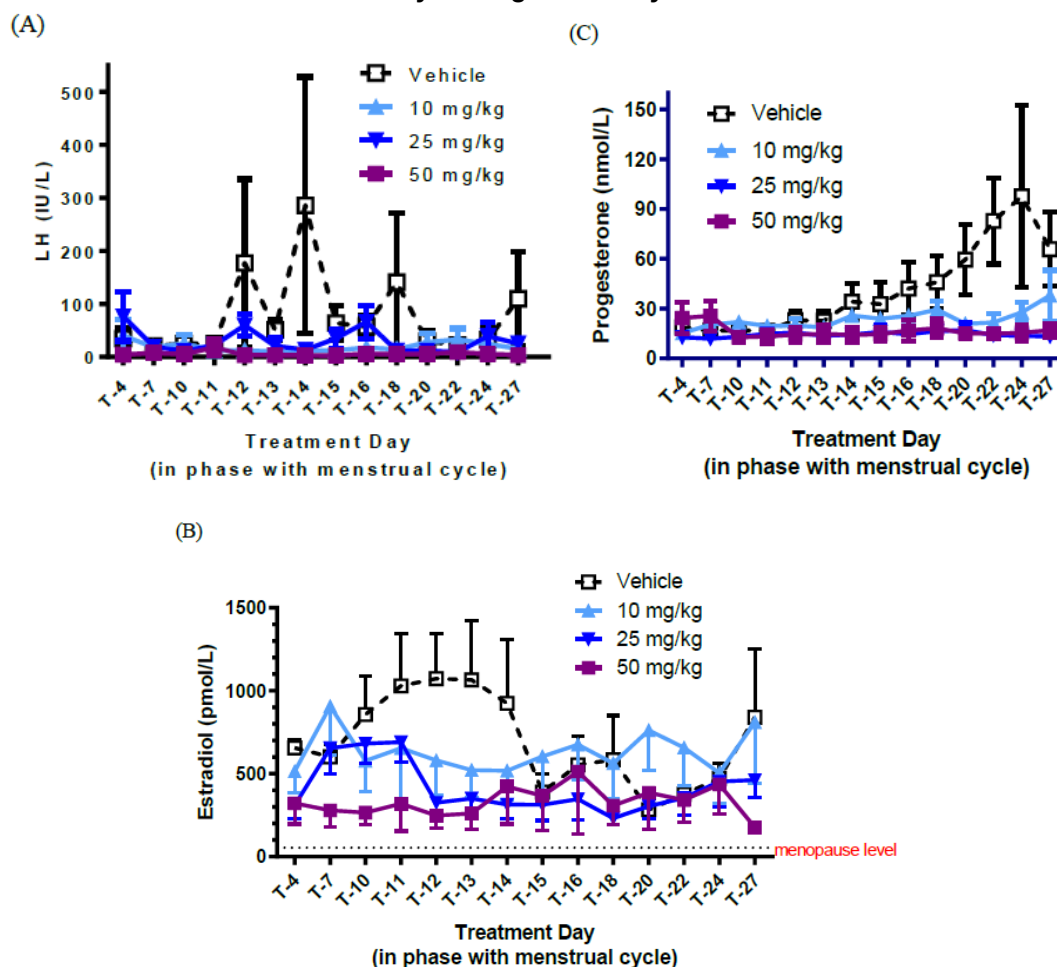
4.2 Secondary Pharmacology

Secondary PD Studies		
Study No	Study Aim	Findings
10002171 In vitro	In vitro target selectivity assay on broad target classes (including other G protein-coupled receptors)	Fezolinetant had selective activity for hNK3 in comparison to all other target classes tested in the screen: 48 receptors, 6 ion channels, 3 transporters and 48 enzymes.
100018051 In vitro	Pharmacological characterization of the major metabolite ES00259564	ES00259564 had no significant off-target activities (as tested on 45 receptors, 7 ion channels, 3 transporters), with biological activity only observed at hNK3.
ESN364-gnrh001 Rat, single dose oral administration @ 0.8, 3, 10, and 20 mg/kg fezolinetant	Fezolinetant effect on circulating LH levels in castrated male rats	Fezolinetant at ≥ 3 mg/kg resulted in a significant decrease in plasma LH of castrated male rats.
ESN364-gnrh047 Rat, single dose oral administration @ 10 mg/kg fezolinetant	Fezolinetant effect on pulsatile LH secretion in ovariectomized female rats	Fezolinetant at 10 mg/kg significantly decreased mean plasma LH levels and pulsatile LH secretion but induced no change in circulating FSH level in OVX female rats.
ESN364-chronic010 Rat, oral administration @ 10 mg/kg fezolinetant (bid) for 10 days	Fezolinetant effect on circulating estradiol level in intact female rats	Fezolinetant at 10 mg/kg/day given twice daily for 10 days decreased estradiol levels and prolonged the estrous cycle in female rats.
49404-17-147 Monkey, single dose oral @ 1, 3, 10 mg/kg fezolinetant,	Fezolinetant effect on circulating LH level in castrated male monkeys	Single dose of fezolinetant via oral or IV administration decreased LH levels in castrated male monkeys.

single dose intravenous (IV) @ 1 mg/kg fezolinetant		
8276020 Monkey, oral @ 0, 10, 25, and 50 mg/kg fezolinetant for 5 weeks with 4-week recovery	Fezolinetant effect on hormonal changes in female monkeys, as evaluated as a part of the 5-week oral tox study.	In females, fezolinetant decreased serum LH levels (including inhibition of the LH surge), decreased estradiol levels during the follicular phase (but not to menopausal levels observed in female monkeys based on historical data), and decreased progesterone levels during the luteal phase. These changes were reversible (4-week after treatment) (Figure 4). In males, fezolinetant decreased LH and testosterone levels at 25 and 50 mg/kg in Weeks 1 and 2.

Figure 4 PD Study No. 8276020. Effect of Fezolinetant on Hormones in Monkeys

Effect of Fezolinetant on LH (A), Estradiol (B) and Progesterone (C) Levels in Intact Female Cynomolgus Monkeys



Figures were prepared by the data in Table 14.14 in Study 8276020. Data represent the mean $\pm$ SD of 4 animals.

LH: luteinizing hormone.

Source: [Study 8276020](#)

4.3 Safety Pharmacology

Safety Pharmacology Studies	
Study No. & Design	Study Findings
8276186 (GLP) CNS, rat, oral gavage @ 0, 25, 125, and 250 mg/kg fezolinetant <u>Endpoints:</u> General activity, behavior, and body temperature	<u>Clinical signs:</u> @ MD: Constricted pupils in 2 males and 2 females. @ HD: Constricted pupils. Signs mostly associated with sedation, including decreased activity, touch escape response and grip strength, decreased visual placing (less frequently), startle response and alertness (less frequently). These findings resolved by 24 hours post dose. No effect on body temperature.

	NOAEL = 25 mg/kg/day (85X MRHD), based on C_{max} = 39100 ng/mL at 30 mg/kg on Day 1, obtained from the 4-week rat study (Study No. 8276179), when compared to human C_{max} = 458 ng/mL at 45 mg QD, obtained from Study No. 2693-PK-0010. <u>Note:</u> The sedation-like CNS findings (e.g., reduced activity) were noted at 300 mg/kg/day in the 4-week rat study and at 30 mg/kg/day in the 13-week rat study (60X MRHD, based on C_{max} = 27400 ng/mL at 30 mg/kg on Day 1 in the 13-week rat study). However, the CNS findings were not observed in 5, 13, and 39-week monkey studies up to 50 mg/kg/day (67X MRHD, based on C_{max} = 30900 ng/mL at 50 mg/kg on Day 1 in the 13-week monkey study).
20120123-1 (GLP) CV, in vitro hERG assay (3 - 300 μmol/L) <u>Endpoint:</u> IC_{50} of hERG current blockade	IC_{50} could not be calculated, as the highest concentration tested (300 μmol/L) had only 43% inhibition rate.
8447894 (GLP) CV, in vitro hERG assay (10 - 837.1 μmol/L) <u>Endpoint:</u> IC_{50} of hERG current blockade	IC_{50} = 231.8 μmol/L (83074.8 ng/mL, 371X MRHD), based on $C_{max, unbound}$ = 224 ng/mL at 45 mg QD, obtained from Study No. 2693-PK-0010.
8276021 (GLP) CV, monkey, oral gavage @ 0, 10, 25, and 50 mg/kg fezolinetant (single dose ascending with 7-day drug withdrawal intervals) <u>Endpoints:</u> General activity, body temperature, blood pressure, heart rate and ECG.	@ All doses: Decreased $+dP/dt_{max}$ and systolic and mean atrial blood pressure, increased heart rate. However, these changes were slight and without statistical significance, hence not considered serious effect on the CV system. No effect on ECG parameters or body temperature. NOAEL = 50 mg/kg/day (59X MRHD), based on C_{max} = 26800 ng/mL at 50 mg/kg on Day 1, obtained from the 5-week monkey study (Study No. 8276020), when compared to human C_{max} = 458 ng/mL at 45 mg QD, obtained from Study No. 2693-PK-0010.
8276187 (GLP) Respiratory, rat, oral gavage @ 0, 40, 80, and 125 mg/kg fezolinetant <u>Endpoints:</u> Tidal volume, minute volume and rate of respiration	@ All doses: Decreased tidal volume and increased respiratory rate and minute volume. NOAEL < 40 mg/kg/day (85X MRHD), based on C_{max} = 39100 ng/mL at 30 mg/kg on Day 1, obtained from the 4-week rat study (Study No. 8276176), when compared to human C_{max} = 458 ng/mL at 45 mg QD, obtained from Study No. 2693-PK-0010. The mechanism underlying these effects is unclear, but no respiratory-related nonclinical findings (in repeat dose rat and monkey studies) nor clinical findings were noted.
20121203-1 (non-GLP) CV, in vitro (3 – 30 μmol/L), isolated rabbit heart <u>Endpoints:</u> Electrophysiological and mechanical parameters	No direct effects on the heart. @ 10 μmol/L: Prolonged RR interval (slowing down of the heart rate) without arrhythmic episode, not concentration dependent.

	NOAEL = 30 μmol/L (10800 ng/mL, 48X MRHD) , based on $C_{max, unbound}$ = 224 ng/mL at 45 mg QD, obtained from Study No. 2693-PK-0010.
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5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

PK Studies: Absorption	
Study No & Design	Study Findings
2693-me-0020 Rat, oral gavage @ 1, 3, 10 mg/kg and IV @ 1 mg/kg fezolinetant, single dose	After a single IV dose of fezolinetant to rats, the plasma concentration of fezolinetant and the ES00259564 metabolite decreased with $t_{1/2}$ = 5.89 and 6.34 hours, respectively. The CL and V_{ss} of fezolinetant = 132 mL/h/kg and 1010 mL/kg, respectively (Table 1). After a single oral dose of fezolinetant to rats, the t_{max} of fezolinetant and ES00259564 was 0.5 and 2.0 hours, respectively. The C_{max} and AUC_{last} of fezolinetant and ES00259564 increased almost dose-proportionally (Table 2). The BA of fezolinetant was 91.9% to 115%.
2693-me-0021 Monkey, oral gavage @ 1, 3, 10 mg/kg and IV @ 1 mg/kg fezolinetant, single dose	After a single IV dose of fezolinetant to monkeys, the plasma concentration of fezolinetant and the ES00259564 metabolite decreased with $t_{1/2}$ of 3.50 and 2.72 hours, respectively. The CL and V_{ss} of fezolinetant were 262 mL/h/kg and 935 mL/kg, respectively (Table 1). After a single oral dose of fezolinetant to monkeys, the t_{max} of fezolinetant and ES00259564 was 1.00 to 2.67 and 1.67 to 2.67 hours, respectively. The C_{max} and AUC_{last} of fezolinetant and ES00259564 increased almost dose-proportionally (Table 2). The BA of fezolinetant was 74.6% to 103%
2693-me-0014 Monkey, oral gavage, single dose of 3 mg/kg radio-labelled fezolinetant	After a single oral dose of [^{14}C]fezolinetant at 3 mg/kg, the radioactivity concentrations in blood and plasma reached C_{max} at 0.67 hours after administration and eliminated with a $t_{1/2}$ of 3.8 and 5.4 hours, respectively. The C_{max} and AUC_{inf} in blood were 3130 ng eq./mL and 14300 ng eq.·h/mL, respectively. The C_{max} and AUC_{inf} in plasma were 3210 ng eq./mL and 15100 ng eq.·h/mL, respectively (Table 3).

Table 1 Summary of Absorption Data: Single IV Dose of Fezolinetant in Rats and Monkeys**Pharmacokinetic Parameters of Fezolinetant and ES259564 after Single Intravenous Administration of Fezolinetant to Rats and Cynomolgus Monkeys**

Dose (mg/kg)	Analyte	t _{1/2} (h)	AUC _{last} (ng·h/mL)	AUC _{inf} (ng·h/mL)	CL (mL/h/kg)	V _{ss} (mL/kg)
Species: Rat						
1	Fezolinetant	5.89	7170	7580	132	1010
	ES259564	6.34	645	694	NA	NA
Species: Cynomolgus monkey						
1	Fezolinetant	3.50 (2.66)	4440 (2610)	4690 (2880)	262 (120)	935 (92)
	ES259564	2.72 (1.14)	1150 (616)	1580†	NA	NA

Rat values were calculated from the mean plasma concentrations of 4 animals/time point.

Cynomolgus monkey values represent the mean (SD) of parameters calculated from the individual plasma concentrations of 3 animals.

NA: not applicable. †: n =2

Source: [Studies 2693-ME-0020](#) and [2693-ME-0021](#)

[Extracted from [Pharmacokinetics Written Summary](#) under Module 2.6.4. Page 11]

Table 2 Summary of Absorption Data: Single Oral Dose of Fezolinetant in Rats and Monkeys**Pharmacokinetic Parameters of Fezolinetant and ES259564 after Single Oral Administration of Fezolinetant to Rats and Cynomolgus Monkeys**

Dose (mg/kg)	Analyte	C _{max} (ng/mL)	t _{max} (h)	t _{1/2} (h)	AUC _{last} (ng·h/mL)	AUC _{inf} (ng·h/mL)	BA (%)
Species: Rat							
1	Fezolinetant	861	0.500	4.46	6790	6960	91.9
	ES259564	61.4	2.00	5.09	550	572	NA
3	Fezolinetant	2710	0.500	5.04	23800	23800	105
	ES259564	178	2.00	5.27	1790	1800	NA
10	Fezolinetant	10400	0.500	4.58	87500	87500	115
	ES259564	681	2.00	5.44	6680	6700	NA
Species: Cynomolgus monkey							
1	Fezolinetant	617 (142)	1.00 (0.00)	3.36 (2.23)	3470 (2600)	3680 (2780)	74.6 (12.7)
	ES259564	217 (132)	1.67 (0.58)	3.11 (1.51)	1040 (497)	1430†	NA
3	Fezolinetant	2220 (588)	1.33 (0.58)	3.95 (3.38)	15400 (14900)	15800 (15000)	98.7 (36.8)
	ES259564	795 (484)	2.00 (0.00)	5.86 (5.35)	4850 (2130)	6080†	NA
10	Fezolinetant	5010 (1240)	2.67 (1.15)	4.41 (2.82)	55300 (53700)	55700 (54300)	103 (41)
	ES259564	2220 (1500)	2.67 (1.15)	5.62 (3.03)	18000 (7640)	18300 (7830)	NA

Rat values were calculated from the mean plasma concentrations of 4 animals/time point.

[Extracted from [Pharmacokinetics Written Summary](#) under Module 2.6.4. Page 11]

Table 3 Summary of Absorption Data: Single Oral Dose of Radio-labelled Fezolinetant in Monkeys

Pharmacokinetic Parameters of Radioactivity Concentration in Blood and Plasma after Single Oral Administration of [¹⁴C]Fezolinetant at 3 mg/kg to Cynomolgus Monkeys

Matrix	C _{max} (ng eq./mL)	t _{max} (h)	t _{1/2} (h)	AUC _{last} (ng eq.·h/mL)	AUC _{inf} (ng eq.·h/mL)
Blood	3130 (120)	0.67 (0.29)	3.8 (1.4)	14300 (3100)	14300 (3100)
Plasma	3210 (170)	0.67 (0.29)	5.4 (0.1)	15000 (3300)	15100 (3300)

Data are expressed as mean (SD) of 3 animals.

Source: [Study 2693-ME-0014](#)

[Extracted from [Pharmacokinetics Written Summary](#) under Module 2.6.4. Page 13]

PK of Fezolinetant (ESN-364) and the ES259564 Metabolite in Human

In human, the major metabolite, ES00259564, was approximately 50% of the total fezolinetant-related plasma [AUC] exposure ([Table 4](#)). In animals, this major metabolite was approximately 8% in rats and 38% in monkeys ([Table 2](#)).

Table 4 Summary of PK for ESN-364 and ES259564 in Healthy Menopausal Female Volunteers

Summary Statistics of Pharmacokinetic Parameters for ESN-364, ES259564, Total Radioactivity in Plasma, and Total Radioactivity in Blood (PK Analysis Set)

Analyte	C _{max} (ng[eq]/mL) ^a	T _{max} (h) ^b	AUC _{0-last} (ng[eq].h/mL) ^a	AUC _{0-inf} (ng[eq].h/mL) ^a	Half-life (h) ^a
ESN-364 in plasma	1528 (1286 - 1831)	0.52 (0.50 - 1.00)	5449 (4049 - 7978)	5463 (4075 - 7987)	5.26 (3.76 - 6.02)
ES259564 in plasma	1425 (1166 - 1777)	1.50 (0.52 - 1.53)	9749 (8341 - 10727)	9778 (8355 - 10770)	6.59 (5.02 - 12.2)
Total radioactivity in plasma	3396 (2858 - 3997)	0.52 (0.50 - 1.00)	18116 (14805 - 22182)	18711 (15547 - 22881)	4.72 (3.90 - 5.08)
Total radioactivity in whole blood	3144 (2552 - 3632)	0.52 (0.50 - 1.00)	15533 (13186 - 19214)	16774 (13812 - 20453)	4.47 (3.77 - 5.59)

PK = pharmacokinetic

a: Geometric mean and range b: Median and range

[Extracted from clinical study report [Clinical Study ESN364-CPK-103 CSR](#), page 54]

Distribution

PK Studies: Distribution	
Study No. & Design	Study Findings
2693-me-0012	The plasma protein binding ratios of [¹⁴ C]fezolinetant at concentrations of 0.2, 5 and 100 µg/mL were 31.6%, 31.0% and 31.0% in mice, 32.0%, 32.6% and 33.6% in rats, 30.0%, 30.5% and 30.6% in rabbits, 31.4%, 30.8% and
In vitro, plasma protein binding of	

[¹⁴C]fezolinetant in mice, rats, rabbits, monkeys, and humans	26.1% in cynomolgus monkeys and 50.8%, 52.4% and 50.2% in humans, respectively. The in vitro plasma protein binding ratios of fezolinetant were almost constant between 0.2 and 100 µg/mL in the tested species.
8355672 Rat (non-pigmented and pigmented), single oral dose @ 30 mg/kg [¹⁴C]fezolinetant	<u>Non-pigmented rats</u> ▪ The administered radioactivity was rapidly absorbed and distributed to the tissues with T_{max} = 1 hr. ▪ The radioactivity concentration: liver (T/P = 4.13) > bile ducts > adrenal cortex > adrenal medulla > urinary bladder wall > choroid plexus > brain. ▪ The radioactivity was eliminated relatively rapidly from all tissues, and the radioactivity concentrations in almost all tissues were BLQ at 96 hours postdosing. <u>Pigmented rats</u> ▪ Similar distribution pattern to that observed in non-pigmented rats. ▪ The radioactivity concentration: liver (T/P = 2.78) > caecum mucosa > nasal mucosa > urinary bladder wall > adrenal medullar > adrenal cortex. ▪ Higher radioactivity concentration in the uveal tract/retina than those in nonpigmented rats. ▪ Fezolinetant-derived components have an association with melanin-containing tissues. <u>Note:</u> Fezolinetant was found to be non-phototoxic (See Section 10 Special Toxicology Studies)
2693-me-0019 Rat (pregnant or lactating) under nonfasted condition, single oral dose @ 30 mg/kg [¹⁴C]fezolinetant on GD14	<u>Pregnant rat (GD14)</u> The radioactivity concentration in the milk was higher than that in the plasma at all the time points, indicating that fezolinetant-derived components transferred to breast milk. <u>Lactating rat</u> The radioactivity concentrations in the infant tissues except for the milk clots in stomach were 0.01 to 0.02 times those in the maternal plasma at 1-hour post-dosing. Thereafter, the radioactivity concentration in the maternal plasma declined with time while the radioactivity concentrations in the infant tissues gradually increased for up to 24 hours post-dosing. At 48 hours post-dosing, the radioactivity concentrations in the infant tissues were 2.05 to 10.06 times higher than those in the maternal plasma. These findings suggested that fezolinetant-derived components transferred to the infant tissues via breast milk (Table 5).

Table 5 Summary of Distribution Data: Single Oral Dose of Radio-labelled Fezolinetant in Lactating Rats

Radioactivity Concentrations in Tissues after Single Oral Administration of [¹⁴C]Fezolinetant at 30 mg/kg to Lactating Female Rats on Day 14 Postpartum

		Radioactivity concentrations (µg eq./g or mL)			
		1 h	4 h	24 h	48 h
Maternal tissue	Plasma	25.5 (1.0) [1.00]	18.8 (1.4) [1.00]	1.47 (0.23) [1.00]	0.168 (0.028) [1.00]
	Blood	21.3 (0.3) [0.84]	15.5 (1.1) [0.82]	1.22 (0.18) [0.83]	0.144 (0.028) [0.86]
	Milk	34.4 (1.1) [1.35]	24.0 (0.9) [1.28]	2.23 (0.39) [1.52]	0.277 (0.062) [1.65]
Infant tissue	Blood	0.164 (0.128) [0.01]	0.389 (0.099) [0.02]	1.19 (0.19) [0.81]	0.493 (0.096) [2.93]
	Plasma	0.194 (0.155) [0.01]	0.439 (0.097) [0.02]	1.36 (0.17) [0.93]	0.538 (0.110) [3.20]
	Milk clots in stomach	21.6 (12.0) [0.85]	31.9 (3.3) [1.70]	15.7 (5.2) [10.68]	1.19 (0.32) [7.08]
	Brain	0.143 (0.122) [0.01]	0.339 (0.096) [0.02]	0.952 (0.114) [0.65]	0.344 (0.098) [2.05]
	Heart	0.205 (0.162) [0.01]	0.472 (0.150) [0.03]	1.48 (0.17) [1.01]	0.564 (0.094) [3.36]
	Lung	0.184 (0.157) [0.01]	0.442 (0.134) [0.02]	1.27 (0.22) [0.86]	0.515 (0.078) [3.07]
	Liver	0.500 (0.429) [0.02]	1.42 (0.52) [0.08]	3.93 (0.60) [2.67]	1.69 (0.28) [10.06]
	Kidney	0.258 (0.218) [0.01]	0.734 (0.222) [0.04]	2.16 (0.27) [1.47]	1.02 (0.10) [6.07]

Data are expressed as mean (SD) of 3 animals.

The value in square parentheses indicates tissue to plasma radioactivity concentration ratio based on maternal plasma.

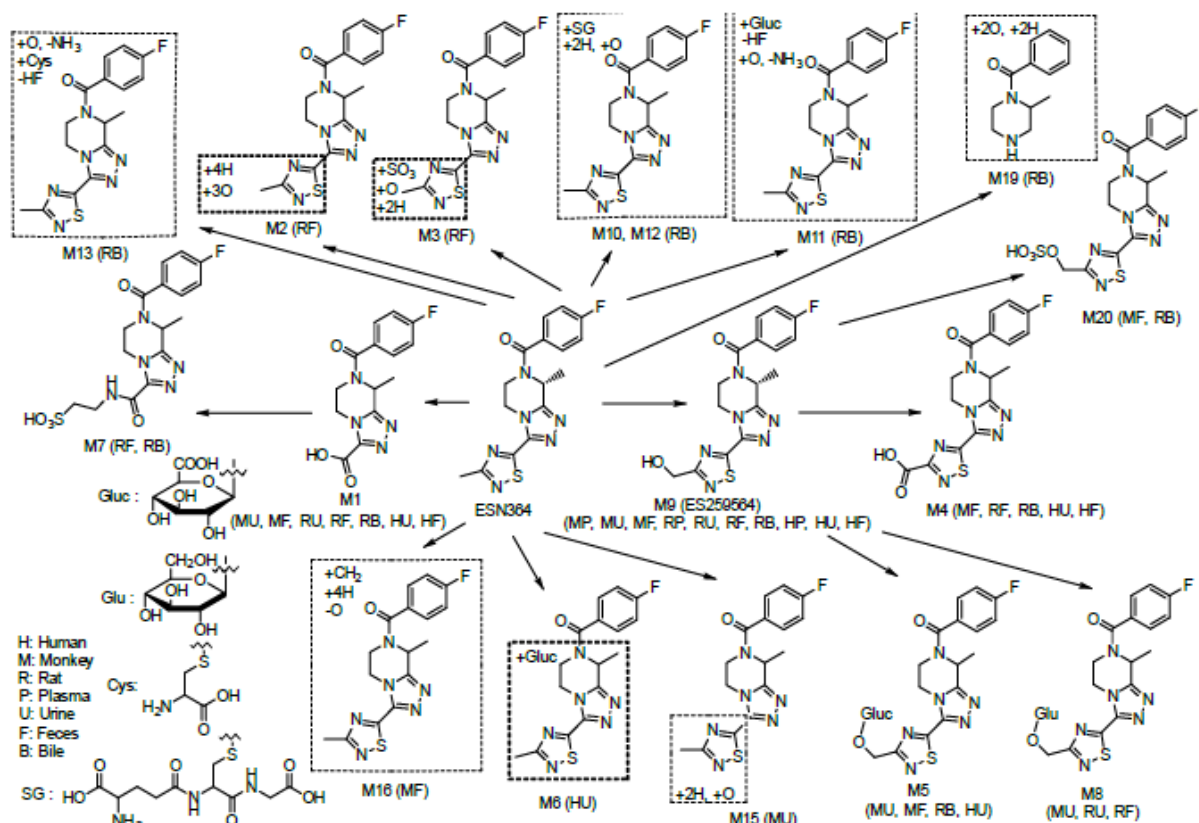
Source: [Study 2693-ME-0019](#)

[Extracted from [Pharmacokinetics Written Summary](#) under Module 2.6.4. Page 22]

Metabolism

PK Studies: Metabolism	
Study No. & Design	Study Findings
Cyp0720-r1 In vitro (cryopreserved hepatocytes) @ 10 µmol/L fezolinetant <u>Metabolite profiling: Hepatocyte</u>	When fezolinetant was incubated with human, rat, and monkey hepatocytes up to 4 hours, 1 metabolite postulated to be a hydroxylated metabolite (ES00259564) was detected in all tested hepatocytes. No human-specific metabolites were formed by hepatocytes.
2693-me-006 Rat and human, single oral dose of [¹⁴ C]fezolinetant @ 30 mg/kg	<u>Rat</u> <u>Plasma (4, 8, and 0-24 h post-dose)</u>: Fezolinetant was the most abundant peak, accounting for 90.3% to 91.8% of radioactivity. Metabolite ES00259564 accounted for 5.0% to 6.4% of radioactivity. <u>Urine (0-96 h post-dose)</u>: ES00259564 was the most abundant peak, accounting for 44.7% of the dose. Fezolinetant accounted for 5.2% of the dose. The other metabolite peaks including M1 and M8 accounted for 2.1% of the dose or less.

<u>Metabolite profiling:</u> Plasma, urine, and feces	▪ <u>Feces (0-72 h post-dose):</u> M3 was the most abundant component, accounting for 13.8% of the dose. Fezolinetant accounted for 5.2% of the dose. ES00259564 accounted for 4.6% of the dose. The other components including M1, M2, M4, M7 and M8 accounted for 3.0% of the dose or less. <u>Human</u> ▪ <u>Plasma (1, 4, and 0–8 h post-dose):</u> Only fezolinetant and the ES00259564 metabolite were detected. ▪ <u>Urine (0–48 h post-dose):</u> ES00259564 was the most abundant peak, accounting for 55.4% of the dose. Fezolinetant accounted for 0.5% of the dose. The other metabolite peaks including M1, M4, M5, and M6 accounted for 6.1% of the dose or less. ▪ <u>Feces (0-144 h post-dose):</u> M4 was the most abundant peak, accounting for 7.4% of the dose. ES00259564 accounted for 1.9% of the dose. Fezolinetant was detected only in the 0–24 h sample, accounting for 0.1% of the dose. The other metabolite peaks including M1 accounted for 1.0% of the dose or less.
2693-me-0015 Rat, monkey, and human, single oral dose of [¹⁴C]fezolinetant <u>Metabolite profiling:</u> Chiral inversion	Chiral inversion of fezolinetant to its enantiomer, AS3472948, was not observed in plasma (4- and 8-hours post-dose), suggesting that chiral inversion of the unchanged drug did not take place after the oral administration of ESN364 to rats, cynomolgus monkeys, and humans.
2693-me-0017 Rat, single oral dose of [¹⁴C]fezolinetant <u>Metabolite profiling:</u> Bile Monkey, single oral dose of [¹⁴C]fezolinetant <u>Metabolite profiling:</u> Plasma, urine, and feces	<u>Rat</u> ▪ <u>Bile (0-48 h post-dose):</u> M10 was the most abundant peak, accounting for 6.5% of the dose. Fezolinetant accounted for 4.5% of the dose. The other metabolite peaks including M1, M4, M5, M7, ES00259564, M11, M12, M13, M17, M18, M19, and M20 accounted for 2.3% or less of the dose. <u>Monkey</u> ▪ <u>Plasma (0.5, 6 h, and 0-24 h post-dose):</u> Fezolinetant was the most abundant peak, accounting for 45.8% to 72.1% of the radioactivity. ES00259564 accounted for 20.7% to 42.1% of the radioactivity. ▪ <u>Urine (0–72 h post-dose):</u> ES00259564 was the most abundant peak, accounting for 67.2% of the dose. Fezolinetant accounted for 0.9% of the dose. The other metabolite peaks including M1, M5, M8, M14, and M15 accounted for 3.1% of the dose or less. ▪ <u>Feces (0-72 h post-dose):</u> ES00259564 was the most abundant peak, accounting for 3.6% of the dose. Fezolinetant accounted for 0.2% of the dose. The other metabolite peaks including M1, M2, M5, M16, and M20 accounted for 3.4% of the dose or less.
<u>In Summary:</u> These results indicated that fezolinetant was metabolized primarily to ES00259564 in rats, cynomolgus monkeys, and humans. Additionally, there were no unique or disproportionate human metabolites. In vivo postulated metabolic pathways of fezolinetant are shown in Figure 5. Postulated metabolic pathways of fezolinetant in rats and cynomolgus monkeys involve at least oxidation of methyl group in methylthiadiazole ring moiety (ES00259564 [also referred to as M9] and M4), glucuronidation (M5 and M11), oxidative ring-opening of the methylthiadiazole ring moiety (M1), glutathione conjugation of oxidized metabolites (M10 and M12), sulfate conjugation of an oxidized metabolite (M3) and glucose conjugation of ES00259564 (M8).	

Figure 5 In Vivo Postulated Metabolic Pathways

[Extracted from [Pharmacokinetics Written Summary](#) under Module 2.6.4. Page 25]

Excretion

PK Studies: Excretion	
Study Title & Design	Study findings
2693-me-0013	After a single oral administration of [^{14}C]fezolinetant to fasted bile-duct cannulated female rats, the excretion of radioactivity in the bile and urine was 24.6% and 67.3% of the dose at up to 48 h post-administration, respectively, resulting in a sum of 91.9%.
Female rats, single dose @ 30 mg/kg [^{14}C]fezolinetant	
Urinary and biliary excretion	Cumulative Excretion of Radioactivity in Urine and Bile after Single Oral Administration of [^{14}C]Fezolinetant at 30 mg/kg to Rats

	<table><tr><th rowspan="2">Time (h)</th><th colspan="3">Cumulative excretion (% of dose)</th></tr><tr><th>Bile</th><th>Urine</th><th>Total</th></tr><tr><td>0 to 6</td><td>7.2 (1.4)</td><td>14.5 (5.4)</td><td>21.7 (4.2)</td></tr><tr><td>0 to 24</td><td>21.7 (4.5)</td><td>56.9 (6.2)</td><td>78.6 (2.8)</td></tr><tr><td>0 to 48</td><td>24.6 (4.8)</td><td>67.3 (5.9)</td><td>91.9 (1.2)</td></tr></table> Data are expressed as mean (SD) of 3 animals. Source: Study 2693-ME-0013 These results indicated that the radioactivity was well absorbed after oral administration.	Time (h)	Cumulative excretion (% of dose)			Bile	Urine	Total	0 to 6	7.2 (1.4)	14.5 (5.4)	21.7 (4.2)	0 to 24	21.7 (4.5)	56.9 (6.2)	78.6 (2.8)	0 to 48	24.6 (4.8)	67.3 (5.9)	91.9 (1.2)												
Time (h)	Cumulative excretion (% of dose)																															
	Bile	Urine	Total																													
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0 to 24	21.7 (4.5)	56.9 (6.2)	78.6 (2.8)																													
0 to 48	24.6 (4.8)	67.3 (5.9)	91.9 (1.2)																													
2693-me-0014 * Monkey, oral gavage, single dose of 3 mg/kg radio-labelled fezolinetant Urinary and <u>biliary</u> excretion	After a single oral administration of [¹⁴C]fezolinetant at 3 mg/kg to cynomolgus monkeys, the dosed radioactivity was almost completely excreted from the body within 120 hours post-dosing. The urinary and fecal excretion of radioactivity up to 120 hours were 81.7% and 14.6%, respectively. The overall recovery ratio up to 120 hours for radioactivity was 96.3%. The administered radioactivity was mainly excreted via urine in monkey. Cumulative Excretion of Radioactivity in Urine and Feces after Single Oral Administration of [¹⁴C]Fezolinetant at 3 mg/kg to Cynomolgus Monkeys <table><tr><th rowspan="2">Time (h)</th><th colspan="3">Cumulative excretion (% of dose)</th></tr><tr><th>Urine</th><th>Feces</th><th>Total</th></tr><tr><td>0 to 6</td><td>47.8 (6.9)</td><td>NS</td><td>NC</td></tr><tr><td>0 to 24</td><td>75.3 (6.5)</td><td>8.2 (1.7)</td><td>83.5 (7.4)</td></tr><tr><td>0 to 48</td><td>78.8 (4.7)</td><td>12.7 (0.4)</td><td>91.5 (4.4)</td></tr><tr><td>0 to 72</td><td>80.4 (3.7)</td><td>13.9 (0.9)</td><td>94.3 (2.9)</td></tr><tr><td>0 to 96</td><td>81.3 (2.9)</td><td>14.5 (1.3)</td><td>95.8 (1.7)</td></tr><tr><td>0 to 120</td><td>81.7 (2.7)</td><td>14.6 (1.3)</td><td>96.3 (1.4)</td></tr></table> Data are expressed as mean (SD) of 3 animals. NC: not calculable; NS: not sampled. Source: Study 2693-ME-0014	Time (h)	Cumulative excretion (% of dose)			Urine	Feces	Total	0 to 6	47.8 (6.9)	NS	NC	0 to 24	75.3 (6.5)	8.2 (1.7)	83.5 (7.4)	0 to 48	78.8 (4.7)	12.7 (0.4)	91.5 (4.4)	0 to 72	80.4 (3.7)	13.9 (0.9)	94.3 (2.9)	0 to 96	81.3 (2.9)	14.5 (1.3)	95.8 (1.7)	0 to 120	81.7 (2.7)	14.6 (1.3)	96.3 (1.4)
Time (h)	Cumulative excretion (% of dose)																															
	Urine	Feces	Total																													
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0 to 96	81.3 (2.9)	14.5 (1.3)	95.8 (1.7)																													
0 to 120	81.7 (2.7)	14.6 (1.3)	96.3 (1.4)																													
8355672 ** Rat (non-pigmented and pigmented), single oral dose @ 30 mg/kg [¹⁴ C]fezolinetant Urinary and <u>biliary</u> excretion	After a single oral administration of [¹⁴C]fezolinetant at 30 mg/kg to both non-pigmented and pigmented rats, the dosed radioactivity was almost completely excreted from the body within 96 hours post-dosing. Across all animals, the urinary and fecal excretion of radioactivity up to 96 hours were 52.3% and 36.1%, respectively. The overall recovery ratio up to 96 hours for radioactivity was 98.5%. The administered radioactivity was mainly excreted via urine in rat.																															

Cumulative Excretion of Radioactivity in Urine and Feces after Single Oral Administration of [¹⁴ C]Fezolinetant at 30 mg/kg to Rats		
Time (h)	Cumulative excretion (% of dose)	
	Urine	Feces
0 to 6	13.7 (7.1)	NS
0 to 24	43.8 (10.0)	22.9 (7.2)
0 to 48	51.3 (6.8)	34.9 (4.4)
0 to 72	52.1 (6.7)	36.0 (4.0)
0 to 96	52.3 (6.7)	36.1 (4.0)
Cage wash†	9.0 (5.4)	
Final cage wash‡	0.6 (0.3)	
Cage debris†	0.1¶	
Expired air§	ND	
Carcass‡	0.7††	
Total recovery†	98.5 (4.0)	

Data are expressed as mean (SD) of 3 animals.
 ND: not detected; NS: not sampled.
 †: up to 96 hours.
 ‡: at 96 hours.
 §: up to 24 hours.
 ¶: n = 2.
 ††: n = 1.
 Source: [Study 8355672](#)

*This study is a part of the absorption PK study.

**This study is a part of the distribution PK study.

5.2 Toxicokinetics

See Section **6.2 Repeat-Dose Toxicity** for TK data obtained from the repeated dose toxicity studies conducted in rats and monkeys.

6 General Toxicology

6.1 Single-Dose Toxicity

None.

6.2 Repeat-Dose Toxicity

Rat

Repeat-Dose Toxicity Studies	
Study No. & Design	Study findings
8276178 (non-GLP, DRF) Rat, oral gavage @ 0, 100, 250, 300, 325, and 750 mg/kg/day fezolinetant for 7 days NOAEL = 100 mg/kg/day.	@ ≥ 325 mg/kg: Death / moribund (males and females) @ ≥ 250 mg/kg: Reduced activity, subdued/sluggish behavior, staggering, loss of righting reflex, prostration, excess salivation and tear production and irregular, shallow or labored respiration, and increased liver and kidney weights.
8276179 (GLP) Rat, oral gavage @ 0, 30, 100, and 300 mg/kg/day fezolinetant for 4 weeks	Mortality: 2 deaths at HD Clinical signs: Lethargy, reduced activity, labored respiration and staggering in HD animals. Body weight: BW loss during first few days of treatment in HD animals.

NOAEL = 100 mg/kg/day based on mortality at HD. Safety margin: 143X MRHD, based on the AUC₂₄ = 551900 ng•h/mL^{&} last dosing when compared to AUC₂₄ = 3855 ng•h/mL obtained from clinical Study No. 2693-PD-0010.	<u>Clinical chemistry:</u> Increased cholesterol in HD males and MD and HD females. Increased urea in HD animals. <u>Hormones:</u> Increased estradiol and decreased testosterone in HD males. <u>Microscopic findings in MD and HD animals:</u> ▪ Bone marrow: Adipocytes. ▪ Liver: Minimal centrilobular hepatocellular hypertrophy. ▪ Lung: Aggregates of alveolar foamy macrophage. ▪ Ovary: Increased number of cystic ovarian follicles. ▪ Prostate: low grade epithelial atrophy. ▪ Uterus: Atrophy. ▪ Correlated to changes in organ weights and macroscopic findings. ▪ Changes in reproductive system were attributed to the exaggerated pharmacological effects of fezolinetant.
8297361* ** (GLP) FEMALE Rat, oral gavage @ 0, 30, 100, and 300 mg/kg/day fezolinetant for 13 weeks NOAEL = Not established (< 30 mg/kg/day) for females based on convulsive-type episodes in all treated animals. Safety margin: < 58X MRHD, based on the AUC₂₄ = 225100^{&} ng•h/mL last dosing when compared to AUC₂₄ = 3855 ng•h/mL obtained from clinical Study No. 2693-PD-0010.	<u>Clinical signs:</u> Related to the CNS system including paddling and slight sedative effects in MD and HD animals. Only constricted pupils in LD animals. Convulsive episodes in treated animals without any dose-dependent trend or concurrent fezolinetant exposure as these observations were (mainly) recorded in the pre-dosing period; the incidence of these convulsive-like episodes was reduced following a change in animal handling procedure in the latter half of the study. <u>Clinical chemistry:</u> Increased total cholesterol in LD, MD, and HD animals (↑17%, ↑41%, ↑47 % respectively), increased urea in MD and HD animals (↑32% and ↑29% respectively), increased ALP in HD animals (↑39%). <u>Organ weights:</u> Increased liver weights in MD and HD females, higher thyroid weights in HD females, lower ovary weights in HD females. <u>Microscopic findings:</u> ▪ Liver: Centrilobular hypertrophy in MD and HD females. ▪ Thyroid: Follicular cell hypertrophy in HD females. ▪ Uterus: Abnormal estrus cycle in HD females.
8331724** *** (GLP) FEMALE Rat, oral gavage @ 0, 30, 100, and 200 mg/kg/day fezolinetant for 26 weeks NOAEL = 200 mg/kg/day for females, based on findings not being considered adverse and attributed to exaggerated pharmacological effect of fezolinetant. Safety margin: 148X, MRHD, based on the AUC₂₄ = 569000 ng•h/mL last dosing, when compared to AUC₂₄ = 3855 ng•h/mL obtained from clinical Study No. 2693-PD-0010 For metabolite ES00259564: 36X MRHD, based on the	<u>Clinical signs:</u> None (see note below) <u>Body weight:</u> Increased body weight gains in all treated groups for Days 8-15. Increased overall body weight gain in LD and MD animals (↑24% and ↑18%, respectively). <u>Food consumption:</u> Increased food consumption in MD and HD animals. <u>Hematology:</u> Decreased red blood cell parameters (↓4 and/or 5%), increased white blood cell count (↑30-67%), due to increased absolute lymphocyte (↑38- 101%) and monocyte counts (↑67%) occurred in HD animals. These changes did not generally progress over time. <u>Clinical chemistry:</u> Decreased total protein (↓5-7%) and albumin (↓8-9%) in MD animals; increased cholesterol (↑24-69%) in LD animals, increased ALP activity (↑27-28%) in HD animals. <u>Organ weights:</u> Decreased uterine weight in all treated animals, increased liver weight in MD and HD animals, increased thyroid in HD animals, increased spleen and kidney weights in MD and HD animals. <u>Microscopic findings:</u> ▪ Cervix: Mucification of the epithelium in MD and HD animals.

AUC₂₄ = 93200 ng•h/mL last dosing when compared to AUC₂₄ = 2570 ng•h/mL obtained from clinical Study No. 2693-PD-0010.	▪ Liver: Centrilobular hepatocellular hypertrophy in MD and HD animals (likely attributed to adaptive change due to an enzyme induction). ▪ Lung: Minimal infiltrates of microphages in the alveolus of the lungs in all treated animals. ▪ Nasal: Increased of minimal or slight eosinophilic globules in the olfactory epithelium of the nasal turbinates in all treated animals. ▪ Thyroid: Follicular cell hypertrophy in all treated animals. ▪ Uterus: Atrophy in all treated animals. ▪ Vagina: Mucification of the epithelium in all treated animals. <u>Note:</u> The CNS-related clinical signs seen in the 4-week and 13-week rat studies were not observed in this 26-week study. This is attributed to poor animal handling in the previous studies.
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& AUC₂₄ at the first and last dose in each toxicity study was converted from nmol/L to ng•h/mL using the molecular weight of 358.39 g/mol.

* PT reviews under IND 130277 in DARRTS dated 05/31/16.

** PT (SPA) review under IND NON-RESPONSIVE in DARRTS dated 12/05/17 when reviewing the 2-year rat SPA.

*** PT review under IND NON-RESPONSIVE in DARRTS dated 07/01/19.

Monkey

Repeat-Dose Toxicity Studies	
Study No. & Design	Study findings
8276019 (non-GLP) Monkey, oral gavage @ 50, 100, 150, and 300 mg/kg/day fezolinetant for 14 days NOAEL = Not established.	@ ≥100 mg/kg (males and females): Moribund sacrificed due to neurological signs (ataxia, uncoordinated movement, hypoactivity, hunched posture and apathy). @ 50 mg/kg: Periods of reduced food consumption and weight loss as well as subacute inflammation of the stomach and erosion at the pylorus/duodenum region.
8276020 (GLP) Monkey, oral gavage @ 0, 10, 25, and 50 mg/kg/day for 5 weeks NOAEL = 25 mg/kg/day, based on loss of body weight noted in 1 HD male and severe microscopic findings noted in 1 HD female. Safety margin: 26X MRHD, based on the AUC₂₄ = 100300 ng•h/mL & last dosing when compared to AUC₂₄ = 3855 ng•h/mL obtained from clinical Study No. 2693-PD-0010.	<u>Clinical signs:</u> Excessive salivation before and after dosing at MD and HD males. <u>Body weight:</u> Loss of body weight in all treated animals. Most apparent in 1 HD male. <u>Hormones:</u> ↓ LH, estradiol, and progesterone in females (dose-dependent). <u>Microscopic findings (noted in 1 HD female):</u> ▪ Bone marrow: Increased hematopoiesis with vacuolation of marrow cells. ▪ Kidney: Perivascular inflammation with hyperplasia of the vascular wall. ▪ Lung: Hyperplasia of lymphocytes ▪ Lymph nodes: Hyperplasia of lymphocytes ▪ Spleen: Hyperplasia of lymphocytes ▪ Uterus: Stromal atrophy without the presence of normal cycle stages in all treated animals (reversible), hyperplasia of the myometrium and inflammation of the vessels in the myometrium.
8297388* (GLP) FEMALE Monkey, oral gavage @ 0, 10, 25, and 50 mg/kg/day fezolinetant for 13 weeks with 4-week recovery	<u>Body weight:</u> Decreased relative weight gain in MD and HD animals. <u>Hematology:</u> Marked decreased platelet counts, associated with a moderate to marked monocytosis (in 2 HD animals) or associated with a moderate temporary regenerative anemia with reticulocytosis, indicating a hemorrhagic event (in 1 HD animal). <u>Vaginal smears:</u> No regular bleeding in all treated animals. <u>Organ weight:</u> Decreased uterus weight.

NOAEL = 25 mg/kg/day, based on hemorrhage and regenerative anemia in 1 HD female Safety margin: 29X MRHD, based on the $AUC_{24} = 111500$ ng•h/mL & last doing when compared to $AUC_{24} = 3855$ ng•h/mL obtained from clinical Study No. 2693-PD-0010.	<u>Microscopic findings:</u> ▪ Cervix: Deceased epithelium and increased mucous secretion in MD and HD animals. ▪ Ovary: Lack of new corpora lutea, indicating absence of ovarian activity and cyclic ovulation in MD and HD animals. ▪ Uterus: stromal atrophy in all treated animals. ▪ Pharmacological action related findings partially recovered as 1 of 2 HD animals had not reassumed ovarian activity and stromal atrophy remained. <u>Note:</u> Blood samples were collected for potential hormone analysis. However, no analysis of these samples was performed.
8331280*** (GLP) FEMALE Monkey, oral gavage @ 0, 10, 25, and 40 mg/kg/day fezolinetant for 39 weeks with 4-week recovery NOAEL = 25 mg/kg/day based on reduced platelets in 2 HD animals resulting in hemorrhage and moribundity in 1 HD animal Safety margin: 43X MRHD, based on the $AUC_{24} = 166000$ ng•h/mL last doing when compared to $AUC_{24} = 3855$ ng•h/mL obtained from clinical Study No. 2693-PD-0010) For metabolite ES00259564: 26X MRHD, based on the $AUC_{24} = 65900$ ng•h/mL last dosing when compared to $AUC_{24} = 2570$ ng•h/mL obtained from clinical Study No. 2693-PD-0010.	<u>Mortality:</u> 1 HD female (acute hemorrhagic anemia due to severe thrombocytopenia) <u>Clinical signs:</u> Salivation and/or emesis in all treated animals. <u>Body weight:</u> Decreased body weight gain and body weight loss in HD animals. <u>Food consumption:</u> Decreased food consumption in HD animals. <u>DEXA scan:</u> Decreased fat content (recoverable), unchanged muscle or bone mass. <u>Hematology:</u> Decreased platelet (recoverable), thrombocytopenia in 2 HD animals. <u>Clinical chemistry:</u> Increased creatinine in MD and HD animals. <u>Hormones:</u> No treatment-related change. <u>Organ weight:</u> Decreased uterus and cervix weight in all treated animals. Decreased ovary weight in LD and HD animals. <u>Microscopic findings:</u> ▪ Lymph nodes (mandibular and mesenteric): Increased lymphocytes with increased germinal centers in few HD animals (reversible). ▪ Mammary gland: Decreased number and size of glandular tissue in all treated animals (present after recovery phase). ▪ Spleen: Increased lymphocytes with increased germinal center in few HD animals (reversible). ▪ Ovary: Absent corpora lutea in all treated animals (reversible). ▪ Uterus: Stromal atrophy in all treated animals (reversible). ▪ Pharmacological action related findings: Interrupted menstrual cycles which correlated with ovarian inactivity (reversible) and stromal atrophy of the uterus (reversible).

& AUC_{24} at the first and last dose in each toxicity study was converted from nmol/L to ng•h/mL using the molecular weight of 358.39 g/mol.

* PT reviews under IND 130277 in DARRTS dated 05/31/16.

** PT (SPA) review under IND NON-RESPONSIVE in DARRTS dated 12/05/17 when reviewing the 2-year rat SPA.

*** PT review under IND NON-RESPONSIVE in DARRTS dated 07/01/19.

7 Genetic Toxicology

Genotoxicity Studies	
Study No. & Design	Study findings
8276182* AMES (up to 5000 µg/plate)	Fezolinetant did not induce gene mutation with or without S-9.
8276184*	Fezolinetant did not induce chromosomal aberrations in vitro under all treatment conditions (3 hr + 17 hr recovery with or without S-9 and 20 hr without S-9).

Chromosomal aberration test in cultured human lymphocytes (up to 258.4 µg/mL, ~ 1 mmol/L)	
8276179 ** Micronucleus test in rats (incorporated in 4-week repeat dose toxicity study)	Fezolinetant did not induce chromosomal aberrations in vivo in rats.

* PT reviews under IND 130277 in DARRTS dated 05/31/16. All genotoxicity studies were determined to be "negative" in the review.

** Analysis was conducted in the 4-week repeat toxicity study in rats (Study No. 826179)

8 Carcinogenicity

Carcinogenicity Studies	
Study No. & Design	Study findings
8375967 ** (GLP) FEMALE rat, oral gavage @ 0, 10, 30, and 100 mg/kg/day fezolinetant for 104 weeks with TK assessment at Week 26 <u>Carcinogenicity: Negative</u> NOEL = 100 mg/kg/day , based on no drug-related tumor findings. <u>Safety Margin: 186X MRHD (female)</u> , based on AUC ₀₋₂₄ = 718000 ng•h/mL on Week 26 when compared to AUC ₂₄ of 3855 ng•h/mL, obtained from clinical Study No. 2693-PK-0010 <u>For metabolite ES00259564: 31X MRHD</u> , based on the AUC ₀₋₂₄ = 79900 ng•h/mL on Week 26 when compared to AUC ₂₄ = 2570 ng•h/mL obtained from clinical Study No. 2693-PD-0010.	<u>Survival:</u> No effect. <u>Tumor findings:</u> The tumor data were analyzed by the FDA statistical reviewer, Dr. Hepei Chen, for dose response relationships across vehicle control group and the treated groups, and pairwise comparisons of each of the three treated groups against the vehicle control group. The FDA statistical review identified statistically significant trend or pairwise relationships for tumor findings; however, no tumor findings was determined to be toxicologically relevant by statistical analysis. The following findings may be considered pharmacologically relevant. ▪ Thyroid: Increased incidence of benign follicular adenomas with increased ESN364 dose (trend only; p-value = 0.0094). A pairwise analysis did not identify a statistical increase in this tumor type between the control and any fezolinetant dose level. ▪ Ovary: Increased incidence of the combination of ovarian epithelial tumors (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma) was noted in MD females only (p=0.0132) when compared to the vehicle control group, without corresponding statistically significant dose response relationship. No statistical significance by trend or pairwise analysis was observed for each individual ovary tumor type or for the combination of ovarian stromal tumors. <u>Non-tumor findings:</u> ▪ Liver: Centrilobular hepatocyte hypertrophy in HD females, ▪ Uterus: Increased incidences of atrophy in the uterus in MD and HD females ▪ Mammary gland: Decreased incidences of hyperplasia in all treated females ▪ Ovary: Decreased incidences of age-related atrophy in all treated females ▪ Cervix: Decreased incidence of squamous metaplasia in all treated females

	▪ Adrenal: Decreased incidence of cystic degeneration of the cortex in all treated females. ▪ These findings, except liver findings, were exaggerated pharmacological effects of fezolinetant.
8375968* (non-GLP, DRF) C57BL mouse, oral gavage Phase I @ 0, 75, 150, 300 fezolinetant for 7 days and Phase II @ 600 mg/kg/day fezolinetant for 2 days NOAEL = 300 mg/kg/day based on severe clinical signs at 600 mg/kg/day. Safety margin: 58X MRHD (female only), based on the $AUC_{12} = 224000$ ng•h/mL on Day 7 when compared to $AUC_{24} = 3855$ ng•h/mL obtained from clinical Study No. 2693-PD-0010) For metabolite ES00259564: 74X MRHD (female only), based on the $AUC_{12} = 190000$ ng•h/mL last dosing when compared to $AUC_{24} = 2570$ ng•h/mL obtained from clinical Study No. 2693-PD-0010.	Fezolinetant was not tolerated at 600 mg/kg/day with adverse clinical observations and moribund conditions by Day 1 or 2 of Phase II. <u>Clinical signs</u> (@ 600 mg/kg/day): Clonic convulsions, rigid stance, tremors, ataxia, hypoactivity, recumbency, irregular or labored respiration, cold to touch, and squinting eyes. <u>Clinical chemistry</u>: Minimally increased urea nitrogen in HD males on Day 8 (Phase 1), suggesting pre-renal dehydration.
8378969* (GLP) RasH2 wild type, oral gavage @ 0, 90, 200, and 450 fezolinetant for 4 weeks NOAEL = 450 mg/kg/day Safety margin: 98X MRHD (female only), based on the $AUC_{12} = 376000$ ng•h/mL on Day 28 when compared to $AUC_{24} = 3855$ ng•h/mL obtained from clinical Study No. 2693-PD-0010) For metabolite ES00259564: 165X MRHD (female only), based on the $AUC_{12} = 425000$ ng•h/mL last dosing when compared to $AUC_{24} = 2570$ ng•h/mL obtained from clinical Study No. 2693-PD-0010.	Fezolinetant was well tolerated. <u>Clinical chemistry</u>: Increased ALP in all treated males and MD and HD females. <u>Organ weight</u>: Increased liver weight @ HD <u>Microscopic findings</u>: ▪ Liver: Hepatocyte vacuolation @ HD (not adverse)
8375970** (GLP) Tg mouse, oral gavage @ 0, 50, 150, and 450 mg/kg/day fezolinetant for 26 weeks with TK assessment at Week 26 Carcinogenicity: Negative	<u>Survival</u>: No effect. <u>Clinical sign</u>: White teeth in MD and HD animals (higher in females). <u>Body weight</u>: Increased mean body weight at HD animals ($\uparrow$ 12.5% in males and $\uparrow$ 25% in females). <u>Food consumption</u>: Increased food consumption in MD and HD males. No tumor identified.

NOAEL = 450 mg/kg/day (female only), based on statistically increased incidences of follicular adenomas at HD. Safety Margin: 48X MRHD, based on $AUC_{0-24} = 183000 \text{ ng}\cdot\text{h/mL}$ on Week 26 when compared to AUC_{24} of 3855 $\text{ng}\cdot\text{h/mL}$, obtained from clinical Study No. 2693-PK-0010 For metabolite ES00259564: 142X MRHD, based on the $AUC_{0-24} = 366000 \text{ ng}\cdot\text{h/mL}$ on Week 26 when compared to $AUC_{24} = 2570 \text{ ng}\cdot\text{h/mL}$ obtained from clinical Study No. 2693-PD-0010.	<u>Non-tumor lesions findings:</u> ▪ Liver: Increased vacuolation in HD males and MD/HD females, centrilobular hepatocyte hypertrophy in both HD males and females ▪ Ovary: Increased incidence of decreased numbers of corpora lutea in HD females. ▪ Vagina: Increased incidence and/or severity of mucosal cornification in HD females.
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* PT (SPA) review under IND NON-RESPONSIVE in DARRTS dated 11/28/18 when reviewing the 6-month tg mouse SPA.

**Final study findings were presented to the ECAC on 10/25/22. The ECAC review for these two studies is in DARRTS dated 10/28/22.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Seg I Studies	
Study No. & Design	Study findings
8331725 Rat, oral gavage @ 0, 10, 30, and 100 mg/kg/day fezolinetant given 14 days prior to mating and through mating period and through GD 7 NOAEL = 100 mg/kg/day	Fezolinetant had no effect on fertility or early embryonic development even though there were altered estrous cyclicity and reduced adjusted mean uterus/cervix/vagina weight noted in HD females. These findings were consistent with the known pharmacological actions of fezolinetant.

9.2 Embryonic Fetal Development

Seg II Studies	
Study No. & Design	Study findings
8297365 (non-GLP, DRF) FEMALE rat, oral gavage @ 0, 25, 100, and 250 mg/kg/day fezolinetant given GD 6-17 NOAEL (maternal) = 100 mg/kg/day NOAEL (fetal) = 25 mg/kg/day	<u>Dams (F_0):</u> ▪ Decreased body weight and food consumption in MD and HD females. ▪ 2 HD females were euthanized on GD 6 or GD 7 after dosing, and 2 HD females were found dead on Day 7 of gestation. <u>Litters (F_1):</u> ▪ Total litter loss in 2 MD females, increased mean percentage pre- and post-implantation loss in MD females, and lower number of live fetuses in MD females. ▪ There were no fetal malformations or treatment-related variations at any doses.
8297366* (GLP, definitive)	<u>Dams (F_0):</u>

FEMALE rat, oral gavage @ 0, 25, 50, and 100 mg/kg/day fezolinetant given GD 6-17 NOAEL (maternal) = 100 mg/kg/day NOAEL (fetal) = 50 mg/kg/day based on embryo lethality in 3 dams at HD. Maternal Safety Margin: 62X MRHD, based on $AUC_{0-24} = 238700^{\circ} \text{ ng}\cdot\text{h/mL}$ on GD 17 when compared to AUC_{24} of 3855 $\text{ng}\cdot\text{h/mL}$, obtained from clinical Study No. 2693-PK-0010.	▪ Dose proportional increase in C_{max} and AUC. ▪ Incomplete clearance on GD 17, with measurable concentration in all animals 24 hrs post dose (6-7% of mean C_{max}) ▪ No accumulation. Low linearity ratios, therefore multi-dose concentrations cannot be well predicted from a single dose. Exposure lower on GD 17 than GD 6. ▪ No clinical observations of note (no signs of convulsion or other CNS-related events - only fur staining). ▪ Total litter loss in 3/24 HD females. For HD females with live fetuses, no change in post-implantation loss compared to control. These findings suggest that litter loss was either total or absent at HD. ▪ These findings are likely attributed to an exaggerated pharmacological effect of fezolinetant (a decrease in LH). <u>Litters (F_1):</u> ▪ No treatment-related malformations or variations. ▪ Zygomatic arch ossification bridge (partially fused) incidence was elevated over historical controls at LD and HD (+0.6%). In the absence of a dose relationship and other associated findings in the skull, this finding was considered incidental.
8297363 (non-GLP, DRF) FEMALE rabbit, oral gavage @ 0, 45, 75, and 125 mg/kg/day fezolinetant given GD 7-19 NOAEL (maternal) = Not Established due to decreased body weight gains and food consumption in all treated animals NOAEL (fetal) = 45 mg/kg/day	<u>Dams (F_0):</u> ▪ Inappetence and low body weight gain in all treated animals <u>Litters (F_1):</u> ▪ Total litter loss in 1 MD female and 1 HD female, increased mean percentage pre-and post-implantation loss in MD and HD females, and slightly lower number of female fetuses in MD and HD females. ▪ There were no fetal malformations and treatment-related variations at any doses.
8297364* (GLP, definitive) FEMALE rabbit, oral gavage @ 0, 45, 75, and 125 mg/kg/day fezolinetant given GD 7-19 NOAEL (maternal) = Not Established due to decreased body weight gains and food consumption in all treated animals NOAEL (fetal) = 45 mg/kg/day based on an increase in late resorption percentage and the slightly lower weight of male fetuses at MD. Fetal Safety Margin: 16X MRHD, based on maternal $AUC_{0-24} = 60600^{\circ} \text{ ng}\cdot\text{h/mL}$ on GD 19 when compared to AUC_{24} of 3855	<u>Dams (F_0):</u> ▪ Single dose showed supra-proportional increases in C_{max} and AUC. ▪ Multiple administrations showed dose proportional increases in C_{max} and AUC from LD to MD but supra-proportional increases between LD and HD. ▪ Saturation of routes of elimination. ▪ Clearance achieved at end of dosing except for HD (measurable concentrations in all 3 animals, 20% and 49% of C_{max}) ▪ Reduced body weight gain, lower food consumption (all doses). Recovered over GD 19-29. ▪ No clinical observations. ▪ 3 HD females terminated before GD 29 due to abortion of their litters ▪ 4 HD had total embryo loss <u>Litters (F_1):</u> ▪ Increased post-implantation loss, early/late resorptions, low uterine weight at HD. ▪ Increased late resorptions at MD. ▪ Decreased fetal weight at HD, slight at MD. Male fetuses smaller than controls.

ng•h/mL, obtained from clinical Study No. 2693-PK-0010	- No treatment-related malformations. - Increase in nodulated ribs spread across litters at MD and HD. - Dumbbell ossification of sternebra and incomplete ossification of thoracic centrum were observed and associated with a slight delay in development due to lower fetal weight at MD and HD.
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& AUC₂₄ at the first and last dose in each toxicity study was converted from nmol/L to ng•h/mL using the molecular weight 358.39.

* PT reviews under IND 130277 in DARRTS dated 05/31/16.

9.3 Prenatal and Postnatal Development

Seg III Studies	
Study No. & Design	Study findings
8381968 (non-GLP, DRF) FEMALE rat, oral gavage @ 0, 10, 30 and 100 mg/kg/day fezolinetant given from GD 6 to LD 4 NOAEL = 100 mg/kg/day	Test article-related, lower mean pup weight on PND 1 and 4 at HD (↓ 4-7%). However, mean body weight gain was similar between PND 1 and PND 4. No effects on pup clinical observations, survival, or development markers were noted; therefore, this slight reduction in pup weight at the HD was considered not adverse. Up to 100 mg/kg per day, no maternal or developmental toxicity was observed.
8382261 (GLP, definitive) FEMALE rat, oral gavage @ 0, 10, 30 and 100 mg/kg/day fezolinetant given from GD 6 to LD 21 NOAEL (maternal) = 30 mg/kg/day, based on delayed parturition. NOAEL (embryotoxicity) = 30 mg/kg/day, based on embryo-lethality at HD Safety Margin: 36X MRHD, based on maternal AUC₀₋₂₄ = 140000 ng•h/mL on LD21 when compared to AUC₂₄ of 3855 ng•h/mL, obtained from clinical Study No. 2693-PK-0010. NOAEL (F1 generation development) = 100 mg/kg/day for females and 10 mg/kg/day for males, based on changes noted during sexual maturation, mating, and fertility. Safety Margin: 204X MRHD (female) and 11 X MRHD (male), based on AUC₀₋₂₄ = 788000 ng•h/mL and AUC₀₋₂₄ = 42500 ng•h/mL on LD 21 when compared to AUC₂₄ of 3855 ng•h/mL, obtained from clinical Study No. 2693-PK-0010.	<u>Dams (F₀)</u> - Extended gestation duration at HD (23.3 vs 22.9 days in the control group). - Early implantation loss in 4/26 HD. <u>Litters (F₁)</u> - Increased sexual maturation time for males at HD. - Incomplete balanopreputial separation at the time of pairing for mating in 6 males at HD. - Reduced mating, fertility, and fecundity indices and a reduced number of pregnant females at HD. - No effect on pregnancies. 1 male at MD: incomplete balanopreputial separation at the time of pairing for mating and did not mate with its female. See Study No. 8382261 in Appendix for study detail.

10 Special Toxicology Studies

Mechanistic Study: Effect on CNS system in female rats	
Study No & Designs	Study Findings
8306209 * (non-GLP) FEMALE rat, oral gavage @ 0, 30, 100 and 300 mg/kg/day fezolinetant for 13 weeks	Test article related clinical signs observed involved changes in muscle tone including twitching, intermittent tremors, and rigid stance. These clinical signs increased in frequency and number of animals affected in a dose dependent manner. No seizures were detected throughout the study based on EEG records. There was no indication that administration of fezolinetant was correlated with increased risk of seizure.

* PT reviews under IND 130277 in DARRTS dated 05/31/16.

Dependence Studies																																																																														
Study No & Designs	Study Findings																																																																													
8416428 (GLP) – physical dependency Rat, Oral gavage @ 0, 10, 30, and 100 mg/kg/day fezolinetant for 4 weeks. NOAEL = 100 mg/kg/day Safety Margin: 108X MRHD (female only) , based on AUC ₀₋₉₆ = 418000 ng•h/mL on Week 4 when compared to AUC ₂₄ of 3855 ng•h/mL, obtained from clinical Study No. 2693-PK-0010. For metabolite ES00259564: 15X MRHD, based on the AUC ₀₋₉₆ = 39200 ng•h/mL on Week 4 when compared to AUC ₂₄ = 2570 ng•h/mL obtained from clinical Study No. 2693-PD-0010.	Fezolinetant administered for 28 days did not produce a withdrawal syndrome. Fezolinetant and ES00259564 were not detected at all dose levels by 72 and 48 hours post dose, respectively. Toxicokinetic Parameters in Rat Plasma following Daily Oral Gavage Administration: Day 28 <table><tr><th colspan="2">ESN-364</th><th colspan="4">ESN-364</th><th colspan="4">ES259564</th></tr><tr><th>mg/kg/day</th><th>Sex</th><th>C_{max} (ng/mL)</th><th>T_{max} (h)</th><th>AUC₀₋₉₆ (h•ng/mL)</th><th>T_{1/2} (h)</th><th>C_{max} (ng/mL)</th><th>T_{max} (h)</th><th>AUC₀₋₉₆ (h•ng/mL)</th><th>T_{1/2} (h)</th></tr><tr><td rowspan="2">10</td><td>M</td><td>5140</td><td>2</td><td>77400</td><td>7.57</td><td>313</td><td>6</td><td>4080</td><td>NR</td></tr><tr><td>F</td><td>4420</td><td>2</td><td>52500</td><td>6.76</td><td>281</td><td>2</td><td>2530</td><td>NR</td></tr><tr><td rowspan="2">30</td><td>M</td><td>14700</td><td>4</td><td>221000</td><td>6.06</td><td>1280</td><td>4</td><td>17100</td><td>NR</td></tr><tr><td>F</td><td>11400</td><td>2</td><td>139000</td><td>6.09</td><td>844</td><td>2</td><td>8830</td><td>NR</td></tr><tr><td rowspan="2">100</td><td>M</td><td>49500</td><td>4</td><td>775000</td><td>NR</td><td>4990</td><td>6</td><td>91500</td><td>NR</td></tr><tr><td>F</td><td>34200</td><td>2</td><td>418000</td><td>4.50</td><td>2890</td><td>4</td><td>39200</td><td>NR</td></tr></table> F = Female; M = Male; NR - Not reportable [Extracted from the study report 8416428 page 10] Codeine at 150 mg/kg/day (positive control) produced a marked withdrawal syndrome which was primarily characterized by decreased body temperature, food consumption, body weight gain, and general activity; increased pain response and vocalization; and thigmotaxis (wall hugging arena location).	ESN-364		ESN-364				ES259564				mg/kg/day	Sex	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₉₆ (h•ng/mL)	T _{1/2} (h)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₉₆ (h•ng/mL)	T _{1/2} (h)	10	M	5140	2	77400	7.57	313	6	4080	NR	F	4420	2	52500	6.76	281	2	2530	NR	30	M	14700	4	221000	6.06	1280	4	17100	NR	F	11400	2	139000	6.09	844	2	8830	NR	100	M	49500	4	775000	NR	4990	6	91500	NR	F	34200	2	418000	4.50	2890	4	39200	NR
ESN-364		ESN-364				ES259564																																																																								
mg/kg/day	Sex	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₉₆ (h•ng/mL)	T _{1/2} (h)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₉₆ (h•ng/mL)	T _{1/2} (h)																																																																					
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	F	34200	2	418000	4.50	2890	4	39200	NR																																																																					
8416427 (GLP, DRF) - self-administration study Rat, IV bolus (single) @ 0.25, 0.5, and 0.75 mg/kg/0.2 mL fezolinetant [prepared in 9% (w/v) HPβCD and 1% DMSO, prepared in 0.9% (w/v) saline]	Formulation analysis revealed 1.0 mg/kg per 0.2 mL was unsuitable for the main study due to the solubility. The highest dose (0.75 mg/kg per 0.2 mL) was determined because the C _{max} was approximately 3-fold the human C _{max} at the MRHD and no noteworthy finding was observed.																																																																													

Summary of the Mean ESN-364 Toxicokinetic Parameters in Rat Plasma							
Dose Group	Dose Level (mg/kg/0.2mL)	Sex	C ₀ (ng/mL)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂ (h*ng/mL)	T _{1/2} (h)
1	0.25	M	468	439	0.0167	338	2.27
		F	525	496	0.0167	448	3.01
		MF	496	467	0.0167	393	2.71
2	0.5	M	869	815	0.0167	795	4.67
		F	803	764	0.0167	810	4.32
		MF	836	790	0.0167	803	4.46
3	0.75	M	1450	1370	0.0167	1370	4.04
		F	1190	1130	0.0167	1110	6.54
		MF	1320	1250	0.0167	1240	5.29
AUC ₀₋₂ = Area under the curve from time 0 to 2 hours; C ₀ = Back-extrapolated concentration at time 0; C _{max} = Maximum observed concentration; T _{1/2} = Elimination half-life; T _{max} = Time of maximum observed concentration.							
[Extracted from the study report 8416427 page 19]							

8416429 (GLP, definitive) - self-administration study Rat, IV self-administration via lever press (in response to a cocaine reward) @ 0.25, 0.5, and 0.75 mg/kg/0.2 mL fezolinetant [prepared in 9% (w/v) HPβCD and 1% DMSO, prepared in 0.9% (w/v) saline]	Fezolinetant did not produce reinforcing effects in the male or female rats. The difference in response pattern was not statistically significant compared with the vehicle control. Intravenous self-administration of 0.05 mg/kg/0.2 mL infusion of amphetamine (at 0.05 mg/kg/0.2 mL infusion as positive control) resulted in a statistically significant, increased, and stable response pattern, compared with the vehicle control. Amphetamine produced the anticipated reinforcing effects.
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Genotoxicity Studies: The Main Metabolite (ES00259564)	
Study No & Designs	Study Findings
8390040 (GLP) In vitro, chromosomal aberration, up to 374.4 µg/mL (Short-term treatment for 3 hours plus recovery for 17 hours with and without S-9 and continuous treatment for 20 hours without S-9)	ES00259564 had no potential to induce chromosomal aberrations.
8390041 (GLP) In vitro, AMES, up to 5000 µg/plate ES00259564	ES00259564 had no potential to induce gene mutation.

Impurities	
Study No & Designs	Study Findings
210013 Two QSAR methods: rule-based and statistical-based.	63 impurities which may be present in fezolinetant drug product were evaluated. The assessment was performed per ICHM7. These impurities were classified into Class 1 to 5, per ICHM7. All impurities have been controlled at acceptable levels. Refer to Section 2.5 Comments on Impurities/Degradants of Concern for details.
501-158 (GLP) Monkey, oral gavage @ (b) (4) mg/kg/day (b) (4) for 13 weeks	No Effect.

NOAEL = (b) (4) mg/kg/day, (b) (4) X the maximum content of (b) (4) ((b) (4) mg/kg per day) at the MRHD assuming a 60 kg human.	
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Phototoxicity	
Study No & Designs	Study Findings
8390039 (GLP) In vitro, concentration tested 0.0316 to 100 µg/mL of fezolinetant. Irradiation Condition: UV-A 1.24 mW/cm ² (5 J/cm ² UV-A) for 67 minutes.	No decrease in cell survival both with and without UV irradiation. Fezolinetant was not phototoxic in in vitro Balb/c 3T3 Neutral Red Uptake assay when tested up to 100 µg/mL.

11 Integrated Summary and Safety Evaluation

Astellas submitted NDA 216578 for fezolinetant (ESN364) as an immediate release tablet to treat moderate to severe VMS associated with menopause. The recommended dose of fezolinetant is 45 mg once daily. Fezolinetant is a NK3 receptor antagonist. By antagonizing NKB/NK3-mediated signaling, fezolinetant modulates KNDy neuronal activity in the thermoregulatory center in the hypothalamus to treat moderate to severe VMS associated with menopause. The Applicant has submitted a full nonclinical program to support their NDA, including pharmacology, safety pharmacology, pharmacokinetics, repeat dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and phototoxicity, as well as impurity assessment.

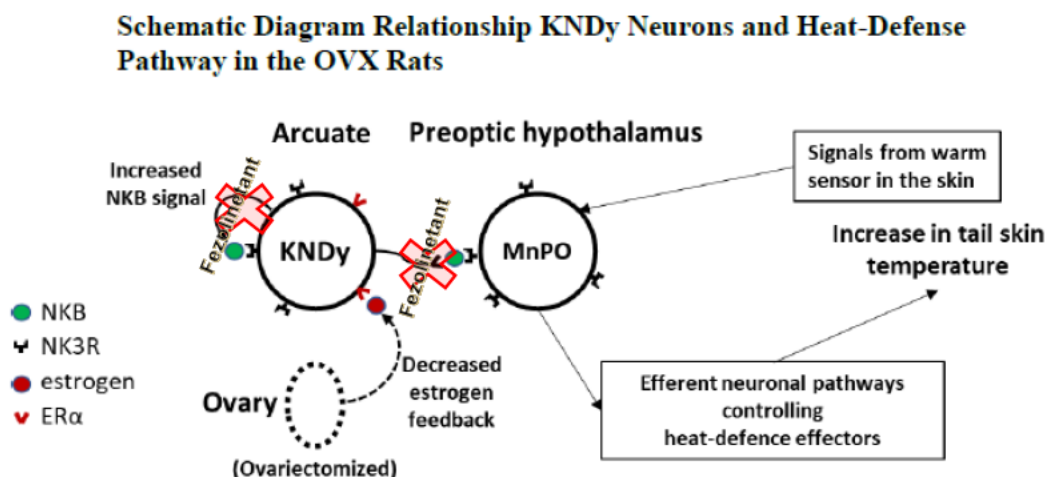
Pharmacology

The in vitro radioligand binding assay was performed on membranes prepared from cells expressing human, rhesus monkey and rat NK3 receptors. Binding studies were also performed on membranes prepared from cells expressing the other members of tachykinin receptor family (hNK1 and hNK2) and other G-protein coupled receptors including those known to be implicated in modulation of the GnRH axis. Fezolinetant was found to be approximately 450X more selective for the hNK3 receptor than for the hNK1 or hNK2 receptors in vitro. Functional activity assays were conducted to examine the agonist and antagonist activities of fezolinetant on the hNK3 receptor expressed in CHO cells. These studies demonstrated that fezolinetant was a potent antagonist of the hNK3 receptor and had no agonist activity on this receptor. Fezolinetant had a similar affinity for human and rhesus monkey NK3 receptors and 8-fold less for rat NK3 receptors. The major metabolite of fezolinetant, ES00259564 was less potent at the hNK3 receptor compared with fezolinetant and has no significant off-target activities.

In vivo effects of fezolinetant on hot flash-like symptoms and activation of MnPO neurons were investigated in OVX female rats, a nonclinical model of postmenopause. Effects of fezolinetant on hormones were investigated in rats and cynomolgus monkeys. These studies demonstrated that OVX rats exhibited several typical menopause symptoms: hyperphagia, increased body weight, significantly decreased plasma estradiol levels, increased LH and FSH levels, and significantly increased tail skin

temperature (**Figure 6**). Increased neuronal activity in the MnPO hypothalamic neurons was also observed in these OVX rats (**Figure 6**). Repeated oral administration of fezolinetant reduced hyperphagia and weight gain, reduced plasma LH levels, without affecting estradiol or FSH levels, and attenuated hot flash-like symptoms in these OVX rats (**Figure 6**). Fezolinetant also inhibited the activation of MnPO neurons (**Figure 6**).

Figure 6 Mechanism of Action of Fezolinetant (as NK3 Receptor Antagonist)



ERα: estrogen receptor α; KNDy: kisspeptin-neurokinin B-dynorphin neuron; MnPO: median preoptic nucleus; NK3R: neurokinin 3 receptor; NKB: neurokinin B

Safety pharmacology

Core battery studies and a cardiovascular follow-up study were also conducted. Except for in vitro cardiovascular follow-up study in isolated rabbit hearts, all the safety pharmacology studies were conducted according to the “Guidelines of the ICH S7A, Safety Pharmacology Studies for Human Pharmaceuticals” in compliance with GLP. These studies showed that 1) fezolinetant poses little CNS risk to patients at the MRHD, 2) fezolinetant has little or no cardiovascular effects at the MRHD, and 3) fezolinetant is unlikely to induce effects on the respiratory system at clinically relevant doses. See **Table 6** for study key findings and safety margins based on exposure.

Table 6 Key Findings and Safety Margin from Safety Pharmacology Studies.

Study	The Effects of Fezolinetant	NOAEL	Safety Margin
CNS in rats @ 0, 25, 125, and 250 mg/kg	- Constricted pupils at 125 mg/kg - Decreased activity, touch escape response and etc. at 250 mg/kg	25 mg/kg C_{max} = 39100 ng/mL at 30 mg/kg on Day 1 in the 4-week rat study	85X*
In vitro hERG @ 3 - 837.1 μ mol/L	Inhibition of potassium currents via the hERG channel in HEK293 cells.	IC_{50} = 231.8 μ mol/L (~ 83074.8 ng/mL)	371X**
CV in monkeys @ 0, 10, 25, and 50 mg/kg	- No effect on ECG $\downarrow$ mean $+dP/dt_{max}$, $\downarrow$ systolic and mean arterial blood pressure, and $\uparrow$ heart rate (not significant)	50 mg/kg C_{max} = 26800 ng/mL at 50 mg/kg on Day 1 in the 5-week monkey study	59X*
In vitro, isolated rabbit hearts @ 3 and 30 μ mol/L	No effect on ECG or mechanical parameters	30 μ mol/L (~ 10800 ng/mL)	59X**
Respiratory in rats @ 0, 40, 80, and 125 mg/kg	$\downarrow$ tidal volume and $\uparrow$ respiratory rate and minute volume at all dose levels in dose dependent manner.	< 40 mg/kg C_{max} = 39100 ng/mL at 30 mg/kg on Day 1 in the 4-week rat study	59X*

* C_{max} = 458 ng/mL at steady state [Report 2693-PK-0010] at the MRHD (45 mg QD).

**Human $C_{max,u}$ = 224 ng/mL at MRHD (45 mg QD), calculated for 450 ng/mL using human protein binding ratio.

Pharmacokinetics

To clarify characteristics of absorption, distribution, metabolism, and excretion of fezolinetant, a series of in vitro and in vivo studies in mice, rats, rabbits and cynomolgus monkeys were conducted using [^{14} C]fezolinetant and non-radiolabeled fezolinetant.

After oral administration in rats, fezolinetant was rapidly and highly absorbed and distributed into all tissues of the body, with the greatest extent of fezolinetant-related material observed in the liver at approximately 1 h after administration. In addition, fezolinetant-derived components were found to pass through the placenta and transfer to the fetus as well as being transferred to breast milk of lactating rats. In rats, cynomolgus monkeys and humans, fezolinetant was primarily metabolized to the hydroxylated metabolite ES00259564, and then excreted mainly via urine, indicating no marked species difference in the major elimination (metabolism and excretion) route.

Since ES00259564 is the major metabolite in human plasma (i.e., approximately 50% of total fezolinetant-related AUC exposure; [Clinical Study ESN364-CPK-103 CSR](#)), the systemic exposure to the metabolite after single oral administration of 45 mg fezolinetant to humans (MRHD) was compared to those in the repeated-dose toxicity studies of fezolinetant in rats (26 weeks) and cynomolgus monkeys (39 weeks). The systemic exposure to ES00259564 in rats and cynomolgus monkeys at NOAEL (200

and 25 mg/kg per day, respectively) was 36X and 26X higher than that in humans, respectively (**Table 7**). Therefore, the safety of ES00259564 was appropriately evaluated in the nonclinical toxicity studies.

Repeated-dose toxicity

Oral gavage studies were conducted in rats and cynomolgus monkeys with fezolinetant (4-, 13- and 26-week administrations in rats; 5-, 13- and 39-week administrations in cynomolgus monkeys). The studies ≥ 13 weeks were conducted in FEMALE rats or monkeys only. See **Table 7** for study key findings and safety margins based on exposure.

Table 7 Key Findings and Safety Margin from Pivotal Repeat Dose Toxicity Studies.

Rat Study	The Effects of Fezolinetant	NOAEL	Safety Margin*
Daily dosing for 26 weeks with TK assessment @ 0, 30, 100, and 200 mg/kg/day fezolinetant	↑ Total cholesterol levels at all doses. ↑ Liver weight at MD and HD. ↑ ALP levels at HD. ↑ Thyroid (included parathyroid) weight at HD. <u>Microscopic Findings:</u> - Liver: ↑ centrilobular hepatocellular hypertrophy at MD and HD (143X and 148X MRHD, respectively), attributed to a rodent specific adaptive effect. - Nasal (eosinophilic globules in the olfactory epithelium), lungs (infiltrates of macrophages in the alveolus), and thyroid (follicular cell hypertrophy, attributed to adaptive reaction) at all doses - Uterus (atrophy) at all doses, vagina (mucification of the epithelium) at all doses, and cervix (mucification of the epithelium) at MD and HD, attributed to exaggerated pharmacological effects of fezolinetant. <u>Note:</u> The sedation-like CNS findings observed in 4-week rat study at 300 mg/kg/day and 13-week rat study at 100 mg/kg/day were not observed in this study. In addition, the convulsive-like episode noted in the 13-week rat study was not present in this study and these episodes were reduced by a change in animal handling procedure.	200 mg/kg <u>Fezolinetant</u> AUC₂₄ = 569000 ng•h/mL on Week 26 <u>ES00259564</u> AUC₂₄ = 93200 ng•h/mL on Week 26	148X 36X

*AUC₂₄ = 3855 (fezolinetant) and 2570 ng•h/mL (ES00259564) at steady state [Report 2693-PK-0010] at the MRHD (45 mg QD).

Monkey Study	The Effects of Fezolinetant	NOAEL	Safety Margin*
Daily dosing for 39 weeks with TK assessment and 4-week recovery @ 0, 10, 25, and 40 mg/kg/day fezolinetant	1 HD animal death (sacrificed due to an acute hemorrhagic anemia due to severe thrombocytopenia, 102X MRHD) ↓ platelet counts in 2 (out of 6) HD animals (reversible). - Thrombocytopenia in 1 HD animal (reversible). ↓ body weight gain, ↓ body weight and ↓ food consumption at HD. - Disturbed or interrupted menstrual cycles and ovarian inactivity (reversible) at all doses. <u>Microscopic findings:</u> Ovary (absence of corpora lutea and follicular cyst, reversible) and uterus (stromal atrophy, reversible) and mammary gland (decreased gland, present after recovery) at all doses, attributed to exaggerated pharmacological effects of fezolinetant. ↓ fat content at HD (recoverable during dosing phase) but no changes in muscle or bone mass (with DEXA scan). <u>Note:</u> Thrombocytopenia was also observed in 13-week monkey study at HD.	25 mg/kg <u>Fezolinetant</u> $AUC_{24} = 166000$ ng•h/mL on Week 39	43X
		<u>ES00259564</u> $AUC_{24} = 65900$ ng•h/mL on Week 39	26X

* $AUC_{24} = 3855$ (fezolinetant) and 2570 ng•h/mL (ES00259564) at steady state [Report 2693-PK-0010] at the MRHD (45 mg QD).

Genotoxicity

Studies were conducted using bacteria (Ames, up to 5000 µg/plate), cultured human lymphocytes (up to 358.4 µg/mL = 1 mmol/L), and rats (up to 300 mg/kg/day). Fezolinetant had no potential to induce gene mutation and chromosomal aberrations in vitro nor in vivo.

Carcinogenicity

The 2-year rat study (female only) and 26-week transgenic study were conducted. See **Table 8** for key study findings and safety margins based on exposure. Both studies showed fezolinetant has no carcinogenic potential.

Study	The Effects of Fezolinetant	NOAE/NOEL (Female Only)	Safety Margin*
2 years, rat @ 0, 10, 30, and 100 mg/kg/day	- No effect on survival. - Clear oral discharge at HD and hypoactivity at MD and HD. ↑ mean body weight gain at MD and HD through Week 38, all doses through Week 26. ↑ mean food consumption at HD through Week 42. ↑ incidences of benign thyroid follicular adenoma (significant by trend analysis only, $p = 0.0094$). Thyroid follicular cell hypertrophy was observed in repeat dose toxicity studies in rats. <i>This was not considered toxicologically relevant by FDA statistical analysis.</i> ↑ incidences of combined ovarian epithelial tumors (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma); significant by pairwise analysis only, $p = 0.0132$) in MD females when compared to vehicle control, with no corresponding statistically significant dose response relationship. <i>This was not considered toxicologically relevant by FDA statistical analysis.</i> - Non-tumor findings were noted in the liver, uterus, mammary gland, ovary, cervix, and adrenal. Except liver (associated with liver enzyme induction as adaptive change), all other changes were considered an exaggerated pharmacological effect of fezolinetant.	100 mg/kg/day (NOEL) Fezolinetant AUC₂₄ = 718000 ng•h/mL on Week 26 ES00259564 AUC₂₄ = 79900 ng•h/mL on Week 26	186X 31X
6 months, transgenic mouse @ 0, 50, 140, and 450 mg/kg/day	- No effect on survival. ↑ mean body weight gain at HD (12.5 and 25% in males and females, respectively). ↑ mean food consumption at MD and HD. - White teeth at MD and HD. - No tumor observed. - Non-tumor findings were noted in liver, ovary, and vagina at MD and HD.	450 mg/kg/day (NOEL) Fezolinetant AUC₂₄ = 183000 ng•h/mL on Week 39 ES00259564 AUC₂₄ = 366000 ng•h/mL on Week 39	47X 142X

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Reproductive and developmental toxicity

Studies were conducted in rats and rabbits. See [Table 9](#) for study key findings and safety margins based on exposure. Fezolinetant caused embryotoxicity in both rats and rabbits and had adverse developmental effect in F₁ generation in rats.

Table 9 Key Findings and Safety Margin from Reproductive and Developmental Toxicity Studies.

Study	The Effects of Fezolinetant	NOAEL	Safety Margin*
Fertility and early embryonic development, rat @ 0, 10, 30, and 100 mg/kg/day	↓ numbers of estrus cycle at HD ↑ mean cycle length at HD ↓ uterus, cervix, vagina weight at HD - No effect on reproductive indices (mating and fertility indices)	100 mg/kg/day (fertility) <u>Fezolinetant</u> AUC ₂₄ = 551900 ng/mL at 100 mg/kg on Day 28 obtained from the 4-week rat study	143X
Embryofetal development, rat @ 0, 25, 50, and 100 mg/kg/day	- No maternal toxicity. - Total litter loss at 3 HD females.	50 mg/kg/day (embryotoxicity) <u>Fezolinetant</u> AUC ₂₄ = 238700 ng•h/mL on GD 17	62X
Embryofetal development, rabbit @ 0, 45, 75, and 125 mg/kg/day	↓ body weight gain and food consumption throughout dosing period at all doses. - Total embryo loss prior to c-section in 4 HD females and litters aborted in 3 HD females. - Red discharge (related to abortion or to loss of pregnancy) in MD females. ↑ % post-implantation loss, early and late resorptions, ↓ uterine weight at HD. ↓ fetal weight in both males and females at HD, slight ↓ fetal weight in males at MD. ↑ numbers of fetuses with nodulated ribs at MD and HD.	45 mg/kg/day (embryotoxicity) <u>Fezolinetant</u> AUC ₂₄ = 60600 ng•h/mL on GD 19	16X

*AUC₂₄ = 3855 (fezolinetant) and 2570 ng•h/mL (ES00259564) at steady state [Report 2693-PK-0010] at the MRHD (45 mg QD). Values based on maternal exposure.

Study	The Effects of Fezolinetant	NOAEL	Safety Margin*
Pre- and post-natal development, rat @ 0, 10, 30, and 100 mg/kg/day	<u>Maternal/Embryo</u> - Early implantation loss in 4/26 HD females. ↑ duration of gestation at HD <u>F₁ Development</u> - Delayed preputial separation in some males at MD and HD. ↑ time to sexual maturation for males with some not achieving complete balanopreputial separation at time of pairing for mating at HD. ↓ number of mated and fertile males and ↓ number of pregnant females but no effect on pregnancies at HD.	30 mg/kg/day (embryotoxicity)	
		<u>Fezolinetant</u> AUC ₂₄ = 140000 ng•h/mL on LD 21	36X
		<u>ES00259564</u> AUC ₂₄ = 14700 ng•h/mL on LD 21	6X
		100 mg/kg/day (F ₁ female, development)	
		<u>Fezolinetant</u> AUC ₂₄ = 788000 ng•h/mL on LD 21	204X
		<u>ES00259564</u> AUC ₂₄ = 91100 ng•h/mL on LD 21	35X
		10 mg/kg/day (F ₁ male, development)	
		<u>Fezolinetant</u> AUC ₂₄ = 42500 ng•h/mL on LD 21	11X
		<u>ES00259564</u> AUC ₂₄ = 4600 ng•h/mL on LD 21	2X

*AUC₂₄ = 3855 (fezolinetant) and 2570 ng•h/mL (ES00259564) at steady state [Report 2693-PK-0010] at the MRHD (45 mg QD). Values based on maternal exposure.

Others

Other toxicity studies included a mechanistic study in rats, drug dependency studies in rats, genotoxicity studies of the major metabolite (ES00259564), a repeated-dose toxicity study of an impurity in cynomolgus monkeys and a phototoxicity study using cultured mammalian cells). Key findings from these studies include:

- Fezolinetant had no potential to induce seizures in a 13-week oral study in rats.
- Fezolinetant did not induce physical dependency in a 4-week oral study and a single dose IV study in rats.
- ES00259564 did not induce gene mutation and chromosomal aberration in vivo.
- An impurity ((b) (4)) showed no toxicity in a 13-week oral study in monkeys at ((b) (4)) X MRHD.
- Fezolinetant did not induce phototoxicity in vivo.

Conclusion

In conclusion, fezolinetant is a small molecule NK3 receptor antagonist which decreases the activity of KNDy neurons by antagonizing NKB/NK3 signaling with the outcomes of relief from VMS. The pharmacology, pharmacokinetics, and toxicology of fezolinetant were characterized in nonclinical studies to support this application for the

treatment of moderate to severe VMS associated with menopause. Pharm/tox supports approval of this application.

12 Appendix/Attachments

Study No. [8382261](#)

Study title: ESN-364: Oral (Gavage) Study of Pre- and Postnatal Development in the Rat

Study no.:	8382261
Study report location:	eCTD 0001, Module 4.2.3.5.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	01/02/2019
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	N12013F, 97.5%

Key Study Findings

- The NOAEL for maternal toxicity and embryotoxicity = 30 mg/kg/day ($AUC_{24} = 140,000 \text{ ng}\cdot\text{h/mL}$ on LD 21, 36X MRHD), based on delayed parturition and in utero embryo-lethality for 4 HD females.
- The NOAEL for F_1 generation development was = 100 mg/kg/day (F1 females, $AUC_{24} = 788000 \text{ ng}\cdot\text{h/mL}$ on LD 21, 204X MRHD) and 10 mg/kg/day (F1 males, $AUC_{24} = 42500 \text{ ng}\cdot\text{h/mL}$ on LD 21, 11X MRHD), based on the changes in fertility mainly due to incomplete balanopreputial separation in MD and HD males.

Methods

Doses:	0 (vehicle), 10, 30, and 100 mg/kg/day
Frequency of dosing:	Once daily, GD 6 to lactation day (LD) 21
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Crl:WI (Han)
Number/Sex/Group:	22 or 26 (Group 4) time-mated females - parental (F_0) generation
Satellite groups:	N/A
Study design:	Assessment of toxicity was based on clinical observations, body weights, food consumption, pregnancy indices, parturition, and offspring development. Pup physical development was evaluated through pinna unfolding, incisor eruption, and eye opening. Functional development was evaluated by surface righting and air righting.

Following weaning, the F_1 selected animals in the fertility subgroup were assessed through maturation for sexual maturation. At approximately 10 weeks of age, the F_1 generation in the fertility subgroup was mated and the outcome of mating was assessed.

Following weaning, the F₁ selected animals in the neurobehavior subgroup were assessed through acoustic startle, motor activity, and learning and memory in the Morris swim maze.

Deviation from study protocol: No impact on integrity or interpretability of the results of the study.

Observations and Results

F₀ Dams

Survival: No effect.
 Clinical signs: No effect.
 Body weight: No effect.
 Feed consumption: No effect.

Uterine content:

- Increased implantation loss at HD. Reduced gestation index at HD. Loss of pregnancy in 1 control, 2 MD and 4 HD females ([Table 10](#)). Note: The Applicant explained that 2 *in utero* loss of pregnancy at MD was only an increase of one over the concurrent controls. In their historical control data between 2015 and 2018, no females were observed with 100% in utero implantation losses, indicating that the concurrent control was unusual and needed to be considered in setting the test article-related effect level. The Applicant further noted that in their embryofetal development rat study (Study No. 8297366), there was no 100% in utero loss at 50 mg/kg/day. Therefore, the Applicant considered that this pregnancy loss in 2 MD females were not test-article related. This justification is reasonable based on the data presented. The loss of pregnancy at the HD is considered treatment related.
- Increased gestation duration at HD (23.3 vs 22.9 days) with 6 dams showed parturition on Day 24 but no correlation to stillborn pups in the litter
- No effect on parturition, the outcome of littering, or pup survival.

Necropsy observation: No effect.
 Toxicokinetics: Exposure, as assessed by ESN364 and ES00259564 C_{max} and AUC₀₋₂₄, increased with the

increase in ESN364 dose level from LD to HD (**Table 11**).

- The increases in C_{max} and AUC_{0-24} values were generally dose proportional, with the exception of ESN364 AUC_{0-24} and ES00259564 C_{max} and AUC_{0-24} values between MD and HD on LD 21, which appeared to be greater than dose proportional.

No accumulation of ESN364 at all dose levels and ES00259564 at LD and MD was observed after multiple doses of ESN364 in rats.

- Potential accumulation of ES00259564 at HD was observed. On Day 6, the metabolite to parent ratios ranged from 0.0624 to 0.0656 for C_{max} and from 0.0617 to 0.0696 for AUC_{0-24} . On LD 21, the metabolite to parent ratios ranged from 0.0718 to 0.107 for C_{max} and from 0.105 to 0.116 for AUC_{0-24} .

Dosing Solution Analysis

Mostly within acceptance criteria, no effect on dosing outcome for the animals.

Other:

N/A

Table 10 F₀ Generation – Summary of Implantation Data

Implantation Data for Animals That Did Not Litter				
Group/Dose Level	Day of Necropsy	Total Number of Corpora Lutea	Total Number of Implantations	Status of Implantations
Group 1: Control				
R0011	GD 26	17	1	Early Resorptions
Group 2: 10 mg/kg/day				
	-	-	-	-
Group 3: 30 mg/kg/day				
R0214	GD 26	10	1	Early Resorptions
R0215	GD 26	15	7	Early Resorptions
Group 4: 100 mg/kg/day				
R0309	GD 26	15	14	Early Resorptions
R0310	GD 26	16	13	Early Resorptions
R0319	GD 26	12	11	Early Resorptions
R0321	GD 26	9	9	Early Resorptions

GD = Gestation Day.

[Extracted from the study report [8382261](#) page 36]

Table 11 Summary of ESN364 and ES00259564 TK Parameters in Female Rat Plasma.**Summary of the ESN-364 Toxicokinetic Parameters in Female Rat Plasma**

Day	Dose Group	Dose Level (mg/kg/day)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (h*ng/mL)	t _{1/2} (h)
GD 6	2	10	6780	1.00	77900	6.21
	3	30	22600	2.00	227000	5.09
	4	100	58100	2.00	884000	7.18
LD 21	2	10	7090	1.00	42500	3.42
	3	30	20800	1.00	140000	2.59
	4	100	84500	4.00	788000	NC

AUC₀₋₂₄ = Area under the concentration-time curve from 0 to 24 hours postdose, estimated by the linear trapezoidal rule; C_{max} = Maximum observed concentration; GD = Gestation Day; LD = Lactation Day; NC = Not calculated; t_{1/2} = Elimination half-life; T_{max} = Time of maximum observed concentration.

[Extracted from the study report [8382261](#) page 32]

Summary of the ES259564 Toxicokinetic Parameters in Female Rat Plasma

Day	Dose Group	Dose Level (mg/kg/day)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (h*ng/mL)	t _{1/2} (h)
GD 6	2	10	423	2.00	4810	NC
	3	30	1470	2.00	14600	NC
	4	100	3810	6.00	61500	NC
LD 21	2	10	571	2.00	4610	NC
	3	30	1500	1.00	14700	NC
	4	100	9010	4.00	91100	NC

AUC₀₋₂₄ = Area under the concentration-time curve from 0 to 24 hours postdose, estimated by the linear trapezoidal rule; C_{max} = Maximum observed concentration; GD = Gestation Day; LD = Lactation Day; t_{1/2} = Elimination half-life; T_{max} = Time of maximum observed concentration; NC = Not calculated.

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F₁ Generation

Survival:

No effect.

Clinical signs:

No effect.

Body weight:

Lower mean body weight gain in HD males during the F₁ maturation phase in the fertility (paring days 7-14) and neurobehavior subgroups (MDs 22-64), attributed to a period of change, activity, and possibly stress.

Feed consumption:

--

Physical development:

No effect.

Neurological assessment:

No effect on motor activity, learning and memory, and acoustic startle.

(Postweaning in the neurobehavior subgroup)

Reproduction:

(Postweaning in the fertility subgroup)

Sexual maturation (characterized by balanopreputal separation)

- Increased number of days to sexual maturation in HD males (PND 57 vs PND 50 control). This was above their historical control of PND 43 to 52.

- Incomplete preputial separation in MD or HD males with no confirmed mating (**Table 12**).
- No effect in females.

Estrous cycle

No effect in the duration of estrous cycles in F₁ females prior to pairing.

Reproductive indices

Reduction in mating, fecundity, and fertility indices in MD and HD males (**Table 13**).

Table 12 Summary of Males with Incomplete Preputial Separation Prior to Mating.

Males Not Demonstrating Complete Balanopreputial Separation at the Time of Mating or Not Showing Evidence of Mating When Paired with a Female				
Group	Animal Number	Incomplete Preputial Separation on Day of Maturation	Comments	Mating and Fertility Outcome
Maternal Dose Level		Phase		
2	R0513	76	Not examined on day of necropsy	Mated; female (Animal R0912) was pregnant.
10 mg/kg/day	R0614	76	Not examined on day of necropsy	No mating was confirmed (Animal R1013); the female was showing estrous cyclicity during the first 3 days of the pairing phase and then went into diestrous.
30 mg/kg/day				
Group 4 100 mg/kg/day	R0708	75	Post-pairing Day 13, Male examined on day of necropsy. Preputial separation still incomplete	No mating was confirmed (Animal R1107). The female was showing estrous cyclicity during the pairing phase.
	R0709	75	Post-pairing Day 13, Male examined on day of necropsy. Preputial separation still incomplete	No mating was confirmed (Animal R1108). The female was showing estrous cyclicity during the pairing phase.
	R0710	75	Post-pairing Day 13, Male examined on day of necropsy. Preputial separation complete	No mating was confirmed (Animal R1109). The female was showing estrous cyclicity during the pairing phase.

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Group	Maternal Dose	Animal Number	Incomplete Preputial Separation on Day of Maturation Phase	Comments	Mating and Fertility Outcome
Group 4	100 mg/kg/day	R0713	76	Not examined on day of necropsy	Male mated with female (Animal R1112), but the female was not pregnant. The female was showing estrous cyclicity during the pairing phase.
(Continued)		R0717	75	Not examined on day of necropsy	Male mated with female (Animal R1116), but the female was not pregnant. The female was showing estrous cyclicity during the pairing phase.
		R0718	75	Not examined on day of necropsy	Male mated with female (Animal R1117), but the female was not pregnant. Mated within one estrous cycle.
		R0707	Recorded as complete on Maturation Day 75		No mating was confirmed (Animal R1106). The female was showing estrous cyclicity during the pairing phase.
		R0712	Recorded as complete on Maturation Day 60		No mating was confirmed (Animal R1120). Female showed estrous cyclicity. This female did not mate with a proven male (Animal R0715).

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Table 13 Summary of Males Reproduction Indices.

Males Reproductive Indices			
Index	Mating Index %	Fecundity Index %	Fertility Index %
Historical Control Range	80-100	84-100	75-100
Concurrent Control	100	95	95
Group 3 Males (30 mg/kg/day)	85	94	80
Group 4 Males (100 mg/kg/day)	75*	67*	50**

* Fisher 1-tail Descending Test significant at the 0.05 level.

** Fisher 1-tail Descending Test significant at the 0.01 level.

[Extracted from the study report [8382261](#) page 43]

F₂ Generation (litters)

No effects on gestation parameters ([Table 14](#)).

Table 14 Summary of C-section Data.

		Summary of Cesarean Section Data - F₁ Generation			
		Group (Fertility Subgroup)			
		Control	10 mg/kg	30 mg/kg	100 mg/kg
Number of females pregnant at cesarean section	N	19	19	18	13
Corpora Lutea	N	19	19	18	13
	Mean	15	14	13	14
	SD	1.6	2.4	2.2	2.2
Implantation Sites	N	19	19	18	13
	Mean	13	11	12	13
	SD	2.1	3.3	2.7	1.6
Pre-implantation Loss	N	19	19	18	13
	Mean	1	3	1	1
	SD	1.7	3.9	1.5	1.5
Pre-implantation Loss (%)	N	19	19	18	13
	Mean	8.1	16.7	8.1	8.3
	SD	10.93	24.70	13.27	8.41
Early Resorptions	N	19	19	18	13
	Mean	1	1	1	1
	SD	1.5	1.5	1.7	0.8

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**PHARMACOLOGY/TOXICOLOGY
SECONDARY REVIEW**

NDA #:	216578 (IND 130277)
Supporting Document #:	SDN 1; eCTD 0001
Received Date:	June 22, 2022
PDUFA Goal Date:	February 22, 2023
Sponsor:	Astellas Pharma Inc.
Drug:	Fezolinetant
Proposed Tradename:	VEOZAH
Pharmacologic Class:	Neurokinin 3 receptor antagonist
Indication:	Moderate to severe vasomotor symptoms (VMS) associated with menopause
Secondary Reviewer:	Andrea Benedict, PhD
Tertiary Reviewer:	Mukesh Summan, PhD, DABT
Primary Reviewer:	Miyun Tsai-Turton, PhD, MS
Pharm/Tox Division:	Division of Pharm/Tox for Rare Diseases, Pediatrics, Urologic and Reproductive Medicine (DPT-RPUM)
Clinical Review Division:	Division of Urology, Obstetrics and Gynecology (DUOG)
Recommendation on Regulatory Action:	Approval
Review Date:	November 22, 2022

Pharmacology/Toxicology Recommendation:

I agree with the nonclinical reviewer that there are no nonclinical concerns for the safety of fezolinetant 45 mg orally once daily in the general population of individuals with moderate to severe vasomotor symptoms (VMS) associated with menopause.

Pharmacology/Toxicology therefore supports the approval of fezolinetant for the treatment of moderate to severe VMS associated with menopause under NDA 216578.

Drug Product Information:

Fezolinetant is a New Molecular Entity (NME) for this gynecological indication. Fezolinetant is a neurokinin 3 (NK3) receptor antagonist. The intended mechanism of action of fezolinetant is to block neurokinin B (NKB) binding on the kisspeptin/neurokinin B/dynorphin (KNDy) neurons in the thermoregulatory center in the hypothalamus. KNDy neurons signal to the thermoregulatory center of the brain and modulate the output to heat dissipating mechanisms in response to input from peripheral temperature sensing neurons. By antagonizing NKB/NK3-mediated signal, fezolinetant modulates KNDy neuronal activity in the thermoregulatory center resulting in attenuated vasomotor symptoms associated with menopause.

Proposed Clinical Population and Dosing Regimen: VEOZAH is formulated as a 45 mg tablet. The intended treatment regimen is 45 mg orally once daily with or without water to the general population of individuals with moderate to severe VMS associated with menopause.

Nonclinical Support of Fezolinetant:

A full nonclinical battery of studies was completed to support the nonclinical safety of fezolinetant as a NK3 receptor antagonist. Relevant observations are summarized in the sections below. The exposure and safety margins presented compare the animal systemic exposures on an AUC (area under the concentration-time curve) basis to the clinical human exposure at the maximum recommended human dose (MRHD) of 45 mg/day.

Pharmacology and Mechanism of Action: Pharmacology studies demonstrated a high affinity of fezolinetant to human, rat, and monkey NK3 receptors, with greater than 400-fold selectivity for the NK3 receptor as compared to NK1 or NK2 receptors. Functional studies showed that fezolinetant was an antagonist of the human NK3 receptor with no agonist activity on this receptor. In a rat ovariectomy model of postmenopause, fezolinetant significantly decreased neuronal activity in the median preoptic nucleus (MnPO) hypothalamic neurons and reduced hyperphagia and weight gain, reduced plasma luteinizing hormone (LH) levels, without affecting estradiol, progesterone, testosterone, or follicle stimulating hormone (FSH) levels and attenuated hot flash-like symptoms. Additionally, in intact female rats and monkeys, fezolinetant was found to lower the circulating sex hormones LH, estradiol, and progesterone, but not FSH or testosterone, demonstrating that antagonism of the NK3 receptor inhibits LH secretion in female animals with functioning ovaries. Therefore, fezolinetant antagonism of the NK3 receptor appears to affect the downstream signaling of KNDy neurons to gonadotropin releasing hormone (GnRH) neurons, modulating the GnRH pulse frequency and consequently the release of LH.

Safety Pharmacology: There were no risks identified in a full battery of safety pharmacology studies with fezolinetant.

Metabolism and Excretion: There were no unique human metabolites. Fezolinetant was primarily metabolized to the hydroxylated metabolite ES00259564, accounting for approximately 6.0%, 21-42%, and 50% of the total fezolinetant-related plasma exposure in rats, cynomolgus monkeys, and humans, respectively. In all species, fezolinetant and ES00259564 were excreted mainly via urine, indicating no marked species difference in the major elimination (metabolism and excretion) route. ES00259564 was 10 to 20 times less potent at the human, rat, and cynomolgus monkey NK3 receptor compared with fezolinetant and had no significant off-target activities. Systemic exposure to ES00259564 in chronic toxicology studies in the female rat and monkey at the No Observed Adverse Effect Level (NOAEL; 200 and 25 mg/kg per day, respectively) indicated a greater than 25X safety margin on an AUC basis at the MRHD of 45 mg/day. Therefore, the safety of ES00259564 was appropriately evaluated in the nonclinical toxicity studies.

Fezolinetant-derived components were found to pass through the placenta and transfer to the fetus as well as being transferred to breast milk of lactating rats. In the lactating

rat, concentrations of fezolinetant-derived components in the breastfeeding infant tissues were 0.01 to 0.02 times those in the maternal plasma at 1-hour post-dosing. Thereafter, the concentrations of fezolinetant-derived components in the maternal plasma declined with time while gradually increasing in the infant tissues up to 24 hours post-dosing. At 48 hours post-dosing, the concentrations of fezolinetant-derived components in the infant tissues were 2.05 to 10.06 times higher than those in the maternal plasma. (b) (4)

General Toxicology: The chronic repeat dose toxicity studies were conducted in the female rat and cynomolgus monkey. Most of the observed effects with fezolinetant in these studies were attributed to its pharmacologic action. Predominant pharmacologic action-related findings of fezolinetant included a decrease in uterine weight, uterine atrophy, and mucification of the epithelium of the vagina and cervix in female rats and decreased uterine, cervix and ovary weights, decreased mammary gland tissue, absent corpora lutea in the ovary, uterine stromal atrophy, and altered menstrual cycles in female monkeys. Pharmacological action related findings of the female reproductive tract and menstrual cycles were reversible in the monkey after 39-weeks of treatment. Reversibility was not examined in the 26-week rat toxicology study. In the 13- and 39-week monkey studies, DEXA scans showed that fat content decreased at the high dose of 50 and 40 mg/kg/day, respectively, and recovered during the dosing phase, but muscle and bone mass were unchanged. In the female monkey, fezolinetant-related thrombocytopenia, sometimes occurring with increased peripheral monocytes, anemia, or hypocellular bone marrow, was observed in both the 13- and 39-week toxicology studies at the high dose of 50 and 40 mg/kg/day, respectively, equating to approximately 61X and 102X the MRHD on an AUC basis. Fezolinetant-related thrombocytopenia was reversible with a drug withdrawal period or recovery period. Fezolinetant-related thrombocytopenia was determined to be an adverse drug-related effect in these studies. In the pivotal 26-week rat and 39-week monkey studies, the mid-dose levels of 200 (rat) and 25 (monkey) mg/kg/day fezolinetant were the NOAELs, with margins of 148X and 43X MRHD based on AUC, respectively. These exposure margins were considered acceptable based on most findings being attributed to the exaggerated pharmacological effect of fezolinetant and the adverse effects on the hematology system being observed only at the high dose level in the 39-week female monkey study at approximately 100X the MRHD based on AUC.

Carcinogenicity: Fezolinetant was found not carcinogenic in both a 2-year female rat bioassay up to 100 mg/kg/day (186X MRHD) and a 26-week Tg.rasH2 mouse bioassay up to 450 mg/kg/day (47X MRHD). While some neoplastic changes were noted in the female rat, including increased incidence of benign follicular adenomas in the thyroid with increased fezolinetant dose (trend only; p-value = 0.0094) and increased incidence of the combination of ovarian epithelial tumors (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma) in mid-dose females only (pair-wise only; p=0.0132; 30 mg/kg/day; 75X MRHD), these findings were not determined to be toxicologically relevant by statistical analysis. Non-neoplastic histopathological findings in the ovary and vagina in mice, and ovary, uterus, cervix, and mammary gland in female rats were considered related to the pharmacologic action of fezolinetant.

Genetic Toxicology: Fezolinetant was not mutagenic based on a standard battery of in vitro and in vivo genotoxicity studies.

Reproductive and Developmental Toxicology: Reproductive and developmental toxicology studies were conducted in rats and rabbits.

In a fertility and early embryonic development study in rats, fezolinetant had no effect on fertility or early embryonic development even though there were altered estrous cyclicity and reduced adjusted mean uterus/cervix/vagina weight noted in females at the high dose of 100 mg/kg/day (143X MRHD).

In rat and rabbit embryo-fetal development studies, total litter loss was observed in both rats and rabbits at 100 and 125 mg/kg/day (128X and 174X MRHD, respectively). In addition, rabbits also showed increased late resorption and reduced fetal weight at 75 mg/kg/day (28X MRHD). Fezolinetant did not show teratogenic potential in either rats or rabbits. The NOAELs for the rat and rabbit embryo-fetal development studies based on embryotoxicity were 50 mg/kg/day (62X MRHD) and 45 mg/kg/day (16X MRHD), respectively.

In pre- and post-natal development studies in the rat, fezolinetant-related total litter loss was observed in 4 high-dose females (100 mg/kg/day; 204X MRHD). Additionally, fezolinetant extended the gestational duration in high-dose females (204X MRHD). Fezolinetant exposure through gestation and lactation period delayed sexual maturation of high-dose group F₁ male rats (100 mg/kg/day; 204X MRHD), characterized as incomplete balanopreputial separation at time of pairing for mating, for 1 male at the mid-dose of 30 mg/kg/day (36X MRHD) and 6 males at the high-dose of 100 mg/kg/day (204X MRHD) leading to unsuccessful mating. High-dose F₁ males (204X MRHD) also had reduced mating, fertility, and fecundity indices and a reduced number of pregnant females. There were no effects on the pregnancies of the high-dose F₁ males that did successfully mate. There were no effects observed in the F₁ generation females. The NOAEL for F₁ generation development was determined to be 100 mg/kg/day in females (204X MRHD) and 10 mg/kg/day (11X MRHD) in males.

Given the intended clinical population for fezolinetant is menopausal women, the risks to pregnancy and fetal development for this drug product are low. However, it could also be presumed that incorrect administration to a pregnant female could carry the risks of early pregnancy loss or delayed maturation of a male fetus. (b) (4)

Central Nervous System and Drug Dependency: The applicant conducted additional studies to investigate the potential for effects on seizure threshold and drug dependency. Fezolinetant was found to have no potential to induce seizures in a 13-week oral study in rats at doses up to 200 mg/kg/day (>100X MRHD) and did not induce physical dependency in a 4-week oral study in rats up to 100 mg/kg/day (108X MRHD).

Pharmacology/Toxicology Conclusion:

The nonclinical data support the clinical safety of fezolinetant. There are no anticipated concerns for use of fezolinetant when used clinically in the intended patient population of females of non-childbearing potential for the proposed indication.

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/s/

ANDREA BENEDICT
11/22/2022 11:48:10 AM

KIMBERLY P HATFIELD
11/22/2022 11:51:57 AM
I concur with the review and recommendations of Dr. Benedict.

Executive Carcinogenicity Assessment Committee Cover Sheet for Final Carcinogenicity Study Reports

P/T Reviewer(s): Miyun Tsai-Turton, PhD, MS
Supervisor/TL: Andrea Benedict, PhD
Division(s): Division of Urology, Obstetrics, and Gynecology
IND/NDA/BLA: NDA 216578/IND 130277
Sponsor/Applicant: Astellas Pharma US, Inc. (Japan)
Drug Name(s): (b) (4) (Fezolinetant)
Drug Code#: ESN364
CAS#: NON-RESPONSIVE
Indication(s): Moderate and severe vasomotor symptoms (VMS) associated with menopause
Pharmacological Class: NK3 (Neurokinin-3) receptor antagonist
Mutagenic/Genotoxic: Negative

Rat Carcinogenicity Study [8375967](#)

Study Duration (weeks): 104 weeks
Study Site: (b) (4)
Carcinogenicity Study Report Date: April 20, 2021
Date of first dose: March 06, 2018
Rat Strain: Rat Wistar
Route: Oral Gavage
Vehicle: 0.5% w/v methylcellulose
Dosing Comments: None

Doses and numbers of animals:

Group		Dose Levels (mg/kg/day)	No. of Females	
			Main Study	TK
1	Vehicle	0	100	4
2	Low	10	100	10
3	Mid	30	100	10
4	High	100	100	10

Basis for Doses Selected: MTD. The high dose was based on mortality at 300 mg/kg/day and AUC exposure > 25-fold the clinical exposure. In a 4-week toxicity rat study, ESN364 (0, 30, 100, or 300 mg/kg/day) in 0.5% methylcellulose was administered by oral gavage (Study No 8276179). The 300 mg/kg/day dose caused the death of one rat, and caused severe clinical signs, and significant body weight loss in other high-dose rats. A 26-week toxicity study up to 200 mg/kg/day did not find any adverse effects.

Prior FDA ExecCAC Dose Concurrence? Yes, under IND 130277, with ECAC SPA Agreement dated December 5, 2017. The study was conducted at the agreed upon doses and in females only.

Rat Carcinogenicity Conclusion: Negative**Rat Tumor Findings (details):**

The tumor data were analyzed by the FDA statistical reviewer, Dr. Hepei Chen, for dose response relationships across vehicle control group and the treated groups, and pairwise comparisons of each of the three treated groups against the vehicle control group.

The FDA statistical review identified a statistically significant dose response relationship in the incidence of benign follicular cell adenoma (common tumor) in the thyroid, without a corresponding statistically significant pairwise increase in the HD group when compared to the vehicle control.

- **Thyroid:**
 - Increased incidence of benign thyroid follicular adenomas with increased ESN364 dose (trend only; p-value = 0.0094).
 - The incidence of benign follicular cell adenomas in HD females (9%, 9/100) was slightly outside the range of the historical control data for female rats, as provided by the Applicant (22.2% and 8.0% were the upper ranges for benign follicular cell adenoma in males and females, respectively).
 - There was no statistically significant ESN364-related change in tumor incidence for the combination of benign thyroid follicular cell adenomas and malignant thyroid follicular cell carcinomas by either trend or pairwise analysis.
- **Ovary:**
 - Increased incidence of the combination of ovarian epithelial tumors (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma) was noted in MD females only (p=0.0132) when compared to the vehicle control group, without corresponding statistically significant dose response relationship.
 - No statistical significance by trend or pairwise analysis was observed for each individual ovary tumor type or for the combination of ovarian stromal tumors.

The high-dose level of 100 mg/kg/day **ESN364** in the female rat has a corresponding AUC₀₋₂₄ of 718000 ng•h/mL (Week 26), representing an exposure margin of 186X MRHD of 45 mg/day based on AUC₂₄ of 3855 ng•h/mL, obtained from clinical Study No. 2693-PK-0010.

The high-dose level of 100 mg/kg/day **ES00259564** (metabolite) in the female rat has a corresponding AUC₀₋₂₄ of 79900 ng•h/mL (Week 26), representing an exposure margin of 31X MRHD of 45 mg/day based on AUC₂₄ of 2570 ng•h/mL, obtained from clinical Study No. 2693-PK-0010.

Rat Study Comments:

ESN364 did not affect the overall survival rate or clinical signs up to the high dose of 100 mg/kg/day. The survival rates were 59%, 65%, 60% and 65%, in the dose groups 0 (vehicle), 10, 30 and 100 mg/kg/day, respectively.

Higher body weights were noted periodically through Week 38 in females at 30 or 100 mg/kg/day and higher body weight gain was noted over the first 26 weeks of the study in females at 10, 30, or 100 mg/kg/day. Increased food consumption was also noted in females at 100 mg/kg/day through Week 42.

Non-tumor lesions were observed in the **liver, uterus, mammary gland, ovary, cervix, and adrenal gland**, including centrilobular hepatocyte hypertrophy in HD females, increased incidences of atrophy in the uterus in MD and HD females, decreased incidences of hyperplasia in the mammary gland of all treated females, decreased

incidences of age-related atrophy in ovary of all treated females, decreased incidence of squamous metaplasia in cervix of all treated females, and decreased incidence of cystic degeneration of the cortex in adrenal gland of all treated females. These findings, except liver findings, were considered exaggerated pharmacological effects of ESN364.

Mouse Carcinogenicity Study [8375970](#)

Study Duration (weeks): 26 weeks
Study site: (b) (4)
Carcinogenicity Study Report Date: May 25, 2021
Date of first dose: January 24, 2019 (males)
 January 25, 2019 (females)
Mouse Strain: Tg.rasH2
Route: Oral gavage
Vehicle: 0.5% w/v methylcellulose (MC) solution
Dosing Comments: None.

Doses and numbers of animals:

Group		Dose Levels (mg/kg/day)	No. of Animals	
			Main Study /sex/group	TK /sex/group
1	Vehicle	0	25	3
2	Low	50	25	18
3	Mid	150	25	18
4	High	450	25	18
5	Positive Control*	75	10	10

* N-methyl-N-nitrosourea (MNU); single intraperitoneal dose on Day 1 of the Dosing phase

Basis for Doses Selected: MTD

Prior FDA ExecCAC Dose Concurrence? Yes, under IND 130277, with ECAC SPA Agreement dated November 20, 2018. The study was conducted at the agreed upon doses.

Mouse Carcinogenicity Conclusion: Negative**Mouse Tumor Findings (details):**

The tumor data were analyzed by the FDA statistical reviewer, Dr. Hepei Chen, for dose response relationships across the vehicle control group, and low, mid, and high dose groups, and pairwise comparisons of each of the three treated groups against the vehicle control group. Two separate analyses were conducted, one in male mice and one in female mice.

No tumor types with a statistically significant dose response relationship in tumor incidences were observed in male or female mice with increased ESN364 dose when the positive control group was excluded. The pairwise comparisons also showed no tumor types with a statistically significant increase in tumor incidences in ESN364 treated groups, when compared to the vehicle control group in either male or female mice when the positive control group was excluded.

The high-dose level of 450 mg/kg/day **ESN364**, in the female mouse has a corresponding AUC₀₋₂₄ of 183000 ng•h/mL (Week 26), representing an exposure margin of 47X MRHD of 45 mg/day based on AUC₂₄ of 3855 ng•h/mL, obtained from clinical Study No. 2693-PK-0010.

The high-dose level of 450 mg/kg/day **ES00259564** (metabolite), in the female mouse has a corresponding AUC₀₋₂₄ of 366000 ng•h/mL (Week 26), representing an exposure margin of 95X MRHD of 45 mg/day based on AUC₂₄ of 3855 ng•h/mL, obtained from clinical Study No. 2693-PK-0010.

Mouse Study Comments:

ESN364 did not affect the overall survival rate or adverse clinical signs up to the high dose of 450 mg/kg/day in males and 450 mg/kg/day in females. The survival rates were 96%, 96%, 96% and 96% for males, and 92%, 92%, 100% and 92% for females, in the dose groups 0 (vehicle), 50, 150 and 450 mg/kg/day, respectively, and 0% and 0% for males and females, respectively, of the positive control group. There were increased mean body weight gain in HD animals (+12.5% and +25%, males and females, respectively) when compared to vehicle control animals. Increased mean body weights correlated with ESN364-related increased mean food consumption for HD males but not HD females. Increased mean food consumption was also noted in MD males.

Non-tumor lesions were observed in the **liver**, **ovary**, and **vagina**, including increased vacuolation in HD males and MD/HD females, centrilobular hepatocyte hypertrophy in both HD males and females, increased incidence of decreased numbers of corpora lutea in the ovary of HD females, and increased incidence and/or severity of mucosal cornification in the vagina of HD females.

Executive CAC Conclusions (refer to the Executive CAC Final Study Minutes document, dated October 27, 2022, for full details)

Rat:

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

Mouse:

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

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 216578
Supporting document/s: [SDN 1 \(eCTD 0001\)](#)
Applicant's letter date: 06/22/2022
CDER stamp date: 06/22/2022
Product: (b) (4) (Fezolinetant) tablet
Indication: Moderate to severe vasomotor symptoms (VMS)
associated with menopause
Applicant: Astellas Pharma Inc.
Review Division: Division of Urology, Obstetrics, and Gynecology
Reviewer: Miyun Tsai-Turton, PhD, MS
Supervisor/Team Leader: Andrea Benedict, PhD
Acting Division Director: Christina Chang, MD
Project Manager: Samantha Bell

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

NDA 216578 is a 505(b)(1) application submitted by Astellas Pharma US for (b) (4) (fezolinetant or ESN364) 45 mg tablets for the treatment of moderate to severe vasomotor symptoms (VMS) associated with menopause. Fezolinetant is a small molecule, non-hormonal, selective NK3 receptor antagonist. It is a first-in class product where its mechanism of action is to block neurokinin B (NKB) binding on the KNDy neuron to modulate neuronal activity in the thermoregulatory center.

The clinical program includes 18 completed studies: There are 11 Phase 1 studies, 4 Phase 2 studies, and 3 Phase 3 studies, comprising 3682 participants across these studies. The nonclinical program includes pharmacodynamic (PD), pharmacokinetic (PK), repeat dose toxicity (up to 26 weeks in rats and 39 weeks in dogs), reproductive and developmental toxicology (in rats and rabbits), genotoxicity, and carcinogenicity studies (in transgenic mice and rats). In January 2022, the Sponsor had a pre-NDA meeting with the FDA and the nonclinical package and nonclinical data supporting the mechanism of action of fezolinetant appeared to be adequate (see more details in the 01/31/22 pharm/tox memo and the 02/24/22 Meeting Minutes in DARRTS). The pre- and post-natal developmental toxicity study and carcinogenicity studies (all submitted under IND NON-RESPONSIVE eCTD 0115) will be reviewed in this NDA submission.

1.2 Brief Discussion of Nonclinical Findings

In vitro **pharmacology** studies showed that fezolinetant is a potent antagonist of the hNK3 receptor. Fezolinetant has a similar affinity for human and monkey NK3 receptors and 8-fold less for rat NK3 receptors. The major metabolite, ES00259564, showed no significant activity at off-target receptors, with biological activity only at hNK3. In vivo pharmacology studies demonstrated fezolinetant attenuated hot flash-like symptoms in ovariectomized rats (a nonclinical model to replicate menopause) and fezolinetant inhibited c-fos expressed in median preoptic nucleus (MnPO) neurons in OVX rats.

Pharmacokinetic studies showed that fezolinetant was rapidly absorbed and widely distributed to the tissues. The bioavailability of fezolinetant was 91.9 to 115% in rats and 74.6-103% in monkeys. The highest levels of fezolinetant in tissues were associated with the kidney cortex, clitoris, uveal tract/retina, adrenal gland (cortex and medulla) and liver. CYP1A2 was the major CYP isozyme implicated in the metabolism of fezolinetant. One oxidated metabolite (ES00259564) was detected in cryopreserved hepatocytes from rats, monkeys, and humans, suggesting that human-specific fezolinetant metabolites were not formed. Fezolinetant and its metabolite were excreted in urine and feces. Additionally, fezolinetant-derived components transferred to the infant tissues via breast milk.

Repeated dose toxicity studies were conducted in rats up to 26 weeks and in monkeys up to 39 weeks.

- o In the **26-week rat study** (Study No 8331724), fezolinetant was administered orally by daily gavage to sexually mature rats at a dose of 0 (vehicle), 30, 100, or 200 mg/kg. No adverse effects on in-life parameters, clinical pathology, or microscopic changes were noted. Uterine atrophy and epithelial mucification of the vagina and cervix were attributed to pharmacological effects of fezolinetant, did not affect the general health of the animals, and were not adverse. The NOAEL was the high-dose of 200 mg/kg/day, with $AUC_{24} = 569000 \text{ ng}\cdot\text{h/mL}$ (148X MRHD of 45 mg with $AUC_{24} = 3855 \text{ ng}\cdot\text{h/mL}$ obtained from clinical Study 2693-PD-0010).
- o In the **39-week monkey study** (Study No 8331280), fezolinetant was administered orally by daily gavage to sexually mature female monkeys at a dose of 0, 10, 25, or 40 mg/kg. High doses of fezolinetant resulted in weight loss and microscopic findings (hyperplasia of lymphocytes in lymph nodes, spleen, and lung, as well as perivascular inflammation in the kidney). Decreased platelet counts (at 40 mg/kg/day in the 39-week study) due to decreased production in bone marrow resulted in hemorrhage and regenerative anemia. This was recoverable. The NOAEL was the mid-dose of 25 mg/kg/day, with $AUC_{24} = 166000 \text{ ng}\cdot\text{h/mL}$ (43X MRHD).

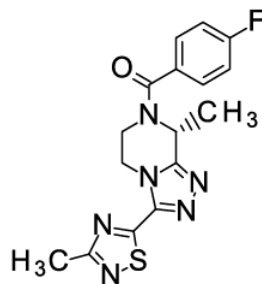
Genotoxicity studies indicated that fezolinetant was not genotoxic.

The carcinogenicity studies are being reviewed here.

2 Drug Information

2.1 Drug

CAS Registry Number	1629229-37-3
Generic Name	Fezolinetant
Code Name	ESN364
Chemical Name	(4-Fluorophenyl)[(8R)-8-methyl-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]methanone
Molecular Formula/Molecular Weight	$C_{16}H_{15}FN_6OS$, 658.39



Structure or Biochemical Description
Pharmacologic Class

Selective NK3 receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 130277 (hot flashes), IND (b) (4) (polycystic ovarian syndrome), IND (b) (4) (uterine fibroids), DMF (b) (4)

2.3 Drug Formulation

Fezolinetant tablets 30 mg are (b) (4) tablets containing 30 mg active ingredient. Fezolinetant tablets 45 mg are round light red film-coated tablets containing 45 mg active ingredient.

Compositions of Fezolinetant Tablets 30 mg and 45 mg

Component	Function	Reference to Quality Standard	Quantity (mg/tablet)	
			30 mg	45 mg
Fezolinetant	Active ingredient	In-house	30.0	45.0
Mannitol	(b) (4)	USP	(b) (4)	
Hydroxypropyl Cellulose		NF		
Low-Substituted Hydroxypropyl Cellulose		NF		
Microcrystalline Cellulose		NF		
Magnesium Stearate		NF		
Total				

NF: National Formulary, USP: United States Pharmacopeia, N/A: Not applicable

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

No concern on impurities or degradants.

2.6 Proposed Clinical Population and Dosing Regimen

The recommended dose of fezolinetant is 45 mg orally once daily with or without food in the general population of individuals with moderate to severe VMS associated with menopause.

2.7 Regulatory Background

Fezolinetant (ESN364) is being developed under INDs 130277 (hot flashes), IND (b) (4) (polycystic ovarian syndrome), and IND (b) (4) (uterine fibroids). It was originally submitted by Ogeda S.A. who conducted the majority of pharmacology,

pharmacokinetics, and toxicology studies. In 2017, Astellas Pharma Inc. acquired Ogeda as a wholly owned subsidiary of Astellas Pharma Inc. In January 2022, the pre-NDA meeting was held. The Agency told the Sponsor at the time that the nonclinical development program appeared to adequately support the marketing registration of fezolinetant for VMS indication. However, the adequacy of the nonclinical data to support approval will be a review issue.

8 Carcinogenicity

Study No. [8375967](#): 2-year rat study

Study title: 104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with ESN364 in Female Wistar Han Rats

Study no.:	8375967
Study report location:	Module 4.2.3.4.1 (under NDA 216578)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	02/26/18
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot No. 171101 (100.15% purity) and Lot No. 180621 (99.62% purity)
CAC concurrence:	Protocol: 12-05-2017 Final study: 04-30-2021

Key Study Findings

Thyroid tumor was identified but was not considered toxicologically relevant. Increased incidence of benign thyroid follicular cell adenomas (common tumor) was noted with increased fezolinetant dose by trend analysis only ($p = 0.0094$).

Combined ovarian epithelial tumors were identified but were not considered toxicologically relevant. Increased incidence of the combination of ovarian epithelial tumors (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma) was noted in MD females only ($p=0.0132$) when compared to the vehicle control group, without corresponding statistically significant dose response relationship.

Non-neoplastic histopathological findings observed in the liver, uterus, ovary, cervix, mammary gland, and adrenal cortex were mostly attributed to the pharmacological action of fezolinetant and aging.

- **Liver:** Centrilobular hepatocyte hypertrophy in HD females, may be attributed to microsomal enzyme induction in the hepatocytes by ESN364.
- **Uterus:** Increased incidence of uterus atrophy in MD and HD females.
- **Ovary:** Decreased incidence of age-related atrophy in all treated females.
- **Cervix:** Decreased incidence and severity of squamous cell metaplasia in all treated females.

- **Mammary gland:** Hyperplasia in all treated females.
- **Adrenal cortex:** Decreased incidence of cystic degeneration in all treated females.

Maximum Clinical Exposure: 45 mg/day, 3855 ng•h/mL (Study 2693-PD-0010). The mid and high doses tested in this 2-year rat study achieved exposures of 75X and 186X the clinical exposure, respectively. $AUC_{24} = 290000$ and 718000 ng•h/mL at 30 and 100 mg/kg/day in females at Week 26, respectively.

Adequacy of Carcinogenicity Study

The Division considers the rat study an adequate assessment of carcinogenic potential because the high dose of ESN364 was based on mortality at 300 mg/kg/day and AUC exposure > 25-fold the clinical exposure.

Appropriateness of Test Models

Yes.

Evaluation of Tumor Findings

Negative

Methods

Doses: 0 (0.5% w/v MC), 10, 30, and 100 mg/kg/day
 Frequency of dosing: Daily
 Dose volume: 5 mL/kg/day
 Route of administration: Oral gavage
 Formulation/Vehicle: 0.5% w/v methylcellulose solution
 Basis of dose selection: MTD; with the high dose based on mortality at 300 mg/kg/day and AUC exposure > 25-fold the clinical exposure. In a 4-week toxicity study in rats, ESN-364 (0, 30, 100, or 300 mg/kg/day) in 0.5% methylcellulose was administered by oral gavage (Study No 8276179). The 300 mg/kg/day dose caused the death of one rat, and caused severe clinical signs, and significant body weight loss in other high-dose rats. A 26-week toxicity study up to 200 mg/kg/day did not find any adverse effects. The ECAC protocol minutes can be found in DARRTS dated 12/05/17 under IND 130277.
 Species/Strain: Rat/Wistar
 Number/Sex/Group: 100 females/group
 Age: 7-8 weeks of age
 Animal housing: Up to 3 animals/sex/cage
 Paradigm for dietary restriction: Allowed free access to pelleted diet and tap water
 Dual control employed: No
 Interim sacrifice: No
 Satellite groups: Yes, TK animals [4 (VH) or 10 (ESN364)/group].
 Deviation from study protocol: No effect on the study findings.

Group Composition

Group	Subgroup	No. of Females	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Vehicle Control) ^a	1 (Carcinogenicity)	100	0	0
	2 (Toxicokinetic)	4 ^b	0	0
2 (Low)	1 (Carcinogenicity)	100	10	2
	2 (Toxicokinetic)	10 ^b	10	2
3 (Mid)	1 (Carcinogenicity)	100	30	6
	2 (Toxicokinetic)	10 ^b	30	6
4 (High)	1 (Carcinogenicity)	100	100	20
	2 (Toxicokinetic)	10 ^b	100	20

^a Group 1 was administered vehicle control article only.

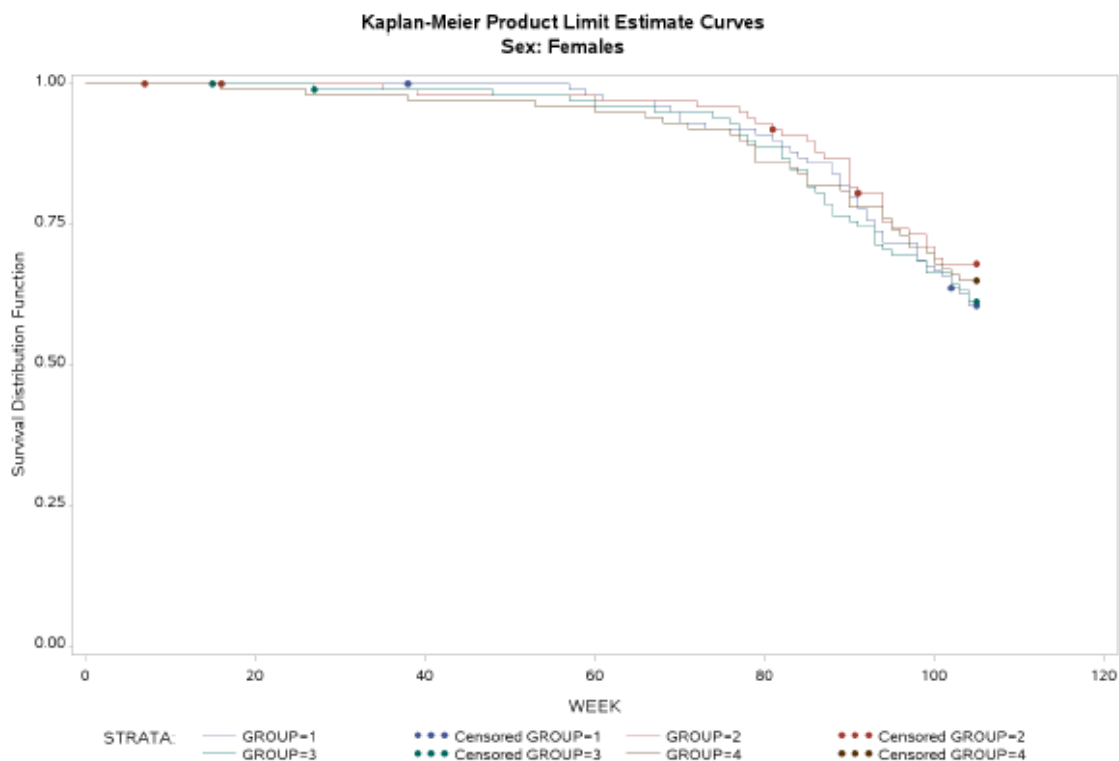
^b One additional animal/group was included in the toxicokinetic subgroup for replacements in the event that an early death/sacrifice would have occurred.

Observations and Results

Mortality

There was no ESN364-related change in survival rate.

- The survival rates were 59%, 65%, 60% and 65% in the dose groups 0 (vehicle), 10, 30 and 100 mg/kg/day, respectively.
- **FDA Statistical reviewer agreed with the Applicant's survival analysis.**



[Extracted from the Study No. 8375967 study report page 2596.]

In addition, ESN364 did not increase the number of deaths attributable to specific neoplasms or non-neoplastic lesions.

Incidence of Selected Causes of Death - Unscheduled Necropsies

Sex		Females			
		ESN364			
Group	1	2	3	4	
Dose Level (mg/kg/day)	0	10	30	100	
Mortality ^a (total each group)	41	35	40	35	
Death Comment					
Pituitary gland neoplasm	13	11	16	10	
Mammary gland neoplasm	9	8	5	0	
Undetermined	1	1	3	6	
Uterus neoplasm	4	1	1	1	
Skin/subcutis neoplasm	2	2	1	3	
Gavage-related death	2	3	0	0	
Brain neoplasm	0	1	1	4	
Thymus neoplasm	0	0	3	2	
Cervix neoplasm	3	2	1	0	
Ovary neoplasm	0	1	3	0	
Tooth neoplasm	0	1	0	2	
Hematopoietic neoplasm	0	2	1	0	
Skin/subcutis erosion/ulceration	0	0	1	2	
Zymbal gland neoplasm	3	0	0	0	
Oral cavity neoplasm	1	1	0	0	
Eye inflammation/atrophy	1	0	1	0	
General debilitation	1	1	0	0	
Adipose tissue neoplasm	0	0	1	0	
Accidental	0	0	1	0	
Gastrointestinal inflammation	0	0	0	1	
Hemorrhage	1	0	0	0	
Limited mobility	0	0	0	1	
Liver necrosis	0	0	0	1	
Sarcoma	0	0	0	1	
Thromboembolism	0	0	1	0	
Urinary tract inflammation	0	0	0	1	

^a Total number of unscheduled necropsy animals.

[Extracted from the Study No. 8375967 study report page 1499.]

Clinical Signs

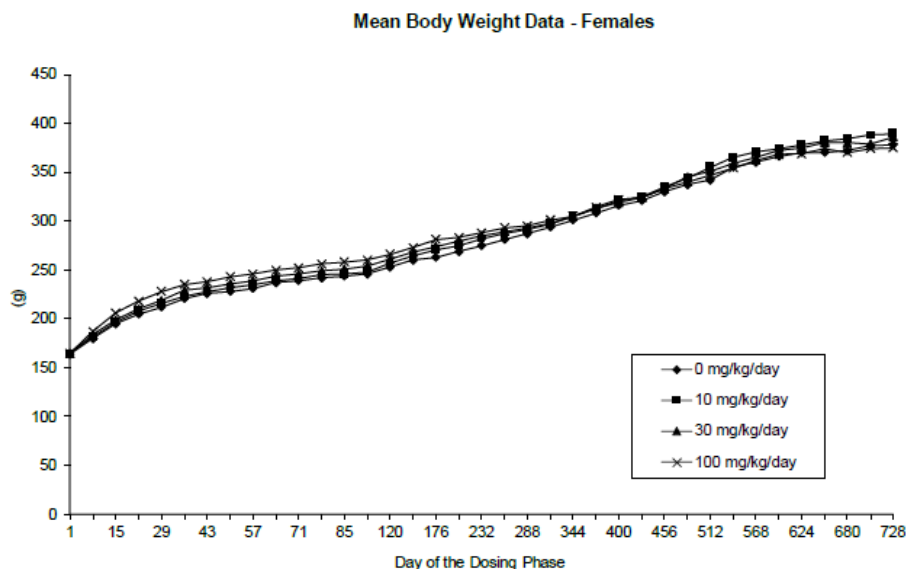
ESN364-related clinical signs included clear oral discharge in HD females and hypoactivity in MD and HD females.

Body Weights

ESN364-related higher mean body weights occurred periodically through Week 38 in MD and HD females when compared to controls. ESN364-related higher mean body weight gain was noted in all treated females when assessed over the first 26 weeks of the study. After Week 42, differences became less, such that over the course of the entire dosing phase, mean body weight gains were not different between groups. Effects on mean body weights were associated with increased mean food consumption in HD females through approximately Week 42.

Body Weights on Day 1 and Day 728

Group	Female BW (g)	
	Day 1	Day 728
Control (vehicle)	164	378
10 mg/kg/day	165	389
30 mg/kg/day	166	386
100 mg/kg/day	165	375



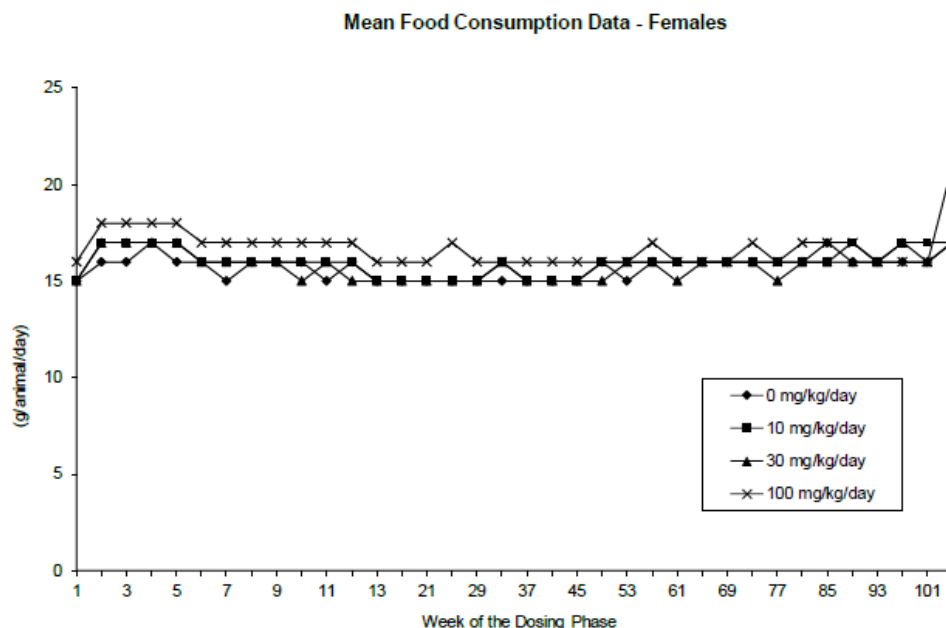
[Extracted from the Study No. 8375967 study report page 57.]

Feed Consumption

ESN364-related increased mean food consumption occurred in HD females (differences being statistically significant in most weekly intervals) through Week 42 and was associated with higher mean body weights in HD females (but not with the body weight effect in LD and MD females). Differences after Week 42 were less and were generally not statistically significant. Mean food consumption at lower dose levels (and at all dose levels after Week 42) was generally similar when compared to controls at most weekly intervals.

Food Consumption on Day 1 and Day 728

Group	Female BW (g)	
	Interval Day 1 - 8	Interval Day 722 - 728
Control (vehicle)	15	17
10 mg/kg/day	15	17
30 mg/kg/day	15	17
100 mg/kg/day	16 (↑ 7%)	21 (↑ 24%)



[Extracted from the Study No. 8375967 study report page 58.]

Clinical Pathology

No effect.

Gross Pathology

ESN364-related macroscopic findings were observed in the pituitary, mammary gland, and adrenal.

- Pituitary: Decreased incidences of large pituitary in all treated females. Decreased incidences of pituitary masses in MD and HD females, correlated with decreased incidence of pituitary adenomas.
- Mammary gland: Decreased incidences of mammary gland masses in MD and HD females, correlated with decreased incidence of mammary gland fibroadenomas and carcinomas.
- Adrenal: Decreased incidences of discolored adrenal in all treated females, which correlated with decreased incidences of cystic degeneration of the adrenal cortex.

: Incidence of ESN364-Related Macroscopic Findings - All Animals

Sex		Females ESN364			
Group		1	2	3	4
Dose Level (mg/kg/day)		0	10	30	100
Number Examined		100	100	100	100
Pituitary					
	Large Mass	8	3	1	2
Mammary Gland					
	Mass	13	11	4	1
Adrenal					
	Discolored	7	2	1	1

All other macroscopic findings were considered spontaneous and/or incidental because they occurred at a low incidence, were randomly distributed across groups (including concurrent controls), and/or were as expected for Crl:WI(Han) rats; therefore, they were considered not ESN364 related.

[Extracted from the Study No. 8375967 study report page 36.]

Histopathology**Peer Review**

Yes

Neoplastic**FDA's statistical reviewer's findings**

Guidance for Industry. Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (Draft Guidance). U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2001.

- For **dose response relationship (trend)** tests, $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors for a submission with one two-year study in one species and one short-term study with another species.
- For **pairwise comparisons** of treated group with control, $\alpha=0.05$ for both common and rare tumors.

Neoplastic tumors were identified in the thymus, thyroid, and ovary as identified in the FDA statistical reviewer's table below.

**Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship
and/or Pairwise Comparisons of Treated Groups and Vehicle control Group in Female Rats**

Organ name	Tumor name	0 mg Vehicle (C) P - Trend	10 mg Low (L) P - C vs. L	30 mg Mid (M) P - C vs. M	100 mg High (H) P - C vs. H
Ovary	B-Adenoma, Rete Ovarii/B-Adenoma, Tubulostromal/B-Cystadenom /M-Carcinoma	0/100 (84) 0.3606	0/100 (84) NC	6/100 (82) 0.0132 \$	1/100 (84) 0.5000
	B-Luteoma/M-Granulosa Cell Tumor, Mal*/M-Malignant Granulosa/Theca*	0/100 (84) 0.2964	1/100 (84) 0.5000	1/100 (82) 0.4940	1/100 (84) 0.5000
Thymus	B-Thymoma/ M-Malignant Thymoma	5/98 (82) 0.0401 @	5/99 (84) 0.3884	8/99 (82) 0.2824	11/99 (84) 0.1025
Thyroid	B-Adenoma, Follicular Cell	3/100 (84) 0.0094 \$	2/100 (84) 0.5000	4/100 (82) 0.4866	9/100 (85) 0.0685
	B-Adenoma, Follicular Cell/ M-Carcinoma, Follicular	6/100 (84) 0.0299 @	2/100 (84) 0.8615	5/100 (82) 0.4832	10/100 (85) 0.2232

* X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.

\$ = Statistically significant at 0.01 level in common tumor for test of dose response relationship, and at 0.05 level in rare or common tumor for test of pairwise comparisons;

@ = Not statistically significant at 0.01 level in common tumor for test of dose response relationship;

Table provided by CDER statistical reviewer

Thyroid

There was a statistically significant dose response relationship in tumor incidences for **benign follicular cell adenomas in the thyroid** with increased ESN364 dose across the vehicle control and the treated groups of female rats (trend; p-value = 0.0094, which is < 0.01 for common tumors). No pairwise significance was identified for this tumor. Since benign thyroid follicular cell adenoma was found to be statistically significant by trend analysis only, it is therefore considered a negative finding. This was also identified by the Applicant.

Additionally, there was no statistically significant ESN364-related change in tumor incidence for the combination of benign follicular cell adenomas and malignant follicular cell carcinomas by either trend (p=0.0299, > 0.01 for common tumor) or pairwise analysis.

Ovary

Ovary tumors were combined in alignment with the guidance for the combination of ovarian tumor types¹. There was a statistically significant increase by pairwise analysis for the **combination of ovarian epithelial tumor types (benign rete ovarii adenoma, benign tubulostromal adenoma, benign cystadenoma, and malignant carcinoma)** in MD females only (p=0.0132, < 0.05 for rare or common tumors) when compared to the vehicle control group, without corresponding statistically significant dose response relationship regardless the tumor classification (rare or common). Therefore, this is considered a negative finding for the combination of ovarian epithelial tumors. No

¹ McConnell, E. E., Solleveld, H. A., Swenberg, J. A., and Boorman, G. A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst 76, 283-289.

statistical significance by trend or pairwise analysis was observed for each individual ovary tumor type or for the combination of ovarian stromal tumors.

Applicant's findings

The Applicant noted that historical control data was used to evaluate the incidence of specific hyperplastic and neoplastic findings as well as a specific degenerative finding in control animals. In order to obtain historical data on animals of comparable strain and age, the data query was limited to Han Wistar rats assigned to 104-week studies. The software used to generate this data did not meet current validation standards and therefore represented a deviation from GLP compliance. Below is the list of significant findings identified by the Applicant with $p < 0.05$.

**Tumor Observed which had Statistically Significant Dose Response Results
When Evaluated at the 5% Level**

Tissue and Lesion	Test (Group)	Unadjusted p-value
Ovary Combined B-Adenoma, rete ovarii/B-Cystadenoma/ B-Luteoma/M-Carcinoma/M-Dysgerminoma/ M-Granulosa cell tumor, malignant/ M-Malignant granulosa/theca cell tumor	Mid v Control (Group 3 v Group 1)	0.0254
Thymus Combined B-Thymoma/M-Malignant thymoma	Trend (Groups 1,2,3,4)	0.0396
Thyroid B-Adenoma, follicular cell	Trend (Groups 1,2,3,4)	0.0075
Thyroid Combined B-Adenoma, follicular cell/ M-Carcinoma, follicular cell	Trend (Groups 1,2,3,4)	0.0277

Thyroid

- The incidence of benign **follicular cell adenomas** in HD females (9%, 9/100) was slightly outside the range of the historical control data provided by the Applicant (22.2% and 8.0% were the upper ranges for benign follicular cell adenoma in males and females, respectively; 10.9% and 1.8% were the upper ranges for malignant follicular cell carcinoma in males and females, respectively).
 - **Statistical significance was recorded by trend test only ($p = 0.0075$).**
 - Increased incidences of benign follicular cell adenomas in HD females were most likely secondary to centrilobular hepatocyte hypertrophy noted in the liver, reported in the published literature (McClain and Rice, 1999; Klaassen and Hood, 2001).
 - **This finding seems to correlate with follicular cell hyperplasia.** A small increased incidence of follicular cell hyperplasia was noted in MD and HD females, with unclear relationship to ESN364.
 - No increased incidence of malignant follicular cell carcinomas was noted in the treated females (increase only observed when combined with benign follicular cell adenoma and the combination did not achieve statistical significance by trend or pairwise analysis).

Summary of Severity of Microscopic Observations					
All animals					
Test Article (dosage)	1	2	3	4	
ESN364 mg/kg/day	0	10	30	100	
Tissue/	Group/Subgroup/Sex: 1/1/F 2/1/F 3/1/F 4/1/F				
Observation	Number of Animals: 100 100 100 100				
Thyroid	Number Examined: 100 100 100 100				
	Unremarkable: 67 73 65 68				
B-Adenoma, C-cell	10	5	8	5	
B-Adenoma, follicular cell	3	2	4	9	
M-Carcinoma, C-cell	0	2	2	0	
M-Carcinoma, follicular cell	3	0	1	2	
Follicle, cystic					
finding not present	94	91	94	92	
minimal	1	4	7	4	
slight	2	2	5	1	
moderate	3	0	0	3	
Total Incidence:	6	9	6	8	
Hyperplasia, C-cell					
finding not present	87	87	87	90	
minimal	1	10	13	9	
slight	2	3	0	1	
Total Incidence:	13	13	13	10	
Test Article (dosage)	1	2	3	4	
ESN364 mg/kg/day	0	10	30	100	
Tissue/	Group/Subgroup/Sex: 1/1/F 2/1/F 3/1/F 4/1/F				
Observation	Number of Animals: 100 100 100 100				
Thyroid	Number Examined: 100 100 100 100				
	Unremarkable: 67 73 65 68				
Hyperplasia, follicular cell					
finding not present	99	99	96	95	
slight	2	1	1	5	
moderate	3	0	0	0	
Total Incidence:	1	1	4	5	
Hypertrophy, follicular cell					
finding not present	100	100	100	99	
slight	2	0	0	1	
Total Incidence:	0	0	0	1	

[Extracted from the Study No. 8375967 study report pages 1621-1622.]

Thymus

- Increased incidence of thymoma (combined benign and malignant) was statistically significant (by trend, $p=0.0396$). These incidences were within the range of the historical control data provided by the Applicant (8.2% was the upper range for benign thymomas in males and females, while 1.9% was the upper range for malignant thymomas in females only).
 - The incidences of combined benign and malignant thymoma were 5/98 (5.1%), 5/99 (5.1%), 8/99 (8.1%), and 11/99 (11.1%) at VH, LD, MD and HD, respectively.
 - All benign thymomas displayed a lymphocyte-rich morphological pattern, which is specific to Han Wistar rats, whereas malignant thymomas had a predominantly squamous to spindle pattern.
 - These common tumors were not considered ESN364 related

Summary of Severity of Microscopic Observations					
All animals					
Test Article (dosage)	1	2	3	4	
ESN364 mg/kg/day	0	10	30	100	
Tissue/Observation	Group/Subgroup/Sex: 1/1/F 2/1/F 3/1/F 4/1/F				
	Number of Animals: 100 100 100 100				
Thymus	Number Examined: 98 99 99 99				
	Unremarkable: 38 66 58 59				
B-Thymoma	5	5	6	10	
F-Hemolymphoreticular tumor	2	3	2	1	
M-Malignant thymoma	0	0	2	1	
N-Carcinoma, metastatic	0	0	1	0	
N-Sarcoma, metastatic	0	0	0	2	
Decreased, lymphocytes					
finding not present	92	96	92	96	
slight	2	5	4	3	
moderate	3	1	2	0	
marked	4	0	1	0	
Total Incidence:	6	3	7	3	

[Extracted from the Study No. 8375967 study report page 1619.]

Ovary

There was a significant increase in combined ovarian tumors in MD females only when compared to control females ($p = 0.0254$). However, the Applicant combined both stromal and germ cell tumor types, which does not align the guidance for the combination of ovarian tumor types. See the discussion of the ovarian tumor findings and appropriate tumor combinations under the FDA's statistical reviewer findings above.

Summary of Severity of Microscopic Observations					
All animals					
Test Article (dosage)	1	2	3	4	
ESN364 mg/kg/day	0	10	30	100	
Tissue/Observation	Group/Subgroup/Sex: 1/1/F 2/1/F 3/1/F 4/1/F				
	Number of Animals: 100 100 100 100				
Ovary	Number Examined: 100 100 100 100				
	Unremarkable: 19 23 16 33				
B-Adenoma, rete ovarii	0	0	1	0	
B-Adenoma, tubulostromal	0	0	3	0	
B-Cystadenoma	0	0	0	1	
B-Luteoma	0	0	1	0	
F-Hemolymphoreticular tumor	0	1	1	0	
M-Carcinoma	0	0	2	0	
M-Dysgerminoma	0	0	1	0	
M-Fibrosarcoma	0	0	0	1	
M-Granulosa cell tumor, malignant	0	1	0	0	
M-Malignant granulosa/theca cell tumor	0	0	0	1	
M-Schwannoma	0	1	0	0	
N-Carcinoma, metastatic	0	1	0	0	
N-Sarcoma, metastatic	0	1	0	2	
Atrophy, age-related					
finding not present	25	31	47	58	
minimal	1	5	8	6	
slight	2	51	32	16	
moderate	3	19	13	20	
Total Incidence:	75	69	53	42	

[Extracted from the Study No. 8375967 study report page 1602.]

Mammary gland

The incidence of carcinoma was decreased in all treated females. The incidence of fibroadenoma was decreased in HD females. These findings had a correlation with a decreased incidence of hyperplasia in all treated females. This was considered a pharmacological effect of ESN364 due to decreased estradiol and progesterone levels.

Test Article (dosage)	1	2	3	4
ESN364 mg/kg/day	0	10	30	100
Tissue/ Observation	Group/Subgroup/Sex: 1/1/F Number of Animals: 100	2/1/F 100	3/1/F 100	4/1/F 100
Mammary Gland	Number Examined: 98	97	97	94
	Unremarkable: 13	26	41	74
B-Adenoma	1	0	0	0
B-Fibroadenoma	18	17	12	4
M-Carcinoma	12	6	3	0
Ectasia, duct				
finding not present	- 95	96	93	93
minimal	1	0	0	0
slight	2	1	1	1
moderate	3	0	3	0
Total Incidence:	3	1	4	1
Hyperplasia				
finding not present	- 38	47	55	78
minimal	1	26	17	8
slight	2	25	23	19
moderate	3	7	10	5
marked	4	2	0	0
Total Incidence:	60	50	42	16

[Extracted from the Study No. 8375967 study report page 1598.]

Pituitary

The incidence of pars distalis adenoma was decreased in HD females. There was no hyperplasia correlate. This was considered a pharmacological effect of ESN364 due to decreased estradiol and progesterone levels.

Test Article (dosage)	1	2	3	4
ESN364 mg/kg/day	0	10	30	100
Tissue/ Observation	Group/Subgroup/Sex: 1/1/F Number of Animals: 100	2/1/F 100	3/1/F 100	4/1/F 100
Pituitary	Number Examined: 100	100	100	100
	Unremarkable: 22	25	25	34
B-Adenoma, pars distalis	56	54	51	39
B-Adenoma, pars intermedia	2	0	1	0
B-Ganglioneuroma, benign	0	1	0	0
M-Carcinoma	0	1	1	0
N-Germ cell tumor, metastatic	0	0	1	0
Cyst				
finding not present	- 100	100	99	100
Present	0	0	1	0
Hyperplasia, pars distalis				
finding not present	- 80	82	79	73
minimal	1	3	1	1
slight	2	12	11	16
moderate	3	4	8	10
marked	4	1	1	0
Total Incidence:	20	18	21	27

[Extracted from the Study No. 8375967 study report page 1608.]

Non-Neoplastic

Non-tumor lesions were observed in the **liver, uterus, ovary, cervix, mammary gland** and **adrenal cortex**. These findings were mostly considered to be a result of the pharmacological effects of ESN364.

Incidence of ESN364-Related Non-Neoplastic Microscopic Findings - All Necropsies

		ESN364			
		Females			
	Sex	0	10	30	100
	Dose (mg/kg/day)				
	Number Examined ^a	100	100	100	100
Liver					
Hypertrophy, centrilobular, hepatocyte					
	Minimal	0	0	0	16
	Slight	0	0	0	12
Uterus					
Atrophy					
	Minimal	0	0	0	1
	Slight	3	3	6	20
	Moderate	0	0	3	10
Ovary					
Atrophy, age-related					
	Minimal	5	16	8	6
	Slight	51	41	32	16
	Moderate	19	12	13	20
Cervix					
Metaplasia, squamous cell					
	Minimal	4	2	1	0
	Slight	16	6	1	0
	Moderate	8	6	1	1
Mammary Gland					
Hyperplasia					
	Minimal	26	17	18	8
	Slight	25	23	19	6
	Moderate	7	10	5	2
	Marked	2	0	0	0
Adrenal, Cortex					
Degeneration, cystic					
	Minimal	31	24	29	33
	Slight	40	41	28	23
	Moderate	9	15	11	4
	Marked	7	0	0	1

^a Number examined unless otherwise specified.

[Extracted from the Study No. 8375967 study report page 39.]

- **Liver:** Centrilobular hepatocyte hypertrophy was noted in HD females. This is a common adaptive response which can be either a result of proliferation of the smooth endoplasmic reticulum and drug metabolizing CYP450 enzyme induction or peroxisome proliferation (Gopinath and Mowat, 2014). The mechanism of centrilobular hepatocyte hypertrophy for this study is unknown but it is possibly a result of microsomal enzyme induction in the hepatocytes by ESN364 based on the histomorphology of the affected hepatocytes.
- **Uterus:** An increased incidence of uterus atrophy was noted in MD females. Uterus atrophy rarely correlated with small uterus (noted macroscopically) and was characterized by diffuse, decreased thickness of the wall.
- **Ovary:** Decreased incidence of age-related atrophy in the ovary was noted in all treated females.
- **Cervix:** Decreased incidence and severity of squamous cell metaplasia in the cervix was noted in all treated females.
- **Mammary gland:** Decreased incidence and severity of hyperplasia was noted in all treated females.
- **Adrenal cortex:** Cystic degeneration in the adrenal cortex was noted in all treated females.

- **Eye:** An increased incidence of retinal atrophy of the eye in HD females was within the historical control data provided by the Applicant (33.3% was the upper range in females). Therefore, this finding was considered not related to ESN364.
- **Thyroid:** A small increased incidence of follicular cell hyperplasia of the thyroid was noted in MD and HD females, with unclear relationship to ESN364.

Test Article (dosage)	1	2	3	4			
ESN364	mg/kg/day	0	10	30	100		

Tissue/	Group/Subgroup/Sex: 1/1/F				2/1/F	3/1/F	4/1/F
Observation	Number of Animals: 100				100	100	100

Eye	Number Examined: 100				100	100	100
	Unremarkable: 80				78	85	71
Atrophy, retina							
finding not present	-	83	79	85	71		
minimal	1	3	1	1	0		
slight	2	8	7	5	11		
moderate	3	6	13	8	16		
marked	4	0	0	1	2		
Total Incidence:	17	21	15	29			

[Extracted from the Study No. 8375967 study report pages 1570.]

Test Article (dosage)	1	2	3	4	
ESN364	mg/kg/day	0	10	30	100

Tissue/	Group/Subgroup/Sex: 1/1/F 2/1/F 3/1/F 4/1/F				
Observation	Number of Animals: 100 100 100 100				

Thyroid	Number Examined: 100 100 100 100				
	Unremarkable: 67 73 65 68				
B-Adenoma, C-cell	10	5	8	5	
B-Adenoma, follicular cell	3	2	4	9	
M-Carcinoma, C-cell	0	2	2	0	
M-Carcinoma, follicular cell	3	0	1	2	
Follicle, cystic					
finding not present	- 94	91	94	92	
minimal	1 4	7	1	4	
slight	2 2	2	5	1	
moderate	3 0	0	0	3	
Total Incidence:	6	9	6	8	
Hyperplasia, C-cell					
finding not present	- 87	87	87	90	
minimal	1 10	12	13	9	
slight	2 3	1	0	1	
Total Incidence:	13	13	13	10	

[Extracted from the Study No. 8375967 study report page 1621.]

Toxicokinetics

The systemic exposure to ESN364 and its metabolite, ES00259564, was demonstrated. The C_{max} and AUC_{0-24} values of ESN364 and ES00259564 increased with increasing dose levels, though less than dose-proportional and dose-proportional, respectively. The values of ES00259564 were markedly lower than those of ESN364. The metabolite:parent ratios ranged from 0.0567 to 0.122 for C_{max} and from 0.0588 to 0.111 for AUC_{0-24} .

Summary of the ESN364 Toxicokinetic Parameters in Female Rat Plasma

Week	Dose Group	Dose Level (mg/kg/day)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (h*ng/mL)	t _{1/2} (h)
1	2	10	7340	2.00	85700	4.94
	3	30	25200	2.00	300000	4.34
	4	100	69100	4.00	971000	NC
5	2	10	8860	1.00	93500	4.68
	3	30	22600	2.00	303000	4.91
	4	100	51100	2.00	633000	3.96
26	2	10	8880	1.00	105000	5.27
	3	30	23200	2.00	290000	5.68
	4	100	54100	4.00	718000	NC

AUC₀₋₂₄ = Area under the concentration-time curve from 0 to 24 hours postdose; C_{max} = Maximum observed concentration; NC = Not calculated due to lack of a distinct elimination phase; t_{1/2} = Half-life; T_{max} = Time of maximum observed concentration.

[Extracted from the Study No. 8375967 study report page 31.]

Summary of the ES00259564 Toxicokinetic Parameters in Female Rat Plasma

Week	ESN364 Dose Group	Dose Level (mg/kg/day)	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (h*ng/mL)
1	2	10	500	2.00	6820
	3	30	1920	2.00	22900
	4	100	5490	4.00	67000
5	2	10	502	2.00	5490
	3	30	1820	2.00	24800
	4	100	5220	4.00	68000
26	2	10	543	2.00	6520
	3	30	1880	4.00	25300
	4	100	6600	4.00	79900

AUC₀₋₂₄ = Area under the concentration-time curve from 0 to 24 hours postdose; C_{max} = Maximum observed concentration; T_{max} = Time of maximum observed concentration.

Note: Half-lives could not be calculated at any interval due to the lack of a distinct elimination phase.

[Extracted from the Study No. 8375967 study report page 31.]

Dosing Solution Analysis

Both the concentration and homogeneity met the acceptance criteria.

Study No. [8375970](#): 26-week transgenic mouse study

Study title: A 26-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with ESN364 in 001178-T (Hemizygous) and 001178-W (Wild Type) RasH2 mice

Study no.: 8375970

Study report location: Module 4.2.3.4.2.

Conducting laboratory and location: (b) (4)

Date of study initiation: 12/11/2018

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: 180621 (99.62%)

CAC concurrence: Protocol: 11-20-2018

Final study: 05-25-2021

Key Study Findings

There were no statistically significant neoplastic findings.

Non-neoplastic lesions were observed in the liver, ovary, and vagina in MD and HD females.

Adequacy of Carcinogenicity Study

The Division considers the transgenic mouse study an adequate assessment of carcinogenic potential because the high dose of ESN364 was based on MTD. A single intraperitoneal dose of N-methyl-N-nitrosourea (MNU) at 75 mg/kg/day was used as a positive control. The lack of survival and occurrence of MNU-related neoplasia (most commonly included malignant lymphoma, squamous cell papilloma on the skin/subcutis and/or stomach, and squamous cell carcinoma on the skin/subcutis) were consistent with the published literature for the RasH2 transgenic mice administered MNU, validating the model for this study.

Appropriateness of Test Models

Yes.

Evaluation of Tumor Findings

Negative

Methods

Doses: 0 (0.5% w/v MC), 50, 150, and 450 mg/kg/day
 Frequency of dosing: Daily
 Dose volume: 10 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: 0.5% w/v methylcellulose solution
 Basis of dose selection: MTD. The ECAC protocol minutes can be found in DARRTS dated 1129/17 under IND 130277.
 Species/Strain: Tg.rasH2
 Number/Sex/Group: 25/sex/group
 Age: 8 weeks of age
 Animal housing: Up to 3 animals/cage
 Paradigm for dietary restriction: Allowed free access to pelleted diet and tap water
 Dual control employed: Yes
 Interim sacrifice: No
 Satellite groups: Yes, TK animals.
 Deviation from study protocol: No effect on the study findings.

Group Composition

Group ^a	Subgroup	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
		Male	Female		
1 (Control)	1 (Carcinogenicity)	25	25	0	0
	2 (Toxicokinetic)	3	3	0	0
2 (Low)	1 (Carcinogenicity)	25	25	50	5
	2 (Toxicokinetic)	18	18	50	5
3 (Mid)	1 (Carcinogenicity)	25	25	150	15
	2 (Toxicokinetic)	18	18	150	15
4 (High)	1 (Carcinogenicity)	25	25	450	45
	2 (Toxicokinetic)	18	18	450	45
5 (Positive Control) ^b	1 (Carcinogenicity)	10	10	75	7.5

^a Group 1 was administered vehicle control article only.

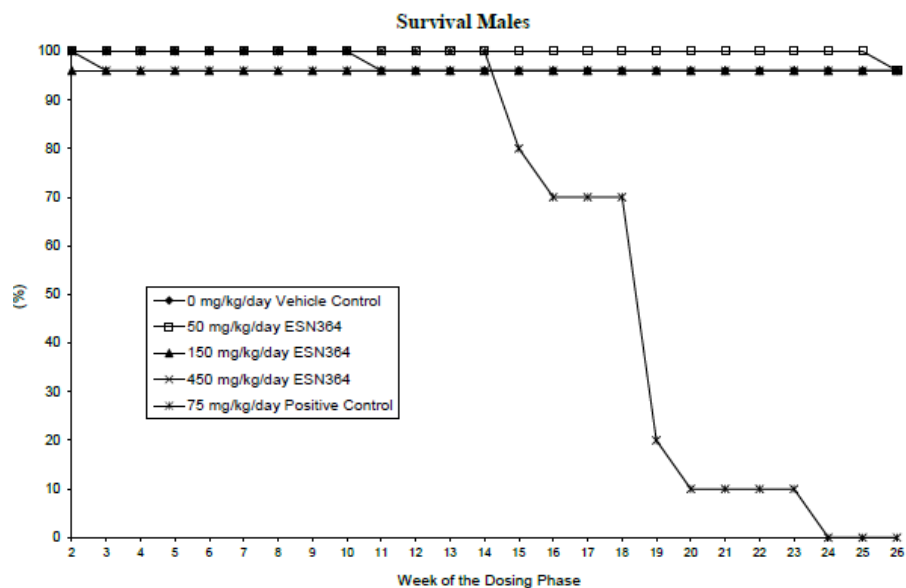
^b Group 5 was administered one intraperitoneal dose of N-methyl-N-nitrosourea (MNU) on Day 1 of the dosing phase. These animals were included as positive controls to ensure animals supplied appropriately expressed oncogenes and responded to carcinogenic insult.

Observations and Results

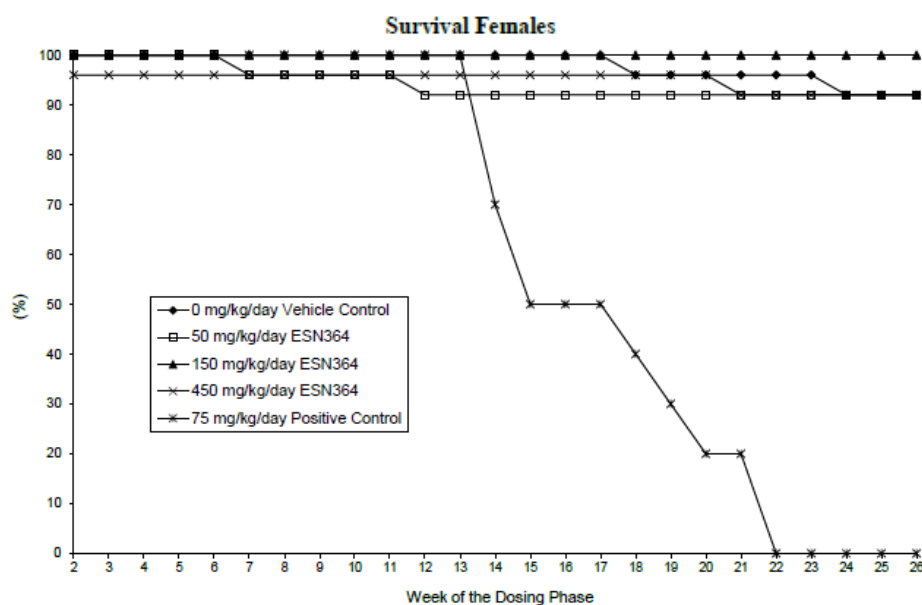
Mortality

There were no test article-related changes in survival rate.

- The survival rates were 96%, 96%, 100% and 96% for males, and 92%, 92%, 100% and 96% for females, in the dose groups 0 (vehicle), 50, 150 and 450 mg/kg/day, respectively, and 0% and 0% for males and females, respectively, of the positive control group.
- FDA Statistical reviewer agreed with the Applicant's survival analysis.**



[Extracted from the Study No. 8375970 study report page 53.]



[Extracted from the Study No. 8375970 study report page 54.]

In addition, ESN364 did not increase the number of deaths attributable to specific neoplasms or non-neoplastic lesions.

Causes of Early Morbidity/Death (Death Comments)

Group	Sex	Animal Number	Dose (mg/kg/day)	Study Day of Death/Sacrifice	Cause of Death/Moribund Condition
1	M	M0025	0	71	Undetermined
2	M	M0121	50	179	Undetermined
4	M	M0321	450	19	Undetermined
1	F	M0505	0	166	Mesothelioma, lung (M)
1	F	M0518	0	124	General debilitation
2	F	M0601	50	47	Undetermined
2	F	M0620	50	78	Hemangiosarcoma, bone (M)
4	F	M0819	450	141	Squamous cell carcinoma, skin (M)

F = Female; M = Male; (M) = Malignant.

[Extracted from the Study No. 8375970 study report page 35.]

Clinical Signs

No effect. No ESN364-related palpable masses were noted in either sex.

ESN364-related effects included clinical observation of white teeth in MD and HD animals. The incidence of white teeth for females was higher than those for males and was associated with macroscopic findings at necropsy for MD and HD females. The cause was not apparent microscopically, and the white teeth observations did not impact the overall health of animals with respect to food consumption, body weight, or survival.

Clinical Sign: White Teeth

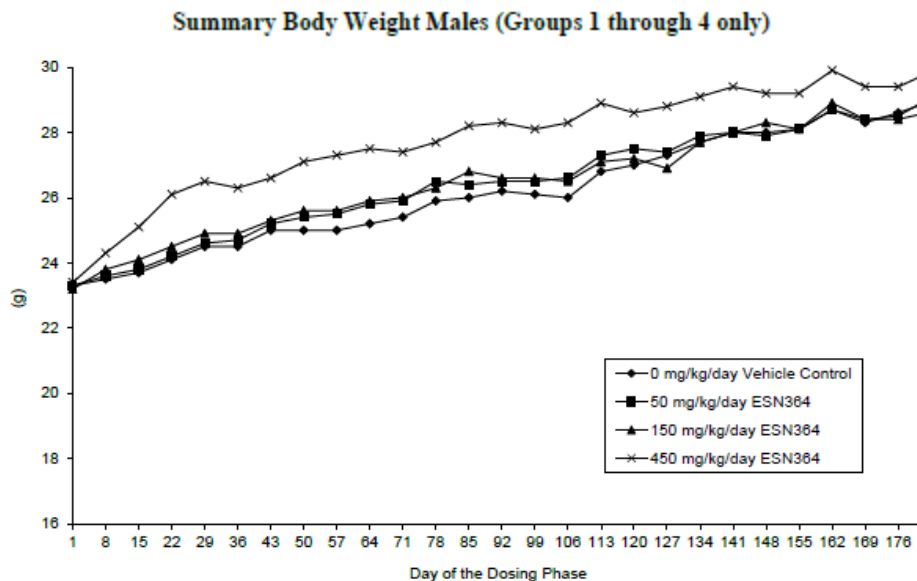
Group	Vehicle Control	ESN364 50 mg/kg/day	ESN364 150 mg/kg/day	ESN364 450 mg/kg/day	Positive Control
Male	0/25	0/25	3/26	1/25	0/10
Female	1/25	0/25	18/25	20/26	2/10

Body Weights

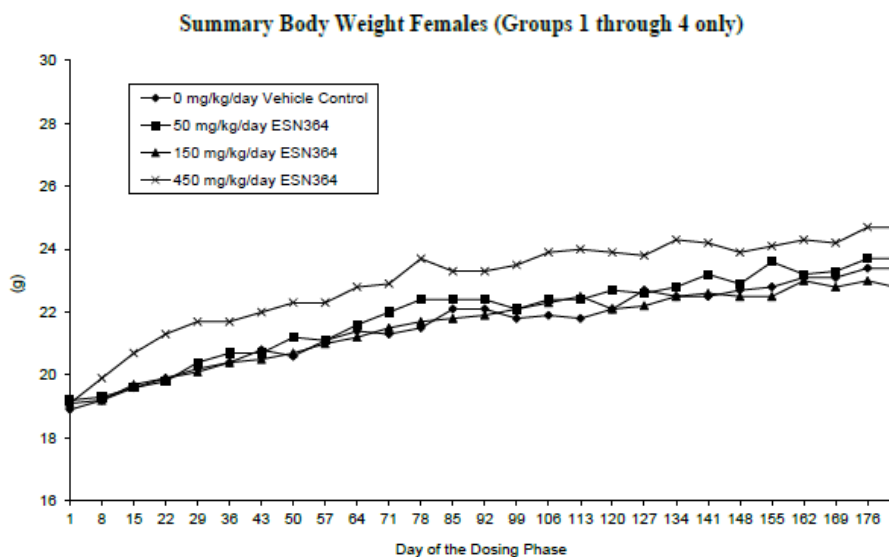
Increased mean body weight gain was observed in HD animals. Over the course of the dosing phase (Days 1 through 182), mean body weight gain in HD animals was 12.5% and 25% (males and females, respectively) higher than those for controls. Increased body weights correlated with ESN364-related increased mean food consumption for HD males, but not HD females.

Body Weights on Day 1 and Day 182

Sex	Male BW (g)		Female BW (g)	
	Day 1	Day 182	Day 1	Day 182
Control (vehicle)	23.3	28.9	18.9	23.4
450 mg/kg	23.4	29.8	19.1	24.7



[Extracted from the Study No. 8375970 study report page 55.]



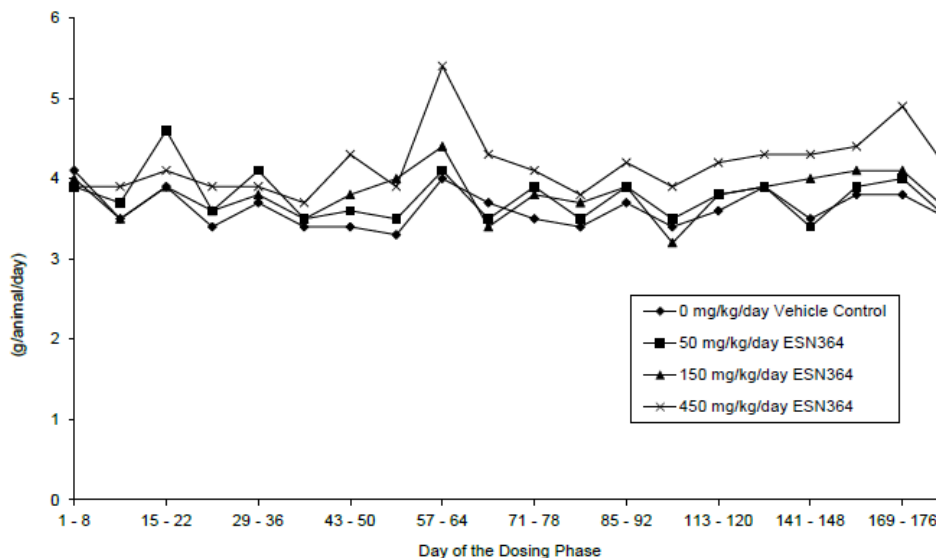
[Extracted from the Study No. 8375970 study report page 56.]

Feed Consumption

An ESN364-related increase in mean food consumption, relative to controls, was noted from Week 3 through to the end of the in-life phase for HD males (5-29%, $p < 0.05$) and at periodic intervals for MD males, which correlated with the increased mean body weights of males during that time. No ESN364-related alterations in mean food consumption were noted for LD males or for all treated females.

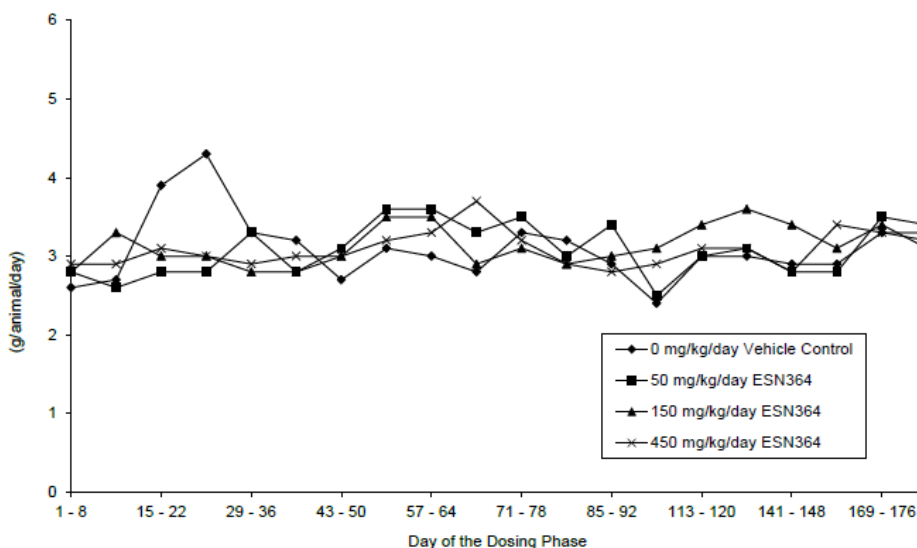
Food consumption				
Sex	Male BW (g)		Female BW (g)	
	Interval 1-8	Interval 176-182	Interval 1-8	Interval 176-182
Control (vehicle)	4.1	3.5	2.6	3.2
450 mg/kg	3.9	4.1 (↑ 17%)	2.9	3.3 (↑ 3%)

Summary Food Consumption Males (Groups 1 through 4 only)



[Extracted from the Study No. 8375970 study report page 57.]

Summary Food Consumption Females (Groups 1 through 4 only)



[Extracted from the Study No. 8375970 study report page 58.]

Gross Pathology

White discoloration of the lower incisor teeth was noted in all treated females, without microscopic correlates, including negative and positive controls, and in one MD male, with increased incidence in MD and HD females. The white teeth discoloration in MD and HD females administered was considered most likely ESN364 related due to the

increased incidence and correlation with in-life observations of white teeth, but its cause was not apparent microscopically.

Histopathology

Peer Review

Yes.

Neoplastic

FDA's statistical reviewer's findings

There were no tumor types with a statistically significant dose response relationship in tumor incidences with increased ESN364. There were also no tumor types with a statistically significant increase in tumor incidences in ESN364 treated groups by the pairwise comparison test, when compared to the vehicle control group in either male or female mice.

Applicant's findings

No statistical significance was observed for tumor incidences.

**Primary Neoplastic Findings in Animals Administered Vehicle
Control Article or ESN364 - Scheduled and Unscheduled Sacrifices and Deaths**

	Sex	Males				Females			
	Dose (mg/kg/day)	0a	50	150	450	0a	50	150	450
	Number of Animals	25	25	25	25	25	25	25	25
Vascular neoplasms									
B-Hemangioma	0	0	2	0	0	2	0	0	
M-Hemangiosarcoma	0	2	0	1	2	1	0	2	
Lung									
B- Adenoma, bronchiolo-alveolar	2	3	0	2	1	0	0	2	
M- Carcinoma, bronchiolo-alveolar	0	0	0	0	0	1	0	0	
M- Mesothelioma	0	0	0	0	1	0	0	0	
Thymus									
B-Thymoma	0	1	1	2	1	2	2	4	
M-Mesothelioma	0	0	0	0	1	0	0	0	
Harderian Gland									
B-Adenoma	0	0	0	0	1	0	0	0	
Stomach, Glandular									
M-Carcinoma	0	0	0	0	0	1	0	0	
Skin/Subcutis									
M- Carcinoma, squamous cell	0	0	0	0	0	0	0	1	

B = Benign; M = Malignant.

a Vehicle control article.

[Extracted from the Study No. 8375970 study report page 39.]

Non-Neoplastic

ESN-364 related non-tumor lesions were observed in the liver of MD and HD females and in the ovary and vagina of HD females.

- **Liver:** Centrilobular hepatocyte hypertrophy was noted in the liver of HD animals. An increased incidence of hepatocyte vacuolation (likely due to increased intracellular glycogen) was noted in MD and HD females and HD males.
- **Ovary:** Decreased numbers of corpora lutea were noted, with increased incidence in the ovary of HD females.

- **Vagina:** Increased incidence and/or severity of cornification of the vaginal mucosa was noted in HD females.

**ESN364-Related Non-Neoplastic Findings in Animals Administered
Vehicle Control Article or ESN364 - Scheduled and Unscheduled Sacrifices and
Deaths**

Sex		Males				Females			
Dose (mg/kg/day)	0 ^a	50	150	450	0 ^a	50	150	450	
Number of Animals	25	25	25	25	25	25	25	25	
Liver									
Vacuolation, increased, hepatocytes									
Minimal	0	1	1	5	0	1	5	7	
Slight	0	0	0	4	0	0	1	15	
Hypertrophy, hepatocyte, centrilobular									
Minimal	0	0	0	8	0	0	0	2	
Ovary									
Corpora lutea, decreased									
Slight	NA	NA	NA	NA	5	2	4	13	
Moderate	NA	NA	NA	NA	0	0	1	0	
Vagina									
Cornification, epithelium									
Minimal	NA	NA	NA	NA	4	4	4	5	
Slight	NA	NA	NA	NA	1	2	2	6	
Moderate	NA	NA	NA	NA	0	0	0	1	

NA = Not Applicable

^a Vehicle control article.

[Extracted from the Study No. 8375970 study report page 40.]

Toxicokinetics

In the toxicokinetics analysis, the systemic exposure to ESN364 and its metabolite, ES00259564, was demonstrated. The C_{max} and AUC_{0-24} values of ESN364 and ES00259564 increased with increasing dose levels, but generally less than dose-proportional. The ES00259564 and ESN364 ratios ranged from 0.264-0.833 for C_{max} and from 0.291-1.31 for AUC_{0-24} .

Summary of the ESN364 Toxicokinetic Parameters in Mouse

Plasma

Interval	Dose Group	Dose Level (mg/kg/day)	Sex	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (h*ng/mL)	t _{1/2} (h)
Day 1	2	50	M	19300	0.500	63500	1.09
			F	20500	1.00	68100	0.869
			MF	19600	1.00	65800	0.986
	3	150	M	48300	2.00	244000	NC
			F	52400	2.00	276000	NC
			MF	50400	2.00	260000	NC
	4	450	M	113000	2.00	1570000	3.01
			F	101000	2.00	749000	2.49
			MF	107000	2.00	1160000	2.84
Day 182	2	50	M	18600	1.00	56800	1.19
			F	17500	0.500	35400	0.865
			MF	16800	0.500	46100	1.04
	3	150	M	56900	1.00	176000	1.39
			F	48200	1.00	108000	0.885
			MF	52500	1.00	142000	1.23
	4	450	M	89600	1.00	350000	1.60
			F	58700	0.500	183000	1.04
			MF	70800	1.00	266000	1.42

NC Not calculated, due to the lack of adequate sampling during the elimination phase, estimation of elimination phase half-life (t_{1/2}) was not attempted at the 150 mg/kg/day dose level on Day 1.

Note: Combined male and female (MF) parameters were calculated by combining concentration data for all animals (male and female) at each dose level on each interval and using these data as a separate composite profile for toxicokinetic analysis. These parameters are not an average of the values calculated for males and females separately.

Summary of the ES00259564 Toxicokinetic Parameters in Mouse

Plasma

Interval	Dose Group	ESN364 Dose Level (mg/kg/day)	Sex	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (h*ng/mL)	t _{1/2} (h)
Day 1	2	50	M	7190	1.00	31100	1.50
			F	7760	1.00	29600	NC
			MF	7480	1.00	30400	1.19
	3	150	M	15100	2.00	100000	NC
			F	14000	2.00	92100	NC
			MF	14500	2.00	96200	NC
	4	450	M	35100	4.00	457000	NC
			F	21200	4.00	217000	NC
			MF	28100	4.00	337000	NC
Day 182	2	50	M	12300	2.00	50800	NC
			F	10800	2.00	35500	NC
			MF	11500	2.00	43200	NC
	3	150	M	26900	2.00	141000	NC
			F	42100	2.00	146000	NC
			MF	34500	2.00	144000	NC
	4	450	M	52000	2.00	334000	2.35
			F	66000	2.00	366000	NC
			MF	59000	2.00	350000	2.84

NC Not calculated, due to the lack of adequate sampling during the elimination phase, estimation of elimination phase t_{1/2} was not attempted for most profiles.

Note: Combined male and female (MF) parameters were calculated by combining concentration data for all animals (male and female) at each dose level on each interval and using these data as a separate composite profile for toxicokinetic analysis. These parameters are not an average of the values calculated for males and females separately.

Dosing Solution Analysis

Both the concentration and homogeneity met the acceptance criteria.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MIYUN M TSAI-TURTON
10/28/2022 10:03:46 AM

ANDREA BENEDICT
10/28/2022 10:09:58 AM