CLINICAL STUDY PROTOCOL

A Randomized, Placebo-Controlled, Double-Blind, Dose-Ranging, Phase 2b Study to Investigate the Efficacy of ESN364 in Postmenopausal Women Suffering From Vasomotor Symptoms (Hot Flashes)

Investigational Product: ESN364
Protocol Number: ESN364_HF_205

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Amendment 3: 01 Aug 2018

SIGNATURE PAGE

STUDY TITLE: A Randomized, Placebo-Controlled, Double-Blind, Dose-Ranging, Phase 2b Study to Investigate the Efficacy of ESN364 in Postmenopausal Women Suffering From Vasomotor Symptoms (Hot Flashes)

I, the undersigned, have read this protocol and agree that it contains all necessary information required to conduct the study.

Signature Date

PPD

INVESTIGATOR AGREEMENT

By signing below I agree that:

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the study as described. I will conduct this study in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the study within the time designated. I will provide copies of this protocol and access to all information furnished by Ogeda S.A. to study personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the study product and study procedures. I will let them know that this information is confidential and proprietary to Ogeda S.A. and that it may not be further disclosed to third parties. I understand that the study may be terminated or enrollment suspended at any time by Ogeda S.A., with or without cause, or by me if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in full accordance with Food and Drug Administration (FDA) Regulations, IRB Regulations, and International Council for Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP).

_____ _____

Investigator's Signature Date

_____

Investigator's Printed Name

SYNOPSIS

TITLE: A Randomized, Placebo-Controlled, Double-Blind, Dose-Ranging, Phase 2b Study to Investigate the Efficacy of ESN364 in Postmenopausal Women Suffering From Vasomotor Symptoms (Hot Flashes)

PROTOCOL NUMBER: ESN364_HF_205

INVESTIGATIONAL PRODUCT: ESN364 (International nonproprietary name: Fezolinetant)

PHASE: 2b

INDICATION: Postmenopausal vasomotor symptoms (hot flashes)

OBJECTIVES:

The primary objective of this study is to evaluate the effect of different doses and dosing regimens of ESN364 on frequency and severity of vasomotor symptoms (hot flashes).

The secondary objectives of this study are the following:

- To evaluate the effect of different doses and dosing regimens of ESN364 on the frequency, severity, and hot flash score of mild, moderate, and severe vasomotor symptoms;
- To evaluate the effect of different doses and dosing regimens of ESN364 on responder rates using variation of responder definitions;
- To evaluate the effect of different doses and dosing regimens of ESN364 on patient-reported outcomes;
- To evaluate the effect of different doses and dosing regimens of ESN364 on pharmacodynamics markers (hormones and bone markers); and
- To evaluate the effect of different doses and dosing regimens of ESN364 on safety and tolerability.

The exploratory objectives of this study are the following:

- [redacted] CCI
- To evaluate the pharmacokinetic (PK) plasma concentrations of ESN364 and metabolite [redacted] CCI

POPULATION:

The population for this study is postmenopausal women >40 years and ≤65 years of age with at least 50 moderate to severe vasomotor symptoms (hot flashes) per week.

Inclusion Criteria:

Subjects who meet all of the following criteria will be eligible to participate in the study:

1. Women >40 years and ≤65 years of age at the screening visit;
2. A body mass index between 18 kg/m^2 to 38 kg/m^2 (extremes included);
3. Spontaneous amenorrhea for ≥12 consecutive months; or spontaneous amenorrhea for ≥6 months with biochemical criteria of menopause (follicle-stimulating hormone [FSH] >40 IU/L); or having had bilateral oophorectomy ≥6 weeks prior to the screening visit (with or without hysterectomy);
4. At least 50 moderate to severe vasomotor symptoms per week (ie, 7 consecutive days), as recorded in the daily diary during the screening period;
5. In good general health as determined on the basis of medical history and general physical examination, including a bimanual clinical pelvic examination and clinical breast examination devoid of relevant clinical findings, performed at the screening visit; hematology and biochemistry parameters, pulse rate and/or blood pressure, and electrocardiogram (ECG) within the reference range for the population studied, or showing no clinically relevant deviations, as judged by the Investigator;
6. Women >40 years of age who have documentation of a normal/negative or no clinically significant findings mammogram (obtained at Screening or within the prior 9 months of trial enrollment). Appropriate documentation includes a written report or an electronic report indicating normal/negative or no clinically significant mammographic findings;
7. Willing to undergo a transvaginal ultrasound to assess endometrial thickness at screening and at Week 12 (end-of-treatment), and for subjects who are withdrawn from the study prior to completion, at the Early Termination (ET) Visit. This is not required for subjects who have had a partial (supracervical) or full hysterectomy;
8. Willing to undergo an endometrial biopsy at screening (in the event that the subject's transvaginal ultrasound shows endometrial thickness ≥4 mm) and at Week 12 (end-of-treatment – all subjects), for subjects with uterine bleeding, and for subjects who are withdrawn from the study prior to completion, at the ET Visit if study drug exposure is ≥10 weeks. This is not required for subjects who have had a partial (supracervical) or full hysterectomy;
9. Negative alcohol breath test and negative urine test for selected drugs of abuse (amphetamines, tricyclic antidepressants, cocaine, or opiates) at the screening visit;
10. Negative urine pregnancy test;
11. Negative serology panel (including hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antibody screens);

12. Informed Consent Form signed voluntarily before any study-related procedure is performed, indicating that the subject understands the purpose of and procedures required for the study and is willing to participate in the study; and
13. Documentation of a normal Pap smear (or equivalent cervical cytology) or of no clinical significance in the opinion of the Investigator within the previous 9 months or at screening.

Exclusion Criteria:

Subjects who meet any of the following criteria will be excluded from participation in the study:

1. Use of a prohibited therapy (hormone therapy, hormonal contraceptive, or vasomotor symptom medication [prescription, over the counter, or herbal]) or not willing to wash out drugs;
2. History (in the past year) or presence of drug or alcohol abuse;
3. Previous or current history of a malignant tumor, except for basal cell carcinoma;
4. Uncontrolled hypertension and a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg;
5. Judged by the Investigator to be unsuited to participate in the study based on findings observed during physical examination, vital sign assessment, or 12-lead ECG;
6. History of severe allergy, hypersensitivity, or intolerance to drugs in general, including the study drug and any of its excipients;
7. Exclusion criterion 7 has been removed in Amendment 1;
8. An unacceptable result from endometrial biopsy (performed when endometrial thickness is ≥4 mm measured by transvaginal ultrasound) of endometrial hyperplasia, endometrial cancer, or inadequate specimen at screening (1 repeat biopsy permitted if technically possible);
9. History of endometrial hyperplasia or uterine/endometrial cancer;
10. History of unexplained uterine bleeding;
11. History of seizures or other convulsive disorders;
12. Medical condition or chronic disease (including history of neurological [including cognitive], hepatic, renal, cardiovascular, gastrointestinal, pulmonary [eg, moderate asthma], endocrine, or gynecological disease) or malignancy that could confound interpretation of the study outcome;
13. Presence or sequelae of gastrointestinal, liver, kidney, or other conditions known to interfere with the absorption, distribution, metabolism, or excretion mechanisms of drugs as judged by the Investigator;
14. Active liver disease or jaundice, or values of alanine aminotransferase and aspartate aminotransferase >1.5 × the upper limit of normal (ULN); or total bilirubin >1.5 × ULN; or creatinine >1.5 × ULN; or estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease formula ≤59 mL/min/1.73 m^2 at the screening visit;

15. Concurrent participation in another interventional study (or participation within 3 months prior to screening in this study);
16. Suicide attempt in the past 3 years;
17. Unable or unwilling to complete the study procedures; or
18. Subject is the Investigator or any sub-Investigator, research assistant, pharmacist, study coordinator, or other staff or relative thereof, who is directly involved in the conduct of the study.

STUDY DESIGN AND DURATION:

This is a 12-week randomized, double-blind, placebo-controlled, dose-ranging, parallel-group, multicenter study to assess the efficacy of ESN364 in postmenopausal women suffering from vasomotor symptoms (hot flashes).

This study will consist of a screening period (Days -35 to -1, including the screening visit [Visit 1] and a minimum 7-day collection of baseline vasomotor symptom frequency and severity assessments), a 12-week treatment period (Day 1 [Visit 2] to Week 12 [Visit 5], including safety visits at Week 2 [Visit 2A], Week 6 [Visit 3A], and Week 10 [Visit 4A]), and a follow-up visit (Week 15 [Visit 6]) approximately 3 weeks after the last dose of study drug, for a total of 9 visits.

The study will be performed on an ambulatory basis.

The screening visit (Visit 1) will occur up to 35 days prior to randomization. Eligibility will be assessed via physical examination, clinical laboratory testing, vital signs, ECG, Pap smear, mammography, and endometrial biopsy. Subjects will receive an electronic diary in which to record daily vasomotor symptoms during the duration of the screening period. Subjects must have ≥7 consecutive days of vasomotor symptom recordings to participate in the study. Subjects are encouraged to continue recording for the duration of the whole screening period. The electronic diary will be reviewed by study site staff on Day 1 (Visit 2) to confirm study eligibility. Subjects may be rescreened 1 time upon approval of the medical monitor.

During the treatment period, subjects will return to the study site every 2 weeks for assessments.

The follow-up visit will occur approximately 3 weeks following the last dose of study drug.

DOSAGE FORMS AND ROUTE OF ADMINISTRATION:

Subjects will be randomized to 1 of 8 treatment groups in an equal ratio:

- ESN364 15 mg twice daily (BID),
- ESN364 30 mg BID,
- ESN364 60 mg BID,
- ESN364 90 mg BID,
- ESN364 30 mg once daily (QD) + placebo QD,
- ESN364 60 mg QD + placebo QD,

- ESN364 120 mg QD + placebo QD, or
- Placebo BID.

ESN364 capsules will be taken orally with a glass of room temperature tap water.

ENDPOINTS FOR EVALUATION:

Co-Primary Efficacy Variables

The primary efficacy objective will require the evaluation of the effect of ESN364 on the following 4 co-primary variables:

- Mean change in the frequency of moderate to severe vasomotor symptoms from baseline to Week 4;
- Mean change in the frequency of moderate to severe vasomotor symptoms from baseline to Week 12;
- Mean change in the severity of moderate to severe vasomotor symptoms from baseline to Week 4; and
- Mean change in the severity of moderate to severe vasomotor symptoms from baseline to Week 12.

Secondary Efficacy Variables

The secondary efficacy variables include the effect of ESN364 on the following:

Vasomotor Symptom Variables:

- Mean change in the frequency of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean change in the frequency of moderate and severe vasomotor symptoms from baseline to each study week;
- Mean change in the severity of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean change in the severity of moderate and severe vasomotor symptoms from baseline to each study week;
- Mean change in the hot flash score of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean change in the hot flash score of moderate and severe vasomotor symptoms from baseline to each study week;
- Mean percent reduction of mild, moderate, and severe vasomotor symptoms from baseline to each study week; and
- Mean percent reduction of moderate and severe vasomotor symptoms from baseline to each study week.

Responder Variables:

- Mean percent reduction of 50%, 70%, 90%, and 100% of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean percent reduction of 50%, 70%, 90%, and 100% of moderate and severe vasomotor symptoms from baseline to each study week;
- Absolute reduction of 2, 3, 4, and 5 in mean number of mild, moderate, and severe vasomotor symptoms per day from baseline to each study week; and
- Absolute reduction of 2, 3, 4, and 5 in mean number of moderate and severe vasomotor symptoms per day from baseline to each study week.

Patient-Reported Outcome Variables:

- Change in Hot Flash-Related Daily Interference Scale (HFRDIS), Leeds Sleep Evaluation Questionnaire (LSEQ), Greene Climacteric Scale (GCS), and Menopause-Specific Quality of Life (MENQoL) questionnaire from baseline to Weeks 4, 8, 12, and 15.

Pharmacodynamic Variables:

- Change over time from baseline to Week 12 in plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and sex hormone-binding globulin (SHBG).

Exploratory Variables

[redacted]

- Level of PK plasma concentrations of ESN364 and metabolite [redacted] at pre-specified timepoints.

Safety Variables

The safety variables include the following:

- Incidence and severity of treatment-emergent adverse events;
- Endometrial health assessment (transvaginal ultrasound ± endometrial biopsy);
- Vital signs (sitting systolic and diastolic blood pressure and pulse rate);
- Laboratory tests (hematology, biochemistry, urinalysis, and coagulation);
- ECG parameters;
- Plasma bone density marker concentrations;
- Physical examinations; and
- Columbia Suicide Severity Rating Scale (C-SSRS).

STATISTICAL ANALYSES:

Analysis Populations

All primary and secondary efficacy endpoints will be analyzed using the Full Analysis Set (FAS). The Per Protocol Analysis Set (PPAS) will be used only for the analysis of the selected endpoints to examine the robustness of the primary analysis.

Safety and tolerability will be analyzed using the Safety Analysis Set.

Safety Analysis Set: The Safety Analysis Set comprises all subjects who receive at least 1 dose of study drug.

Full Analysis Set: The FAS comprises the subset of the Safety Analysis Set who have a baseline and post-baseline efficacy evaluation.

Per Protocol Analysis Set: The PPAS comprises the subset of subjects from the FAS who are treated according to the protocol without any major deviations. A full list of inclusion criteria will be decided and assessed prior to database lock and unblinding.

Pharmacokinetic Analysis Set: The PK Analysis Set comprises the subset of the Safety Analysis Set who provide evaluable PK assessments.

Efficacy Analysis

Primary Efficacy Analysis: The 4 co-primary efficacy endpoints are the mean change in the frequency of moderate to severe vasomotor symptoms and the mean change in severity of vasomotor symptoms from baseline to Week 4 and Week 12.

For each of the 4 co-primary efficacy endpoints, an analysis of covariance model will be used with treatment group, pooled center, and smoking status as factors, with baseline weight and baseline measurement as covariates. Pairwise comparisons between the active doses and placebo will be calculated based on least-squares mean contrasts using a 2-tailed 95% confidence interval (CI). For subjects in the efficacy analysis populations with missing primary efficacy endpoints, multiple imputation by fully conditional specification methods will be used. The imputation model will use subject demographics (age, sex, race, baseline weight, and smoking status) and baseline and post-baseline mean number and severity of vasomotor symptoms. Sensitivity analyses will be performed with other multiple imputation methods and mixed model for repeat measurements.

Since the study design requires the comparison of 7 active dose groups with placebo for 4 co-primary efficacy variables, a two-tier closed testing procedure will be used to control the family-wise error. A step-down testing procedure will be followed. The following testing order of doses will be employed:

a. Placebo versus 90 mg BID (daily dose 180 mg),
b. Placebo versus 60 mg BID (daily dose 120 mg),
c. Placebo versus 120 mg QD (daily dose 120 mg),
d. Placebo versus 30 mg BID (daily dose 60 mg),
e. Placebo versus 60 mg QD (daily dose 60 mg),
f. Placebo versus 15 mg BID (daily dose 30 mg), and
g. Placebo versus 30 mg QD (daily dose 30 mg).

The 4 co-primary efficacy endpoints will be compared at each dose level. Only when ESN364 is proved to be significantly better than placebo at a 2-sided alpha level of 0.05 for all 4 co-primary efficacy endpoints will this dose level be claimed to be superior to placebo. A failure to prove the significance for 1 or more co-primary efficacy endpoints will lead to a failure to claim superiority to placebo at this dose level, and the other dose levels after this one in the sequence will not be tested.

The primary efficacy analysis will be based on the FAS. A supportive analysis will be carried out for the co-primary efficacy variables based on the PPAS to examine the impact of premature dropouts and/or major protocol deviations.

Secondary Efficacy Analysis: Efficacy will be further assessed based on the secondary endpoints described above using the FAS.

Safety Analysis

Safety will be assessed by examining the incidence of adverse events, physical examination findings, C-SSRS, vital signs, ECGs, clinical laboratory tests, and bone density marker concentrations over time using the Safety Analysis Set.

SAMPLE SIZE DETERMINATION:

A total of 352 subjects are planned to be randomized, 44 subjects in each treatment arm. This is based on the observed data for severity and frequency of moderate and severe vasomotor symptoms from the Phase 2a study. Sample size calculations from statistical analysis software were based on 2-sample t-tests, assuming 2-sided 5% alpha and power of >90% for expected effect sizes.

SITES: Approximately 55 sites in the United States

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Abbreviation	Definition
ADME	Absorption, distribution, metabolism, and excretion
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BALP	Bone alkaline phosphatase
BID	Bis in die; twice daily
CI	Confidence interval
CRA	Clinical Research Associate
CRO	Contract Research Organization
C-SSRS	Columbia Suicide Severity Rating Scale
CTX	Carboxy-terminal telopeptide of type I collagen
DILI	Drug-Induced Liver Injury
DXA	Dual-energy x-ray absorptiometry
E2	Estradiol
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
ePRO	Electronic Patient-Reported Outcome
ET	Early Termination
FAS	Full Analysis Set
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GCS	Greene Climacteric Scale
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GnRH	Gonadotropin-releasing hormone
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HFRDIS	Hot Flash Related Daily Interference Scale
HIV	Human immunodeficiency virus
HPG	Hypothalamic-pituitary-gonadal
HRT	Hormone replacement therapy
ICF	Informed Consent Form
ICH	International Council for Harmonisation
INR	International Normalized Ratio

Abbreviation	Definition
IRB	Institutional Review Board
IRT	Interactive Response Technology
KNDy	Kisspeptin/neurokinin B/dynorphin
LH	Luteinizing hormone
LSEQ	Leeds Sleep Evaluation Questionnaire
MENQoL	Menopause-Specific Quality of Life
MTD	Maximum tolerated dose
NIH	National Institutes of Health
NK3	Neurokinin 3
NK3R	Neurokinin 3 receptor
NKB	Neurokinin B
NOAEL	No observed adverse effect level
P1NP	Procollagen type 1 amino-terminal propeptide
P4	Progesterone
PD	Pharmacodynamic(s)
PGX	Pharmacogenomic(s)
PK	Pharmacokinetic(s)
PPAS	Per Protocol Analysis Set
QD	Quaque die; once daily
SAE	Serious adverse event
SHBG	Sex hormone-binding globulin
SD	Standard deviation
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal

1 INTRODUCTION AND BACKGROUND INFORMATION

1.1 Background Information

1.1.1 Vasomotor Symptoms (Hot Flashes)

Vasomotor symptoms (hot flashes) are the most common complaint among women entering menopause and, for many women, may continue to occur for up to 5 years (although about 20% of women may have them for up to 15 years).[1–3] Approximately 75% of peri-menopausal women will experience vasomotor symptoms (hot flashes), with 10% to 20% of those enduring severe symptoms.[4] Today, more than 25 million women in the United States alone experience symptoms of vasomotor symptoms (hot flashes), and 4 million women report severe symptoms.[5]

Vasomotor symptoms (hot flashes) can have a significant negative impact on the quality of life and are therefore a major reason for menopausal women to seek medical attention. Despite the vast numbers of individuals affected, the physiology of vasomotor symptoms (hot flashes) is not fully understood; although, a disturbance in normal thermoregulatory function is thought to be the main underlying cause.

The primary symptom of vasomotor symptoms (hot flashes) is a subjective and transient sensation of heat, flushing, and sweating that usually lasts 4 to 10 minutes and may be accompanied by palpitations, feelings of anxiety, irritability, and in rare occurrence, panic.[4]

The most effective and commonly used pharmacological treatment for vasomotor symptoms (hot flashes) is hormone replacement therapy (HRT), but a Women's Health Initiative study raised questions about the long-term safety of this treatment. Current guidelines recommend a limited duration of HRT due to associated risks of breast cancer, coronary artery disease, stroke, and thromboembolism.[1,6] Furthermore, the current safety data do not support the use of HRT in many patients (eg, patients with breast/endometrial cancer or liver disease).

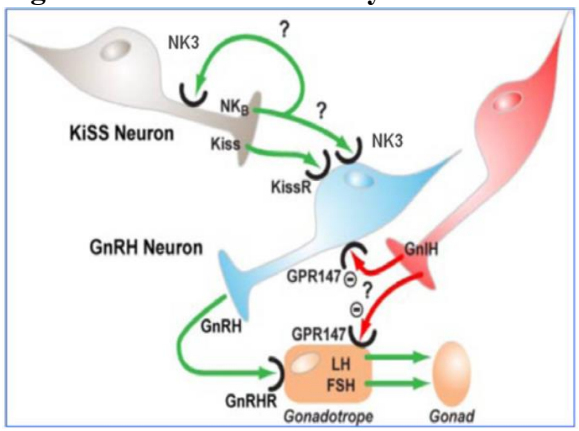
The perceived limitations of HRT coupled with the lack of efficacy and the adverse effects observed with non-hormonal therapies have led clinicians to search for other treatment options for vasomotor symptoms. Recent studies of venlafaxine and fluoxetine in women with a prior history of breast cancer have suggested that certain antidepressants with the ability to inhibit serotonin reuptake may significantly reduce vasomotor symptoms of menopause.[7–9] However, the clinical benefit of these antidepressants is not as great as that observed for estrogen.

1.1.2 Introduction to ESN364 (International Nonproprietary Name: Fezolinetant)

ESN364 is an orally-available, potent, and selective small molecule neurokinin 3 receptor (NK3R) antagonist shown to significantly modulate the hypothalamic-pituitary-gonadal (HPG) axis to lower the circulating levels of sex hormones in nonclinical studies and in clinical trials. The mechanism-of-action of ESN364 is to decrease the luteinizing hormone (LH) pulse frequency, a response indicative of a decrease in the gonadotropin-releasing hormone (GnRH) pulse frequency.[10] Moreover, the finding that the drug action selectively affected LH but not follicle-stimulating hormone (FSH) provides additional evidence that ESN364 does not block GnRH signaling but merely decreases the GnRH pulse frequency. The selective effect of ESN364 to lower LH but not FSH is consistent in rats, sheep, monkeys, and male and female humans.[10,11]

Recent advances in the field have demonstrated that the GnRH pulse frequency is modulated by the Kisspeptin/neurokinin B (NKB)/dynorphin (KNDy) neurons (also known as "KiSS Neuron") in the arcuate nucleus of the hypothalamus.[12] Neuroanatomical studies have shown that these neurons are sensitive to NKB/NK3R signal.[13] These data support the hypothesis that ESN364 antagonism of NK3R on the KNDy neuron decreases the GnRH pulse frequency as the basis of the downstream effects observed on LH, FSH, and the sex hormones. The schematic of the neurokinin 3 (NK3) modulatory role on GnRH neurons to inhibit GnRH release and consequent release of sex hormones is presented in Figure 1.

Figure 1. NK3 Modulatory Role on GnRH Neurons



Select abbreviations: FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; KiSS = kisspeptin/neurokinin B/dynorphin; LH = luteinizing hormone; NK3 = neurokinin 3.
Source: Investigator's Brochure[14]

Furthermore, the proposed pharmacological action of ESN364 is consistent with the findings of a landmark publication revealing that a human population identified to have a loss-of-function mutation in NK3R presents a phenotype of reduced circulating levels of sex hormones, but with the important distinction that the levels of these hormones are above castration levels in women.[15] A follow-up article demonstrates this point clearly by showing that patients with a loss-of-function mutation in NK3 present very low levels of LH but that FSH levels are not significantly altered. In contrast, patients expressing a GnRH receptor loss-of-function mutation exhibit castration levels of both LH and FSH.[16] Luteinizing hormone and FSH function independently to stimulate estradiol (E2) release from the ovaries. In total, these human mutant studies predict that ESN364 antagonism of NK3R will diminish levels of E2 due to reduction of the LH pulse frequency but will not affect FSH levels. Therefore, there is a natural safety margin in place that protects against over-inhibition of E2 and the attenuation side effects of chemical castration such as the maintenance of bone density as well as maintenance of a normal, healthy sex life. This safety margin is based on the NK3-selective modulation of LH, but not FSH, and this is the anticipated advantage of ESN364 over the existing GnRH products currently prescribed (eg, Lupron®, Zoladex®, etc.).

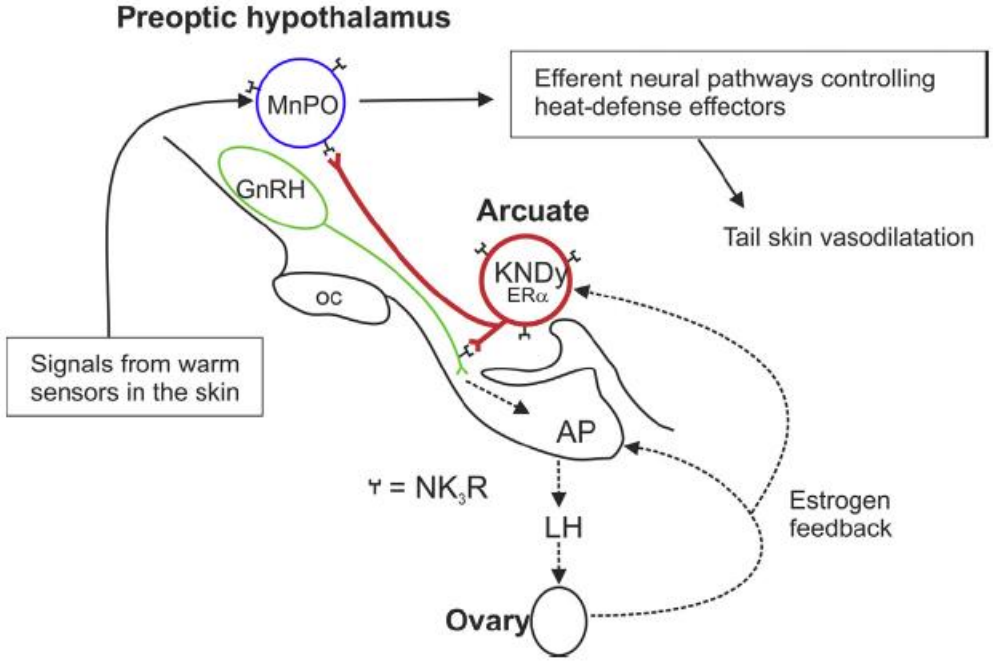
In addition to the precedent established by the human population study, an additional clinical precedence has been established by the historic testing of NK3 antagonists in clinical studies by various pharmaceutical companies. The therapeutic goal at the time was the treatment of schizophrenia, wherein all compounds failed due to lack of convincing efficacy in Phase 2 studies. Importantly, these development programs report dose-related changes in LH, testosterone, and various reproductive organs (testes, epididymis, and prostate) in male dogs and a

testosterone-lowering effect in male volunteers.[17] This history indicates that NK3 antagonism is a target concept for drug development with clinical precedence to reversibly lower sex hormones.

As described above, the KNDy neurons are sensitive to NKB/NK3R signaling that is susceptible to pharmacological blockade by ESN364 to decrease KNDy neuron activity. Importantly, estrogen also acts directly on the alpha estrogen receptor expressed on KNDy neurons to similarly decrease KNDy neuron activity.[18,19] Thus, ESN364 and estrogen have common pharmacological effects to decrease KNDy neuron activity.

In physiological situations where there is a change toward lower estrogen levels, such as in menopause or in the case of therapies used to lower estrogen to treat estrogen-dependent cancers, estrogen feedback control of KNDy neuron activity is lost. The KNDy neuron is known to synapse at 2 sites: (1) the GnRH neuron, as explained earlier, and (2) the median preoptic nucleus which is an area of the brain critical for autonomic thermoregulation.[20] Furthermore, additional evidence linking NK3R signaling on KNDy neuron activity to vasomotor symptom/thermoregulation includes the observations that (1) administration of NKB (NK3R agonist) provoked vasomotor symptoms in premenopausal women,[21] (2) administration of ESN364 (NK3 antagonist) prevented body temperature changes in ovariectomized ewe,[10] and (3) the demonstration of a significant association between risk of vasomotor symptoms and genetic variation in tachykinin receptor 3[22] (see Figure 2).

Figure 2. Relationship Between KNDy Neurons, GnRH Neurons, and the Heat-Defense Pathway in Rat



Select abbreviations: ERα = estrogen receptor alpha; GnRH = gonadotropin-releasing hormone; KNDy = kisspeptin/neurokinin B/dynorphin; LH = luteinizing hormone; MnPO = median preoptic nucleus; NK3R = neurokinin 3 receptor.
Source: Rance NE[20]

1.2 Summary of Nonclinical Studies

A series of non-Good Laboratory Practice (GLP) and GLP nonclinical studies were carried out in order to define the pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of the test product ESN364. Compound affinity to human NK3R and to rat and monkey NK3R orthologs were confirmed via in vitro studies. Receptor-ligand affinity for ESN364 has been confirmed as essentially equipotent for the human and monkey NK3R orthologs. Also, the metabolic stability of the test item was determined to be similar in human and monkey liver microsomes. These preliminary studies justified the use of rat and monkey species in nonclinical testing. Since castrate animals exhibit high and stable levels of plasma LH, castrated rats and monkeys were used as the animal model of choice to measure the PD response to ESN364 in the modulation of plasma LH. Rats and monkeys were used for GLP toxicity studies, and monkeys, as the most sensitive species, were used to define the no observed adverse effect level (NOAEL) and to determine the starting dose in the Phase 1 clinical study.

In the nonclinical toxicology studies in rats and monkeys, ESN364 was well tolerated and the NOAEL was considered to be 25 mg/kg/day in Cynomolgus monkeys, the most relevant species; drug exposure (ie, AUC) at this dose level in Cynomolgus monkey was similar to drug exposure levels measured in premenopausal women dosed at 540 mg/day. The main events that were observed in the nonclinical studies were considered to be related to the pharmacology of ESN364, including reduction of the ovarian activity in female monkeys. Adverse events were only observed at the very high doses used in the nonclinical studies. The NOAEL exceeded the intended clinical doses by a factor of 20. In monkeys, high doses of ESN364 resulted in weight loss and histopathological changes such as hyperplasia of lymphocytes in lymph nodes, spleen, and lung, as well as perivascular inflammation in the kidney. In addition, a reduction in platelet counts considered to be due to decreased production in bone marrow resulted in observations of hemorrhage and regenerative anemia; these effects were recoverable with discontinuation of dosing. In rats, very high dose levels were associated with death and marked clinical signs (including lethargy, reduced activity, labored respiration, and staggering), and body weight loss during the first few days of treatment.

In a 3-month toxicology study in rats, convulsive episodes were reported with no dose- or time-dependent trends or concurrent ESN364 exposure. Convulsive episodes were reduced significantly by changing the handling procedures. In an independent assessment of the convulsive episodes in this study, it was concluded that the event was not linked to the test drug ESN364, but rather a stress-and-fear reaction (expert report from Brunner Naga Health Science Consulting, Switzerland, available upon request). In order to evaluate the potential of ESN364 to produce seizures in rats, a long-term central nervous system toxicity study was conducted. In this study, no seizures were detected throughout the study based on review of video-electroencephalogram records. In addition, no convulsions were observed in a subsequent 6-month study in rat conducted at exactly the same dose levels (NOAEL of 200 mg/kg/d). Therefore, it was concluded that the convulsive episodes observed in the 3-month study were unlikely to be related to ESN364 administration. Notably, no signs of convulsive activity were observed in 3-month and 9-month toxicology studies in nonhuman primates, nor in any of the clinical studies conducted.

Reproductive toxicology studies on both rats and rabbits demonstrated significant litter loss in both animal species at high dose levels; however, the surviving embryos did not show any adverse effect on development. The litter loss in this case is to be regarded as a pharmacologic effect on the

hormonal and reproductive status. A fertility and early embryonic development study was also completed in rat without any reported adverse events (NOAEL of 100 mg/kg/d).

For more detailed information on ESN364 and the nonclinical toxicology studies, see the current Investigator's Brochure.[14]

1.3 Clinical Studies

1.3.1 Summary of Clinical Studies

[redacted]

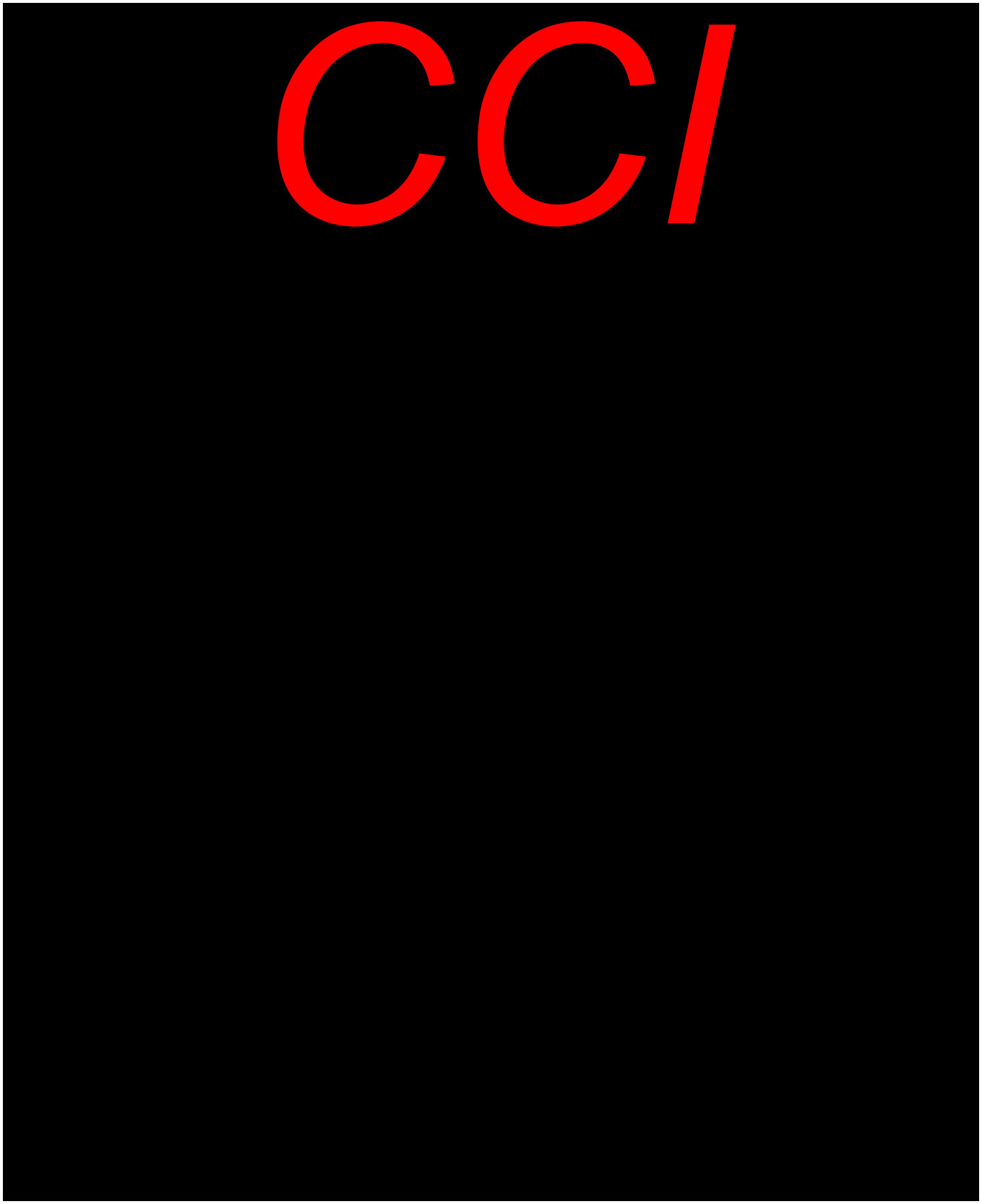
A second Phase 1 clinical trial exploring the maximum tolerated dose (MTD) of ESN364 in healthy female and healthy male volunteers has been recently completed (ESN-364-CPK-102). Dose ranges between 180 mg and 900 mg have been tested as single doses. Doses up to 720 mg have been given in a multiple dose fashion for 7 consecutive days. It was decided that 900 mg ESN364 was to be the MTD because of the occurrence of headache and facial paresthesia (a feeling of numbness around the mouth and face). This complaint was reported at 540 mg (reported start times around 40 minutes postdose). The reported paresthesia tended to become more pronounced and prolonged with increasing doses. The apparent dose-dependent nature of the event, together with the occurrence of some moderate headaches, led to the decision to stop further escalation. The subsequent 7-day multiple dose part was conducted using 540 mg and 720 mg of ESN364 once daily (QD) in healthy female volunteers. These dose levels were well tolerated and safe.

Three Phase 2a trials (proof-of-concept) have been initiated in 3 separate therapeutic indications: polycystic ovary syndrome (ESN-364-PCO-201), heavy menstrual bleeding due to uterine fibroids (ESN-364-UF-02), and [redacted]

Based on the collected clinical and nonclinical safety data, it can be concluded that doses up to 360 mg/day can be safely administered over a 3-month dosing period. Dose ranges that cover an appropriate 10-fold range between 20 mg/day and 180 mg/day will allow exploration of a dose response in the patients.

For more detailed information on ESN364 and the clinical studies, see the current Investigator's Brochure.[14]

1.3.2 Effects of ESN364 (Fezolinetant)



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Two other Phase 2a trials (proof-of-concept) have been initiated in 2 separate therapeutic indications: polycystic ovary syndrome (ESN-364-PCO-201) and heavy menstrual bleeding due to uterine fibroids (ESN-364-UF-02). The results of these Phase 2 clinical trials are not yet available.

Please see the most current version of the Investigator's Brochure for additional details.

1.4 Rationale

ESN364 has been selected as the development candidate molecule based on its superior PK profile, oral bioavailability, and selective target potency. The summary of clinical studies supports the rationale for the current trial to refine both the dose-level and dosing regimen in order to define the minimal effective dose level of fezolinetant to treat moderate and severe vasomotor symptoms.

1.5 Risk/Benefit

ESN364 is being developed for the treatment of women's health disorders such as menopausal vasomotor symptoms, endometriosis, polycystic ovarian syndrome, and uterine fibroids. The compound may also be developed for sex hormone disorders affecting men, such as benign prostate hyperplasia.

Due to recent advances in science, there are reasons to believe that ESN364 can be an effective treatment for the symptoms and morbidity associated with menopause.

A key feature of NK3 antagonism is the selective decrease of LH secretion without compromising FSH secretion by decreasing the firing rate of the KNDy neuron.

When given to normally cycling healthy women, ESN364 is capable of altering the menstrual cycle and decreasing the circulating levels of E2, LH, progesterone (P4), and testosterone. Since this study aims to include postmenopausal volunteers, these effects will become of less importance because of the physiological changes that happen in the climacterium (anovulation with loss of P4 and E2 production, and consequently increase of LH/FSH).

In terms of hormonal changes, a mild to moderate decrease of the already elevated LH and FSH plasma levels is anticipated. There are no known risks associated with this decrease of the gonadotropins in menopause.

Treatment with ESN364 can cause adverse effects or other symptoms. Adverse effects that can be expected are those adverse events that presented in ESN364 clinical trials in healthy male and female volunteers as well as in menopausal women.

Details of the adverse event profile from the proof-of-concept study are presented in Section 1.3. All recorded treatment-related TEAEs were mild to moderate in severity. All adverse events resolved spontaneously without sequelae. The reported palpitations were short-lived and were not accompanied by other signs/symptoms of cardiac involvement (vital signs and electrocardiogram [ECG] findings were normal). Current knowledge of the side effect profile of ESN364 is still limited.

[redacted]

Indirectly, subjects could benefit from participating in this clinical trial because of the intense medical follow-up demanded by the protocol for the duration of the trial.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to evaluate the effect of different doses and dosing regimens of ESN364 on frequency and severity of vasomotor symptoms (hot flashes).

2.2 Secondary Objectives

The secondary objectives of this study are the following:

- To evaluate the effect of different doses and dosing regimens of ESN364 on the frequency, severity, and hot flash score of mild, moderate, and severe vasomotor symptoms;
- To evaluate the effect of different doses and dosing regimens of ESN364 on responder rates using variation of responder definitions;
- To evaluate the effect of different doses and dosing regimens of ESN364 on patient-reported outcomes;
- To evaluate the effect of different doses and dosing regimens of ESN364 on PD markers (hormones and bone markers); and
- To evaluate the effect of different doses and dosing regimens of ESN364 on safety and tolerability.

2.3 Exploratory Objectives

The exploratory objectives of this study are the following:

- CCI [redacted]
- To evaluate the PK plasma concentrations of ESN364 and metabolite CCI [redacted]

3 STUDY DESCRIPTION

3.1 Summary of Study Design

This is a 12-week, randomized, double-blind, placebo-controlled, dose-ranging, parallel-group, multicenter study to assess the efficacy of ESN364 in postmenopausal women suffering from vasomotor symptoms (hot flashes).

This study will consist of a screening period (Days -35 to -1, including the screening visit [Visit 1] and a minimum 7-day collection of baseline vasomotor symptom frequency and severity assessments), a 12-week treatment period (Day 1 [Visit 2] to Week 12 [Visit 5], including safety visits at Week 2 [Visit 2A], Week 6 [Visit 3A], and Week 10 [Visit 4A]), and a follow-up visit (Week 15 [Visit 6]) approximately 3 weeks after the last dose of study drug, for a total of 9 visits.

The study will be performed on an ambulatory basis.

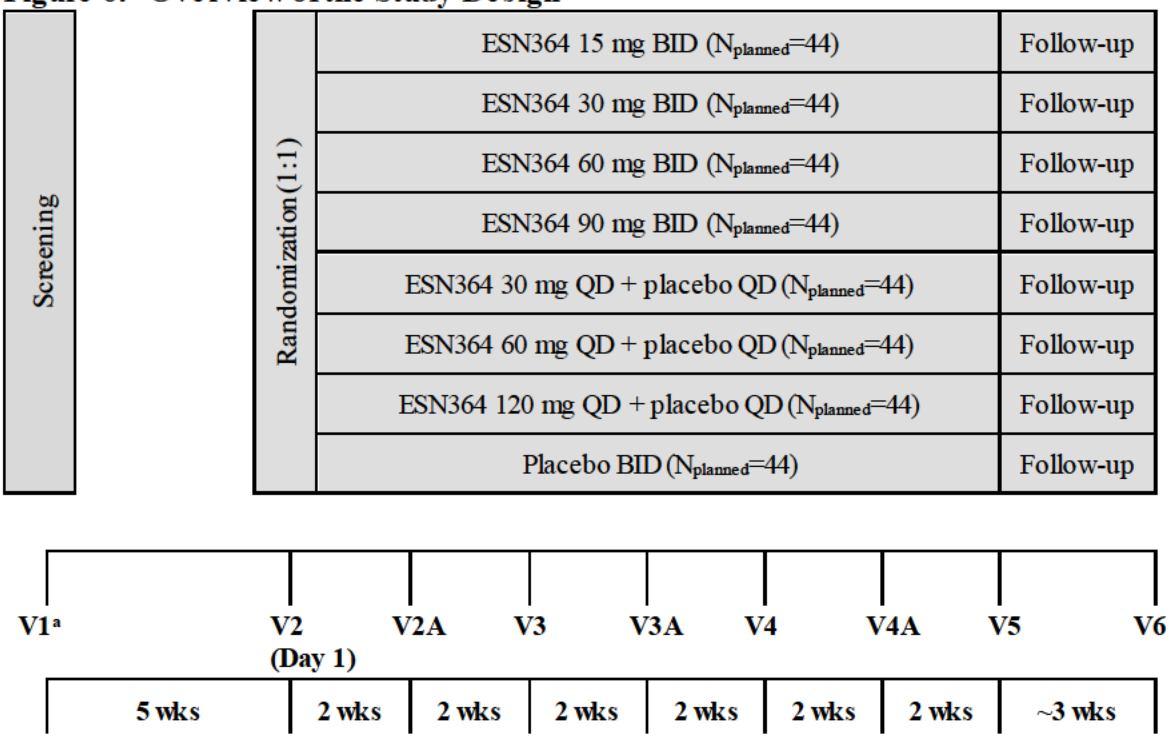
The screening visit (Visit 1) will occur up to 35 days prior to randomization. Eligibility will be assessed via physical examination, clinical laboratory testing, vital signs, ECG, Pap smear, mammography, and endometrial biopsy. Subjects will receive an electronic diary in which to record daily vasomotor symptoms during the duration of the screening period. Subjects must have ≥7 consecutive days of vasomotor symptom recordings to participate in the study. Subjects are encouraged to continue recording for the duration of the whole screening period. The electronic diary will be reviewed by study site staff on Day 1 (Visit 2) to confirm study eligibility. Subjects may be rescreened 1 time upon approval of the medical monitor (see Section 6.2).

A total of 352 subjects will be enrolled in the study. Subjects will be randomized to 1 of 8 treatment groups (ie, 44 subjects in each arm).

Subjects and study personnel will be blinded to the treatment (placebo or ESN364), dosage (30 mg, 60 mg, etc.), and dosing regimen (QD or twice daily [BID]).

For a schematic overview of the study design, see Figure 6.

Figure 6. Overview of the Study Design



a. Screening is to be performed up to 35 days prior to randomization, with a minimum of 7 days to allow for baseline data collection of vasomotor symptom frequency and severity.

BID = twice daily; QD = once daily; V = visit; wks = weeks.

3.2 Study Indication

The indication for this study is the treatment of postmenopausal vasomotor symptoms.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Inclusion Criteria

Subjects who meet all of the following criteria will be eligible to participate in the study:

1. Women >40 years and ≤65 years of age at the screening visit;
2. A body mass index between 18 kg/m^2 to 38 kg/m^2 (extremes included);
3. Spontaneous amenorrhea for ≥12 consecutive months; or spontaneous amenorrhea for ≥6 months with biochemical criteria of menopause FSH >40 IU/L); or having had bilateral oophorectomy ≥6 weeks prior to the screening visit (with or without hysterectomy);
4. At least 50 moderate to severe vasomotor symptoms per week (ie, 7 consecutive days), as recorded in the daily diary during the screening period;
5. In good general health as determined on the basis of medical history and general physical examination, including a bimanual clinical pelvic examination and clinical breast examination devoid of relevant clinical findings, performed at the screening visit; hematology and biochemistry parameters, pulse rate and/or blood pressure, and ECG within the reference range for the population studied, or showing no clinically relevant deviations, as judged by the Investigator;
6. Women >40 years of age who have documentation of a normal/negative or no clinically significant findings mammogram (obtained at screening or within the prior 9 months of trial enrollment). Appropriate documentation includes a written report or an electronic report indicating normal/negative or no clinically significant mammographic findings;
7. Willing to undergo a transvaginal ultrasound to assess endometrial thickness at screening and at Week 12 (end-of-treatment), and for subjects who are withdrawn from the study prior to completion, at the Early Termination (ET) Visit. This is not required for subjects who have had a partial (supracervical) or full hysterectomy;
8. Willing to undergo an endometrial biopsy at screening (in the event that the subject's transvaginal ultrasound shows endometrial thickness ≥4 mm) and at Week 12 (end-of-treatment – all subjects), for subjects with uterine bleeding, and, for subjects who are withdrawn from the study prior to completion, at the ET Visit if study drug exposure is ≥10 weeks. This is not required for subjects who have had a partial (supracervical) or full hysterectomy;
9. Negative alcohol breath test and negative urine test for selected drugs of abuse (amphetamines, tricyclic antidepressants, cocaine, or opiates) at the screening visit;
10. Negative urine pregnancy test;
11. Negative serology panel (including hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antibody screens);

12. Informed Consent Form (ICF) signed voluntarily before any study-related procedure is performed, indicating that the subject understands the purpose of and procedures required for the study and is willing to participate in the study; and
13. Documentation of a normal Pap smear (or equivalent cervical cytology) or of no clinical significance in the opinion of the Investigator within the previous 9 months or at screening.

4.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from participation in the study:

1. Use of a prohibited therapy (hormone therapy, hormonal contraceptive, or vasomotor symptom medication [prescription, over the counter, or herbal]) or not willing to wash out drugs;
2. History (in the past year) or presence of drug or alcohol abuse;
3. Previous or current history of a malignant tumor, except for basal cell carcinoma;
4. Uncontrolled hypertension and a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg;
5. Judged by the Investigator to be unsuited to participate in the study based on findings observed during physical examination, vital sign assessment, or 12-lead ECG;
6. History of severe allergy, hypersensitivity, or intolerance to drugs in general, including the study drug and any of its excipients;
7. Exclusion criterion 7 has been removed in Amendment 1;
8. An unacceptable result from endometrial biopsy (performed when endometrial thickness is ≥4 mm measured by transvaginal ultrasound) of endometrial hyperplasia, endometrial cancer, or inadequate specimen at screening (1 repeat biopsy permitted if technically possible);
9. History of endometrial hyperplasia or uterine/endometrial cancer;
10. History of unexplained uterine bleeding;
11. History of seizures or other convulsive disorders;
12. Medical condition or chronic disease (including history of neurological [including cognitive], hepatic, renal, cardiovascular, gastrointestinal, pulmonary [eg, moderate asthma], endocrine, or gynecological disease) or malignancy that could confound interpretation of the study outcome;
13. Presence or sequelae of gastrointestinal, liver, kidney, or other conditions known to interfere with the absorption, distribution, metabolism, or excretion (ADME) mechanisms of drugs as judged by the Investigator;
14. Active liver disease or jaundice, or values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) >1.5 × the upper limit of normal (ULN); or total bilirubin >1.5 × ULN; or creatinine >1.5 × ULN; or estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease formula ≤59 mL/min/1.73 m^2 at the screening visit;
15. Concurrent participation in another interventional study (or participation within 3 months prior to screening in this study);

16. Suicide attempt in the past 3 years;
17. Unable or unwilling to complete the study procedures; or
18. Subject is the Investigator or any sub-Investigator, research assistant, pharmacist, study coordinator, or other staff or relative thereof, who is directly involved in the conduct of the study.

4.3 Study Drug Interruption

If a subject develops a liver function test abnormality, promptly investigate with repeat biochemical and physical evaluation and notify the CRA immediately.

Interrupt study drug administration for any subject who also experiences any symptoms of abdominal pain, worsening or new fatigue, anorexia, nausea, rash, vomiting, or diarrhea. If prompt evaluation is not possible, then drug administration should be interrupted until this investigation has been done.

Once the subject has been evaluated physically and liver function tests have returned to normal or baseline values, study drug can be restarted and the normal follow-up should be installed.

If a subject requires to stop study drug for more than 7 days, this should be discussed with the medical monitor and/or sponsor with regards to re-starting study drug treatment.

4.4 Withdrawal Criteria

Subjects have the right to withdraw from the study at any time for any reason, including personal reasons. A subject can withdraw without giving a reason. This will not affect the subject's future care. The Investigator should, however, try to find out why a subject has withdrawn from the study and document the reason for withdrawal in the source documents and on the electronic Case Report Form (eCRF).

A subject **must** be withdrawn from the study or treatment for any of the following reasons:

- Withdrawal of informed consent;
- Lost to follow-up;
- If, for safety reasons, including meeting the requirement for interruption of study drug, it is in the best interest of the subject that she be withdrawn, in the Investigator's opinion;
- Development of a medical condition that requires concomitant treatment with a prohibited therapy (see Section 5.6);
- Development of seizures or other convulsive disorders;
- Breaking of the randomization code during administration of the study drug by the Investigator or by a member of the site staff. If the code is broken by the Sponsor for safety reporting purposes or early timepoint analysis, the subject may remain in the study;
- Confirmed decrease in platelets below 75,000 mm^3, which does not normalize after 7 days;
- ALT or AST >8 × ULN;
- ALT or AST >5 × ULN for more than 2 weeks;

- ALT or AST >3 × ULN **AND** total bilirubin >2 × ULN or International Normalized Ratio (INR) >1.5 × ULN; or
- ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

See Appendix D and Section 9.14 for the procedure to follow in case of suspected drug-induced liver injury (DILI).

In the event that a subject is withdrawn from the study drug, the Medical Monitor and Sponsor or designee should be informed. In the event of withdrawal due to a serious adverse event (SAE), the Sponsor should be notified within 24 hours (for details on adverse event reporting see Section 9.3). In the event of withdrawal for other reasons, the Sponsor or designee should be notified within 2 days of the event.

Subjects who are withdrawn from the study prior to completion of the scheduled study procedures for any reason (adverse event, withdrawal of consent, etc.) should be invited to complete the ET assessments (see Section 6.5). As long as the subject consents, all relevant assessments of the day on which the subject withdrew from the study should be completed (at a minimum those related to safety) and the subject should return for a safety follow-up visit approximately 3 weeks after the last intake of study drug. As long as the subject consents, she will be asked to continue the completion of the eDiary up to the safety follow-up visit. In case of an adverse event, the appropriate follow-up will be done.

Subjects who are withdrawn from the study will not be replaced.

Study drug assigned to a withdrawn subject must not be assigned to another subject.

5 STUDY TREATMENTS

5.1 Treatment Groups

Subjects will be randomized to 1 of 8 treatment groups in an equal ratio (see Table 1).

Table 1. Treatment Groups

Dose, Regimen, Route	Number of Subjects	Total ESN364 Daily Dose
ESN364 15 mg BID, oral	44	30 mg
ESN364 30 mg BID, oral	44	60 mg
ESN364 60 mg BID, oral	44	120 mg
ESN364 90 mg BID, oral	44	180 mg
ESN364 30 mg QD + placebo QD, oral	44	30 mg
ESN364 60 mg QD + placebo QD, oral	44	60 mg
ESN364 120 mg QD + placebo QD, oral	44	120 mg
Placebo BID, oral	44	N/A
BID = twice daily; N/A = not applicable; QD = once daily.		

5.2 Rationale for Dosing

Luteinizing hormone secretion is a surrogate for the activity of the KNDy neuron. In the first-in-human Phase 1 study, the mid-range dose of ESN364 (60 mg/day) was demonstrated to be associated with a submaximal LH suppression, and thus a submaximal suppression of the KNDy neuron. ESN364 given as a dose of 180 mg/day was also tested in the first-in-human Phase 1 study and was demonstrated to be safe and well tolerated. Luteinizing hormone suppression was maximal with this dose level. Hence, the expectation is that the 180 mg dose represents the dose associated with maximal suppression of the KNDy neuron.

During menopause, the negative feedback by E2 does not occur due to the cessation of estrogen production in the ovaries. The loss of negative E2 feedback causes the KNDy neuron to become hyperactive in an attempt to restore the needed E2 levels via the increased release of LH/FSH. When the KNDy neuron is in the hyperactive state, as it is presumed to be in the postmenopausal period, a QD morning dose of ESN364 is unlikely to yield the desired suppression of KNDy at night to prevent night sweats. For this reason, a high dose of ESN364 (total daily dose of 180 mg) given as 90 mg BID will be tested to investigate the possibility of completely suppressing LH secretion.

The other doses are based on their ability to significantly decrease LH levels in healthy female volunteers, [redacted]. The aim is to demonstrate an ESN364 dose response using BID dosing as well as QD dosing in the morning and to select the optimal dose for Phase 3.

Placebo control will be used to establish the frequency and magnitude of changes in clinical endpoints that may occur in the absence of active treatment. Placebo control will be used to maintain blinding as well as to investigate the placebo effect that is widely seen in vasomotor symptom trials.

5.3 Randomization and Blinding

In total, 352 subjects will be randomized to 1 of 8 treatment groups in an equal ratio. Randomization will be used to avoid bias in the assignment of subjects to treatment, to increase the likelihood that known and unknown subject attributes (eg, demographic and baseline characteristics) are evenly balanced across treatment arms, and to enhance the validity of statistical comparisons across treatment arms.

Subjects and study personnel will be blinded to the treatment (placebo or ESN364), dosage (30 mg, 60 mg, etc.), and dosing regimen (QD or BID).

Randomization will be based on a computer-generated randomization schedule prepared by Medpace, Inc. Biostatistics using SAS® software (SAS Institute Inc., Cary, NC, USA) prior to the start of the study. The randomization will be balanced using randomly permuted blocks across the treatment arms. The randomization list will be retained by Medpace Biostatistics until the end of the study (database lock). A copy will be sent in a sealed envelope to the bioanalytical laboratory responsible for plasma drug and serum hormone determination before the start of the study as applicable. Because of a planned early timepoint analysis (see Section 10.2.3) and because PK/PD modeling will start prior to database hard-lock, specific assigned Sponsor staff involved in these activities (or delegates, if outsourced to an external vendor) will be unblinded after recruitment has been completed. Details of the unblinding will be documented in a separate charter to ensure the direct study team remains blinded and that unblinded results are restricted to a very small group independent of the study team.

Subjects will be assigned a randomization number from the Interactive Response Technology (IRT) system upon enrollment. The randomization list will be uploaded into the IRT system. Based on this randomization list, the study drug will be packaged and labeled. Medication numbers will be preprinted on the study drug labels. The study team, including those involved in data management, clinical, medical, or statistical review of the study data, will also be blinded during the study as they will not have access to the randomization list. Medpace, the Contract Research Organization (CRO) performing data management and statistical activities, will receive a copy of the randomization list at database lock. The study drug administered to a subject can be identified by the IRT system. The IRT system will allow rapid access to the treatment allocation codes when relevant for site or CRO personnel.

5.4 Breaking the Blind

The randomization list will not be available to the Investigator or other employees of the study site, subjects, monitors, or blinded Medpace and Sponsor personnel before unblinding of the data, unless in case of emergency. Blinding will be maintained unless unblinding is considered mandatory because of a life-threatening situation for management of medical treatment. In such case, the date at which breaking of the code occurs, the person who breaks the code, and the reason why the code is broken will need to be documented in the subject's file. Sites will use IRT to break the blind. The Medpace IRT team will supply a passcode (6 digits) in a sealed envelope for each site in order to break the blind in the IRT system. Site personnel should inform the Medical Monitor immediately. If the Investigator accidentally breaks the code, the Medical Monitor and the Clinical Trial Manager must be also informed as soon as possible. If the code is broken by the Investigator or by someone of his/her clinical staff, the subject must be withdrawn from the study and must be

followed as appropriate. If the code is broken by the Sponsor or designee for safety reporting purposes or the planned early timepoint analysis, the subject may remain in the study.

Because of a planned early timepoint analysis (see Section 10.2.3) and because PK/PD modeling will start prior to database hard-lock, specific assigned Sponsor staff involved in these activities (or delegates, if outsourced to an external vendor) will be unblinded after recruitment has been completed. Details of the unblinding will be documented in a separate charter to ensure the direct study team remains blinded and that unblinded results are restricted to a very small group independent of the study team.

5.5 Drug Supplies

5.5.1 Formulation and Packaging

ESN364 and placebo will be manufactured, packaged, and labeled under the responsibility of Ogeda S.A. The study drug and placebo are manufactured under current Good Manufacturing Practice (GMP), as required by the current Good Clinical Practice (GCP), at Pharmavize N.V. (Mariakerke, Belgium). The packaging and labeling of study drug will be in accordance with applicable local regulatory requirements.

The ESN364 and placebo capsules will be packaged in high-density polyethylene bottles with polypropylene closure. Subjects will be assigned 2 bottles as a kit (1 bottle labeled “morning” and 1 bottle labeled “evening”) at Day 1 (Visit 2), Week 4 (Visit 3), and Week 8 (Visit 4). Each bottle will contain 34 capsules of ESN364 or placebo.

Labels will be printed in the local language of the countries in which the study takes place in accordance with applicable local regulations and will contain the following:

- Protocol number;
- Medication number;
- Visit number;
- Batch number;
- Storage conditions;
- Contents;
- The statements “Keep out of reach and sight of children,” “For oral route only,” and “For clinical study use only”; and
- Sponsor (or sponsor representative) name.

ESN364 will be supplied as immediate-release Coni cap size 0 white opaque hard gelatin capsules for oral administration in active dosage strength ranges of 15 mg to 180 mg. Capsules will be prepared according to the dosing scheme in Table 1. A copy of the certificate of analysis will be provided to study sites. The qualitative composition of the ESN64 capsules is provided in Table 2.

Table 2. Qualitative Composition of ESN364 Hard Gelatin Capsules

Component	Function
ESN364	Active pharmaceutical ingredient
Starch, pregelatinized	Filler
Croscarmellose sodium	Disintegrant
Silicon dioxide	Glidant
Magnesium stearate	Lubricant

Placebo will be supplied as hard gelatin capsules that are visually indistinguishable from the investigational product. The placebo capsules contain the same excipients as ESN364 and will be supplied in capsules shells identical to ESN364.

5.5.2 Study Drug Preparation and Dispensing

ESN364 and placebo are manufactured under current GMP, as required by the current GCP, at Pharmavize N.V. (Mariakerke, Belgium). Study drug will be assigned by the IRT to study sites/subjects.

5.5.3 Study Drug Administration

Subjects will be assigned 2 bottles of study drug as a kit (1 bottle labeled "morning" and 1 bottle labeled "evening") at Day 1 (Visit 2), Week 4 (Visit 3), and Week 8 (Visit 4). Each bottle will contain 34 capsules of ESN364 or placebo. Study drug should be dispensed under the supervision of the Investigator, a qualified member of the clinical staff, or the hospital/clinic pharmacist.

All study visits should be conducted in the morning. Subjects should be fasted (defined as nothing by mouth except for water [up to 1 hour before study drug intake] for at least 10 hours prior to study visit). Day 1 (Visit 2) should be planned to allow for first intake of study drug ("morning" dose) at the study site between 7:00 AM and 10:00 AM. Following Day 1 (Visit 2), each subsequent visit should be planned at the same time of day (morning) for each subject.

Study drug intake will be done with a glass of room temperature tap water. The first intake of study drug will take place at the study site on Day 1 (Visit 2) between 7:00 AM and 10:00 AM under the supervision of the study staff. On study visit days (Day 1 [Visit 2], Week 4 [Visit 3], and Week 8 [Visit 4], only), the morning dose of study drug will be taken at the study site under the supervision of the study staff, after collection of predose blood samples. Subjects will be instructed to arrive at the study site fasted (defined as nothing by mouth except water [up to 1 hour prior to study drug intake] for at least 10 hours prior to study visit). On all other days throughout the treatment period, subjects will be instructed to take their morning dose of study drug at home, around the same time of the day and spaced as near to 12 hours apart as possible (preferably between 7:00 AM and 10:00 AM and 7:00 PM and 10:00 PM). Intake of study drug should be preferably 1 hour before or 2 hours after meals.

Subjects need to record all home study drug intake in the electronic Patient-Reported Outcome (ePRO) diary.

In the event a subject accidentally misses a scheduled dose of the study drug, the missed dose should be skipped and the subject should resume the usual dosing schedule.

Any deviation from the treatment regimen defined in the protocol must be documented in the eCRF.

5.5.4 Treatment Compliance

Subjects will be assigned 2 bottles of study drug as a kit (1 bottle labeled "morning" and 1 bottle labeled "evening") at Day 1 (Visit 2), Week 4 (Visit 3), and Week 8 (Visit 4). Each bottle will contain 34 capsules of ESN364 or placebo.

On visit days during the treatment period (Day 1 [Visit 2], Week 4 [Visit 3], and Week 8 [Visit 4], only), the morning dose of study drug will be taken at the study site under the supervision of the clinical staff. All other study drug intakes (evening dose and morning dose on all days without visits) will take place at home. When taking study drug at home, subjects will be asked to record their home study drug intake in the ePRO diary. Subjects will also be asked to return all unused study drug.

Compliance of study drug intake will be assessed by counting returned study drug in addition to reviewing ePRO entries of study drug intake. Any discrepancies between returned study drug number and dosing in the ePRO diary will be discussed with the subject for whom a discrepancy was seen and recorded in the source documents and the eCRF. If a subject demonstrates continued non-compliance with study drug dosing (≤85% compliance) despite educational efforts, the Investigator should contact the Sponsor to discuss withdrawal of the subject from the study.

5.5.5 Storage and Accountability

The Investigator (or his/her designee) is responsible for the safe storage of all study drug assigned to the study site. Study drug will be stored in a locked, secure storage facility with access limited to those individuals authorized to dispense the study drug and maintained within the appropriate ranges of temperature. All study drug must be stored as specified at delivery and in the original packaging. Instructions for the subjects regarding the storage and handling of the study drug will also be provided to the study sites.

ESN364 must be stored at ambient conditions (15°C to 30°C or 59°F to 86°F), should not be exposed to freezing temperatures, and should be protected from light during storage at the study site. Study drug is stable up to 24 months at 25°C with 60% relative humidity.

Daily temperature logging of the study drug storage room at the study site should be performed. In the event a deviation in storage conditions should occur, the site must discontinue dispensing the affected study drug and notify the Clinical Research Associate (CRA). The same instruction applies for temperature deviations occurring during shipment of study drug to the sites.

The Investigator is responsible for ensuring that all study drug received at the study site is inventoried and accounted for throughout the study. As misallocations of study drug will have an impact on study execution and will jeopardize the validity of the data obtained, utmost care should be taken to correctly dispense the study drug as assigned by the randomization system.

Subjects will be assigned 2 bottles of study drug as a kit (1 bottle labeled "morning" and 1 bottle labeled "evening") at Day 1 (Visit 2), Week 4 (Visit 3), and Week 8 (Visit 4). Each bottle will contain 34 capsules of ESN364 or placebo. Study drug should be dispensed under the supervision of the Investigator, a qualified member of the clinical staff, or the hospital/clinic pharmacist. The Investigator must maintain accurate records demonstrating date and amount of study drug supplied to whom and by whom. Study drug will be supplied only to subjects participating in the study.

The site monitor will periodically check the supplies of study drug held by the Investigator or pharmacist to ensure accountability and appropriate storage conditions of all study drug used.

Subjects must be instructed to return all unused study drug at Day 1 (Visit 2), Week 4 (Visit 3), Week 8 (Visit 4), and Week 12 (Visit 5)/ET. A record of any returned study drug will be kept for each subject. Returned study drug must not be relabeled or dispensed again.

Unused study drug and study drug returned by the subject (if applicable) must be available for verification by the site monitor during on-site monitoring visits. Any discrepancies between returned and expected returned study drug should be explained and documented.

After the last visit of the last subject in the study (Last Subject Last Visit), any unused study drug (including any study drug returned by the subject) will be returned to the Sponsor or designee.

5.6 Prior and Concomitant Medications and/or Procedures

5.6.1 Excluded Medications and/or Procedures

Current use of hormonal medications such as hormone therapy or hormonal contraception or any treatment for vasomotor symptoms (prescription, over the counter, or herbal) is not allowed during the study. Prescription medications for the treatment of vasomotor symptoms should be washed out after consultation with the prescribing physician and as per package insert guidance to ensure clinical safety. A minimum of 5 half-lives is required prior to screening. For women who recently discontinued hormone therapy, the therapy must have been discontinued for at least the following durations prior to the screening visit:

- 1 week or longer for prior vaginal hormonal products (rings, creams, gels, inserts);
- 4 weeks or longer for prior transdermal estrogen alone or estrogen/progestin products;
- 8 weeks or longer for prior oral estrogen and/or progestin therapy;
- 8 weeks or longer for prior intrauterine progestin therapy;
- 3 months or longer for prior progestin implants and estrogen alone injectable drug therapy; or
- 6 months or longer for prior estrogen pellet therapy or progestin injectable drug therapy.

5.6.2 Restricted Medications and/or Procedures

Any medications, other than those excluded by the protocol (see Section 5.6.1), that are considered necessary for a subject's welfare or will not interfere with the study drug may be given at the discretion of the Investigator.

Analgesics are permitted on an as-needed basis. Long-term or prophylactic use is allowed if stable for >3 months.

The use of stable doses (defined as no relevant dose changes within 3 months prior to screening) of thyroid replacement therapy is allowed.

The use of stable doses (defined as no relevant dose changes within 3 months prior to screening) of antihypertensive medication (except clonidine) is allowed on the condition that hypertension is well controlled.

The use of stable doses (defined as no relevant dose changes within 3 months prior to screening) of antidepressants is allowed.

Occasional use (defined as less than 3 times per week) of benzodiazepine or Z-drugs can be allowed on an as-needed basis.

Restricted use (≤7 days) of topically applied corticosteroid containing cream/ointments is allowed.

Active prescription for opiates is allowed at the discretion of the Investigator.

The Sponsor or designee must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

For any medication for which the Investigator is uncertain if it might interfere with the screening assessments or study drug, the Medical Monitor should be consulted.

5.6.3 Documentation of Prior and Concomitant Medication Use

All therapies (prescriptions, over the counter, and herbal), other than the study drug, administered from informed consent until the last study-related activity must be recorded in the source documents and in the concomitant therapy section of the eCRF (name of the drug, dosage, route, and dates of administration). If a concomitant medication is started later than 2 weeks after the last intake of study drug, it will only be recorded when linked to an adverse event.

In the event of observed liver enzyme elevations during the study, additional therapies will be recorded. All therapies (prescriptions, over the counter, and herbal) administered from 90 days prior to informed consent up to informed consent will be recorded in the source documents and in the concomitant therapy section of the eCRF (name of the drug, dosage, route, and dates of administration).

6 STUDY PROCEDURES

The screening visit (Days -35 to -1 [Visit 1]), Day 1 (Visit 2), Week 4 (Visit 3), Week 8 (Visit 4), Week 12 (Visit 5)/ET, and Week 15 (Visit 6) assessments should be conducted in the morning. Subjects should be fasted (defined as nothing by mouth except for water [up to 1 hour before study drug intake] for at least 10 hours prior to study visit). Day 1 (Visit 2) should be planned to allow for the first intake of study drug at the study site between 7:00 AM and 10:00 AM. Following Day 1 (Visit 2), each subsequent visit (excluding Visits 2A, 3A, and 4A) should be planned at the same time of day (morning) for each subject.

Subjects may schedule Week 2 (Visit 2A), Week 6 (Visit 3A), and Week 10 (Visit 4A) assessments at their convenience within the visit window (see Section 6.3). Fasting is not required for these visits.

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[redacted]

An overview of the timing of study drug intake and assessments is given in the Schedule of Procedures (Appendix A).

All assessments will be completed predose, except at Week 2 (Visit 2A), Week 6 (Visit 3A), and Week 10 (Visit 4A) and for postdose blood sampling for PK and PD assessments at Week 4 (Visit 3). Administration of questionnaires will be completed at the respective visits prior to any invasive procedures.

During the postdose assessment period, the subject may eat and drink, provided this is done at least 1 hour after dosing.

6.1 Informed Consent

Signed informed consent will be collected at or before the screening visit (Days -35 to -1 [Visit 1]) for all subjects before any study-related procedures are done. A separate signature will be collected for pharmacogenomics (PGX) sampling. Subjects who do not consent to PGX sampling are not excluded from participating in the study. The PGX sample can be taken at any time during the study following informed consent. Any PGX sample taken during screening will be destroyed in the event of a screen failure.

6.2 Screening Visit (Days -35 to -1 [Visit 1])

The screening visit is to occur within 35 days of randomization (Day 1 [Visit 2]), with a minimum of 7 days to allow for baseline data collection of vasomotor symptom frequency and severity assessments. Subjects will receive an electronic diary in which to record daily vasomotor symptoms during the duration of the screening period. Subjects must have ≥7 consecutive days of vasomotor symptom recordings to participate in the study. Subjects are encouraged to continue recording for the duration of the whole screening period. Subjects will be instructed to arrive to the study site fasted (defined as nothing by mouth except water [up to 1 hour prior to study drug intake] for at least 10 hours). Subjects may be rescreened 1 time with approval of the Medical Monitor. The following assessments do not need to be repeated at the rescreen provided they still fall within the acceptable time window as outlined below: transvaginal ultrasound, endometrial biopsy, mammogram, CCI, ECG, and Pap smear.

The following procedures will be performed at the screening visit (Days -35 to -1 [Visit 1]):

- Collect signed ICFs (see Section 6.1);
- Determine eligibility (inclusion/exclusion criteria);
- Perform mammogram (only in the event the subject does not have a normal/negative or no clinically significant findings mammogram from previous 9 months on record);
- Perform Pap smear (only in the event the subject does not have a normal/negative or no clinically significant findings Pap smear from previous 9 months on record);
- Perform transvaginal ultrasound;
- Perform endometrial biopsy (only in the event the transvaginal ultrasound shows an endometrial thickness ≥4 mm);
- Review medical history and concomitant diseases;
- Collect demographic data;
- Perform physical examination, including bimanual clinical pelvic and clinical breast examination, height, weight, and waist circumference;
- Collect urine sample for:
  - Urine drug screen,
  - Urine pregnancy test, and
  - Urinalysis;
- Collect blood sample for:
  - Clinical laboratory tests (biochemistry, coagulation, and hematology);
  - Endocrinology; and
  - Serology (HBsAG, HCV antibodies, and HIV antibodies);
- Perform alcohol breath test;

- Collect vital signs, including oral/tympanic temperature, blood pressure (sitting), and pulse rate (supine after 5 minutes rest);
- Perform triplicate 12-lead ECG (supine after 10 minutes of rest);
- Provide ePRO training;
- [redacted]
- Begin recording vasomotor symptom frequency and severity; and
- Review concomitant medications.

6.3 Treatment Period (Visits 2 through 5)

Following randomization to study drug, subjects will return to the study site for visits and procedures to occur within ±3 days of the scheduled time. Except for Visits 2A, 3A, and 4A, subjects will be instructed to arrive to the study site fasted (defined as nothing by mouth except water [up to 1 hour prior to study drug intake] for at least 10 hours). Administration of questionnaires will be completed at the respective visits prior to any invasive procedures.

6.3.1 Day 1 (Visit 2)

The following procedures will be performed at Day 1 (Visit 2):

- Review inclusion/exclusion criteria (collect and review ePRO diary);
- Review recorded vasomotor symptom frequency and severity;
- Record Hot Flash Related Daily Interference Scale (HFRDIS);
- Record baseline Leeds Sleep Evaluation Questionnaire (LSEQ);
- Record Greene Climacteric Scale (GCS);
- Record Menopause-Specific Quality of Life (MENQoL) Questionnaire;
- Record Columbia Suicide Severity Rating Scale (C-SSRS);
- Perform physical examination, including weight and waist circumference;
- Collect blood sample for:
  - Clinical laboratory tests (including biochemistry and hematology);
  - PD (predose); and
  - Bone turnover markers (including bone alkaline phosphatase [BALP], procollagen type 1 N-terminal propetide [P1NP], and carboxy-terminal telopeptide of type I collagen [CTX]);
- Collect urine sample for urinalysis;

- Collect vital signs, including oral/tympanic temperature, blood pressure (sitting), and pulse rate (supine after 5 minutes of rest);
- Perform triplicate 12-lead ECG (supine after 10 minutes of rest);
- Randomize subjects;
- Dispense study drug;
- Begin assessing study drug compliance and accountability; and
- Review concomitant medications and assess adverse events.

6.3.2 Week 2 (Visit 2A)

The following procedure will be performed at Week 2 (Visit 2A):

- Collect blood sample for clinical laboratory tests (including biochemistry and hematology).

6.3.3 Week 4 (Visit 3)

The following procedures will be performed at Week 4 (Visit 3):

- Review recorded vasomotor symptom frequency and severity;
- Record HFRDIS;
- Record post-baseline LSEQ;
- Record GCS;
- Record MENQoL;
- Perform limited physical examination (weight and waist circumference only);
- Collect blood sample for:
  - Clinical laboratory tests (including biochemistry and hematology);
  - PD (predose and 3 h postdose). The Week 4 (Visit 3) 3 h postdose PD sample should be shifted to a later date in cases where the subject cannot accommodate the PK sampling schedule at that visit. The PD sample should be taken at the same time as the 3 h postdose PK sample (see Section 8.2); and
  - PK (predose and 1 h [±30 min], 3 h [±30 min], 5 h [-1 h/+30 min], and 7 h [-1 h/+2 h] postdose). The indicated time windows for Week 4 (Visit 3) PK sampling will allow for flexibility;
- Collect urine sample for urinalysis;
- Collect vital signs, including oral/tympanic temperature, blood pressure (sitting), and pulse rate (supine after 5 minutes of rest);
- Perform triplicate 12-lead ECG (supine after 10 minutes of rest);
- Dispense study drug;

- Assess study drug compliance and accountability; and
- Review concomitant medications and assess adverse events.

6.3.4 Week 6 (Visit 3A)

The following procedure will be performed at Week 6 (Visit 3A):

- Collect blood sample for Clinical laboratory tests (including biochemistry and hematology).

6.3.5 Week 8 (Visit 4)

The following procedures will be performed at Week 8 (Visit 4):

- Review recorded vasomotor symptom frequency and severity;
- Record HFRDIS;
- Record post-baseline LSEQ;
- Record GCS;
- Record MENQoL;
- Perform limited physical examination (weight and waist circumference only);
- Collect blood sample for:
  - Clinical laboratory tests (including biochemistry and hematology), and
  - PD (predose);
- Collect urine sample for urinalysis;
- Collect vital signs, including oral/tympanic temperature, blood pressure (sitting), and pulse rate (supine after 5 minutes of rest);
- Perform triplicate 12-lead ECG (supine after 10 minutes of rest);
- Dispense study drug;
- Assess study drug compliance and accountability; and
- Review concomitant medications and assess adverse events.

6.3.6 Week 10 (Visit 4A)

The following procedure will be performed at Week 10 (Visit 4A):

- Collect blood sample for clinical laboratory tests (including biochemistry and hematology).

6.3.7 Week 12 (Visit 5)

The following procedures will be performed at Week 12 (Visit 5):

- Review recorded vasomotor symptom frequency and severity;
- Record HFRDIS;

- Record post-baseline LSEQ;
- Record GCS;
- Record MENQoL;
- Record C-SSRS;
- Perform physical examination, including weight and waist circumference;
- Collect blood sample for:
  - Clinical laboratory tests (including biochemistry, coagulation, and hematology);
  - PD (predose);
  - PK (predose);
  - PGX (can be taken at any time during the study following signed informed consent [see Section 6.1]); and
  - Bone turnover markers (including BALP, P1NP, and CTX);
- Collect urine sample for urinalysis;
- Collect vital signs, including oral/tympanic temperature, blood pressure (sitting), and pulse rate (supine after 5 minutes of rest);
- Perform triplicate 12-lead ECG (supine after 10 minutes of rest);
- Perform transvaginal ultrasound;
- Perform endometrial biopsy. If the Week 12 (Visit 5) biopsy is abnormal, subjects will have a repeat biopsy 4 weeks later, if clinically indicated;
- [redacted]
  - [redacted];
- Assess study drug compliance and accountability; and
- Review concomitant medications and assess adverse events.

6.4 Follow-Up Visit (Week 15 [Visit 6])

The follow-up visit should occur approximately 3 weeks after the last intake of study drug. For subjects requiring a repeat biopsy, the follow-up visit will occur 4 weeks after Week 12 (Visit 5). The following procedures will be performed at the follow-up visit (Week 15 [Visit 6]):

- Review recorded vasomotor symptom frequency and severity;
- Record HFRDIS;
- Record post-baseline LSEQ;
- Record GCS;
- Record MENQoL;

- Record C-SSRS;
- Perform physical examination, including weight and waist circumference;
- Collect blood sample for:
  - Clinical laboratory tests (including biochemistry and hematology);
  - PD;
  - PGX if not collected at previous visit (can be taken at any time during the study following signed informed consent [see Section 6.1]), and
  - Bone turnover markers (including BALP, P1NP, and CTX);
- Collect urine sample for urinalysis;
- Collect vital signs, including oral/tympanic temperature, blood pressure (sitting), and pulse rate (supine after 5 minutes of rest);
- Perform triplicate 12-lead ECG (supine after 10 minutes of rest); and
- Review concomitant medications and assess adverse events.

6.5 Early Termination Visit and Withdrawal Procedures

The end-of-treatment for subjects completing the study is Week 12 (Visit 5). For subjects who are withdrawn from the study prior to completion, all Week 15 (Visit 6) procedures will be performed at an ET visit. These procedures include the following:

- Review recorded vasomotor symptom frequency and severity;
- Record HFRDIS;
- Record post-baseline LSEQ;
- Record GCS;
- Record MENQoL;
- Record C-SSRS;
- Perform physical examination, including weight and waist circumference;
- Collect blood sample for:
  - Clinical laboratory tests (including biochemistry, coagulation, and hematology);
  - PD;
  - PK;
  - PGX if not collected at previous visit (can be taken at any time during the study following signed informed consent [see Section 6.1]); and
  - Bone turnover markers (including BALP, P1NP, and CTX);
- Collect urine sample for urinalysis;

- Collect vital signs, including oral/tympanic temperature, blood pressure (sitting), and pulse rate (supine after 5 minutes of rest);
- Perform triplicate 12-lead ECG (supine after 10 minutes of rest);
- Perform transvaginal ultrasound;
- Perform endometrial biopsy (only if exposure to study drug is ≥10 weeks or to assess adverse events of special interest [see Section 9.15]);
- [redacted]
- Assess study drug compliance and accountability; and
- Review concomitant medications and assess adverse events.

6.6 Unscheduled Visits

Unscheduled visits can be planned outside the scheduled visits. The following are examples of reasons for unscheduled visits:

- To obtain additional information to ensure safety of the subject. Additional blood and urine samples may be taken at the discretion of the Investigator;
- To follow up on any abnormal liver function test result during the course of the study;
- To collect an additional blood sample for PK assessment and/or if a subject discontinues the study drug due to an adverse event;
- To assess, confirm, and follow up on an out-of-range clinical laboratory test, vital sign, or ECG value that will determine a subject's eligibility, or in case of a positive drug screen at screening. Results of this retest must be available prior to randomization. The result of the retest will be considered for subject eligibility;
- To investigate undiagnosed uterine bleeding after randomization (see Section 9.15); or
- To follow up on further evaluation of abnormal transvaginal ultrasound, endometrial biopsy, or mammogram findings during the screening period.

Findings made during unscheduled visits should be reported in the source documents and in the designated sections of the eCRF.

7 ENDPOINTS FOR EVALUATION

7.1 Co-Primary Efficacy Variables

The primary efficacy objectives will require the evaluation of the effect of ESN364 on the following 4 co-primary variables:

- Mean change in the frequency of moderate to severe vasomotor symptoms from baseline to Week 4;
- Mean change in the frequency of moderate to severe vasomotor symptoms from baseline to Week 12;
- Mean change in the severity of moderate to severe vasomotor symptoms from baseline to Week 4; and
- Mean change in the severity of moderate to severe vasomotor symptoms from baseline to Week 12.

7.2 Secondary Efficacy Variables

The secondary efficacy variables include the effect of ESN364 on the following:

Vasomotor Symptom Variables:

- Mean change in the frequency of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean change in the frequency of moderate and severe vasomotor symptoms from baseline to each study week;
- Mean change in the severity of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean change in the severity of moderate and severe vasomotor symptoms from baseline to each study week;
- Mean change in the hot flash score of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean change in the hot flash score of moderate and severe vasomotor symptoms from baseline to each study week;
- Mean percent reduction of mild, moderate, and severe vasomotor symptoms from baseline to each study week; and
- Mean percent reduction of moderate and severe vasomotor symptoms from baseline to each study week.

Responder Variables:

- Mean percent reduction of 50%, 70%, 90%, and 100% of mild, moderate, and severe vasomotor symptoms from baseline to each study week;
- Mean percent reduction of 50%, 70%, 90%, and 100% of moderate and severe vasomotor symptoms from baseline to each study week;
- Absolute reduction of 2, 3, 4, and 5 in mean number of mild, moderate, and severe vasomotor symptoms per day from baseline to each study week; and
- Absolute reduction of 2, 3, 4, and 5 in mean number of moderate and severe vasomotor symptoms per day from baseline to each study week.

Patient-Reported Outcome Variables:

- Change in Hot Flash-Related Daily Interference Scale (HFRDIS), Leeds Sleep Evaluation Questionnaire (LSEQ), Greene Climacteric Scale (GCS), and Menopause-Specific Quality of Life (MENQoL) questionnaire from baseline to Weeks 4, 8, 12, and 15.

Pharmacodynamic Variables:

- Change over time from baseline to Week 12 in plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and sex hormone-binding globulin (SHBG).

7.3 Exploratory Variables

[redacted]

- Level of PK plasma concentrations of ESN364 and metabolite [redacted] at pre-specified timepoints.

7.4 Safety Variables

Safety variables will include the following:

- Incidence and severity of TEAEs;
- Endometrial health assessment (transvaginal ultrasound ± endometrial biopsy);
- Vital signs (sitting systolic and diastolic blood pressure and pulse rate);
- Laboratory tests (hematology, biochemistry, urinalysis, and coagulation);
- ECG parameters;
- Plasma bone density marker concentrations;
- Physical examinations; and
- Columbia Suicide Severity Rating Scale (C-SSRS).

8 EFFICACY AND OTHER ASSESSMENTS

8.1 Efficacy Assessments

8.1.1 Hot Flash Frequency and Severity

Subjects will record vasomotor symptom frequency and severity of each vasomotor symptom on an ad hoc basis, but at a minimum twice daily (morning and evening), via the ePRO diary from the screening visit (Days -35 to -1 [Visit 1]) through the follow-up visit (Week 15 [Visit 6]). At the screening visit, subjects will receive the ePRO diary in which to record daily vasomotor symptoms during the screening period. Subjects must have ≥7 consecutive days of vasomotor symptom recordings to participate in the study. Data on vasomotor symptom frequency and severity collected during the screening period (Days -35 to -1) will serve as baseline for vasomotor symptom frequency and severity. Vasomotor symptoms will be recorded by calendar day (ie, 1 day is midnight [12:00 AM (00:00 hours)] to 11:59 PM [23:59 hours]).

Night sweats (vasomotor symptoms that disrupt the subject's night sleep) should be recorded no later than in the morning upon awakening to start a new day. Subjects should take note of the time of the night sweat and record it on the appropriate calendar day (ie, before or after midnight).

The severity of vasomotor symptoms is defined clinically as follows (according to the FDA and European Medicines Agency Guidances for Industry[23,24] and the National Institutes of Health [NIH] Hot Flash Workshop[25]):

- Mild: Sensation of heat without sweating/dampness. If at night, subject does not wake up but later notices damp sheets or clothing.
- Moderate: Sensation of heat with sweating/dampness, but able to continue activity. If at night, subject wakes up because she is feeling hot and/or is sweating, but no action is necessary other than rearranging the bed sheets.
- Severe: Sensation of intense heat with sweating, causing disruption of activity. If at night, subject wakes up hot and is sweating and needs to take action (eg, remove layers of clothes, open the window, or get out of bed).

The definition of the main parameters are as follows:

Frequency

The frequency is the number of vasomotor symptoms per day.

Severity

Severity of moderate to severe vasomotor symptoms per day is calculated as follows:

[(number of moderate vasomotor symptoms ×2) + (number of severe vasomotor symptoms ×3)]/ number of daily moderate/severe vasomotor symptoms

Severity is zero for subjects that have no moderate or severe vasomotor symptoms.

A weekly average severity is the average of the daily severity scores.

Severity of vasomotor symptoms per day is calculated as follows:

[(number of mild vasomotor symptoms × 1) + (number of moderate vasomotor symptoms × 2) + (number of severe vasomotor symptoms × 3)]/ number of daily mild/moderate/severe vasomotor symptoms

Severity is zero for subjects that have no hot flashes.

A weekly average severity is the average of the daily severity scores.

Hot Flash Score

Hot flash score per day is calculated as follows:

(number of moderate vasomotor symptoms × 2) + (number of severe vasomotor symptoms × 3)

Frequency, severity, and hot flash score will be assessed by taking the mean of the data over 7 days.

8.1.2 Hot Flash Related Daily Interference Scale

Perceived vasomotor symptom interference on daily activities will be evaluated at Day 1 (Visit 2), Week 4 (Visit 3), Week 8 (Visit 4), Week 12 (Visit 5)/ET, and the follow-up visit (Week 15 [Visit 6]) using the HFRDIS. The HFRDIS will be paper-based and administered at the study site prior to any invasive procedures.

The HFRDIS is a 10-item scale that measures a woman's perceptions of the degree to which vasomotor symptoms interfere with 9 daily life activities (work, social activities, leisure, sleep, mood, concentration, relations with others, sexuality, and enjoying life); the 10th item measures interference with overall quality of life.[26] This scale was modeled after items on the Brief Pain Inventory[27] and Brief Fatigue Inventory,[28] which assess the extent to which pain or fatigue interfere with daily life. Subjects will be asked to rate the extent to which vasomotor symptoms have interfered with each item during the previous 2-week time interval using a 0 (do not interfere) to 10 (completely interfere) scale. Recent structural equation modeling suggests this is a unidimensional scale best represented by an overall mean score (sum of items/10).[29]

8.1.3 Leeds Sleep Evaluation Questionnaire

Subject sleep quality will be evaluated at Day 1 (Visit 2), Week 4 (Visit 3), Week 8 (Visit 4), Week 12 (Visit 5)/ET, and at the follow-up visit (Week 15 [Visit 6]) using the LSEQ. A baseline questionnaire will be used at Day 1 (Visit 2) and a post-baseline questionnaire will be used at all other visits. The questionnaire will be paper-based and administered at the study site prior to any invasive procedures.

The LSEQ is a 10-item self-rated questionnaire that assesses a subject's aspects of sleep and early morning behavior. The questions are grouped into 4 chronological areas: ease of getting to sleep, perceived quality of sleep, ease of awaking from sleep, and integrity of early morning behavior following wakefulness.[30] The LSEQ is a visual analogue scale that requires respondents to place marks on a group of 10 cm lines representing the changes they have experienced in a variety of symptoms compared to usual (baseline version) versus since the beginning of treatment (post-baseline version). Lines extend between extremes like "more difficult than usual" and "easier than usual." Responses are measured using a 100 mm scale and are averaged to provide a score for each domain.

8.1.4 Greene Climacteric Scale

Changes in climacteric symptoms will be evaluated at Day 1 (Visit 2), Week 4 (Visit 3), Week 8 (Visit 4), Week 12 (Visit 5)/ET, and at the follow-up visit (Week 15 [Visit 6]) using the GCS. The questionnaire will be paper-based and administered at the study site prior to any invasive procedures.

The GCS is a 21-item scale that provides a brief but comprehensive and valid measure of climacteric symptomatology.[31] Each item is rated by the subject according to its severity using a 4-point rating scale from 0 (none) to 3 (severe). The first 20 items of the scale combine into 3 main independent symptom measures by summing up the individual item scores: psychological symptoms (items 1 to 11; score 0 to 33), physical symptoms (items 12 to 18; score 0 to 21), and vasomotor symptoms (items 19 to 20; score 0 to 6). Item 21 is a probe for sexual dysfunction. The total score can range from 0 to 63. Higher scores indicate worse symptoms.

8.1.5 Menopause-Specific Quality of Life

The MENQoL questionnaire will be recorded at Day 1 (Visit 2), Week 4 (Visit 3), Week 8 (Visit 4), Week 12 (Visit 5)/ET, and at the follow-up visit (Week 15 [Visit 6]). The questionnaire will be paper-based and administered at the study site prior to any invasive procedures.

The MENQoL questionnaire was introduced in 1996 as a tool to assess health-related quality of life in the immediate postmenopausal period. An inherent assumption of the MENQoL is that disease states and conditions like menopause produce symptoms that may disrupt emotional, physical, and social aspects of an individual's life, and must be considered concomitantly with treatment decisions. The MENQoL improves upon several instruments used to assess the impact of menopausal symptoms on quality of life, including the Kupperman Index and the General Well-Being Scale, in the following ways: 1) specificity to the condition of menopause; 2) item development based upon women's own qualitative and quantitative accounts of menopausal symptoms; 3) inclusion of all pertinent domains of the menopause experience, including sexual symptoms; and 4) demonstrated reliability and validity.

The MENQoL is self-administered and consists of a total of 29 items in a Likert-scale format. Each item assesses the impact of 1 of 4 domains of menopausal symptoms, as experienced over the last month: vasomotor (items 1 to 3), psychosocial (items 4 to 10), physical (items 11 to 26), and sexual (items 27 to 29). Items pertaining to a specific symptom are rated as present or not present, and if present, how bothersome on a 0 (not bothersome) to 6 (extremely bothersome) scale.[32,33] Means are computed for each subscale by dividing the sum of the domain's items by the number of items within that domain.[33] Non-endorsement of an item is scored a "1" and endorsement a "2," plus the number of the particular rating, so that the possible score on any item ranges from 1 to 8.[34]

8.1.6 Body Composition

[redacted]

[redacted]

8.2 Pharmacokinetic Assessments

Venous blood samples will be collected for PK analysis of ESN364 and metabolite [redacted] in plasma according to the timepoints defined below and in the Schedule of Procedures. Subjects should be fasted (defined as nothing by mouth except water [up to 1 hour before study drug intake] for at least 10 hours prior to study visit. During the postdose assessment period, the subject may eat and drink, provided this is done at least 1 hour after dosing.

Pharmacokinetic samples will be taken at Week 4 (Visit 3) predose and 1 (±30 min), 3 (±30 min), 5 (-1 h/+30 min), and 7 hours (-1 h/+2 h) postdose as well as predose at Week 12 (Visit 5). The indicated time windows for PK sampling will allow for flexibility. For the samples before the morning dose on Week 4 (Visit 3) and Week 12 (Visit 5) the time of the morning and evening dose of the previous day should be recorded. For the samples after the morning dose on Week 4 (Visit 3) the time of the morning dose should be recorded. If the subject is not fasted, PK assessments should still be completed and the time of last meal documented in the eCRF.

In the event that 1 or more PK samples are missed at Week 4 (Visit 3) or if the subject cannot accommodate this schedule, efforts should be made to collect PK samples for the missed timepoints at Week 8 [Visit 4] or Week 12 [Visit 5] or by means of an unscheduled visit. The time of dosing relative to these samples should be recorded as well. In the event that the subject cannot accommodate this schedule on Week 4 (Visit 3), this PK sampling may be shifted to a later visit.

The exact date and time of the PK sampling must be recorded in the source documents and on the eCRF as well as the exact time of last drug intake before the samples were taken. This means that for a predose blood sample, the time of the morning and evening drug intake of the day before needs to be recorded, and for the postdose samples, the exact time of the morning dose on the very same day needs to be recorded.

Blood samples of approximately 2 mL will be collected by venipuncture or indwelling cannula in the forearm into vacuum tubes containing lithium heparin (Venoject green top or equivalent). Immediately after blood collection, the plasma will be separated in a refrigerated centrifuge (4°C) for 10 minutes at approximately 1500 g and transferred with a sterile plastic pipette into 2 polypropylene tubes with at least 400 µL of plasma per tube. In case immediate centrifuge is not possible, the sample should be chilled in an ice bath for a maximum of 30 minutes after blood collection until centrifugation.

After appropriate labeling, the plasma samples will be stored below -20°C at the clinical site. Thereafter, the frozen plasma samples will be transported/shipped on dry ice to a central laboratory, where they will be collected and kept frozen below -20°C until shipment to the bioanalytical laboratory.

Further procedures for sample collection, shipment, processing, and storage are described in the laboratory manual.

8.3 Pharmacodynamic Assessments

Venous blood samples will be collected for PD assessments at Day 1 (Visit 2), Week 4 (Visit 3), Week 8 (Visit 4), Week 12 (Visit 5)/ET, and at the follow-up visit (Week 15 [Visit 6]). Subjects should be fasted (defined as nothing by mouth except water [up to 1 hour before study drug intake] for at least 10 hours prior to study visit. During the postdose assessment period, the subject may eat and drink, provided this is done at least 1 hour after dosing.

Samples will be taken predose at Day 1 (Visit 2), Week 8 (Visit 4), and Week 12 (Visit 5)/ET, as well as predose and 3 hours postdose at Week 4 (Visit 3). The Week 4 (Visit 3) 3-hour postdose PD sample should be shifted to a later date in cases where the subject cannot accommodate the PK sampling schedule at that visit. The PD sample should be taken at the same time as the 3-hour postdose PK sample (see Section 8.2). A PD sample will be taken at the follow-up visit (Week 15 [Visit 6]) as well. Markers include LH, FSH, E2, and SHBG.

The exact date and time of blood sampling must be recorded in the source documents and on the eCRF. Plasma will be collected and handled as specified in the central laboratory manual. After appropriate labeling, the plasma samples will be stored below -70°C at the study site. Thereafter, the frozen plasma samples will be transported/shipped on dry ice to the central laboratory for collection and storage below -70°C until analysis.

Further procedures for sample collection, shipment, processing, and storage are described in the laboratory manual.

8.4 Pharmacogenomic Assessments

Optional pharmacogenomic sampling is scheduled to occur at Week 12 (Visit 5)/ET; however, sampling can occur any time after subjects sign the PGX ICF and are enrolled into the study. Pharmacogenomic samples will be stored for potential future analysis. Any PGX sample taken during screening will be destroyed in the event of a screen failure.

9 SAFETY ASSESSMENTS

Safety variables will include adverse events, C-SSRS, endometrial health assessment (transvaginal ultrasound ± endometrial biopsy), clinical laboratory tests, plasma bone density marker concentrations, ECGs, vital signs, and physical examinations, as described in the following sections.

9.1 Adverse Events

An adverse event is defined as any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product. All adverse events, including observed or volunteered problems, complaints, or symptoms, are to be recorded on the appropriate eCRF.

Adverse events, which include clinical laboratory test variables, will be monitored and documented from the time of informed consent until study participation is complete. Subjects should be instructed to report any adverse event that they experience to the Investigator. Investigators should make an assessment for adverse events at each visit (excluding Visits 2A, 3A, and 4A) and record the event on the appropriate adverse event eCRF.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Investigator and recorded on the eCRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, it should be recorded as a separate adverse event on the eCRF. Additionally, the condition that led to a medical or surgical procedure (eg, surgery, endoscopy, tooth extraction, or transfusion) should be recorded as an adverse event, not the procedure.

Any medical condition already present at the screening visit will be recorded as medical history and should not be reported as an adverse event. Any medical condition or signs or symptoms present at screening that change in severity or seriousness at any time during the study should be reported as an adverse event.

Clinically significant abnormal laboratory or other examination (eg, ECG) findings that are detected during the study or are present at the screening visit and significantly worsen during the study should be reported as adverse events. The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant abnormal laboratory values occurring during the clinical study will be followed until repeat tests return to normal, stabilize, or are no longer clinically significant. Any abnormal test that is determined to be an error does not require reporting as an adverse event.

9.1.1 Unexpected Adverse Drug Reaction

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

9.1.2 Adverse (Drug) Reaction

All noxious and unintended responses to a medicinal product related to any dose should be considered an adverse drug reaction. "Responses" to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, ie, the relationship cannot be ruled out.

9.1.3 Assessment of Adverse Events by the Investigator

The Investigator will assess the severity (intensity) of each adverse event as mild, moderate, or severe, and will also categorize each adverse event as to its potential relationship to study drug using the categories of yes or no.

Assessment of Severity:

Mild – An event that is easily tolerated and generally not interfering with normal daily activities.

Moderate – An event that is sufficiently discomforting to interfere with normal daily activities.

Severe – An event that is incapacitating with inability to work or perform normal daily activities.

Causality Assessment:

The relationship of an adverse event to the administration of the study drug is to be assessed according to the following definitions:

No (unrelated, not related, no relation) – The time course between the administration of study drug and the occurrence or worsening of the adverse event rules out a causal relationship and another cause (concomitant drugs, therapies, complications, etc.) is suspected.

Yes (related) – The time course between the administration of study drug and the occurrence or worsening of the adverse event is consistent with a causal relationship and no other cause (concomitant drugs, therapies, complications, etc.) can be identified.

The definition implies a reasonable possibility of a causal relationship between the event and the study drug. This means that there are facts (evidence) or arguments to suggest a causal relationship.

The following factors should also be considered:

- The temporal sequence from study drug administration-
  - The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event.
- Underlying, concomitant, intercurrent diseases-
  - Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant drug-
  - The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be recognized to cause the event in question.

- Known response pattern for this class of study drug-
  - Clinical and/or nonclinical data may indicate whether a particular response is likely to be a class effect.
- Exposure to physical and/or mental stresses-
  - The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event.
- The pharmacology and PK of the study drug-
  - The known pharmacologic properties (ADME) of the study drug should be considered.

9.2 Serious Adverse Events

An adverse event or adverse reaction is considered serious if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening adverse event,
  - NOTE: An adverse event or adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death.
- Requires hospitalization or prolongation of existing hospitalizations,
  - NOTE: Any hospital admission with at least 1 overnight stay will be considered an inpatient hospitalization. An emergency room visit without hospital admission will not be recorded as a SAE under this criterion, nor will hospitalization for a procedure scheduled or planned before signing of informed consent. However, unexpected complications and/or prolongation of hospitalization that occur during elective surgery should be recorded as adverse events and assessed for seriousness. Admission to the hospital for social or situational reasons (ie, no place to stay, live too far away to come for hospital visits) will not be considered inpatient hospitalizations.
- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions,
- A congenital anomaly/birth defect, or
- An important medical event.
  - NOTE: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalizations, or the development of drug dependency.

9.3 Serious Adverse Event Reporting − Procedures for Investigators

Initial Reports

All SAEs occurring from the time of informed consent (see Section 9.1) until 30 days following the last administration of study drug must be reported to Medpace Clinical Safety within 24 hours of the knowledge of the occurrence (this refers to any adverse event that meets any of the aforementioned serious criteria). All SAEs that the Investigator considers related to study drug occurring after the 30-day follow-up period must be reported to the Sponsor.

To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study. When the form is completed, Medpace Safety personnel will be notified electronically and will retrieve the form. If the event meets serious criteria and it is not possible to access the EDC system, send an email to Medpace Safety at medpace-safetynotification@medpace.com or call the Medpace SAE hotline (phone number listed below), and fax the completed paper SAE form to Medpace (fax number listed below) within 24 hours of awareness. When the EDC system becomes available, the SAE information must be entered within 24 hours of the system becoming available.

Safety Contact Information: Medpace Clinical Safety
Medpace SAE hotline – USA:
Telephone: +1-800-730-5779, dial 3 or +1-513-579-9911, dial 3
Fax: +1-866-336-5320 or +1-513-579-0444
E-mail: medpace-safetynotification@medpace.com

Follow-Up Reports

The Investigator must continue to follow the subject until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the subject dies.

Within 24 hours of receipt of follow-up information, the Investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (eg, redacted subject discharge summary or autopsy reports) to Medpace Clinical Safety via fax or e-mail. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs.

9.4 Stopping Rules or Discontinuation Criteria

The study may be stopped for any of the following reasons:

- If ≥3 subjects are withdrawn from the study due to a confirmed study-related increase in:
  - ALT or AST >8 × ULN;
  - ALT or AST >5 × ULN for >2 weeks;
  - ALT or AST >3 × ULN **AND** total bilirubin >2 × ULN or INR >1.5 × ULN; or
  - ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever rash, and/or eosinophilia (>5%);
- If ≥3 subjects are withdrawn from the study due to a confirmed study-related decrease in platelets below 75,000 mm^3, which does not normalize after 7 days;

- If ≥2 subjects develop seizures or other convulsive disorders that are not readily attributable to another underlying disease (eg, brain tumor, head trauma); or
- If an increased frequency of unanticipated deaths or other SAEs is observed.

9.5 Pregnancy Reporting

If the subject becomes pregnant during the study or within 30 days of discontinuing study drug, the Investigator should report the pregnancy to Medpace Clinical Safety within 24 hours of being notified. Medpace Clinical Safety will then forward the Exposure In Utero form to the Investigator for completion.

A subject becoming pregnant while on study drug will immediately be withdrawn from the study and ET study procedures will be performed.

The subject should be followed by the Investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the Investigator should notify Medpace Clinical Safety/Sponsor. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (ie, postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for reporting an SAE.

9.6 Expedited Reporting

The Sponsor will report all relevant information about suspected unexpected SAEs that are fatal or life-threatening as soon as possible to the FDA, and in any case no later than 7 days after knowledge by the Sponsor of such a case, and that relevant follow-up information will subsequently be communicated within an additional 8 days.

All other suspected unexpected serious adverse reactions will be reported to the FDA as soon as possible but within a maximum of 15 days of first knowledge by the Sponsor.

The Sponsor will also inform all Investigators as required.

9.7 Clinical Laboratory Evaluations

Blood samples for clinical laboratory tests will be collected at each visit. Urine samples for urinalysis will be collected at each visit (excluding Visits 2A, 3A, and 4A).

Blood samples will be collected and handled as specified in the central laboratory manual. Except for Visits 2A, 3A, and 4A, all blood samples for clinical laboratory tests should be taken in a fasted state (defined as nothing by mouth except water [up to 1 hour before study drug intake] for at least 10 hours).

Standard laboratory tests will be performed by the central laboratory. Further procedures for sample collection, shipment, processing, and storage are described in the laboratory manual provided by the central laboratory.

The following clinical laboratory tests will be performed on the samples (see Appendix B):

- Biochemistry: sodium, potassium, uric acid, creatinine, calcium, AST, ALT, blood urea nitrogen, eGFR, chloride, inorganic phosphorus, bicarbonate, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl aminotransferase, albumin, glucose, total bilirubin, creatine kinase, and total protein;
- Coagulation (at the screening visit and Week 12 [Visit 5]/ET only): INR and activated partial thromboplastin time; and
- Hematology: white blood cell count with differential (neutrophils, lymphocytes, eosinophils, monocytes, and basophils), hemoglobin, hematocrit, red blood cell count, and platelets.

Serology (HBsAG, HCV, and HIV antibodies) will be completed at the screening visit.

Urinalysis will be performed on site using a commercial urine dipstick. In the event the dipstick result is positive, a urine sample must be shipped to a central laboratory for urine sediment analysis (see Appendix B for list of urinalysis analytes).

Urine drug screen for amphetamines, benzodiazepines, tricyclic antidepressants, cannabinoids, cocaine, or opiates will be performed at the screening visit. Note that testing positive for cannabinoids will not exclude a subject from participating the study. A subject with an active prescription for opiates may also participate in the study at the discretion of the Investigator.

The Investigator must review the laboratory report, document this review, and record any change occurring during the study considered to be clinically relevant in the source documents and in the adverse events section of the eCRF. Laboratory values outside the laboratory normal range will be flagged and their clinical relevance will be assessed by the Investigator. A copy of all laboratory reports must be filed in the subject's medical records.

Samples that remain after protocol-specific assessments have been performed may be used for further exploratory work on PK, metabolites, plasma protein binding, protein analysis, and biochemistry.

9.8 Bone Turnover Markers

Plasma concentrations of the bone turnover markers BALP, P1NP, and CTX will be assessed at Day 1 (Visit 2), Week 12 (Visit 5)/ET, and follow-up visit (Week 15 [Visit 6]).

Blood samples for the assessment of BALP, P1NP, and CTX in plasma will be collected and handled as specified in the central laboratory manual.

After appropriate labeling, the plasma samples will be stored below -70°C at the study site. Thereafter, the frozen plasma samples will be transported/shipped on dry ice to the central laboratory for collection and storage below -70°C until analysis.

Further procedures for sample collection, shipment, processing, and storage are described in the laboratory manual.

9.9 Vital Signs

Vital sign parameters will be assessed at each study visit (excluding Visits 2A, 3A, and 4A).

The vital sign parameters that will be assessed are body temperature (oral/tympanic), blood pressure (sitting), and pulse rate (supine after 5 minutes of rest). These parameters will be measured using a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values will be registered on a built-in recorder so that measurements are observer-independent.

Any change from baseline in vital sign values occurring during the study that is considered to be clinically relevant or that requires concomitant medication, as judged by the Investigator, should be recorded in the source documents and the adverse event section of the eCRF. Refer to Appendix E for normal ranges for vital sign parameters.

9.10 Electrocardiograms

Twelve-lead digital ECGs will be obtained at each study visit (excluding Visits 2A, 3A, and 4A) after 10 minutes in a supine position. Whenever vital signs, 12-lead ECGs, and blood draws are scheduled for the same nominal time, the assessments should occur in the following order: 12-lead ECG, vital signs, and blood draws.

Skin preparation should be thorough and electrodes should be placed according to standard 12-lead ECG placement.

Electrocardiograms will be digitally recorded and printed on paper. All 12-lead ECGs will be captured in triplicate (ie, 3 separate 12-lead ECGs at least 1 to 2 minutes apart within a 5-minute window). The printed paper ECGs will be used for "real time" bedside ECG assessment by the Investigator (or designee) who will be responsible for the overall interpretation and determination of the clinical significance of any potential ECG findings. Digital ECGs will be submitted to the ECG core laboratory, which will perform the digital ECG analysis and interpretation in this study using standard methodology. If the central reader identifies an abnormality, per the study alert criteria, the Investigator will be notified who will review the ECG and report any adverse events as necessary.

The following variables will be reported: HR, RR, PR, QRS, QT, QTcB, and QTcF intervals. The Investigator may add additional 12-lead ECG safety assessments if there are any abnormal findings or if the Investigator considers it is required for any other safety reason. Refer to Appendix E for normal ranges for ECG parameters.

9.11 Physical Examinations

Physical examinations will be performed at the screening visit (Days -35 to -1 [Visit 1]), Day 1 (Visit 2), Week 12 (Visit 5)/ET, and the follow-up visit (Week 15 [Visit 6]). A limited physical examination (weight and waist circumference only) will be performed at Week 4 (Visit 3) and Week 8 (Visit 4).

The physical examination includes height (at screening only), weight, and waist circumference. To obtain the actual body weight, subjects must be weighed lightly clothed. The height should be measured barefoot. Waist circumference will be measured according to NIH guidelines (see Appendix C).

Demographic data will be collected at screening only. This includes age, race, sex, and smoking status (smoker/non-smoker).

A clinical breast examination will be performed at the screening visit only.

A bimanual clinical pelvic examination will be performed at screening and at any time during the study where clinically indicated.

Any change in physical examination occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the source documents and the adverse event section of the eCRF.

9.12 Pap Smear

Pap smears will be performed at the screening visit (Days -35 to -1 [Visit 1]) only in the event that the subject does not have documentation of a normal/negative or no clinically significant findings Pap smear (or equivalent cervical cytology) within the prior 9 months. Pap smears must show no clinically significant findings in order for subjects to be included in the study (refer to guidance[35]). Samples will be analyzed at a central laboratory. For details on collection, handling, and shipment instructions, refer to the laboratory manual.

9.13 Mammograms

Mammograms will be performed at the screening visit (Days -35 to -1 [Visit 1]) only in the event that the subject does not have documentation of a normal/negative or no clinically significant findings mammogram within the prior 9 months of trial enrollment. Mammograms must show no clinically significant findings in order for subjects to be included in the study.

9.14 Abnormal Liver Function Monitoring and Drug-Induced Liver Injury Monitoring

Subjects will be monitored for liver function tests throughout the study, including transaminases, total bilirubin, complete blood count, serum alkaline phosphatase, prothrombin time/INR, and creatine kinase. If a subject develops a liver function test abnormality, promptly investigate with repeat biochemical and physical evaluation and notify the CRA immediately (refer to Section 4.3 for detailed procedures).

9.14.1 Abnormal Liver Function

Weekly monitoring of liver function tests is required under the following conditions:

- Subject with a non-elevated liver function test at baseline is found to have any abnormal liver function test elevation that does not meet the stopping rules or discontinuation criteria (Section 9.4) or withdrawal criteria (Section 4.4); or
- Subject who is enrolled with a baseline liver function test that is ≤1.5 × ULN and subsequently has an elevation 2 × that of the baseline value.

Weekly monitoring shall continue for these subjects until their liver function tests return to below baseline value(s).

9.14.2 Suspected Drug-Induced Liver Injury

Subjects will be monitored for DILI from randomization to 21 days following the last administration of study drug (refer to Appendix D for detailed procedures). Subjects must be immediately withdrawn from the study and follow the evaluation for suspected DILI in Appendix D, if they meet any of the following criteria:

- ALT or AST >8 × ULN;
- ALT or AST >5 × ULN for more than 2 weeks;
- ALT or AST >3 × ULN **AND** total bilirubin >2 × ULN or INR >1.5 × ULN; or
- ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

9.15 Adverse Events of Special Interest

Adverse events of special interest are adverse events the Sponsor may wish to carefully monitor. These adverse events may be serious or non-serious and are not considered SAEs unless they meet the SAE definition in Section 9.2. Adverse events of special interest should be reported on the eCRF as such. Adverse events of special interest in this study will include:

- Uterine bleeding,
- Oral/facial paresthesia, and
- Elevation in ALT and/or AST >3 × ULN.

9.16 Columbia Suicide Severity Rating Scale

The C-SSRS will be collected at Day 1 (Visit 2), Week 12 (Visit 5)/ET, and the follow-up visit (Week 15 [Visit 6]).

The C-SSRS will be used to assess all suicidal events and provide a summary of suicidal ideation and behavior. It assesses the lethality of attempts and other features of ideation (frequency, duration, controllability, reasons for ideation, and deterrents), all of which are significantly predictive of completed suicide. The questionnaire will be paper-based and administered at the study site prior to any invasive procedures.

9.17 Transvaginal Ultrasound

Subjects will undergo transvaginal ultrasound at screening, Week 12 (end-of-treatment), and, for subjects who are withdrawn from the study prior to completion, at the ET Visit. The endometrium should be measured in the long axis or sagittal plane. The measurement is of the thickest echogenic area from one basal endometrial interface across the endometrial canal to the other basal surface. Care should be taken not to include the hypoechoic myometrium in this measurement. All transvaginal ultrasounds will be read by a local reader to determine endometrial biopsy eligibility followed by a central reading.

In the event a subject has endometrial fibroids, the subject must undergo endometrial biopsy. Subjects with endometrial fibroids may be included in the study provided the endometrial biopsy

result at screening is satisfactory and the Investigator is confident no treatment will be required during the study.

9.18 Endometrial Biopsy

Subjects will undergo endometrial biopsy (Pipelle) at the following timepoints:

- At screening, if endometrial thickness is ≥4 mm determined by transvaginal ultrasound;
- At Week 12 (end-of-treatment) – all subjects;
- At the ET Visit for subjects who are withdrawn from the study prior to completion, if exposure to study drug is ≥10 weeks; and
- In all cases of uterine bleeding.

Although the transvaginal ultrasounds (see Section 9.17) will be read centrally, a local transvaginal ultrasound reading at screening should guide the decision to perform endometrial biopsy or not. In the event the local reader measures an endometrial thickness ≥4 mm, a single biopsy will be taken from the thickest part of the endometrium. Refer to the FDA draft guidance.[23] In the event an inadequate specimen is obtained at screening, 1 repeat biopsy may be performed if technically possible. If the biopsy is abnormal at Week 12 (end-of-treatment), subjects will have a repeat biopsy 4 weeks later, if clinically indicated. All biopsies will be read by centrally by a blinded pathologist.

In the event that subject's biopsy indicates hyperplasia, biopsies will be assessed by 2 additional blinded pathologists. The participating study pathologists will be from different institutions with independent fiduciary and organizational reporting and will be blinded to each other's assessments. Concurrence of 2 of the 3 pathologists will be accepted as the final diagnosis. If the 3 pathologists do not agree, the most severe pathologic diagnosis would be used as the final diagnosis.

10 STATISTICS

All statistical methods shall be detailed in the Statistical Analysis Plan that will be finalized before database lock.

All data collected in the study will be documented using summary tables, figures, and subject data listings.

10.1 Analysis Populations

All primary and secondary efficacy endpoints will be analyzed using the Full Analysis Set (FAS). The Per Protocol Analysis Set (PPAS) will be used only for the analysis of the selected endpoints to examine the robustness of the primary analysis.

Safety and tolerability will be analyzed using the Safety Analysis Set.

10.1.1 Safety Analysis Set

The Safety Analysis Set comprises all subjects who receive at least 1 dose of study drug.

10.1.2 Full Analysis Set

The FAS comprises the subset of the Safety Analysis Set who have a baseline and post-baseline efficacy evaluation.

10.1.3 Per Protocol Analysis Set

The PPAS comprises the subset of subjects from the FAS who are treated according to the protocol without any major deviations. A full list of inclusion criteria will be decided and assessed prior to database lock and unblinding.

10.1.4 Pharmacokinetic Analysis Set

The PK Analysis Set comprises the subset of the Safety Analysis Set who provide evaluable PK assessments.

10.2 Statistical Methods

10.2.1 Analysis of Efficacy

10.2.1.1 Primary efficacy analysis

Primary Efficacy Analysis: The 4 co-primary efficacy endpoints are the mean change in the frequency of moderate to severe vasomotor symptoms and the mean change in severity of vasomotor symptoms from baseline to Week 4 and Week 12.

For each of the 4 co-primary efficacy endpoints, an analysis of covariance model will be used with treatment group, pooled center, and smoking status as factors, with baseline weight and baseline measurement as covariates. Pairwise comparisons between the active doses and placebo will be calculated based on least-squares mean contrasts using a 2-tailed 95% confidence interval (CI). For subjects in the efficacy analysis populations with missing primary efficacy endpoints, multiple imputation by fully conditional specification methods will be used. The imputation model will use subject demographics (age, sex, race, baseline weight, and smoking status) and baseline and

post-baseline mean number and severity of vasomotor symptoms. Sensitivity analyses will be performed with other multiple imputation methods and mixed model for repeat measurements.

Since the study design requires the comparison of 7 active dose groups with placebo for 4 co-primary efficacy variables, a two-tier closed testing procedure will be used to control the family-wise error. A step-down testing procedure will be followed. The following testing order of doses will be employed:

a. Placebo versus 90 mg BID (daily dose 180 mg),

b. Placebo versus 60 mg BID (daily dose 120 mg),

c. Placebo versus 120 mg QD (daily dose 120 mg),

d. Placebo versus 30 mg BID (daily dose 60 mg),

e. Placebo versus 60 mg QD (daily dose 60 mg),

f. Placebo versus 15 mg BID (daily dose 30 mg), and

g. Placebo versus 30 mg QD (daily dose 30 mg).

The 4 co-primary efficacy endpoints will be compared at each dose level. Only when ESN364 is proved to be significantly better than placebo at a 2-sided alpha level of 0.05 for all 4 co-primary efficacy endpoints will this dose level be claimed to be superior to placebo. A failure to prove the significance for 1 or more co-primary efficacy endpoints will lead to a failure to claim superiority to placebo at this dose level, and the other dose levels after this one in the sequence will not be tested.

The primary efficacy analysis will be based on the FAS. A supportive analysis will be carried out for the co-primary efficacy variables based on the PPAS to examine the impact of premature dropouts and/or major protocol deviations.

10.2.1.2 Secondary efficacy analysis

Efficacy will be further assessed based on the secondary endpoints described in Section 7.2 using the FAS.

10.2.1.3 Pharmacodynamic analysis

Individual plasma hormone concentration values and actual sampling times relative to study drug intake will be listed. Descriptive statistics on the actual values and changes from baseline values will be summarized by assessment timepoint and by treatment arm.

Descriptive statistics will include number of subjects, (arithmetic) mean, standard deviation (SD), median, minimum, and maximum, and a 95% CI. Frequency tabulations will show counts and percentages. Pharmacodynamic data and efficacy data may be evaluated by a population PD or population PK/PD approach. All details of population analyses will be described in a separate analysis plan and a separate report will be written. When deemed necessary, data from this study may be combined with data from other studies.

10.2.1.4 Exploratory efficacy analysis

[redacted] CCI [redacted]. Pharmacokinetics may be

evaluated by a population PK approach. All details of population analyses will be described in a separate analysis plan and a separate report will be written. When deemed necessary, data from this study may be combined with data from other studies.

10.2.2 Analysis of Safety

Safety will be assessed by examining the incidence of adverse events, physical examinations findings, C-SSRS, vital signs, ECGs, clinical laboratory tests, and bone density marker concentrations over time using the Safety Analysis Set.

10.2.3 Interim Analysis

An early timepoint analysis is planned 4 weeks after the last randomization. The unblinded results will be restricted to a very small group independent of the study team. No changes to the study or the statistical analysis plan will be made as a result of this analysis (which is intended to assist with internal decision making only). Full details will be documented in a separate charter to ensure the integrity of the trial blinding; all study team personnel, including all those involved in data management, clinical, medical, or statistical review of the data, will remain blinded until database hard-lock (see Section 5.3).

10.2.4 Sample Size Determination

A total of 352 subjects are planned to be randomized, 44 subjects in each treatment arm.

In the Phase 2a study, the observed least-squares mean difference between ESN364 90 mg BID (highest dose of this Phase 2b study) and placebo in change from baseline to Week 12 in mean daily frequency of moderate to severe vasomotor symptoms was -5.0 (95% CI: -6.8, -3.3) with similar results at Week 4. For any given pairwise comparison for a 2-sample t-test at a 2-sided 5% alpha, 40 subjects provides the following power to detect the following effect sizes assuming an SD of 5:

Assumed treatment difference in mean daily frequency	Power for pairwise test
3.3	83%
3.5	87%
4	94%
4.5	97%
5	>99%

For change from baseline to Week 12 in mean severity of moderate to severe vasomotor symptoms, the observed mean treatment different in the Phase 2a study was -1.12 (95% CI: -1.5, -0.74) with similar results at Week 4. For any given pairwise comparison for a 2-sample t-test at a 2-sided 5% alpha, 40 subjects provides the following power to detect the following effect sizes assuming a SD of 1:

Assumed treatment difference in mean severity	Power for pairwise test
0.64	80%
0.75	91%
1	>99%

Note that the combined power for testing all 4 co-primary endpoints will be lower than the power for each considered individually. Assuming approximately 10% of subjects discontinue prematurely, the number of 40 subjects is increased to 44 subjects per arm.

11 DATA MANAGEMENT AND RECORD KEEPING

11.1 Data Management

11.1.1 Data Handling

Data will be recorded at the site on eCRFs and reviewed by the CRA during monitoring visits. The CRAs will verify data recorded in the EDC system with source documents. All corrections or changes made to any study data must be appropriately tracked in an audit trail in the EDC system. An eCRF will be considered complete when all missing, incorrect, and/or inconsistent data has been accounted for.

11.1.2 Computer Systems

Data will be processed using a validated computer system conforming to regulatory requirements.

11.1.3 Data Entry

Data must be recorded using the EDC system as the study is in progress. All site personnel must log into the system using their secure user name and password in order to enter, review, or correct study data. These procedures must comply with Title 21 of the Code of Federal Regulations (21 CFR Part 11) and other appropriate international regulations. All passwords will be strictly confidential.

11.1.4 Medical Information Coding

For medical information, the following thesauri will be used:

- Latest version of Medical Dictionary for Regulatory Activities for adverse events, and
- World Health Organization Drug Dictionary for prior and concomitant medications.

11.1.5 Data Validation

Validation checks programmed within the EDC system, as well as supplemental validation performed via review of the downloaded data, will be applied to the data in order to ensure accurate, consistent, and reliable data. Data identified as erroneous, or data that are missing, will be referred to the investigative site for resolution through data queries.

The eCRFs must be reviewed and electronically signed by the Investigator.

11.2 Record Keeping

Records of subjects, source documents, monitoring visit logs, eCRFs, inventory of study product, regulatory documents, and other Sponsor correspondence pertaining to the study must be kept in the appropriate study files at the site. Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the evaluation and reconstruction of the clinical study. Source data are contained in source documents (original records or certified copies). These records will be retained in a secure file for the period as set forth in the Clinical Study Agreement. Prior to transfer or destruction of these records, the Sponsor must be notified in writing and be given the opportunity to further store such records.

12 INVESTIGATOR REQUIREMENTS AND QUALITY CONTROL

12.1 Ethical Conduct of the Study

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve human subjects. Compliance with this standard provides public assurance that the rights, safety, and wellbeing of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical study data are credible.

12.2 Institutional Review Board

The Institutional Review Board (IRB) will review all appropriate study documentation in order to safeguard the rights, safety, and wellbeing of subjects. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, ICF, advertisements (if applicable), written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the Investigator.

Federal regulations and the International Conference on Harmonisation (ICH) require that approval be obtained from an IRB prior to participation of subjects in research studies. Prior to study onset, the protocol, any protocol amendments, ICFs, advertisements to be used for subject recruitment, and any other written information regarding this study to be provided to a subject or subject's legal guardian must be approved by the IRB.

No drug will be released to the site for dosing until written IRB authorization has been received by the Sponsor.

12.3 Informed Consent

The ICF and any changes to the ICF made during the course of the study must be agreed to by the Sponsor or designee and the IRB prior to its use and must be in compliance with all ICH GCP requirements, local regulatory requirements, and legal requirements.

The Investigator must ensure that each study subject is fully informed about the nature and objectives of the study and possible risks associated with participation and must ensure that the subject has been informed of her rights to privacy. The Investigator will obtain written informed consent from each subject before any study-specific activity is performed and should document in the source documentation that consent was obtained prior to enrollment in the study. The original signed copy of the ICF must be maintained by the Investigator and is subject to inspection by a representative of the Sponsor, their representatives, auditors, the IRB, and/or regulatory agencies. A copy of the signed ICF will be given to the subject.

12.4 Study Monitoring Requirements

It is the responsibility of the Investigator to ensure that the study is conducted in accordance with the protocol, Declaration of Helsinki, ICH GCP, and applicable regulatory requirements, and that valid data are entered into the eCRFs.

To achieve this objective, the monitor's duties are to aid the Investigator and, at the same time, the Sponsor in the maintenance of complete, legible, well organized, and easily retrievable data. Before the enrollment of any subject in this study, the Sponsor or their designee will review with

the Investigator and site personnel the following documents: protocol, Investigator's Brochure, eCRFs and procedures for their completion, informed consent process, and the procedure for reporting SAEs.

The Investigator will permit the Sponsor or their designee to monitor the study as frequently as deemed necessary to determine that data recording and protocol adherence are satisfactory. During the monitoring visits, information recorded on the eCRFs will be verified against source documents and requests for clarification or correction may be made. After the eCRF data is entered by the site, the CRA will review the data for safety information, completeness, accuracy, and logical consistency. Computer programs that identify data inconsistencies may be used to help monitor the clinical study. If necessary, requests for clarification or correction will be sent to Investigators. The Investigator and his/her staff will be expected to cooperate with the monitor and provide any missing information, whenever possible.

All monitoring activities will be reported and archived. In addition, monitoring visits will be documented at the investigational site by signature and date on the study-specific monitoring log.

12.5 Disclosure of Data

Data generated by this study must be available for inspection by the FDA, the Sponsor or their designee, applicable foreign health authorities, and the IRB as appropriate. Subjects or their legal representatives may request their medical information be given to their personal physician or other appropriate medical personnel responsible for their welfare.

Subject medical information obtained during the study is confidential and disclosure to third parties other than those noted above is prohibited.

12.6 Retention of Records

To enable evaluations and/or audits from regulatory authorities or the Sponsor, the Investigator will keep records, including the identity of all participating subjects (sufficient information to link records, eg, eCRFs and hospital records), all original signed ICFs, copies of all eCRFs, SAE forms, source documents, and detailed records of treatment disposition. The records should be retained by the Investigator according to specifications in the ICH guidelines, local regulations, or as specified in the Clinical Study Agreement, whichever is longer. The Investigator must obtain written permission from the Sponsor before disposing of any records, even if retention requirements have been met.

If the Investigator relocates, retires, or for any reason withdraws from the study, the Sponsor should be prospectively notified. The study records must be transferred to an acceptable designee, such as another Investigator, another institution, or to the Sponsor.

12.7 Publication Policy

Following completion of the study, the data may be considered for publication in a scientific journal or for reporting at a scientific meeting. Each Investigator is obligated to keep data pertaining to the study confidential. The Investigator must consult with the Sponsor before any study data are submitted for publication. The Sponsor reserves the right to deny publication rights until mutual agreement on the content, format, interpretation of data in the manuscript, and journal selected for publication are achieved.

12.8 Financial Disclosure

Investigators are required to provide financial disclosure information to the Sponsor to permit the Sponsor to fulfill its obligations under 21 CFR Part 54. In addition, Investigators must commit to promptly updating this information if any relevant changes occur during the study and for a period of 1 year after the completion of the study.

13 STUDY ADMINISTRATIVE INFORMATION

13.1 Protocol Amendments

Any amendments to the study protocol will be communicated to the Investigators by Medpace or the Sponsor. All protocol amendments will undergo the same review and approval process as the original protocol. A protocol amendment may be implemented after it has been approved by the IRB, unless immediate implementation of the change is necessary for subject safety. In this case, the situation must be documented and reported to the IRB within 5 working days.

13.2 Address List

13.2.1 Sponsor

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PPD

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APPENDIX A. SCHEDULE OF PROCEDURES

Assessments	Screening Visit	Treatment Period							Follow-Up Visit
Study Visit	Visit 1	Visit 2	Visit 2A	Visit 3	Visit 3A	Visit 4	Visit 4A	Visit 5/ET	Visit 6
Time of Visit	Days -35 to -1[a,b]	Day 1[a]	Week 2[c]	Week 4[a]	Week 6[c]	Week 8[a]	Week 10[c]	Week 12[a]	Week 15[a,d]
Informed consent[e]	X								
Informed consent PGX[e]	X								
Inclusion/exclusion criteria	X	X							
Medical history/concomitant diseases	X								
Screening mammogram[f]	X								
Demographic data[g]	X								
Physical examination[h]	X	X		X[i]		X[i]		X	X
Urine drug screen	X								
Urine pregnancy test	X								
Clinical laboratory[j] and urinalysis	X	X	X[k]	X	X[k]	X	X[k]	X	X
Alcohol breath test	X								
Vital signs[l]	X	X		X		X		X	X
12-lead ECG[m]	X	X		X		X		X	X
Pap smear[n]	X								
Transvaginal ultrasound	X							X	
Endometrial biopsy[o]	X							X	
Serology[p]	X								
Blood PD sample[q]		X		X		X		X	X
Blood PK sample[r]				X				X	
Blood PGX sample[s]								X	
Vasomotor symptom diary[t]	X	X		X		X		X	X
HFRDIS[u]		X		X		X		X	X
LSEQ[u]		X[v]		X[v]		X[v]		X[v]	X[v]
GCS[u]		X		X		X		X	X
MENQoL[u]		X		X		X		X	X
C-SSRS[u]		X						X	X
[redacted][w]	X							X	
Bone turnover markers[x]		X						X	X
ePRO training	X								
Randomization		X							

Assessments	Screening Visit	Treatment Period							Follow-Up Visit
Study Visit	Visit 1	Visit 2	Visit 2A	Visit 3	Visit 3A	Visit 4	Visit 4A	Visit 5/ET	Visit 6
Time of Visit	Days -35 to -1[a,b]	Day 1[a]	Week 2[c]	Week 4[a]	Week 6[c]	Week 8[a]	Week 10[c]	Week 12[a]	Week 15[a,d]
Dispense study drug[y]		X		X		X			
Study drug compliance and accountability[z]		X		X		X		X	
Concomitant medications and adverse events[aa]	X	X		X		X		X	X

a. Study visits should be conducted in the morning. Subjects should be fasted (defined as nothing by mouth except for water [up to 1 hour before study drug intake] for at least 10 hours prior to study visit). All assessments will be completed predose, except for postdose blood sampling for PK and PD assessments at Week 4 (Visit 3). Administration of questionnaires will be completed at the respective visits prior to any invasive procedures. Day 1 (Visit 2) should be planned to allow for the first intake of study drug at the study site between 7:00 AM and 10:00 AM. Following Day 1 (Visit 2), each subsequent visit should be planned at the same time of day (morning) for each subject. Following randomization to study drug, subjects will return to the study site for visits and procedures to occur within ±3 days of the scheduled time. Unscheduled visits can be planned outside the scheduled visits (see Section 6.6).

b. The screening visit is to occur within 35 days of randomization (Day 1 [Visit 2]), with a minimum of 7 days to allow for baseline data collection of vasomotor symptom frequency and severity assessments. Subjects will receive an electronic diary in which to record daily vasomotor symptoms during the screening period. Subjects must have ≥7 consecutive days of vasomotor symptom recordings to participate in the study. Subjects may be rescreened 1 time upon approval of the Medical Monitor. The following assessments do not need to be repeated at the rescreen provided they still fall within the acceptable time window: transvaginal ultrasound, endometrial biopsy, mammogram, [redacted], ECG, and Pap smear.

c. Subjects may schedule Week 2 (Visit 2A), Week 6 (Visit 3A), and Week 10 (Visit 4A) assessments at their convenience within the visit window (±3 days). Fasting is not required for these visits.

d. The follow-up visit will occur approximately 3 weeks following the last dose of study drug. For subjects requiring a repeat biopsy, the follow-up visit will occur 4 weeks after Week 12 (Visit 5).

e. Signed informed consent will be collected for all subjects before any study-related procedures are done. A separate signature will be collected for PGX sampling. Subjects who do not consent to PGX sampling are not excluded from participating in the study.

f. Only in the event the subject does not have a normal/negative or no clinically significant findings mammogram from previous 9 months on record.

g. Includes age, race, sex, and smoking status (smoker/non-smoker).

h. Includes height (at the screening visit only), weight and waist circumference. A bimanual clinical pelvic and clinical breast examination will be performed at the screening visit. A bimanual clinical pelvic examination can be performed at any time in the study where clinically indicated.

i. Weight and waist circumference only.

j. Includes biochemistry, coagulation (at the screening visit and Week 12 [Visit 5]/ET only), and hematology panel. Blood samples for clinical laboratory tests should be taken in a fasted state (defined as nothing by mouth except water for 10 hours), except for Week 2 (Visit 2A), Week 6 (Visit 3A), and Week 10 (Visit 4A).

k. Includes biochemistry and hematology, only.

l. Includes oral/tympanic temperature, sitting blood pressure, and pulse rate (supine after 5 minutes of rest).

m. All 12-lead ECGs will be captured in triplicate (ie, 3 separate 12-lead ECGs at least 1 to 2 minutes apart within a 5-minute window). The subject should rest in supine position for at least 10 minutes prior to the first ECG.

n. Only in the event the subject does not have a normal/negative or no clinically significant findings Pap smear from previous 9 months on record.

o. Subjects will undergo endometrial biopsy at screening (if endometrial thickness is ≥4 mm), at Week 12 (end-of-treatment)-(all subjects), at the ET Visit for subjects who are withdrawn from the study prior to completion (if study drug exposure is ≥10 weeks), and in all cases of uterine bleeding. If the uterine biopsy is abnormal at Week 12 (end-of-treatment), subjects will have a repeat biopsy 4 weeks later, if clinically indicated.

p. For HBsAG, anti-HCV antibodies, and anti-HIV antibodies.

q. Samples will be taken predose at Day 1 (Visit 2), Week 8 (Visit 4), and Week 12 (Visit 5)/ET, as well as predose and 3 hours postdose at Week 4 (Visit 3). The Week 4 (Visit 3) 3 hours postdose PD sample should be shifted to a later date in cases where the subject cannot accommodate the PK sampling schedule at that visit. The PD sample should be taken at the same time as the 3-hour postdose PK sample (see Section 8.2). A PD sample will be taken at the follow-up visit (Week 15 [Visit 6]) as well. Markers include LH, FSH, E2, and SHBG.
r. Samples to be taken Week 4 (Visit 3) predose and 1 h (±30 min), 3 h (±30 min), 5 h (-1 h/+30 min), and 7 h (-1 h/+2 h) postdose as well as predose Week 12 (Visit 5). The indicated time windows for Week 4 (Visit 3) PK sampling will allow for flexibility.
s. While scheduled for Week 12 (Visit 5), the PGX sample can be taken at any time during the study following signed informed consent and enrollment into the study.
t. The vasomotor symptom diary will be kept by subjects. Subjects will record the vasomotor symptom frequency and severity of each vasomotor symptom in the ePRO diary on an ad hoc basis, but at a minimum twice daily (morning and evening), from the screening visit (Days -35 to -1 [Visit 1]) through the follow-up visit (Week 15 [Visit 6]). Night sweats should be recorded no later than in the morning upon awakening to start a new day.
u. Paper-based, administered at the study site prior to any invasive procedures.
v. A baseline LSEQ assessment will be collected at Day 1 (Visit 2). A post-baseline LSEQ assessment will be collected for all other indicated visits.
w. [redacted]
x. Includes BALP, P1NP, and CTX.
y. Subjects will be assigned 2 bottles of study drug as a kit (1 bottle labeled "morning" and 1 bottle labeled "evening") at Day 1 (Visit 2), Week 4 (Visit 3), and Week 8 (Visit 4). Each bottle will contain 34 capsules of ESN364 or placebo. Study drug intake will be done with a glass of room temperature tap water. The first intake of study drug will take place at the study site on Day 1 (Visit 2) between 7:00 AM and 10:00 AM, under the supervision of the study staff. On study visit days (Day 1 [Visit 2], Week 4 [Visit 3], and Week 8 [Visit 4], only), the morning dose of study drug will be taken at the study site, under the supervision of the study staff, after collection of predose blood samples. Subjects will be instructed to arrive at the study site fasted (nothing by mouth except water [up to 1 hour prior to study drug intake] for at least 10 hours). On all other days throughout the treatment period, subjects will be instructed to take their morning dose of study drug at home, around the same time of the day spaced as near to 12 hours apart as possible (preferably between 7:00 AM and 10:00 AM and 7:00 PM and 10:00 PM). Intake of study drug should be preferably 1 hour before or 2 hours after meals. Subjects need to record all home study drug intake in the ePRO diary.
z. Includes compliance of study drug intake and ePRO completion. Subjects will be asked to return all unused study drug. Compliance of study drug intake will be assessed by counting returned study drug in addition to reviewing ePRO entries of study drug intake. Any discrepancies between returned study drug number and dosing in the ePRO diary will be discussed with the subject for whom a discrepancy was seen and recorded in the source documents and the eCRF.
aa. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity.

BALP = bone alkaline phosphatase; C-SSRS = Columbia Suicide Severity Rating Scale; CTX = carboxy-terminal telopeptide of type I collagen; [redacted]; E2 = estradiol; ECG = electrocardiogram; eCRF = electronic Case Report Form; ET = early termination; ePRO = electronic Patient-Reported Outcome; FSH = follicle-stimulating hormone; GCS = Greene Climacteric Scale; HBsAG = hepatitis B virus surface antigen; HCV = hepatitis C virus; HFRDIS = Hot Flash Related Daily Interference Scale; HIV = human immunodeficiency virus; LH = luteinizing hormone; LSEQ = Leeds Sleep Evaluation Questionnaire; MENQoL = Menopause-Specific Quality of Life; P1NP = procollagen type 1 amino-terminal propeptide; PD = pharmacodynamic; PGX = pharmacogenomic; PK = pharmacokinetic; SHBG = sex hormone-binding globulin.

APPENDIX B. CLINICAL LABORATORY ANALYTES

Biochemistry

- Alanine aminotransferase
- Albumin
- Alkaline phosphatase
- Bicarbonate
- Aspartate aminotransferase
- Calcium
- Blood urea nitrogen
- Creatine kinase
- Chloride
- Estimated glomerular filtration rate
- Creatinine
- Glucose
- Gamma-glutamyl transferase
- Lactate dehydrogenase
- Inorganic phosphorus
- Potassium
- Sodium
- Total bilirubin
- Total protein
- Uric acid

Endocrinology

- Estradiol
- Follicle-stimulating hormone
- Luteinizing hormone
- Sex hormone-binding globulin

Hematology

- Hematocrit
- Hemoglobin
- Platelets
- Red blood cell count
- White blood cell count and differential [1]

1. Neutrophils, lymphocytes, eosinophils, monocytes, and basophils. Manual microscopic review is performed only if white blood cell count and/or differential values are out of reference range.

Urinalysis

- Bilirubin
- Blood
- Glucose
- Ketones
- Leukocyte esterase
- Microscopy [1]
- Nitrite
- pH
- Protein
- Specific gravity
- Urobilinogen

1. Microscopy is performed only as needed based on positive dipstick test results.

Coagulation

- International normalized ratio
- Activated partial thromboplastin time
- Prothrombin time

Serology

- Hepatitis B virus surface antigen
- Hepatitis C virus
- Human immunodeficiency virus

Bone Markers

- Procollagen type 1 amino-terminal propeptide
- Bone alkaline phosphatase
- Carboxy-terminal telopeptide of type 1 collagen

Urine Drug Screen

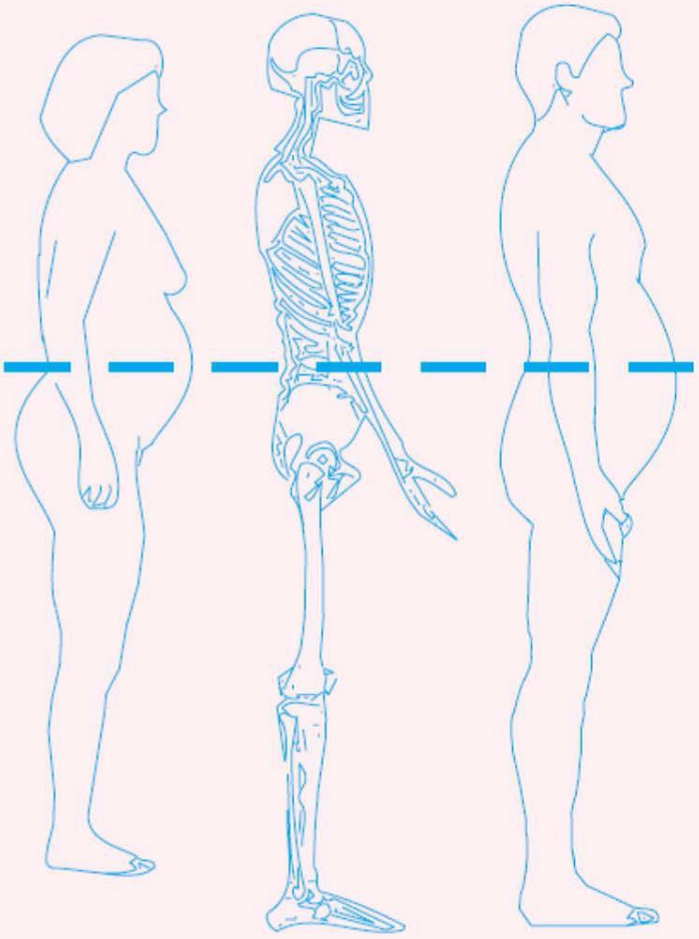
- Amphetamines
- Cannabinoids
- Opiates
- Tricyclic antidepressants
- Cocaine

APPENDIX C. WAIST CIRCUMFERENCE MEASUREMENT

The National Institutes of Health provided a protocol for the measurement of waist circumference.

Waist Circumference Measurement

To measure waist circumference, locate the upper hip bone and the top of the right iliac crest. Place a measuring tape in a horizontal plane around the abdomen at the level of the iliac crest. Before reading the tape measure, ensure that the tape is snug, but does not compress the skin, and is parallel to the floor. The measurement is made at the end of a normal expiration.



Measuring-Tape Position for Waist (Abdominal) Circumference in Adults

Source: Adapted from the National Heart, Lung, and Blood Institute as a part of the National Institutes of Health and the U.S. Department of Health and Human Services. Available at: http://www nhlbi nih.gov/guidelines/obesity/prctgd_c.pdf

APPENDIX D. EVENTS OF CLINICAL INTEREST GUIDANCE FOR POTENTIAL DRUG-INDUCED LIVER INJURY

Event of Clinical Interest (ECI) Guidance for Potential DILI (Drug-Induced Liver Injury) in Clinical Trials

Site Guidance Document for Assessment and Follow-Up

EUROSCREEN

Site Guidance Document for Assessment of Potential DILI

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of this document is to provide guidance to enable the investigator/study coordinator to provide clinical follow-up and systematically gather and report data on potential

DILI. The data collected will be used by the Sponsor to create narratives for regulatory agency reporting.

2.0 INTRODUCTION

Hepatotoxicity is injury or damage to the liver that may be associated with impaired liver function (Navarro and Senior 2006). Drug-induced hepatotoxicity is one of the most common causes of termination of drug development, a major reason for refusal of market authorization and for restricted use, and the single most important cause of the withdrawal of market authorization for products (Björnsson 2006). Thus, drug-induced hepatotoxicity is a major concern during the discovery, development to post-authorization phases of the product life cycle (excerpted from Draft Guidance Document, Hepatotoxicity of Health Products, Ministry of Public Health, Canada, December 2010).

As stated in the United States Food and Drug Administration (FDA) "Guidance for Industry - Drug-Induced Liver Injury: Premarketing Clinical Evaluation"; hepatocellular injury (usually detected by serum aminotransferase elevations [AT]) can be caused by drugs that rarely, if ever, cause severe DILI (e.g., aspirin, tacrine, statins, and heparin), as well as by drugs that do cause such injury. The frequency of serum AT elevations also is not a good indicator of a potential for severe DILI because drugs such as tacrine (not a cause of severe DILI) can cause AT elevations in as many as 50 percent of patients. Very high levels of observed ATs may be a somewhat better indicator of potential for severe DILI, but the most specific indicator is evidence of altered liver function accompanying or promptly following evidence of hepatocellular injury.

The single clearest (most specific) predictor found to date of a drug's potential for severe hepatotoxicity, is the occurrence of hepatocellular injury (AT elevation) accompanied by increased serum total bilirubin (TBL) not explained by any other cause, such as viral hepatitis or exposure to other hepatotoxins, and without evidence of cholestasis, together with an increased incidence of AT elevations in the overall trial population compared to control. Increased plasma prothrombin time, or its international normalized ratio (INR), a consequence of reduced hepatic production of Vitamin K-dependent clotting factors, is another potentially useful measure of liver function that might suggest the potential for severe liver injury.

Recognition of the importance of altered liver function, in addition to liver injury, began with Hyman Zimmerman's observation that drug-induced hepatocellular injury (i.e., AT elevation) accompanied by jaundice (i.e., TBL elevation) had a poor prognosis, with a 10 to 50 percent mortality from acute liver failure (in pretransplantation days) (Zimmerman 1978, 1999). This became known as "Hy's Law". This document describes the recommended process for monitoring and evaluation of subjects meeting the laboratory criteria for potential DILI defined as:

- an elevated alanine transaminase (ALT) or aspartate transaminase (AST) lab value that is greater than or equal to three times (3X) the upper limit of normal (ULN) and
- an elevated TBL lab value that is greater than or equal to two times (2X) ULN and
- at the same time, an alkaline phosphatase (ALP) lab value that is less than 2X ULN,

as a result of within-protocol-specific testing or unscheduled testing.

The protocol identifies these laboratory criteria for potential DILI as ECIs. ECIs are selected adverse experiences that must be reported to the Sponsor within 24 hours. The Principal Investigator should record these ECIs on the Adverse Experience Case Report Forms (CRFs) and complete pertinent adverse experience fields as outlined in the Data Entry Guidelines (DEGs).

3.0 CLOSE OBSERVATION RECOMMENDATIONS

The following steps should be taken when a subject is observed to meet one of the following conditions:

- Elevation in ALT and/or AST >3 x ULN
- Suspected DILI, defined as:
  - ALT or AST >8 × ULN;
  - ALT or AST >5 × ULN for more than 2 weeks;
  - ALT or AST >3 × ULN **AND** total bilirubin >2 × ULN; or International Normalized Ration (INR) >1.5 x ULN
  - ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

Initiate **close observation,** defined below, and continue performing **follow-up to resolution.**

Close observation is defined as follows:

- Repeat liver enzyme and serum bilirubin tests two (2) or three (3) times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or study drug has been discontinued and the subject is asymptomatic.
- Obtain a more detailed history of symptoms and prior or concurrent diseases. (See Section 5).

- Obtain a history of concomitant medication use (including prescription and nonprescription medications, herbal and other dietary supplements), alcohol use, recreational drug use and special diets. (See Section 4 for details.)
- Obtain a history of exposure to chemical agents or other environmental toxins.
- Obtain additional history and complete Stage 1 work-up to attempt to rule out other potential causes of the transaminase elevation, including but not limited to the following: acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis (NASH); hypoxic/ischemic hepatopathy; and biliary tract disease. (See Section 5.5 for details.)
- Consider gastroenterology or hepatology consultation.

In general, treatment with study therapy should be stopped if the laboratory criteria for potential DILI are met. Please refer to the specific discontinuation criteria in the protocol as appropriate.

4.0 FACTORS TO CONSIDER IN ASSESSING POTENTIAL DILI

When there is a potential DILI, it is important to thoroughly assess the subject's history, hepatic risk factors, clinical condition and hepatic function until resolution (normal or baseline levels).

Answers to the following questions should be recorded in source documents and in appropriate CRFs as outlined in the DEGs.

4.1 Study Medication

Considerations should include the following: What was the time interval between administration of study medication and the laboratory abnormality(ies)? What is the status of study medication use- Continuing? Interrupted? Discontinued? Was the subject re-challenged with study medication?

4.2 Treatment

Record any concomitant treatments.

4.3 Signs and Symptoms (associated with the potential DILI event)

Does the subject have a concomitant illness? Does the subject currently exhibit signs or symptoms of hepatitis/DILI? What are the subject's signs and symptoms (see examples below)? What are the pertinent findings from medical history, physical/laboratory examination (e.g., fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia > 5%, hepatomegaly, splenomegaly, etc.) that could suggest DILI?

Category	Examples of Signs and Symptoms
Blood/lymphatic	Eosinophilia, coagulopathy, susceptibility to bleeding/bruising
Circulatory	Varicose veins, edema
Constitutional	Fever, fatigue, malaise, weight gain, other (identify).
Digestive/hepatic	Anorexia, diarrhea, bloody or black stool, light-colored stools, nausea, vomiting, hematemesis, upper quadrant abdominal pain, upper quadrant tenderness, hepatomegaly, jaundice, splenomegaly, ascites, cholestasis
Endocrine/reproductive	Loss of libido
Integumentary	Rash, pruritus
Muscular	Myalgia
Nervous	Changes in mental status or level of consciousness
Urinary	Dark urine

4.4 Confounding Variables

What are the relevant medical history and findings? What is the differential diagnosis? What risk factors does the subject have for hepatic injury? (See examples below.) Provide onset of risk factor and duration.

Category	Examples of Confounding Variables
Subject medical history	Autoimmune disorder, cancer, Gilbert's syndrome, obesity, Wilson's disease
Substance use/abuse	Alcohol, illegal drugs, illegal intravenous (IV) drugs
Prior & Concomitant Medications: Review all non-study medications and therapies, including: over-the- counter (OTC), as well as prescription. Ask the subject to bring products/packaging to site and review contents.	History of recent concomitant acetaminophen (APAP)/paracetamol use, excessive nonsteroidal anti-inflammatory drug (NSAID) intake, use of non-study drug or therapy that can cause liver damage or idiosyncratic adverse drug reactions
Herbal and nutritional supplements	Herbal, complementary therapies, and nutritional supplements
Adulteration of products	History of previous exposure to the product or a similar product, and information on potential contamination or adulteration of products
Chemical exposure	Occupational or in other situations
Potential exposure to infectious agents	Infectious hepatitis, transfusion, travel, tattoos, sexually transmitted diseases, new sexual partner, shared needles
Special Diet	Special diet started since randomization
Other	Recent physical trauma, excessive exercise, or other prolonged physical exertion
Family history	Autoimmune disorder, cancer, Gilbert's syndrome, Wilson's disease

4.5 Evaluation algorithm for potential DILI if there are no other clinical reasons

Note: If clear etiology for the laboratory abnormalities has been confirmed, additional testing may not be required. In this case, consultation with the Sponsor is recommended.

The following should be performed within 48-72 hours:

- Toxicology screen for drugs of abuse (including ethanol) and for acetaminophen/paracetamol level should also be sent. Investigators may order additional toxicology tests as clinically indicated.
- Evaluate subject for the following signs and symptoms: fatigue, nausea, vomiting, right upper quadrant abdominal pain or tenderness, fever, rash.
- Obtain the following additional history and assessment for associated risk/confounding factors:
  - ✓ More detailed history of symptoms and prior or concurrent illness
  - ✓ Aminotransferase values obtained prior to the study or administration of study medication
  - ✓ Alcohol consumption (recent and historical)
  - ✓ Alcohol, opioid and other substances of abuse
  - ✓ Acetaminophen (APAP)/paracetamol use
  - ✓ New prescription, concomitant, or non-prescription (including herbal and other dietary supplements) medications
  - ✓ Unusual foods (e.g. mushrooms) or special diets. Consumption of seasonal foods.
  - ✓ Recreational drug use
  - ✓ Prior history of liver injury or disease, including but not limited to Gilbert's syndrome, autoimmune disorders, cancer, Wilson's disease, NASH, alcoholic or infectious hepatitis, biliary tract disease, hypoxic/ischaemic hepatopathy
  - ✓ Obesity/abdominal adiposity (record weight, height, and waist circumference)
  - ✓ Occupational history and history of exposure to chemical agents or other environmental toxins
  - ✓ Recent travel (last three [3] years)
  - ✓ Transfusion history

- Perform the following required laboratory tests:

  - ✓ Blood Chemistry including at least
    - ALT
    - AST
    - Bilirubin: total, direct, indirect
    - Alkaline phosphatase (ALP)
    - Creatine phosphokinase (CPK)
    - GGT
    - LDH

- Albumin

✓ Coagulation
- Prothrombin Time (PT)/international normalized ratio (INR)

✓ Complete Blood Count
- Eosinophils (percentage and absolute; obtain manual count if automated count is elevated)
- Manual eosinophil count (if automated count was elevated)

✓ Folate
✓ Thiamine
✓ Viral hepatitis serologies (obtain appropriate consent prior to testing, if required locally)
- A (IgG, IgM)
- B (HepBs Ag, Hep Bs Ab, Hep Bc Ab, Hep Be Ag)
- C (RNA)
- D (requires concomitant hepatitis B infection)
- E (IgG and IgM, obtain appropriate consent prior to testing, if required locally)

✓ Human Immunodeficiency Virus (HIV) testing (obtain appropriate consent prior to testing, if required locally)
✓ Evaluation for autoimmune hepatitis:
- Serum gamma globulin levels/ serum protein electrophoresis
- Antinuclear antibody (ANA)
- Anti-mitochondrial antibody

✓ Anti-smooth muscle antibody
✓ Anti-liver-kidney microsomal antibody
✓ Anti-soluble liver antigen
✓ Serologies for the following:
- Cytomegalovirus (CMV) (IgG, IgM)
- Epstein-Barr Virus (EBV) (IgG, IgM)
- Herpes simplex
- Toxoplasmosis
- Varicella
- Parvovirus

✓ Ceruloplasmin
✓ Serum alpha-1 anti-trypsin
✓ Iron Studies:
- serum ferritin
- serum iron
- total iron binding capacity

✓ Genetic test for hemochromatosis. Ensure appropriate subject consent is obtained for this test

- ✓ Genetic test for Gilbert's disease if there is a suspicious history. Ensure appropriate subject consent is obtained for this test.

- Obtain a right upper quadrant ultrasound
- Consider referral to hepatologist/gastroenterologist
- Consider screen for celiac disease and cystic fibrosis if clinically indicated
- If laboratory tests or ultrasound evidence of biliary tract obstruction, consider obtaining Endoscopic Retrograde Cholangiopancreatography (ERCP) or Magnetic Resonance Cholangiopancreatography (MRCP)

If applicable, request copies of hospital discharge summaries, consultation reports, pathology reports, special studies (e.g. imaging or biopsy), etc.

4.6 Potential diagnosis

What diagnosis do the history, clinical course, and laboratory tests suggest?

4.7 Overall clinical impression

What are the investigator's overall clinical impressions (e.g., differential diagnosis, potential alternative causes)?

4.8 Treatment plan

What is the plan for treatment and follow-up?

5.0 PREAPPROVED TESTS

For the blood volume requirements, please refer to the trial laboratory manual. The following tests have been pre-approved by the Sponsor:

- Anti-liver-kidney microsomal antibody
- Anti-mitochondrial antibody (if alkaline phosphatase or total bilirubin > ULN)
- Antinuclear antibody (ANA)
- Anti-smooth muscle antibody
- Anti-soluble liver antigen
- Ceruloplasmin
- Chemistry Panel (as specified in protocol)
- Complete blood count (CBC), with manual differential if absolute eosinophil count is > ULN
- Hepatitis tests: Hepatitis A IgG AB, Hepatitis A IgM AB, Hepatitis B Core AB, Hepatitis Be AG, Hepatitis B surf AB, Hepatitis B surf AG, Hepatitis C AB, Hepatitis C Qualitative RNA, Hepatitis D AB, Hepatitis E Ab IgM, Hepatitis E IgG (obtain consent prior to testing, if required locally)
- HIV antibody (obtain consent prior to testing, if required locally)
- Genetic test for hemochromatosis

- Iron studies : Serum ferritin Serum iron Total iron binding capacity
- Lactic Acid dehydrogenase (LDH)
- PT / INR
- Serum albumin and total protein
- Serum alpha-1 anti-trypsin
- Serum creatine kinase (CK)
- Serum gamma globulin levels/ serum protein electrophoresis
- Serum gamma-glutamyl transferase (GGT)
- Toxicology screen (including ethanol and acetaminophen/paracetamol level) - tests not on the standard screen may be ordered, if clinically indicated
- IgM, IgG for both CMV and EBV-VCA Antibody, herpes simplex IgM, toxoplasma AB IgG, IgM, varicella zoster AB IgG, IgM, parvovirus IgM, IgG (if clinically relevant)
- screen for celiac disease and cystic fibrosis (if clinically indicated and appropriate consent obtained for cystic fibrosis screen)
- UGT1A tests for Gilbert's syndrome (appropriate consent should be obtained)

Additional lab tests and/or procedures – The investigator may order additional tests after consultation with the Sponsor.

6.0 CONTACTS

If you have any questions, please refer to your Sponsor contact list for the following Euroscreen personnel:
- Clinical Research Associate or Subsidiary Monitor
- Medical Director

7.0 REFERENCES

- Draft Guidance Document, Hepatotoxicity of Health Products, Ministry of Public Health, Canada, December 2010
  http://www.hc-sc.gc.ca/dhp-mps/alt_formats/pdf/consultation/drug-medic/draft_ebauche_hepatotox_guide_ld-eng.pdf

- FDA Guidance for Industry - Drug-Induced Liver Injury: Premarketing Clinical Evaluation, July 2009
  www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

APPENDIX E. NORMAL RANGES FOR VITAL SIGNS AND ELECTROCARDIOGRAMS

Normal Ranges for Vital Sign Parameters

Systolic blood pressure (SBP) (mmHg)	Diastolic blood pressure (DBP) (mmHg)	Pulse rate (bpm)	Oral/tympanic temperature (°C)
90 ≤ SBP ≤ 160	45 ≤ DBP ≤ 90	40 ≤ pulse ≤ 100	35.0 ≤ t° ≤ 37.5

These normal ranges are applicable in supine position (after 5 minutes rest).

Normal Ranges for Electrocardiogram Parameters

PR (ms)	QRS (ms)	QTcF (ms)	Heart rate (bpm)
120 ≤ PR ≤ 220	QRS ≤ 120	QTcF ≤ 450	40 ≤ HR ≤ 100