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EUROSCREEN

Clinical Study Protocol

Pilot/Phase IIa Trial to Investigate the Effect of ESN364 in Early Postmenopausal Women Suffering From Hot Flashes

Product	ESN364
Protocol Number	ESN364_HF_204
EudraCT Number	2015-002578-20
Clinical Phase	IIa
Clinical Indication	Hot flashes
Issue Date (Version)	10-December-2015 (Final 2.0)

Sponsor	Euroscreen S.A. 47 Rue Adrienne Bolland, 6047 Gosselies, Belgium
Sponsor Representative	PPD

This study will be conducted in compliance with this protocol, the ICH Note for Guidance on Good Clinical Practice (CMPM/ICH/135/95) and with the applicable regulatory requirement(s).

SIGNATURES

Signature of Sponsor Representative

Title: Pilot/Phase IIa Trial to Investigate the Effect of ESN364 in Early Postmenopausal Women Suffering From Hot Flashes

Name: PPD

'This Clinical Study Protocol has been reviewed and approved by the Sponsor in order to ensure compliance with Good Clinical Practice.'

Signature: PPD

Date:

Signature of Medical Expert

Title: Pilot/Phase IIa Trial to Investigate the Effect of ESN364 in Early Postmenopausal Women Suffering From Hot Flashes

Name: PPD

Affiliation:

Address:

'This Clinical Study Protocol has been reviewed and approved by a medical expert in order to ensure that the protocol and any amendments cover relevant clinical matters clearly and accurately.

Signature: PPD

Date:

Signature of Study Statistician

Title: Pilot/Phase IIa Trial to Investigate the Effect of ESN364 in Early Postmenopausal Women Suffering From Hot Flashes

Name: PPD

Affiliation:

Address:

'This Clinical Study Protocol has been reviewed and approved by the study statistician in order to ensure that the protocol and any amendments cover all relevant statistical matters clearly and accurately, using technical terminology as appropriate.'

Sign PPD

Date

Signature of Coordinating Investigator

Title: Pilot/Phase IIa Trial to Investigate the Effect of ESN364 in Early Postmenopausal Women Suffering From Hot Flashes

Name: PPD

Affiliation:

Address:

'I have read this Clinical Study Protocol and agree that it contains all information necessary for proper conduct of the study. I will carry out the study as outlined herein and will complete the study within the designated time.'

Sig PPD

Da

Signature of Principal (Site) Investigator

Title: Pilot/Phase IIa Trial to Investigate the Effect of ESN364 in Early Postmenopausal Women Suffering From Hot Flashes

Name:

Affiliation:

Address:

'I have read this Clinical Study Protocol and agree that it contains all information necessary for proper conduct of the study. I will carry out the study as outlined herein and will complete the study within the designated time.'

Signature:

Date:

PROTOCOL HISTORY

Protocol History
Euroscreen S.A. – ESN364_HF_204

Document	Issue Date	Amendment Type	Comments
Initial Clinical Study Protocol	25-June-2015	-	This document
Revised Clinical Study Protocol	10-December-2015	General	The overall reason for the revision is to liberalize the participant selection criteria to facilitate recruitment, and to allow for a lower number of subjects to be included in the interim analysis. For a detailed overview of the changes, please refer to the Protocol Amendments.

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PROTOCOL AMENDMENTS

AMENDMENT 1 – REVISED CLINICAL STUDY PROTOCOL VERSION 2.0

The overall reason for the revision is to liberalize the participant selection criteria to facilitate recruitment, and to allow for a lower number of subjects to be included in the interim analysis.

Description and Rationale of Change

- Change of the inclusion criteria with respect to age requirements, early post-menopause definitions and minimum frequency of hot flashes or night sweats.
- Change of concomitant therapy restrictions.
- Affected sections:
    - Section 4.1: Inclusion Criteria (inclusion criteria 1, 2, 3 and 5).
    - Section 6.1: Permitted Concomitant Therapies
    - Section 6.2: Prohibited Concomitant Therapies

Description and Rationale of Change

- Reduction of the number of subjects to be included in the interim analysis, to speed up the availability of interim analysis results.
- Affected sections:
    - Section 3.1: Overview of Study Design
    - Section 5.6: Blinding
    - Section 9.1: Statistical Analysis
    - Section 9.1.6: Interim Analysis
    - Section 12.6: Data Management

Description and Rationale of Change

- Addition of dual-energy X-ray absorptiometry (DEXA) parameters to the list of endpoints analyzed during the interim analysis, to gather as much proof as possible to substantiate the conclusions of the interim analysis.
- Affected sections:
    - Section 3.1: Overview of Study Design
    - Section 5.6: Blinding
    - Section 9.1: Statistical Analysis
    - Section 9.1.6: Interim Analysis
    - Section 12.6: Data Management

Description and Rationale of Change

- Removal of benzodiazepines from the drugs of abuse list, since they are listed as permitted concomitant therapy (on an as-needed basis).
- Affected sections:
  - Section 4.1: Inclusion Criteria
  - Section 7.5.1.2: Clinical Laboratory Tests

Description and Rationale of Change

- The protocol stated that certificates of analysis would accompany the study drug to the site. This statement was deleted since certificates of analysis are not provided to the sites because this is a blinded study and based on the certificates of analysis assumptions could be made about the nature of medication kits.
- Affected sections:
  - Section 5.1: Physical Description of the Study Drug

Description and Rationale of Change

- Change of project manager.
- Affected section:
  - Study Administrative Structure and Investigators

PROTOCOL SYNOPSIS

Study Title	**Pilot/Phase IIa Trial to Investigate the Effect of ESN364 in Early Postmenopausal Women Suffering From Hot Flashes**		
Product	ESN364	**Clinical Phase**	IIa
Protocol Number	ESN364_HF_204	**Indication**	Hot flashes
Eudract Number	2015-002578-20		

Sponsor	Euroscreen S.A.
Sponsor Representative	PPD MD
Clinical Center(s)	Multi-center study in Belgium

Objectives:

The primary objective of the study is to evaluate the effect of ESN364 on the severity and frequency of hot flashes in early postmenopausal women suffering from hot flashes, in terms of changes in weekly Hot Flash Score from baseline to Week 12.

Secondary objectives are to evaluate in early postmenopausal women suffering from hot flashes:

- the effect of ESN364 on the severity and frequency of hot flashes, in terms of changes in weekly Hot Flash Score from baseline to Weeks 4 and 8, and at follow-up;
- the effect of ESN364 on the frequency of hot flashes, in terms of changes in weekly Hot Flash Frequency from baseline to Weeks 4, 8, and 12, and at follow-up;
- the effect of 12-week administration of ESN364 on hot flash interference on daily life, in terms of changes from baseline over time in Hot Flash Related Daily Interference Scale (HFRDIS);
- the effect of 12-week administration of ESN364 on climacteric symptoms, in terms of changes from baseline over time in Leeds Sleep Evaluation Questionnaire (LSEQ), Greene Climacteric Scale (GCS), and Sheehan Disability Scale (SDS);
- the pharmacodynamic (PD) effect of 12-week administration of ESN364, in terms of changes over time in plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), sex hormone-binding globulin (SHBG), leptin, insulin, C-peptide, and glycated hemoglobin (HBA1c);
- safety and tolerability of 12-week administration of ESN364, in terms of adverse events (AEs), physical examination, vital signs, electrocardiogram (ECG), clinical laboratory tests, and plasma bone density marker concentrations (bone alkaline phosphatase [BALP] and carboxy-terminal telopeptide of type I collagen [CTX]).

Exploratory objectives are to:

- evaluate the effect of 12-week administration of ESN364 on body composition (lean mass, fat mass, bone mass/density) assessed by dual-energy X-ray absorptiometry (DEXA);
- assess ESN364 plasma concentrations from sparse pharmacokinetic (PK) sampling.

Overview of Study Design:

This is a 12-week double-blind, placebo-controlled, parallel-group, multicenter, proof-of-concept study to assess the effect of 12-week administration of ESN364 in early postmenopausal women suffering from hot flashes.

The study will consist of a screening period of maximum 4 weeks and a 12-week double-blind treatment period (Weeks 1-12), followed by a follow-up visit 2-3 weeks after the last study drug intake.

The study will be performed on an ambulatory basis. During the treatment period, subjects will be asked to come to the clinical site every 4 weeks (Day 1 and Weeks 4, 8, 12; Visits 2-5).

Study Population:

In total 80 subjects are planned to be enrolled. Subjects will be randomized to one of 2 treatment arms in a 1:1 ratio as per pregenerated randomization list: ESN364 90 mg twice daily (b.i.d.) (40 subjects) or placebo (40 subjects).

Eligibility Criteria:

Inclusion Criteria:

Subjects meeting all of the following criteria are eligible to participate in this study:

1. Women, between 40 and 65 years old (extremes included) at screening;
2. Spontaneous amenorrhea for at least 12 consecutive months; or spontaneous amenorrhea for at least 6 months with biochemical criteria of menopause (FSH >40 IU/L); or spontaneous amenorrhea for at least 3 months with biochemical/physical criteria of menopause (FSH >40 IU/L and E2 <0.21 nmol/L); or having had bilateral oophorectomy at least 6 weeks prior to screening (with or without hysterectomy);
3. At least 49 moderate or severe hot flashes or night sweats over a period of 7 consecutive days, as recorded in the daily diary during the screening period, with at least 4 of those days with 7 or more moderate or severe hot flashes per day;
4. In good general health as determined on the basis of medical history and general physical examination performed at screening; Hematology and chemistry 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;
5. Negative urine test for selected drugs of abuse (amphetamines, tricyclic antidepressants, cannabinoids, cocaine, tetrahydrocannabinol, or opiates) at screening;
6. Negative serology panel (including hepatitis B surface antigen [HBsAg], anti-hepatitis C virus [HCV] and human immunodeficiency virus (HIV) antibody screens);
7. Negative urine pregnancy test at screening;
8. 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.

Exclusion Criteria:

Subjects meeting any of the following criteria are excluded from participation in this study:

1. Use of a prohibited therapy or not willing to wash-out drugs as mentioned in the prohibited therapies section of the protocol;
2. History (in the past year) or presence of drug or alcohol abuse;
3. Suicide attempt in the past 3 years;
4. Previous or current history of a malignant tumor, except basal cell carcinoma;
5. Active liver disease or jaundice, or out-of-range values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST); or total bilirubin >1.3 times the upper limit of normal (ULN); or creatinine >1.5 times the ULN; or estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) formula <60 mL/min/1.73 m^2 at screening;
6. Medical condition or chronic disease (including history of neurological [including cognitive], hepatic, renal, cardiovascular, gastrointestinal, pulmonary [e.g., moderate asthma], or endocrine disease) or malignancy that could confound interpretation of the study outcome;
7. Any psychological disorder according to the criteria indicated in the Diagnostics and Statistical Manual of Mental Disorders (DSM, 4^{th} edition) within one year prior to screening. Such disorders include but are not limited to current major depression, alcohol (more than 3 glasses of wine, beer, or equivalent/day) or substance abuse/dependence;
8. 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;
9. History of severe allergy, hypersensitivity, or intolerance to drugs in general, including the study drug and any of its excipients;

10. Presence or sequellae 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;
11. Concurrent participation in another interventional study (or participation within 3 months prior to screening in this study);
12. History of poor compliance in clinical studies;
13. Unable or unwilling to complete the study procedures;
14. 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.

Test Product, Dose, Mode of Administration:

ESN364 capsules to be taken orally b.i.d. with a glass of tap water at room temperature. The dose level of ESN364 that will be investigated is 90 mg b.i.d. (total daily dose of 180 mg)

Reference Product, Dose, Mode of Administration:

Matching placebo capsules to be taken orally b.i.d. with a glass of tap water at room temperature.

Study/Treatment Duration:

The total duration of involvement for each subject, screening through study exit, will be at most 19 weeks: the study will consist of a screening period (screening is to be performed within 4 weeks prior to the first intake of study drug, with a minimum of 7 days to allow for baseline data collection of Hot Flash Score and Hot Flash Frequency) and a 12-week double-blind treatment period (Weeks 1-12). A follow-up visit will be scheduled 2-3 weeks after the last study drug intake.

Criteria for Evaluation:

Efficacy

Primary endpoint:

- Change from baseline to Week 12 in weekly Hot Flash Score

Secondary endpoints:

- Changes from baseline over time in weekly Hot Flash Score and Hot Flash Frequency;
- Change from baseline over time in HFRDIS score;
- Changes from baseline over time in LSEQ, GCS, and SDS.

Exploratory endpoint: the change from baseline to Week 12 in body composition (lean mass, fat mass, bone mass/density) will be explored.

Pharmacokinetics

Assessment of plasma ESN364 concentrations will be exploratory.

Pharmacodynamics

Changes in plasma concentrations of LH, FSH, E2, SHBG, leptin, insulin, C-peptide, and HBA1c from baseline over time will be evaluated.

Safety

- AE frequency and severity from first dose of study drug through the last visit;
- Changes from baseline over time in hematology and biochemistry concentrations;
- Changes from baseline over time in vital sign and ECG assessments;
- Changes from baseline over time in BALP and CTX levels.

Statistical Methods:

Sample size

As this study is only the first proof-of-concept Phase IIa study in this therapeutic area, no formal power calculations have been done. All the statistical analyses involving testing for significance will be exploratory in nature and will serve as a basis for designing and powering a subsequent (dose-ranging) Phase IIb study.

One (and actually the main reason) for the absence of such power calculation is the fact that this study is unprecedented. There are no references available that can give an indication of observed effect size of

NK3 antagonism on Hot Flash Score and Hot Flash Frequency.

In a review article by Sloan et al., the authors propose the following general guideline regarding set-up of studies using hot flash diary data (severity and frequency):

- For Phase III placebo-controlled studies, 50 patients per treatment arm seem appropriate to provide sufficient power specifications to detect a clinically meaningful change in hot flash activity.
- For Phase II trials, 25 patients per study seem to provide reasonable estimates of eventual hot flash efficacy to screen potential agents for more definitive testing.

Since the review article typically considers paroxetine and hormones, we would, in our specific case, expect the effect size to be larger and would therefore propose to be sufficiently powered to conduct a successful Phase II proof-of-concept study, while leaving some room for drop-outs and interim analysis.

Efficacy

Primary endpoint:

Changes from baseline in Hot Flash Score will be analyzed using an Analysis of Covariance (ANCOVA) model with treatment as fixed effect and baseline as covariate. If a significant difference between the treatment arms is present, differences between each active treatment arm and placebo will be tested, correcting for multiplicity.

Secondary endpoints:

Hot Flash Score and Hot Flash Frequency at all time points will be evaluated by means of descriptive statistics (actual values and changes from baseline) by treatment arm.

The percentage of subjects with at least 70% reduction from baseline in Hot Flash Score will be compared across the treatment arms, using chi-square or Fisher exact test, as appropriate.

The percentage of subjects with at least 50% reduction in the number of moderate and severe hot flashes will be compared across the treatment arms, using chi-square or Fisher exact test, as appropriate.

Questionnaires will be evaluated by means of descriptive statistics (actual values and changes from baseline) by treatment arm. HFRDIS, GCS, SDS and LSEQ total scores and subscores (if applicable) are to be reported.

Change in lean body mass, fat mass, and bone mass/density will be explored by means of descriptive statistics on the actual values, at each assessment time point and by treatment arm. Changes from baseline to Week 12 will be summarized by treatment arm.

Pharmacokinetics

Individual plasma ESN364 concentration values and actual sampling times relative to study drug intake will be listed. Descriptive statistics will be summarized by assessment time point.

Pharmacodynamics

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

Safety

Safety parameters will be tabulated and analyzed descriptively.

TIME AND EVENTS SCHEDULE

Assessments	Visit 1: Screening[b]	Treatment Period				Visit 6: Follow-up[c]
Study Visit		Visit 2	Visit 3	Visit 4	Visit 5	
Time of Visit (Weeks)[a]		W1	W4	W8	W12	
Informed consent[d]	X					
In-/exclusion criteria	X	X				
Medical history/concomitant diseases	X					
Demographic data	X					
Physical examination[e]	X	X	X	X	X	X
Urine pregnancy test	X					
Urine drug screen	X					
Serology[f]	X					
Dual-energy X-ray absorptiometry[g]		X			X	
Sampling for pharmacokinetic assessments[h]		X	X	X	X	
Sampling for pharmacodynamic assessments[h,i]	X	X	X	X	X	X
Hot Flashes Severity and Frequency[j]	X	X	X	X	X	X
Hot Flash Related Daily Interference Scale[k]		X	X	X	X	X
Leeds Sleep Evaluation Questionnaire[l]		X	X	X	X	X
Greene Climacteric Scale[l]		X	X	X	X	X
Sheehan Disability Scale[l]		X	X	X	X	X
Clinical laboratory tests and urinalysis[m]	X	X	X	X	X	X
Bone density markers[n]		X			X	
Vital signs[o]	X	X	X	X	X	X
ECG[p]	X	X	X		X	X
Randomization		X				
ePRO training	X					
Dispensing of study drug[q]		X	X	X		
Intake of study drug[r]		X	X	X	X	
Compliance[s]			X	X	X	
Concomitant therapies[t]	X	X	X	X	X	X
Adverse events[t]	X	X	X	X	X	X

Footnotes on the next page.

a. All visits need to be planned in the morning, fasted (for unbiased glucose, insulin, and C-peptide determination). Visit 2 should be planned to allow for first intake of study drugs at the clinical site, between 7:00 and 10:00 AM. For each subject, all visits during the treatment period subsequent to Visit 2 should be planned at the same time of the day, in the morning. A deviation of ±1 day is allowed on the visits during the treatment period subsequent to the first study drug intake (Visits 3-5).
b. Screening is to be performed within 4 weeks prior to the first intake of study drug, with a minimum of 7 days to allow for baseline data collection of Hot Flash Score and Hot Flash Frequency.
c. Two to 3 weeks after the last intake of study drug.
d. No study-related assessments are to be carried out before signing of the informed consent form.
e. Includes height, weight, and waist circumference (height at screening only).
f. Includes HBsAg, and anti-HCV and HIV antibodies.
g. For practical reasons, the timing of DEXA may vary from the actual time of the visit, depending on DEXA availability (DEXA appointment).The Visit 2 DEXA can be performed prior to Visit 2 during the screening period, as well as at Visit 2 but must be performed before randomization. The Visit 5 DEXA can be performed ±4 days from the actual time of Visit 5. It is however to be performed before the last dose of the study drug; in case the DEXA appointment related to Visit 5 is scheduled later in time than Visit 5, study drug intake must be continued until DEXA has been performed.
h. Predose at Visits 2, 3, and 4; predose and 3 hours postdose at Visit 5.
i. Includes LH, FSH, E2, SHBG, leptin, insulin, C-peptide, and HBA1c (HBA1c at Visits 2 and 5 only). Additional back-up samples will be collected at every visit and stored for future, exploratory use.
j. Recorded in the ePRO diary on an ad hoc basis, but at a minimum twice-daily (morning/evening) from screening to follow-up. Night sweats should be recorded no later than morning upon awakening to start a new day.
k. Completed in the ePRO diary at the clinical site at any time during the visit.
l. Paper-based, administered at the clinical site at any time during the visit.
m. Includes biochemistry, coagulation, and hematology panel (coagulation panel only at screening). Blood samples for clinical laboratory tests should be taken in a fasted state (overnight fast for at least 10 h) for unbiased glucose, insulin, and C-peptide determination.
n. Includes BALP and CTX.
o. Includes body temperature (oral), sitting blood pressure and pulse rate (supine after 5 minutes of rest).
p. Supine 12-lead after 5 minutes of rest.
q. Study drugs will be dispensed to the subjects at Visits 2, 3, and 4 (one bottle containing 68 capsules at every visit).
r. The first intake of study drugs (Day 1; Visit 2) will be done at the clinical site (between 7:00 and 10:00 AM), under the supervision of the clinical staff. Study drug intake will be done with a glass of tap water at room temperature. Also on other days of visits, the morning dose of study drug will be taken at the clinical site, under the supervision of the clinical staff, after collection of predose blood samples. Subjects will be instructed not to consume food/fluids for at least 10 hours before their visit to the clinical site (except water intake until 1 hour before). On all other days throughout the treatment period, subjects will be asked to take their morning dose of study drug at home, around the same time of day (preferably between 7:00 and 10:00 AM). Morning intake at home may be done together with a light meal. All evening doses of study drug will be taken at home (preferably between 7:00 and 10:00 PM). Subjects need to record all home study drug intakes in the ePRO diary.
s. Includes compliance towards study drug intake and ePRO completion. Subjects will be asked to return all unused study drugs at every visit during the treatment period and compliance towards study drug intake will be assessed by counting returned dosage units in addition to ePRO entries upon study drug intake. Any discrepancies between returned dosage units and dosing in the ePRO diary will be discussed with the subject for whom a discrepancy was seen.
t. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

List of Abbreviations

ADME	Absorption, distribution, metabolism, or excretion
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC_{∞}	Area under the plasma concentration-time curve extrapolated to infinity
b.i.d.	Bis in die; twice daily
BALP	Bone alkaline phosphatase
BPH	Benign prostate hyperplasia
C_{max}	Maximum plasma concentration
CRO	Contract Research Organization
CTX	carboxy-terminal telopeptide of type I collagen
DBP	Diastolic blood pressure
DEXA	Dual-energy X-ray absorptiometry
DNA	Human deoxyribonucleic acid
E2	Estradiol
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ePRO	Electronic Patient Reported Outcome
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GCS	Greene Climacteric Scale
GnRH	Gonadotropin-releasing hormone
HBA1c	Glycated hemoglobin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
hERG	Human ether-à-go-go-related gene
HFRDIS	Hot Flash Related Daily Interference Scale
HIV	Human immunodeficiency virus
*h*NK	Human neurokinin
HPG	Hypothalamic-pituitary-gonadal
HR	Heart rate
HRT	Hormone replacement therapy
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITT	Intent-to-treat
IWRS	Interactive Web Response System
KNDy	Kisspeptin/neurokinin B/dynorphin
LH	Luteinizing hormone
LPLV	Last Patient Last Visit

LSEQ	Leeds Sleep Evaluation Questionnaire
NK	Neurokinin
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
P4	Progesterone
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PP	Per-protocol
q.d.	Quaque die; once daily
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected according to Fridericia's formula
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SDO	Secure Data Office
SDS	Sheehan Disability Scale
SHBG	Sex hormone-binding globulin
TEAE	Treatment-emergent adverse event
t_{max}	Time to maximum plasma concentration
ULN	Upper limit of normal

Definitions of Terms

BMI	Weight in kilogram divided by the square of height in meters
QTcF	QT interval corrected according to Fridericia's formula: $QTcF = QT/(1000/RR)^{1/3}$
Study drug	All drugs administered during the 12-week treatment period, i.e., the Investigational Medicinal Product (ESN364) and placebo

Study Administrative page removed as out of scope

1. INTRODUCTION

1.1 BACKGROUND INFORMATION

1.1.1 *Hot Flashes*

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) [1,2,3]. Approximately 75% of perimenopausal women will experience 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 hot flashes, and 4 million women report severe symptoms [5].

Hot flashes can have a significant negative impact on the quality of life, causing hot flashes to be a major reason for menopausal women to seek medical attention. Despite the vast numbers of individuals affected, the physiology of 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 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, a rare occurrence, panic [2].

The most effective treatment of hot flashes is hormone replacement therapy (HRT), but a Women's Health Initiative study raised questions about the long-term safety of this treatment. Hormone replacement therapy is also the most commonly used pharmacological treatment for hot flashes. However, 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 (e.g., breast/endometrial cancer, liver disease).

The perceived limitations of HRT, coupled with the lack of efficacy and adverse effects observed with non-hormonal therapies, have led clinicians to search for other treatment options for hot flashes. 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 [7,8,9]. The clinical benefit of these antidepressants is not as great as that observed for estrogen however.

1.1.2 *Introduction to ESN364*

ESN364 is an orally-available small molecule, potent and selective Neurokinin (NK) 3 antagonist that significantly modulates the hypothalamic-pituitary-gonadal (HPG) axis to lower the circulating levels of sex hormones in animal models. The 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 the NK3 receptor 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 [10]. 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 luteinizing hormone

(LH) but that follicle-stimulating hormone (FSH) levels are not significantly altered; in contrast, patients expressing a gonadotropin-releasing hormone (GnRH) receptor loss-of-function mutation exhibit castration levels of both LH and FSH [11]. LH and FSH function independently stimulate estradiol (E2) release from the ovaries. In total, these human mutant studies predict that ESN364 antagonism of the NK3 receptor will diminish levels of E2 due to reduction of the LH pulse frequency, but will not affect FSH levels and therefore there is a natural safety margin in place that protects against over-inhibition of E2 and the attendant 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 (e.g., Lupron®, Zoladex®, etc.).

The proposed mechanism of action is that neurokinin B (NKB), the endogenous neuropeptide agonist for the NK3 receptor, acts at the level of the hypothalamus to regulate LH pulse frequency. NK3 receptor antagonism blocks tonic, endogenous NKB signaling to thereby decrease LH pulse frequency and consequently lower plasma E2 levels. The schematic NK3 modulatory role on GnRH neurons to inhibit GnRH release and consequent release of sex hormones is presented in Figure 1.

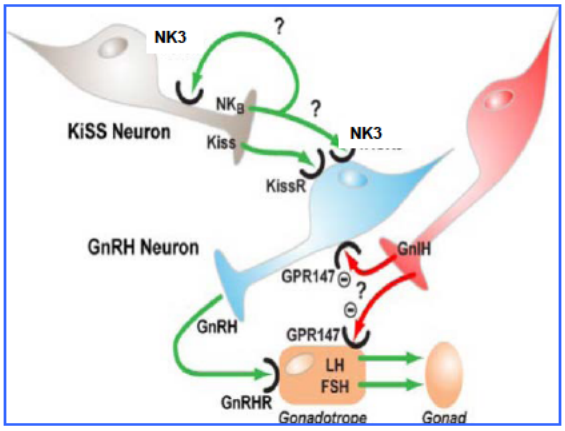


Figure 1: Modulatory Role of NK3 on GnRH Neurons (Figure From [12])

In addition to the precedence 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 II studies. Importantly, these development programs report dose-related changes LH, testosterone and various reproductive organs (testes, epididymis, and prostate) in male dog and a testosterone-lowering effect in male volunteers [13]. This history indicates that NK3 antagonism is a target concept for drug development with clinical precedence to reversibly lower sex hormones. The inhibitory effect of NK3 antagonist AZD2624 (Astra Zeneca) on testosterone levels in male volunteers is shown in Figure 2.

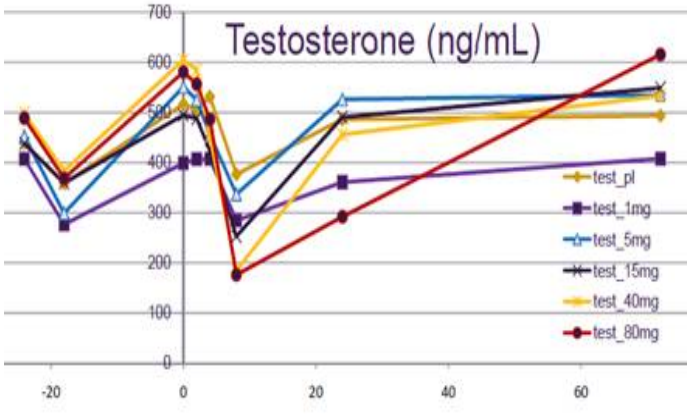


Figure 2: Inhibitory Effect of NK3 Antagonist on Testosterone Levels in Male Volunteers (Figure From [13])

1.2 NON-CLINICAL STUDIES

1.2.1 *Summary of Pharmacodynamics*

Pharmacological assays characterized ESN364 as an NK3 receptor antagonist with activity to lower sex hormones via a reduction in LH, indicative of a centrally-mediated effect. *In vitro* studies demonstrated that ESN364 is a potent full inhibitor of human neurokinin (*h*NK) 3 receptor and is highly selective for *h*NK3 in comparison to the other members of tachykinin receptor family (*h*NK1 and *h*NK2) and other G-protein coupled receptors including the ones known to be implicated in modulation of GnRH axis. Further i*n vitro* studies demonstrated that ESN364 have similar affinity for *human* and *monkey* NK3 receptors. The ESN364 affinity for *rat* NK3 receptor was high, however 8-fold less than the affinity for *human* NK3 receptor. These data indicated that rat and specially monkey are useful animal models for *in vivo* non-clinical testing.

In vivo studies demonstrated that ESN364 significantly reduces plasma LH levels in castrate male rats at a dose range of 3 to 20 mg/kg. At a dosage of 3 mg/kg, ESN364 significantly reduced circulating testosterone levels in intact male rats. ESN364 effectively reduced prostate size without concomitant effects to reduce testis weight in rat model of benign prostate hyperplasia (BPH) at dosages of 10 and 30 mg/kg (once daily [q.d.]; oral administration) after 21 days of treatment.

In ovariectomized female rats, ESN364 significantly reduced the mean plasma levels and pulsatile LH secretion frequency and amplitude at 10 mg/kg dosage. ESN364 treatment at the dosage of 10 mg/kg twice daily (b.i.d.) for a period of 10 days reduced E2 levels and appeared to prolong the estrous cycle in adult intact female rats. ESN364 significantly reduced circulating LH levels in castrate male monkeys following single and 5-day repeated oral dosing at 5 mg/kg/day. After five consecutive days of dosing, ESN364 had no effect on plasma FSH levels, demonstrating that antagonism of the NK3 receptor is a means to selectively inhibit LH, but not FSH, as a novel and precise approach to modulate the HPG axis for the treatment of sex hormone disorders.

ESN364 suppressed plasma LH levels 2 hours following bolus injection in ovariectomized female ewes at the 1 mg/kg dosage; pulsatile LH secretion restored

2-4 hours after the injection. ESN364 injection had no effect on FSH secretion in ovariectomized female ewes.

ESN364 significantly reduced LH, E2, and progesterone (P4) levels in intact Cynomolgus female monkeys after oral administration at the dosages of 10, 25 and 50 mg/kg/day during a treatment period of 35 days. The reduction in E2 was not to castration levels. With the same dosages and duration in female monkeys, ESN364 had no effect on FSH and testosterone levels. The LH, E2, and P4 levels were recovered in all compound treated animals after 28 days following cessation of treatment. In intact male Cynomolgus monkeys, following oral administration of ESN364 at the dosages of 25 and 50 mg/kg/day, there was a small trend towards decrease levels of mean testosterone, LH and E2, whereas no test item-related effects were seen on male FSH levels.

1.2.2 *Summary of Pharmacokinetics*

Pharmacokinetic (PK) and absorption, distribution, metabolism, and elimination (ADME) studies have been performed to demonstrate that ESN364 has a suitable profile for continued advancement through preclinical development as an oral product for the treatment of sex-hormone related disorders.

Following oral administration of ESN364 at a dose range of 3 to 20 mg/kg in castrate male rats, plasma concentrations increased linearly with dose and remained stable up to 300 minutes. ESN364 in methylcellulose formulation (0.5%) showed a good oral absorption in intact male rats at dosage of 3 mg/kg. In a BPH rat model, administration of ESN364 at dosages of 3, 10, 30 mg/kg q.d. (oral administration) for 21 days showed no significant accumulation, high absorption variability with relatively well-respected absorption ratios between the doses.

ESN364 had a rapid oral absorption after 1.0 hour postdose with a maximum plasma concentration within 5.0 hours postdose in monkeys at dosages of 10, 25 and 50 mg/kg. There was little or no accumulation of ESN364 following repeated dose administration for 25 days. There were no notable trends in the plasma concentrations or toxicokinetic parameters which could be related to gender.

ESN364 was devoid of significant inhibition of cytochrome P (CYP) 450 enzymes (>40 μM, standard panel). Plasma protein binding was low (<50%) and microsome stability was high ($t_{1/2}$ >1000 min) with consistent values in matrices from rat, monkey and human. Also, metabolic stability in hepatocytes proved to be similar across species (rat, monkey, human).

ESN364 had a good PK profile in rats with a low clearance, a good exposure, an elimination half-life longer than 4.0 hours, and bioavailability consistent with potential use via oral administration. Pharmacokinetic studies in rats and monkeys indicated that ESN364 undergoes low clearance and exhibits high oral exposure. In rats and monkeys, a single metabolite was predominantly observed resulting from an oxidation. The main route of excretion in both species was in the urine, with the oxidated metabolite being more rapidly excreted. Thus, the PK and excretory pathways appeared similar in rats and monkeys, the two species selected as models for the human safety profiling of this product.

High brain penetration was demonstrated in rat, consistent with the putative central mechanism of action of ESN364 to regulate LH release.

Toxicokinetic studies in rats showed that mean plasma concentrations are at a maximum at between 1.0 and 8.0 hours postdose for both males and females in all dosage groups (30, 100, 300 mg/kg/day; oral administration). Exposure of ESN364 indicated no accumulation and appeared to be similar in males and females on both sampling occasions and at all dose levels.

Toxicokinetic studies in monkeys demonstrated that ESN364 had a rapid oral absorption after 1.0 hour postdose (10, 25, 50 mg/kg/day; oral administration) with a maximum plasma concentration within 5.0 hours postdose. There was little or no accumulation of ESN364 following repeated dose administration for 25 days. There were no notable trends in the plasma concentrations or toxicokinetic parameters which could be related to gender.

1.2.3 *Summary of Toxicology*

No cytotoxicity was observed for ESN364 using HEPG2 cells. ESN364 did not induce mutation in 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA102) in the absence and in the presence of a rat liver metabolic activation system S9. ESN364 did not induce chromosome aberrations in cultured human peripheral blood lymphocytes up to a concentration equivalent to 1 mM for 3+17 hours in the absence and presence of S9 and for 20+0 hours in the absence of S9.

ESN364 did not exhibit any significant inhibition of human ether-à-go-go-related gene (hERG) channels nor did it significantly modulate either the electrical or the mechanical properties of the heart in a Langendorff model (rabbit) at tested concentrations up to 30 μM. In the human embryonic kidney (HEK) 293 cells transfected with hERG, ESN364 exhibited a hERG inhibition liability which was more than 8000-fold lower than that of the reference compound (terfenadine).

A study to investigate the effect of ESN364 on general activity, behavior and body temperature, oral administration of ESN364 to male and female Han Wistar rats demonstrated signs associated with sedation together with constricted pupils at the dose level of 250 mg/kg. At the dose level of 125 mg/kg, constricted pupils was the only sign seen and was present in 50% of the animals. These signs were judged to be of mild intensity and showed complete recovery at 24 hours postdose. No significant effect on body temperature was observed.

Measurement of respiratory parameters using whole body plethysmography in rats showed a clear dose-related response in both male and female rats with minimal transient effects at the low dose (40 mg/kg) and increasing marked effects on tidal volume with increasing dose levels (80 and 125 mg/kg). In the context of this study, and the absence of respiratory-related clinical signs, the respiratory effects were not considered to be adverse.

Electrocardiographic investigations in conscious male Cynomolgus monkeys showed that single oral doses of ESN364 at 10, 25, and 50 mg/kg were well-tolerated and elicited no significant changes. A slight decrease in mean $+dP/dt_{max}$ and systolic and mean arterial blood pressure as well as a slight increase in heart rate (HR) were observed. No electrocardiogram (ECG) waveform morphologic abnormalities on qualitative

assessments and no effects on PR, QRS, and QTc (QT interval corrected for heart rate) interval following oral doses of ESN364 were noted.

Repeated-dose toxicity studies in male and female HsdHan™: Wist rats demonstrate that daily oral administration of ESN364 for 28 days at dose levels of 30 and 100 mg/kg was well-tolerated. At the highest daily dosage of 300 mg/kg, two deaths and marked clinical signs (lethargy, reduced activity, labored respiration and staggering) and body weight loss during the first few days of treatment were observed. There were histopathological findings in the 100 and 300 mg/kg dosing groups in the lung, liver, bone marrow, uterus, ovary and prostate and some with corresponding changes in weight and macroscopic evaluation. These changes were considered to be adaptive or related to the pharmacology of the test article rather than being an adverse effect. Based on the findings from this study, the No observed adverse effect level (NOAEL) was considered to be 100 mg/kg/day in rats.

Repeated-dose toxicity studies in male and female Cynomolgus monkeys showed that daily oral administration of ESN364 for 5 weeks up to a dose of 50 mg/kg/day was tolerated with no mortality and no test item-related effects on hematology, clinical chemistry, urinalysis, respiratory rate, neurobehavioral observations, ophthalmic examinations, cardiovascular investigations and organ weights. Following oral administration of ESN364, a loss of body weight was observed, especially in male monkeys. One female monkey in the 50 mg/kg dosing group showed hyperplasia of lymphocytes in lymph nodes, spleen and lung and perivascular inflammation in the kidney. Therefore, the NOAEL for ESN364 was defined to be 25 mg/kg/day in monkeys.

For more detailed information on ESN364 and the conducted long-term (13-week) toxicology studies, refer to the current Investigator's Brochure [14].

1.3 CLINICAL STUDIES

To date, the development program of ESN364 includes only one recently completed study, conducted in 65 subjects. A dose range between 3 and 180 mg as single dose in fasted and fed conditions, and up to 21 days of dosing in a multiple dose setting was tested. Both sexes were included in this study. Male healthy volunteers have been dosed up to 10 consecutive days and healthy female subjects of childbearing potential have been dosed up to 21 consecutive days in accordance with their menstrual cycle. The highest dose in both multiple dose parts was 180 mg q.d.

The results of the Phase I study demonstrated that administration of single and multiple ascending doses up to 180 mg of ESN364 were safe and well tolerated.

No deaths occurred during the study. The majority of reported treatment-emergent adverse events (TEAEs) were mild to moderate in severity. A severe TEAE was reported in 1 (7.1%) subject in the placebo treatment group. One serious adverse event (SAE) (foot fracture) was reported in 1 (5.9%) subject during the follow-up period and led to withdrawal of the subject from the study.

The most frequently reported TEAEs (i.e., those reported in >2 subjects per treatment group) following multiple ascending dosing for 21 days in healthy female subjects were abdominal pain in 3 (50.0%) subjects each in the placebo and 180 mg ESN364 treatment group, nausea in 3 (50.0%) subjects in the 20 mg ESN364 treatment group, headache in 3 (50.0%) and 4 (66.7%) subjects in the 60 mg and 180 mg ESN364 treatment groups,

respectively, and dry skin in 3 (50.0%) subjects in the 180 mg ESN364 treatment group. Nausea and headache were only reported in the 20 mg, 60 mg, and 180 mg ESN364 treatment groups and not in the placebo treatment group.

All subjects had recovered or were recovering from the TEAEs by the end of the study.

All treatment-emergent laboratory abnormalities (worst-case) were observed in at most 2 subjects per treatment group, except for some isolated elevated abnormally high potassium levels and isolated low levels of total bilirubin. None of the laboratory abnormalities were considered to be of clinical relevance, and as a consequence none of the laboratory abnormalities were reported as adverse event (AE).

No clinically relevant trends or changes were observed in median vital sign values and ECG parameters over time. None of the observed ECG related abnormalities were reported as AE. Vital sign abnormalities were reported as a AE in 2 subjects (palpitations in one subject after single dose administration and palpitations and orthostatic hypertension in one subject after multiple dose administration).

None of the subjects were observed with new (i.e., not present at screening) abnormal findings during physical examination in the study.

In female subjects, a dose-dependent decrease in detectable ovulations and a delay of the mid-cycle LH surges was observed, resulting in a prolonged or absent menstrual cycle. A 84-day follow-up period showed a recovery of the normal menstrual pattern once the treatment with ESN364 had finished.

Following single ascending dose administration of ESN364, the PK of ESN364 was dose proportional in terms of maximum plasma concentration (C_{max}) and plasma exposure (AUC_{∞}) over the 3 to 180 mg dose range. Median time to maximum plasma concentration (t_{max}) ranged between 1.25 to 2.00 hours, being longer in the 90 mg dose group compared to the 3 and 12 mg dose groups. Mean terminal half-life ranged between 2.39 and 4.19 hours. No significant food effect was observed on ESN364 exposure and absorption rate measured in the 23 mg dose group.

Single dose administration of ESN364 in the dose range from 3 to 180 mg induced a dose-dependent decrease in total and free testosterone, FSH and LH plasma concentrations within the 24 hours after administration, compared to placebo. Both minimum concentration (i.e., maximum effect) and plasma exposure were decreased after different single doses of ESN364. No clear effect on sex hormone-binding globulin (SHBG) plasma concentration was observed compared to placebo.

Following multiple dose administration for 10 days in healthy males, it could be concluded that steady-state in ESN364 plasma concentrations was reached quickly, by Day 2, so after the first administration of study drug. The mean total amounts of ESN364 excreted unchanged in urine over the 0-24 hour period were similar on Day 1 and Day 10 (with a range of 1.53 to 2.16% of the dose). The bioavailability of ESN364 increased proportionally with the dose from 20 to 180 mg q.d, as measured by peak and plasma exposure. Time to peak concentration was similar after single and multiple administrations and for all tested doses, with median t_{max} of 2.50 or 3.00 hours. From descriptive data, the apparent elimination half-life seems to be comparable between the studied dose groups as well between single dosing and steady-state with mean values ranging between 2.84 and 4.20 hours. No accumulation of ESN364 was observed after

10 days of 60 or 180 mg q.d. administration. Renal clearance seems to be similar in the 60 or 180 mg q.d. groups, with mean values of 11 and 9.93 mL/min, respectively.

Single and multiple administration of ESN364, in the dose range from 20 to 180 mg q.d., induced a similar dose-dependent decrease in total and free testosterone within the 24 hours after administration, compared to placebo. A decrease of a similar extent throughout the dose range (20 mg to 180 mg q.d.) was observed in LH and FSH plasma concentrations after single and multiple administration of ESN364, compared to placebo. After a single administration of ESN364 (Day 1), total testosterone plasma concentrations <5.2 nmol/L were found for 16.7% of the subjects at the dose of 20 mg q.d. (for a median extent of time of 5.58 hours), for 66.7% of the subjects at a dose of 60 mg q.d. (for a median extent of time of 6.52 hours) and for 66.7% of the subjects at a dose of 180 mg q.d. (for a median extent of time of 11.5 hours). After multiple administration (Day 10) of ESN364 (Day 10), total testosterone plasma concentrations <5.2 nmol/L were found for 50% of the subjects at the dose of 20 mg q.d. (for a median extent of time of 1.77 hours), for 50% of the subjects at a dose of 60 mg q.d. (for a median extent of time of 4.21 hours) and for 66.7% of the subjects at a dose of 180 mg q.d. (for a median extent of time of 7.5 hours). After single and multiple dose administration of ESN364, no clear effect on plasma SHBG concentrations was observed, compared to placebo. Relatively high between-subject variability was observed for pharmacodynamic (PD) parameters.

Following multiple dose administration for 21 days in healthy females, it could be concluded that steady-state in ESN364 plasma concentrations was reached quickly, by Day 2, so after the first administration of study drug. The bioavailability of ESN364 increased dose proportionally between 20 and 60 mg q.d. but was above the dose proportionality for the highest 180 mg q.d. compared to 60 mg q.d., as measured by peak and plasma exposure, and this independently from PK sampling day (Day 1 and Day 21). No significant dose and day effects were observed on t_{max} with median t_{max} of 3.00 to 4.00 hours. Plasma ESN364 exposure was higher at steady-state (Day 21) compared to Day 1. From descriptive data, the apparent elimination half-life seems to be comparable between the studied dose groups as well between single dosing and steady-state (mean half-life ranged between 4.22 and 4.84 hours), with the exception of the highest dose group where the half-life was prolonged on Day 1 (7.06 hours) and Day 21 (6.34 hours) compared to the other dose groups. No relevant accumulation of ESN364 was observed after 21 days of administration of 60 or 180 mg q.d. administration, with mean accumulation ratio (R_{ac}) values of 1.23 and 1.18, respectively. Renal clearance seems to be comparable in the 60 or 180 mg q.d. groups, with mean values of 6.83 and 5.34 mL/min, respectively. When comparing ESN364 exposure after single dosing or at steady-state following multiple dose administration for 10 days (healthy males) and 21 days (healthy women), peak and plasma exposures were significantly lower in males. Time to peak exposure was similar for both genders.

Based on plasma concentrations monitored from the first administration (Day 1) to 24 hours after the last administration (Day 22) (i.e., over the time extent of the menstrual cycle during presence of study drug in the body), the time to reach the peak concentration in LH was lengthened with the 60 and 180 mg q.d. dosages (median time of ≈17 days) compared to placebo (median time of ≈ 8 days). No clear difference between active treatments and placebo could be evidenced in FSH plasma concentrations. The time to reach the peak concentration in total and free estradiol was lengthened with the 60 and

180 mg q.d. dosages (median time of between 17 and 19 days) compared to placebo (median time of ≈ 9 days). A dose-dependent decrease in P4 plasma concentration was observed with the 60 and 180 mg q.d. dosages, compared to placebo, and the 20 mg q.d. dosage; the calculated median time to reach P4 peak concentration was between around 18 days for the 60 and 180 mg q.d. dosages, compared to 14 days for placebo and the 20 mg q.d. dosage. However, for a majority of subjects in the 60 and 180 mg q.d. dose groups, the menstrual peak in P4 was not yet reached at Day 22.

1.4 OVERALL RATIONALE FOR THE STUDY

Since 2012, there is an increasing number of articles and papers pointing towards a direct link between kisspeptin/neurokinin B/dynorphin (KNDy) neurons and the occurrence of hot flashes [15,16,17]. Mittelman-Smith and Rance linked the modulation of body temperature, estradiol and KNDy neurons to the occurrence of hot flashes [15,16]. Jayasena et al. demonstrated a direct causal relationship between the injection of NKB and the occurrence of hot flashes in the study participants [17].

Euroscreen did an experiment in 10 ovarectomized ewe (non-GLP study Monash 001) where the ewes were injected with either 1 mg/kg ESN364 or placebo. The study was used to demonstrate the suppression of the LH pulse frequency by ESN364. When analyzing the safety data from the ewes, there was a significant difference in core body temperature between the treated versus the placebo ewes, as shown in Figure 3.

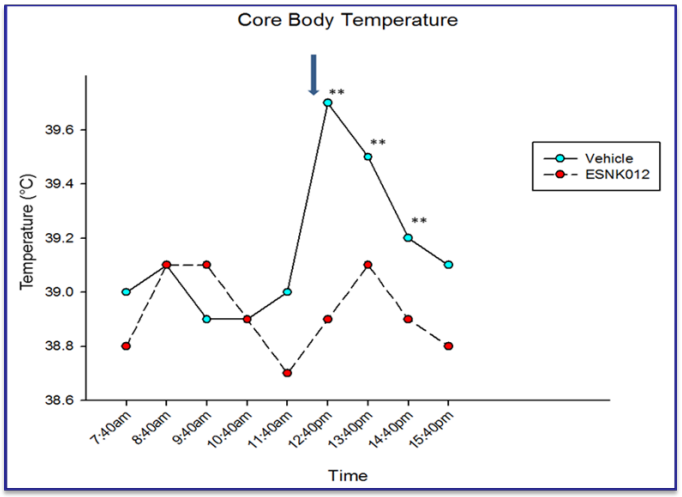


Figure 3: Core Body Temperature in Ovarectomized Ewes Injected With 1 mg/kg ESN364 or Placebo

Our current hypothesis is that in menopause, the lack of E2 feedback to the KNDy neuron leads to an increased firing frequency of that neuron which in turn leads to the increase in plasma LH and FSH concentrations typically seen in postmenopause.

Because of the projecting nerve endings from the KNDy neuron to the preoptic hypothalamus, this increase firing of the KNDy neuron also results in the generation of hot flashes. Our hypothesis is that ESN364 - via NK3 antagonism - will decrease this firing frequency of the KNDy neuron to normal 'premenopausal' levels thus eliminating the occurrence of hot flashes.

A schematic diagram showing the anatomic relationship between KNDy neurons, GnRH neurons, and the heat-defense pathway in the rat is shown in Figure 4.

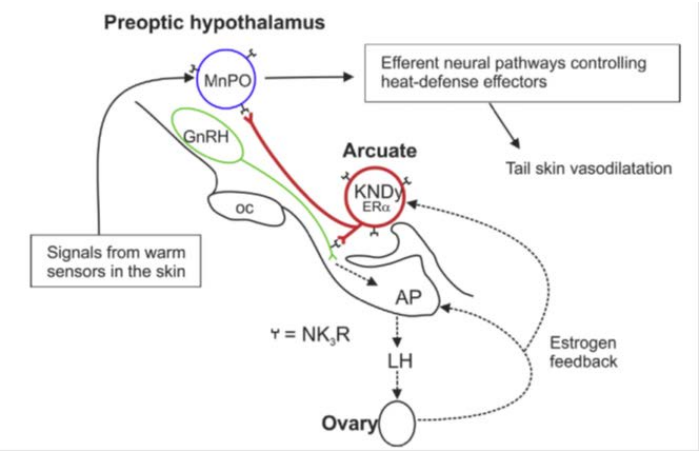


Figure 4: Schematic Diagram Showing the Relationship Between KNDy Neurons, GnRH Neurons and the Heat-Defense Pathway in the Rat. KNDy Neurons Branch and Project to GnRH Terminals in the Median Eminence and Preoptic Structures That Regulate Body Temperature (Figure from [18])

1.5 RISK BENEFIT ANALYSIS

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

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, P4 and testosterone. Since in this study we are aiming at including 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 we do anticipate a moderate decrease of the already elevated LH and FSH plasma levels, maybe even to premenopausal levels. There are no known risks associated with this quasi normalization of the gonadotropins.

Treatment with ESN364 can cause adverse effects or other symptoms. Adverse effects that can be expected as derived from the AE profile of the first-in-human study in male and female healthy volunteers (see also Section 1.3) are as follows:

During the Phase I study, following side effects have been recorded in the 41 male healthy volunteers who received ESN364:

- Very Frequent (i.e., in more than 1/10 participants): none;
- Frequent (i.e., in more than 1 in 100 but fewer than 1 in 10 participants): nausea and headache (both in 3/41 participants) and palpitations, abdominal discomfort, diarrhea, oral paresthesia (tingling sensation in the mouth), dizziness,

hyperhidrosis (increased sweating), abnormal skin odor, irritability, and fatigue (each in 1/41 participants).

During the Phase I study, following side effects have been recorded in the 18 female healthy volunteers who received ESN364:

- Very Frequent (i.e., in more than 1/10 participants): headache (7/18 participants), dry skin (3/18 participants), abdominal pain, metrorrhagia, vaginal discharge, and acne (each in 2/18 participants);
- Frequent (i.e., in more than 1/100 but fewer than 1/10 participants): dry mouth, dysphagia (pain during swallowing), nausea, sensation of pressure, dizziness, breast pain, vulvovaginal itching, oropharyngeal pain, generalized itchy feeling, abnormal skin odor, palpitations, dry eye, abdominal discomfort, and abdominal distension (all in 1/18 participants).

All recorded treatment-related AEs were mild to moderate in severity and had a short duration. All AEs resolved spontaneously without sequellae. Only 3 AEs were rated moderate in severity, being abdominal pain, headache and palpitations. The reported palpitations were short-lived and were not accompanied by other signs/symptoms of cardiac involvement (vital signs and ECG were normal).

However, given the stage of drug development, our current knowledge of the side effect profile of ESN364 is still limited.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of the study is to evaluate the effect of ESN364 on the severity and frequency of hot flashes in early postmenopausal women suffering from hot flashes, in terms of changes in weekly Hot Flash Score from baseline to Week 12.

2.2 SECONDARY OBJECTIVES

Secondary objectives are to evaluate in early postmenopausal women suffering from hot flashes:

- the effect of ESN364 on the severity and frequency of hot flashes, in terms of changes in weekly Hot Flash Score from baseline to Weeks 4 and 8, and at follow-up;
- the effect of ESN364 on the frequency of hot flashes, in terms of changes in weekly Hot Flash Frequency from baseline to Weeks 4, 8, and 12, and at follow-up;
- the effect of 12-week administration of ESN364 on hot flash interference on daily life, in terms of changes from baseline over time in Hot Flash Related Daily Interference Scale (HFRDIS);
- the effect of 12-week administration of ESN364 on climacteric symptoms, in terms of changes from baseline over time in Leeds Sleep Evaluation Questionnaire (LSEQ), Greene Climacteric Scale (GCS), and Sheehan Disability Scale (SDS);
- the PD effect of 12-week administration of ESN364, in terms of changes over time in plasma concentrations of LH, FSH, E2, SHBG, leptin, insulin, C-peptide, and glycated hemoglobin (HBA1c);
- safety and tolerability of 12-week administration of ESN364, in terms of AEs, clinical laboratory tests, plasma bone density marker concentrations (bone alkaline phosphatase [BALP] and carboxy-terminal telopeptide of type I collagen [CTX]), vital signs, ECG, and physical examination.

Exploratory objectives are to:

- evaluate the effect of 12-week administration of ESN364 on body composition (lean mass, fat mass, bone mass/density) assessed by dual-energy X-ray absorptiometry (DEXA);
- assess ESN364 plasma concentrations from sparse PK sampling.

3. STUDY DESIGN

3.1 OVERVIEW OF STUDY DESIGN

This is a 12-week double-blind, placebo-controlled, parallel-group, multicenter, proof-of-concept study to assess the effect of 12-week administration of ESN364 in early postmenopausal women suffering from hot flashes.

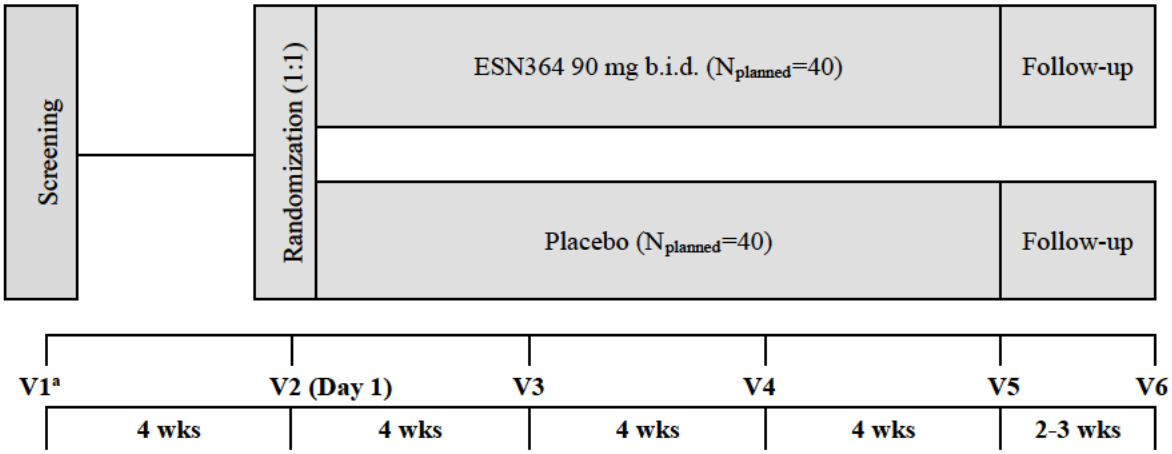
A total of 80 subjects are planned to be included in the study, i.e., 40 subjects in the active treatment arm and 40 subjects in the placebo arm. Each subject will be administered an oral dose of 90 mg ESN364 b.i.d. or placebo b.i.d., as per pregenerated randomization list.

The study will consist of a screening period of maximum 4 weeks and a 12-week double-blind treatment period (Weeks 1-12), followed by a follow-up visit 2-3 weeks after the last study drug intake.

The study will be performed on an ambulatory basis. During the treatment period, subjects will be asked to come to the clinical site every 4 weeks (Day 1 and Weeks 4, 8, 12; Visits 2-5).

A schematic overview of the study design is shown in Figure 5. The assessments performed are summarized per visit in the Time and Events Schedule.

Figure 5: Schematic Overview of the Study



[a] Screening is to be performed within 4 weeks prior to the first intake of study drug, with a minimum of 7 days to allow for baseline data collection of Hot Flash Score and Hot Flash Frequency.

An interim analysis on the primary endpoint, DEXA parameters and safety data will be performed once the first 16 subjects will have completed 12 weeks of treatment and the follow-up visit (or have discontinued earlier). The final analysis will be performed once all subjects will have completed 12 weeks of treatment and the follow-up visit (or have discontinued earlier).

3.2 DISCUSSION OF STUDY DESIGN

Dose Selection

LH secretion is a surrogate for the activity of the KNDy neuron. In the first-in-human Phase I study (see Section 1.3), 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 I study and was demonstrated to be safe and well tolerated. LH suppression was maximal with this dose level. Hence, we expect that the 180-mg dose represents the dose associated with maximal suppression of the KNDy neuron.

During menopause, the negative feedback by E2 is unexisting, due to the cessation of estrogen production in the ovaries. The lacking negative estradiol feedback will cause the KNDy neuron to become hyperactive in an attempt to restore the needed estradiol levels via the increased release of LH/FSH. When dealing with an hyperactive state of the KNDy neuron, as assumedly is the case in the postmenopause period, a once-daily morning dose of ESN364 will probably not yield the desired suppression of KNDy at night, preventing night sweats. For this reason, a high dose of ESN364 (total daily dose of 180 mg) given as 90 mg b.i.d. will be tested to investigate the possibility to completely suppress LH secretion.

Placebo Control

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.

Randomization and Blinding

Randomization will be used to avoid bias in the assignment of subjects to treatment, to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment arms, and to enhance the validity of statistical comparisons across treatment arms.

Blinded treatment will be used to reduce potential bias during data collection and evaluation of clinical endpoints.

4. SELECTION OF STUDY POPULATION

Screening for eligible subjects will be performed at a screening visit that is to be planned within 4 weeks prior to the first intake of study drug (with a minimum of 7 days to allow for baseline data collection of Hot Flash Score and Hot Flash Frequency).

In total 80 subjects are planned to be enrolled. Subjects will be randomized to one of 2 treatment arms in a 1:1 ratio: ESN364 90 mg b.i.d. (40 subjects) or placebo (40 subjects).

In- and exclusion criteria will be checked during screening (Visit 1) and reassessed prior to randomization (Visit 2).
Note: in case of 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, a retest can be done. Results of this retest must be available prior to randomization. The result of the retest will be considered for subject eligibility.

4.1 INCLUSION CRITERIA

Subjects meeting all of the following criteria are eligible to participate in this study:

1. Women, between 40 and 65 years old (extremes included) at screening;
2. Spontaneous amenorrhea for at least 12 consecutive months; or spontaneous amenorrhea for at least 6 months with biochemical criteria of menopause (FSH >40 IU/L); or spontaneous amenorrhea for at least 3 months with biochemical/physical criteria of menopause (FSH >40 IU/L and E2 <0.21 nmol/); or having had bilateral oophorectomy at least 6 weeks prior to screening (with or without hysterectomy);
3. At least 49 moderate or severe hot flashes or night sweats over a period of 7 consecutive days, as recorded in the daily diary during the screening period, with at least 4 of those days with 7 or more moderate or severe hot flashes per day;
4. In good general health as determined on the basis of medical history and general physical examination performed at screening; hematology and chemistry 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;
5. Negative urine test for selected drugs of abuse (amphetamines, tricyclic antidepressants, cannabinoids, cocaine, tetrahydrocannabinol, or opiates) at screening;
6. Negative serology panel (including hepatitis B surface antigen [HBsAg], anti-hepatitis C virus [HCV] and human immunodeficiency virus (HIV) antibody screens);
7. Negative urine pregnancy test at screening;
8. 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.

4.2 EXCLUSION CRITERIA

Subjects meeting any of the following criteria are excluded from participation in this study:

1. Use of a prohibited therapy or not willing to wash-out drugs as mentioned in the prohibited therapies section (Section 6.2);
2. History (in the past year) or presence of drug or alcohol abuse;
3. Suicide attempt in the past 3 years;
4. Previous or current history of a malignant tumor (except basal cell carcinoma);
5. Active liver disease or jaundice, or out-of-range values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST); or total bilirubin >1.3 times the upper limit of normal (ULN); or creatinine >1.5 times the ULN; or estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) formula <60 mL/min/1.73 m^2 at screening;
6. Medical condition or chronic disease (including history of neurological [including cognitive], hepatic, renal, cardiovascular, gastrointestinal, pulmonary [e.g., moderate asthma], or endocrine disease) or malignancy that could confound interpretation of the study outcome, as judged by the Investigator;
7. Any psychological disorder according to the criteria indicated in the Diagnostics and Statistical Manual of Mental Disorders (DSM, 4^{th} edition) within one year prior to screening. Such disorders include but are not limited to current major depression, alcohol (more than 3 glasses of wine, beer, or equivalent/day) or substance abuse/dependence;
8. 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;
9. History of severe allergy, hypersensitivity, or intolerance to drugs in general, including the study drug and any of its excipients;
10. Presence or sequellae of gastrointestinal, liver, kidney or other conditions known to interfere with the ADME mechanisms of drugs, as judged by the Investigator;
11. Concurrent participation in another interventional study (or participation within 3 months prior to screening in this study);
12. History of poor compliance in clinical studies;
13. Unable or unwilling to complete the study procedures;
14. 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.

5. TREATMENT(S)

Manufacturing, packaging, and labeling of the study drug, ESN364, will be done under the responsibility of the Sponsor.

The ESN364 and placebo capsules are manufactured under current Good Manufacturing Practice (GMP), as required by the current Good Clinical Practice (GCP), at Pharmavize N.V. (Mariakerke, Belgium).

5.1 PHYSICAL DESCRIPTION OF THE STUDY DRUG

The Investigational Medicinal Product (IMP), ESN364 capsules, is being developed as an immediate-release hard gelatin capsule for oral administration in an active dosage strength range of 2.25 mg to 180 mg. In this clinical study, only the 90 mg strength will be tested. The qualitative composition of the ESN364 capsules is provided in Table 1.

Table 1: 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
Coni snap size 0 white opaque hard gelatin capsule (opaque white cap/opaque white body)	Capsule shell

5.2 OTHER MEDICATION ADMINISTERED IN THE STUDY

Placebo is supplied as hard gelatin capsules that match the IMP. Placebo capsules contain the same excipients as the IMP. The capsule shells used for manufacturing of placebo capsules are identical to the capsule shells used for manufacturing of ESN364 capsules.

5.3 PACKAGING AND LABELING

The packaging and labeling of study drugs will be in accordance with applicable local regulatory requirements.

The ESN364 and placebo capsules will be packaged in high-density polyethylene (HDPE) bottles with polypropylene closure. Each bottle will contain 68 capsules of ESN364 or placebo. A patient kit containing 3 bottles of study drug will be foreseen for each subject.

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

- EudraCT number
- Protocol number
- Medication number
- Visit number
- Date of manufacture and/or expiration date

- Batch number
- Storage conditions
- Contents
- The statements ‘Keep out of reach and sight of children’, ‘For oral route only’, ‘For clinical trial use only’
- Sponsor (or sponsor representative) name

5.4 STORAGE AND DRUG ACCOUNTABILITY

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

ESN364 must be stored at ambient conditions (15-30°C or 59-86°F), should not be exposed to freezing temperatures, and should be protected from light during storage at the clinical site.

Daily temperature logging of the study drug storage room at the clinical site should be performed. In case a deviation in storage conditions should occur, the site must not further dispense the affected study drug and notify the Clinical Research Associate (CRA) or Pharmavize N.V.

For the available stability data of ESN364 capsules and placebo, please refer to the Investigational Medicinal Product Dossier (IMPD).

The Investigator is responsible for ensuring that all study drugs received at the clinical site are inventoried and accounted for throughout the study. As misallocations of study drugs may have a detrimental effect on subjects' safety and/or the study drugs’ efficacy and are a potential source of bias, utmost care should be taken to correctly dispense the study drugs as assigned by the randomization system.

Study drugs will be dispensed to the subjects at Visits 2, 3, and 4 (one bottle containing 68 capsules at every visit). Study drugs 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 drugs supplied to whom and by who. Study drugs will be supplied only to subjects participating in the study.

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

Subjects must be instructed to return all unused study drugs at every visit. A record of any returned study drugs will be kept for each subject. Returned study drugs must not be relabeled or dispensed again, not even to the same subject.

Unused study drugs and study drugs 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 drugs should be explained and documented.

After the last visit of the last subject in the study (Last Patient Last Visit [LPLV]), any unused study drug will be returned to the Sponsor.

5.5 RANDOMIZATION

In total 80 subjects are planned to be randomized to one of 2 treatment arms in a 1:1 ratio: ESN364 90 mg b.i.d. (40 subjects) or placebo (40 subjects).

Randomization will be based on a computer-generated randomization schedule prepared by the Secure Data Office (SDO) Department of SGS Life Science Services 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 uploaded into the randomization module of the Electronic Data Capture (EDC) system. Based on this randomization list, the study drugs will be packaged and labeled. Medication numbers will be preprinted on the study drug labels.

At the clinical site, through the randomization module of the EDC system, subjects will automatically be assigned a randomization number as soon as they qualify for the study.

5.6 BLINDING

Blinding will be achieved by the double-dummy method with placebo identical in smell, taste, and appearance.

The randomization list will be retained by SDO 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 determination before the start of the study.

The randomization list will not be available to the Investigator or other employees of the clinical site, subjects, monitors, or personnel of SGS Life Science Services departments other than SDO before unblinding of the data, unless in case of emergency. The Sponsor's clinical team will also be blinded during the study as they will not have direct access to the randomization list. The Clinical 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 Interactive Web Response System (IWRS). IWRS will allow rapid access to the treatment allocation codes when relevant for site or CRO personnel. However, codes should only be broken if it is considered as 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. Site personnel should inform the Medical Monitor (SGS Life Science Services) immediately. If the Investigator accidentally breaks the code, the Medical Monitor 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 the subject may remain in the study.

At the time of the interim analysis, which will be conducted on the primary endpoint, DEXA parameters and safety data once the first 16 subjects will have completed 12 weeks of treatment and the follow-up visit (or have discontinued earlier), the interim analysis subjects' treatment arm will be unblinded. All other subjects will remain blinded.

5.7 DOSE AND ADMINISTRATION

A rationale for the dose of study drug selected in this study is provided in Section 3.2.

Subjects will be randomly, sequentially, and in a blinded fashion assigned to one of 2 treatments arms (i.e., ESN364 90 mg b.i.d. [total daily dose of 180 mg] or placebo) in a 1:1 ratio according to the randomization schedule. An overview of the treatment in each of the treatment arms is provided in Table 2.

Table 2: Treatment Overview

Treatment Arm	Number of Subjects	Treatment
ESN364 90 mg b.i.d.	40	oral dose of 90 mg ESN364 b.i.d. for 12 weeks
Placebo	40	oral dose of placebo b.i.d. for 12 weeks

All visits need to be planned in the morning, fasted (for unbiased glucose, insulin, and C-peptide determination). Visit 2 should be planned to allow for first intake of study drugs at the clinical site, between 7:00 and 10:00 AM. For each subject, all visits during the treatment period subsequent to Visit 2 should be planned at the same time of the day, in the morning.

Study drugs will be dispensed to the subjects at Visits 2, 3, and 4 (one bottle containing 68 capsules at every visit).

The first intake of study drug (Day 1; Visit 2) will be done at the clinical site (between 7:00 and 10:00 AM), under the supervision of the clinical staff. Study drug intake will be done with a glass of tap water at room temperature. Also on other days of visits, the morning dose of study drug will be taken at the clinical site, under the supervision of the clinical staff, after collection of predose blood samples. Subjects will be instructed not to consume food/fluids for at least 10 hours before their visit to the clinical site (except water intake until one hour before). On all other days throughout the treatment period, subjects will be asked to take their morning dose of study drug at home, around the same time of the day (preferably between 7:00 and 10:00 AM). Morning intake at home may be done together with a light meal.

All evening doses of study drug will be taken at home (preferably between 7:00 and 10:00 PM).

Subjects need to record all home study drug intakes in the Electronic Patient Reported Outcome (ePRO) diary.

In case a subject accidentally misses a scheduled dose of the study drug and this is noticed within 12 hours of the time of scheduled intake, the missed dose should be taken

as soon as possible, with or without food. The subject may then continue her usual dosing schedule. In case a subject accidentally misses a scheduled dose of the study drug and this is noticed more than 12 hours after the time of scheduled intake, the missed dose should be skipped and the subject should simply resume her usual dosing schedule.

Any deviation from the treatment regimen defined in the protocol must be documented in the electronic Case Report Form (eCRF).

5.8 TREATMENT COMPLIANCE

Study drugs will be dispensed to the subjects at Visits 2, 3, and 4 (one bottle containing 68 capsules at every visit).

On days of visits during the treatment period, the morning dose of study drug will be taken at the clinical site, under the supervision of the clinical staff. All other study drug intakes (evening dose and morning dose on all days without visits) will be done at home. When taking their 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 drugs.

Compliance towards study drug intake will be assessed by counting returned dosage units in addition to ePRO entries upon study drug intake. Any discrepancies between returned dosage units 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, despite educational efforts, the Investigator should contact the Sponsor to discuss withdrawal of the subject from the study.

6. PRIOR AND CONCOMITANT THERAPY

The use of concomitant therapies should be kept at a minimum throughout the study. All therapies (prescriptions and over-the-counter medications) other than the study drug administered from informed consent until the last study visit 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 a protocol-related (S)AE.

6.1 PERMITTED CONCOMITANT THERAPIES

Any medications, other than those excluded by the protocol (see Section 6.2), 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.

The use of analgesics is permitted on an as-needed basis. Prophylactic use is not allowed due to potential impact on the efficacy endpoints of this study.

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 is allowed on the condition that hypertension is under control.

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

6.2 PROHIBITED CONCOMITANT THERAPIES

Prophylactic use of analgesics is not allowed due to potential impact on the efficacy endpoints of this study.

Current use of hormonal medications such as hormone therapy or hormonal contraception or any treatment for hot flashes (prescription, over-the-counter, or herbal) is not allowed during the study. These treatments must be discontinued at least 6 weeks prior to screening.

Other medications that should be washed-out prior to screening and are not allowed during the study are:

- 12 weeks wash-out:
  - ✓ tricyclic antidepressants
  - ✓ selective noradrenaline reuptake inhibitors
  - ✓ selective serotonin reuptake inhibitors (SSRIs)
  - ✓ lithium
  - ✓ oral neuroleptics
  - ✓ all sedatives
  - ✓ all hypnotics

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

- 4 weeks wash-out:
  - ✓ fluoxetine
  - ✓ monoamine oxidase inhibitors (MAOIs)

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

Any medication that is not explicitly covered by the permitted and non-permitted medication sections can only be allowed after discussion with the Medical Monitor.

7. ASSESSMENTS

7.1 TIMING OF ASSESSMENTS

All visits need to be planned in the morning, fasted (for unbiased glucose, insulin, and C-peptide determination). Visit 2 should be planned to allow for first intake of study drugs at the clinical site between 7:00 and 10:00 AM. For each subject, all visits during the treatment period subsequent to Visit 2 should be planned at the same time of the day, in the morning, fasted. A deviation of ± 1 day is allowed on the visits during the treatment period (Visits 3-5).

At Visits 2 and 5, DEXA will be performed. For practical reasons, the timing of DEXA may vary from the actual time of the visit, depending on the DEXA availability (DEXA appointment):

- The Visit 2 DEXA can be performed prior to Visit 2 during the screening period, as well as at Visit 2 but must be performed before randomization;
- The Visit 5 DEXA can be performed ±4 days from the actual time of Visit 5. It is however to be performed before the last dose of the study drug; in case the DEXA appointment related to Visit 5 is scheduled later in time than Visit 5, study drug intake must be continued until DEXA has been performed (study drug intake at home and recorded in the ePRO diary).

An overview of the timing of study drug intake and assessments is given in the Time and Events Schedule.

All assessments will be done predose, except for 3 hours postdose blood sampling for PK and PD assessments at Visit 5 and administration of questionnaires, which can be done at any time during the visit.

7.1.1 *Screening Period (Visit 1)*

Subjects will be given a full explanation of the nature of the study and written informed consent (approved by the local ethics committee) will be obtained according to local requirements before any study-related assessment will be carried out.

Screening (Visit 1) will be performed within 4 weeks prior to the first intake of study drug (with a minimum of 7 days to allow for baseline data collection of Hot Flash Score and Hot Flash Frequency). The visit will consist of screening assessments for eligibility purposes (physical examination, thorough medical history, demographic data, vital signs, ECG, blood/urine samples for PD measurements, clinical laboratory tests, serology, urine drug screen, and urine pregnancy test). In addition, subjects will be trained in the use of the ePRO diary to record their hot flashes severity and frequency, to administer HFRDIS, and to register their study drug intake.

Collection of hot flashes severity and frequency will be started on an ad hoc basis, but at a minimum twice-daily (morning and evening), via ePRO diary. Data on hot flash severity and frequency collected during the screening period will serve as baseline for Hot Flash Score and Hot Flash Frequency.

An overview of the assessments to be performed at the screening visit is provided in the Time and Events Schedule.

Adverse events and concomitant therapies will be collected from signing of informed consent onwards (screening) through the follow-up visit.

All results from the screening procedure that are needed to evaluate eligibility, including the clinical laboratory results, must be available prior to randomization (Visit 2). Any abnormal assessment at the screening visit will be assessed according to its clinical relevance, and if found relevant, the subject will not be included in the study.

Unscheduled visits may be planned 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 urine drug screen. Results of this retest must be available prior to randomization. The result of the retest will be considered for subject eligibility. Findings made during unscheduled visits should be reported in the source documents and in the designated sections of the eCRF.

For screening failures, demographic data, the date of screening and signature of the ICF, and the reason(s) for screening failure will be recorded on the eCRF.

7.1.2 *Treatment Period (Visits 2-5; Weeks 1-12)*

Visit 2 should be scheduled in the morning between 7:00 and 10:00 AM, fasted. In- and exclusion criteria will be reassessed. Eligible subjects will be randomized in a 1:1 ratio to one of the 2 treatment arms (ESN364 90 mg b.i.d. or placebo). Predose assessments for efficacy (questionnaires), PK (ESN364 plasma concentrations), PD (hormone concentrations), and safety (clinical laboratory testing, bone density marker concentrations, vital signs, ECG, and physical examination) parameters will be made to serve as baseline (except for Hot Flash Score and Hot Flash Frequency, for which baseline will be data collected during the screening period).

Baseline body composition will be assessed by means of DEXA. For practical reasons, the timing of DEXA may vary from the actual time of the visit, depending on the DEXA availability (DEXA appointment). Baseline DEXA can be performed prior to Visit 2 during the screening period, as well as at Visit 2 but must be performed before randomization.

Treatment will be dispensed (first of 3 bottles each containing 68 capsules) and the subjects will start taking their study drug in a q.d. regimen for 12 weeks. The first intake of study drug (Day 1; Visit 2) will be done at the clinical site (between 7:00 and 10:00 AM), under the supervision of the clinical staff. Also on other days of visits, the morning dose of study drug will be taken at the clinical site, under the supervision of the clinical staff. All other study drug intakes (evening dose and morning dose on all days without visits) will be done at home. When taking their study drug at home, subjects will be asked to record their home study drug intake in the ePRO diary. Also see Section 5.7 for dosing instructions.

After the start of treatment, the subjects will be asked to return to the clinical site every 4 weeks (Weeks 4, 8, 12; Visits 3, 4, 5) to follow-up on compliance, efficacy, PK, PD, and safety parameters. A second and third bottle of study drug will be dispensed at Visits 3 and 4, respectively.

Hot flashes severity and frequency will be assessed on an ad hoc basis, but at a minimum twice-daily (morning and evening), via ePRO at home from screening (Visit 1) to follow-

up (Visit 6). Other efficacy questionnaires will be administered at the clinical site on paper (LSEQ, SDS, and GCS) or in the ePRO diary (HFRDIS) at every visit from Visit 2 onwards. The questionnaires can be administered at any time during the visit.

Blood sampling for PK assessments (ESN364 plasma concentrations) will be taken at every visit during the treatment period, i.e., at Visits 2, 3, and 4 predose and at Visit 5, predose and 3 hours postdose.

Blood sampling for PD assessment (LH, FSH, E2, SHBG, leptin, insulin, C-peptide, and HBA1c) will also be performed at every visit (at Visits 2, 3, and 4 predose and at Visit 5, predose and 3 hours postdose; HBA1c at Visits 2 and 5 only). Additional back-up samples will be collected at every visit and stored for future, exploratory use.

Blood samples for clinical laboratory tests will be taken at every visit and should be taken in a fasted state (overnight fast for at least 10 hours) for unbiased glucose, insulin, and C-peptide determination. Physical examination and vital sign assessments will also be made at every visit. An ECG will be recorded at every visit except Visit 4. Levels of the bone density markers BALP and CTX will be assessed at Visits 2 and 5 only.

DEXA will be repeated at Visit 5, before the last dose of the study drug. For practical reasons, the timing of DEXA may vary from the actual time of the visit, depending on the DEXA availability (DEXA appointment). The Visit 5 DEXA can be performed ±4 days from the actual time of Visit 5. It is however to be performed before the last dose of the study drug; in case the DEXA appointment related to Visit 5 is scheduled later in time than Visit 5, study drug intake must be continued until DEXA has been performed (study drug intake at home and recorded in the ePRO diary).

Adverse events and concomitant therapies will be collected from signing of informed consent onwards (screening) through the follow-up visit.

Compliance towards study drug intake and ePRO completion will be checked at every visit during the treatment period. Compliance towards study drug intake will be assessed by counting returned dosage units in addition to ePRO entries. Any discrepancies between returned dosage units and dosing in the ePRO diary will be discussed with the subject for whom a discrepancy was seen.

An overview of the assessments to be performed during the treatment period is also provided in the Time and Events Schedule.

7.1.3 *Follow-up Period (Visit 6)*

Two to 3 weeks after the last intake of the study drug, subjects will be asked to come to the clinical site for a follow-up visit.

An overview of the assessments to be performed at the follow-up visit is provided in the Time and Events Schedule.

7.1.4 *Unscheduled Visits*

Unscheduled visits can be planned for instance:

- to obtain additional information to ensure safety to the subject. Additional blood and urine samples may be taken at the discretion of the Investigator.

- to collect an additional blood sample for PK assessment if a subject discontinues the study drug due to an AE;
- 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.

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

7.2 PHARMACOKINETIC EVALUATIONS

7.2.1 *Blood Sample Collection and Handling*

Venous blood samples will be collected for analysis of ESN364 in plasma according to the time points defined in the Time and Events Schedule. The exact date and time of blood sampling must be recorded in the source documents and on the eCRF.

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 ca 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 centrifugation is not possible, the samples 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 the central laboratory (BARC, Gent, Belgium), where they will be collected and kept frozen below -20°C until shipment to the bioanalytical laboratory (SGS, Wavre, Belgium).

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

7.2.2 *Bioanalysis*

Samples for determination of plasma ESN364 concentrations will be analyzed by a qualified vendor (SGS, Wavre, Belgium) under the responsibility of the Sponsor, using a validated analytical method (liquid chromatography with tandem mass spectrometric [LC-MS/MS]).

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. No human deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) analysis will be performed.

7.2.3 *Pharmacokinetic Endpoints*

Assessment of plasma ESN364 concentrations will be exploratory.

7.3 PHARMACODYNAMIC EVALUATIONS

7.3.1 *Blood Sample Collection and Handling*

Blood samples for PD assessments will be drawn at the time points indicated in the Time and Events Schedule. The exact date and time of blood sampling must be recorded in the source documents and on the eCRF.

Blood samples for the assessment of LH, FSH, E2, SHBG, insulin, and leptin, C-peptide, and HBA1c (HBA1c at Visits 2 and 5 only) in plasma will be collected and handled as specified in the central laboratory manual.

Additional back-up samples will be collected at every visit and stored for future, exploratory use.

After appropriate labeling, the plasma samples will be stored below -70°C at the clinical site. Thereafter, the frozen plasma samples will be transported/shipped on dry ice to the central laboratory (BARC, Gent, Belgium), where they will be collected and kept frozen below -70°C until analysis.

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

7.3.2 *Bioanalysis*

Plasma samples for PD assessments will be analyzed by BARC (Gent, Belgium), using a validated analytical method.

After completion of the analytical study, the study samples will be kept deep frozen until three months after issuing of the final Clinical Study Report, or six months after issuing of the draft Clinical Study Report, whichever comes first. Then, upon agreement with the Sponsor Representative, the study samples will either be transferred to the sponsor facilities, or kept deep frozen at BARC (Gent, Belgium) for a longer period of time (as to be agreed with the Sponsor) at -70°C ± 6°C, or destroyed at BARC (Gent, Belgium).

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. No human DNA or RNA analysis will be performed.

7.3.3 *Pharmacodynamic Endpoints*

Changes in plasma concentrations of LH, FSH, E2, SHBG, leptin, insulin, C-peptide, and HBA1c from baseline over time will be evaluated.

7.4 EFFICACY EVALUATIONS

7.4.1 *Efficacy Variables*

Efficacy evaluation in this study will be based on hot flash severity and frequency as well as HFRDIS, assessed via ePRO diary, and the following paper-based questionnaires: LSEQ, SDS, and GCS, as described in the following sections. All questionnaires used in the study will be validated and available in the local language of the clinical sites.

7.4.1.1 *HOT FLASH FREQUENCY AND SEVERITY*

Hot flashes severity and frequency will be assessed on an ad hoc basis, but at a minimum twice-daily (morning and evening), via ePRO diary from screening (Visit 1) through the follow-up visit (Visit 6). Data on hot flash severity and frequency collected during the screening period will serve as baseline for Hot Flash Score and Hot Flash Frequency.

Night sweats should be recorded no later than morning upon awakening to start a new day.

The severity of hot flashes is defined clinically as follows (according to the FDA and EMA Guidance for Industry [19,20] and the National Institutes of Health (NIH) Hot Flash Workshop [21]):

- **Mild:**
  Sensation of heat without sweating/dampness. If at night, patient doesn't wake up but later notices damp sheets or clothing.
- **Moderate:**
  Sensation of heat with sweating/dampness, but able to continue activity. If at night, patient 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, patient wakes up hot and is sweating and needs to take action (e.g., removing layers of clothes, open the window, or get out of bed).

The Hot Flash Score (based on severity and frequency) is calculated as follows [22]:

(number of mild hot flashes/day × 1) + (number of moderate hot flashes/day × 2) + (number of severe hot flashes/day × 3)

Higher scores indicate worse symptoms. There is no maximum score since the score is patient-dependent for both number and severity.

7.4.1.2 *HOT FLASH RELATED DAILY INTERFERENCE SCALE (HFRDIS)*

Perceived hot flash interference on daily activities will be evaluated every 4 weeks from the start of study drug intake (Visit 2) through the follow-up visit (Visit 6) using the HFRDIS. The HFRDIS will be completed at the clinical site in the ePRO diary, at any time during the visit.

The HFRDIS is a 10-item scale which measures a woman's perceptions of the degree to which hot flashes interfere with 9 daily life activities (work, social activities, leisure, sleep, mood, concentration, relations with others, sexuality, enjoying life); the 10^{th} item measures interference with overall quality of life [23]. This scale was modeled after items on the Brief Pain Inventory [24] and Brief Fatigue Inventory [25] both of which assess the extent to which pain or fatigue interfere with daily life. Subjects will be asked to rate the extent to which hot flashes have interfered with each item during the previous 4-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) [26].

Scoring of each item of the HFRDIS is illustrated in Attachment 1.

7.4.1.3 *LEEDS SLEEP EVALUATION QUESTIONNAIRE (LSEQ)*

Subjects' sleep quality will be evaluated every 4 weeks from the start of study drug intake (Visit 2) through the follow-up visit (Visit 6) using the LSEQ. The questionnaire will be paper-based, administered at the clinical site at any time during the visit.

The LSEQ is a 10-item self-rated questionnaire which assesses subjects' aspects of sleep and early morning behavior. The questions are grouped into 4 chronological areas: the ease of getting to sleep, the perceived quality of sleep, the ease of awaking from sleep, and the integrity of early morning behavior following wakefulness [27]. The LSEQ is a visual analogue scale which requires respondents to place marks on a group of 10-cm lines representing the changes they have experienced in a variety of symptoms since the beginning of treatment. 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.

A copy of the LSEQ is provided in Attachment 2.

7.4.1.4 *GREENE CLIMACTERIC SCALE (GCS)*

Changes in climacteric symptoms will be evaluated every 4 weeks from the start of study drug intake (Visit 2) through the follow-up visit (Visit 6) using the GCS. The questionnaire will be paper-based, administered at the clinical site at any time during the visit.

The GCS is a 21-item scale which provides a brief but comprehensive and valid measure of climacteric symptomatology [28]. Each item is rated by the subject according to its severity using a four-point rating scale from 0 (none) to 3 (severe). The first 20 items of the scale combine into three main independent symptom measures: 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), by summing up the individual item scores. Item 21 is a probe for sexual dysfunction. The total score can range from 0 to 63. Higher scores indicate worse symptoms.

Scoring of each item of the GCS is illustrated in Attachment 3.

7.4.1.5 *SHEEHAN DISABILITY SCALE (SDS)*

Subjects' functional impairment of life will be evaluated every 4 weeks from the start of study drug intake (Visit 2) through the follow-up visit (Visit 6) using the SDS. The questionnaire will be paper-based, administered at the clinical site at any time during the visit.

The SDS is a composite of 3 self-rated items designed to measure the extent to which 3 major sectors in a patient's life are impaired by panic, anxiety, phobic, or depressive symptoms [29]. The patient rates the extent to which his/her 1- work/school, 2- social life, and 3- family life are impaired by his/her symptoms on a 10-point visual analog scale. The 3 items may be summed into a single dimensional measure of global functional impairment that ranges from 0 (unimpaired) to 30 (highly impaired). It is recommended that clinicians pay attention to patients who score 5 or greater on any of the 3 scales, because such high scores are associated with significant functional impairment [30].

A copy of the SDS is provided in Attachment 4.

7.4.1.6 *BODY COMPOSITION*

Lean body mass, fat mass, muscle mass, bone mass/density will be assessed by DEXA at Visits 2 and 5.

7.4.2 *Efficacy Endpoints*

7.4.2.1 *PRIMARY ENDPOINT*

- Change from baseline to Week 12 in weekly Hot Flash Score

7.4.2.2 *SECONDARY ENDPOINTS*

- Changes from baseline over time in weekly Hot Flash Score and Hot Flash Frequency;
- Change from baseline over time in HFRDIS score;
- Changes from baseline over time in LSEQ, GCS, and SDS score.

7.4.2.3 *EXPLORATORY ENDPOINT*

The change from baseline to Week 12 in body composition (lean mass, fat mass, bone mass/density) will be explored.

7.5 SAFETY EVALUATIONS

Safety evaluation in this study will be based on AEs, clinical laboratory tests, plasma bone density marker concentrations, ECG, vital signs, and physical examination, as described in the following sections.

7.5.1 *Safety Variables*

7.5.1.1 *ADVERSE EVENTS*

Adverse events will be monitored continuously from informed consent until the last study-related activity. At regular intervals during the study, subjects will be asked non-leading questions to determine the occurrence of any AEs. All AEs reported spontaneously during the course of the study will be recorded as well.

For detailed definitions and reporting procedures of AEs, see Section 10.

7.5.1.2 *CLINICAL LABORATORY TESTS*

Blood and urine samples will be collected at the time points indicated in the Time and Events Schedule.

Blood samples will be collected and handled as specified in the central laboratory manual. Biochemistry and hematology testing will be performed on these samples, as well as coagulation and serology (HbsAg, anti-HCV and HIV antibodies) on the sample from screening. All blood samples for clinical laboratory tests should be taken in a fasted state (overnight fast for at least 10 hours; intake of water will be allowed until one hour before study drug intake) for unbiased glucose, insulin, and C-peptide determination.

Standard laboratory tests will be performed by the central laboratory BARC (Gent, Belgium). 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:

- Biochemistry: sodium, potassium, uric acid, creatinine, calcium, AST, ALT, urea, alkaline phosphatase (ALP), gamma glutamylaminotransferase (GGT), albumin, glucose (fasted for at least 10 hours) total bilirubin (and direct bilirubin in case >1.3 x ULN), creatine kinase (CK), and total protein;
- Coagulation: international normalized ratio (INR), activated partial thromboplastin time (aPTT) (at screening only);
- Hematology: white blood cell (WBC) count with differential (neutrophils, lymphocytes, eosinophils, monocytes, basophils), hemoglobin, hematocrit, red blood cell (RBC) count, platelet count.

Urinalysis will be performed on site using a commercial urine dipstick (dipstick will be provided by BARC to the clinical sites). In case the dipstick result is positive, a urine sample has to be shipped to BARC for urine sediment analysis.

A urine pregnancy test will be performed at screening, as well as a urine drug screen for amphetamines, tricyclic antidepressants, cannabinoids, cocaine, tetrahydrocannabinol, or opiates.

The Investigator must review the laboratory report, document this review, and record any change occurring during the study he/she considers to be clinically relevant in the source documents and in the AE 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. No human DNA or RNA analysis will be performed.

7.5.1.3 *BONE DENSITY MARKERS*

Plasma concentrations of the bone density markers BALP and CTX will be assessed at Visits 2 and 6.

Blood samples for the assessment of BALP 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 clinical site. Thereafter, the frozen plasma samples will be transported/shipped on dry ice to the central laboratory (BARC, Gent, Belgium), where they will be collected and kept frozen below -70°C until analysis.

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

7.5.1.4 *VITAL SIGNS*

Vital sign parameters will be assessed after 5 minutes in supine position at the time points indicated in the Time and Events Schedule.

The vital sign parameters that will be assessed are body temperature (oral), supine systolic and diastolic blood pressure (SBP and DBP, respectively) and pulse rate. 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 AE section of the eCRF.

7.5.1.5 *ELECTROCARDIOGRAM*

Twelve-lead ECGs will be recorded after 5 minutes in supine position at the time points indicated in the Time and Events Schedule.

The interpretations of the ECGs will be performed by the Investigator or his/her designee at the clinical site. Any change from baseline ECG occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the source documents and the AE section of the eCRF.

A local ECG reader will be used.

7.5.1.6 *PHYSICAL EXAMINATION*

Physical examination will be performed at the time points indicated in the Time and Events Schedule.

Physical examination includes height, weight, and waist circumference (height at screening only). 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 guideline (see Attachment 5).

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 AE section of the eCRF.

7.5.2 *Safety Endpoints*

Safety endpoints are:

- AE frequency and severity from first dose of study drug through the last visit;
- Changes from baseline over time in hematology and biochemistry concentrations;
- Changes from baseline over time in vital sign and ECG assessments;
- Changes from baseline over time in BALP and CTX levels.

7.6 APPROPRIATENESS OF MEASUREMENTS

The assessments that will be made in this study are standard, and are generally recognized as reliable, accurate, and relevant.

8. STUDY TERMINATION/COMPLETION

8.1 STUDY COMPLETION

A subject will be considered to have completed the study if she has completed the 12-week treatment period.

8.2 REMOVAL OF SUBJECTS FROM THE STUDY OR TREATMENT

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 eCRF.

A subjects **may** be withdrawn from the study or treatment in the event of:

- An AE, including clinically significant new or worsening existing conditions as judged by the Investigator (documented on the AE Form of the eCRF);
- Subject request (subject moved, schedule conflicts, etc);
- A major protocol violation which would confound interpretation of the results;
- Other: this reason should only be used if the reason for withdrawal is not better accounted for by one of the other categories.

A subjects **must** be withdrawn the study or treatment in the event of:

- Withdrawal of informed consent;
- Lost to follow-up;
- For safety reasons, it being 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 6.2);
- Breaking of the randomization code during administration of the study drug by the Investigator or by a member of his/her staff. If the code is broken by the Sponsor, for safety reporting purposes, the subject may remain in the study.
- QT interval corrected according to Fridericia's formula (QTcF) increases to >500 ms, as confirmed by 2 additional ECG recordings obtained within 10 minutes of the initial recording;
- Confirmed decrease in thrombocytes below 75.000, which does not normalize after 7 days;
- ALT or AST >8x ULN;
- ALT or AST >5x ULN for more than 2 weeks;
- ALT or AST >3x ULN and total bilirubin >2x ULN;
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

See Attachment 6 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, the monitor and Sponsor should be informed: in case of withdrawal due to an SAE (for details on AE reporting see Section 10), the Sponsor should be notified within 24 hours; in case of withdrawal for other reasons, the Sponsor should be notified within 2 days from the event.

If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned.

Subjects who are withdrawn from the study prior to completion of the scheduled study procedures for any reason (AE, withdrawal of consent, etc.) should be invited to complete the assessments as much as possible: as long as the subject consents, all relevant assessments of the day on which the subject withdrew from the study should be completed, at least those related to safety, and the subject should come for a safety follow-up visit 2-3 weeks after the last intake of study drug. In case of an AE, the appropriate follow-up will be done.

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

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

8.3 STOPPING RULES OR DISCONTINUATION CRITERIA

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

- If 2 or more subjects on the study drug are withdrawn from the study due to QTcF interval increases >500 ms, as confirmed by 2 additional ECG recordings obtained within 10 minutes of the initial recording;
- If 2 or more subjects on the study drug are withdrawn from the study due to a confirmed increase in ALT or AST >5x ULN for more than 2 weeks;
- If 2 or more subjects on the study drug are withdrawn from the study due to a confirmed decrease in thrombocytes below 75.000, which does not normalize after 7 days.

9. STATISTICAL METHODS

9.1 STATISTICAL ANALYSIS

All statistical analyses will be performed by SGS Life Science Services using SAS® (SAS Institute Inc., Cary, NC, USA; version 9.2 or higher) software for statistical computations.

All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalized before database lock.

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

Descriptive statistics will include number of subjects, (arithmetic) mean, standard deviation (SD), median, minimum and maximum, and a 95% confidence interval (CI). Frequency tabulations will show counts and percentages.

All statistical tests will be interpreted at the 5% significance level (2-tailed).

An interim analysis on the primary endpoint, DEXA parameters and safety data will be performed once the first 16 subjects will have completed 12 weeks of treatment and the follow-up visit (or have discontinued earlier). The final analysis will be performed once all subjects will have completed 12 weeks of treatment and the follow-up visit (or have discontinued earlier).

The following populations will be considered for analysis:

- Safety population: defined as all subjects who are randomized into the study and received at least one dose of the study drug. All safety tables and listings will be based on the safety population.
- Intent-to-treat (ITT) population: defined as all randomized subjects who received at least one dose of the study drug and who have post-baseline efficacy data. Unless specified otherwise, the ITT population will be used for efficacy analysis.
- Per-protocol (PP) population: defined as a subset of the ITT population, excluding those subjects who have major protocol deviations and violations. All deviations and violations will be reviewed prior to database lock and classified as either minor or major.

9.1.1 *Initial Characteristics of the Subject Sample*

Demographic data (age, race, body weight, height and Body Mass Index [BMI]) will be summarized. Demographic data will be presented for the safety population. In case the ITT and/or PP population(s) deviate by more than 5% from the safety population, demographic data will also be presented for the ITT and/or PP population(s). The evaluation consists of descriptive statistics and frequency tabulations, as appropriate.

Subject disposition data such as study termination reasons, visit adherence, and compliance to ePRO completion will be tabulated.

Demographic data and disposition data will be presented overall and per treatment arm.

Findings for medical and surgical history and concomitant diseases will be tabulated per treatment arm.

The use of study drugs will be summarized by treatment arm.

Prior and concomitant medications will be coded using the World Health Organization (WHO)_DRUG dictionary and will be summarized.

Protocol deviations will be tabulated.

9.1.2 *Pharmacokinetic Data*

Individual plasma ESN364 concentration values and actual sampling times relative to study drug intake will be listed. Descriptive statistics will be summarized by assessment time point.

9.1.3 *Pharmacodynamic Data*

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 time point and by treatment arm.

9.1.4 *Efficacy Data*

For a list of efficacy endpoints, see Sections 7.4.2.

Primary Endpoint

Changes from baseline in Hot Flash Score will be analyzed using an Analysis of Covariance (ANCOVA) model with treatment as fixed effect and baseline as covariate. If a significant difference between the treatment arms is present, differences between each active treatment arm and placebo will be tested, correcting for multiplicity.

Rules for defining valid diary days and imputing missing scores will be elaborated in the SAP.

Secondary Endpoints

Hot Flash Score and Hot Flash Frequency at all time points will be evaluated by means of descriptive statistics (actual values and changes from baseline) by treatment arm.

The percentage of subjects with at least 70% reduction from baseline in Hot Flash Score will be compared across the treatment arms, using chi-square or Fisher exact test, as appropriate.

The percentage of subjects with at least 50% reduction in the number of moderate and severe hot flashes will be compared across the treatment arms, using chi-square or Fisher exact test, as appropriate.

Questionnaires will be evaluated by means of descriptive statistics (actual values and changes from baseline) by treatment arm. HFRDIS, GCS, SDS and LSEQ total scores and subscores (if applicable) are to be reported.

Change in lean body mass, fat mass, and bone mass/density will be explored by means of descriptive statistics on the actual values, at each assessment time point and by treatment arm. Changes from baseline to Week 12 will be summarized by treatment arm.

9.1.5 *Safety Data*

Safety parameters will be tabulated and analyzed descriptively.

Adverse Events

The original terms used in the source documents and the designated sections of the eCRFs by the Investigator to identify AEs will be fully described and coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

The reported AEs will be allocated to phases based on their start date. All AEs will be listed. All AEs with onset during the treatment phase (i.e., TEAEs) will be summarized by treatment arm by System Organ Class (SOC) and Preferred Term (PT).

Special attention will be paid to those subjects who died, discontinued the study drug due to an AE, or experienced a severe or serious AE. Summaries, listings, and narratives (also see Section 12.11) may be provided, as appropriate.

Clinical Laboratory Tests

Each continuous biochemistry and hematology laboratory test will be evaluated by means of descriptive statistics on the actual values, at each assessment time point and by treatment arm. Changes from baseline will be summarized by assessment time point and by treatment arm.

Laboratory tests will be categorized according to the normal ranges of the clinical laboratory (below, within, or above normal). A shift table versus baseline will be created. The worst post-baseline value will be summarized as well.

A listing of subjects with any clinical laboratory test result outside the normal range of the clinical laboratory will be provided.

Vital Signs

Body temperature, pulse rate, SBP, and DBP will be evaluated by means of descriptive statistics (actual values and changes from baseline) and shift tables according to the normal ranges (as defined in Attachment 7) at each assessment time point and by treatment arm.

Electrocardiogram

Electrocardiogram variables will be evaluated by means of descriptive statistics (actual values and changes from baseline) and shift tables according to the normal ranges (as defined in Attachment 7) at each assessment time point and by treatment arm.

The ECG variables that will be analyzed are HR, PR interval, QRS interval, QT interval. Values for QTc will be derived. QTcF will be the primary correction parameter [31]. Categorical assessment will be performed on absolute QTcF interval prolongation (>450, >480, >500 ms) and on changes from baseline (increase >30 and >60 ms).

Physical Examination

Abnormal findings in physical examination will be listed.

9.1.6 *Interim Analysis*

In order to get a preliminary view on efficacy and safety results, an interim analysis is planned once the first 16 subjects will have completed 12 weeks of treatment and the follow-up visit (or have discontinued earlier). The interim analysis will be limited to the primary efficacy endpoint, DEXA parameters and safety data. At the time of the interim analysis, the interim analysis subjects' treatment arm will be unblinded. All other subjects will remain blinded.

9.2 DETERMINATION OF SAMPLE SIZE

As this study is only the first proof-of-concept Phase IIa study in this therapeutic area, no formal power calculations have been done. All the statistical analyses involving testing for significance will be exploratory in nature and will serve as a basis for designing and powering a subsequent (dose-ranging) Phase IIb study.

One (and actually the main reason) for the absence of such power calculation is the fact that this study is unprecedented. There are no references available that can give an indication of observed effect size of NK3 antagonism on Hot Flash Score and Hot Flash Frequency.

In a review article by Sloan et al. [22], the authors propose the following general guideline regarding set-up of studies using hot flash diary data (severity and frequency):

- For Phase III placebo-controlled studies, 50 patients per treatment arm seem appropriate to provide sufficient power specifications to detect a clinically meaningful change in hot flash activity.
- For Phase II trials, 25 patients per study seem to provide reasonable estimates of eventual hot flash efficacy to screen potential agents for more definitive testing.

Since the review article typically considers paroxetine and hormones, we would, in our specific case, expect the effect size to be larger and would therefore propose to be sufficiently powered to conduct a successful Phase II proof-of-concept study, while leaving some room for drop-outs and interim analysis.

10. ADVERSE EVENT REPORTING

10.1 DEFINITIONS

Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the study drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal result of diagnostic procedures, including clinical laboratory test abnormalities.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose meets any of the following conditions:

- results in death;
- is life-threatening, i.e., the subject was at risk of death at the time of the event (e.g., ventricular fibrillation and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing inpatient hospitalization:
  Hospitalization refers to an overnight admission into hospital for the purpose of investigating and/or treating the AE. Hospitalization for an elective procedure, or routinely scheduled treatment for a pre-existing condition that has not worsened, is not an SAE.
- results in persistent or significant disability/incapacity, i.e., causing substantial disruption of the subject's ability to conduct normal life;
- is a congenital anomaly/birth defect;
- is medically significant, i.e., may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject's health or may require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that does not result in hospitalization or development of drug dependency or drug abuse.

Unlisted (Unexpected) Adverse Event

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information (see Investigator's Brochure [14]).

10.2 INTENSITY OF ADVERSE EVENTS

The severity of each AE must be rated as follows:

Grade 1:

Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2:

Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily life (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3:

Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated, disabling; limiting self-care activities of daily life (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

10.3 CAUSALITY ASSESSMENT

The causality of each AE must be assessed as follows:

Not related/unlikely related:

The AE is considered not related to the use of the study drug.

Possibly related:

The AE may be due to the use of the study drug. An alternative explanation e.g., concomitant drug(s), concomitant disease(s) is inconclusive. The relationship of the AE with study drug administration in time is reasonable, therefore, the causal relationship cannot be excluded.

Very likely related/related:

The AE is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s), concomitant disease(s). The relationship of the event with study drug administration in time is very suggestive (e.g., it is confirmed by dechallenge and rechallenge).

10.4 ACTION TAKEN REGARDING THE STUDY DRUG

The action taken towards the study drug of each AE must be described as follows:

- Permanently discontinued;
- Stopped temporarily;
- No action taken;
- Unknown;
- Not applicable.

10.5 OUTCOME

The outcome of each AE must be rated as follows:

- Recovered/resolved;
- Recovered with sequelae/resolved with sequelae;
- Recovering/resolving;
- Not recovered/not resolved;
- Fatal;
- Unknown.

10.6 RECORDING OF ADVERSE EVENTS

All (S)AEs occurring during the clinical investigation must be documented in the source documents and on the AE forms of the eCRF.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the (S)AE to the study drug in the source documents and on the eCRF. All measures required for (S)AE management must be recorded in the source documents and reported according to Sponsor's instructions.

All AEs occurring at any time during the study (including the follow-up period) will be followed by the Investigator until satisfactory resolution (e.g., value back to baseline value) or stabilization or until final database lock. If necessary, in order to obtain additional information to ensure safety to the subject, additional blood and urine samples may be taken at the discretion of the Investigator. Certain long-term AEs related to therapy cannot be followed until resolution within the setting of this study. In these cases follow-up will be the responsibility of the treating physician.

10.7 REPORTING OF SERIOUS ADVERSE EVENTS TO SGS LIFE SCIENCE SERVICES MEDICAL AFFAIRS

All SAEs, independent of the circumstances or suspected cause, must be reported on a Serious Adverse Event Form by the Investigator to the SGS Life Science Services Medical Affairs Department within 24 hours of their knowledge of the event, preferably by fax (+32 15 29 93 94) or by e-mail (be.life.saefax-ma@sgs.com).

The SAE form should include a clearly written narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

Follow-up and outcomes should be reported for all subjects who experience an SAE.

It is critical that the information provided on the Serious Adverse Event Form matches the information recorded in the source documents and on the eCRF, for the same event.

Copies of additional laboratory tests, consultation reports, post-mortem reports, hospital case reports, autopsy reports, and other documents should be sent when requested and applicable. Follow-up reports relative to the subject's subsequent course must be

submitted to SGS Life Science Services Medical Affairs Department until the event has subsided, or, in case of permanent impairment, until the condition stabilizes.

10.8 REPORTING OF SERIOUS ADVERSE EVENTS TO COMPETENT AUTHORITIES/ETHICS COMMITTEES

The SGS Life Science Services Medical Affairs Department assumes responsibility for appropriate reporting of AEs to the regulatory authorities. The SGS Life Science Services Medical Affairs Department will also report to the Investigator all SAEs that are unlisted (unexpected) and associated with the use of the study drug. The Investigator (or the SGS Life Science Services Medical Affairs Department where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

Adverse events reporting, including suspected unexpected serious adverse reactions (SUSARs), will be carried out in accordance with applicable local regulations.

After completion of the clinical study (determined as LPLV), any unexpected safety issue that changes the risks benefit analysis and is likely to have an impact on the subjects who have participated in the study, will be reported by the Sponsor or designee to the competent authority(ies) concerned as soon as possible.

11. ETHICAL ASPECTS

11.1 STUDY-SPECIFIC DESIGN CONSIDERATIONS

Potential subjects will be fully informed of the nature of the study and of the risks and requirements of the study before any study-related assessment will be carried out. During the study, subjects will be given any new information that may affect their decision to continue participation. They will be informed that their participation in the study is voluntary and that they may withdraw from the study at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and who provide their consent voluntarily will be enrolled.

11.2 REGULATORY ETHICS COMPLIANCE

11.2.1 *Investigator Responsibilities*

The Investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the study, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB/IEC, or the regulatory authority(ies).

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current International Conference on Harmonization (ICH) guidelines on GCP, and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles originating from the Declaration of Helsinki (1964 and revisions), and that the clinical study data are credible.

11.2.2 *Independent Ethics Committee or Institutional Review Board (IEC/IRB)*

An IRB/IEC should safeguard the rights, safety, and well-being of all study subjects. Special attention should be paid to studies that may include vulnerable subjects.

Before the start of the study, the Investigator (or Sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- final protocol and, if applicable, amendments;
- Sponsor-approved ICF (and any updates or any other written materials to be provided to the subjects);
- Sponsor-approved subject recruiting materials;
- Investigator's Brochure (or equivalent information) and addenda;
- available safety information;

- information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable;
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by the IEC/IRB);
- information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects;
- any other documents that the IEC/IRB may require to fulfill its obligation.

This study will be undertaken only after the IEC/IRB has given full written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials, and any other written information to be provided to the subjects, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study, the Investigator (or Sponsor where required) will send the following documents and updates to the IEC/IRB for its review and approval, where appropriate:

- protocol amendments;
- revision(s) of the ICF and any other written materials to be provided to the subjects;
- new or revised subject recruiting materials approved by the Sponsor, if applicable;
- revisions of compensation for study-related injuries or payment to subjects for participation in the study;
- Investigator's Brochure addenda or new edition(s);
- summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually);
- reports of AEs that are serious, unlisted, and associated with the IMP;
- new information that may adversely affect the safety of the subjects or the conduct of the study;
- deviations from or changes to the protocol to eliminate immediate hazard to the subjects;
- report of death of any subjects under the Investigator's care;
- notification if a new Investigator is responsible for the study at the clinical site;
- Development Safety Update Report, Short-Term Study Specific Safety Summary and Line Listings, where applicable;
- any other requirements of the IEC/IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study subjects. If a deviation from or a change to the protocol was implemented to eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IEC/IRB as soon as possible.

The Investigator (or Sponsor where required) will notify the IEC/IRB about the study completion within 90 days after the end of the study (defined as LPLV).

11.2.3 *Informed Consent*

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and the reviewing IEC/IRB. The informed consent should be in accordance with the principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before enrollment in the study, the Investigator or an authorized member of the clinical staff must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may refuse to participate or withdraw consent to participate at any time, without penalty or loss of benefits to which the subject was entitled. Finally, they will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access, and agrees to allow her study physician to recontact her for the purpose of obtaining consent for additional safety evaluations, if needed.

The language about the study used in the oral and written information, including the ICF, should be non-technical and practical and should be understandable to the subject or the subject’s legal representative. The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained consent, a copy of the ICF must be given to the subject.

If a subject is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the ICF after the oral consent of the subject is obtained, if permitted by local law.

11.2.4 *Privacy of Personal Data*

The collection and processing of personal data from subjects enrolled in the study will be limited to those data that are necessary to investigate the safety, quality, and utility of the IMP used in the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose

responsibilities require access to personal data need to agree to keep the identity of the study subjects confidential.

The informed consent obtained from the subjects includes explicit consent for the processing of personal data and for the Investigator to allow direct access to subjects' original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

12. ADMINISTRATIVE REQUIREMENTS

12.1 PROTOCOL AMENDMENTS

Neither the Investigator nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the Sponsor and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IEC/IRB approval nor when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazard to the subjects, in which case an amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IEC/IRB must be provided to the Sponsor or his designee. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

12.2 SUBJECT IDENTIFICATION, ENROLLMENT, AND SCREENING LOGS

The Investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure subject confidentiality, no copies will be made. All reports and communications related to the study will identify subjects by initials and/or assigned number only.

The Investigator must also complete a subject screening log which reports on all subjects who were seen to determine eligibility for inclusion in the study.

12.3 SOURCE DOCUMENTATION

At a minimum, source documentation must be available for the following to confirm data collected on the eCRF: subject identification, eligibility, and study identification; study discussion and date of informed consent, dates of visits, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow-up of AEs, concomitant medication, study drug receipt/dispensing/return records, study drug administration information, laboratory and ECG printouts, date of study completion, and reason for early discontinuation of study drug or withdrawal from the study, if applicable.

Direct access to source documentation must be allowed for the purpose of verifying that the data recorded on the eCRF are consistent with the original source data.

It is recommended that the author of an entry in the source documents be identifiable.

At a minimum, the type and level of detail of source data available for a study subject should be consistent with that commonly recorded at the clinical site as a basis for standard medical care. Specific details required as source data for the study will be reviewed with the Investigator before the study and will be described in the monitoring guidelines (or other equivalent document). The nature and location of all source documents will be identified in the Source Document Identification Form. Data that will be recorded directly on the eCRF are specified in the Source Document Identification Form.

12.4 CASE REPORT FORM COMPLETION

Electronic Data Capture will be used for this study. The study data will be transcribed by study personnel from the source documents onto an eCRF, and transmitted in a secure manner to the Sponsor. The electronic file will be considered to be the eCRF.

All eCRF entries, corrections, and alterations must be made by the Investigator or other authorized study site personnel.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheet will become part of the subject's source documentation. Such worksheet should not resemble an eCRF. All data related to the study must be recorded on the eCRFs prepared by the Sponsor. Data must be entered on the eCRFs in English. Designated study site personnel must complete the eCRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

The Investigator must verify that all data entries on the eCRFs are accurate and correct.

12.5 MONITORING

The monitoring of the study will be done under the responsibility of the Sponsor by SGS Life Science Services.

The monitor will perform on-site monitoring visits as frequently as necessary. The monitor will record the dates of the visits in a study site visit log that will be kept at the clinical site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered onto the eCRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the Sponsor and study site staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the clinical site, the method of verification must be discussed with the clinical staff.

Direct access to source documentation must be allowed at all times for the purpose of verifying that the data recorded on the eCRF are consistent with the original source data. Findings from this review of eCRFs and source documents will be discussed with the clinical staff. During on-site monitoring visits (notified and agreed upfront with the clinical staff), the relevant clinical staff will be available, the source documentation will be accessible, and a suitable environment for review of study-related documents will be provided. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

12.6 DATA MANAGEMENT

Data management of the study will be performed under the responsibility of the Sponsor by SGS Life Science Services.

After the monitor has reviewed the data entered onto the eCRFs for completeness and accuracy and the data are released by the Investigator, data will be uploaded into the clinical database to perform cleaning activities. Computerized data cleaning checks will be used in addition to manual review, including listings review, to check for discrepancies and to ensure consistency and completeness of the data.

If necessary, queries will be generated in the EDC tool. The Investigator or an authorized member of the clinical staff must adjust the eCRF (if applicable) and complete the query. If corrections to an eCRF are needed after the initial entry into the eCRF, this can be done in 3 different ways: 1- site personnel can make corrections in the EDC tool at their own initiative or as a response to an auto query (generated by the EDC tool), 2- the site manager can generate a query (field data correction form [DCF]) for resolution by the clinical staff, and 3- the clinical data manager can generate a query for resolution by the clinical staff.

The clinical database will be locked as soon as it is considered clean. Only authorized and well-documented updates to the study data are possible after database lock. The locked database is used in the final statistical analysis for study reporting. Measures will be undertaken to protect subject data handed over by the Investigator to the data management department and during inspections against disclosure to unauthorized third parties. Subject confidentiality will be maintained at all times.

An interim lock is planned once the first 16 subjects will have completed 12 weeks of treatment and the follow-up visit (or have discontinued earlier) to allow for an interim analysis on the primary endpoint, DEXA parameters and safety data.

12.7 DATA QUALITY ASSURANCE

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor or designate.

Written instructions will be provided for the collection, preparation, and shipment of plasma samples. Instruction booklets for completion of eCRFs will be provided and reviewed with the study personnel prior to the start of the study.

The Sponsor or his designee will review the eCRFs system for accuracy and completeness during (on-site) monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. After upload of the data into the clinical study database, their accuracy is verified using appropriate validation programs.

In accordance with Good Clinical Research Practice Guidelines and Recommendations, the Sponsor will be entitled to audit the facilities used in the clinical and laboratory parts of the study, as well as to access all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.8 ON-SITE AUDITS

Representatives of the Sponsor’s clinical quality assurance department may visit the clinical site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eCRFs. Subject privacy must, however, be respected. The

Investigator and clinical staff are to be present and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or his designees.

Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.9 STUDY TERMINATION

The Sponsor has the right to terminate the study at any time. In case of an early termination of the study for safety reasons, or temporary halt by the Sponsor, the IEC/IRB should be notified within 15 calendar days and should be provided with a detailed written explanation for the termination/halt.

An end-of-study declaration will be submitted to the regulatory authorities and the IEC/IRB after the complete study has ended. This notification will be submitted within 90 days after the end of the study.

12.10 RECORD RETENTION

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eCRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

12.11 USE OF INFORMATION AND PUBLICATION

All information, including but not limited to, information regarding ESN364 or the Sponsor's operations (e.g., patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the Sponsor to the Investigator and not previously published, and any data generated as a result of this study are considered confidential and remain the sole property of the Sponsor. The Investigator agrees to maintain this information in confidence, to use this information only to accomplish this study, and not to use it for other purposes without the Sponsor's prior written consent.

The Investigator understands that the information generated in this clinical study will be used by the Sponsor in connection with the continued development of the study drug, and thus may be disclosed as required to other clinical Investigators or regulatory agencies. To permit information derived from the clinical studies to be used, the Investigator is obliged to provide the Sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated under the responsibility of the Sponsor and will contain eCRF data from all clinical sites that participated in the study. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating Investigator.

Clinical narratives may be written for the following events:

- All deaths (irrespective of drug relationship);
- All other SAEs during treatment with the study drug;
- All discontinuations of the study drug due to AEs (irrespective of drug relationship);
- At the discretion of the team and after statistical analysis of the data, certain discontinuations not related to AEs or treatment failure, i.e., related to lost to follow-up or withdrawal of consent (irrespective of treatment arm);
- Any events of special interest explicitly requested by the regulatory agencies.

The coordinating Investigator will sign off the final version of the Clinical Study Report. A summary of this final version will be provided to the Investigators, the applicable regulatory authorities, and the IECs/IRB, if required by the applicable regulatory requirements, within one year after the end of the study (LPLV).

The Sponsor shall have the right to publish study data and information without approval from the Investigator. If an Investigator wishes to publish information from the study, a copy of the manuscript must be provided to the Sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the Sponsor in writing, the Investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the Sponsor will review these issues with the Investigator. The Sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicenter study designs and substudy approaches, results may need to be published in a given sequence (e.g., substudies should generally not be published before the primary endpoints of a study have been published).

Similarly, Investigators will recognize the integrity of a multicenter study by not publishing data derived from an individual clinical site until the combined results from the completed study have been published in full, within 12 months after conclusion, abandonment, or termination of the study at all clinical sites, or the Sponsor confirms there will be no multicenter study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

12.12 REGISTRATION OF CLINICAL STUDIES AND DISCLOSURE OF RESULTS

The Sponsor will register the existence of a clinical study and disclose the results as required by law.

12.13 INVESTIGATOR INDEMNITY

The Sponsor holds and will maintain an adequate insurance policy covering damages arising out of Euroscreen-sponsored clinical research studies.

The Sponsor will indemnify the Investigator and hold him/her harmless for claims related to damages arising from the investigation, provided that the study drug was administered under the Sponsor's or deputy's supervision and in strict accordance with accepted medical practice and the study protocol.

The Investigator must notify the Sponsor immediately upon notice of any claims or lawsuits.

12.14 CONFIDENTIALITY

All study documents are provided by the Sponsor to the Investigator and appointed clinical staff in confidence. None of this material may be disclosed to any party not directly involved in the study without the Sponsor's written permission.

The Investigator must assure that subjects' anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the subjects' study numbers, names, addresses, and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from the Sponsor.

13. REFERENCES

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Hot Flash Related Daily Interference Scale (HFRDIS):

Perceived hot flash interference will be evaluated at each of the assessment points using the HFRDIS. This 10-item scale measures a woman's perceptions of the degree to which hot flashes interfere with nine daily life activities; the tenth item measures interference with overall quality of life[1]. This scale was modeled after items on the Brief Pain Inventory[2]2 and Brief Fatigue Inventory[3], which assess the degree pain or fatigue interfere with similar activities. Participants rate the degree to which hot flashes have interfered with each item during the previous week 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)[4].4 As of March 2015, the original publication of this scale had been cited over 130 times by national and international colleagues, referenced in books on menopause, and translated into 12 different languages (Afrikaans, Danish, Farsi, Flemish, French, Italian, Mandarin, Norwegian, Portuguese, Spanish, Swedish, and Taiwanese). The HFRDIS is included as a psychometrically sound outcome measure for use in treatment trials within the National Cancer Institute's Physician Data Query Cancer Information Summaries for Supportive and Palliative Care[5,6].

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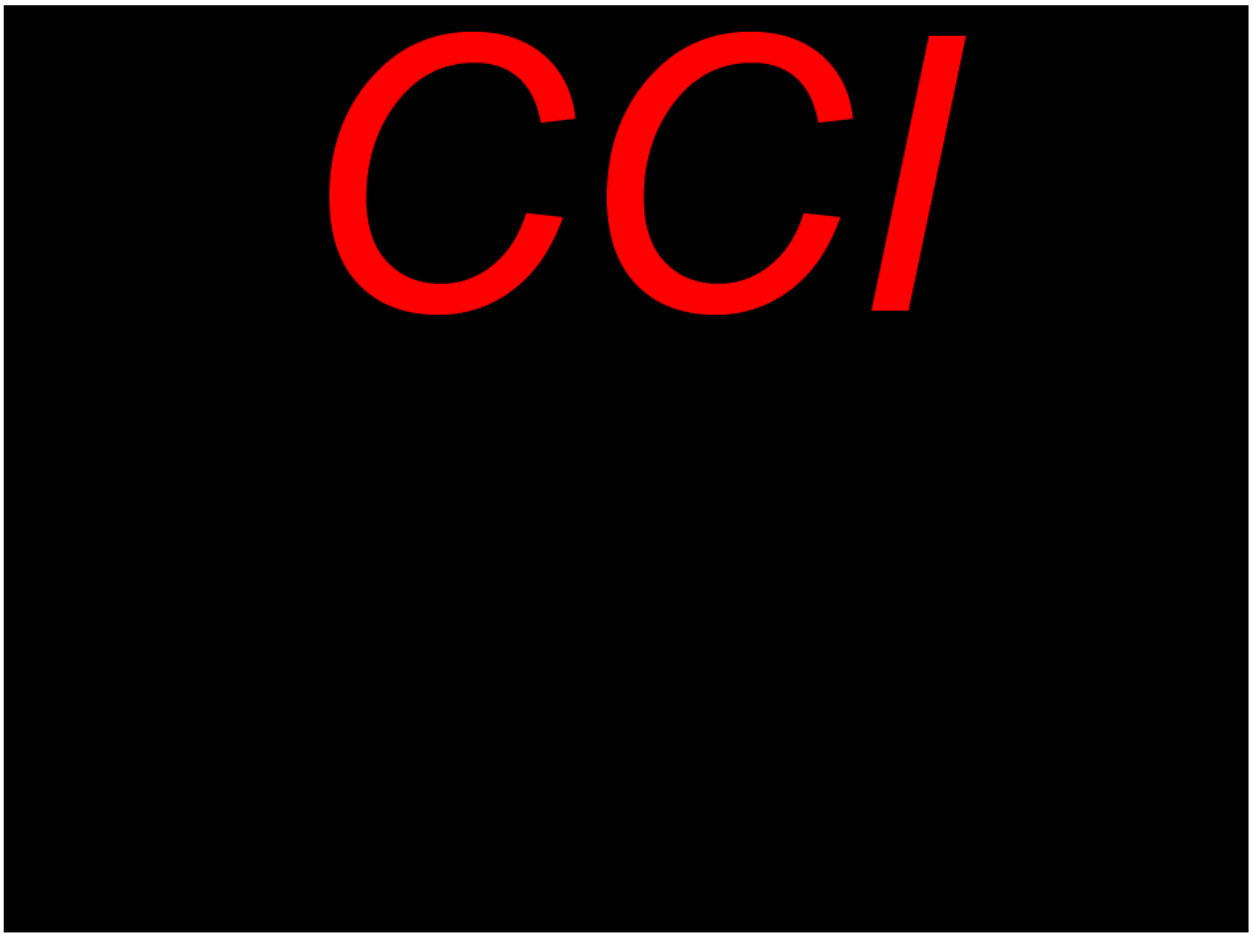
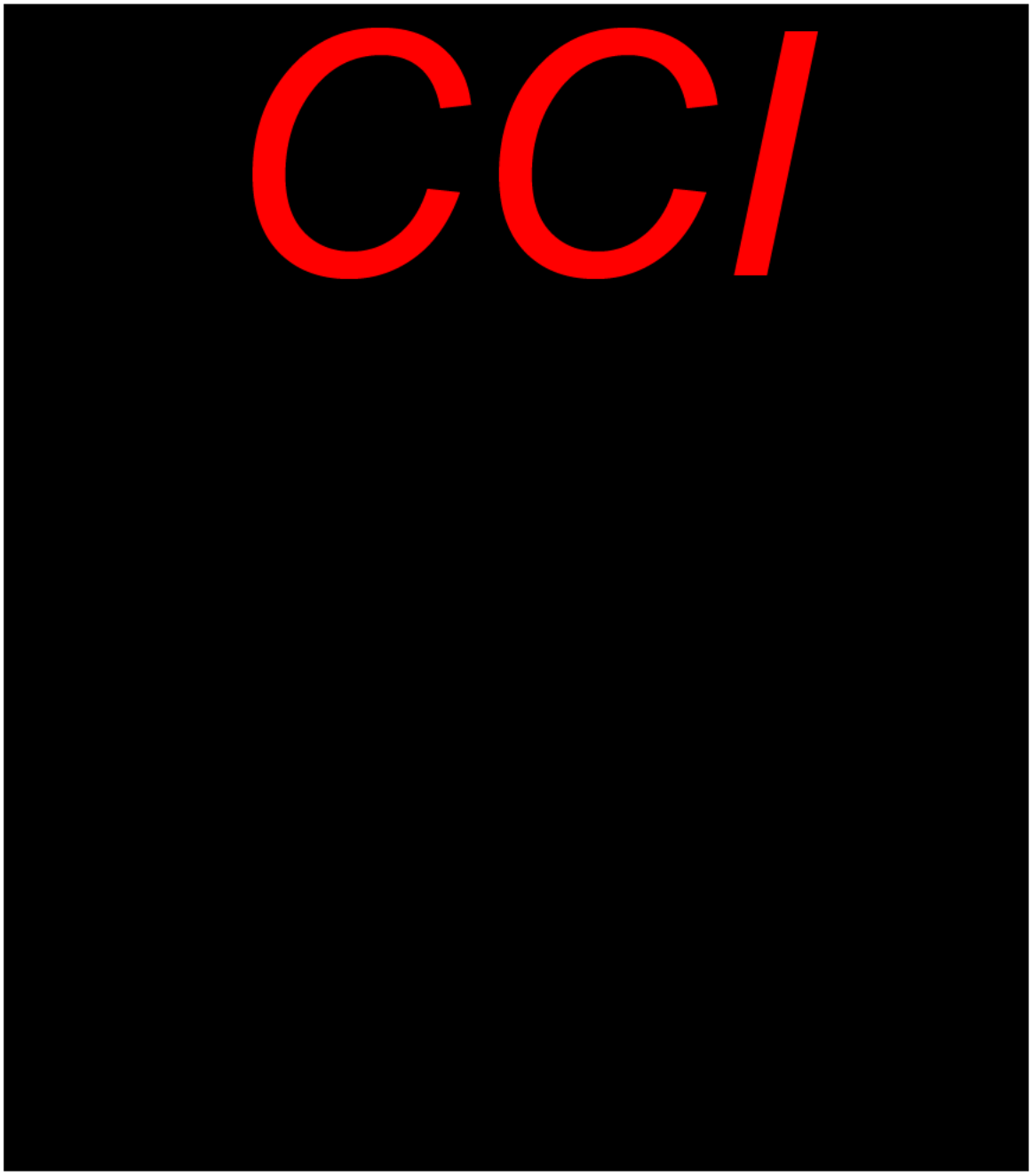
ATTACHMENT 2: LEEDS SLEEP EVALUATION QUESTIONNAIRE



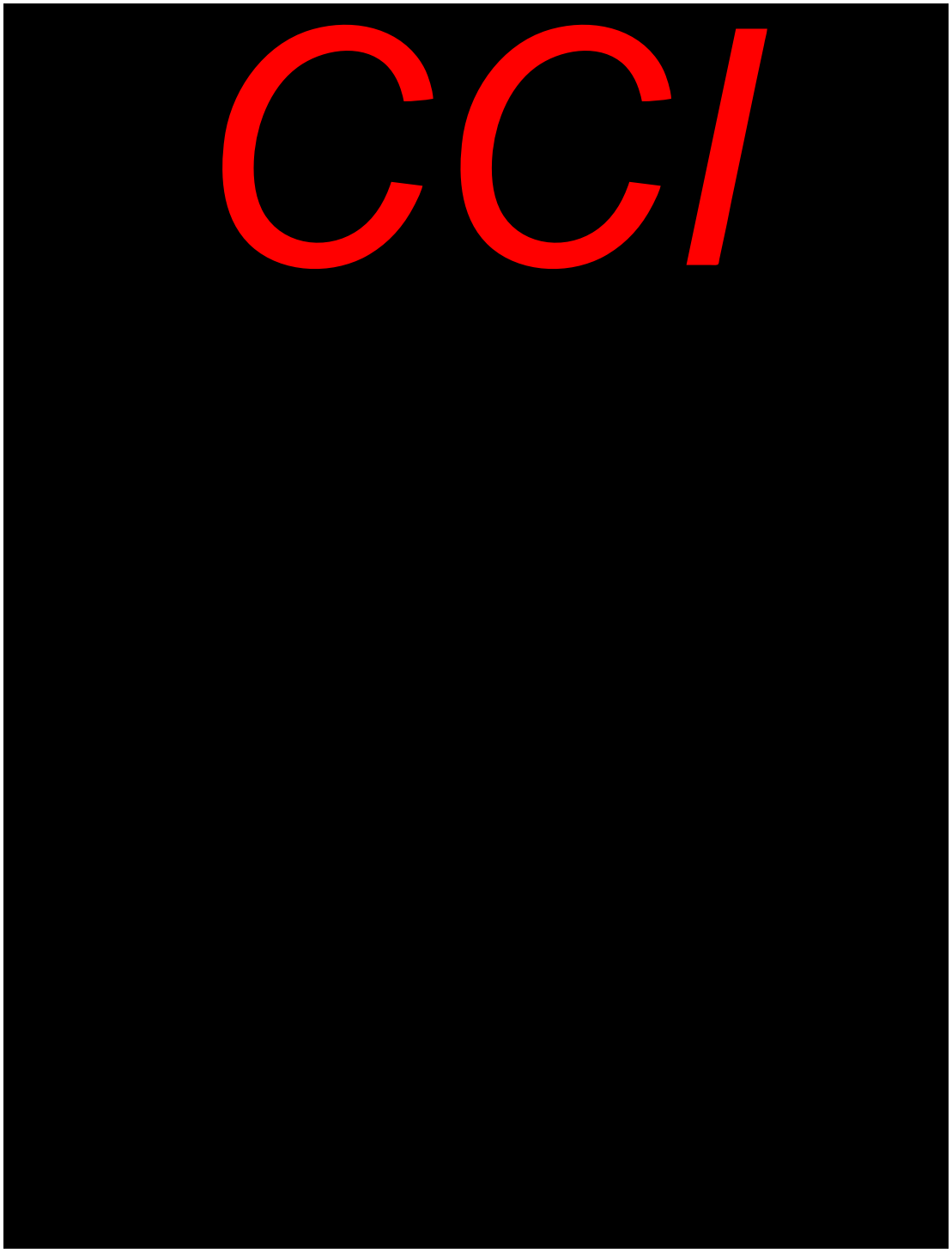
ATTACHMENT 3: GREENE CLIMACTERIC SCALE



GCS - United Kingdom/English
GCS_TS1.0_eng-GBen.doc

The GCS was reproduced with kind permission from Dr Greene.

ATTACHMENT 4: SHEEHAN DISABILITY SCALE

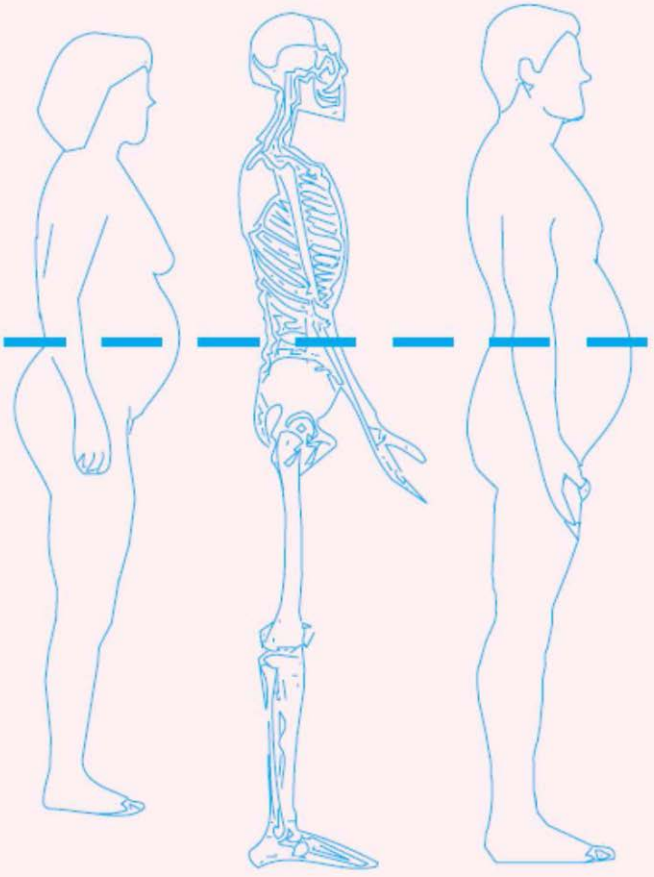


ATTACHMENT 5: WAIST CIRCUMFERENCE MEASUREMENT

The NIH 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

Adapted from the National Heart, Lung, and Blood Institute asa 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

ATTACHMENT 6: EVENTS OF CLINICAL INTEREST GUIDANCE FOR POTENTIAL DRUG-INDUCED LIVER INJURY

EUROSCREEN

Site Guidance Document for Assessment of Potential DILI

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

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EUROSCREEN

Site Guidance Document for Assessment of Potential DILI

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 have an elevated AST or ALT lab value that is greater than or equal to 3X ULN and an elevated TBL lab value that is greater than or equal to 2X ULN and, at the same time, an ALP lab value that is less than 2X ULN, as a result of within-protocol-specific testing or unscheduled testing.

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

Close observation is defined as follows:

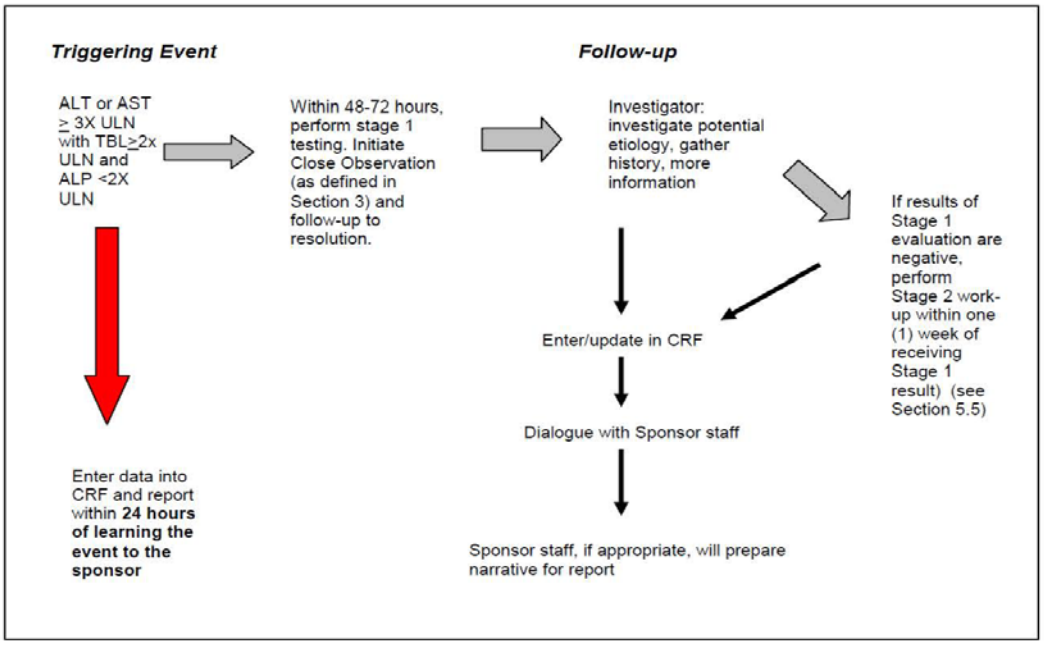
- Repeat liver enzyme and serum bilirubin tests two (2) or three (3) times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or study drug has been discontinued and the subject is asymptomatic.
- Obtain a more detailed history of symptoms and prior or concurrent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



5.0 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.

5.1 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?

5.2 Treatment

Record any concomitant treatments.

5.3 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

5.4 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

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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

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

Note: If clear etiology for the laboratory abnormalities has been confirmed, Stage 1 and 2 testing may not be required. In this case, consultation with the Sponsor is recommended.

Stage 1 work-up should be performed within 48-72 hours:

- ALT
- AST
- Bilirubin: total, direct, indirect
- Alkaline phosphatase (ALP)
- Prothrombin Time (PT)/international normalized ratio (INR)
- Creatine phosphokinase (CPK)
- Manual eosinophil count (if automated count was elevated)
- 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:
- dede
  - ✓ 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)
  - ✓ 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.

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- ✓ 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:

  - ✓ Albumin
  - ✓ Eosinophils (percentage and absolute; obtain manual count if automated count is elevated)
  - ✓ 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)
  - ✓ 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 (if ALP or TBL >ULN)
  - ✓ If AST/ALT ratio is greater than one (1) with suspicions of increased alcohol intake, perform the following:
    - Gamma-glutamyl transferase (GGT)

- Obtain a right upper quadrant ultrasound

Stage 2 work-up tests should be drawn within one (1) week of receiving the Stage 1 work-up results and the results of Stage 1 evaluation are negative.

Note: A specific test may be performed earlier if the investigator determines that the clinical presentation leads to a certain diagnosis.

Stage 2 work-up:

- Perform the following laboratory tests:
  - ✓ Genetic test for Gilbert's disease if there is a suspicious history. Ensure appropriate subject consent is obtained for this test.
  - ✓ Viral hepatitis E (IgG and IgM, obtain appropriate consent prior to testing, if required locally)
  - ✓ 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
  - ✓ Genetic test for hemochromatosis. Ensure appropriate subject consent is obtained for this test
  - ✓ Iron Studies:
    - serum ferritin
    - serum iron
    - total iron binding capacity
- 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.

5.6 Potential diagnosis

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

5.7 Overall clinical impression

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

5.8 Treatment plan

What is the plan for treatment and follow-up?

6.0 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.

7.0 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

8.0 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

ATTACHMENT 7: NORMAL RANGES FOR VITAL SIGNS AND ECG

Normal Ranges for Vital Sign Parameters

Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Pulse rate (bpm)	Oral 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 ECG Parameters

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