Document Type:	Statistical Analysis Plan
Official Title:	Evaluation of the Pharmacokinetics and Safety of NT-814 in Post-Menopausal Women With Vasomotor Symptoms
NCT Number:	NCT02865538
Document Date:	Statistical Analysis Plan 27 February 2017 Statistical Analysis Plan for Pharmacokinetics 05 May 2017

Appendix 16.1.9 Documentation of Statistical Methods

- Statistical Analysis Plan (Final Version 2.0) 27 February 2017
- Statistical Analysis Plan for Pharmacokinetics (Final Version 1.0) 05 May 2017

STATISTICAL ANALYSIS PLAN

EVALUATION OF THE PHARMACOKINETICS AND SAFETY OF NT-814 IN POST-MENOPAUSAL WOMEN WITH VASOMOTOR SYMPTOMS

SAP Version 2.0
FINAL
Date: 27 February 2017

for

Protocol No. RELENT-1

Submitted to:
NeRRe Therapeutics Ltd,
SBC, Incubator Building,
Gunnels Wood Rd.,
Stevenage,
SG1 2FX
UK

Prepared by:
ICON Early Phase Services
820 West Diamond Avenue, Suite 100
Gaithersburg, Maryland 20878 USA
Phone: PPD
Fax: PPD

ICON CLINICAL RESEARCH LLC (ICON) SIGNATURE PAGE (all section except section 8.11.7)

ICON Authors:

PPD
PPD
06 Mar 2017 16:20:023+0000
I approve this document.

PPD

Date

ICON Reviewers:

PPD
PPD
07 Mar 2017 03:39:024+0000
I have reviewed this document and have no comments that require addressing.

PPD
PPD

Date

PPD
PPD
PPD
06 Mar 2017 20:28:017+0000
I approve this document.

Date

PPD
PPD

PPD
PPD
08 Mar 2017 15:08:014+0000
I approve this document.

PPD
PPD

Date

ICON Approvals:

PPD
PPD
06 Mar 2017 16:28:042+0000
I approve this document.

PPD

Date

DocUUID : 9f96b4ca-f974-49e1-a41d-e26a27b92b87

ICARDIAC SIGNATURE PAGE (SECTION 8.11.7)

PPD

4 March, 2017
Date

6 Mar, 2017
Date

DocuSign Envelope ID: 9f9b84ca-f974-49e1-a41d-e26a27b92b87

NERRE THERAPEUTICS SIGNATURE PAGE

NeRRe Therapeutics Approvals:

PPD

03 MAR 2017
Date

3 MAR 2017
Date

DocuSign Envelope ID: 9f96b4ca-f974-49e1-a41d-e26a27b92b87

TABLE OF CONTENTS

1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	**Explanation**
ACTH	adrenocorticotropic hormone
AE	adverse event
AUC	area under the concentration-time curve
AUC_{0-24}	AUC from time 0 to 24 hours
AUC_{0-8}	AUC from time 0 to 8 hours
$AUC_{0-\infty}$	AUC from time 0 to infinity
AUC_{0-t}	AUC from time 0 to the time of the last quantifiable concentration
$AUC_{0-\tau}$	AUC during a dosage interval
BP	blood pressure
CI	confidence interval
C_{max}	maximum observed concentration
CPU	Clinical Pharmacology Unit
CRF	case report form
CYP	cytochrome P450
DMP	Data Management Plan
ECG	electrocardiogram
eCRF	electronic case report form
FSH	follicle stimulating hormone
HR	heart rate
ICH	international conference on harmonization
IMP	investigational medicinal product
LH	luteinizing hormone
MedDRA	medical dictionary for regulatory activities
N.B.	nota bene
No.	number
NTA	night time awakening
PK	pharmacokinetic

PR	atrioventricular nodal delay
PT	preferred term
QD	once daily
QRS	ventricular depolarization
QT	electrical depolarization and repolarization of the ventricles
QTcB	corrected QT by Bazett's formula
QTcF	corrected QT by Fridericia's formula
RR interval	time between heart beats used to calculate heart rate
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SE	standard error
SOC	system organ class
TEAE	treatment-emergent adverse event
T3	triiodothyronine
T4	thyroxine
t½	terminal elimination half-life
T_{max}	the time that C_{max} was observed
TSH	thyroid-stimulating hormone

2 INTRODUCTION

This statistical analysis plan (SAP) is consistent with the statistical methods section of the study protocol (version 4.0 dated 13 December 2016) and includes additional detail of safety and efficacy summaries to be included in the clinical study report. The bioanalyses will be performed by Aptuit Medicines Research Centre and the pharmacokinetic (PK) analyses will be done by Parexel and described in a separate document.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

The primary objective is to evaluate the PK and safety profile of multiple ascending dose levels of NT-814, compared to placebo, by once daily administration of each dose for 14 days.

3.2 Exploratory Objectives

The exploratory objectives are as follows:

- To assess effects of multiple, single daily doses of NT-814, compared to placebo, on hot flash frequency and severity using objective and subjective measures.
- To investigate the relationship between PK exposure and hot flash measures, if data permit.
- To explore the relationship between plasma concentrations and placebo-corrected change from baseline QTcF and QTcB values using concentration effect modeling, depending on the results observed.

3.3 Pharmacokinetic Endpoints

The PK endpoints include:

- peak concentration (C_{max})
- time of occurrence of C_{max} (T_{max})
- area under the curve (AUC) from time zero to the time of the last quantifiable concentration (AUC_{0-t})
- AUC in the dosing interval ($AUC_{0-\tau}$, synonymous with AUC_{0-24})
- AUC from time zero extrapolated to infinity ($AUC_{0-\infty}$)
- apparent terminal phase half-life ($t_{½}$)
- apparent clearance (CL/F)

The PK parameters will be derived on Day 1, Day 7, and Day 14, as appropriate.

3.4 Safety Endpoints

The safety endpoints are as follows:

- Physical examinations

- Analysis for arrhythmias by continuous Holter monitoring from Day -1 to Day 8 and Day 14 (24 hours)
- Abnormalities on 12-lead electrocardiograms (ECGs)
- Change from baseline in ECG variables (heart rate [HR], RR, PR, QRS, QT, QTcF, and QTcB)
- Change from baseline in clinical laboratory assessments, including lipids and coagulation profile
- Change from baseline in hormones including estradiol, testosterone, follicle stimulating hormone (FSH), adrenocorticotropic hormone (ACTH), cortisol, thyroid-stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4)
- Change from baseline in vital signs including oxygen saturation, oral temperature, and postural blood pressure (BP) changes
- Adverse events (AEs) of dehydration (supported by changes in serum urea and creatinine, urinary sediment and weight and vital signs)
- Nature and severity of AEs
- Withdrawals due to an AE

3.5 Exploratory Endpoints

The exploratory endpoints are as follows:

- Change in frequency of hot flashes from predose (Day -1) as assessed by skin conductance
- Change from baseline in frequency of moderate to severe hot flashes, hot flash average severity, and hot flash severity score, as measured by twice daily diary throughout the study
- Change from baseline in frequency of hot flashes as measured by continuous day time diary from Day -1 predose to Day 7 and to Day 14
- Change from baseline in night time awakenings (NTA) secondary to hot flashes as measured by twice daily diary
- Change in luteinizing hormone (LH) AUC_{0-8} hours on Day-1 versus Day 1 and Day 7

- To explore the relationship between plasma concentrations and placebo-corrected, change from baseline QTcF and QTcB values using concentration effect modeling, depending on the results observed.

4 STUDY DESIGN

4.1 General

This is a multicenter, double-blind, randomized, placebo-controlled multiple ascending dose trial in postmenopausal women with vasomotor symptoms.

Subjects will be recruited into a maximum of 5 sequentially-dosed cohorts; Cohort 1 (50 mg NT-814), Cohort 2 (100 mg NT-814), Cohort 3 (150 mg NT-814), and, if needed, 2 further cohorts up to 400mg NT-814. Refer to Figure 1 for the study design schematic.

For each cohort, subjects will enter a 4 week screening period to determine eligibility. Twenty eligible subjects will be randomized per cohort (15 active and 5 placebo).

During screening, each subject will be required to complete twice-daily diaries. Eligible subjects will demonstrate, at the baseline visit, an average 24-hour moderate to severe hot flash frequency of ≥ 7 and ≤ 20, based upon the average daily frequency of all available readings (at least 4 days) in the final week of screening (Week -1). If, at the baseline visit, subjects have an average 24-hour hot flash frequency < 7/ day or > 20/day, they will be discontinued from the study. In addition, subjects who have a change of $\geq 50\%$ in average 24-hour hot flash frequency between the first and second week of screening (i.e., Week -2 and Week -1) will also be discontinued. Subjects will also be provided with the continuous daily diary during screening and instructed to start completing this on the morning of Day -1 after waking, prior to admission to the clinic, and to continue to complete this throughout Day -1.

It may be necessary to extend a patient's screening period beyond 28 days (with the approval of the sponsor) when a subject is held over from one cohort to the next because the original cohort into which they would have been enrolled is complete. If this occurs, the following safety procedures must be repeated such that the most recent evaluation occurs within 28 days of the rescheduled randomization:

- Biochemistry and hematology
- Vital signs
- 12-lead ECG
- Physical examination

If the subject has completed (or partially completed) the screening twice daily Hot Flash Diary this must still be repeated and a new diary started on the new planned Day -15 and completed for the 2 weeks up to Day -2. Eligibility will be assessed on the repeated diary, not the initial one. If the subject had not started completing their screening diary at the time screening was put on hold, they will start this screening diary on the re-scheduled Day -15.

Eligible subjects will be admitted to the Clinical Pharmacology Units (CPUs) as inpatients on the morning of Day -1 and will be fitted with and instructed on use of a sternal skin conductance monitor and Holter monitor, which will be worn continuously until discharge from the clinic on Day 8. Both the Holter monitor and sternal skin conductance monitor will be refitted on return to the CPU on Day 14. Routine safety monitoring will be carried out to form the baseline for comparisons for the remainder of the study.

On Day 1, the subjects will be randomized in a 3:1 ratio to once daily (QD) morning doses of NT-814 or placebo, to be taken for a total of 14 days. Subjects will be monitored as inpatients from Day -1 to Day 8, and will undergo blood draws for intensive PK sampling, safety monitoring, blood hormonal profiling, 24-hour skin conductance measurements, and continuous completion of hot flash diaries.

Subjects will be discharged from the clinic on Day 8 without any monitoring devices, providing there are no safety concerns and will undergo dosing for a further 5 days as an outpatient. Subjects will be instructed to continue recording details of the hot flashes experienced, using the twice daily diary. They will be provided with the continuous daily diary and instructed to start completing this upon waking on the morning of Day 14 prior to admission to the clinic. They will be re-admitted to clinic on the morning of Day 14 for the final dose and undergo the same assessments with the same devices, including Holter and skin conductance, as Day 1. Subjects will be discharged following the 24-hour post Day 14 assessments (Day 15). Subjects will return to the clinic, as outpatients, for a single PK sample on the mornings of Days 16 and 17 (48 hours and 72 hours after the Day 14 dose respectively).

Throughout the study, subject safety will be closely monitored for safety including assessments of AEs, 12-lead ECGs, arterial oxygen saturation using pulse oximetry, vital signs (sitting and standing BP, HR, oral body temperature, and respiration rate), clinical labs and physical examinations.

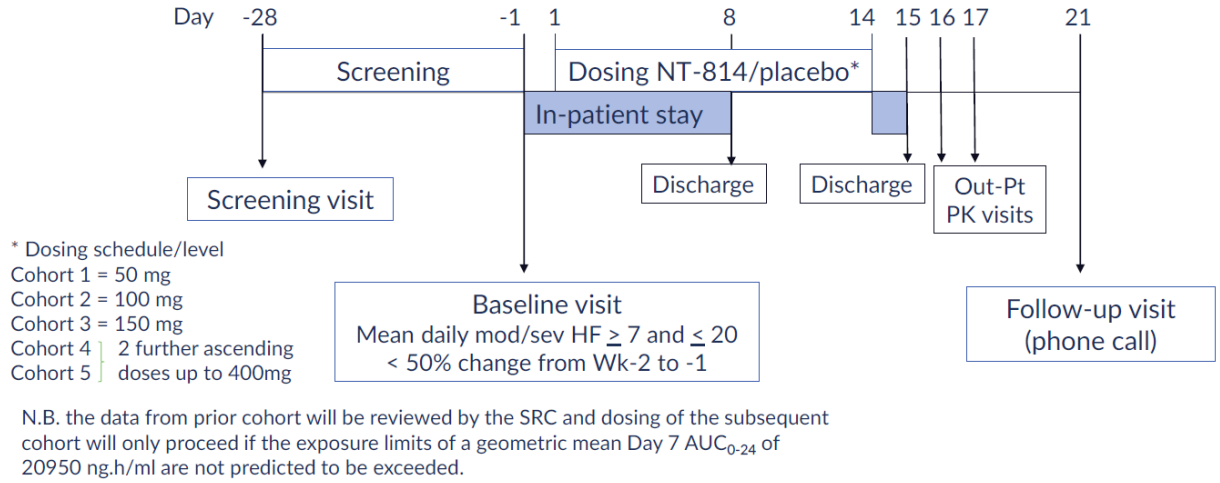
At the end of each cohort, a blinded review of PK and safety data from all subjects will be conducted by the Safety Review Committee to confirm it is safe to proceed to the next dose. This review must comprise complete data up to Day 17 from a minimum of 14 subjects per cohort.

In the case of any safety concern or ambiguous data from any one of the cohorts, either individual subjects or the entire cohort may be unblinded to enable decision making. If this happens, a separate unblinded Safety Review Committee will be used to maintain the integrity of following cohorts if needed.

Subjects may not participate in more than one cohort.

A final follow-up visit will be conducted by phone one week after completion of dosing for each cohort, unless a CPU visit is deemed more appropriate by the Investigator to follow-up on any ongoing AEs.

Figure 1 Study Schematic



4.2 Study Population

The study population will include postmenopausal female subjects, 40 to 65 years of age. Subjects will have an average of ≥ 7 and ≤ 20 moderate to severe hot flashes/day during the last week's twice daily diary during screening and have a change of < 50% in average 24-hour hot flash frequency between the first and second week of screening period twice daily diary (Week -2 and Week -1).

4.3 Evaluations at Screening and Check-in

4.3.1 Screening

Screening procedures (Day -28 to Day -1) will include the following:

- Informed consent
- Medical history and concomitant disease evaluation
- Physical examination
- Evaluation of study entry criteria
- Vital signs (sitting and standing BP, HR, oral body temperature, arterial oxygen saturation, respiration rate)

- Body weight
- 12-lead ECG
- Hot flash twice daily diary: Subjects will complete the twice daily diary for the 2 weeks prior to the baseline visit to ascertain eligibility. Subjects will record NTAs. The diary will be provided to subjects during screening.
- Recording of AEs and serious adverse events (SAEs)
- Clinical laboratory sampling (for chemistry and hematology)

4.3.2 Check-in

Check-in procedures (Day -1) will include the following:

- Medical history and concomitant disease evaluation
- Evaluation of study entry criteria
- Vital signs (sitting and standing BP, HR, oral body temperature, arterial oxygen saturation, respiration rate)
- Body weight
- 12-lead ECG
- Hot flash twice daily diary and day time continuous hot flash paper diary: Subjects will record the occurrence and severity of the flash (scale 1 to 3) in a continuous diary. Subjects will be instructed to start completing it after waking on the morning of Day -1 prior to admission and throughout the day and night.
- Sternal skin conductance monitor fitting
- Holter monitor fitting
- Recording of AEs and SAEs
- Clinical laboratory sampling (for chemistry and hematology) and urinalysis
- Sampling for LH, estradiol, testosterone, FSH, ACTH, cortisol, TSH, T3, and T4

4.4 Randomization and Treatment Assignments

Following screening and confirmation of eligibility, subjects in each cohort will be randomized on Day 1 in a 3:1 ratio to receive either NT-814 or placebo. The investigational medicinal

product (IMP) that each subject will receive will be allocated by an Interactive Web Response Services tool provided by the Clinical Research Organization on behalf of the Sponsor.

4.5 Study Drug Administration

Subjects will be administered study medication in the morning, according to the randomization schedule, at the CPU on Day 1 through Day 8. On Day 8, subjects will be dispensed study medication to be self-administered on Days 9 to 13 (1-2 days' overage should be included). On Day 14, subjects will be administered study medication on the morning of admission to the CPU. Subjects will be reminded to take the study medication at the same time each morning and to return any unused medication when they return to CPU on Day 14. Subjects will be required to fast overnight prior to study drug administration on Day 1 through Day 14.

4.6 Concomitant Medications

All concomitant medications taken during the study will be recorded in the electronic case report form (eCRF) with indication, dose information, and dates of administration. Subjects using the following medications will be excluded from the study:

- Any antidepressants (e.g., selective serotonin reuptake inhibitor, serotonin-norepinephrine reuptake inhibitor) within 4 weeks of screening
- Neurokinin receptor antagonists within 2 weeks of screening
- Women on tamoxifen (or other estrogen modulating drugs) or receiving chemotherapy/radiation therapy within 4 weeks of screening or planned antineoplastic chemotherapy/radiation therapy
- Drugs or herbal medications that could be associated with hot flash-like symptoms including nicotinic acid, calcium channel blockers, clonidine, opioid drugs, and black cohosh within 2 weeks of screening
- Drugs that are highly protein bound (e.g., warfarin and digoxin) within 4 weeks of screening visit, use of non-steroidal anti-inflammatory drugs from the time of signing informed consent
- Use of non-steroidal anti-inflammatory drugs from the time of signing the informed consent
- Use of any of the following hormonal treatments within the washout period stated before screening:
    - Within 1 week for prior vaginal hormonal products (e.g., rings, creams, gels)

  - Within 4 weeks for prior transdermal estrogen alone or estrogen/progestin products
  - Within 12 weeks for prior oral estrogen and/or progestin therapy
  - Within 3 months for prior progestin implants and estrogen alone injectable drug therapy
  - Within 6 months for prior estrogen pellet therapy or progestin injectable drug therapy
- Subjects using the following medications within 2 weeks prior to first dosing (or within 5 times the half-life of that medication, whichever is longer) will be excluded from the study:
  - Inhibitors of cytochrome P450 (CYP) 3A4 (including but not limited to macrolide antibiotics, human immunodeficiency virus protease inhibitors, azole antifungal drugs, cyclosporine, calcium channel inhibitors, and cimetidine)
  - Inducers of CYP3A4 (including but not limited to rifampicin, carbamazepine, efavirenz, bosentan, modafinil, and St. John's wort)
  - Known P-glycoprotein inhibitors (including but not limited to amiodarone, azithromycin, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir, ritonavir, quinidine, ranolazine, and verapamil)
- Known CYP3A4 substrates with a narrow therapeutic range are not allowed from screening until up to 5 times the half-life after the last dose of NT-814 is administered (including but not limited to alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus)

As the inhibition potential of NT-814 with breast cancer resistance protein transporter has not yet been evaluated *in vitro*, an interaction with statins cannot be ruled out. Subjects receiving statins can be allowed into the study, but should be monitored for signs and symptoms of rhabdomyolysis and will be withdrawn from the study if these are observed.

Subjects will be required to inform the study investigator of any regularly taken medications (prescription or over the counter), including hormone replacement therapy, natural and herbal products, vitamins, minerals, or dietary supplements taken within 2 weeks (or less than 5 times the half-life of that medication, whichever is longer) prior to the first dose. Use of such medications should be reviewed with the medical monitor and permitted on a case by case basis.

Subjects must refrain from eating grapefruit, grapefruit juice, Seville oranges, and pomelos within 7 days prior to Day -1 until after their final follow-up visit. Other than this restriction subjects should maintain their usual diet during outpatient periods of the study.

4.7 Compliance

In accordance with regulatory requirements, the Investigator or designated site staff must document the amount of study medication dispensed and/or administered to study subjects, the amount returned by study subjects, and the amount received from and returned to the Sponsor (or representative), when applicable.

4.8 Evaluation of Efficacy

4.8.1 Hot Flash Diaries

Subjects will complete two different diaries during the study to record the incidence and severity of hot flashes and NTAs. A twice daily diary will be completed throughout the study and a Continuous Diary will be completed on days subjects are resident in the clinical pharmacology unit.

4.8.1.1 Twice Daily Diaries

Subjects will document the number of individual hot flashes experienced and rate the severity of each on a scale of mild, moderate or severe (1, 2, or 3). The diaries will be completed twice daily (evening and morning). The diaries will be completed for the 2 weeks prior to the baseline visit to ascertain eligibility, and from screening until the morning of Day 15 (the last entry will be the information from overnight). Subjects will also record the number of NTAs.

4.8.1.2 Continuous Diaries

While in the clinic on Days -1, 1 through 7, and Day 14, subjects will record in the hot flash continuous diary each hot flash and its severity (3 point scale of mild, moderate, or severe), as they occur during the day and night. Subjects will also be required to push a button on the skin conductance monitor when they sense a hot flash, and then record the event in the continuous diary. For Days -1 and 14, subjects will be provided with the continuous diary at the previous visit and instructed to start completing it upon waking in the morning on Days -1 and 14.

4.8.2 Sternal Skin Conductance Monitors

Studies have shown that rather than over-reporting hot flashes when they do not occur, women tend to under report them when asked to complete hot flash diaries, thus leading to inaccuracy. Objective assessment is therefore useful and can be achieved using a sternal skin conductance monitor. Sternal skin conductance monitors will be fitted on Day -1 (24 hours ± 1 hour prior to

the planned study drug administration on Day 1) and will remain in place until after the Day 7, 24 hour assessments are completed (Day 8).

The sternal skin conductance monitors will be refitted around 30 minutes prior to study drug administration on Day 14 and remain in place until after the 24 hour assessments are completed on Day 15.

When fitted with the monitor, subjects will be required to push a button on the skin conductance monitor when they sense a hot flash, and then provide details of the hot flash in the continuous hot flash diary.

4.8.3 Luteinizing Hormone Analysis

Blood samples for analysis of LH will be collected every 60 minutes from time 0 (predose) to 8 hours on Days 1 and 7. The same sampling schedule will also be followed on Day -1, starting at a similar time to that planned for Days 1 and 7.

Luteinizing hormone samples will be collected as close to the nominal time point as possible. The allowable window for LH sample collection relative to dosing will be ± 5 minutes.

4.9 Pharmacokinetic Sampling Schedule

Blood samples for PK analysis will be collected at the following time points on Days 1, 7, and 14: predose, and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, and 24.0 hours postdose. For Cohorts 3 onwards, additional samples will be collected at 48.0 and 72.0 hours after the Day 14 dose.

The predose sample will be collected within 30 minutes prior to dose administration.

Pharmacokinetic samples will be collected as close to the nominal time point as possible. Allowable windows for PK sample collection relative to dosing will be as follows: ± 2 minutes through 2 hours postdose, ± 5 minutes through 8 hours postdose, ± 10 minutes through 24 hours postdose and ± 30 minutes for the 48 and 72 hour postdose samples.

4.10 Evaluation of Treatment Safety

4.10.1 Adverse Events

All AEs will be recorded from the time the informed consent is signed until study exit, and SAEs occurring up to 30 days after the last dose of study medication should be reported.

4.10.1.1 Definitions

An AE is any untoward medical occurrence in a subject or clinical trial subject administered a medicinal product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory

finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All events considered being untoward and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility (i.e., the relationship cannot be ruled out).

An SAE is and AE that at any dose:

- Results in death
- Is life-threatening (i.e., the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalizations are defined as initial or prolonged admissions that include an overnight stay; hospitalization or prolonged hospitalization for technical, practical, or social reasons, in the absence of an AE, is not an SAE.)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is considered to be an important medical event (based on medical and scientific judgment, important medical events that may not be immediately life-threatening, or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed)

An additional blood sample for the determination of NT-814 plasma levels should be taken in case of SAEs with reasonable possibility of relatedness to the study drug. The analysis of such PK samples will be conducted as soon as possible after the occurrence of the SAE.

Pregnancy itself is not considered an AE. However, any pregnancy complication, spontaneous or elective abortion (for medical reasons), still birth, neonatal death, or congenital anomaly will be recorded as an AE or SAE.

An unexpected adverse drug reaction is any adverse reaction, the nature or severity of which is not consistent with applicable product information (eg, Investigator's Brochure for an unapproved IMP). Reports that add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious adverse reaction constitute unexpected events.

A suspected unexpected serious adverse reaction is an SAE suspected to be related to the administered medicinal product; the nature or severity of the event is not consistent with applicable product information.

4.10.1.2 Severity and Relatedness Assessment

Severity will be classified as follows:

- Mild: Awareness of sign or symptom, but easily tolerated
- Moderate: Sign or symptom causes discomfort, but does not interfere with normal activities
- Severe: Sign or symptom of sufficient intensity to interfere with normal activities

The likely relationship of each AE to the medicinal product will be assessed according to the definitions below:

- Unrelated
- Possibly
- Probably
- Definitely

4.10.2 Clinical Laboratory Assessments

Clinical chemistry/ biochemistry and hematology sampling will be performed at screening and on Days -1, 8, 14, and early termination. The following clinical chemistry/ biochemistry parameters will be assessed: sodium, potassium, glucose, urea, creatinine, creatine kinase, albumin, calcium, phosphate, bilirubin (total), alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, bicarbonate, magnesium, chloride, total protein, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides. The following hematology parameters will be assessed: red blood cells, white blood cells, hematocrit, hemoglobin, mean corpuscular volume, platelets, white blood cell differentials, prothrombin time, and activated partial thromboplastin time.

Urinalysis sampling will be performed on Days -1, 8, and 14. Urinalysis will include glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrites, leucocytes, and sedimentation.

Blood samples will be collected to assess estradiol, testosterone, FSH, ACTH, cortisol, TSH, T3, and T4 when the subjects are admitted to the unit on Day -1 and again prior to discharge on Day 15.

4.10.3 Vital Signs

Vital signs will include sitting and standing BP, HR, arterial oxygen saturation, oral body temperature, respiration rate, and weight. Vital sign assessments will be performed on Day -1 at a similar time to the same assessments that are planned predose on Day 1. The assessments will then be performed postdose for all other days in clinic and at a similar time on Day 15.

4.10.4 Twenty-four-hour Holter Monitoring

Subjects will be fitted with Holter monitors upon admission to the CPU on Day -1. These will remain in place until after the Day 7, 24-hour assessments are completed (on Day 8). Subjects will be fitted with Holter monitors again upon admission to the CPU on Day 14. These will remain in place until after the Day 14, 24-hour assessments are completed (on Day 15).

4.10.5 Electrocardiograms

On Days 1 and 14, resting 12-lead ECG data will be collected at the following timepoints: predose and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 12.0 and 24.0 hours (all +/- 15 mins).

The same assessment schedule is also required on Day -1, starting at a time similar to that planned for Days 1 and 14. The Day -1 and Day 1, 24 hour ECGs must be performed prior to the dose of study medication the following morning (on Days 1 and 2, respectively).

Once-daily ECG recordings will be just prior to dosing (approx. 30 mins) on all other days during the inpatient stay (Days 3 to 8).

4.10.6 Physical Examinations

A full physical examination will be performed at screening and Days 1, 8, and 15, and is composed of a review of the following body systems:

- General appearance
- Skin
- Head, eyes, ears, nose and throat
- Respiratory
- Cardiovascular
- Abdomen (including liver and kidneys)
- Musculoskeletal
- Neurological

Any abnormalities that are identified at screening will be documented on the medical history eCRF page. Any changes (including new and worsening findings) between screening and the final study visit should be captured as AEs on the AE eCRF page.

If an improvement/resolution of a physical examination finding documented in the subject's medical history occurs during the study, it should be recorded in the source document. If there is resolution of a physical examination finding previously noted as an AE, then the event resolution and stop date should be recorded on the AE eCRF page and documented in the subject's notes.

4.10.7 Medical History and Concomitant Diseases

The investigator must record all medically and clinical relevant information regardless of the time since the date of diagnosis. The history should include (but is not limited to):

- All current and past medications taken during 3 months before screening
- Relevant history of respiratory, cardiovascular, renal, gastrointestinal, hepatic, endocrine, hematological, neurological, psychiatric, and any other diseases

4.11 Protocol Deviation Reporting

Procedural deviations found by the clinical research associate during monitoring visits and data deviations captured on the eCRF and found through programming and examining the database will be collected. Examples of deviations are documented in the Protocol Deviation Criteria Form.

5 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSIS

The conduct of the study and planned analyses are consistent with the study protocol (Version 4.0, dated 13 December 2016). The exposure response analysis on QTcB will not perform since this correction method is not regarded as appropriate ; per the Q&A E14, question 1.5 (available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Q_As_R3__Step4.pdf).

6 QUALITY CONTROL AND QUALITY ASSURANCE METHODS FOR DATA ANALYSIS

Clinical source data will be entered into eCRFs, which will then be monitored by an independent Monitor designated by the Sponsor. All eCRF data will be processed according to the ICON Study Specific Procedure SSP DM-RELENT-1.01 Data Management Plan (DMP). The DMP describes eCRF data processing, edit checks, data query management, medical dictionary coding, SAE reconciliation, data transfers, and data quality review through database lock or any necessary reopening of the database. After database lock, the data will be retrieved from the database and analysed using SAS® 9.3 or higher.

7 PHARMACOKINETIC ASSESSMENTS

7.1 Pharmacokinetic Assessments

The bioanalyses for plasma NT-814 concentrations will be performed by Aptuit Medicines Research Centre and the PK analyses will be done by Parexel and described in a separate document.

8 STATISTICAL METHODS

8.1 General

The statistical analysis will be conducted following the principles specified in the International Council for Harmonization, formerly the International Conference on Harmonization, Topic E9 Statistical Principles for Clinical Trials (CPMP/ICH/363/96).

All statistical tabulations and analyses will be done using SAS®, Version 9.3 or higher.

Unless otherwise noted, continuous variables will be summarized using number of non-missing observations, arithmetic mean, standard deviation (SD), median, minimum, and maximum; categorical variables will be summarized using the frequency count and the percentage of subjects in each category.

In the data listings, study day relative to first dose of study drug may be presented. Study day relative to first dose will be calculated as: event date – first dose date (+ 1 if event date ≥ first dose date). That is, there is no Day 0.

Baseline will be the latest available measurement prior to the first study drug administration in the study, unless otherwise specified.

For safety summaries, the unscheduled and repeat assessments will not be summarized; however, all results will be included in the data listing.

All subject level data, including those derived, will be presented in the individual subject data listings.

Unless otherwise noted, the analyses will be done by treatment (50 mg NT-814, 100 mg NT-814, 150 mg NT-814, etc… (XX mg NT-814), Total NT-814 (if applicable), placebo). Data for the placebo subjects will be combined across cohorts prior to any summaries.

8.2 Handling of Dropouts or Missing Data

For the calculation of the luteinizing hormone AUC_{0-8} at any visit,

- If more than one time point is missing, then the AUC won't be calculated and will be considered as missing.
- If one of the following time points is missing (T1, T2, T3, T4, T5, T6, T7) then the AUC will be calculated without the missing time point.
- If one of the following time points is missing (T0 or T8), then the AUC won't be calculated and will be considered as missing.

For the derivation of the daily scores in the efficacy section, the daily derivation will not be calculated and will be treated as missing if the results of the corresponding day are incomplete:

Twice daily diary

- The hot flash data are collected twice (day time and night time) and if either period is not complete, then the whole day will be considered incomplete and therefore missing for the corresponding daily variable derived. For each period of the diary to be considered incomplete, there must be no hot flashes of any severity recorded and the box indicating that there were no hot flashes must also not have been completed.

Continuous diary

- For the hot flash data, the diary will be considered incomplete if there are no hot flashes of any severity recorded and the box indicating that there were no hot flashes has also not been completed.

- For NTA not associated with hot flashes, the diary will always be considered complete

Skin Conductance

- If the skin conductance device has not recorded the hot flashes for a continuous period (e.g. due to battery failure) on a given day, then the whole day is considered incomplete and therefore missing for the corresponding daily variable derived.

For the calculation of the weekly scores, at least 4 complete days are needed for the derivations of the weekly values. The results from the complete days will then be averaged to give the weekly score.

Summary statistics will be based primarily on non-missing values. For hypothesis tests, estimates and confidence intervals (CIs) of the exploratory efficacy endpoints, missing values for efficacy endpoints will not be directly imputed. Missing values for endpoints analyzed via likelihood methods (e.g. mixed models) are handled within the analysis itself, under the assumption that the model specification is correct and that the data is missing at random. Sensitivity analyses may be conducted to check the robustness of the analysis results under alternative assumptions with regards to missing data.

8.3 Multicenter Studies

This is a multi-center study.

8.4 Examination of Subgroups

No pre-specified subgroup analysis is planned.

8.5 Analysis Populations

The following data sets will be used for the statistical analysis.

1. All Subjects Set: all subjects who signed the informed consent.
2. All Randomized Set: all subjects who were randomized.
3. Safety Set: all subjects who receive at least 1 dose of double-blind study drug (any dose of NT-814 or matching placebo). Subjects will be analyzed according to treatment received.
4. Full Analysis Set: all randomized subjects who received at least 1 dose of double-blind study drug and for whom the efficacy data are considered sufficient and interpretable. Subjects will be analyzed according to randomized treatment.

Analysis sets will be identified prior to the unblinding of the study data.

8.6 Subject Accountability

Summaries of analysis populations and subject disposition will be presented by treatment based on the All Randomized Set and will contain the following information:

- Number and percent of subjects who were dosed.
- Number and percent of subjects who completed the study (completed the Day 21 follow-up call)
- Number and percent of subjects who discontinued early and reason for early discontinuation
- Number and percent of subjects in the Safety and Full Analysis Set

Subject disposition will be presented in listings.

The screening outcomes will be listed and summarized based on the All Subjects Set.

The randomization allocation and the reason for exclusion from each population will also be listed.

8.7 Protocol Deviation Reporting

Protocol deviations determined to have significant or potential impact on the safety or integrity of the study data will be listed only.

8.8 Subject Demographics and Baseline Characteristics

Demographics (sex, age [in years, at time of signing informed consent], race, and ethnicity) and physical measurements (height, weight, and body mass index) will be listed and summarized by treatment based on the safety set.

8.9 Study Drug Exposure and Compliance

Duration of study drug exposure (in days) will be calculated as: last dose date – first dose date + 1 day, regardless of study drug interruption. Exposure summaries will be based on the safety set.

Dosing administration will be presented in an individual subject data listing.

8.10 Analysis of Efficacy Data

All efficacy analyses will be performed using the Full Analysis Set and are considered as exploratory. Any statistical hypothesis tests and/or CIs will be two-sided, using a type I error rate of 0.1. No adjustments will be made for multiplicity in this early phase study. An unstructured covariance matrix is planned for the analyses that use linear mixed-effects models. However, alternative covariance matrices may be used if the unstructured matrix causes model convergence issues.

8.10.1 Twice Daily Hot Flash Paper Diaries

8.10.1.1 Frequency of Hot Flashes

Frequency by day

Daily (24-hour) frequency of hot flashes for Day Y will be equal to the number of hot flashes recorded on Day Y (day time hot flushes recorded in the evening + night time hot flushes recorded the following morning).

The daily frequency of mild, moderate, severe, moderate/severe, and total hot flashes will be summarized for each day. The mean (±SE) frequency of mild, moderate, severe, moderate/severe (moderate and severe combined), and total hot flashes will be displayed graphically by day (Day -8 onwards).

Average daily frequency by week

The average daily frequency of hot flashes for each of Week -1, Week 1, and Week 2 will be equal to the average of the daily frequency of hot flashes from Day -8 to Day -2 inclusive, from Day 1 to Day 7 inclusive, and from Day 8 to Day 14 inclusive, respectively.

The average daily frequency by week and the change from Week -1 (for Week 1 and Week 2) of mild, moderate, severe, moderate/severe, and total hot flashes will be summarized descriptively by treatment.

Statistical analysis

The analysis will test the treatment difference of change in the average daily frequency of moderate/severe hot flashes from Week -1 to each of Week 1 and Week 2.

The analysis will be based on a linear mixed-effects model with the change from Week -1 as a dependent variable, treatment, week, treatment-by-week interaction as fixed effects, subject as a random effect, and baseline (frequency of moderate/severe hot flashes at Week -1) and baseline-by-week interaction as covariates. Subjects dosed with placebo will be analyzed as a pooled group. An unstructured covariance matrix will be specified for the repeated measures at postdose timepoints for each subject. From this analysis, the least-squares (LS) mean and 2-sided 90% CIs will be calculated for the contrast "NT-814 versus placebo" for each treatment and each postdose week, separately.

Following is the SAS code for reference:

```
proc mixed data=dataset ;
class TRT <50 mg NT-814, 100 mg NT-814, 150 mg NT-814, etc… , placebo> WEEK <1,2> USUBJID;
model CHG = TRT WEEK TRT*WEEK BSL BSL*WEEK / s ddfm=kr;
random USUBJID;
repeated WEEK / type=un subject=USUBJID;
lsmeans TRT*WEEK / cl diff alpha=0.1;
run;
```

The analysis assumes a constant average treatment difference on the original scale. The possibility of a proportional treatment effect will also be explored as it is not certain whether the continuous endpoints will be normally or log-normally distributed, and/or whether an absolute or proportional change in endpoints will be observed. If deemed necessary, data will be log transformed prior to the analysis. The subsequent results (mean differences and corresponding CIs) will be back transformed and hence reported in terms of ratios of geometric means.

8.10.1.2 Hot Flashes Average Severity

Severity by day

Daily (24-hour) hot flashes average severity for Day Y will be equal to the (number of mild hot flashes recorded on Day Y + number of moderate hot flashes recorded on Day Y x 2 + number of severe hot flashes recorded on Day Y x 3) / total number of hot flashes recorded on Day Y. As

the hot flashes are recorded twice daily, the number of hot flashes recorded on Day Y is equal to the sum of the Day Y day time hot flushes recorded in the evening + night time hot flushes recorded following morning.

The daily hot flashes average severity will be summarized and displayed graphically (Day -8 onwards) for each day.

Average daily severity by week

The average daily hot flashes severity for each of Week -1, Week 1, and Week 2 will be equal to the average of the daily hot flashes average severity from Day -8 to Day -2 inclusive, from Day 1 to Day 7 inclusive, and from Day 8 to Day 14 inclusive, respectively. The average hot flashes severity by week and the change from Week -1 (for Week 1 and Week 2) will be summarized by treatment.

The same statistical analyses as described in Section 8.10.1.1 will be done for the average daily severity by week and change from Week -1 in hot flashes average severity.

8.10.1.3 Hot Flashes Severity Score

Severity score by day

The hot flashes severity score for Day Y will be equal to the number of mild hot flashes recorded on Day Y+ number of moderate hot flashes recorded on Day Y x 2 + number of severe hot flashes recorded on Day Y x 3. As the hot flashes are recorded twice daily, the number of hot flashes recorded on Day Y is equal to the sum of the Day Y (day time hot flushes recorded in the evening + night time hot flushes recorded following morning).

The daily hot flashes severity score will be summarized and displayed graphically (Day -8 onwards) for each day.

Average daily severity score by week

The average daily hot flashes severity score for each of Week -1, Week 1, and Week 2 will be equal to the average of the daily hot flashes average severity scores from Day -8 to Day -2 inclusive, from Day 1 to Day 7 inclusive, and from Day 8 to Day 14 inclusive, respectively. The daily average hot flashes severity scores by week and the change from Week -1 (for Week 1 and Week 2) will be summarized descriptively by treatment.

The same statistical analyses as described in Section 8.10.1.1 will be done for the average change in severity score from Week -1 hot flashes severity score.

8.10.1.4 Night Time Awakenings

8.10.1.4.1 Night Time Awakenings Secondary to Hot Flashes

The number of NTA secondary to hot flashes will be the sum of the number of moderate and severe night time hot flashes recorded the following morning.

The NTA secondary to hot flashes will be summarized for each day and displayed graphically by day.

The average daily NTA secondary to hot flashes by week for each of Week -1, Week 1, and Week 2 will be equal to the average of the number of NTA secondary to hot flashes per day from Day -8 to Day -2 inclusive, from Day 1 to Day 7 inclusive, and from Day 8 to Day 14 inclusive, respectively. The weekly average number of NTA secondary to hot flashes per day and change from Week -1 will be summarized by treatment.

The same statistical analyses as described in Section 8.10.1.1 will be done for the weekly average change from Week -1 NTA secondary to hot flashes.

8.10.1.4.2 Night Time Awakenings – Total

The total number of night time awakenings for Day Y will be equal to the number of NTA recorded the following morning.

The total NTA will be summarized for each day and displayed graphically by day.

The weekly average NTA per day for each of Week -1, Week 1, and Week 2 will be equal to the average of the number of NTA per day from Day -8 to Day -2 inclusive, from Day 1 to Day 7 inclusive, and from Day 8 to Day 14 inclusive, respectively. The weekly average number of NTA per day and change from Week -1 will be summarized by treatment.

The same statistical analyses as described in section 8.10.1.1 will be done for the weekly average change from Week -1 NTA.

8.10.2 Continuous Hot Flash Diary

8.10.2.1 Frequency of Hot Flashes

<u>Frequency by day</u>

Daily (24-hour) frequency of hot flashes for Day Y for the continuous hot flash diary will be equal to the number of hot flashes recorded on Day Y (day period + night period)

The daily frequency of mild, moderate, severe, moderate/severe, and total hot flashes as measured by continuous day-time diary will be summarized for each day. The mean (+-SE) in frequency of mild, moderate, severe, moderate/severe, and total hot flashes will be displayed graphically by day. The change from Day -1 will also be summarized.

Average daily frequency by week

The average daily frequency of hot flashes for Week 1 will be equal to the average of the daily frequency of hot flashes from Day 1 to Day 7, inclusive.

The Week 1 average frequency of mild, moderate, severe, moderate to severe, and total hot flashes will be summarized by treatment.

Statistical analysis

The analysis will test the treatment difference of daily frequency of moderate/severe hot flashes from Day -1 to Day 7 and Day 14.

The analysis will be based on a linear mixed-effects model with the change from Day -1 as a dependent variable, treatment, day and treatment-by-day interaction as fixed effects, subject as a random effect, and the number of moderate/severe hot flashes at Day -1 and the interaction with day as covariates. Subjects dosed with placebo will be analyzed as a pooled group. An unstructured covariance matrix will be specified for the repeated measures at postdose timepoints for each subject. From this analysis, the LS mean and 2-sided 90 % CIs will be calculated for the contrast "NT-814 versus placebo" at each treatment and each postdose day, separately.

Following is the SAS code for reference:

```
proc mixed data=dataset ;
class TRT <50 mg NT-814, 100 mg NT-814, 150 mg NT-814, etc…, placebo> DAY <7,14> USUBJID;
model CHG = TRT DAY TRT*DAY BSL BSL*DAY / s ddfm=kr;
random USUBJID;
repeated DAY / type=un subject=USUBJID;
lsmeans TRT*DAY / cl diff alpha=0.1;
run;
```

8.10.2.2 Night Time Awakenings

8.10.2.2.1 Night Time Awakenings Secondary to Hot Flashes

Night time awakenings secondary to hot flashes for Day Y = the sum of the number of moderate and severe night time hot flashes recorded on the continuous diary (as these are defined as resulting in a night time awakening) .

Frequency by day

The NTA secondary to hot flashes will be summarized for each day and displayed graphically by day. The change from Day -1 will also be summarized.

Average daily frequency by week

The average daily NTA secondary to hot flashes for Week 1 will be equal to the average of the NTA from Day 1 to Day 7. The average daily NTA secondary to hot flashes for Week 1 will be summarized by treatment.

The same statistical analyses for the daily scores as described in section 8.10.2.1 will be done based on the change from Day -1 NTA.

8.10.2.2.2 Night Time Awakenings - Total

The total NTA for Day Y = number of awakenings recorded on the continuous diary that are recorded as an awakening that is not accompanied by a hot flash + the number of night time moderate and severe hot flashes recorded.

The total NTA will be summarized for each day and displayed graphically by day. The change from Day -1 will also be summarized.

8.10.3 Sternal Skin Conductance Monitors

Frequency by day

The Day -1 frequency of hot flashes will be the number of hot flashes detected by the device from 24 hours before dosing on Day 1 to the dosing on Day 1. If there is >24 hours of recorded data, then only the 24-hour prior to the time of dosing on Day 1 will be used. If there is <24 hours of data recorded, then all the recorded data will be used and the actual recording duration will be used to derive a 24 hour value.

Postbaseline values will be based on 24-hour periods from the dose time on Day 1. If the recording time on Day 7 (or an earlier day in the event of early withdrawal) is <24 hours, then all the recorded data will be used and the actual recording duration will be used to derive a 24-hour value.

The number of algorithm-detected hot flashes (Bahr et al *Physiol Meas*. 2014 February ; 35(2): 95–110. doi:10.1088/0967-3334/35/2/95; algorithm commercial property of SimplexScientific LLC) will be summarized and displayed graphically for each day by treatment. The change from Day -1 will also be summarized.

Average daily frequency by week

The average daily frequency of hot flashes detected by the device for Week 1 will be equal to the average of the daily frequency of hot flashes detected by the device from Day 1 to Day 7 inclusive.

The average daily frequency for Week 1 will be summarized by treatment.

Statistical analysis

The same statistical analyses as described in Section 8.10.2.1 will be done for the change from Day -1 in the frequency of hot flashes detected by the device.

8.10.4 Luteinizing Hormone

The observed values on Day -1, Day 1, and Day 7, and change from Day -1 (for Day 1 and Day 7) data for continuous LH AUC_{0-8} values will be summarized by treatment at each visit. The AUC_{0-8} will be derived using the trapezoidal method and based on the actual times.

An analysis will be based on a linear mixed-effects model with the change from Day -1 as a dependent variable, treatment, day and treatment-by-day interaction as fixed effects, subject as a random effect and the AUC_{0-8} value at Day -1 and the interaction with day as covariates. Subjects dosed with placebo will be analyzed as a pooled group. An unstructured covariance matrix will be specified for the repeated measures at postdose timepoints for each subject. From this analysis, the LS mean and 2-sided 90 % CIs will be calculated for the contrast "NT-814 versus placebo" at each treatment and each postdose day, separately.

Following is the SAS code for reference:

```
proc mixed data=dataset ;
class TRT <50 mg NT-814, 100 mg NT-814, 150 mg NT-814, XX mg NT-814(2 further ascending doses up to 400mg), placebo>
DAY <1,7 > USUBJID;
model CHG = TRT DAY TRT*DAY BSL BSL*DAY / s ddfm=kr;
random USUBJID;
repeated DAY / type=un subject=USUBJID;
lsmeans TRT*DAY / cl diff alpha=0.1;
run;
```

8.10.5 Relationship Between PK Exposure and Responses

The relationship between PK exposure and efficacy and pharmacodynamic responses will be investigated depending on what is seen in the response data. These analyses will be described in a separate document.

8.11 Analysis of Safety Data

8.11.1 General

All analyses will be performed using the safety set.

8.11.2 Adverse Events

All AEs will be coded to system organ class (SOC) and preferred term (PT) using MedDRA, Version 19.0, and presented by subject in data listings.

A treatment-emergent adverse event (TEAE) is defined as an AE that was not present prior to treatment with investigational product, but appeared following treatment or was present at treatment initiation but worsened during treatment. An AE that was present at treatment initiation but resolved and then reappeared while the subject was on treatment is a TEAE (regardless of the intensity of the AE when the treatment was initiated). Programmatically, an AE will be classified as a TEAE if the start date and time occurs on or after the start date and time of first investigational product dosing. Any change in severity will be recorded as a new AE in the eCRF. An AE with an incomplete start or end date will be considered a TEAE unless it is clear from the incomplete start and end date that the AE started before start of dosing.

The overall incidence of TEAEs (number and percentage of subjects with one or more TEAEs) as well as the number of events will be summarized by treatment received. Separate summaries by treatment received will also be presented showing TEAE severity, causally related TEAE and SAEs, TEAEs leading to study or treatment discontinuation, all SAEs, life-threatening SAEs, SAEs resulting in death and TEAEs of dehydration (changes in serum urea and creatinine, urinary sediment and weight and vital signs that the PI classifies to be an AE).

The TEAEs will be summarized and tabulated at both the subject (number [%] of subjects) and event (number of events) level:

- By treatment, SOC, and PT
- By treatment, SOC, PT, and reported severity
- By treatment, SOC, PT, and relationship to study drug

For the incidence at the subject level by SOC and PT, if a subject experiences more than one event within the same SOC and PT, the patient will only be included once in the incidence.

For the incidence at the subject level by SOC, PT, and severity, if a subject experiences more than one event within the same SOC, PT, the patient will only be included once in the incidence and will be assigned the worst severity.

For the incidence at the subject level by SOC, PT, and relationship to study drug; if a subject experiences more than one event within the same SOC, PT, to study drug, the patient will only be included once in the incidence and will be assigned the most conservative relationship.

Any SAEs, SAEs with outcome of death, AEs resulting in discontinuation of study or treatment, and TEAEs of dehydration will also be listed separately.

8.11.3 Clinical Laboratory Assessments

Observed and change from baseline of continuous clinical laboratory values (chemistry, hematology, lipids, coagulation, and hormones including estradiol, testosterone, FSH, ACTH, cortisol, TSH, T3 and T4) for each parameter will be summarized by treatment at each time point. The number and percentage of subjects with shift changes from baseline based on the laboratory normal ranges will be tabulated.

Laboratory data will be listed by subject at each time point. Clinical laboratory values that are out of normal ranges will be presented in a separate listing.

Urinalysis laboratory tests will be presented in data listings only.

8.11.4 Vital Signs

Vital signs data will be listed by subject at each time point. Observed and change from baseline vital signs values (pulse rate, respiration rate, arterial oxygen saturation, systolic and diastolic BP, oral temperature, and weight) will be summarized by treatment at each time point period.

8.11.5 Safety Electrocardiograms

Observed values and change from baseline of 12-lead ECG parameters (HR, RR, PR, QRS, QT, QTcB and QTcF intervals) will be summarized descriptively by treatment at each scheduled time point collected. Individual ECG data will be listed. The baseline for the assessments done on Day 1 and 14 for each scheduled post-treatment timepoint will be the time-matched assessment on Day -1. The baseline for the assessments done on Day 3 to 8 will be the last assessment done before dosing on Day 1.

12-lead ECGs that the investigator flags as being abnormal (both clinically significant and not clinically significant) will be summarized separately.

In addition, the number and percentage of subjects by maximum baseline and post baseline value of QTcB and QTcF intervals, categorized as ≤450 msec, >450 msec and ≤480 msec, >480 msec and ≤500 msec, and >500 msec, as well as the daily maximum category, will be summarized. In addition, the same summaries (overall maximum and daily maximum) for change from baseline value of QTcB and QTcF intervals, categorized as ≤0 msec, >0 msec and ≤30 msec, >30 msec and ≤60 msec, and >60 msec will be tabulated based on 12-lead ECG measurements.

8.11.6 24-hour Holter Monitoring

The 24-hour Holter Monitoring data will be listed. The arrhythmias analysis by continuous Holter monitoring will be summarized. All findings will be summarized on the basis of incidence rates for the study overall (by baseline and post-baseline) and by day. A subject will be counted only once for a particular morphological abnormality if the subject experiences more than one incidence of that event.

8.11.7 Relationship Between Plasma Concentrations and Placebo-Corrected, Change from Baseline QTcF

The analyses described in this section may be conducted depending on the results observed. They will be based on the QT/QTc and PK/QTc Analysis Set.

QT/QTc Analysis Set will include all subjects in the Safety Set with measurements at baseline as well as on-treatment with at least one postdose timepoint with a valid ΔQTcF value. The QT/QTc Analysis Set will be used for the analysis of ECG parameters.

PK/QTc Analysis Set will include all subjects in both the QT/QTc and PK analysis sets with at least one pair of postdose PK and QTcF data from the same timepoint. The PK/QTc Analysis Set will be used for the exposure-response analysis.

Baseline: For all ECG parameters, baseline is defined as the measured QTc intervals from the time-matched predose timepoint on Day 1.

Exposure-Response Analysis

The relationship between NT-814 plasma concentration and ΔQTcF (change-from-baseline QTcF) will be quantified using a linear mixed-effects model with ΔQTcF as the dependent variable, NT-814 plasma concentration as a continuous covariate (zero for placebo), treatment (active or placebo) and time since drug administration as categorical factors, and a random intercept per subject. The degrees of freedom for the model estimates will be determined by the Kenward-Rogers method. From the model, the slope (i.e., the regression parameter for the concentration) and the treatment effect (defined as the difference between active and placebo) will be estimated together with 2-sided 90% CI. The estimates for the time effect will be reported with degrees of freedom and standard error (SE).

The geometric mean of the individual C_{max} values for subjects in each of the NT-814 dose groups will be determined. The predicted effect and its 2-sided 90% CI for ΔΔQTcF (placebo-corrected change-from-baseline QTcF) (ie, the product with the slope estimate + treatment effect) at the geometric mean C_{max} will be obtained for each NT-814 dose, separately. If the upper bound of the 90% confidence interval of the predicted effect at this plasma level is below 10 ms, it will be

concluded that NT-814 does not cause clinically relevant QTc prolongation. These analyses will be performed using the PK/QTc population.

The plot of the observed median-quantile NT-814 concentrations and associated mean ΔΔQTcF (90% CI) adjusted for diurnal effects together with the regression line presenting the predicted ΔΔQTcF (90% CI) (as described in the publication by Tornoe et al[1]) will be used to evaluate the adequacy of the model fit to the assumption of linearity and the impact on quantifying the exposure response relationship. Additional exploratory analyses (via graphical displays and/or model fitting) will include accounting for a delayed effect (hysteresis) and the justification for the choice of pharmacodynamic model (linear versus nonlinear) as follows.

Investigation of hysteresis

If a QTc effect (ΔΔQTcF) exceeding 10 msec cannot be excluded in the by-timepoint analysis, hysteresis, i.e., difference in peak QT response and C_{max}, will be explored through visual inspection of overlaid NT-814 concentration and ΔΔQTc time curves and through so called hysteresis loops for each dose of NT-814.

Appropriateness of a linear model

To assess the appropriateness of a linear model, normal QQ-plots for the residuals and plots of weighted residuals versus concentration and vs. fitted values will be produced. The scatter plot of residuals versus concentration by Loess (i.e., locally weighted scatterplot smoothing as described in the publication by Cleveland[2]) fitting will be also produced with an optimal smoothing parameter selected by the Akaike information criterion with a correction (AICC[3]). In addition, a model with a quadratic term in concentration will be fitted and the quadratic term will be tested on the two-sided 5 % level. If there is an indication that a linear model is inappropriate, additional models will be fitted, in particular:

- An E-max model;
- A log-linear model where the plasma concentration (C) is replaced by $\log(C/C_0)$, C_0 is the limit of quantification of the assay used to determine C and all values below C_0 are replaced by C_0 (i.e., $\log[C_0/C_0] = 0$).

The Exposure-Response analysis will then be repeated for the model found to best accommodate the nonlinearity detected.

By-Timepoint Analysis:

[1] Tornoe C et al. J Clin Pharmacol 2011; 7: 1035-42
[2] Cleveland WS. J Amer Statist Assoc 1979; 74: 829-36
[3] Hurvich, CM, Simonoff, JS, and Tsai, CL 1998; J. Roy. Statist. Soc. Ser. B, 60: 271–93.

The analysis for QTcF will be based on a linear mixed-effects model with change-from-baseline QTcF (ΔQTcF) as the dependent variable, time (categorical), treatment (each NT-814 dose group and all placebo group), and time-by-treatment interaction as fixed effects, and baseline QTcF as a covariate. Baseline will be the derived QTc intervals from the ECG timepoint recorded predose on Day 1. Subjects dosed with placebo will be analyzed as a pooled group. An unstructured covariance matrix will be specified for the repeated measures at postdose timepoints for each subject. If the model with unstructured covariance matrix fails to converge, other covariance matrix such as autoregressive and compound symmetry will be considered. From this analysis, the LS mean and 2-sided 90 % CIs will be calculated for the contrast "NT-814 versus placebo" at each treatment and each postdose timepoint, separately. These analyses will be performed using the QT/QTc population.

For HR, PR, QTcB, QT, RR, and QRS intervals, the analysis will be based on the change-from-baseline post-dosing (ΔHR, ΔPR, ΔQTcB, ΔQT, ΔRR, and ΔQRS). The same model will be used as described for QTcF. The LS mean, SE and 2-sided 90% CI from the statistical modeling for both change-from-baseline and placebo-corrected change-from-baseline values will be listed in the tables and graphically displayed.

Categorical Analyses

The analyses results for categorical outliers, T-wave morphology and U-wave presence will be summarized in frequency tables with counts percentages for both number of subjects and number of timepoints. For categorical outliers, the number (percentage) of subjects as well as timepoints who had increases in absolute QTc (QTcF and QTcB) values >450, 480, and 500 msec, and changes from predose baseline of >30 or >60 msec; increase in PR from predose baseline >25% to a PR >200 msec; increase in QRS from predose baseline >25% to a QRS >120 msec; decrease in HR from predose baseline >25% to a HR <50 bpm; and increase in HR from predose baseline >25% to a HR >100 bpm will be determined. For T-wave morphology and U-wave presence, the analyses will be focused on change from baseline (i.e., treatment-emergent changes). These analyses will be performed using the QT/QTc population.

8.11.8 Physical Examinations

Physical examination findings will be presented in a subject listing.

8.11.9 Prior and Concomitant Medications and Procedures

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (Version March 2017) and classified according to ATC codes levels 2 (therapeutic sublevel) and 4 (chemical sublevel). Prior medications are defined as all medications with start date before first dose date. Concomitant medications are defined as all medications with start

date on or after the first dose date. Medications with an incomplete start or end date will be considered concomitant unless it is clear from the incomplete start and end date that the medication started before the first dose date. Prior and concomitant medication data will be listed.

The concomitant procedures will also be listed.

8.12 Sample Size

The planned sample size is 20 subjects per cohort, with 15 active and 5 placebo. This is deemed enough to provide sufficient separation of exposures between dose groups and to allow for an assessment of safety versus dose group/exposure. Assuming a SD on the log scale of 0.413 for plasma AUC_{0-24} (based on Day 7 mean AUC_{0-24} data for 200 mg from study MNK111857), 15 active subjects per cohort provides close to 80% power at a type I error rate of 0.1 to detect a difference from one in the ratio of geometric means between the 2 highest possible dose levels (and greater than 90% power for the middle 2 dose levels) given the previously seen slightly greater than dose proportionality increase in systematic exposure. The placebo subjects are included primarily to provide a reference group for comparison of safety in the target population.

8.13 General Conventions for Tables, Listings and Figures

For summary tables, unless otherwise specified, the number of decimal places provided in the SAS output will be based on the accuracy of the least accurate value in the raw data as follows:

- n — integer
- Arithmetic mean — 1 decimal place more than the least accurate number in the raw data
- SD — 2 decimal places more than the least accurate number in the raw data
- Median — 1 decimal place more than the least accurate number in the raw data
- Minimum — same number of decimal places as raw data
- Maximum — same number of decimal places as raw data

9 TABLES, FIGURES, AND LISTINGS

Table/Figure Number	Table/Figure Name
Section 14.1	**Demographic and Subject Characteristics Data Summaries**
Table 14.1.1	Summary of Screening Outcome (All Subjects Set)
Table 14.1.2	Summary of Subject Disposition (All Randomized Set)
Table 14.1.3	Summary of Analysis Populations (All Randomized Set)
Table 14.1.4	Summary of Demographic Data (Safety Set)
Table 14.1.5	Summary of Baseline Physical Measurements (Safety Set)
Table 14.1.6	Summary of Study Drug Exposure (Safety Set)
Section 14.2	**Efficacy/Pharmacokinetic/Pharmacodynamic Data Summaries**
Section 14.2.1	**Efficacy Data Summaries**
Table 14.2.1.1.1	Summary of Daily Frequency of Hot Flashes - Twice Daily Diary (Full Analysis Set)
Table 14.2.1.1.2	Summary of Average Daily Frequency of Hot Flashes By Week and Change from Week -1 - Twice Daily Diary (Full Analysis Set)
Table 14.2.1.1.3	Statistical Analysis of Change from Week -1 in Average Daily Frequency of Moderate/Severe Hot Flashes - Twice Daily Diary (Full Analysis Set)
Table 14.2.1.2.1	Summary of Hot Flashes Average Severity - Twice Daily Diary (Full Analysis Set)
Table 14.2.1.2.2	Summary of Average Daily Severity of Hot Flashes by Week and Change from Week -1 - Twice Daily Diary (Full Analysis Set)
Table 14.2.1.2.3	Statistical Analysis of Change from Week -1 in Hot Flashes Average Daily Severity - Twice Daily Diary (Full Analysis Set)
Table 14.2.1.3.1	Summary of Hot Flashes Severity Score - Twice Daily Diary (Full Analysis Set)
Table 14.2.1.3.2	Summary of Average Daily Severity Score of Hot Flashes by Week and Change from Week -1 - Twice Daily Diary(Full Analysis Set)
Table 14.2.1.3.3	Statistical Analysis of Change from Week -1 in Hot Flashes

Table 14.2.2.3.1	Summary of Night Time Awakenings (Total) - Continuous Hot Flash Diary (Full Analysis Set)
Table 14.2.2.3.2	Summary of Average Daily Night Time Awakenings (Total) by Week - Continuous Hot Flash Diary (Full Analysis Set)
Table 14.2.2.3.3	Statistical Analysis of Change from Day -1 in Daily Night Time Awakenings (Total) - Continuous Hot Flash Diary (Full Analysis Set)
Table 14.2.3.1	Summary of Daily Frequency of Hot Flashes Detected by the Device - Sternal Skin Conductance (Full Analysis Set)
Table 14.2.3.2	Summary of Average Daily Frequency of Hot Flashes Detected by the Device by Week - Sternal Skin Conductance (Full Analysis Set)
Table 14.2.3.3	Statistical Analysis of Change from Day -1 in Daily Frequency of Hot Flashes Detected by the Device - Sternal Skin Conductance (Full Analysis Set)
Table 14.2.4.1	Summary of Luteinizing Hormone AUC0-8 and Change from Day -1 (Full Analysis Set)
Table 14.2.4.2	Statistical Analysis of Luteinizing Hormone AUC0-8 Change from Day -1 (Full Analysis Set)
Figure 14.2.1.1.1	Mean (+/- SE) Daily Frequency of Hot Flashes - Twice Daily Diary (Full Analysis Set)
Figure 14.2.1.2.1	Mean (+/- SE) Daily Hot Flashes Average Severity - Twice Daily Diary (Full Analysis Set)
Figure 14.2.1.3.1	Mean (+/- SE) Daily Hot Flashes Severity Score - Twice Daily Diary (Full Analysis Set)
Figure 14.2.1.4.1	Mean (+/- SE) Night Time Awakenings Secondary to Hot Flashes - Twice Daily Diary (Full Analysis Set)
Figure 14.2.1.5.1	Mean (+/- SE) Night Time Awakenings (Total) - Twice Daily Diary (Full Analysis Set)
Figure 14.2.2.1.1	Mean (+/- SE) Daily Frequency of Hot Flashes - Continuous Hot Flash Diary (Full Analysis Set)
Figure 14.2.2.2.1	Mean (+/- SE) Night Time Awakenings Secondary to Hot Flashes

	(Safety Set)
Table 14.3.5.2	Absolute and Change from Baseline for Laboratory Hormones Results (Safety Set)
Table 14.3.5.3	Laboratory Shift in Normal Range Indicator by Category (Chemistry, Hematology, Lipids and Coagulation) (Safety Set)
Table 14.3.5.4	Laboratory Shift in Normal Range Indicator of Hormones Results (Safety Set)
Table 14.3.5.5	Absolute and Change from Baseline Vital Signs (Safety Set)
Table 14.3.5.6	Summary Statistics of 12-Lead ECG Values and Change from Baseline (Safety Set)
Table 14.3.5.7.1.1	Summary of Daily Maximum QTcB and QTcF Interval Results (Safety Set)
Table 14.3.5.7.1.2	Summary of Overall Maximum Post-Baseline QTcB and QTcF Interval Results (Safety Set)
Table 14.3.5.7.1.3	Summary of Overall Maximum Predose QTcB and QTcF Interval Results (Safety Set)
Table 14.3.5.7.2.1	Summary of Daily Maximum Change From Baseline QTcB and QTcF Interval Results (Safety Set)
Table 14.3.5.7.2.2	Summary of Overall Maximum Change From Baseline QTcB and QTcF Interval Results (Safety Set)
Table 14.3.5.7.3	Abnormal Interpretation for 12-Lead ECG (Safety Set)
Table 14.3.5.8.1	Analysis for Arrhythmias by Continuous Holter Monitoring by Day (Safety Set)
Table 14.3.5.8.2	Analysis for Post-Baseline Arrhythmias by Continuous Holter Monitoring (Safety Set)

Listing Number	**Listing Name**
Section 16.1.7	**Randomization**
Listing 16.1.7	Randomization Allocation (All Randomized Set)

Section 16.2.1	**Discontinued Subjects**
Listing 16.2.1.1	Screening Outcome (All Subjects Set)
Listing 16.2.1.2	Subject Disposition (Safety Set)
Section 16.2.2	**Protocol Deviations**
Listing 16.2.2	Protocol Deviations (All Randomized Set)
Section 16.2.3	**Subjects Excluded from Analysis**
Listing 16.2.3	Exclusions from Analysis Populations (All Randomized Set)
Section 16.2.4	**Demographic Data**
Listing 16.2.4.1	Demographics Data (Safety Set)
Listing 16.2.4.2	Physical Measurements (Safety Set)
Listing 16.2.4.3	Inclusion/Exclusion Criteria (Safety Set)
Listing 16.2.4.4	Consent Information (Safety Set)
Listing 16.2.4.5	Medical and Surgical History (Safety Set)
Listing 16.2.4.6	Prior and Concomitant Medications (Safety Set)
Listing 16.2.4.7	Concomitant Procedures (Safety Set)
Listing 16.2.4.8	Visit Dates (Safety Set)
Section 16.2.5	**Compliance and/or Drug Concentration Data**
Listing 16.2.5.1	Study Drug Dosing (Safety Set)
Section 16.2.6	**Individual Efficacy Data**
Section 16.2.6.1	**Individual Efficacy Response Data**
Listing 16.2.6.1.1.1	Hot Flash Twice Daily Diaries Data (Full Analysis Set)
Listing 16.2.6.1.1.2	Hot Flash Twice Daily Diaries Data - Daily Derivation (Full Analysis Set)
Listing 16.2.6.1.1.3	Hot Flash Twice Daily Diaries Data - Weekly Derivation (Full Analysis Set)
Listing 16.2.6.1.2.1	Continuous Hot Flash Diary Data (Full Analysis Set)
Listing 16.2.6.1.2.2	Continuous Hot Flash Diary Data - Daily Derivation (Full Analysis Set)

VERSION HISTORY

Version	Date	List principal changes from previous version
2.0	**27FEB2017**	- Section 4.1 and 4.10.5 has been changed to take into account the changes in the protocol version 4.0 dated 13DEC2016. - The night time awakenings analyses are now separated in 2 groups: NTA secondary to hot flashes and NTA, Total - The baseline for ECG is now defined as the time matched assessment done at Day -1 - The maximum QTcB and QTcF values are analysed daily and overall.
1.0	**01DEC2016**	NA. Initial Version

STATISTICAL ANALYSIS PLAN FOR PHARMACOKINETICS

Protocol RELENT-1

EVALUATION OF THE PHARMACOKINETICS AND SAFETY OF NT-814 IN POST-MENOPAUSAL WOMEN WITH VASOMOTOR SYMPTOMS

Version: Final 1.0

Date: 5 May 2017

REVISION HISTORY

Version	Version Date	Author	Summary of Changes Made
Draft 1.0	April 10, 2017	PPD	New Document
Final 1.0	May 5, 2017	PPD	

SIGNATURE PAGE - NERRE THERAPEUTICS LTD.

Declaration

The undersigned has/have reviewed and agree to the statistical analyses and procedures of this clinical study, as presented in this document.

PPD

Date (DD Mmm YYYY)

8th May 2017

Date (DD Mmm YYYY)

SIGNATURE PAGE - NERRE THERAPEUTICS LTD.

Declaration

The undersigned has/have reviewed and agree to the statistical analyses and procedures of this clinical study, as presented in this document.

PPD

12 MAY 2017

Date (DD Mmm YYYY)

Date (DD Mmm YYYY)

SIGNATURE PAGE - PAREXEL

Declaration

The undersigned agree to the statistical analyses and procedures of this clinical study.

If this document has been signed electronically, signature(s) and date(s) are present at the end of the document:

Document prepared and approved by:

PPD

05 MAY 2017

Date (DD Mmm YYYY)

TABLE OF CONTENTS

ABBREVIATION AND ACRONYM LIST

Abbreviation / Acronym	Definition / Expansion
$\%AUC_{ex}$	Percentage of $AUC_{0\text{-}inf}$ obtained by extrapolation
$AUC_{0\text{-}24}$	Area under the concentration-time curve from time zero to 24 hours postdose
$AUC_{0\text{-}\infty}$	AUC from time zero extrapolated to infinity
$AUC_{0\text{-}t}$	Area under the concentration-time curve from time zero to the time of the last quantifiable concentration postdose
$AUC_{0\text{-}\tau}$	AUC over the dosing interval
BLQ	Below the limit of quantification
CL/F	Apparent total systemic clearance
C_{last}	Last quantifiable concentration at t_{last}
C_{max}	Peak concentration
C_{min}	Minimum observed concentration at steady state
CRF	Case report form
CSP	Clinical study protocol
C_{τ}	Trough concentration observed predose during multiple dosing
CV%	Coefficient of variation
GCV%	Geometric coefficient of variation
GM	Geometric mean
LLOQ	Lower limit of quantification
PD	Pharmacodynamic
PK	Pharmacokinetic
PKAS	Pharmacokinetic analysis set
PTF	Peak to trough fluctuation
QCD	Quantitative Clinical Development
$R_{ac}(AUC)$	Accumulation ratio calculated using AUC
$R_{ac}(C_{max})$	Accumulation ratio calculated using C_{max}
R^2 adjusted	Adjusted correlation coefficient
SAP	Statistical Analysis Plan
SD	Standard deviation
$t_{½}$	Apparent terminal phase half-life
t_{last}	Time of last quantifiable concentration

Abbreviation / Acronym	Definition / Expansion
TLFs	Tables, listings and figures
T_{max}	Time of occurrence of C_{max}
WNL	WinNonlin
λ_z	Terminal elimination rate constant

STATISTICAL ANALYSIS PLAN

This Statistical Analysis Plan (SAP) details the methodology to be used in analyzing the pharmacokinetic (PK) data and outlines the PK Tables, Listings and Figures (TLFs). It describes the variables and populations, anticipated data transformations and manipulations and other details of the analyses not provided in the Clinical Study Protocol (CSP).

The PK analyses described are based on the final CSP Version 4.0, dated 13 December 2016. The SAP will be finalized prior to database lock and describes the pharmacokinetic analysis as it is foreseen when the study is being planned. If circumstances should arise during the study rendering this analysis inappropriate, or if improved methods of analysis should arise, updates to the analyses may be made. Any deviations from the SAP after database lock, reasons for such deviations and all alternative or additional statistical analyses that may be performed, will be described in a SAP Addendum.

1. PRIMARY OBJECTIVE

To evaluate the PK and safety profile of multiple ascending dose levels of NT-814 compared to placebo, by once daily administration of each dose for 14 days

2. PRIMARY PHARMACOKINETIC ENDPOINTS

The PK endpoints are:

- peak concentration (C_{max})
- time of occurrence of C_{max} (T_{max})
- area under the curve (AUC) from time zero to the time of the last quantifiable concentration (AUC_{0-t})
- AUC in the dosing interval ($AUC_{0-\tau}$, synonymous with AUC_{0-24})
- AUC from time zero extrapolated to infinity ($AUC_{0-\infty}$)
- apparent terminal phase half-life ($t_{½}$)
- apparent clearance (CL/F)

The PK parameters will be derived on Day 1, Day 7, and Day 14, as appropriate.

3. STUDY DESIGN

This is a multi-center, double-blind, randomized, placebo-controlled multiple ascending dose trial in post-menopausal women with vasomotor symptoms. Subjects will be recruited into sequentially dosed cohorts; Cohort 1 (50 mg NT-814 or placebo), Cohort 2 (100 mg NT-814 or placebo), Cohort 3 (150 mg NT-814 or placebo) and Cohort 4 (300 mg NT-814 or placebo). For each cohort, subjects will enter a 4-week screening period to determine eligibility. Twenty eligible subjects will be randomized per cohort (15 active and 5 placebo).

On Day 1, the subjects will be randomized in a 3:1 ratio to once daily (morning) doses of NT-814 or placebo, to be taken for a total of 14 days. Subjects will be monitored as an inpatient from Day -1 to Day 8 and will undergo blood draws for intensive PK sampling, safety monitoring and for impact on blood hormonal profiling as well as 24-hour skin conductance measurements and continuous completion of hot flash diaries.

Subjects will be discharged from the clinic on Day 8 without any monitoring devices, providing there are no safety concerns and will undergo dosing for a further 5 days as an outpatient. Subjects will be instructed to continue recording details of the hot flashes experienced, using the twice daily diary. They will be provided with the continuous daily diary and instructed to start completing this upon waking on the morning of Day 14 prior to admission to the clinic. They will be re-admitted to clinic on the morning of Day 14 for the final dose and undergo the same assessments with the same devices including Holter and skin conductance as Day 1. Subjects will be discharged following the 24-hour post Day 14 assessments (Day 15). Subjects will return to the clinic, as outpatients, for a single PK sample on the mornings of Days 16 and 17 (48 hours and 72 hours after the Day 14 dose respectively).

Throughout the study, subjects will be closely monitored for safety including assessments of AEs, 12-lead ECGs, arterial oxygen saturation using pulse oximetry, vital signs (sitting and standing BP, heart rate, oral body temperature, and respiration rate), clinical labs and physical examinations.

Blood samples for PK analysis will be collected at the following time points on Day 1, Day 7 and Day 14: Pre-dose, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 24.0 hours. Additional sampling times of 48 and 72 hours will be collected following the Day 14 dose. The pre-dose sample will be collected within 30 minutes prior to dose administration.

4. STUDY POPULATION

Post-menopausal female subjects 40-65 years of age

5. STATISTICAL BASIS FOR SAMPLE SIZE

The planned sample size is 20 subjects per cohort, with 15 active and 5 placebo. This is deemed enough to provide sufficient separation of exposures between dose groups and to allow for an assessment of safety versus dose group/exposure. Assuming a standard deviation on the log scale of 0.413 for plasma AUC_{0-24} (based on Day 7 mean AUC_{0-24} data for 200 mg from study MNK111857), 15 active subjects per cohort provides close to 80% power at a type I error rate of 0.1 to detect a difference from one in the ratio of geometric means between the two highest possible dose levels (and greater than 90% power for the middle two dose levels) given the previously seen slightly greater than dose proportionality increase in systemic exposure. The placebo subjects are included primarily to provide a reference group for comparison of safety in the target population.

6. RANDOMIZATION

On Day 1, the subjects will be randomized in a 3:1 ratio to once daily (morning) doses of NT-814 or placebo, to be taken for a total of 14 days.

7. ANALYSIS POPULATIONS

The following data sets will be used for the analysis:

- **Safety Analysis Set**: All subjects who receive at least one dose of double-blind study drug. Subjects will be analyzed according to treatment received.
- **Pharmacokinetic Analysis Set (PKAS):** All subjects who received at least one dose of active study drug and for whom the PK data are considered sufficient. Subjects or data may be excluded from the PKAS at the discretion of the PK Scientist, including, but not limited to the following:
  - Concomitant medication which could render the plasma concentration-time profile unreliable

Any data excluded will be discussed in the CSR. Any excluded subject will have their concentration and PK parameter data listed only. Subjects who receive placebo will not be part of the PKAS.

Analysis Sets will be identified prior to the unblinding of the study data.

8. STATISTICAL ANALYSIS CONVENTIONS

8.1 Pharmacokinetic Variables

Derivation of PK parameters will be the responsibility of Quantitative Clinical Development (QCD), PAREXEL International. The PK parameters will be determined for NT-814 in plasma following single and multiple dose administration.

8.1.1 Handling of Values Below the Limit of Quantification

All plasma concentrations below the lower limit of quantification (LLOQ) or missing data will be labeled as below the limit of quantification (BLQ) or missing, respectively, in the concentration data listings.

Considerations in the handling of BLQ data for the generation of plasma PK Parameters

BLQ values will be imputed in the PK concentration dataset used for the derivation of PK parameters. The following rules will be applied:

- BLQs at the beginning of a subject profile (i.e. before the first incidence of a measurable concentration) will be assigned to zero.
- BLQs at the end of a subject profile (i.e. after the last incidence of a measurable concentration) will be set to missing.
- Single BLQs which fall between two measurable concentrations will be set to missing.
- Consecutive BLQs which fall between measureable concentrations will be set to missing. Measurable concentrations after consecutive BLQs will also be set to missing.

Considerations in the handling of BLQ data for plotting of individual plasma PK concentration data

BLQ values will be imputed for individual concentration-time plots according to the following rules:

- BLQs at the beginning of a subject profile (i.e. before the first incidence of a measurable concentration) will be assigned to zero.
- BLQs at the end of a subject profile (i.e. after the last incidence of a measurable concentration) will be set to missing.
- Single BLQs which fall between two measurable concentrations will be set to missing.

- Consecutive BLQs which fall between measureable concentrations will be set to zero. Measurable concentrations after consecutive BLQs will be retained.

For linear plots, zero concentration value(s) will be included in the plot.

For log-linear plots, zero concentration value(s) will be assigned a missing value.

A reference line indicating LLOQ may be included on plots.

Considerations in the handling of BLQ data for summarizing plasma PK concentration data

BLQ values will be imputed for mean/median concentration-time plots and tabular summaries according to the following rules:

- All BLQ values will be set to zero, except when an individual BLQ falls between two measurable values, in which case it will be set to missing.
- All BLQ values will be substituted with the LLOQ for calculation of geometric means and geometric CVs
- Measurable concentrations after consecutive BLQs will be set to missing.

Zero mean or median values will be included in tabular summaries.

A high proportion of BLQ values may affect derivation of standard deviation; if more than 30% of values at a time-point are imputed, then standard deviation (SD) will not be displayed.

Tabular summaries for concentration-time data will report N (number of subjects who received treatment) and n (number of subjects with non-missing values.

8.1.2 Pharmacokinetic Parameter Calculation Methods

PK parameters will be calculated by non-compartmental analysis methods from the concentration-time data using Phoenix® WinNonlin® (WNL) Professional (Version 6.3 or higher) following these guidelines:

- Actual sampling times relative to dosing rather than nominal times will be used in the calculation of all derived PK parameters.
- There will be no imputation of missing data.
- Any subjects with missing concentration data will be included in the PK analysis set provided that at least C_{max} and AUC_{0-t} can be reliably calculated.

PK parameters will be estimated according to the following guidelines:

- C_{max} will be obtained directly from the concentration-time data.
- T_{max} is the time at which C_{max} is observed.
- λ_Z will be estimated at terminal phase by linear regression after log-transformation of the concentrations:
  - Only those data points that are judged to describe the terminal log-linear decline will be used in the regression.
  - A minimum number of three data points in the terminal phase will be used in calculating λ_z with the line of regression starting at any post-C_{max} data point (C_{max} should not be part of the regression slope). The adjusted correlation coefficient (R^2 adjusted) in general should be greater than 0.90. Any value < 0.90 but ≥ 0.80 will be flagged but may be used at the PK Scientist's best knowledge and judgment; any value < 0.80 will be excluded from the analysis.
  - The interval used to determine λz should be equal or greater than 1.5-fold the estimated half-life or otherwise flagged and excluded from the analysis.
- $t_{½}$ will be calculated as $\ln 2/\lambda_Z$.
- AUC is calculated by the linear up/log down method (linear method will be employed for all incremental trapezoids arising from increasing concentrations and the logarithmic method will be used for those arising from decreasing concentrations.

  $AUC_{0-t} = \int_0^t C(t)dt$

  $AUC_{0-inf} = \int_0^t C(t)dt + \int_t^\infty C(t)dt = AUC_{0-t} + C_t/\lambda_z$

  $AUC_{0\ \tau,ss}$ will be calculated at steady state during the time interval between consecutive doses
- C_t is last observed quantifiable concentration.
- $\%AUC_{ex}$ will be calculated as $(1 - [AUC_{0-t}/AUC_{0-inf}]) \times 100$. The $\%AUC_{ex}$ should not exceed 20% for each individual profile. If the $\%AUC_{ex}$ is more than 20%, the individual result should be flagged and mentioned in the report as well as all parameters depending on AUC_{0-inf}. If the $\%AUC_{ex}$ is greater than 30% the value and all dependent parameters should be flagged and excluded from the analysis.
- CL/F will be calculated as dose/AUC_{0-inf}, parent drug only.

The following PK parameters may also be derived.

- $R_{ac}(AUC)$ Accumulation ratio is calculated as: $AUC_{0-\tau}$ (last dose interval)/ $AUC_{0-\tau}$ (first dose interval).
- $R_{ac}(C_{max})$ Accumulation ratio is calculated as: C_{max} (last dose interval)/ C_{max} (first dose interval).

8.2 Statistical Analysis Methods

8.2.1 Software

Statistical analysis of PK parameters will be performed using Phoenix® WinNonlin® (WNL) Professional (Version 6.3 or higher) and will be based on the PK analysis set (PKAS).

8.2.2 Missing Data

There will be no imputation of missing data. The details on handling PK data below the lower level of quantification (LLOQ) are given in Section 8.1.2.

8.2.3 Pharmacokinetic Concentrations and Variables

Pharmacokinetic plasma concentration data will be listed by cohort and subject including actual sampling times relative to dosing. Plasma concentrations will be summarized by cohort, day and nominal time point. The following descriptive statistics will be presented for plasma concentrations obtained at each nominal time point: n, arithmetic mean, SD, coefficient of variation (CV%), geometric mean (GM), geometric coefficient of variation (GCV%), median, minimum and maximum values.

Plasma pharmacokinetic parameters will be listed by cohort, subject and day, and summarized by cohort and day. Descriptive statistics for calculated PK parameters will include: n, arithmetic mean, SD, CV%, GM, GCV%, median, minimum and maximum values. For T_{max}, only median, minimum and maximum values will be presented. No descriptive statistics will be determined when fewer than three individual PK parameters are available.

Individual plasma concentration versus actual times will be plotted by cohort and day in linear and semi-logarithmic scale. Combined individual plasma concentrations versus actual times will be plotted by cohort and day.

Arithmetic mean plasma concentrations versus nominal times will be presented by day on a linear scale and semi-logarithmic scale. All treatments will be overlaid on the same plot.

8.2.4 Presentation of PK Data, Descriptive Statistics and PK Assessment

The following rules will be followed with regards to the number of decimal places and presentation of data in the tables and listings of concentration data:

- The individual concentrations will be reported to the same precision as the source data (for example, if the source data is presented to five significant digits, the individual values will be presented to five significant digits).
- The mean, standard deviation (SD), geometric mean and median will be tabulated to one more significant digit compared to the source data, but with a maximum of four significant digits.
- Minimum and maximum values will be tabulated to the same precision as the source data, but with a maximum of four significant digits.
- Coefficient of variation (CV%) and geometric coefficient of variation (GCV)% will be presented to one decimal place.

The following rules will be followed with regards to the number of decimal places and presentation of data in the tables and listings of PK parameters:

- Individual PK parameters will be presented to four significant digits, with the exception of T_{max}, which will be presented to two decimal places. In addition, parameters directly derived from source data (e.g. C_{max}) shall be reported with the same precision as the source data (if this is not four significant digits).
- The mean, geometric mean, median and SD values will be reported to four significant digits, all other descriptive statistics will be reported to three significant digits except for CV% and GCV%, which will be presented to one decimal place. For Tmax, the minimum and maximum will be presented to two decimals places and the rest of the descriptive statistics to three decimal places.
- P-values will be presented to four decimal places.
- Estimates and confidence intervals in the form of percentages will be presented to two decimal places.

Source data shall be used in all derived PK parameter calculations without prior rounding.

9. REFERENCES

1. WinNonlin Professional Software Version 6.3. http://www.pharsight.com

10. TABLES TO BE INCLUDED IN THE CLINICAL STUDY REPORT

Pharmacokinetic Data

Table 1.1	Individual and Summary of Plasma Concentrations of NT-814 versus Nominal Sampling Times by Cohort and Day (Pharmacokinetic Population)
Table 1.2	Individual and Summary of Plasma Pharmacokinetic Parameters of NT-814 by Cohort and Day (Pharmacokinetic Population)
Table 1.3	Power Model for Dose Proportionality (Pharmacokinetic Population)

11. FIGURES TO BE INCLUDED IN THE CLINICAL STUDY REPORT

Figure 1.1	Individual Profiles for NT-814 Plasma Concentration Time Data (Linear and Semi-logarithmic Scale) (Pharmacokinetic Population)
Figure 1.2	Mean Profiles for NT-814 Plasma Concentration Time Data Overlaid by Cohort and Day (Linear and Semi-logarithmic Scale) (Pharmacokinetic Population)
Figure 1.3	Assessment of NT-814 Dose Proportionality (Pharmacokinetic Population)

12. LISTINGS TO BE INCLUDED IN THE CLINICAL STUDY REPORT

Listing 1.1	Individual Blood Sampling Times and Concentrations of NT-814
Listing 1.2	Individual Pharmacokinetic Parameters of NT-814 in Plasma